FIRST DUTCH - JAPANESE WORKSHOP ON THE TREATMENT OF MUNICIPAL WASTE WATER

8-11 April 1991, Heelsum, The Netherlands

Part II

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STABILITY OF PHOSPHORUS REMOVAL AND POPULATION OF BIO-P-BACTERIA UNDER SHORT TERM DISTURBANCES IN SEQUENCING BATCH REACTOR ACTIVATED SLUDGE PROCESS

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Abstract-----Laboratory-scale sequencing batch reactor(SBR) activated sludge processes were operated to investigate the stability of phosphorus removal capacity and population of bio-P-bacteria under short term disturbances (2 to 5 days) and characterize the structure and dynamics of bacterial population of activated sludge for phosphorus removal. The performance on phosphorus removal deteriorated in 3 days, whereas it took more than 1 week for the recovery and the time required prolonged with the length of disturbances. The responses of phosphorus removal activity and quinone profiles suggested the deterioration and slow recovery was dependent not on the decrease in the activity of each bio-P-bacteria but on the decrease in their population, i.e. species succession of bacteria. The isolated strains of *Acinetobacter* and *Pseudomonas*. were seemed to be predominant species in the total bacterial population in the activated sludge. These strains showed high activity of phosphorus removal and low specific growth rate

Key words-----activated sludge population dynamics, phosphorus removal, rainwater intrusion, quinone profile, *Acinetobacter*, *Pseudomonas*

INTRODUCTION

Biological phosphorus removal processes are studied widely to prevent water pollution caused by artificial eutrophication. Although most studies have been carried out by continuous flow reactors (CFS), sequencing batch reactor, SBR, activated sludge processes with anaerobic and aerobic operations have also been accepted to be one of the promising processes for phosphorus removal (Okada *et al.*, 1991).

Both in SBR and CFS systems for phosphorus removal, it is widely known that activated sludge must be subjected cyclically to anaerobic and aerobic conditions. An adequate supply of volatile fatty acids was also shown to be a key factor in successful phosphorus removal (Mostert *et al.*, 1989; Jones *et al.*, 1987). In addition to these environmental or operational conditions, it is also essential to have a specific group of bacteria, bio-P-bacteria, that is able to accumulate polyphosphate and, hence, to remove phosphate in wastewater (Okada *et al.*, 1987; Wentzel *et al.*, 1986). Selection, accumulation, or enrichment of bio-P-bacteria are regarded to be the most important factor for starting up and stable operation of effective biological phosphorus removal processes (Bowker and Stensel, 1987).

Among numerous bacteria in activated sludge ecosystem, *Acinetobacter* spp. were claimed to be responsible for phosphorus removal. Actually, many strains of the genus *Acinetobacter* have been isolated from activated sludge processes for biological phosphorus removal and was regarded to be a dominant polyphosphate-accumulating species in the sludge (Fuhs and Chen, 1975; Hao and Chang, 1987; Murphy and Lotter, 1986; Lotter *et al.*, 1986). Numerous studies to elucidate the metabolic control mechanisms involved in the enhanced removal of phosphorus, therefore, have been reported presupposing a strain of *Acinetobacter* as a model organism (Lotter and Dubery, 1987; Groenestijn and Deinema, 1987; Groenestijn *et al.*, 1989).

However, *Acinetobacter* spp. were found to form a large fraction (40%) of isolated strains not only in the activated sludge for phosphorus removal but also in conventional activated sludge with fully aerobic condition (Lotter *et al.*, 1986). Those strains isolated from conventional sludge were equivalent in terms of substrate utilization, polyphosphate and polyhydroxybutyrate (PHB) accumulations with those isolated from phosphorus removal sludge.

It was also demonstrated that a strain of *Acinetobacter calcoaeticus* did not exhibit typical features of phosphorus removal sludge, i.e. anaerobiosis did not accelerate the rate of phosphorus uptake, and no significant uptake of acetate was observed in anaerobic condition (Ohtake *et al.*, 1985). Another strain of *Acinetobacter* sp. isolated from activated sludge for phosphorus removal did not release phosphate under anaerobic condition fed with acetate (Hao and Chang, 1987). The aeaerobiosis also reduced viability of the strain in spite of the reported fact that prolonged anaerobic condition in a Phoredox system had no detrimental effect on sludge characteristics and improved phosphorus removal capacity.

The efforts to identify or isolate other strains than *Acinetobacter* spp. revealed that such genus as *Pseudomonas* and *Klebsiera* might be regarded as bio-P-bacteria (Suresh *et al.*, 1984; Gersberg and Allen, 1984). The isolation of new strains, however, does not always assure that total population of bacteria in the activated sludge was examined. It is practically impossible to recover all kinds of bacteria present in activated sludge (Pike and Curds, 1971). In fact, the number of aerobic heterotrophic bacteria detected as "viable colonies" only accounts for small percentages of total population. The isolation and pure culture studies provides only limited information on the "countable and cultivable" bacterial population rather than the total population.

Recent development of methods for *in situ* identification and enumeration of specific microorganisms presented quantitative information on the role of *Acinetobacter*. By using electron dispersive micro-analysis of X-rays, Cloete and Steyn (1988) estimated that a maximum of 34% of the observed phosphorus removal could be removed by *Acinetobacter* as polyphosphate. Also, *in situ* counts of *Acinetobacter* by the fluorescent antibody technique indicated that they constituted less than 10% of total bacterial count by the acridine orange staining, whereas the common API-20E identification system based on cultivation indicated that *Acinetobacter* was the dominant species.

Although the above mentioned methods give us quantitative information on *Acinetobacter* or a selected species of bacteria, still they do not provide little information on the total population. The microbial chemotaxonomy has been applied to mixed bacterial populations such as activated sludge for their *in situ* characterization on total population (Hiraishi, 1988). The analyses of isoprenoid quinones in the activated sludge both for conventional and phosphorus removal revealed that there were no significant difference in the structure of bacterial community, nor any evidence on the dominancy of *Acinetobacter* (Hiraishi *et al.*, 1989).

Practical applications and pilot plant studies in Japan on biological phosphorus removal during the decade pointed out that disturbances such as rapid decrease in temperature and inflow of rainwater, i.e. hydraulic shock loading and/or dilution of wastewater, may cause serious damage to plant performance (JSWA, 1988; Minagawa *et al.*, 1989). It was recommended, in addition to prevent rainwater intrusion, to utilize primary sludge as an agent to increase inflow organic concentration and stabilize effluent quality and activated sludge bacterial population.

The purpose of this study is to investigate the stability of phosphorus removal and population of bio-P-bacteria under short term disturbances such as rainwater intrusion and to characterize the structure and dynamics of bacterial population in activated sludge for phosphorus removal. Laboratory systems of SBR were given disturbances from 2 to 5 days by introducing full-time aeration or diluted wastewater and the responses of bacterial population in activated sludge was monitored by phosphorus removal activity, quinone profiles, and species identification in addition to plant performance.

MATERIALS AND METHODS

Configuration and operation of SBR activated sludge systems

Two laboratory-scale SBR activated sludge processes were operated in an air-conditioned room of 20 ± 2 deg. C. Each reactor consisted of a 5 l acrylic vessel with facilities for wastewater inflow, mixing , aeration, effluent discharge and excess sludge removal (Okada, *et al.*,1991). Acetate was used as a carbon source (TOC = 320 mg l^{-1}) and inorganic nutrients were added to have T-N and T-P concentrations of 106 mg l^{-1} and 15 mg l^{-1}, respectively. Actually, concentrated wastewater was prepared and fed into reactors with tap water for dilution after autoclaving it for 15 min at 120 deg. C. BOD loading was 0.36 kg BOD m^{-3} d^{-1}. BOD concentration of the wastewater was 720 mg $^{-1}$ and the ratio of BOD : N : P were 100 : 14.7 : 2.1. Hydraulic retention time (HRT) was kept at 2 days. MLSS was maintained at around 4,000 mg l^{-1}.

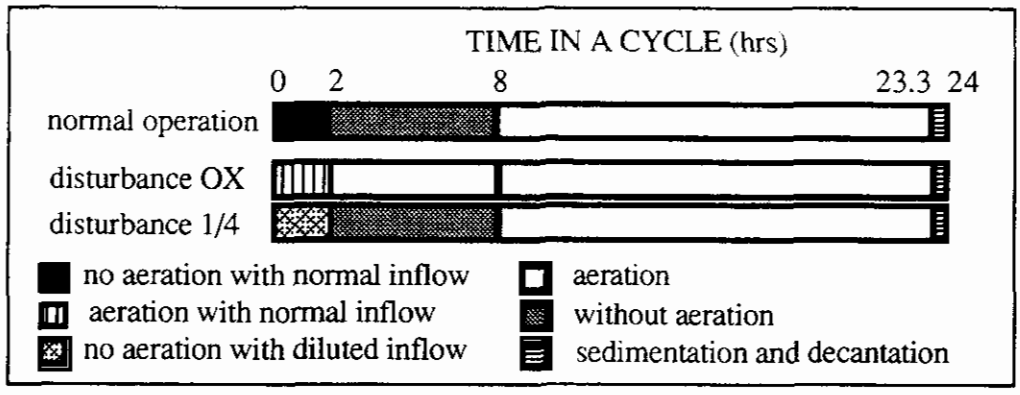

Fig. 1. Operational schedules for normal operation and disturbances.

The SBR systems were operated in a cycle of 24 h. Fig. 1 shows operational schedules of normal operation and two types of disturbances, i.e. OX and 1/4. The SBR was operated on the schedule of normal operation for more than one month to confirm steady-state operation both in effluent quality and activated sludge microbial ecosystem. Anaerobic/aerobic operations in the normal operation was changed into fully aerobic operation throughout a cycle, i.e. continuous aeration from the beginning to 23.5 h, with the same wastewater inflow for 2 to 5 days and returned to the normal operation in the disturbance OX. In the disturbance 1/4, the wastewater was diluted down to 1/4 of the original concentration without any change in the operational schedule.

The SBR operations were monitored by MLSS, SVI and effluent water quality, i.e. dissolved organic carbon (DOC), volatile fatty acids (VFA), orthophosphate (PO_4-P), redox potential (ORP) and dissolved oxygen concentration (DO). All the chemical analyses were carried out according to Standard Methods (APHA, 1985).

Activity of phosphorus removal

The activity of phosphorus removal by activated sludge in the reactor was determined by separated batch cultivations. A sample of activated sludge taken from the reactor was washed by the same synthetic wastewater as in the reactor and separated by centrifugation to remove remained organic substrate and phosphorus. The sludge was suspended into the synthetic wastewater. The mixed liquor was kept anaerobic for 8 h after purging DO less than 0.2 mg l^{-1} by nitrogen gas. The amount of phosphorus release from the activated sludge was determined in 8 h. Based on the fact that the activity of phosphorus removal in anaerobic condition is in proportion to that of phosphorus release, the activity of phosphorus removal was defined as the amount of phosphorus release for 8 hours per unit mass of MLSS, mg phosphorus mg^{-1} MLSS (Okada *et al.*, 1991).

Isolation of bio-P-bacteria and determination of phosphorus removal activity

Plate culture was used to isolate bacteria in the SBR systems using the medium with the same composition as the synthetic wastewater. Actually the concentration was increased up to 5 times of that of original. After the number of colonies became maximum, typical and representative colonies in shape and color were purified. The strain was cultivated under aerobic condition at 20 deg. C in a liquid culture of the same medium. Specific growth rate of the strain was estimated from turbidometric determinations of the increase in the cell mass concentration. When the growth of bacteria levelled off, the culture was turned into anaerobic condition for 8 h and the activity of phosphorus removal by the strain was determined.

Quinone profile analysis in activated sludge

Activated sludge was collected by centrifugation and washed with distilled water and freeze-dried. Quinones were extracted from the dried sludge (0.2 - 0.5 g) by chloroform-methanol (vol:vol = 2:1). The extract was purified by thin-layer chromatography developed with benzene. Quinone components were separated by reverse-phase high performance liquid chromatography (Shimazu LC-6A, column ; Zobax ODS) and then identified by comparing their retention times with those of standard quinones (Katayama *et al.*, 1980; Hiraishi *et al.*, 1984; Hiraishi, 1988). Identified quinones were ubiquinones and menaquinones with n isoprene units and expressed as Q-n and MK-n, respectively.

RESULTS AND DISCUSSION

Responses of reactor performance to the disturbance

Profiles of DO in a cycle of operation before and during the disturbance of 5 days are shown in Fig. 2. Changes in DO in a cycle before the disturbance was typical in SBR operation with anaerobic/aerobic condition. As shown in Fig. 3(a), no DO was observed for 8 hours. Although not shown here, ORP dropped from +100 mV down to -100 mV and nitrate nitrogen remained in the beginning of the cycle was removed by denitrification indicating anoxic and anaerobic reactions. Corresponding profiles of orthophosphate are shown in Fig. 3(a). Remarkable release of phosphorus was noted during anaerobic period. The released phosphorus was taken up in the following aerobic reaction.

Both the disturbances changed DO profile as shown in Fig. 2(b). The system was changed into aerobic condition throughout a cycle in the disturbance OX. Although no aeration was carried out in the disturbance 1/4 for 8 hours at the beginning of each cycle, the diluted wastewater could not turn the system into ananerobic condition. The aerobic condition suppressed phosphorus release in the beginning of each cycle and subsequent luxury uptake of phosphorus as shown in Fig. 3(b). Effluent phosphorus concentration, therefore, increased as shown in Fig. 4 (see later on).

The shown profiles were observed in day 3, whereas similar profiles were observed from the beginning to the end of the disturbances. It must be noted that it took more than 3 weeks to recover the same profile of phosphorus release and uptake as that shown in Fig. 3(a). Both in the disturbances for 2 and 3 days, similar profiles of DO and PO_4-P were noted. In case of 2 days disturbance, however, effluent phosphorus concentration was kept unchanged.

Fig. 4 shows responses of effluent quality and phosphorus release activity in the disturbances of 5 days. Effluent phosphorus concentration was kept low for two days both for the disturbances of OX and 1/4. It increased remarkably after day 3 and showed maximum values at day 5. The disturbances were removed at day 5 and returned to the normal operation after that, whereas it took more than three weeks to recover the same performance as that in the steady-state condition in the normal operation. Significant differences were not noted between disturbances of OX and 1/4.

differences were not noted between disturbances of OX and 1/4.

The disturbance of 3 days showed very similar responses to those of 5 days. Effluent phosphorus concentration, however, returned to the normal level within 1 week (not shown here). Taking the fact that the hydraulic retention time was kept at 2 days throughout the operation, it is most probable that phosphorus removal activity of the activated sludge was damaged during the disturbances. No deterioration in effluent quality was noted in the disturbances for 2 days. The longer disturbances seemed to have resulted in the more serious damage.

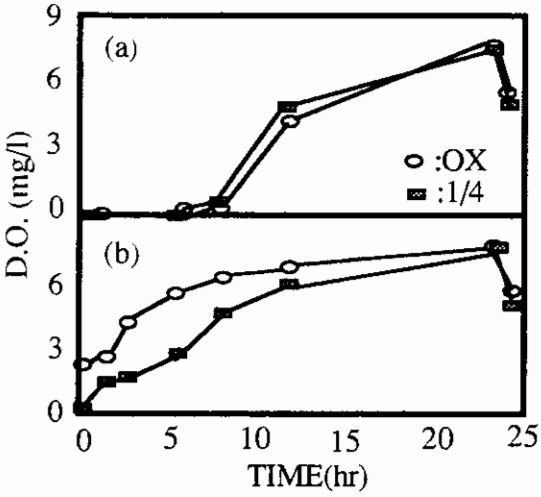

Fig. 2. Profiles of dissolved oxygen concentration (DO) in a cycle of operation before (a) and during (b: day 3) the disturbances for 5 days.

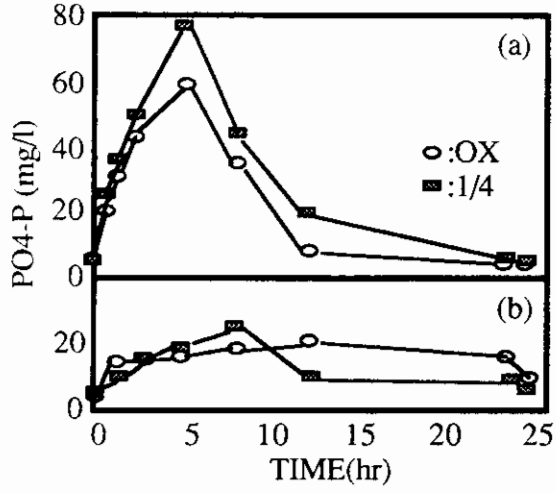

Fig. 3. Profiles of orthophosphate concentration (PO4-P) in a cycle of operation before (a) and during (b: day 3) the disturbances for 5 days.

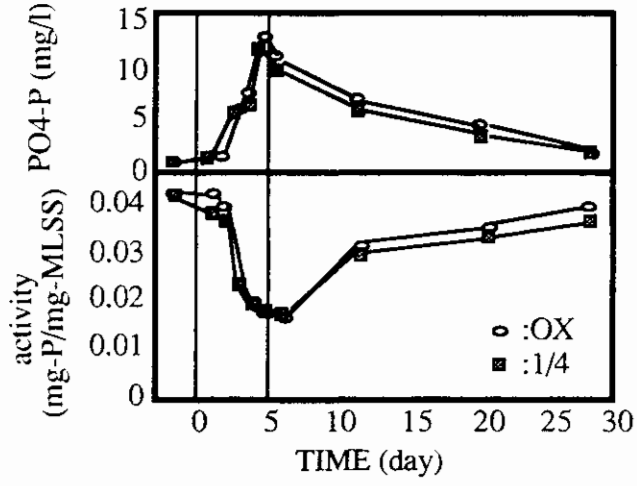

Fig. 4. Response of effluent quality (upper) and phosphorus removal activity (lower) for the disturbance of 5 days from day 0 to day 5.

Responses of activated sludge

As shown in Fig. 4, phosphorus removal activity was kept unchanged for 2 days and decreased after day 3 of disturbance. It decreased less than 50 % of the activity in the steady-state in day 5 and took more than 3 weeks to recover the original level. Similar to effluent phosphorus, the activity decreased at day 3 and took 1 week to recover the original level in the disturbance of 3 days. There was no change in the activity, however, for the disturbance of

2 days. These behavior are well coincident with that of effluent phosphorus concentration. Phosphorus removal capacity, therefore, seemed to be dependent on the activity of phosphorus removal by activated sludge.

SVI increased from 40 to 60 by the disturbance OX, whereas it was kept unchanged throughout the disturbance 1/4. Thus MLSS did not washout during the disturbances and MLSS was kept between 3,000 and 4,000 mg l. SRT estimated from excess sludge removal was more than 40 days during these operations. Taking the facts that phosphorus removal activity dropped during the disturbance and total biomass, i.e. MLSS, was kept unchanged without significant removal of biomass, the deterioration of plant performance can be referred to degradation of phosphorus removing bacteria in the activated sludge.

Responses of bacterial population

Quinone profiles in the activated sludge was determined before (day -2), during, and after the disturbances. Ubiquinone contents per unit mass of MLSS are shown in Fig. 5 with the corresponding phosphorus removal activity. The major ubiquinone detected in the sludge with high activity of phosphorus removal (day -2; steady-state operation) had 9 isoprene units, i.e. ubiquinone-9 (Q-9). The second was Q-8. Although small amount of Q-10 was detected, other quinone homologs were negligible.

Representative bacteria which contain Q-9 as a major constituent are *Acinetobacter* and *Pseudomonas* (HIraishi and Morisawa, 1990). It is most probable, therefore, that the dominant bacteria in the sludge studied are one or both of these two genus. This supports the most common understandings based on isolated strains that *Acinetobacter* and/or *Pseudomonas* are predominant in activated sludge for phosphorus removal (Fuhs and Chen, 1975; Hao and Chang, 1987; Murphy and Lotter, 1986; Lotter *et al.*, 1986; Suresh *et al.*, 1984; Gersberg and Allen, 1984).

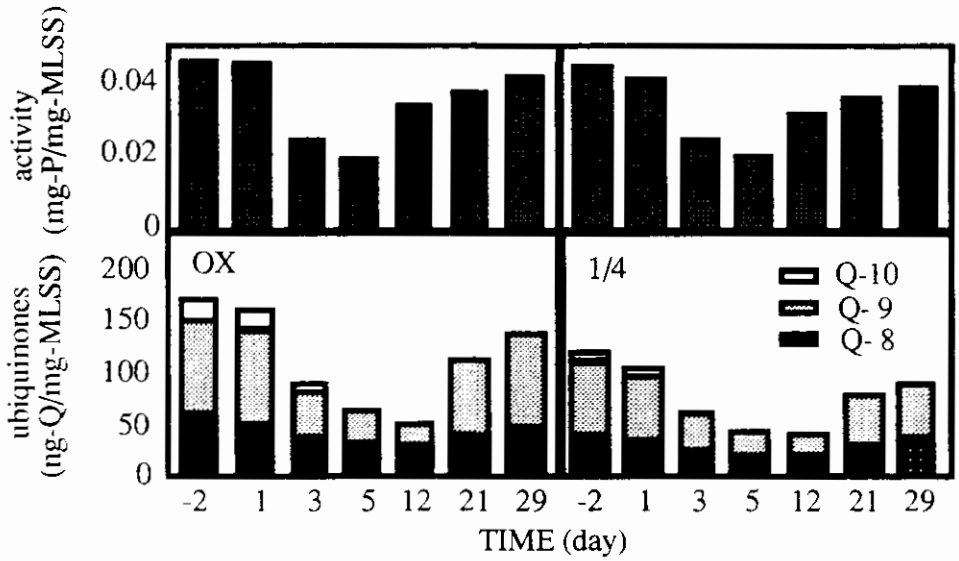

Fig. 5. Ubiquinone contents and phosphorus removal activity in the activated sludge for the disturbance of 5 days from day 0 to day 5.

On the contrary, Hiraishi *et al.*(1989) reported that Q-8 was present as the predominant ubiquinone, Q-10 was the second most common type, and Q-9 and other homologs occurred as minor component irrespective of anaerobic/aerobic or aerobic operation, i.e. activated sludge for phosphorus removal do not have different microbial population from conventional activated sludge processes. This discrepancy may partly due to the substrate used. Although not shown here, Q-9 was dominant even in fully aerobic activated sludge if it was fed with acetate. In our system, peptone, the substrate Hiraishi *et al.* used, changed the structure of bacterial community. Major constituent was Q-8 when the sludge was operated under

aerobic condition. Cyclic anaerobic and aerobic reactions, however, increased Q-9 to be the predominant.

Fig. 6 shows menaquinone contents in the activated sludge for the same disturbances of 5 days. Major menaquinones detected were MK-7 and MK-8. The other homologs were not significant. This is in agreement with the previous reports on activated sludge (Hiraishi, 1988; Hiraishi *et al.*, 1989).

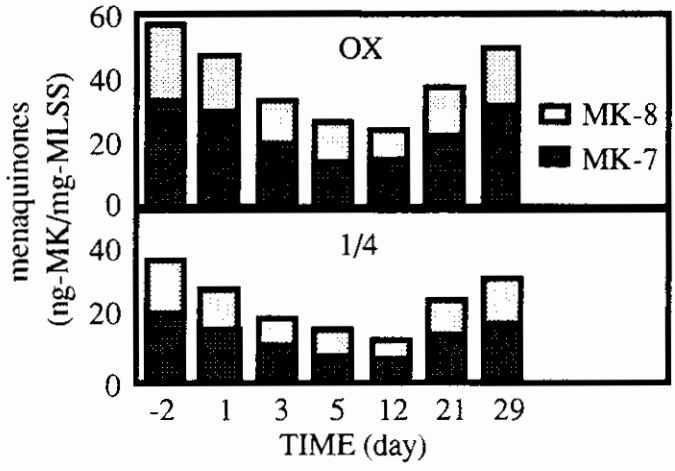

Fig. 6. Menaquinone contents in the activated sludge for the disturbances of 5 days.

The disturbances of 5 days decreased quinone contents in the activated sludge. Although the decrease were not significant on day 1, both ubiquinone and menaquinone decreased remarkably after day 3 and took more than 3 weeks to recover their original contents. It is well known that quinone contents of bacteria are species specific and, in most cases, constant irrespective of environmental conditions. In fact, no significant differences in quinone profiles of isolated strains of *Pseudomonas* from this sludge (strain 2-15-W1 and 2-17-W3, see Table 1) were noted between aerobic and anaerobic conditions.

Thus it is most likely that the decrease in the activity of phosphorus removal was dependent not on the decrease in the activity of each bio-P-bacteria but on the decrease in their population. The facts that it took longer time to recover phosphorus removal activity than to lose it and the longer time was necessary if the duration of disturbances were longer may also support the decrease in bio-P-population.

Homologs of ubiquinones were shown in Fig. 7 by percentages out of total ubiquinones. Although little change in percentages of menaquinone homologs was noted (not shown here), those for ubiquinone showed significant change, i.e. Q-9 decreased during the disturbances. This may correspond to the decrease in the population of bacterial species with high Q-9 contents.

Previous studies on quinone profiles of activated sludge suggested that the introduction of anaerobic conditions into totally aerobic operation has little influence on its bacterial community structure (Hiraishi *et al.*, 1989; Hiraishi and Morishima, 1990). Also a conventional study on isolation and enumeration of activated sludge bacteria revealed that the cyclic change in anaerobic and aerobic conditions did not appear to stimulate microbial succession to a new species of *Acinetobacter*, but rather to stimulate the ability for the accumulation of polyphosphate and PHB inherent in the strains already present (Lotter *et al.*, 1986).

The present study, however, suggested completely different response of bacterial community. Although further studies would be necessary, not only the quinone profile but also slow responses in plant performance and phosphorus removal activity may support the change in bacterial community rather than physiological changes in bacterial community.

Fig. 8 shows relationships between specific growth rate of isolated strains from the sludge in steady-state operation and their activity of phosphorus removal. Similar to the

previous study (Okada *et al.*, 1991), strains with high activity of phosphorus removal were low in specific growth rate. This results also support the above mentioned slow succession of bacterial population and recovery of phosphorus removal after the disturbances.

Table 1 shows specific growth rate, phosphorus removal activity and ubiquinone homologs of representative strains isolated. The bacteria with high activity of phosphorus removal and low specific growth rate were identified as genus of *Acinetobacter* or *Pseudomonas*. The fact that major ubiquinone detected was Q-9 suggests that these strains were predominant species in the total bacterial population of activated sludge. Their high activity also suggests that they played major role for phosphorus removal. Their slow rate of growth may explain the slow recovery of bacterial population and activity. The strain with low activity and high specific growth rate, however, had Q-8 indicating not to play major role for phosphorus removal.

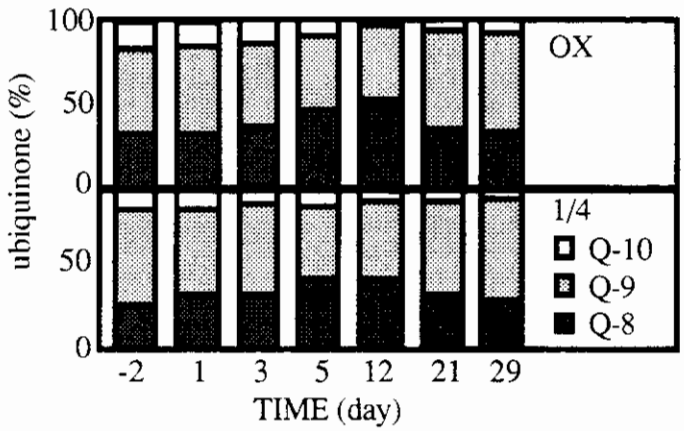

Fig. 7. Ubiquinone homologs as percentages out of total in the disturbances of 5 days.

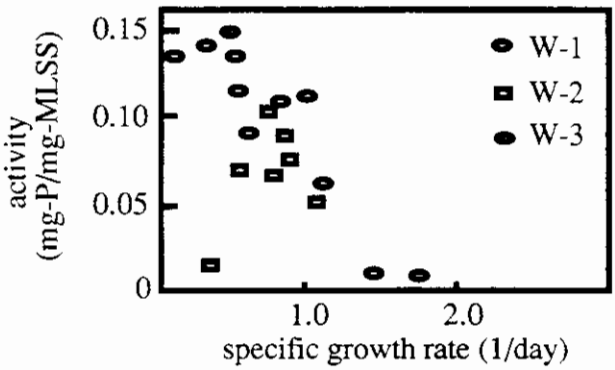

Fig. 8. Relationships between specific growth rate of isolated strains of bacteria and their phosphorus removal activities.

Table 1. Specific growth rate, phosphorus removal activity and ubiquinone homologs of isolated strains.

code	species	specific growth rate (day^{-1})	activity (mg-P mg^{-1}MLSS)	ubiquinone (%) Q-8	Q-9
2-12-W1	*Acinetobacter lwoffi*	0.66	0.18	8	88
2-13-W1	*Acinetobacter anitratus*	0.06	0.19	-	89
2-15-W1	*Pseudomonas aeruginosa*	0.50	0.18	33	61
2-17-W3	*Pseudomonas maltophilia*	0.80	0.18	-	91
4-08-W1	*Pseudomonas aeruginosa*	0.50	0.13	14	88
4-18-W1	*Pseudomonas aeruginosa*	0.43	0.15	7	92
2-08-W2	*Pseudomonas maltophilia*	0.80	0.07	93	-
4-02-W1	*Enterobacter agglomerans*	1.94	0.01	92	-
4-03-W1	*Enterobacter agglomerans*	1.84	0.01	92	-

REFERENCES

APHA (1985) *Standard Methods for the Examination of Water and Wastewater.* American Public Health Association, Washington, D.C.

Bowker, R. P. G. and Stensel, H. D. (1987) Biological phosphorus removal mechanism. In *Design Manual - Phosphorus Removal*, pp.15-19, U.S.EPA.

Cloete, T. E. and Steyn, P. L. (1988) A combined membrane filter-immunofluorescent technique for the *in situ* identification and enumeration of *Acinetobacter* in activated sludge. *Water Res.* **22**, 961-969.

Cloete, T. E. and Steyn, P. L. (1988) The role of *Acinetobacter* as a phosphorus removing agent in activated sludge. *Water Res.* **22**, 971-976.

Fuhs, G. W. and Chen, M. (1975) Microbiological basis of phosphate removal in the activated sludge process for the treatment of wastewater. *Microbial Ecol.* **2**, 119-138.

Groenestijn, J. W. and Deinema, M. H.(1987) The utilization of polyphosphate as an energy reserve in *Acinetobacter* sp. and activated sludge. In *Biological Phosphate Removal from Wastewaters* (Edited by Ramadori, R.), pp.1-6, Pergamon Press, Oxford.

Groenestijn, J. W., Bentvelsen, M. M. A., Deinema, M. H. and Zehnder, A. J. B. (1989) Polyphosphate-degrading enzymes in *Acinetobacter* spp. and activated sludge. *Appl. Environ. Microbiol.* **55**, 219-223.

Hao, O. J. and Chang, C. H. (1987) Kinetics of growth and phosphate uptake in pure culture studies of *Acinetobacter* species. *Biotechnol. Bioeng.* **29**, 819-831.

Hiraishi, A., Hoshino, Y. and Kitamura, H. (1984) Isoprenoid quinone composition in the classification of *Rhodospirillaceae. J. Gen. Appl. Microbiol.* **30**, 197-210.

Hiraishi, A. and Morishima, Y. (1990) Capacity for polyphosphate accumulation of predominant bacteria in activated sludge showing enhanced phosphate removal. *J. Ferment. Bioeng.* **69**, 368-371.

Hiraishi, A., Masamune, K. and Kitamura, H. (1989) Characterization of the bacterial population structure in an anaerobic-aerobic activated sludge system on the basis of respiratory quinone profiles. *Appl. Environ. Microbiol.* **55**, 897-901.

Hiraishi, A. (1988) Respiratory quinone profiles as tools for identifying different bacterial populations in activated sludge. *J. Gen. Appl. Microbiol.* **34**, 39-56.

Jones, P. H., Tadwalkar, A. D. and Hsu, C. L. (1987) Enhanced uptake of phosphorus by activated sludge ---Effect of substrate addition. *Water Res.* **21**, 301-308.

JSWA (1988) The 2nd report on the evaluation of biological processes for nitrogen and phosphorus removal -Phosphorus removal by anaerobic/aerobic activated sludge processes- (in Japanese). Japan Sewage Works Agency.

Katayama, Y., Tsuzaki, N. and Kuraishi, H. (1982) Ubiquinone, fatty acid and DNA base composition determination as a guide to the taxonomy of the genus *Thiobacillus. J. Gen. Microbiol.* **128**, 1599-1611.

Lotter, L. H., Wentzel, M. C., Lowenthal, R. E., Ekama, G. A. and Marais, Gv. R. (1986) A study of selected characteristics of *Acinetobacter* spp. isolated from activated sludge in anaerobic/anoxic/aerobic and aerobic systems. *Water S. A.* **12**, 203-207.

Lotter, L. H. and Dubery, I. A.(1987) Metabolic control in polyphosphate-accumulating bacteria and its role in enhanced biological phosphate removal. In *Biological Phosphate Removal from Wastewaters* (Edited by Ramadori, R.), pp.7-14, Pergamon Press, Oxford.

Minagawa, C., Yuasa, Y. and Takagi, T. (1989) Effects of rainfall on the operation of A2O process and utilization of primary sludge for the recovery (in Japanese). *Jour. Japan Sewage Works Assoc.* **26**(301), 43-53.

Mostert, E. S., Gerber, A. and van Riet, C. J. J. (1989) A comparative study on the effects of feedstock composition on enhanced biological phosphate removal in modified activated sludge systems. *Environ. Technol. Letters* 10, 9-22.

Murphy, M. and Lotter, L. H. (1986) The effect of acetate on polyphosphate formation and degradation in activated sludge with particular reference to Acinetobacter calcoaceticus: a microscopic study. *Water S. A.* **12**, 63-66.

Ohtake, H., Takahashi, K., Tsuzuki, Y. and Toda, K. (1985) Uptake and release of phosphate by a pure culture of *Acinetobacter calcoaceticus*. *Water Res.* **19**, 1587-1594.

Okada, M., Murakami, A. and Sudo, R. (1987) Ecological selection of phosphorus-accumulating bacteria in sequencing batch reactor activated sludge processes for simultaneous removal of phosphorus, nitrogen and organic substances. In *Biological Phosphate Removal from Wastewaters* (Edited by Ramadori, R.), pp.147-154, Pergamon Press, Oxford.

Okada, M., Murakami, A., Lin, C. K., Ueno, Y. and Okubo, T. (1991) Population dynamics of bacteria for phosphorus removal in sequencing batch reactor (SBR) activated sludge processes. *Water Sci. Technol.* **23**, 755-763.

Pike, E. B. and Curds, C. R. (1971) In *Microbial Aspects of Pollution* (Edited by Sykes, G. and Skinner, F. A.), Academic Press, New York.

Wentzel, M. C., Lotter, L. H., Lowenthal, R. E. and Marais, Gv. R. (1986) Metabolic behavior of *Acinetobacter* spp. in enhanced biological phosphorus removal - a biological model. *Water S. A.* **12**, 209-224.

P-REMOVAL: STATE OF THE ARTS IN THE NETHERLANDS

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P-removal: state of the art in the Netherlands.

Introduction.

The care for the quality of Dutch surface-water rests, in the execution of it with 31 water quality authorities.

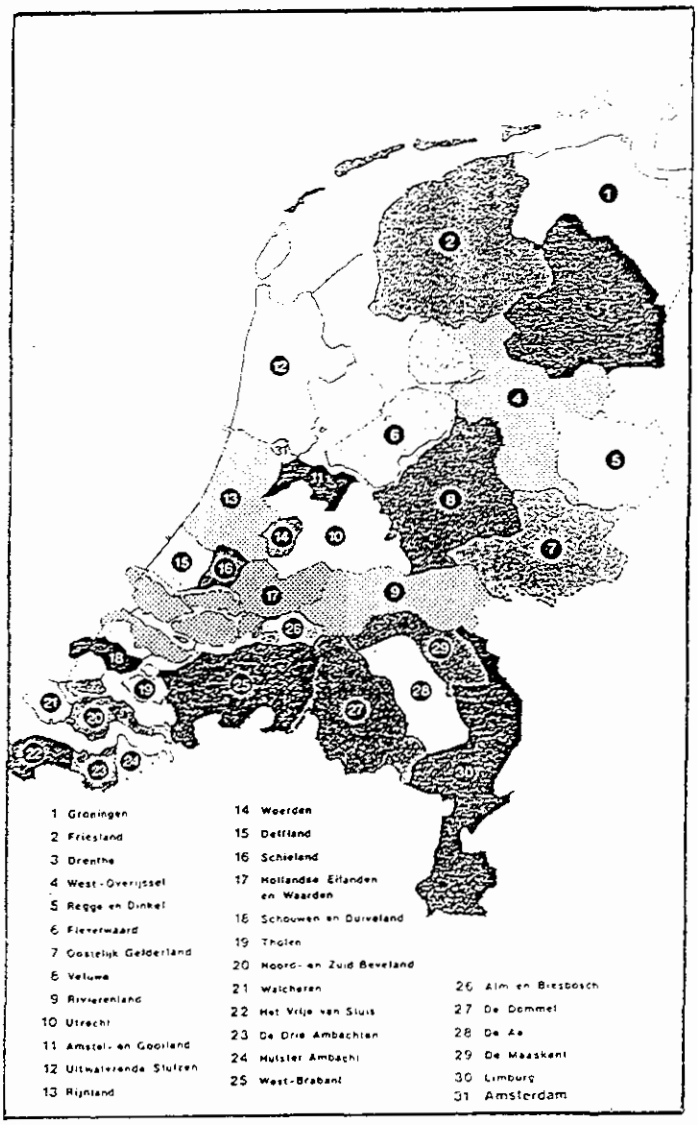


Figure 1. Waterquality authorities in the Netherlands

Besides phosphate-containing fertilizers wahing from the soil and direct, unpurified discharges, the load with phospates is determined for a considerable part by residue-discharges from waste-water treatment plants (WTPs). All these streams ultimately end up in the North Sea.
Besides that, water-management in the Netherlands is determined for an important part by influences of border-transgressing rivers: a considerable part flows via the Rhine and the Meuse directly to the North Sea, whereas an other part is first guided by way of surface-water.
Since the Rhine and the Meuse flow through a number of European countries, making

agreements to arrive at a reduction of P-load on the North Sea (and partly in the Netherlands) is an international affair.
In 1986 the total load with phospate of Dutch surface- and wastewater amounted to about 44 million kilos P per annum, coming from national contributions. Via border-transgressing rivers 46 million kilos P reached our country. From this amount a part is removed (in WTPs) or accumulated in dredge or bottom, after which the total load of the North sea amounted to about 42 million kilos P (figure 2).

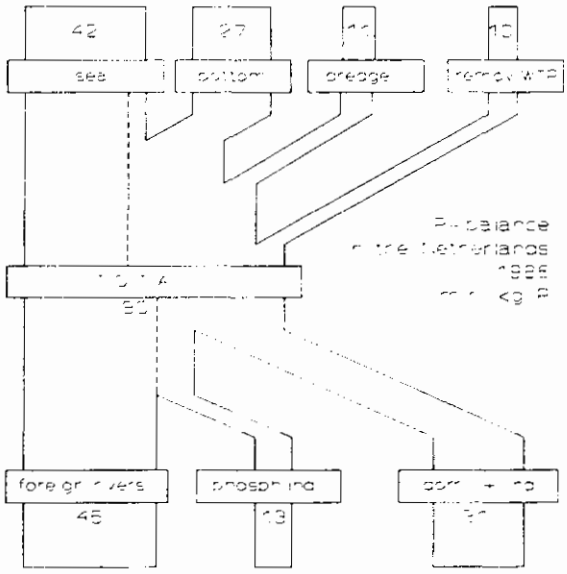


Figure 2. P-load on Dutch surface waters

Plan-modelling.
During an assembly of Rhine-border states in October 1987, agreements were made to reduce the load of the North Sea for a great number of matters/chemicals, amongst others phosphates.
A month later the conference of Ministers of countries on the North Sea confirmed the agreements made.
In 1995 the load with respect to phosphor and other chemicals should be reduced by 50 per cent in comparison to the 1985 situation. Percentages for some chemicals may even be higher.
These agreements were incorporated by the Dutch government into the Third Note for Water-management, in which plans and water quality aims are embedded.
The aim for the quality of Dutch surface-water in the long run is set for phospate at 0.15 mg P-total/l
An integral approach has been chosen upon, in which all important sources are tackled. With respect to industrial discharges with phosphate, notably the fertilizer industry and the intensive stock-farms, reduction by 50 per cent have been laid down. The same reduction-claim holds good for discharges from waste water treatment plants and the international agreements have been translated into a policy in which effluent-requirements are applied.
The above mentioned requirement depends on the capacity of the WTP and will ultimately be 1, respectively 2 mg/l total P.

Table I Limits for phosphate concentrations in effluent of WTPs

	Limit mg P/l	New WTPs from	Existing WTPs from
For WTPs > 100,000 p.e.	1	1/7/1990	1/1/1995
For WTPs between 20,000 p.e. and 100,000 p.e.	2	1/7/1990	1/1/1995
For WTPs < 20,000 p.e.	2	1/1/1995	1/1/1995

The water quality authorities are, however, also allowed to opt for an elaboration in which they see to it that within their area, 75 per cent of all incoming quantities of phospates are removed. By doing so the requirements set are also met.
In the rest of this lecture attention will be paid only to those measures which will be taken at WTPs in order to meet these requirements

Technical possibilities.

* Reduction at the source.

Reducing phosphate quantities from waste water is the most effective source measure.
As far as discharge of industrial waste water with high degrees of phosphate is concerned, reduction will be aimed at by way of granting licences.
For domestic discharges the most important possibility will be the reduction in the use of P-containing detergents.

* Chemical dephosphatation.

Chemical dephosphatation is of all the techniques currently available the one that is being applied on the widest scale. This process relies on chemical additives which react with phosphate in the water phase to produce an insoluble phosphate-salt. There are three basic types of chemical dephosphatation methods.
The first is pre-precipitation. This involves dosing of chemicals to the waste water entering the primary clarifier. The compound commonly used is ferric chloride. Iron(II) salt can not be used. Besides removal of phosphate also reduction of BOD is normally achieved depending on dosing quantities. Therefore this technique is sometimes used as a means of reducing the load for the biological part of the installation.
The second method is simultaneous-precipitation. Compounds used are ferrous chloride, ferric chloride and aluminum salts. When ferrous chloride is used, the iron ions have to be oxidized first by the oxygen in the treatment plant.
The disadvantage of simultaneous dephosphatation is the large increase of sludge amount that is produced in the treatment plant.
The third method is that of post-treatment. This involves treating the biologically purified effluent in a further processing stage. The chemicals used are generally the same as those applied during simultaneous dephosphatation, except ferrous chloride. One advantage of the post-treatment process is that the phosphate sludge is kept separate from the rest of the sewage sludge. This factor is of particular importance when considering the potential recycling of materi-

als.
On the whole, simultaneous dephosphatation is more commonly applied than a post-treatment stage because of the lower costs involved. Experience has shown that, on average, phosphate levels of 1-2 mg/l total P are achievable with this technique.

* Biological dephosphatation

Biological dephosphatating techniques are based on the principle that, given the right conditions, certain bacteria are able to remove phosphates from water. These bacteria, which comprise among others the Acinetobacter genus, are able to accumulate more phosphate than is required to maintain the cell-structure thereby reducing the phosphate content in the water phase. Acinetobacter organisms need volatily acids to grow, which means that an anaerobic zone must always be provided within the sewage treatment plant. Phosphates are removed from the waste water during the subsequent aerobic phase.

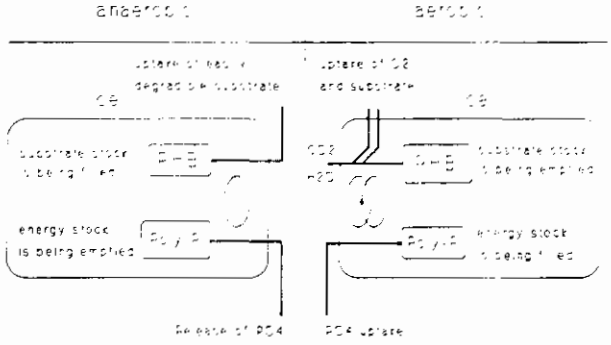
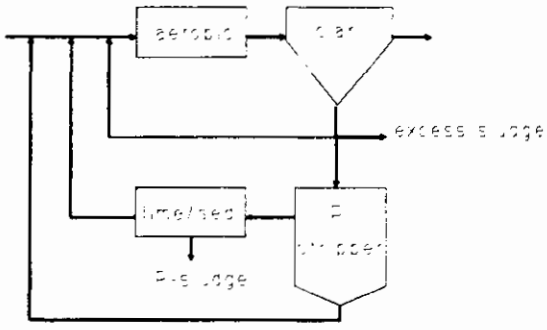


Figure 3. Biological uptake and release of phosphate

In this process, inorganic phosphates are converted to energy-rich polyphosphates, which accumulate in the cells of the bacteria. If the bacteria are moved to an anaerobic environment, the stored energy is released as the phosphates are returned to the water phase (figure 3).
In a normal conventional aerobic treatment process some 30-40% of all phosphates are being removed from the waste water. Biological dephosphating techniques can do more than 80%.



At the moment much attention is being paid to the research and development of the pho-strip process and other biological dephosphating processes in the Netherlands (figure 4).
More about this subject will be presented in another lecture by Mr Rensink.

Figure 4. Phostrip process

* Fluid-bed pellet reactor

A new dephosphating method is the use of the so-called fluid-bed pellet reactor. This process does not result in sludge but in pellets of calcium or sodium phosphate. These pellets can be used in the phosphor-industry thus bringing the phosphate into a cycle.

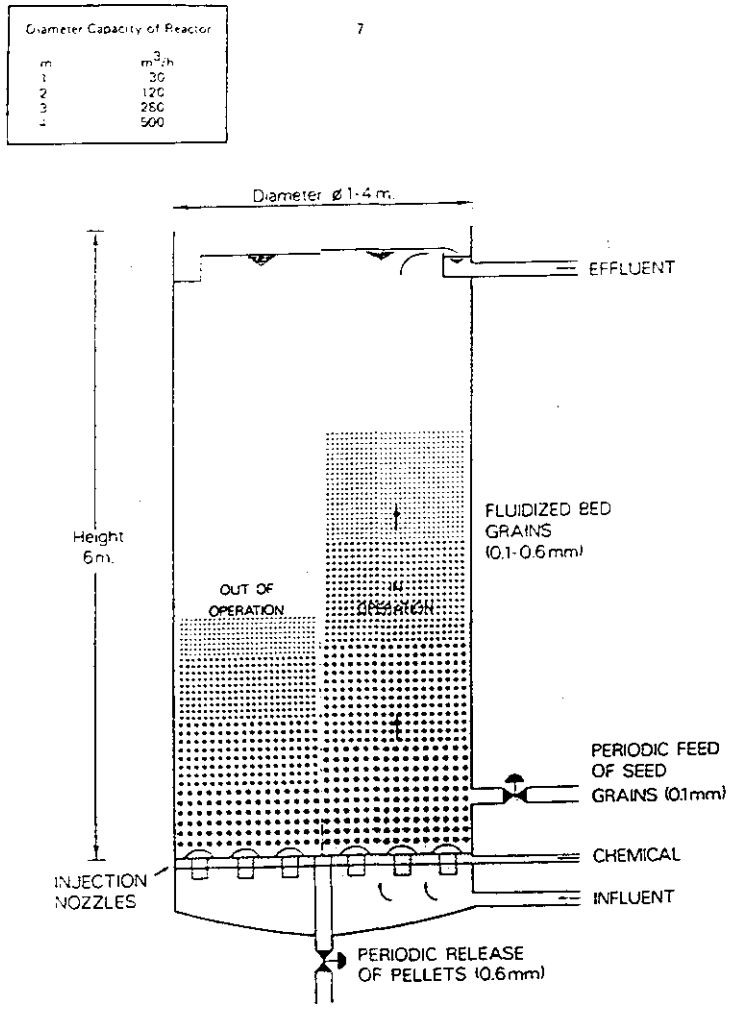
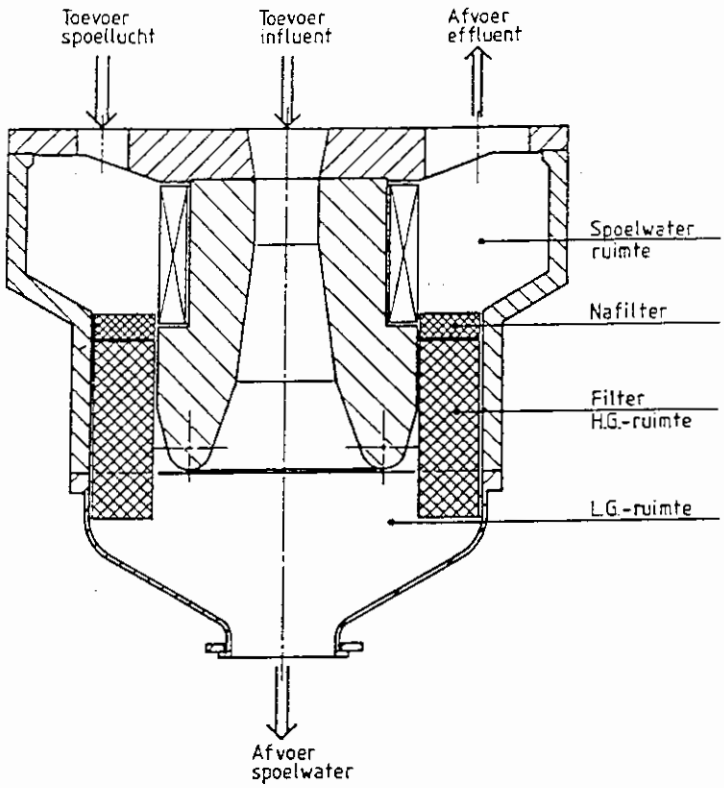


Figure 5. The fluid-bed pellet reactor

The priciple of the fluid-bed reactor is as follows: a part of the reactor is filled with a proper basis for the pellets, such as sand. The effluent of the waste water treatment plant is brought in the fluid-bed in an up-flow direction. With the addition of calcium or sometimes sodium ions the process conditions are adjusted to form phosphate pellets with the calcium ions and the sand as a nucleus. When the pellets are big enough, they are removed from the system.
This technology is capable of bringing total phosphate-P content in the effluent down to about 1 mg/l or lower. Normally, filtration of the effluent using a sand-anthracite filter will be necessary to hold back phosphate in suspended form (figure 5).
The first fluid-bed granular reactors to operate at full scale have been introduced in Denmark and the Netherlands.

* Magnetic separation

Magnetic separation was originally thought to be suitable only for removing certain metallic elements from waste water. However, it soon became clear that non-magnetic materials could also be separated in a similar way. In the latter case it is necessary to add magnetite, a magnetic ferric oxide. The magnetite becomes adsorbed onto certain particles, present in the water phase, which allows them to be removed under the influence of a magnetic field. Adsorption is enhanced by the addition of small amounts of polymer. Under these circumstances it is also possible to remove phosphate (figure 6).



Separatie magneet type AQUAMAG

Figure 6. Magnetic separation

For sake of economics it is essential to recover the magnetite from the residual fraction. Recoveries of about 99% have been achieved on a pilot-plant scale. In trials it has become clear that phosphate levels in the effluent down below 1 mg/l are possible. If the effluent quality has already been brought down to a level of 1 mg/l by means of preliminary treatment, the effluent quality after magnetic separation may reach 0.2 mg total P/l or lower.
Combining chemical or biological techniques with magnetic separation can therefore yield extremely favourable results.
In the Netherlands two full-scale magnetic separation systems for municipal waste water are now under construction. They will be in operation from April 1991.

* Sand filtration

Sand filtration should not be regarded as a dephosphating technique in its own right, as it is always applied in combination with other methods. The effectiveness of this technique in reducing the amount of phosphate in waste water has already been demonstrated in many practical applications, particularly in Switzerland. It has been shown that levels in the order of 0.2 mg/l total P can be achieved. Futhermore, sand filtration can be considered as an extremely useful way of protecting the quality of surface waters.

Costs.

Next to the height of costs per unity, there are also differences in the structure of costs to be discriminated, when dephosphating.
Within the structure of costs a subdivision can be made into principal parts such as capital costs, running costs (amongst others chemicals) and the costs of sludge disposal.
In table II waste water treatment plants having different capacities, namely 50.000 and 100.000 p.e., have been compared as far as costs are concerned for four different implementation situations.

Table II. Costs of dephosphatation (Dfl/(p.e. a.))

	simultaneous precipitation		pho-strip process		fluid bed pelletreact		magnetic separation	
treatment plant cap.	I	II	I	II	I	II	I	II
Capital costs	1.1	0.6	3.7	2.8	12.1	9.7	7.8	6.3
Running costs	2.1	2.0	3.6	3.5	7.1	6.7	6.9	6.8
Sludge disposal	4.7	4.7	2.2	2.2	-	-	2.4	2.4
Total	7.9	7.3	9.5	8.5	19.2	16.4	17.1	15.5

I: capacity 50.000 p.e.
II: capacity 100.000 p.e.

The figures in this index originate from a desk study. In the influent a concentration of 10 mg/l total P is assumed, while effluent concentration is set upon 1-2 mg/l.

Research

In the Netherlands especially biological dephosphatation is being employed in order to do research in different places.
A number of these research-projects is being subsidized by various authorities (e.g. RIZA and STORA) and a national commitee that pursues and steers its progress.
In the next table you will find a brief survey of these research-projects.
At the same time it is indicated whether it concerns a laboratory, a pilot-plant or a full-scale experiment.

plant	size	research-scale	principle
Eindhoven	750.000	full sc.(partial)	phostrip/simultaneous
Bennekom	25	lab.	phostrip+pellet reactor
Bergambacht	7.000	full sc.	phostrip
Bennekom	16.500	full sc.	simultaneous
Ede	100.000	pilot pl.	biodenipho/phostrip
Oosthuizen	7.000	full sc.	biological
Kralingse Veer	300.000	pilot pl.	phostrip+pellet reactor
Almelo-Vissedijk	130.000	full sc.	biological
Lichtenvoorde	48.000	full sc.	biological/chemical

The index shows that in many experiments a lot of attention is being paid to biological dephosphatation, whether or not in a side stream treatment. In a number of cases the pellet reactor is used for the treatment of side streams.
The term of these experiments varies from several months up to one year and is in some cases followed up by an adaption on a full-scale.

Full-scale situation.

* Source-approach.

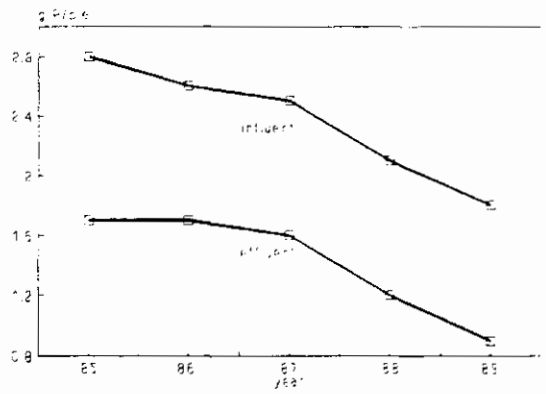


Figure 7. Influence of P-free detergents on P-concentration in wastewater

A considerable reduction of phosphates-supplies in the waste water treatment plants has been established in the years 1985-1989 mainly as a result of the mass switch-over by families to phosphate-free detergents (figure 7). As a result of this supplies have dropped to about 35 %.
In the years to come, a further reduction is to be expected, because the market-portion of phosphate-free detergents has not yet reached its full capacity.
The average concentration of P-total in influent dropped from about 15 mg/l to about 10 mg/l in this period.
An even further reduction may be expected.

* P-removal techniques.

The number of waste water treatment plants in the Netherlands, where next to the normal P-reduction by biological processes, extra attention is paid to a further reduction of phosphate is still limited, but it will rapidly increase in the next few years. In 1988 about 23.5 million p.e. 's were handled in municipal waste water treatment plants, of which 1.4 million p.e.'s underwent a dephosphating treatment.
In the table below the number of waste water treatment plants is indicated where dephosphating is used in some way or other. At the same time the techniques used have been indicated.

Dephosphating techniques	Number of WTPs
Chemical: simultaneous	22
pre-precipitation	8
Biological *	± 15
Pellet reactor	1
Magnetic dephosphatation	2
Total	± 48

Total number of WTPs about 470

* Most waste water treatment plants show spontaneous biological dehosphatation as a result of special process-circumstances or the composition of waste water.

Postscript.

At present dephosphatation, mainly chemical, is being applied on a modest scale in Dutch WTPs.
Since 1987 when international agreements were made with regard to reducing discharges with a great number of chemicals, amongst others phosphate, all water quality authorities in the Netherlands have been working out plans to attain a 50 % reduction with respect to the 1985 situation.
In doing so a lot of attention in research-projects is paid to biological dephosphatation and new techniques such as the pellet reactor and magnetic dephosphatation.
Additional phosphate-removal is realised in about 10 % of the WTPs besides the normal removal as a result of the biological purification-process.
The number of p.e.'s which is involved in this is still small and amounts to about 5 % of the total that is dealt with in municipal waste water treatment plants in the Netherlands. On the other hand the aim of 50 per cent reduction of phosphate discharges to surface water in comparison with the situation in 1985 has already come in sight for many WTPs because of the massive switch-over to phosphate-free detergents. Therefore only moderate extra efforts have to be undertaken for additional measures.

References

* Kwartaalberichten Milieu (CBS)
1988/4 (in Dutch)
* Starkenburg, W.v. Nutrient reduction in waste water. Review of treatment options.
RIZA (1990)
* Dijk, J.C. van. Defosfatering door kristallisatie (cursus tertiaire behandeling van afvalwater).
PATO, Delft (1985) (in Dutch)
* Effect van de vervanging van wasmiddelen-P op de kosten van fosfaatverwijdering.
RIZA (1990) (in Dutch)
* Vries, P.J.R. de. Invloed van het gebruik van fosfaatvrije wasmidellen op de fosfaatgehalten in afvalwater.
* Waterschapsbelangen 14 (1991), p 478-481 (in Dutch)

PRINCIPLES OF PHOSPHORUS AND NITROGEN REMOVAL PROCESSES, INCORPORATED IN ACTIVATED SLUDGE TREATMENT SYSTEMS

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PRINCIPLES OF PHOSPHORUS AND NITROGEN REMOVAL PROCESSES, INCORPORATED IN ACTIVATED SLUDGE TREATMENT SYSTEMS.

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INTRODUCTION

It has been proved that biological nutrient removal of wastewater by the activated sludge process is a great succes. In many cases phosphorus and nitrogen removal processes can be easily incorporated in existing activated sludge treatment plants.
This paper reviews the principles of biological nitrogen and phosphorus removal from municipal wastewater by the activated sludge process. Introduction of these processes in modified activated sludge systems will be presented.

BIOLOGICAL P-REMOVAL

Anaerobic zone

It has been generally known that the genus Acinetobacter is the most important representative of luxury phosphorus uptake in activated sludge. The bacterium Acinetobacter is normally present in activated sludge, but in minority due to the low growth rate. To promote the growth of Acinetobacter in activated sludge, the growth conditions of this bacterium must be well-known. Acinetobacter prefers as substrate low molecular compounds such as low fatty acids - especially acetate- and ethanol. The production of low fatty acids from wastewaters in an activated sludge system can be realized by incorporating an anaerobic zone, mostly at the beginning of the aeration tank, where the return sludge meets the incoming wastewater. See Figure 1. The overall content of low fatty acids is the result of low fatty acids already present and produced in the sewerage system and production of low fatty acids in the anearobic zone by facultative anaerobic bacteria.
Owing to the typical energy metabolism of Acinetobacter this

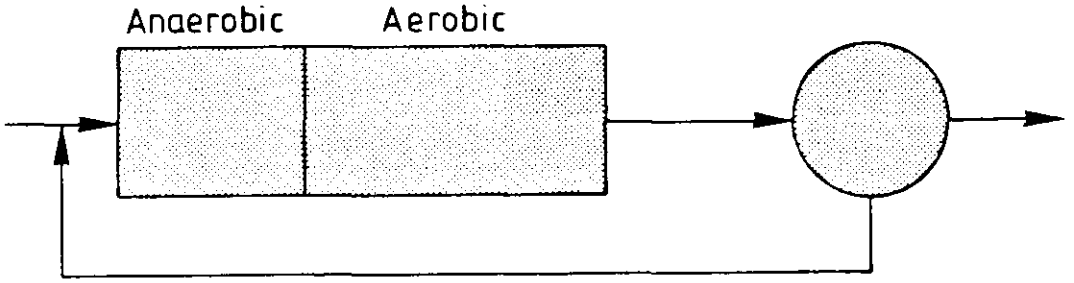

Fig. 1. Introduction of an anaerobic zone for biological P-removal

bacterium is capable to consume low fatty acids under anaerobic circumstances and to form poly-ß-hydroxybutyrate and higher fatty acids. The energy need for this process has been derived from the conversion of polyphosphate to phosphate - the so-called release of phosphate- by way of ATP. During the following aerobic period Acinetobacter metabolizes the storing products via oxidation and synthesis of new cells. Phosphate from the waterphase is accumulated in the cell as polyphosphate. The degree of P-removal from the waste water corresponds with the net growth of Acinetobacter. The more low fatty acids in the anaerobic zone, the more growth of Acinetobacter and as a consequence the more P-removal. A simplyfied metabolic pathway is given in Figure 2.
Although an anaerobic zone is a prerequisite for biological P-removal many factors can affect positively or negatively the degree of P-removal as described below.

Factors affecting biological P-removal performance.

1. Wastewater characteristics

The composition of the wastewater plays an imporant role in the biological phosphorus removal process, especially to have organic fermentation products available for the phosphorus-accumulating organisms. The greater the amount of low fatty acids made available to the P-removal system, the greater will be the amount of phosphorus removal. As the fermentation products in the

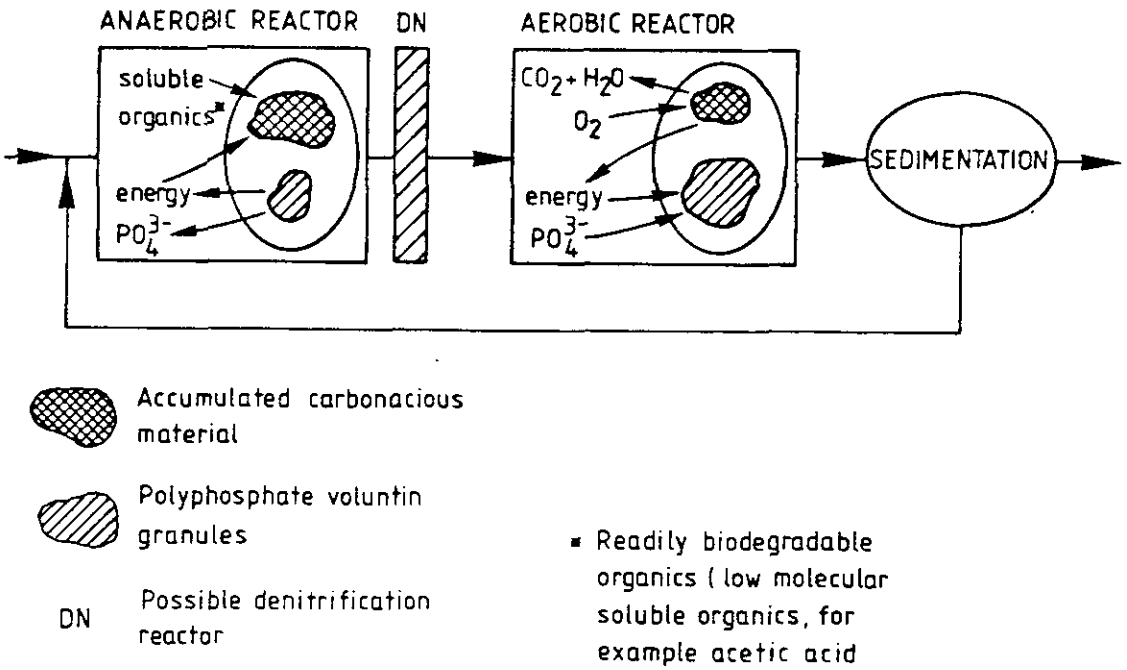

Fig. 2. Metabolic pathway of Acinetobacter

anaerobic zone are assimilated by the P-accumulating micro-organisms about as fast as they are produced, it is not possible to directly measure the fermentation substrate available in a given wastewater. Therefore this makes it difficult to define the P-removal capacity for a given wastewater. Attempts to characterize the wastewater has been made by the BOD/P and BOD/N_{Kj}-ratio. The higher these ratio's, the greater the P-removal degree. The soluble BOD may be a better measure of substrate that is available for low fatty acids production than the total influent BOD, because not all of the influent BOD is considered to be readily fermented.

The other part of the BOD - present in the primary sludge - can be encouraged to low fatty acids production by seperate fermentation of the sludge. By adding this influent enriched with low fatty acids to the biological phosphorus treatment plant, the effluent phosphorus will decrease to lower values.

Domestic wastewater containing also wastewater from e.g. dairy factories and slaughterhouses with easy degradable organic

material, increases the quantity of fermentation products in the anaerobic zone and as a consequence higher P-removal.
Normally the detentiontime of the mixed liquor in the anaerobic zone amounts to 1 - 2 hours by using settled wastewater, but is strongly influenced by the low fatty acids concentration in the influent. By using raw wastewater longer detentiontimes are needed to fermentate organic material to low fatty acids.

2. Oxygen

Alternation of an anaerobic period with an aerobic period is a prerequisite for biological phosphorus removal. The oxygenation capacity (OC) must be so high that sufficient oxygen is available in the aerobic zone for the phosphorus-accumulating micro-organisms. If the oxygenation capacity is not sufficient the P-uptake will not be optimal. A complete or nearly complete nitrification indicates an optimal P-uptake. Also the sludge detentiontime in the second clarifier affects the degree of P-release during the settling period. In practice the sludge detentiontime does not exceed two hours.

3. Sludge loading/sludge age

Pilot plant studies with settled domestic wastewater have led to results presented in Table 1. The higher the sludge loading the greater the amount of P-removal. This must be attributed to the sludge age and the degree of nitrification. At a high sludge loading, a maximum of P-uptake is possible. At lower sludge loadings and higher sludge ages the degree of nitrification and sludge mineralisation increases, which has an adverse effect on the biological phosphorus removal. See Table 1.

4. Nitrate

Nitrate inhibits the biological phosphorus removal process in the anaerobic zone, because denitrification consumes low fatty acids to transform nitrate into N_2, N_2O and NO. When nitrate is present in the anaerobic zone, the redoxpotential is too high for production of low fatty acids by facultative anaerobic bacteria. As soon as the nitrate has been removed the release of phosphate

Table 1 P-removal at different sludge loadings

Sludge loading gCOD/kg sludge/day	P-removal in %	Sludge age in days	% P of dry sludge*
140	80	30	--
280	50	17	5,0
400	87,5	5,3	6,8
600	91	4,0	6,5

* P-influent 18 mg P/l, 1983

takes place as a sign of low fatty acids production. The degree of nitrate inhibition is estimated by the quantity of nitrate present in the return sludge. To lower the nitrate concentration in the return sludge, denitrification have to take place in an anoxic zone downstream in the activated sludge plant. The reduction of nitrate is especially important for biological phosphorus removal in the mainstream.

5. Feed back of phosphorus by sludge treatment

Sludge treatment has an adverse effect on the biological phosphorus removal process. The release is strongly influenced by the way of sludge treatment. Two extremes can be considered, viz. removal of excess sludge directly from the aeration tank and sludge disposal via anaerobic digestion. In the first case no release of phosphorus takes place, in the second case anaerobic digestion can lead to a high feed back of phosphorus to the sewerage system and consequently to the plant. The internal recirculation can be blocked by chemical treatment of these enriched flow streams.

6. Effluent suspended solids

Small quantities of suspended solids are discharged with the effluent over the weir of the second clarifier. Normally the limits of suspended solids in effluents are values lower than 30

mg/l. These solids always contains some phosphorus, which is included in the total phosphorus content of the effluent. However in biological phosphorus removal systems, the phosphorus content of the effluent with suspended solids can become a significant fraction of the total phosphorus discharged. If for example an effluent contains 20 mg suspended solids per liter with a phosphorus content of 5 percent, the effluent has already a concentration of phosphorus of 1 mg/l, which is only the result of the suspended solids.

7. Wastewater temperature

The temperature does not appear to play a significant role in biological phosphorus removal. If anything, an improvement of the phosphorus removal process at extremely low temperature has been observed, possibly due to a shift in microbial population from mesophilic to psychrophilic, inclusive a higher cell yield. Although the wastewater temperature may not significantly affect biological phosphorus removal, the rate of biochemical reactions, especially hydrolysis, are reduced.

8. The composition of the micro-organisms in the activated sludge

Although an anaerobic zone is a prerequisite for the growth of Acinetobacter it may be possible that due to a wrong feed pattern of the anaerobic zone filamentous bacteria will predominate. A completely mixed anaerobic zone can lead to filamentous growth, as a plug flow system or a plug flow selector just suppress growth of filamentous bacteria.

BIOLOGICAL NITRIFICATION AND DENTRIFICATION

Nitrification

Municipal wastewater of predominantly domestic origin contains nitrogen in the organic and ammonium forms, which originate from protein metabolism in the human body. Arriving at sewage treatment plants an important part of the organic nitrogen has been already converted to the ammonium form.

Nitrification is the biological oxidation of ammonia to nitrate with nitrate as an intermediate. This conversion is the action of the autotrophic species Nitrosomonas and Nitrobacter, which carry out the reaction in two steps:

$$2\ NH_4^+ + 3\ O_2 \xrightarrow{\text{Nitrosomonas}} 2\ NO_2^- + 2\ H_2O + 4\ H^+ + \text{new cells.}$$

$$2\ NO_2 + O_2 \xrightarrow{\text{Nitrobacter}} 2\ NO_3^- + \text{new cells}$$

The overall reaction is:

$$NH_4 + 2O_2 \longrightarrow NO_3 + 2H^+ + H_2O + \text{new cells}$$

The net growth of biomass from Nitrosomonas and Nitrobacter is roughly 0,15 g NVSS/ g NH_3.N oxidized. The empirical overall reaction including assimilation and oxidation is:

$$NH_4^+ + 1.83\ O_2 + 1.98\ HCO_3^- \longrightarrow 0.98\ NO_3^- + 0{,}021\ C_7H_5NO_2 + 1.88\ H_2CO_3 + 1.04\ H_2O$$

From this equation it can be concluded that 1 gram of ammonium-nitrogen means:

4.33 g of O_2 are consumed,
0,15 g of new cells are formed,
7.1 g of alkalinity (as $CaCO_3$) are destroyed,
0,08 g of inorganic carbon are consumed.

Factors affecting nitrification

In activated sludge processes nitrification can be affected by the following factors:

1. Sludge loading and sludge age

The degree of nitrification is dependent on the sludge loading or sludge age. Above a sludge loading of 0.10 - 0.15 kg BOD_5/kg

MLSS.day the degree of nitrification will be lowered. This corresponds with a sludge age of less than 5 - 6 days.

2. Temperature

The optimum temperature for nitrification is generally placed in the range of 28 to 32^0 C. There is a significant reduction as the temperature reduces. It is generally assumed that for all practical purposes nitrification ceases to proceed below 5° C. The required detentiontime for succesfull nitrification may be several times greater in the winter than in the summer for adaequate nitrification. This means higher sludge ages in the winter.

3. Oxygen

Oxygen is of great importance for the nitrification process. There is no apparent inhibition of nitrification at dissolved oxygen levels exceeding 1.5 mg/l. Dissolved oxygen levels below 0.5 mg/l will have a negative influence on nitrification as the micro-organisms are obligatory aerobic.

4. PH

The pH range of ammonia oxidation to nitrate declines in the acid range. The rate of nitrification begins to decrease somewhere at pH of 6.3. to 6.7. Generally the rate is assumed constant in the range of 7.2 to 8.0.

5. Alkalinity

Alkalinity is destroyed by the oxidation of ammonia to nitrate. Approximately 7.1 g of alkalinity as $CaCO_3$ is destroyed per gram NH_3 - N oxidized. In wastewater with low alkalinity and/or high ammonia concentration, alkalinity may have to be added in order to maintain the pH at the optimum level for nitrification. Bicarbonate or lime can be used for this purpose.

6. Inhibition of nitrification

The autotrophic nitrifiers are extremely sensitive organisms. Compounds which are most noted for toxity are heavy metals,

cyanides, halogen compounds, phenols, mercaptans and thiourea.

Denitrification.

Denitrification is the biological conversion of nitrate-nitrogen to more reduced forms as N_2, N_2O and NO. The process is carried out by a variety of facultative heterotrophs which can utilize nitrate instead of oxygen as the final electron acceptor. Denitrification depends on the carbonaceous matter involved. The reaction with methanol - electron donor - is as follows:

$$6\ NO_3^- + 5\ CH_3OH \longrightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^-$$

Including cell synthesis the reaction is:

$$NO_3^- + 1.08\ CH_3OH + 0.24\ H_2CO_3 \longrightarrow 0.056\ C_5H_7NO_2 + 0.47\ N_2 + 1.68\ H_2O + HCO_3^-$$

The reaction expresses that for 1 gram of nitrate-nitrogen:

2.47 g of methanol are consumed,
0.45 g of new cells are produced,
3.57 g of alkalinity are formed.

In activated sludge the most applied system concerns the single sludge system with mixed liquor recycle, because this system needs no external carbon source, lower neutralization chemical requirements and lower oxygen requirements. For denitrification an anoxic zone is incorporated into the activated sludge system. The recycled mixed liquor amounts to 3 - 5 times influent flow. See Figure 3.

Factors affecting denitrification

As in nitrification, there are factors which influence both rate and extend of denitrification.

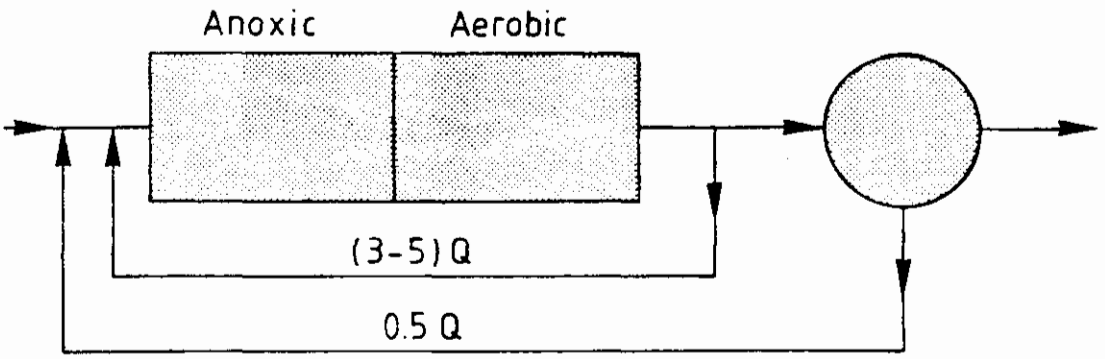

Fig. 3. Denitrification in a single sludge system

1. Temperature

As in all biochemical reactions, denitrification is temperature dependant. Denitrification does occur in the 5 to 10 °C range although slow. The decreased rate can be compensated by the increase of highest possible MLVSS.

2. Oxygen

Presence of oxygen is not toxic for denitrification but it is in competition with nitrate. Absent of oxygen is desirable. On that reason mixed liquor recycle must contain a minimal oxygen concentration.

3. pH

The optimal pH range for denitrification is from 6.5. to 8. Denitrification tends to raise the pH of the system by producing alkalinity from the organic electron donor as demonstrated in the reaction:

$$5\ CH_3OH + 6\ NO_3^- \longrightarrow 5\ CO_2 + 3\ N_2 + 6\ OH^- + 7\ H_2O$$

It has been estimated that alkalinity gained via denitrification is approximately half the loss in which occurs during nitrification.

4. Carbon source

The selection of the organic carbon source depends in reactivity with nitrate consumption, availability and costs. The use of raw wastewaters for denitrification is possible the most economical and effective way of achieving denitrification.

Under anoxic conditions a portion of the wastewater BOD will be used as a carbon source for denitrification. In comparison with biological phosphorus removal Acinetobacter only use low fatty acids while denitrifiers can consume higher components as well. The uptake of low fatty acids by denitrifiers is faster than by Acinetobacter.

ACTIVATED SLUDGE SYSTEMS WITH COMBINED PHOSPHORUS/NITROGEN REMOVAL

Many plants are faced with effluent limits on both phosphorus and nitrogen. It has been pointed out, that the principles of phosphorus and nitrogen removal processes can be introduced into the standard activated sludge treatment. This chapter reviews different modifications of activated systems for biological nutrient removal.

AA/O process

The AA/O process is a modification of the A/O process which stands for anaerobic/oxic. The A/O process is one of the simpliest of the biological phosphorus removal systems, being very similar to a standard activated sludge process. The mixed liquor passes an anaerobic zone followed by an aerobic zone. Finally the mixed liquor passes a secondary clarifier where the phosphorus-enriched sludge is settled from the process and returned to the anaerobic zone. The removed phosphorus from the wastewater is wasted by the excess sludge. For both phosphorus and nitrogen removal the plant is expanded with an anoxic zone between the anaerobic and aerobic zone for denitrification of the nitrified nitrogen. See Figure 4. The mixed liquor recycled from the aerobic zone to the anoxic zone amounts to 100-300 percent of the influent flow.

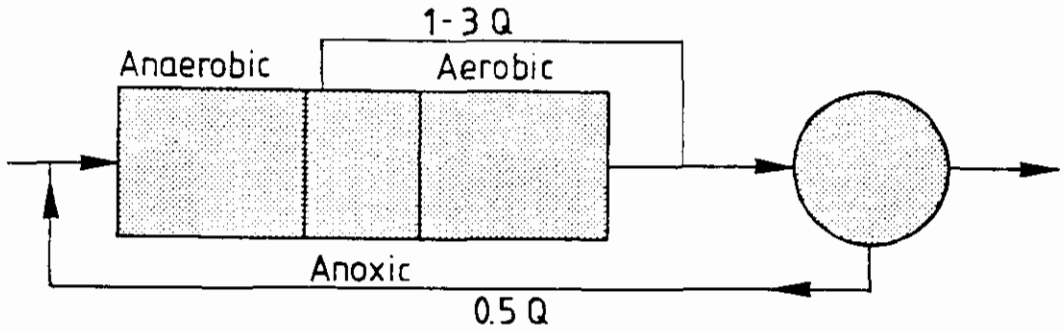

Fig. 4. The AA/O process

Modified or five-stage Bardenpho process.

The five-stage Bardenpho process is a modification of the four-stage Bardenpho process by including an anaerobic zone at the beginning of the four-stage Bardenpho process. The modified process is shown in Figure 5. The anaerobic zone encourages the production of low fatty acids for the growth of Acinetobacter. The phosphorus uptake takes place in the first aerobic zone. The mixed liquor recycled from the first aerobic zone to the first anoxic zone is 400 percent of the influent flow. The function of the second anoxic zone is to denitrify the incoming nitrate from the first aerobic zone by using the endogenous carbon source. The final aeration zone is provided for sedimentation to stimulate the release of nitrogen gas and to improve sludge settleability.

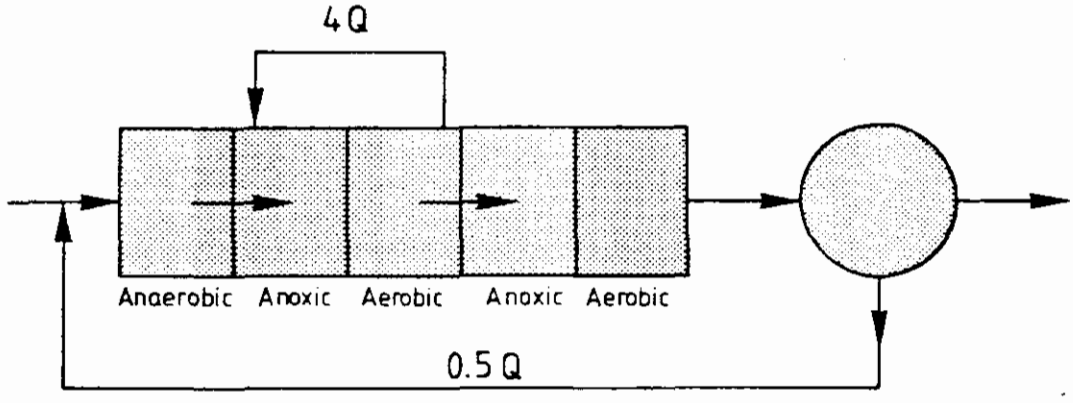

Fig. 5. The modified Bardenpho process

UCT process

The UCT process stands for University of Cape Town and is derived from the modified Bardenpho process. The modification of this process lowers extensively the nitrate concentration flowing to the anaerobic zone by extra internal recirculation of mixed liquor as demonstrated in Figure 6. In the UCT process the recycle of nitrate from the aerobic reactor must be controlled so that the anoxic zone is underloaded with nitrate to minimize the recycle of nitrate back to the anaerobic zone. The mixed liquor recycles amount to 100-200 percent of the influent flow. Owing to the long sludge retention time in the anoxic zone the sludge inclines to bulking sludge.

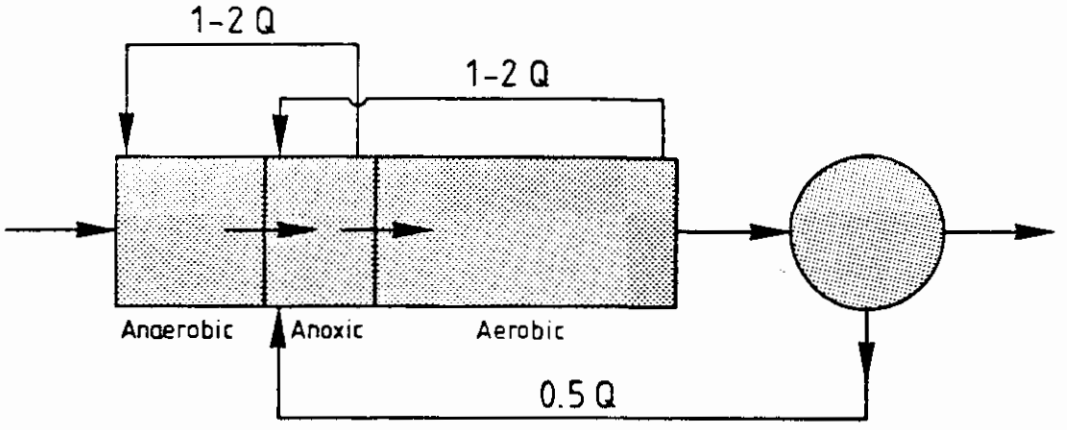

Fig. 6. The UCT process

Modified UCT process

To shorten the anoxic retention time of the mixed liquor the anoxic zone has been changed in two anoxic zones. See Figure 7. The return sludge is sent to the first anoxic zone to denitrify only the nitrate in the return sludge. The second anoxic zone serves where the most nitrate is denitrified. The mixed liquor streams are 100-200 percent of the influent flow.

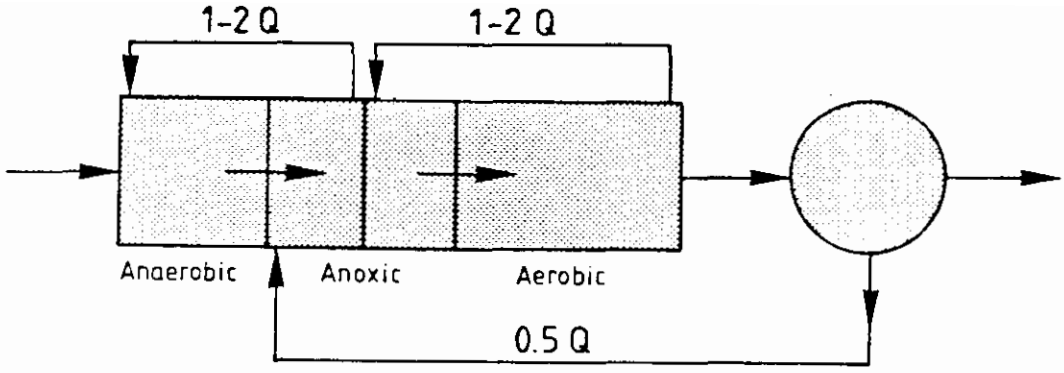

Fig. 7. The modified UCT process

Biodenipho process

The Biodenipho process is based on the Biodenitro process, which exists of two coupled aeration tanks with intermittend aeration. By alternating wastewater supply, optimal nitrification and denitrification occur as well. Because both processes take place in the same tank, recirculation of nitrified water to the anoxic zone has been excluded. The Biodenipho process (see Figure 8) has been equiped with an anaerobic zone in front of the plant to encourage the biological phosphorus processes. The anaerobic zone receives the incoming water and the return sludge.

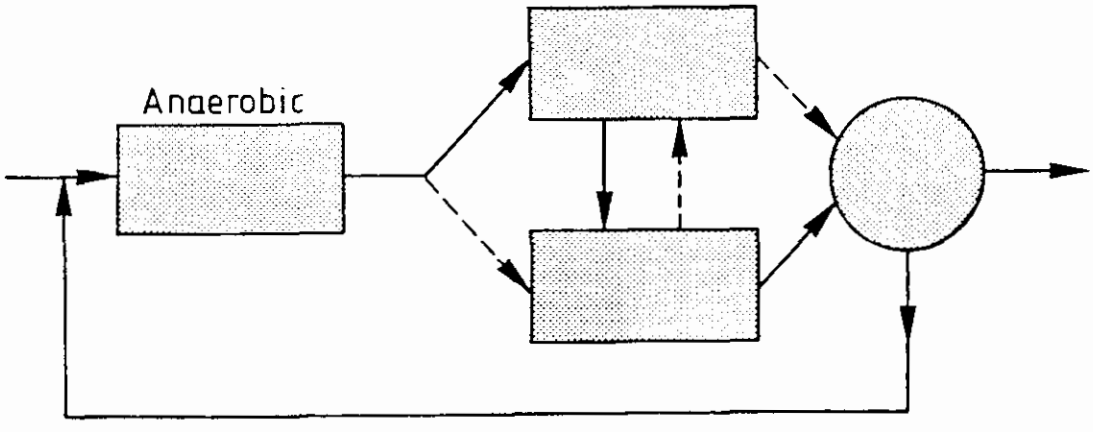

Fig. 8. The biodenipho process

BB process

The BB process, which stands for the villages Bunnik and Bunschoten where this modification initially was introduced, is a two staged activated sludge process with an alternating aeration. This process includes the same basic anaerobic/anoxic/ aerobic components as other modifications but the zones are spatial and temporal alternation. See Figure 9. During the anaerobic/anoxic phase the mixed liquor is not mixed but settles on the bottom of the first aeration tank. The supernatant enriched with nitrate doesn't inhibit the fermentation process in the sludge layer, which results finally in a higher degree of phosphorus removal. The nitrification and denitrification takes place in the first and second aeration tank. By periods of aeration in the first and second aeration tank Acinetobacter accumulates phosphorus. The process is easily controlled by an oxygen electrode.

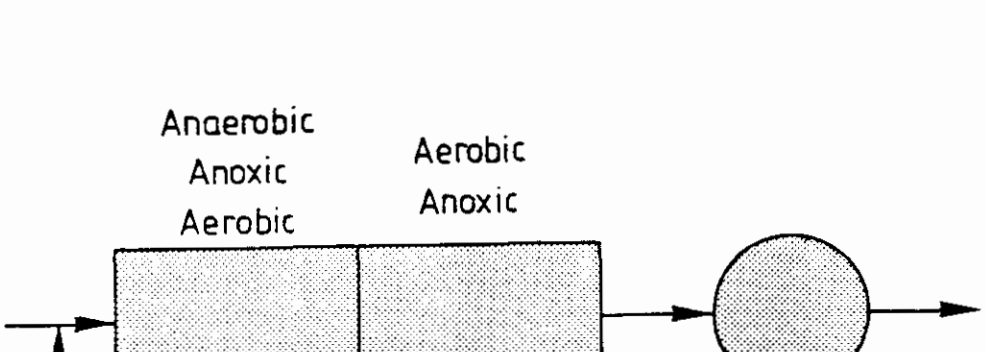

Fig. 9. The BB process

Modified carrousel and oxidation ditch

In a carrousel or oxidation ditch the activated mixed liquor flows continuously around a loop-type channel. An aerator system cares for the aeration of the activated sludge. By a low oxygenation capacity it is possible to create an aerobic zone capable of nitrification immediately downstream of the aerator

and an anoxic zone upstream of the aerator for some distance. By discharging the influent at the upstream limit of the anoxic zone, some of the wastewater carbon source is used for denitrification. By placing an anaerobic zone in front of the ditch, where the return sludge meets the influent an optimal combination of phosphorus and nitrogen can be attained. See Figure 10.

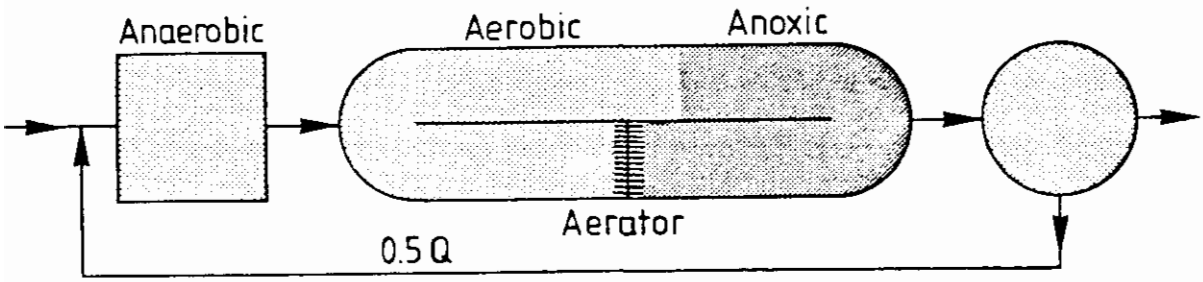

Fig. 10. The modified oxidation ditch

Renpho process or modified Phostrip process

The Phostrip is characterized by a standard activated sludge plant with a stripper (an anaerobic thickener) in the sidestream. So a part of the return sludge has been sent to the anaerobic stripper tank to release the phosphorus from the sludge. The stripped sludge is sent back to the activated sludge plant for phosphorus uptake, while the enriched supernatant is treated with lime. The precipitated calciumphosphate is sent to the primary clairifier for settlement and removal from the plant. To stimulate the release process in the stripper tank acetate can be added. To remove the phosphorus from the sludge area elutration water is pumped through the stripper.

The modified Phostrip process is based on incorporation of anaerobic/anoxic/aerobic zones into the mainstream to remove the nitrogen. See Figure 11. To eliminate nitrate sludge recirculation for denitrification, a part of the influent is led to the anoxic zone.

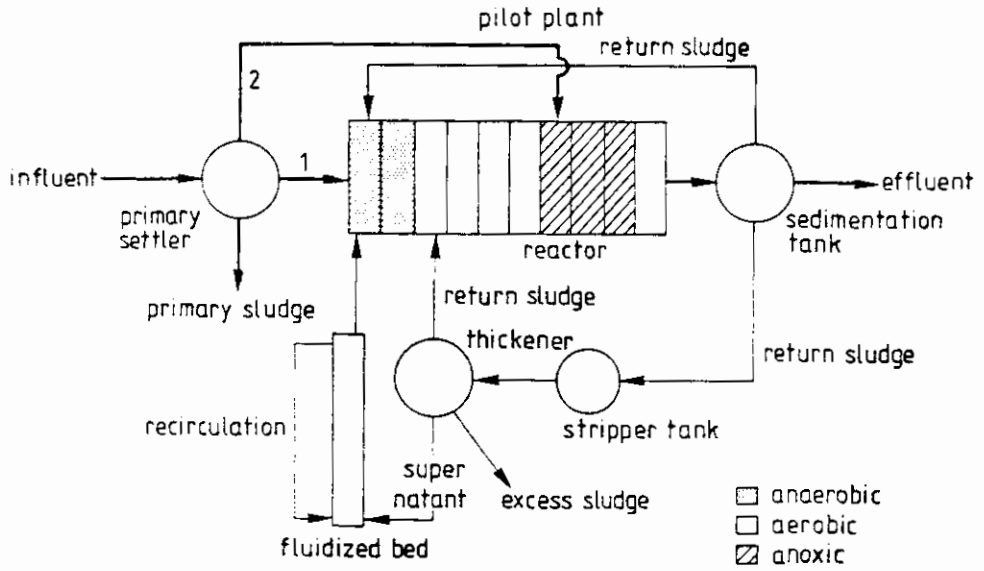

Fig. 11. The Renpho process

BIOLOGICAL NUTRIENT REMOVAL; WWTP BENNEKOM

P.J. Tessel
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BIOLOGICAL NUTRIENT REMOVAL WWTP BENNEKOM

1 Introduction

The Waste Water Treatment Plant (WWTP) Bennekom was constructed in 1970. The WWTP was designed as an oxidation ditch without a primary settling tank with a BOD-sludge loading of 0.05 kg/(kg x day). The final settling of the effluent took place discontinuously within the oxidation ditch. The original lay-out of the WWTP is given in appendix I.

2 Rebuild in 1989

Due to an increasing amount of sewage, in 1989 the WWTP was rebuild mainly by increasing the maximum hydraulic capacity and constructing a new continuously operating final settling tank. To introduce a system for biological phosphorus removal, about 1/6 part of the original oxidation ditch volume has been changed in an anaerobic compartment. The capacity of the WWTP now is 22,000 population equivalent with a maximum hydraulic capacity of 600 m^3/hr.

The new lay-out of the WWTP is given in appendix II.
The design data of the 1989 rebuild are given in appendix III.

In December 1989 the new parts of the WWTP were taken into operation. After some start up problems during the first quarter of 1990, now the WWTP is in stable operation. The excess sludge of the WWTP Bennekom is pumped to the nearby large WWTP in Ede through a 5 km long pipeline. This prevents the potential recycling of phosphorus in the WWTP Bennekom as a result of phosphorus release out of the activated sludge.

3 Comparison of results 1989 and 1990

The 1989 rebuild had to result in a more efficient P-total removal in a biological way without adding any chemicals. Besides that, some increase of the N-total removal efficiency was expected, due to the increase of anoxic volume in the oxidation ditch.

In the next table the removal rates in 1990 are given as well as those in 1989. For more details see appendix IV.

Parameter	*1989* *% removal*	*1990* *% removal*
COD	94	94
BOD	98	98
N-Kjeldahl	90	90
N-total	78	81
P-total	66	87

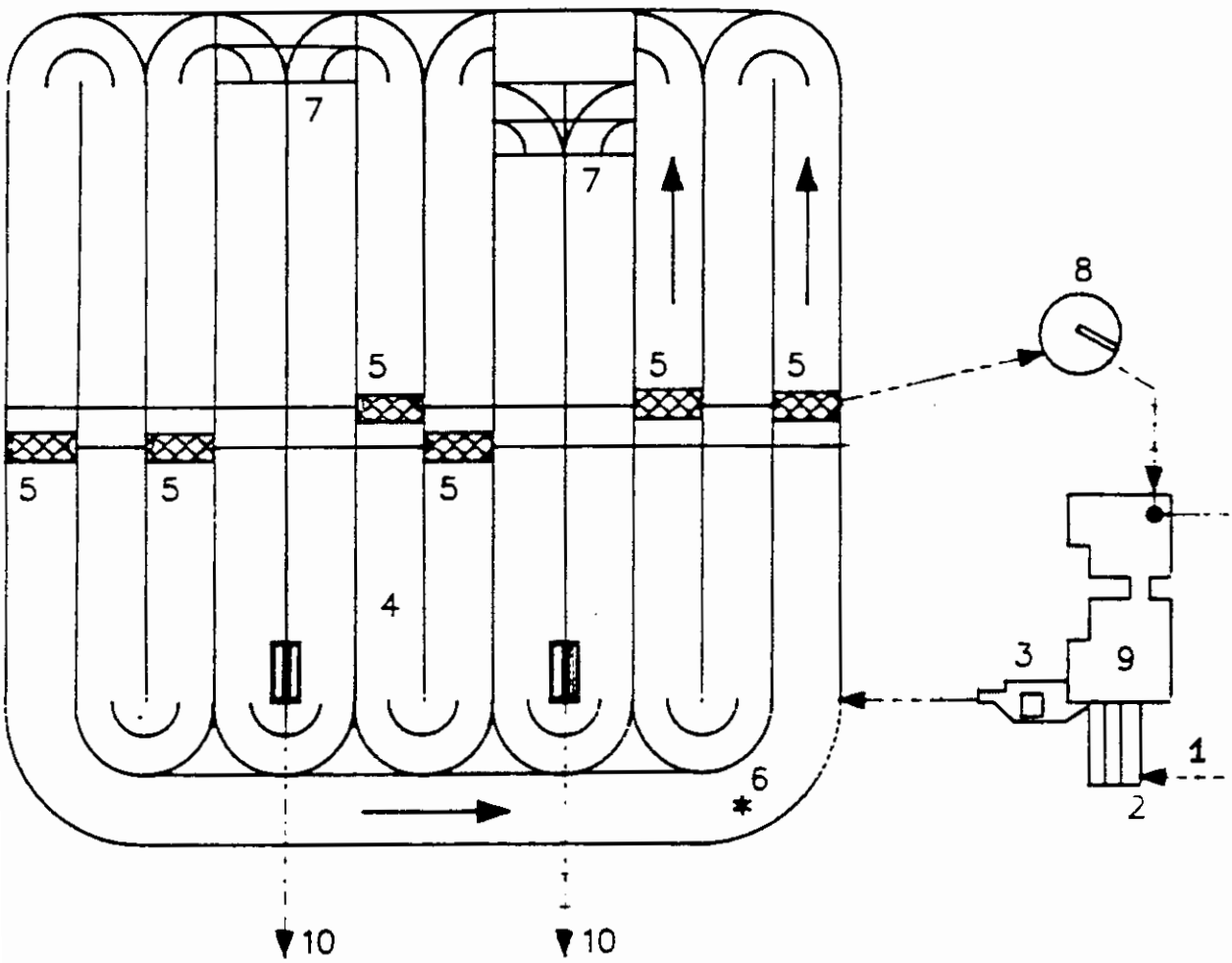
Before the rebuild, the Sludge Volume Index (SVI) had an average value of 175 ml/g. In 1990 the average SVI was 128 ml/g.

4 Discussion

The results are satisfactory in relation with the expectations. In 1990, including some start up problems, the average P-total content in the effluent was 1.06 mg/l with an average N-total concentration of 9.2 mg/l.

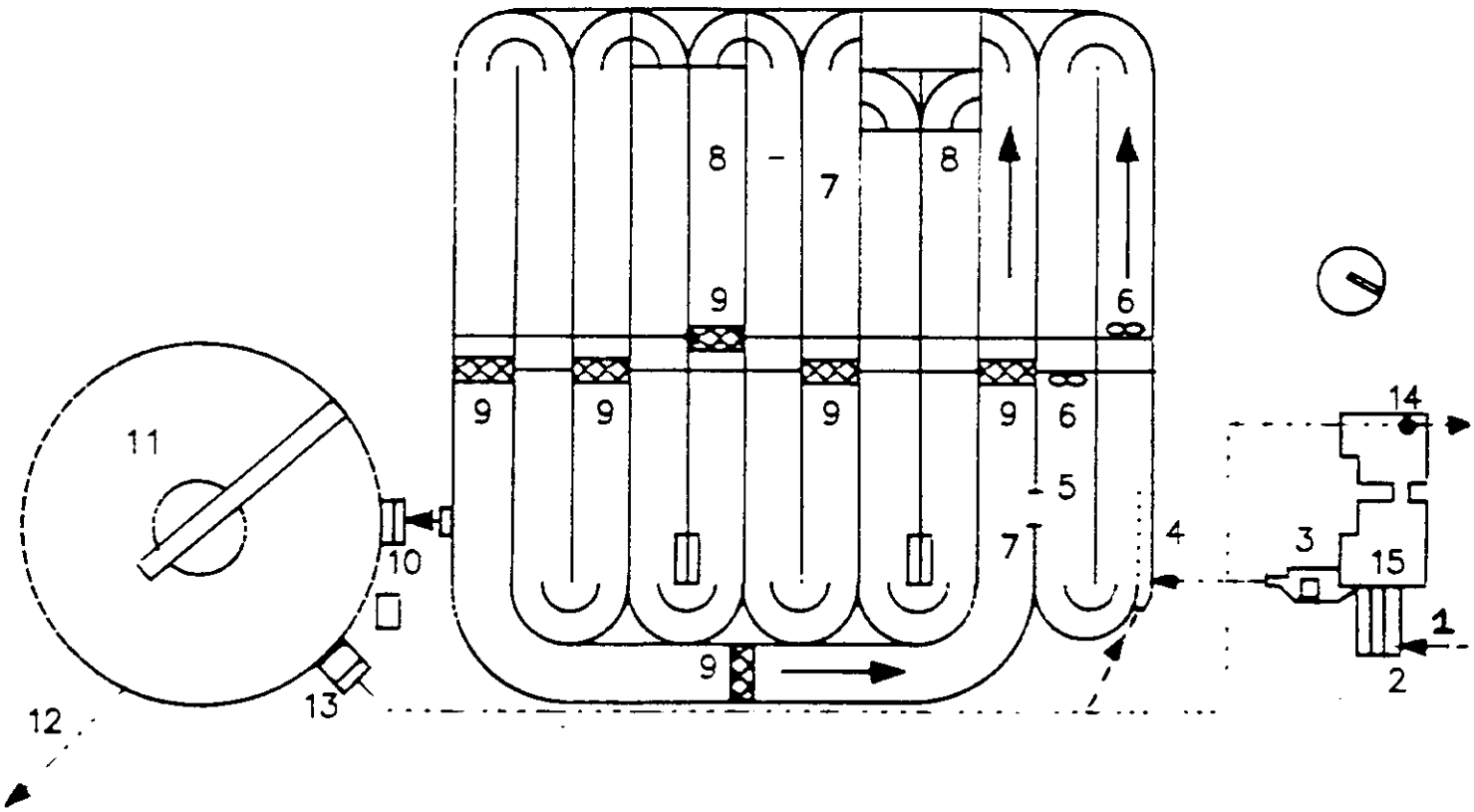
It can be concluded that on the long term, the new effluent standards in the Netherlands of 1.0 mg/l P-total and 10 mg/l N-total can be reached.

Appendix I: WWTP Bennekom before rebuild 1989



1. Sewer
2. Influent pumping station
3. Macerator
4. Aerobic compartment
5. Brush aerators
6. Oxygen measuring device
7. Discontinuous settling volumes
8. Excess sludge thickener
9. Operating building
10. Effluent discharge

Appendix II: WWTP Bennekom after rebuild 1989



1. Sewer
2. Influent pumping station
3. Static screen with press unit and container
4. Plugflow inlet compartment
5. Anaerobic compartment
6. Mechanical mixers
7. Aerobic compartment
8. Discontinuous settling volumes (no longer in operation)
9. Brush aerators
10. Outlet from aerobic compartment
11. Continuous settling tank
12. Effluent discharge
13. Sludge return pumping station
14. Excess sludge pumping station
15. Operation building

Appendix III

Design data WWTP Bennekom, rebuild 1989

Sewage load		
population equivalents	22,000	p.e.
dry weather flow	2,500	m^3/day
COD-load	2,100	kg/day
BOD-load	800	kg/day
TKN-load	190	kg/day
Storm water tanks (4 units)		
total volume	7,500	m^3
Influent pumping station		
1 archimedean screw	100	m^3/hr
1 " "	200	m^3/hr
1 " "	300	m^3/hr
maximum flow through WWTP	600	m^3/hr
Screening installation		
screening distance	6	mm
press unit and container		
Inlet compartment		
total volume	18	m^3
plug flow without mixing		
Anaerobic compartment		
total volume	850	m^3
water depth	0.9	m
mechanical mixers	2	units
Aerobic compartment		
total volume	4,850	m^3
water depth	0.9	m
dry solids content	3.3	kg/m^3
BOD-sludge loading	0.05	kg/(kg x day)
brush aerators (6 m length)	6	units

Appendix III

Final settling tank		
designed for future influent flow of	850	m^3/hr
diameter 41.5	m	
surface 1,350	m^2	
surface loading at 600 m^3/hr	0.44	$m^3/(m^2 \times hr)$
surface loading at 850 m^3/hr	0.63	$m^3/(m^2 \times hr)$
Sludge return pumping station		
designed for future influent flow of	850	m^3/hr
centrifugal pumps	2	units
capacity each pump	380	m^3/hr
Excess-sludge pump		
capacity 12	m^3/hr	

Appendix IV: Details WWTP Bennekom

Parameter	*Influent/effluent/removal*		*1989 before rebuild*	*1990* after rebuild*
COD	Influent	(mg/l)	594	581
	Effluent	(mg/l)	33	34
	Removal	(%)	94	94
BOD	Influent	(mg/l)	209	187
	Effluent	(mg/l)	3.2	3.1
	Removal	(%)	98	98
N-Kjeldahl	Influent	(mg/l)	54	49
	Effluent	(mg/l)	5.4	5.0
	Removal	(%)	90	90
N-total**	Influent	(mg/l)	54	49
	Effluent	(mg/l)	11.9	9.2
	Removal	(%)	78	81
P-total	Influent	(mg/l)	9.6	8.1
	Effluent	(mg/l)	3.3	1.06
	Removal	(%)	66***	87***
SS	Effluent	(mg/l)	9	7

* incl. start up ** Sum of N-Kjeldahl and $N\text{-}NO_3^-$ *** No use of chemicals

SIMULTANEOUS NITRIFICATION AND DENITRIFICATION FOR N-REMOVAL

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Kluyver Laboratory for Biotechnology, Delft University of Technology

SIMULTANEOUS NITRIFICATION AND DENITRIFICATION FOR N-REMOVAL

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INTRODUCTION

Nitrification (the oxidation of reduced nitrogen compounds, generally ammonia, to nitrite, nitrate or other nitrogen oxides) and denitrification (the reduction of oxidized nitrogen compounds to gases; N_2O, N_2) are two of the dominant driving forces in the biological nitrogen cycle. Thus far, there are only a limited number of species known to be able to grow at the expense of energy generated by nitrification, the best studied of which are members of the genera *Nitrosomonas* (which oxidize ammonia to nitrite via hydroxylamine) and *Nitrobacter* (which oxidize nitrite to nitrate). These Gram negative bacteria are obligate chemolithoautotrophs and depend on CO_2 fixation for biosynthetic carbon. In denitrification, the nitrogen oxides serve as terminal electron acceptors rather than donors, and the denitrifiers are considerably more diverse, as is illustrated by the examples shown in table 1. There are both Gram negative and Gram positive denitrifiers with representatives from most physiological (e.g. from obligate chemolithoautotrophs to chemoheterotrophs) and morphological types. The complete reduction of nitrate proceeds via nitrite, nitric oxide and nitrous oxide, but not all denitrifiers can carry out the complete reduction from nitrate to N_2, enzymes most commonly missing being nitrate reductase or nitrous oxide reductase.

TABLE 1. Examples of denitrifying reactions, and some of the bacteria which employ them (adapted from Kuenen & Robertson, 1987).

Reaction	Denitrifying species
nitrate to nitrite	*Thiobacillus thioparus, Lysobacter antibioticum*
nitrate to nitrous oxide	*Aquaspirillum itersonii*, pseudomonads
nitrate to nitrogen	*Paracoccus denitrificans, Thiobacillus denitrificans, Alcaligenes eutropha, Hyphomicrobium* sp., *Halobacterium* sp.
nitrite to nitrogen	*Neisseria* and *Flavobacterium* sp. *Alcaligenes faecalis* strain TUD
nitrous oxide to nitrogen	*Vibrio succinogenes*

Because of their respective natural roles in returning dinitrogen originally fixed in (organic) nitrogenous compounds by nitrogen fixing bacteria to the atmosphere, and also because of their importance in nitrogen removal in today's wastewater treatment systems, both the nitrifiers and the denitrifiers have been the subject of considerable research effort over the years, both in the laboratory and in the field. This research has been extensively reviewed (Stouthamer, 1988; Payne, 1981; Kuenen & Robertson, 1987; Winkler, 1981; Wood, 1986). An examination of the history of research into the nitrogen cycle, especially where denitrification and nitrification are concerned, reveals a number of difficulties and sometimes even controversies which are only becoming resolved as methodology and equipment improve. Among the topics which have controversial aspects are heterotrophic nitrification with aerobic denitrification, and anaerobic nitrification. As both of these processes begin with ammonia and end with gaseous nitrogen, they have obvious attractions for single stage nitrogen removal.

Simultaneous nitrification and denitrification

Aerobic denitrification

For over 100 years, the occurrence of "aerobic denitrification" (i.e. active denitrification in the presence of significant amounts of oxygen) has been a matter of dispute. Despite regular reports of aerobic denitrification, the difficulties of making accurate measurements of parameters such as dissolved oxygen and gas production made the production of convincing results difficult (for a review of the historical arguments see Robertson & Kuenen, 1984a; 1990). However, the availability of modern oxygen and ion-specific electrodes, well-aerated bioreactors vessels and analytical techniques has made it possible to establish that a group of denitrifiers are able to simultaneously utilize oxygen and nitrate or nitrite in completely homogenous cultures, even when the dissolved oxygen concentration approaches air saturation. Indeed, these bacteria simultaneously use both electron acceptors (Kuenen & Robertson, 1987). One of the best-studied members of this group is *Thiosphaera pantotropha*, a mixotrophic colourless sulphur bacterium which was isolated from a denitrifying, sulphide-oxidizing wastewater treatment plant (Robertson & Kuenen, 1983).

During comparisons of the newly-isolated strain with known denitrifiers (e.g. *Thiobacillus versutus,* and *Paracoccus denitrificans*) in anaerobic respirometry experiments, it was observed that aerobically grown *Tsa. pantotropha* began to denitrify immediately when it was supplied with substrate and nitrate. Similarly-grown cultures of the other strains required 2 to 4 hours to induce their denitrifying enzymes (Robertson & Kuenen, 1984b). Oxygen and nitrate electrodes were used to monitor the activity of these cultures, and simultaneous nitrate and oxygen removal in *Tsa. pantotropha* suspensions were clearly observed (Robertson et al., 1986). When grown in batch cultures (Robertson & Kuenen 1984b) with acetate as their substrate, *Tsa. pantotropha* cultures provided with both oxygen (at a dissolved oxygen concentration of 80% air saturation) and nitrate grew more rapidly than similar cultures which had only one electron acceptor (table 2). Moreover, the protein yield at the end of the experiments with the cultures supplied with nitrate and oxygen was lower than that obtained when oxygen was the sole electron acceptor (table 2), and higher than when nitrate served this function in anaerobic cultures (40 mg per l). As denitrification tends to generate less energy, and thus lower biomass yields, than oxygen respiration this observation indicated that both pathways were in simultaneous use by the culture. Sufficient nitrate had disappeared from the oxygen/nitrate cultures to account for half of the acetate dissimilated. Obviously, the other half of the acetate was dissimilated with oxygen as the terminal electron acceptor. A possible explanation of the higher growth rates obtained when both electron acceptors were supplied could be that *Tsa. pantotropha* has a rate-limiting step in the transfer of electrons from its substrate to oxygen. The provision of a second electron acceptor, in this case nitrate, would allow it to use an additional branch in the electron transport chain, overcoming the problem.

Table 2. Comparison of the maximum specific growth rates (μ_{max}), final protein concentrations and nitrate reduced from aerobic batch cultures growing with acetate as substrate and ammonia as the nitrogen source. Results obtained with a *Paracoccus denitrificans* strain which does not denitrify aerobically have been added for comparison (data from Robertson et al., 1989a).

Organism	μ_{max} (h^{-1})			Protein (mg/l)		Δ nitrate
	O_2	O_2/NO_3^-	NO_3^-	O_2	O_2/NO_3^-	(mM)
Pseudomonas sp.	0.1	0.41	0.15	78	68	5.0
A. faecalis	0.17	0.25	0.01	30	14	4.1
Ps. aureofaciens	0.19	0.21	0.07	66	66	5.0
Tsa. pantotropha	0.28	0.34	0.25	81	60	5.5
Pa. denitrificans	0.28	0.28	-	92	88	<1

Since these batch culture experiments indicated that the denitrifying enzymes of *Tsa. pantotropha* were not only constitutive, but indeed active during aerobic growth (Robertson & Kuenen, 1984b, Robertson et al., 1986) a further detailed analysis of this phenomenon was made, from both ecological and physiological points of view. In order to make accurate nitrogen balances, aerobic denitrification was quantified using chemostat cultures and carefully defined environmental conditions. As soon as these studies were initiated, it became

apparent that *Tsa. pantotropha* was also able to nitrify, provided that an organic substrate was available, an ability termed heterotrophic nitrification.

Heterotrophic nitrification.

During the batch culture experiments to discover whether *Tsa. pantotropha* was denitrifying aerobically, nitrite replaced nitrate in a series of experiments, and it was observed that the nitrite concentration increased before eventually decreasing to 0. This phenomenon only occurred in the presence of an organic substrate, ammonia and oxygen, indicating that *Tsa. pantotropha* is a heterotrophic nitrifier. In other words, *Tsa. pantotropha* can catalyze the oxidation of ammonia to nitrite provided that an organic electron donor (in this case acetate) is available. Subsequent experiments revealed that the nitrifying enzymes of *Tsa. pantotropha* were similar to those of autotrophic nitrifiers such as *Nitrosomonas europaea* in that the ammonia monooxygenase required NAD(P)H, the hydroxylamine oxidoreductase was light sensitive (Robertson & Kuenen 1988), and the nitrification was sensitive to the same chemicals which inhibit autotrophic nitrification (e.g. allylthiourea, nitrapyrin, reduced sulfur compounds). Nitrite only accumulated in the presence of nitrite or an inhibitor of nitrite reductase, and it became clear that *Tsa. pantotropha* was simultaneously reducing all or most of the nitrite to N_2 (Kuenen & Robertson 1987, Robertson et al., 1988).

As it has proved impossible to separate the two phenomena, aerobic denitrification and heterotrophic nitrification will be considered further together.

Heterotrophic nitrification and aerobic denitrification - a combined process.

Chemostat experiments carried out with acetate-limited *Tsa. pantotropha* cultures (Robertson et al., 1988) revealed that both the nitrification and the denitrification rates increased as the growth rate increased (table 3) and as the dissolved oxygen concentration decreased. For example, a culture growing at a dilution rate of 0.04 h^{-1} in the presence of ammonia and nitrate at a dissolved oxygen concentration of 25% air saturation nitrified and denitrified at rates of 12 and 107 nmol min^{-1} mg $protein^{-1}$, respectively. In similar cultures maintained at only 5% air saturation, these rates had risen to 33 and 393 nmol min^{-1} mg $protein^{-1}$, respectively. Moreover, the presence of nitrate or nitrite in the medium reduced the amount of nitrification taking place (table 3). If nitrate or nitrite were not provided, and ammonia oxidation was inhibited in some way (e.g. by the presence of hydroxylamine in the medium), *Tsa. pantotropha* synthesized large amounts of poly-*B* hydroxybutyrate (PHB). These observations, considered with the higher growth rates observed during growth in batch on oxygen and nitrate together, led to the hypothesis that both aerobic denitrification and heterotrophic nitrification are mechanisms by which a rate limiting step in the flow of electrons to oxygen can be overcome. The model is shown as a flow chart in figure 1. It is assumed that denitrification has priority over heterotrophic nitrification because the latter costs, rather than generates, energy.

Table 3. Nitrification and denitrification rates obtained with chemostat cultures of *Tsa. pantotropha* growing aerobically (dissolved oxygen = 80% air saturation) on acetate with different nitrogen compounds in the medium (adapted from Robertson et al., 1988). *Nitrification and denitrification rates as nmol ammonia and* nitrate oxidized per minute per mg protein, respectively.

N-compounds	Dilution rate (h^{-1})	Nitrification rate.	Denitrification rate.
ammonia	0.02	13	13
	0.05	43	43
	0.10	94	94
ammonia/nitrate	0.02	8	38
	0.04	12	107
	0.18	26	507
ammonia/nitrite	0.02	10	13
	0.04	21	41
	0.17	44	177

***Tsa. pantotropha* is not unique.**

It was also important to establish whether *Tsa. pantotropha* was unique in its ability to respire oxygen

and denitrify simultaneously. A second line of research was therefore to screen other denitrifiers for the ability to use oxygen and nitrate or nitrite simultaneously (Robertson et al., 1989a; 1989b). The results of the initial experiments with known heterotrophic nitrifiers, where stimulation of growth rate and depression of aerobic protein yields were the parameters tested, are shown in table 2. It can be seen that, with the exception of the negative control, *Paracoccus denitrificans* (a strain which does not nitrify significantly and requires anaerobiosis for denitrification) all of the strains tested had used a significant amount of nitrate. *Pseudomonas aureofaciens* did not show the expected lower yields, and its growth rate was only slightly stimulated by the presence of both electron acceptors. This species is atypical in other features as its denitrification pathway terminates at N_2O, and further work is required in order to understand its physiology.

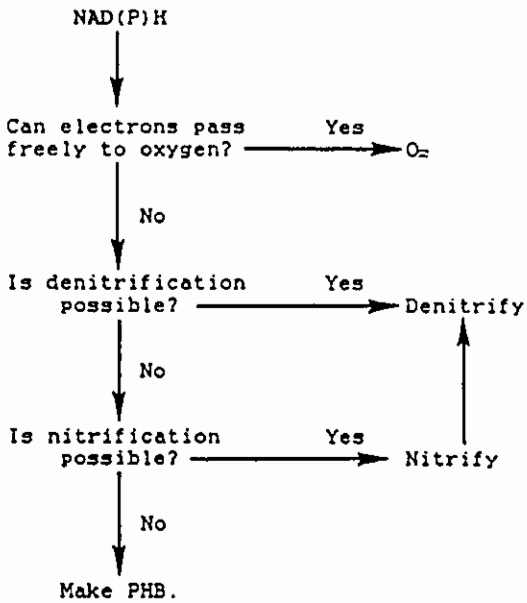

Figure 1. Flow chart to show the hypothesis worked out to explain the physiological controls behind aerobic denitrification and heterotrophic nitrification by *Tsa. pantotropha*. Each of the Yes/No decisions would be controlled by the degree of reduction of the cytochromes involved (from Robertson et al., 1988).

Because they were simultaneously denitrifying, only insignificant amounts of nitrite accumulated in these cultures, indicating that the reported low activities of heterotrophic nitrifiers might, at least in some cases, be underestimates of *in vivo* nitrification rates. Nitrification is traditionally judged from the amounts of nitrification products which accumulate, and these results serve to emphasize a need for the making of element balances for cultures in order to measure nitrogen losses and assess both nitrification and denitrification activity. Aerobic denitrification has now been reported for a number of other species (e.g. Bazylinski & Blakemore, 1983; Krul, 1976; Lloyd et al., 1987; Strand et al., 1988; Trevors & Starodub, 1987), and it will be interesting to investigate whether these are also heterotrophic nitrifiers.

Ecological significance

The compliation of out own and published data shpown in figure 2 emphasizes that the ability to denitrify aerobically is strongly dependent on the organism and the environmental oxygen concentration, with the denitrifying pathways of different strains having differing "cutoff" levels of oxygen above which denitrification becomes impeded. Most of these bacteria are also heterotrophic nitrifiers, and this property, if present, is likely to show a similarly wide response to environmental parameters in various species. Further work is required to determine the scope of these phenomena, and their inter-relationship. Heterotrophic nitrification is slower than autotrophic nitrification when specific activities are compared (by a factor 10^2 - 10^3 rather than the factor

10^4 previously believed), but when the vastly superior numbers of heterotrophs present in, for example, soil is considered, it can be seen that these organisms can make a significant contribution to nitrogen turnover rates.

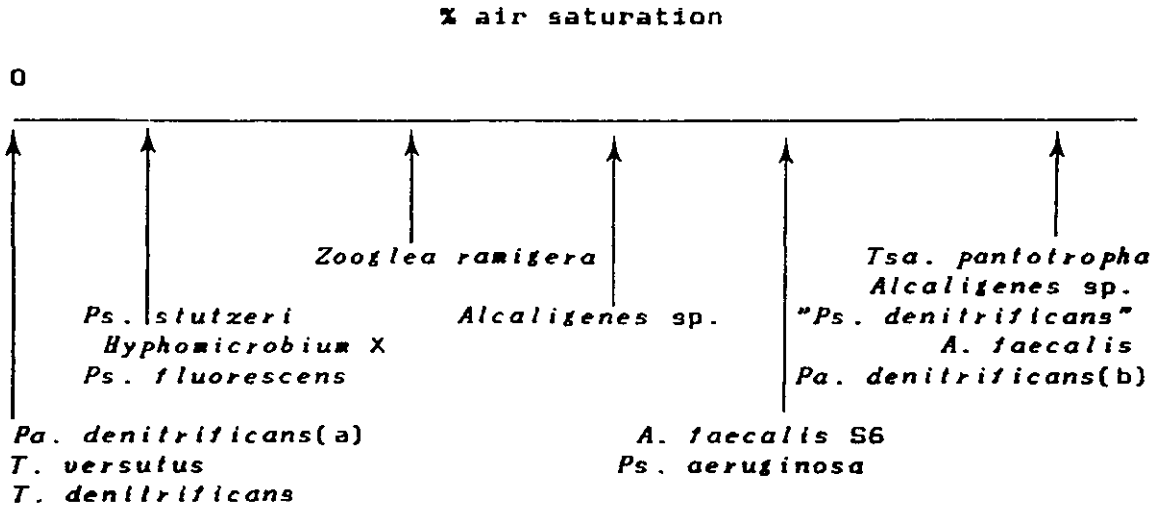

Figure 2. "Spectrum" indicating the thresholds at which denitrification by different bacteria begins to be affected by the dissolved oxygen concentration. % air saturation is shown rather than oxygen concentrations to allow for the different growth temperatures of the various bacteria (from Robertson & Kuenen, 1990).

The contention that one must view the combination of heterotrophic nitrification and aerobic denitrification as a processwhich is multi-dimensional in terms of species, space, time and environmental conditions ios supported by recent work in our and other laboratories. For example, it has been shown that two other nitrifying/denitrifying species show similarities to each other and to *Tsa. pantotropha*, but that they also differ in some respects (Robertson et al., 1989a; van Niel et al., 1990). For example, all of the aerobic denitrifiers thus far tested have the copper-based nitrite reductase rather than cytochrome cd (Robertson et al. 1989a; Robertson & Kuenen, 1990). *Pseudomonas* sp. was very similar to *Tsa. pantotropha*, except that its denitrifying nitrate reductase was inducible. The remainder of its denitrification pathway was constitutive (Robertson et al., 1989a). Denitrification by *Alcaligenes faecalis* strain TUD proved to be partially inhibited by oxygen at concentrations of 50% air saturation, and completely lacked a nitrate reductase (van Niel at al., 1990).

In order to gain an impression of the potential of heterotrophic nitrifers, compared with autotrophic nitrifiers, *Tsa. pantotropha* and *N. europaea* were co-cultured in chemostats under different dissolved oxygen concentrations (figure 3) and C:N ratios (figure 4) (van Niel, 1991). *Tsa. pantotropha* was limited by acetate, and the two bacteria competed for ammonia (as a source of energy as well as nitrogen for *N. europaea*, as the primary source of nitrogen and a substrate for heterotrophic nitrification in *Tsa. pantotropha*. On the basis of the results of these experiments, combined with physiological data from axenic cultures, mathematical models were made to describe the behaviour of the two types of nitrifier (solid lines on figures 3 and 4). Under most conditions, the two species co-existed, with *N. europaea* as the primary nitrifier. However, *N. europaea* was unable to maintain itself at low oxygen concentrations and high C:N ratios, when *Tsa. pantotropha* dominated the culture, and was responsible for most of the ammonia oxidation. Similarly, *Tsa. pantotropha* had a much higher μ_{max} than *N. europaea*, and therefore did better at higher dilution rates. *N. europaea* was not inhibited by the acetate concentrations used, and actually appeared to be able to assimilate a small amount as a carbon supplement.

As yet, there is insufficient data available to allow the evaluation of the activity of bacteria capable of heterotrophic nitrification and simultaneous aerobic denitrification in the field. Inhibitors are frequently used in order to estimate microbial activity in a particular situation. The use of inhibitors can prove valuable. For example, acetylene can block N_2O reduction. As N_2O is much easier to measure than N_2, this can make the

measurement of denitrification rates in, for example, soil or water samples, much more convenient and accurate. However, care should be take in the interpretation of results as these inhibitors are seldom completely specific, and all necessary controls must be done. For example, acetylene also inhibits autotrophic nitrification, and therefore it is essential that NO_x is already present in, or added to the test system because it will not be generated biologically once acetylene has been added to the experimental system. Another example of the potential pitfalls is that a commonly used inhibitor of nitrification, nitrapyrin, is not soluble in water, and is frequently used as a solution in acetone. Hall (1984) showed that in some cases, the acetone, alone, can be as inhibitory as the nitrapyrin solution. This has proved to be the case not only with the obligate autotrophs, but also with heterotrophic nitrifiers such as *Thiosphaera pantotropha*. A final example of a potential problem associated with inhibitors involves the so-called autotroph-specific inhibitors such as nitrapyrin and allylthiourea. For a long time, it was believed that these compounds only inhibited autotrophic nitrification, and thus only the activity which was not inhibited by them was considered to be due to heterotrophic nitrifiers. Recent research (Robertson et al., 1989b) has shown that nitrification by many heterotrophic bacteria is, in fact, also sensitive to nitrapyrin, allylthiourea and thiosulfate. It seems likely that at least some heterotrophic nitrifying activity may have been included in estimates of autotrophic activity.

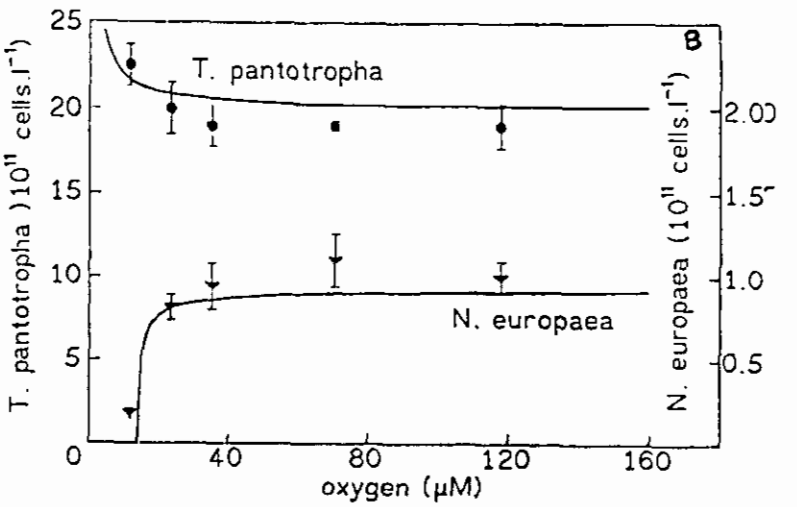

Figure 3. The effect of the dissolved oxygen concentration on *Tsa. pantotropha* (circles) and *N. europaea* (triangles) when competing for ammonia in chemostat cultures. The solid lines show the results predicted by the model (from van Niel, 1991).

This, of course, leaves the problem of how to distinguish between heterotrophic and autotrophic activity in the field. Most heterotrophic nitrifiers appear to also be aerobic denitrifiers (see below), and it might therefore be profitable to measure the production of ^{15}N labelled gases from ^{15}N labelled ammonia in the presence of an organic substrate. Alternatively, ammonia-stimulated CO_2 fixation would give a measure of autotrophic activity. A less direct method might involve the determination of the type of nitrite reductase dominant in a denitrifying community. All of the aerobic denitrifiers thus far tested have possessed a copper-based nitrite reductase rather than the more usual cytochrome cd (Kuenen & Robertson, 1987; Robertson et al., 1989a). A larger number of species must be screened before it is certain whether this is universal, but the two nitrite reductases are sensitive to different inhibitors (copper chelators and azide, respectively) and it might be possible to use this fact to detect the presence of bacteria capable of aerobic denitrification and heterotrophic nitrification in a community. Last, but certainly not least, there is the wide range of modern tools which are becoming available to taxonomists, including fluorescent antibody stains and DNA fingerprinting. The most obvious drawback to the use of such methods in natural systems is the need for extensive databases (derived from known, pure cultures) in order to interpret experimental results. This information is gradually accumulating, and the drawback is therefore only transient.

After sucessfully isolating a range of at least 10 different heterotrophic nitrifier/aerobic denitrifiers from

wastewater treatment systems, van Rijn, Wirenfeldt-Kruse, Robertson & Kuenen (in preparation) were able to show simultaneous nitrification and aerobic denitrification in intact sludge, in the laboratory. Ammonia and nitrate or nitrite were added to the sludge which was then incubated in Kluyver flasks at a dissolved oxygen concetration above 50% air saturation. By means of frequent sampling and analysis of the different nitrogen compounds, it could be shown that nitrate and nitrite both disappeared from these aerobic cultures, despite the presence of ammonia (figures 5 and 6, respectively). A certain amount of nitrification is evident in the culture to which nitrite was added (figure 6) as the nitrate concentration increased, but this appears to have only involved about half of the nitrite, the rest disappeared, presumably to nitrogen.

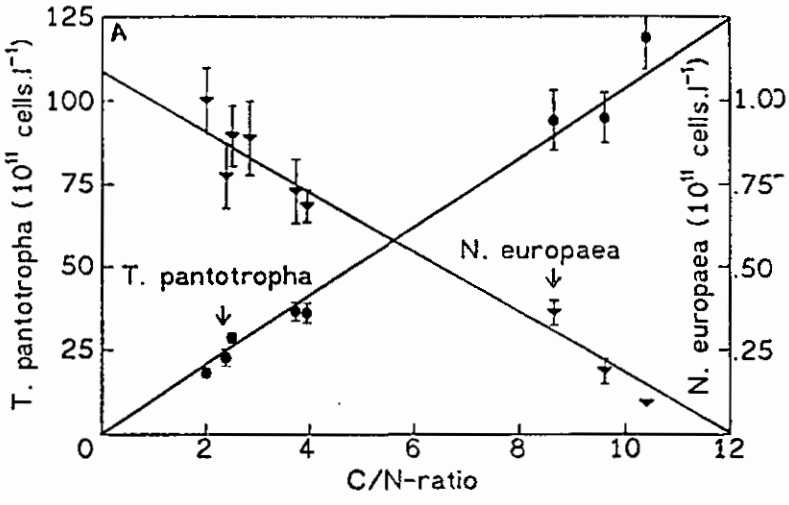

Figure 4. The impact of different (organic)C:N ratios supplied to chemostat cultures of *Tsa. pantotropha* (circles) and *N. europaea* (triangles) competing for ammonia. The solid line shows the results predicted by the rnodel (from van Niel, 1991).

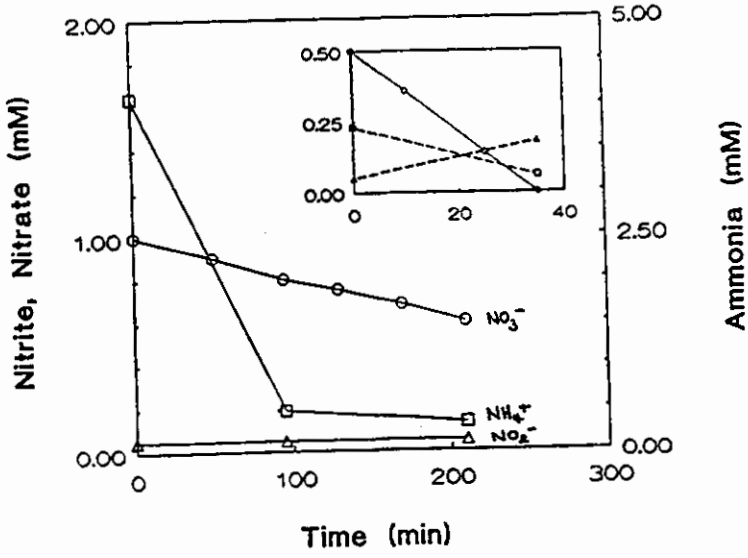

Figure 5. The change in the concentrations of various nitrogen compounds during aerobic incubation of sludge from a Pasveer ditch after the addition of ammonia and nitrate. Circles = nitrate, triangles = nitrite, squares = ammonia. The insert at the top right shows the anaerobic control experiments. From van Rijn, Wirenfeldt-Kruse, Robertson & Kuenen (in preparation)

"Anaerobic nitrification".

Following the confirmation that aerobic denitrification takes place, at least under some conditions, it did not, perhaps, come as a total surprise that anoxic nitrification would also be found to occur. Using *Nitrosomonas, Nitrosococcus* and *Nitrosolobus* species, Poth (1986) showed the production of $^{15}N_2O$ and $^{15}N_2$ from $^{15}NO_2^-$ under oxygen stress. It was postulated during this work that the $^{15}NO_2^-$ was serving as an electron acceptor, so that any available oxygen could be used by the ammonia monooxygenase. However, it has since been reported (van de Graaf et al., 1990) that a mixed culture from a wastewater treatment system is capable of nitrification under fully anaerobic conditions provided that nitrate or nitrite is present, implying that ammonia monooxygenase may not be the sole ammonia oxidizing enzyme available to these bacteria. Poth suggested that it should be possible to grow autotrophic nitrifiers anaerobically, while denitrifying, provided that they were provided with hydroxylamine rather than ammonia, but this limitation need possibly not apply for the bacteria involved in this reaction.

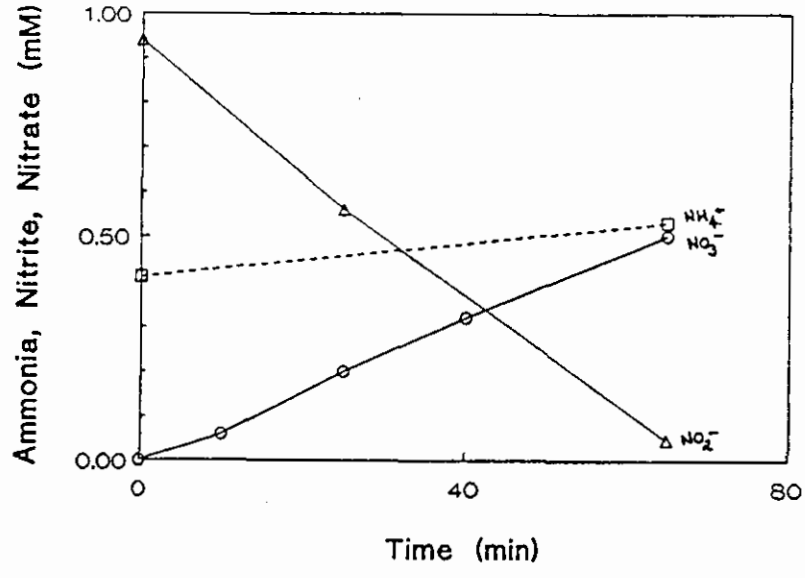

Figure 6. The change in the concentrations of various nitrogen compounds in aerobically incubated Pasveer sludgeto which ammonia and nitrite had been added. circles = nitrate, triangles = nitrite, squares = ammonia. From van Rijn, Wirenfeldt-Kruse, Robertson & Kuenen (in preparation)

It should be mentioned here that it has been shown that some nitrite-oxidizing *Nitrobacter* species can grow anaerobically as heterotrophs, with nitrate serving as the terminal electron acceptor (Bock et al., 1986). This finding stresses, once again, that the traditional concepts of nitrification and denitrification need rigorous adjustment.

Implications for wastewater treatment and the atmosphere

Nitric oxide and nitrous oxide are both important pollutants of the atmosphere. At a recent meeting organised at the University of Georgia (USA) organised by the Environmental Protection Agency, it was claimed that at least 25% of the nitric and nitrous oxides in the atmosphere come from badly managed wastewater treatment plants. It is thus the responsibility of the operators and the designers of novel treatment systems to ensure that their systems are not only operating as efficiently as possible, but that they are fully understood so that the response of the systems to stresses can be predicted. If aerobic denitrification is as widespread in the field as it appears to be in the laboratory, this implies that denitrification is not limited to anaerobic zones caused by water-saturation or large soil aggregates resulting in anaerobic microsites. Although only a few denitrifying species are known to terminate their denitrification at N_2O, it has been shown in a number of cases that N_2O reductase is one of the first denitrifying enzymes to be inhibited by increasing amounts of oxygen. It is therefore not unlikely that some aerobic denitrification may result in the emission of N_2O rather than N_2, particularly in poorly controlled wastewater treatment plants. As some nitrifying bacteria have also been shown to produce N_2O under "oxygen stress", sewage and effluent treatment systems are both

potential sources of atmospheric NO_x.

If NO_x emission into the atmosphere is to be controlled, a two-pronged attack on the problem is clearly needed. Firstly, accurate measurements of the actual release of N_2O and NO from likely sources such as marshes, sulfide-rich sediments (sulfide also inhibits nitrous oxide reductase) and water treatment plants is necessary. For example, Sorensen (1987) showed that sulfide can inhibit N_2O reduction by marine bacteria, resulting in measurable concentrations of N_2O in sea water. Secondly, more laboratory studies, with controlled conditions, are needed in order to reveal the mechanisms which determine whether N_2O, NO or N_2 is released from a particular system. The factors which determine whether NO remains enzyme-bound or is released, especially, require a great deal of further research.

REFERENCES

Bazylinski D.A. and Blakemore R.P. (1983) Denitrification and assimilatory nitrate reduction in *Aquaspirillum magnetotacticum*. Appl. Env. Microbiol. 46:1118-1124.

Bock, E., Koops, H.-P. and Harms, H. (1986). Cell biology of nitrifying bacteria. IN *Nitrification*. J.I. Prosser (editor). IRL Press, Oxford. pp.17-38.

Hall, G. (1984). Measurement of nitrification rates in lake sediments: comparison of the nitrification inhibitors nitrapyrin and allylthiourea. Microb. Ecol. 10:25-36.

Krul, J.M. (1976) Dissimilatory nitrate and nitrite reduction under aerobic conditions by an aerobically and anaerobically grown *Alcaligenes* sp. and by active sludge. J. Appl. Bacteriol. 40:245-260.

Kuenen, J.G. and Robertson, L.A., (1987) Ecology of nitrification and denitrification. IN *The Nitrogen and Sulphur Cycles* J.A. Cole and S. Ferguson (editors) Cambridge University Press. pp.162-218.

Lloyd, D., Boddy, L. and Davies, K.J.P. (1987) Persistence of bacterial denitrification capacity under aerobic conditions: the rule rather than the exception. FEMS Microbiol. Ecol. 45:185-190.

Payne, W.J. *Denitrification* John Wiley & Sons.

Poth, M. (1986) Dinitrogen production from nitrite by a *Nitrosomonas* isolate. Appl. Env. Microbiol. 52:957-959.

Robertson, L.A. and Kuenen, J.G. (1983) *Thiosphaera pantotropha* gen. nov. sp. nov., a facultatively anaerobic, facultatively autotrophic sulphur bacterium. J. Gen. Microbiol. 129:2847-2855.

Robertson, L.A. and Kuenen, J.G. (1984a) Aerobic denitrification -old wine in new bottles? Antonie van Leeuwenhoek 50:525-544.

Robertson, L.A. and Kuenen, K.G. (1984b) Aerobic denitrification: a controversy revived. Arch. Microbiol. 139:351-354.

Robertson, L.A. and Kuenen, J.G. (1988) Heterotrophic nitrification in *Thiosphaera pantotropha* - oxygen uptake and enzyme studies. J. Gen. Microbiol. 134:857-863.

Robertson, L.A. and Kuenen, J.G. 1990. Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaera pantotropha* and other bacteria. Antonie van Leeuwenhoek 57:139-152.

Robertson, L.A., van Kleeff, B.H.A. and Kuenen, J.G. (1986) A microcomputer-based method for semi-continuous monitoring of biological activities J.Microbiol. Methods 5:237-242.

Robertson, L.A., van Niel, E.W.J., Torremans, R.A.M. and Kuenen, J.G. (1988) Simultaneous nitrification and denitrification in aerobic chemostat cultures of *Thiosphaera pantotropha*. Appl. Env. Microbiol. 54:2812-2818.

Robertson, L.A., Cornelisse, R., de Vos, P., Hadioetomo, R. and Kuenen, J.G. (1989a) Aerobic denitrification in various heterotrophic nitrifiers. Antonie van Leeuwenhoek 56:289-299.

Robertson, L.A., Cornelisse, R., Zheng, R. and Kuenen, J.G. (1989b) The effect of thiosulfate and other inhibitors of autotrophic nitrification on heterotrophic nitrifiers. Antonie van Leeuwenhoek 56:301-309.

Sorensen, J. Nitrate reduction in marine sediment: pathways and interactions with iron and sulfur cycling. Geomicrobiol. J. 5:401-421.

Strand, S.E., McDonnell, A.J. and Unz, R.F. (1988) Oxygen and nitrate reduction kinetics of a non-flocculating strain of *Zooglea ramigera*. Antonie van Leeuwenhoek 54:245-255.

Stouthamer A.H. 1988. Dissimilatory reduction of oxidized nitrogen compounds. IN *Environmental Microbiology of Anaerobes*. A.J.B. Zehnder (editor). John Wiley and Sons. pp. 245-303.

Trevors, J.T. and Starodub, M.E, (1987) Effect of oxygen
concentration on denitrification in freshwater sediment. J.Basic Microbiol. 27:387-391.

Van de Graaf, A.A., Mulder, A., Slijkhuis, H., Robertson, L.A. and Kuenen, J.G. (1990) Anoxic ammonium oxidation. Proceeding s of the 5th European Congress on Biotechnology. In Press.

van Niel, E.W.J. (1991) PhD Thesis, Delft University of Technology.

van Niel, E.W.J., Braber, K.J., Robertson, L.A. and Kuenen, J.G. (1990) Heterotrophic nitrification and aerobic

denitrification in *Alcaligenes faecalis* strain TUD. Antonie van Leeuwenhoek. submitted.
Winkler, M. 1981. *Biological Treatment of Waste-Water*. Ellis Horwood, Publishers.
Wood, P. 1986. Nitrification as a bacterial energy source. IN *Nitrification*. J.I. Prosser (editor). IRL Press, Oxford. pp.39-62.

ACCUMULATION OF POLYPHOSPHATE BY *ACINETOBACTER* SP.: PHYSIOLOGY, ECOLOGY AND APPLICATION

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ACCUMULATION OF POLYPHOSPHATE BY *ACINETOBACTER* SP.: PHYSIOLOGY, ECOLOGY, AND APPLICATION

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ABSTRACT. *Acinetobacter* strain 210A accumulates intra-cellularly polyphosphate. Its concentration in the cell remains almost constant during all stages of growth. Under optimal conditions up to 36% of the cell's dry weight consists of phosphate. The amount of polyphosphate in the cells depends on the energy stage of the cell and the phosphate availability. The polyphosphate concentration is highest at low growth rates (0.1 h^{-1}) and low temperatures (< 15°C). Polyphosphate can serve as source of energy, cations (in particular magnesium), and of course phosphate. Polyphosphate can be used for ATP generation by the combined action of two enzymes, namely polyphosphate: AMP phosphotransferase and adenylate kinase. In case energy generation is not possible, a certain ATP level is conserved in the cell by polyphosphate degradation. As a consequence, phosphate is released into the medium. When ATP can be formed, by e.g. respiratory processes, phosphate is taken up again and polyphosphate synthezised. Though acinetobacters are strict aerobic organisms, they are enriched in wastewater treatment plants running in anaerobic/aerobic cycles. There, they take up phosphate during the aerobic phase and store it as polyphosphate. Subsequently phosphate is released during "energy starvation" in the anaerobic phase. In treatment plants in which the waste water passes first through an anaerobic and then through an aerobic phase, large amounts of phosphate can be removed by these microorganisms.

1. Introduction

Already in 1959 Srinath et al. have reported that wastewater treatment plants spontaneously removed more phosphate than expected. From studies with full scale plants it appeared that biological phosphate removal took place in treatment plants

with plug flow aeration reactors in which the return sludge and influent were added at the beginning of the reactor (Vacker et al. 1967, Milbury et al. 1971, Garber 1972, Yall et al. 1972). Barnard (1976) discovered that treatment plants designed for nitrogen elimination were able to remove up to 97% of phosphate as well. The presence of an anaerobic zone in the reactor was compulsory (Daveiaar et al. 1976). The most simple design was a reactor with an anaerobic zone at the beginning to which influent and return sludge were added, and an aerobic zone at the end. In the anaerobic zone the sludge released large amounts of phosphate, while in the aerobic zone phosphate was taken up almost completely.

It had been argued for many years whether this process of phosphate release and subsequent uptake is of chemical or biological nature. Evidence for a biologically catalyzed reaction was presented by Levin and Shapiro (1965). They showed that 2,4-dinitrophenol addition to the sludge inhibited completely phosphate uptake. In 1975 Fuhs and Chen isolated several *Acinetobacter* strains from phosphate removing activated sludge. All these organisms were able to store phosphate intracellularly as polyphosphate granules. Pure cultures accumulated phosphorus up to 50 mg per gram dry biomass under aerobic conditions. Acinetobacters appeared to be the dominant genus in wastewater treatment plants where phosphate was removed biologically (Nicholls and Osborn 1979), however also other species with polyphosphate granules were found in sludge flocs (Streichan et al. 1990). Up to 66% of the bacterial cells in this type of activated sludge were acinetobacters (Buchan 1983). In an acetate fed pilot plant with alternating aerobic and anaerobic conditions acinetobacters were also found to be the dominant organisms (Wentzel et al. 1987).

In the following some aspects of phosphate accumulation in *Acinetobacter*, in particular strain 210A, will be discussed together with new developments in biological phosphate removal technology from waste water.

2. *Polyphosphate in Acinetobacter*

2.1 POLYPHOSPHATE ACCUMULATION

Acinetobacter strain 210A grows best between a pH of 6 and 9. The polyphosphate content of the biomass remained around 60 mg phosphorus per gram dry cells at all pH's. In batch at pH 7.0 and 25°C the amount of phosphorus in the cells was about the same during the entire growth period. No specifically higher accumulation could be observed during either the lag-, the log-, or the stationary phase (Fig.1). Continous cultivation at μ_{max}(0.69 h^{-1}), pH 7.0 and 25°C resulted in the same phosphorus content as in batch cultures. However, at lower growth rates the amount of polyphosphate increased, reaching its maximum of 83 mg phosphorus per gram biomass dry weight at a growth rate of 0.11 h^{-1}. The highest amount of phosphorus in the cell (88 mg) was found when the cells were grown under sulphur limitation at a rate of 0.04 h^{-1} (van Groenestijn et al. 1989b). Concomitantly with orthophosphate, magnesium and potassium were taken up by *Acinetobacter* strain 210A. Although

magnesium was the preferred ion, it could be replaced by calcium. The presence of potassium was essential. In its absence phosphate uptake was strongly reduced. Magnesium acts as counterion in polyphosphate (van Groenestijn et al. 1988), whereas potassium is probably necessary for the energy metabolism of *Acinetobacter* (Neijssel and Tempest 1984).

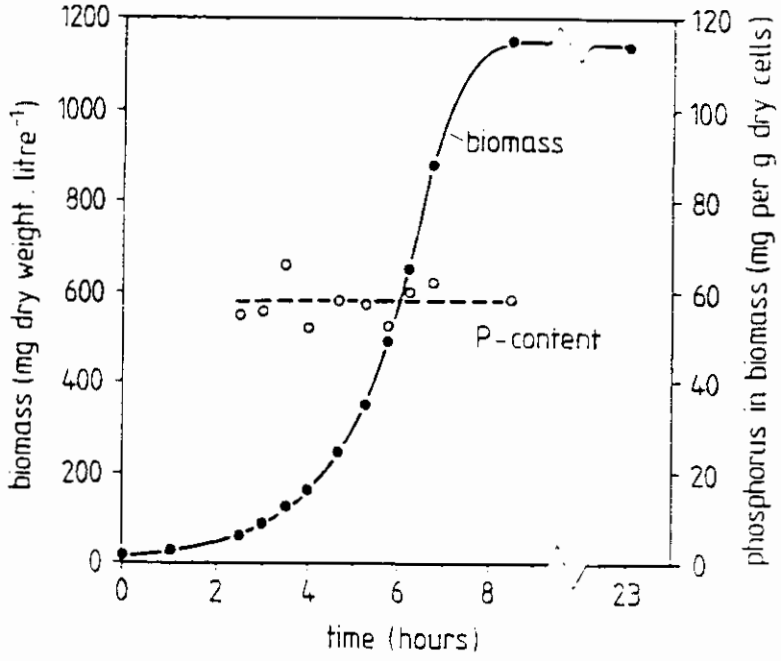

Figure 1. Growth and accumulation of phosphorus by *Acinetobacter* strain 210A in batch culture at 25°C and pH 7.0; from van Groenestijn et al. 1989.

2.2 POLYPHOSPHATE DEGRADATION

Acinetobacter strain 210A is a strict aerobic bacterium. When incubated anaerobically, it degrades polyphosphate which was accumulated during aerobic growth (Fig.1). In fact, any manipulation which prevented *Acinetobacter* strain 210A of ATP generation induced the release of phosphate (van Groenestijn 1988). Besides anaerobiosis (lack of electron acceptor), also the absence of an electron donor (e.g. acetate), or inhibitors of ATP synthesis, such as α-dinitrophenol (DNP), N,N'-dicyclohexylcarbodiimide (DCCD), etc., triggered the release of phosphate. Phosphate release was stoichiometrically paralleled by an excretion of magnesium. However, potassium extruded from the cell at a higher initial rate than phosphate and magnesium once energy conversion by respiration ceased. Some release of ammonium could also be observed after the potassium concentration outside the cell remained constant (van Groenestijn et al. 1988).

3. ATP production from polyphosphate

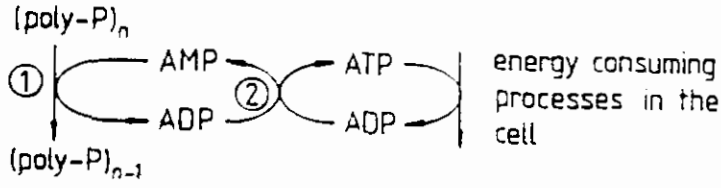

Figure 2. ATP regeneration from polyphosphate by cell-free extracts from *Acinetobacter* 210A by the combined action of polyphosphate:AMP phosphotransferase (1) and adenylate kinase (2); from van Groenestijn and Deinema (1987).

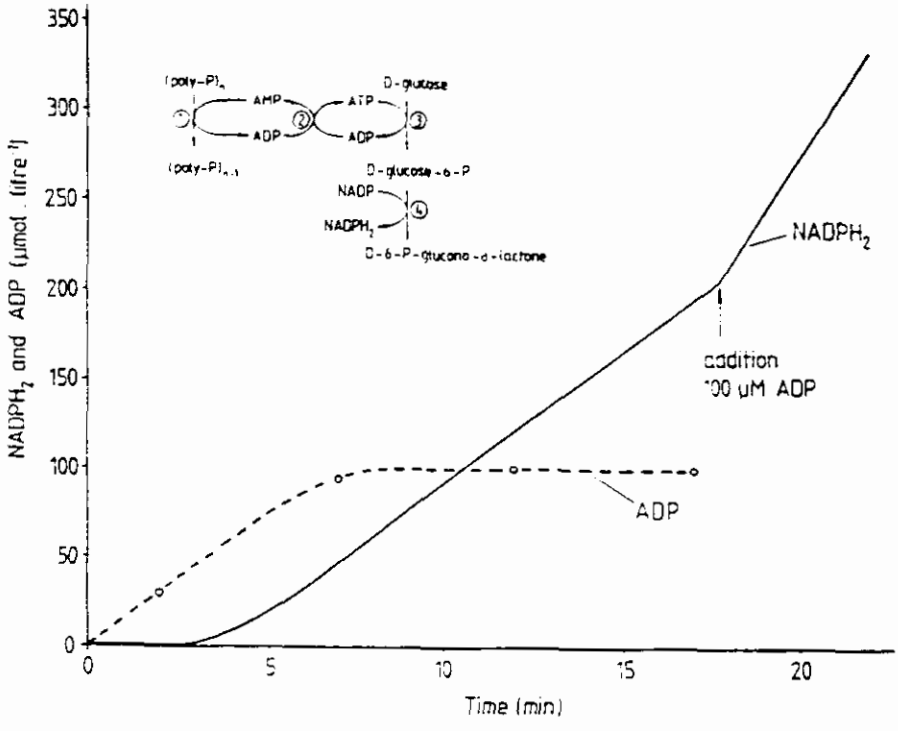

Figure 3. $NADPH_2$ (——) and ADP (_ _ _) production during the polyphosphate:AMP phosphotransferase assay. Hexokinase and glucose-6-P-dehydrogenase were coupled to polyphosphate:AMP phosphotransferase and adenylate kinase (see insert). $NADPH_2$ was measured continously with a spectrophotometer. At 18 minutes 100 nmol ADP was added per ml reaction mixture to demonstrate that the activities of the enzymes in this system were not saturated. Modified from van Groenestijn et al. (1987).

Cell-free extracts of *Acinetobacters* strain 210A contain polyphosphate:AMP phosphotransferase and adenylate kinase. Polyphosphate glucokinase and polyphosphate dependent NAD kinase were not detected. With the first two enzymes ATP can be formed according to Figure 2. The specific activity of polyphosphate:AMP phosphotransferase in this strain is 43 nmol min^{-1} mg^{-1} protein in presence of 1 m*M* AMP. The adenylate kinase has an equilibrium constant of 0.7 and an activity of 54 nmol min^{-1} mg^{-1} protein. The combined action of these two enzymes could produce ATP in cell-free extracts from chemically prepared polyphosphate, the so-called Graham's salt (van Groenestijn et al. 1987). ATP production could continously be monitored as formation of $NADPH_2$ by coupling ATP formation to the hexokinase and glucose-6-P-dehydrogenase reaction (Fig.3).
The measured activities of polyphosphate:AMP phosphotransferase and adenylate kinase are in accordance with the rates by which phosphate is released by pure cultures of *Acinetobacter* sp., namely 1 nmol PO_4-*P* min^{-1} mg^{-1} dry weight as found by Adamse et al. (1984) or 0.6 nmol PO_4-P min^{-1} mg^{-1} dry weight in activated sludge reported by Rensink et al. (1981).

4. Polyphosphate degrading enzymes in activated sludge

TABLE 1. Activity of polyphosphate: AMP phosphotransferase and adenylate kinase in various activated sludges from treatment plants with different capacities for biological phosphorus removal

Type of activated sludge	Phosphorus removal[a]	ADP production[b]	Adenylate kinase[c]
Phosphorus accumulating sludge:			
pilot plant P1	100	64	146
pilot plant Bunschoten	70	16	87
Renkum train 3	55	13	78
Conventional sludge:			
Renkum train 2	16	5	40
conventional pilot plant	n.r.[d]	2	13

[a] Percentage phosphorus removal from waste water.
[b] ADP production from AMP and Graham's salt as a measure for polyphosphate:AMP phosphotransferase activity. The numbers are given as nmol ADP formed per mg protein in 2 hours.
[c] Acitivity given as nmol min^{-1}(mg protein)$^{-1}$.
[d] No removal detected.

The activities of polyphosphate:AMP phosphotransferase and adenylate kinase in cell-free extracts of activated sludge correlated well with the capacity of the sludge to remove biologically phosphate from waste water (Table 1). The activity of polyphospate:AMP phosphotransferase was measured as ADP formation from AMP and Graham's salt (van Groenestijn et al. 1989a). The good correlation and the easy essay for adenylate kinase, makes this enzyme a suitable indicator for the potential of activated sludge to reduce the phosphate concentration in the waste water by biological means.

5. Recent advancements in biological phosphate removal

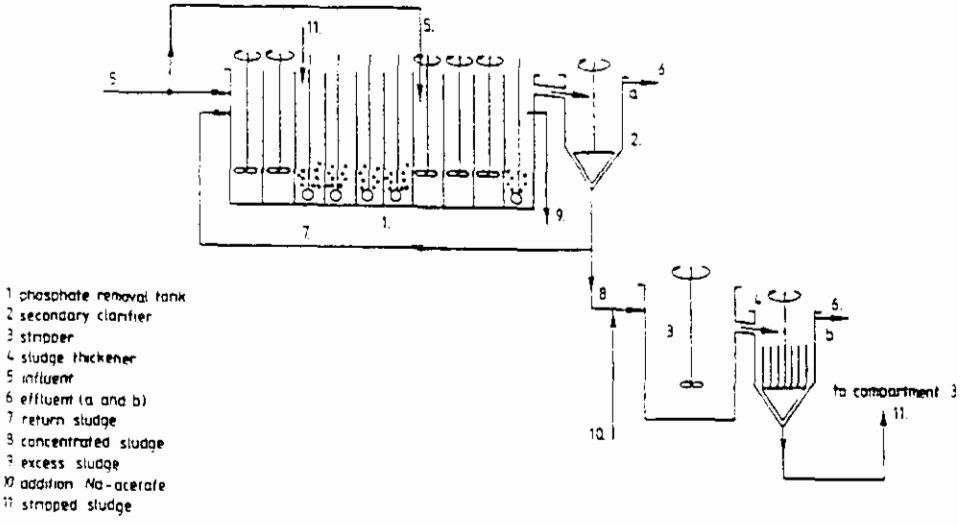

Figure 4. Flow sheet of the Renpho process (van Groenestijn 1988).

Based on the fundamental research with *Acinetobacter* and the extended investigation done by engineers, two new processes have been developed for biological phosphate removal. They are the Phostrip process (Levin and Sala 1987) and the Renpho process (Rensink et al. 1988). The Renpho process reflects almost ideally what was found with *Acinetobacter* strain 210A and therefore this process is discussed in more details. The Renpho process (Fig.4) is able to remove to a great extend both phosphorus and nitrogen from waste water (Fig.5). Influent and return sludge are added into an anaerobic zone in which phosphate is released. The mixed liquor flows subsequently into an aerobic zone in which phosphate is taken up and nitrification takes place. In a third anoxic zone nitrate is denitrified. A part of the influent is directly added to the denitrification zone, because here the original influent is already devoid of degradable organic carbon compounds. The fresh influent adds the

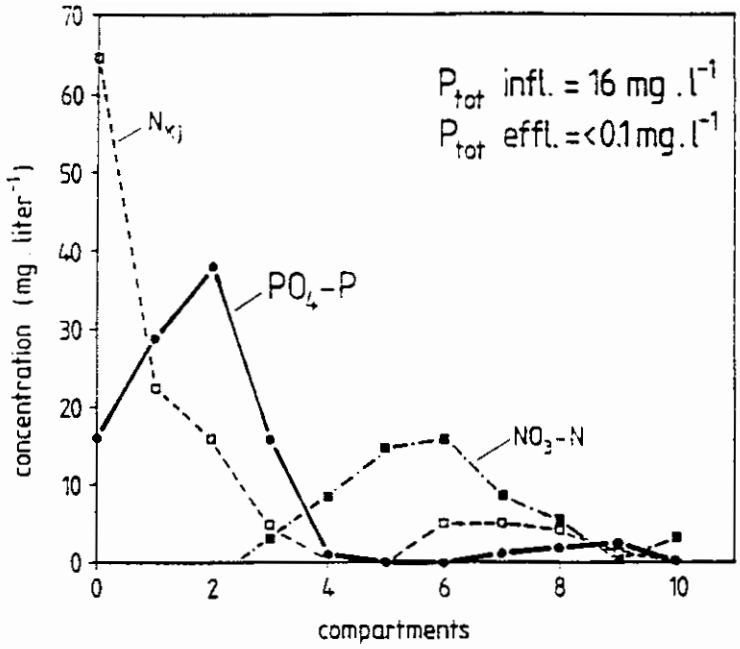

Figure 5. Horizontal phosphate and nitrogen (N_{kj} = Kjeldahl nitrogen) profile through the phosphate removal tank in the Renpho process. Modified from Rensink et al. (1988).

necessary electrons for denitrification. Phosphate is only released in minor amounts, since denitrification inhibits phosphate release (Appeldoorn et al., in preparation). At the end of the reactor an additional aerobic zone creates the environment for the removal of the last traces of biodegradable organic pollutants and of course also the last traces of phosphate. After settling, the sludge is devided into two parts: One part is returned directly to the beginning of the plant. The second part of the concentrated sludge is made anaerobic with some fresh influent and as a consequence phosphate is released. After the stripping, this second part is returned to the first aerated zone. In essence, in this whole process phosphate at low concentrations is removed biologically from a large volume of waste water and is released during stripping into a small volume of water, resulting in a high concentration of phosphate. Phosphate at high concentrations can then be chemically precipitated as calcium salt (Eggers 1988) and be re-used as raw material.

6. Concluding remarks

The combined efforts of microbiologists and engineers in the last decade on the fundamental and applied aspects of biological phosphate removal has led to the development of an alternative process for the chemical phosphate precipitation in

waste water. This is an example how basic microbiological knowledge can help to optimize an empirically found process and how engineers can stimulate research on the level of microorganisms and their metabolism. The central role of the biopolymer polyphosphate and the regulation of its formation and degradation in biological phosphate removal is not yet entirely elucidated. The finding that this polymer can act as an ATP reserve in a microorganism might be of importance in understanding the survival strategies of bacteria and should add to our understanding of the evolution of biological energy conserving systems.

7. References

Adamse, A.D., Deinema, M.H., and Zehnder, A.J.B. 1984. Studies on bacterial activities in aerobic and anaerobic wastewater purification. Antonie van Leeuwenhoek 50:665-682.

Barnard, J.L. 1976. A review of biological phosphorus removal in activated sludge process. Water SA 2:136-144.

Buchan, L. 1983. Possible biological mechanism of phosphorus removal. Wat. Sci. Technol. 15:87-103.

Davelaar, D., Davies, T.R., and Wiechers, S.G. 1978. The significance of an anaerobic zone for the biological removal of phosphate from waste water. Water SA 4:54-59.

Eggers, E. 1988. Toepassingsmogelijkheden van de korreireactor. H_2O 21:231-233.

Fuhs, G.W. and Chen, M. 1975. Microbiological basis of phosphate removal in the activated sludge process for the treatment of waste water. Microb. Ecol. 2:119-138.

Garber, W.F. 1972. Phosphorus removal by chemical and biological mechanisms. In: Applications of new concepts of physical chemical waste water treatment. Vanderbilt University Conference. Pergamon Press, Oxford.

Groenestijn, J.W. van. 1988. Accumulation and degradation of polyphosphate in *Acinetobacter* sp. Ph.D. Thesis Wageningen Agricultural University.

Groenestijn, J.W. van, Bentvelsen, M.M.A., Deinema, M.H., and Zehnder A.J.B. 1989a. Polyphosphate-degrading enzymes in *Acinetobacter* spp. and activated sludge. Appl. Environ. Microbiol. 55:219-223.

Groenestijn, J.W. van and Deinema, M.H. 1987. The utilization of polyphosphate as an energy reserve in *Acinetobacter* sp. and activated sludge. In: R. Ramadori, Ed. Biological phosphate removal from waste waters. Proc. IAWPRC Specialized Conference, Rome 1987, Pergamon Press, Oxford, pp. 1-6.

Groenestijn, J.W. van, Vlekke, G.J., Anink, D.M.E., Deinema, M.H., and Zehnder, A.J.B. 1988. Role of cations in accumulation and release of phosphate by *Acinetobacter* strain 210A. Appl. Environ. Microbiol. 54:2894-2901.

Groenestijn, J.W. van, Zuidema, M., Worp, J.J.M. van der, Deinema, M.H. and Zehnder, A.J.B. 1989b. Influence of environmental parameters on polyphosphate accumulation in *Acinetobacter* sp. Antonie van Leeuwenhoek 55:67-82.

Levin, G.V. and Sala, U.D. 1987. Phostrip process-a variable answer to eutrophication of lakes and coastal sea waters in Italy. In: R. Ramadori, Ed. Biological phosphate removal from waste waters. Proc. IAWPRC Specialized Conference, Rome 1987. Pergamon Press, Oxford, pp. 249-260.

Levin, G.V. and Shapiro, J. 1965. Metabolic uptake of phosphorus by wastewater organisms. J. Water Pollut. Control Fed. 37:800-821.

Milbury, W.F., McCauly, D., and Hawthorne, C.H. 1971. Operation of conventional activated sludge for maximum phosphorus removal. J. Water Pollut. Control Fed. 43:1890-1901.

Nicholls, A. and Osborn, D.W. 1979. Bacterial stress: Prerequisite for biological removal of phosphorus. J. Water Pollut. Control Fed. 51:557-569.

Rensink, J.H. 1981. Biologische defosfatering en procesbepalende factoren. In: Defosfateren, nieuwe ontwikkelingen en praktijkervaringen in Nederland en Zweden. NVA-Symposium pp. 1-20.

Rensink, J.H., Donker, H.J.G.W., and Anink, D.M.E. 1988. Vergaande P- en N-verwijdering uit huishoudelijk afvalwater met terugwinning van fosfaat. H_2O 21:240-242.

Srinath, E.G., Sastry, C.A., and Pillai, S.C. 1959. Rapid removal of phosphorus from sewage by activated sludge. Experientia 15:339.

Streichan, M., Golecki, J.R., and Schon, G. 1990. Polyphosphate-accumulating bacteria from sewage plants with different processes for biological phosphorus removal. FEMS Microbiol. Ecol. 73:113-124.

Tempest, D.W. and Neijssel, O.M. 1984. The status of Y_{ATP} and maintenance energy as biologically interpretable phenomena. Ann. Rev. Microbiol. 38:459-486.

Vacker, D. and Wells, W.N. 1967. Phosphate removal through municipal wastewater treatment at San Antonio, Texas. J. Water Pollut. Control Fed. 39:750-771.

Wentzel, M.C., Dold, P.L., Loewenthal, G., Ekama, G.A. and Marais, G.v.R. 1987. Experiments towards establishing the kinetics of biological excess phosphorus removal. In: R. Ramadori, Ed. Biological phosphate removal from waste waters. Proc. IAWPRC Specialized Conference, Rome 1987. Pergamon Press, Oxford, pp. 79-97.

STUDY ON PRACTICABILITY OF U-TUBE AERATION

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Study on Practicability of U-Tube Aeration

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1. Preface

The biological wastewater treatment processes,a typical representative of which is activated sludge process, are widely applied for treatment of organic wastewaters such as sewage, night soil, wastewater of food factory, etc. At present many atempts are being made to apply these biological treatment processes not only to decomposition removal of carbohydrates but also to oxidation and reduction of nitrogen compounds in wastewaters, removal of phosporic compounds, etc. On the other hand, from viewpoint of resources saving and energy saving, efforts are being made to develop more effective treatment processes. As to the activated sludge process, many studies have been made, aiming at developing the low aeration power process.

The major part of the running cost of conventional activated sludge process was occupied by that of blower. Hence, the low cost oxygen supplying system has been required to be developed. The unique super-deep aeration process (so-called U-tube aeration) developed by our company is an energy saving process featuring increased solubility of oxygen, formation of finer air bubbles and prolonged contact time.

Before putting this process in practical use, we conducted

1) hydraulic experiment by using small-size column, and

2) hydraulic experiment on the pilot plant to examine the hydraulic characteristics.

Below is reported the relation between design conditions and operating conditions, parameters of which are diameter of outer tube, downflow velocity, gas voidage fraction, and area ratio which were determined on the basis of the executed experiments.

2. U-tube Aeration

2.1 Conception of U-tube Aeration

Oxygen transfer in the aerobic wastewater treatment process such as activated sludge process is realized in the following two steps, i.e.

1) solution from gas phase to liquid phase,

2) utilization of oxygen dissolved in liquid phase by activated sludge.

This is mathematically expressed as follows;

$$\frac{dC_L}{dt} = K_{La}(C_S - C_L) - r_r \quad \text{.....(1)}$$

where

C_S = concentration of saturation dissolved oxygen (mg/ℓ)

C_L = actual amount of dissolved oxygen in liquid (mg/ℓ)

K_{La} = overall oxygen transfer coefficient expressed by gas-liquid mixing condition and gas-liquid contact area, etc. (1/h)

r_r = biological respiration rate (mg O_2/ℓh)

If a steady state is taken into consideration in equation(1), we get $K_{La}(C_S - C_L) = r_r$ .....(2), since $\frac{dC_L}{dt} = 0$.

Accordingly, to increase r_r, i.e. to increase the amount of pollutants to be treated by activated sludge, it is necessary to increase the oxygen transfer rate to liquid phase $K_{La}(C_s - C_L)$.
Therefore, many of conducted studies on the activated sludge process have been intended to increase the oxygen transfer rate.
Accordingly, in most cases the examinations have been made in the following directions:

(1) To increase K_{La} by improving contact between gas phase and liquid phase
(2) To increase concentration of saturation dissolved oxygen Cs.

As is well known from Henry's law, the solubility of gas in liquid proportionally increases as pressure rises.

Bruijin[1], Speece[2] et al. reported the results of study and actual application of the U-tube aeration process which employs the vertical dual aeration tank incorporating the upflow and downflow tube as well as a great water depth affording high oxygen transfer rate.
Figure 1 shows the schematic diagram of Bruijin's U-tube aeration process.

Speece pointed out the advantages of the U-tube aeration process as follows.

(1) Owing to hydrostatic pressure the concentration of saturation dissolved oxygen increases, hence the amount of dissolved oxygen increases.
(2) The coefficient of renewal of gas-liquid interphase increases due to effect of turbulence in the tube, as a result of which the oxygen transfer rate increases.
(3) Since air is blown in the upper part of the downflow tube, air blowing-in power is low.

Moreover, Babbitt[3] and Mavinic[4] reported the result of research in which attemp was made to apply circulation with pump to wastewater treatment. The method for circulation through deep upflow and downflow tubes has two versions[5], i.e. pump-aided circulation and circulation employing air. The pump-aided circulation is more practicable because of its high maneuverability in practical wastewater treatment.

2.2 Mechanism of U-tube Aeration Process

The U-tube aeration developed by the authors utilizes a vertical aeration tank incorporating a downflow tube and upflow tube of 30 to 100 m or above in depth and solid-liquid separation tank as shown in Fig. 2. Activated sludge mixed liquor is made to flow downward through the downflow tube at a velocity of about 1 to 2 m/sec., and air is blown in near the water level of the downflow tube. This allows the blower discharge pressure to be reduced. The activated sludge mixed liquor descends, carrying blown-in air, and since hydrostatic pressure is applied, a large quantity of oxygen is dissolved in the mixed liquor according to Henry's law. Next, the mixed liquor transferred to the upflow tube rises up to the intermediate tank where the mixed liquor is sent to the downflow tube through the head tank by the circulation pump together with raw water and return sludge. While the liquid is circulated in such a circulation system, the dissolved oxygen is consumed by the activated sludge. A part of mixed liquor is transferred to the sloid-liquid separation tank where sludge and treated water are separated.

Thus, a sufficient mixing is ensured for the mixed liquor and air during circulation, and high solubility is obtained based on pressure depending on water depth. This enables to cope with high value of r_r and save aeration power.

3. Hydraulic Characteristics

The U-tube aeration process shown in Fig. 2 employs the vertical dual tube aeration tank. In the case when gas-liquid mixture flows through such a vertical tube, flows are classified into bubble flow, slug flow, froth flow, annular flow, and mist flow[6]. Accordingly, unlike the conventional aeration tank, it is necessary to examine especially carefully the hydraulic characteristics, which requires the following conditions to be made clear.

1) Entrainment of air by circulating liquid in downflow tube.
2) Blown-in air becomes bubble flow owing to turbulence, thus forming stable two-phase flow.

So as to confirm the abovementioned conditions, we conducted the hydraulic experiments with small-size column and on the pilot plant.

3.1 Experiment with Small-size Column

3.1.1 Experimental Equipment and Method

We performed the experiment, using the U-shaped transparent acryl column of 130 mm in diameter and 10 m in depth as shown in Fig. 4. Fresh water filled in the column was circulated according to the experiment conditions shown in Table 1, the difference in water level of the intermediate tank and head tank in each condition was determined, and pressure change was measured with the aid of the manometers P1 to P7 provided on the column shown in Fig. 4.

The flow pattern during experiment was checked by photographic measurement. The experiments were arranged depending on shape of the air discharge pipe as follows.

Run I : Straight tube, diameter of outlet 20 mm ϕ 1 pc.

Run II : Filling of static mixer in Run I.

3.1.2 Results of Experiments, Discussion

Figure 5 shows the relation of downflow velocity, gas voidage fractron {amount of air A/(amount of air A + amount of circulating water W)} and bubble forming zone in Run I. Figure 6 shows the relation between downflow velocity and total pressure loss in the circulating system at different air blow-in conditions in Run I. As seen from the obtained results, the critical gas voidage fraction allowing air to be entrained by the downflow increases as the downflow velocity rises. This is followed by increase of pressure loss.

Figure 7 shows change of head at P3 position when the gas voidage fraction is changed. When the gas voidage fraction is 0.05, change of head is very little, and stable flow is obtained. As the gas voidage fraction grows to 0.09 and 0.13, the width of fluctuation range and the total pressure loss increase simultaneously, although air flows as dispersed air bubbles.

In Run II, we used the static mixer shown in Fig. 8 at the air outlet. In this case, the critical gas voidage fraction is 0.3 when the downflow velocity is 0.63 m/s as shown in Fig. 5. The result obtained in Run II is significantly better than that of Run I. Figure 9 and Figure 10 show state near the air outlet in Run I and Run II.

The results revealed that to form stable air bubble flow it is necessary to take into consideration the downflow velocity, gas voidage fraction and air dischrge conditions.

3.2 Experiment on Pilot Plant

3.2.1 Experimental Equipment and Method

Water depth of the pilot plant was set to 100 m corresponding to full-scale condition, and ductile cast iron tube of 0.8 m in diameter and steel tube of 0.3 m in diameter were used as outer tube and inner tube, respectively. To measure pressure, the pressure gauges were fitted at inlet, outlet or air flow-in part, etc. of the circulation pump, blower and U-tube. In this experiment, we confirmed the relation between gas voidage fraction and flow characteristics obtained in the cloumn experiment, measured pressure loss in full-scale condition, compared the measured values with the calculated values, performed calculation of oxygen transfer efficiency, etc.

3.2.2 Result of Experiment, Discussion

1) Pressure loss in one-phase flow

Figure 11 shows pressure loss in one-phase flow of fresh water.

The U-tube aeration process employs concentric circle vertical dual tubes shown in Fig. 2. Since the activated sludge is allowed to circulate between the dual tubes, in practical use it is necessary to check pressure loss caused by circulation. Here, we determined friction loss by using the formula of Hazan-Williams[7].

$$h = 10.667 \cdot C_H^{-1.85} \cdot D^{-4.87} \cdot Q^{1.85} \cdot l \quad \text{.....(3)}$$

where

h = friction loss head (m)

C_H = coefficient of flow velocity

D = I.D. of tube (m)

Q = flow rate (m^3/s)

l = total length of tube (m)

The term C_H of the formula above varies depending on material of tube, duration of its use, etc. Usually, value of about 60 to 150 is applied. The value of C_H measured for the test plant was about 120 to 130 as shown in Fig. 11. Change of the value of C_H of the equipment with the lapse of time will be examined further.

2) Total pressure loss in air bubble flow

Figure 12 shows measured values of total pressure loss in air bubble flow. Here, assuming that the total pressure loss is sum of friction loss and static head loss, we made convension from single-phase flow to air bubble flow as to friction loss by using the correlation of Martinelli and Davis[8] shown below.

Martinelli defined the parameter X for the two-phase flow. In the case when liquid and gas are turbulent flows,the ratio of parameter X is defined by the following foumula.

$$Xtt = \left(\frac{W\ell}{Wg}\right)^{0.9} \cdot \left(\frac{pg}{p\ell}\right)^{0.5} \cdot \left(\frac{\mu\ell}{\mu g}\right)^{0.1} \quad \text{.....(4)}$$

where

$W\ell$ = flow rate of liquid (kg/hr)

$p\ell$ = density of liquid (kg/m^3)

$\mu\ell$ = viscosity of liquid (cp)

Wg = flow rate of gas (kg/hr)

pg = density of gas (kg/m^3)

μg = viscosity of gas (cp)

On the other hand, the friction loss $(\Delta PF)tp$ of air bubble flow is expressed as follows:

$$(\Delta PF)tp = \phi^2 tt \cdot (\Delta PF)\ell \quad \cdots\cdots(5)$$

where

$(\Delta PF)\ell$ = friction loss (mAq) in single-phase flow

$$\phi tt^2 = 1 + \frac{2}{Xtt} + \left(\frac{1}{Xtt}\right)^2 \quad \cdots\cdots(6)$$

The Martineli's correlation given above was solved for the horizontal two-phase flow. Since the pilot plant was vertical tube, we used Daviss correlation employing X'tt in place of Xtt.

$$\frac{1}{X'tt} = 5.26 \left(\frac{1}{Xtt}\right) \cdot (Fr)^{-0.185} \quad \cdots\cdots(7)$$

where

Fr = Froude number in the case when no slip occurs between two-phase flows.

Static head loss occurs due to difference in gas hold-up of downflow tube and upflow tube which is caused by air bubble flow. Since air bubble in the downflow tube tends to rise against flow, it moves more slowly than water flow, whilst air bubble in the upflow tube rises in the direction of flow of liquid with a speed higher than that of liquid flow. Accordingly, at the same water level, the gase voidage fraction in the upflow tube is smaller than that the downflow tube. At air blow-in depth of h_1(m), water pressure P_1 and P_2 in downflow tube and upflow tube is expressed as follows:

$$P_1 = \int_{h_1}^{L} P(g/gc)(1-\varepsilon_1)d\ell + P^* + h_1 P(g/gc) \quad \cdots\cdots(8)$$

$$P_2 = \int_{h}^{L} P(g/gc)(1-\varepsilon_2)d\ell + P^* \quad \cdots\cdots(9)$$

where

ε_1, ε_2 = gas voidage fraction in downflow tube and upflow tube (%)

P^* = atmospheric pressure (kg/cm^2)

h = head difference (m)

g = gravity acceleration (m/h^2)

gc = gravity conversion factor (kg.m/kg/s)

* In this case the friction loss is assumed to be equal to zero.

Accordingly, if stable state is established in the system at $P_1 = P_2$, we get

$$h_1 + \int_{h_1}^{L} (1 + \varepsilon_1)d\ell = \int_{h}^{L} (1 - \varepsilon_2)d\ell \quad \text{.....(10)},$$

hence static pressure difference is obtained.

The solid line of Fig. 12 shows the relation between the total pressure loss and the gas voidage fraction which was obtained by calculation. Accordingly, the calculated values almost match with the measured values, and it is possible to determine total pressure loss according to the operating conditions.

3) Oxygen transfer efficiency

We measured concentration of dissolved oxygen in the pilot plant, and determined transfer efficiency. Figure 13 shows distribution of dissolved oxygen in the downflow tube of pilot plant. Amount of dissolved oxygen in the intermediate tank was 13.8 mg/ℓ. Based on these data we determined the oxygen transfer efficiency, using the following formula.

$$O_E = \frac{(DO_B - DO_R) \times 100}{(A/W)K \times 1000}$$

where

O_E = Oxygen transfer efficiency (%)

DO_B = Concentration of dissolved oxygen at tube bottom (ppm)

DO_R = Concentration of dissolved oxygen in intermediate tank (ppm)

A/W = Amount of air/amount of circulation water : gas-liquid ratio

K = $0.2 \times 32/22.4$; Amount of oxygen in air of 1 mol (g/ℓ)

Hence, the transfer efficiency is about 76%. Accordingly, it is possible to take extremely high value as compared to efficiency of 5 to 10% in the usual aeration tank. Moreover, since this measurement was made for the mixed liquor containing activated sludge, the actual transfer efficiency will be higher.

As to K_{La} we supposed as follows. Assuming that circulation in the equipment is plug flow and liquid flow velocity is U, we obtain a formula expressing depthwise change of dissolved oxygen from the equation(1).

$$U \cdot \frac{dC}{d\ell} = K_{La} \cdot (Cs - C) - r_r \quad \text{.....(12)}$$

Although Cs changes depending on molar fraction of oxygen in air bubble and pressure, we used the following formula, considering that it can be expressed as primary linear approximation against water depth.

$$Cs = \alpha\ell + \beta \quad \text{.....(13)}$$

where

α, β = constant

Making a comparison with the measured amount of dissolved oxygen by using the equations(12) and (13), we presumed an average value of K_{La} of tank. The obtained result was also plotted by solid line in Fig. 12.

4. Estimation of Total Pressure Loss and Oxygen Supply

Comprehending the hydraulic properties in this experiment is necessary not only to obtain stable air bubble flow but also to plan and make economical equipment featuring low running cost and construction cost. In practical treatment of wastewater the specification of equipment must be changed depending on kind of wastewater to be treated, since the decomposability varies depending on kind of wastewater. Hence, the total pressure loss and oxygen supplying capacity were calculated by using the following parameters:

1) Area ratio of upflow chamber and downflow chamber
2) Downflow velocity
3) Gas voidage fraction
4) Diameter of outer tube

Below is given one example.

The total pressure loss was determined by the method stated in item 3.2.2.(2). The pressure loss is minimum at the area ratio of 3 to 4. Following is the result of calculation made for the area ratio of 3. As the downflow velocity increases, the static head loss reduces but the friction loss increases. Accordingly, if the diameter of outer tube is small as shown in Fig. 14, influence of flow velocity is remarkable. If the diameter of outer tube is large, the total pressure loss becomes minimum at flow velocity of 1 to 2 m/s. The total pressure loss tended to increase as the gas voidage fraction increased.

The pump power was determined from circulation flow rate and the required pump head which was equivalent to the total pressure loss. The sum of pump power and blower power was taken to be total power. The value obtained by dividing the blower delivery by total power was taken to be oxygen supply.

The result is shown in Fig. 15. As the diameter of outer tube increases, oxygen supply per unit power increases in any condition. As regards the downflow velocity, the optimum velocity varies depending on the diameter of outer tube. When the diameter is 1 m, the optimum velocity is 1 m/s, and when the diameter is 4 m, the optimum velocity is 1.5 m/s. As to the diameter being within the abovementioned range, the best conditions lie also within the abovementioned range of velocity. The oxygen supply capacity tended to increase as the gas voidage fraction increased.

Thus, the optimum value can be obtained for each condition, hence it is possible to determine the optimum specification by making simulation for each wastewater.

5. Examination of Treatment Capacity

5.1 Treating Experiment on Test Plant

Wastewaters of company I which were taken as objects of treatment are cone starch production wastewater and sugar refining wastewater. A factory to be newly built requires treatment of 7500 m^3 of wastewater per day. Hence, we performed a treating experiment on the test plant equiped with aeration tank of 75 mm in diameter and water depth of 100 m(capacity of about 1 m^3). The obtained result is shown in Fig. 16. We obtained BOD removal of higher than 96% in the range of BOD load of 10 to 34 kg/m^3.d.

5.2 Application to Full-scale Plant

5.2.1 Result of Treatment

Based on the result of treatment on the test plant, we errected a U-tube aeration plant (having outer tube of 2.8 m in diameter, dual tube type ensuring water depth of 100 m, capacity of 615 m^3) which can treat wastewater at BOD load of 27 kg/m^3.d in the conditions that amount of water to be treated is 7500 m^3/d, raw water BOD is 2200 mg/ℓ, total BOD is 16.5 t/d, and raw water COD is 810 mg/ℓ, total COD is 6.1 t/d. This plant was put into

operation in February, 1979. Table 2 shows yearly treatment record in 1980, and Figure 17 shows daily change of treatment. The amount of raw water was 3800 to 5500 m^3/d, and raw water COD was within 360 to 790 mg/ℓ, i.e. lower than those designed. Moreover, pH varies within the range of 2.0 to 11.5. As a result of treatment we attained average COD removal of 89% (average COD load of 3.4 $kg/m^3.d$, daily fluctuation of 0.6 to 24.8 $kg/m^3.d$), and a high value of dissolved oxygen in the intermediate tank, i.e. 10 mg/ℓ in the average.

5.2.2 Amount of Oxygen Utilized

$$O_2(kg/d) = a \times \Delta BOD(kg/d) + b(1/d) \times MLSS(kg) \quad \ldots\ldots(14)$$

From the oxygen consumption determined from the removed BOD, amount of activated sludge in system and parameter r_r, we get the parameters "a" and "b" following the equation given above, i.e. a = 0.227, b = 0.079.

Amount of oxygen necessary for quantity, quality and MLSS of present typical raw water is evaluated by the aid of the equation(14) as follows:
Amount of required oxygen ≒ 1400 kg/d.
In the case when the designed BOD is loaded, amount of required oxygen is as follows:
Required amount of oxygen ≒ 4200 kg/d.

6. Conclusion

So as to solve one of the important problems concerning the aerobic wastewater treating with microorganism, i.e. oxygen supply capacity, we have developed a unique U-tube aeration system which enables 1) to increase concentration of saturation dissolved oxygen and 2) to reduce bubble size and prolong gas-liquid contact time. Since this process needs formation of uniform bubble flow, the gas voidage fraction becomes an important factor. The results of small scale column experiment revealed that the critical gas voidage fraction allowing to entrain the blown-in air increases with the increase of liquid flow velocity in the downflow chamber. Moreover it was revealed that the critical gas voidage fraction is increased by improving the gas-liquid mixing condition at the air outlet.

In the hydraulic experiment conducted on the pilot plant, the measured values well coincided with the evaluated values obtained by using Hazan-Williams's formula, gas-liquid two-phase correlation of Matinelli and Davis and from the static head loss caused by difference in gas hold-up.

On the basis of the obtained results of experiments we determined the total pressure loss and the oxygen supply capacity, using area ratio, downflow velocity, air voidage fraction, and diameter of outer tube as parameters. In the treatment of corn starch production wastesater and sugar refinery wastewater with the full scale plant which had been designed with due regard to the results of experiment, we obtained the expected results. Hence, it was proven that the oxygen necessary to decompose substrate can be sufficiently supplied.

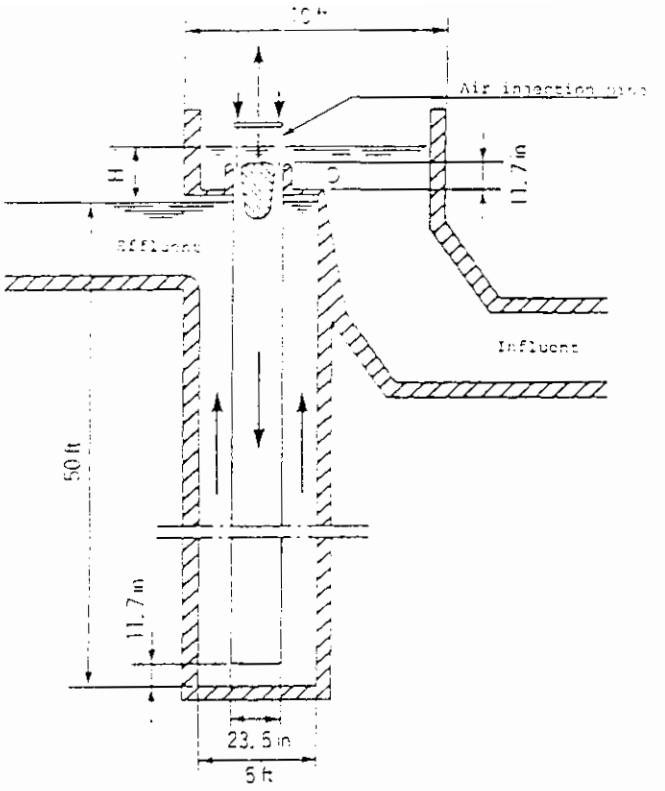


Fig. 1 U-tube Aeration by Bruijn

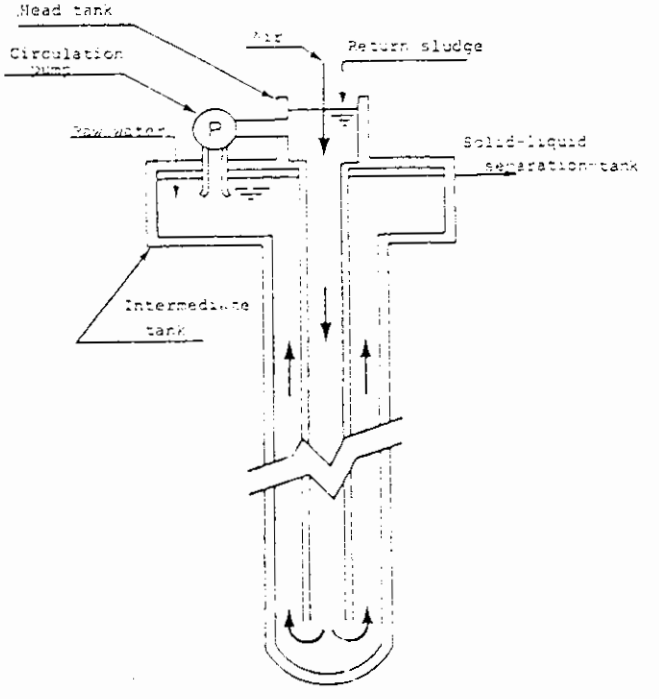


Fig. 2 Schematic Diagram of U-tube Aeration

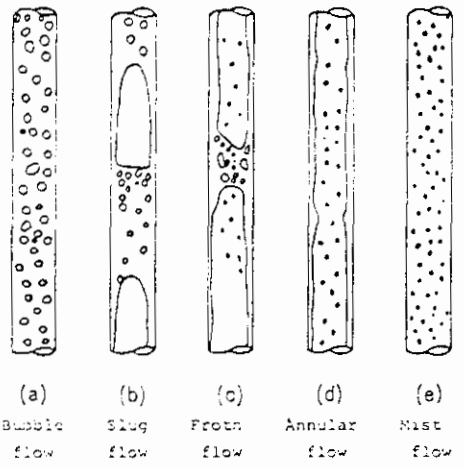


Fig. 3 Two-phase Flow Patterns

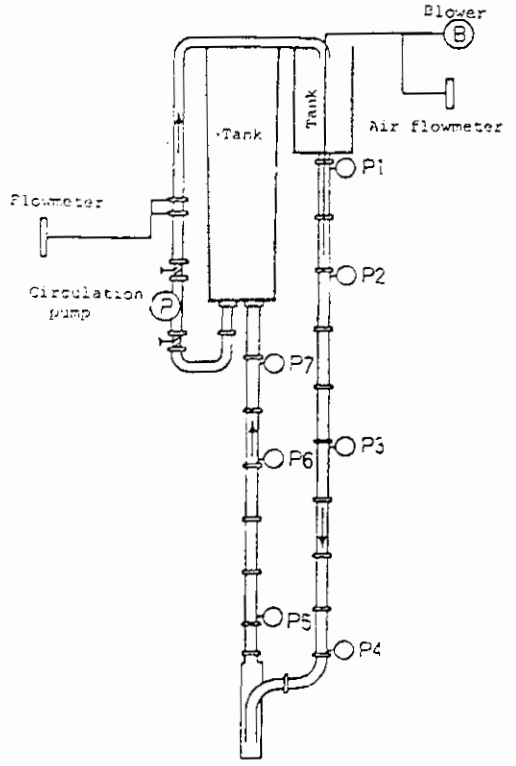


Fig. 4 Experimental Equipment

Table 1 Conditions of Column Test

Circulating water (m^3/min)	0.5, 1.0, 1.5, 2.0
Downflow velocity (m/s)	0.63, 1.3, 1.0, 2.5
Gas-liquid ratio (A/W)	0, 0.05, 0.1, 0.15, 0.2, 0.3

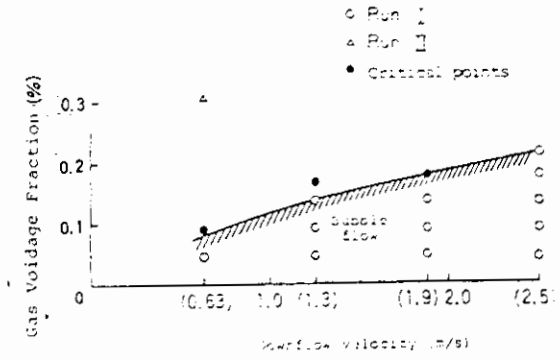


Fig. 5

Downflow Velocity vs.
Critical Gas Voidage Fraction

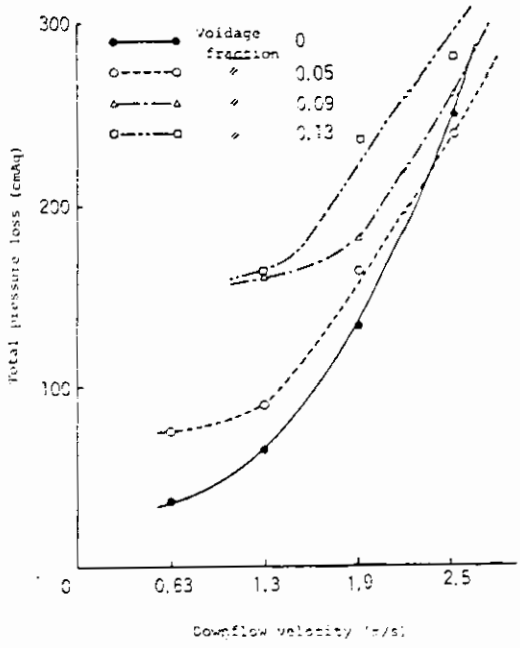


Fig. 6

Gas Voidage Fraction vs.
Total Pressure Loss

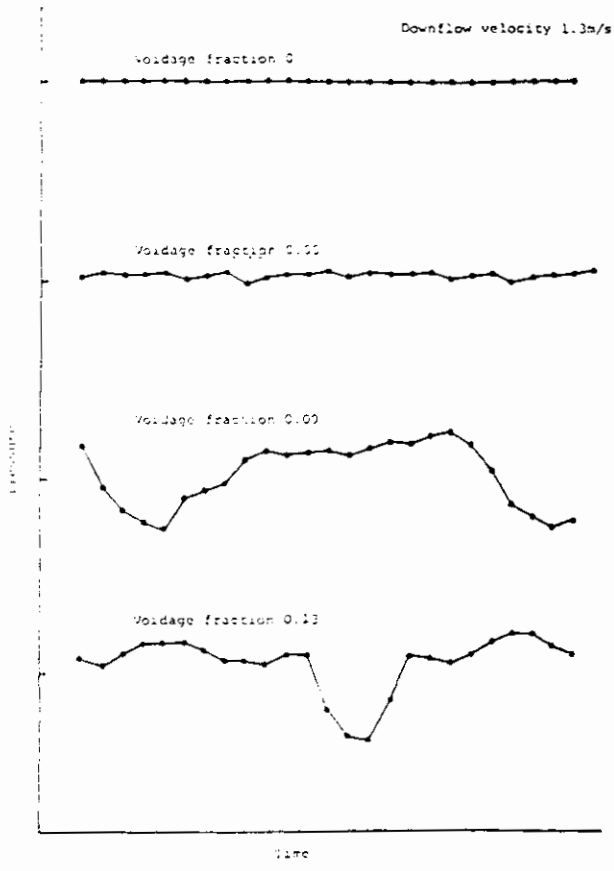


Fig. 7 Change of Head at P3 Position

Fig. 8. Static mixer

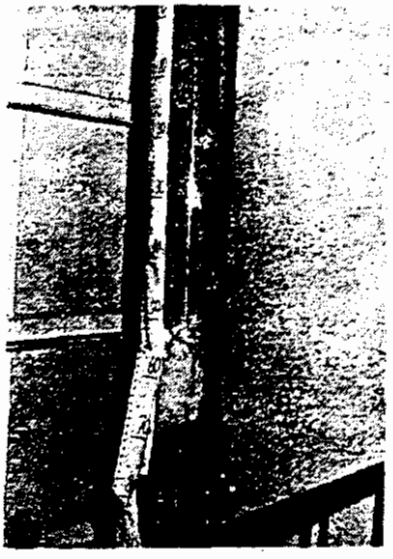


Fig. 9. Flow pattern(Run I)

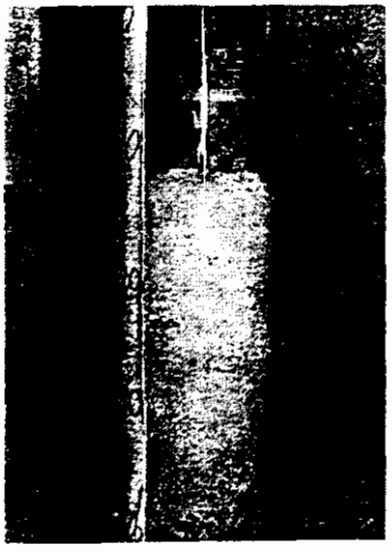


Fig. 10. Flow pattern(Run II)

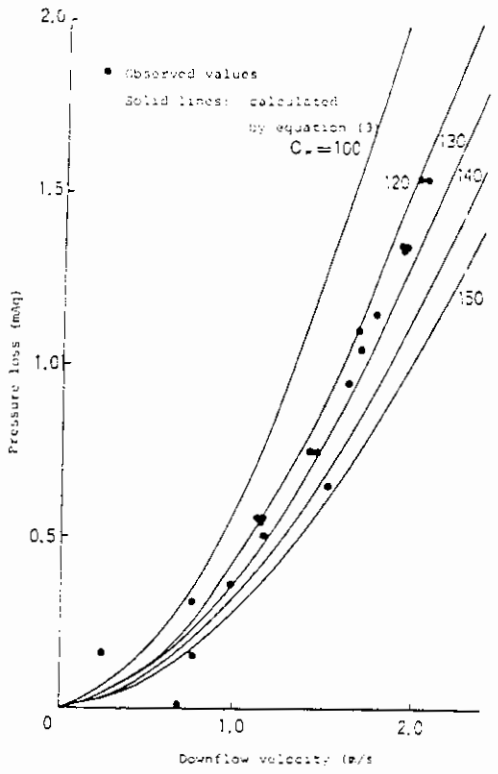


Fig. 11 Pressure Loss of One-phase Flow (Pilot Plant)

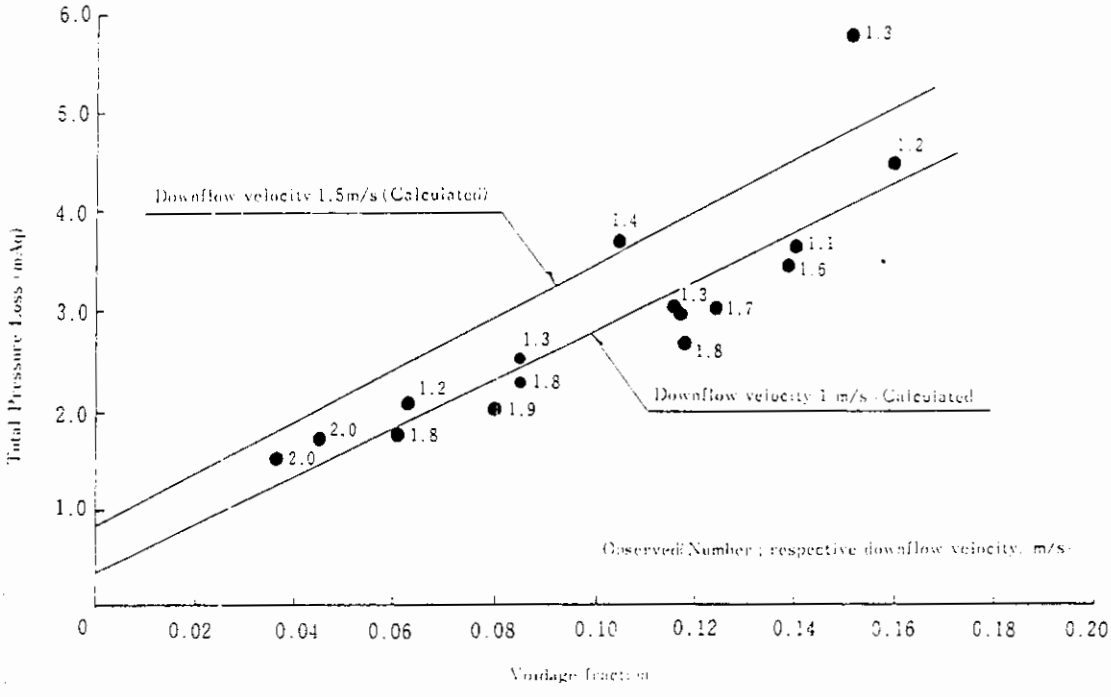


Fig. 12. Gas Voidage Fraction and Total Pressure Loss (Pilot Plant)

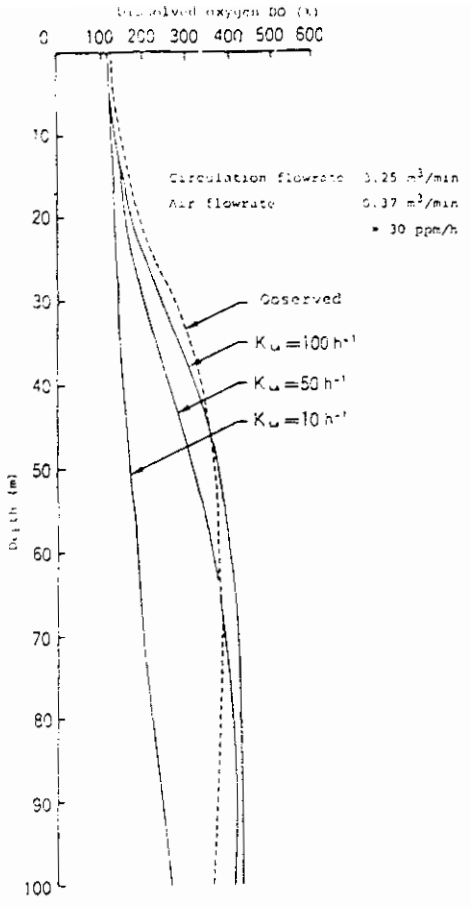


Fig. 13 Distribution of Dissolved Oxygen in Downflow Tube vs. KLa Value (Pilot Plant)

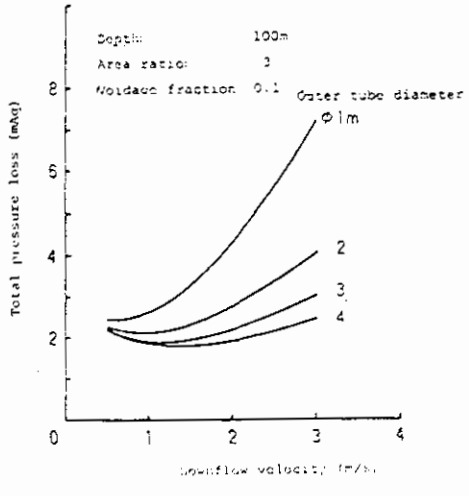


Fig. 14 Downflow Velocity vs. Total Pressure Loss

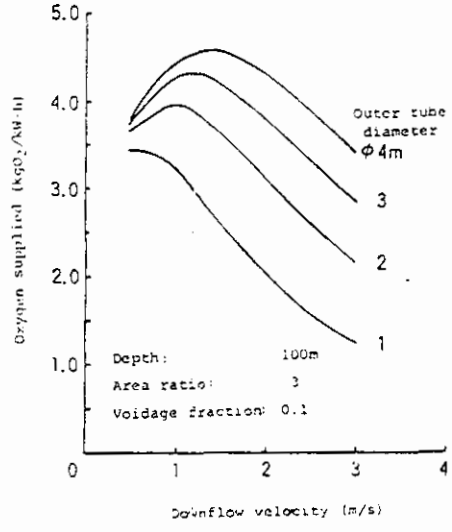


Fig. 15 Downflow Velocity vs. Oxygen Supply Capacity

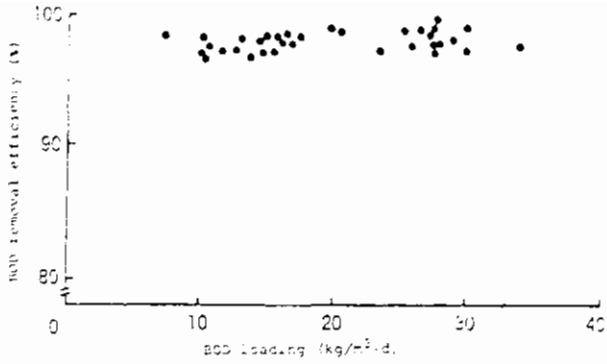


Fig. 16 BOD Loading vs. BOD Removal (Pilot Plant)

Table 2 Average Analytical Data (Full-scale Plant)

Month	Raw Water			Treated Water			In-tube Operating Conditions	
	Amount of Water	COD	pH	COD	pH	MLSS	DO	Water Temperature
	m^3/d	mg/ℓ	--	mg/ℓ	--	mg/ℓ	mg/ℓ	°C
1	4190	540	2.5∿11.4	60.7	--	4510	11.9	23.6
2	4416	789	4.2∿11.4	71.4	7.3	4740	8.99	26.3
3	3786	450	4.6∿ 8.4	49.6	7.3	4650	8.91	27.4
4	4054	442	2.3∿10.4	45.6	7.3	6920	6.30	33.5
5	4838	469	3.7∿ 7.7	55.7	7.2	8930	6.23	36.7
6	5518	380	2.6∿11.2	48.2	7.2	6770	6.20	35.5
7	4710	377	2.1∿10.7	36.5	7.2	6260	7.86	34.6
8	5066	360	3.8∿ 6.4	35.0	7.1	5050	11.7	33.1
9	4509	416	2.0∿10.5	38.6	6.9	6380	11.0	32.2
10	5445	403	3.9∿ 7.4	35.5	7.0	6910	10.9	30.3
11	5397	434	2.8∿11.1	42.7	7.0	6970	12.7	25.9
12	4180	481	2.7∿11.5	42.9	7.0	6510	14.3	23.1
Annual average	4675	462	3.1∿ 9.8	46.9	7.1	6220	9.7	30.1

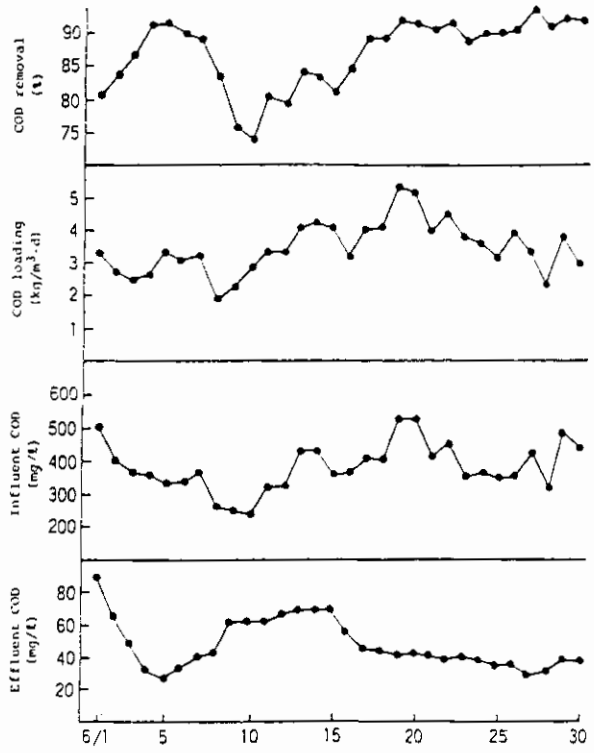


Fig. 17 Result of Treatment at Full-scale Plant

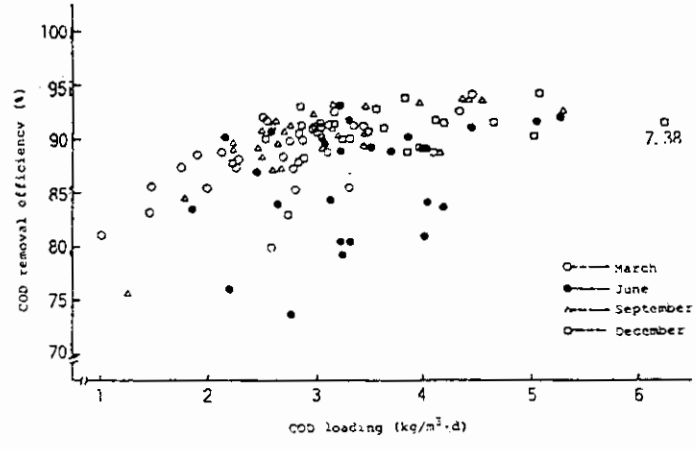


Fig. 18 COD Loading vs. COD Removal (Full-scale Plant)

MULTIREACTOR AND DEEP-SHAFT SYSTEMS; FEASIBILITY FOR SEWAGE TREATMENT IN THE NETHERLANDS

E. Eggers
DHV Water B.V.

Abstract

The Multireactor and Deep Shaft are systems for the biological treatment of waste waters. Industrial waste water treatment at higher concentrations were the principal application areas until now. As treatment schemes with reduced land requirements and decreased environmental nuisances are of increasing importance in the densely populated urban areas in Europe, Japan and elsewhere a feasibility study has been performed to assess the position of both technologies in the future treatment of domestic waste water. It is shown that in the present trend of stricter environmental guidelines including requirements for efficient removal of nitrogen and phosphorous compounds from the waste water the Deep Shaft system is applicable under specific conditions. The Deep Shaft requires relatively high investment costs compared with alternative technologies, making it less relevant as a State-of-the-Art treatment system for the year 2000. The Multireactor is still under study so that definite feasibility statements can not be made.

1. INTRODUCTION AND DESCRIPTION OF THE SYSTEMS

Due to its compactness the Deep Shaft and the Multireactor system are thought to be relevant in the future of sewage treatment in the Netherlands. Both systems have in common that they are subsurface constructions with a relatively small diameter to depth ratio of 1:10 to 1: 20 and more so that the land requirements for these schemes are theoretically very low compared with the conventional activated sludge systems.

Therefore a further assessment of the future role of these systems in the sewage systems in the Netherlands has been initiated by RIZA and STORA in the framework of the "rwzi 2000 programme". The study has been performed by DHV Water b.v. (formerly DHV Consulting Engineers).

As far as the Deep Shaft system is concerned information about design data , operational figures and experiences of the system has been gathered from a number of sources, such as literature, discussions with the proprietors of the process, ICI Ltd, and interviews and discussions with the users of the process in Germany, the United Kingdom and Japan.

The Multireactor is a system with applications in the food industry in the Netherlands. The Multireactor has been developed and marketed by the Multireactor b.v, the Netherlands. The very good performance of the process and the positive properties as minimum land requirement, minimal odour emission and effective aeration system prompted RIZA and STORA to include this reactor in the study. As there are no experiences with the Multireactor in the field of sewage treatment available a pilot scale project has been started to investigate the possibilities to apply the reactor also for sewage treatment purposes. At this moment no definite conclusions of the pilot scale investigations can be presented.

In the sections dealing with experiences, evaluation and conclusions the emphasis therefore will be on the Deep Shaft system.

Description of the Deep Shaft system

The Deep Shaft consists of a vertical shaft, 30 to 150 m deep with a diameter of 0,4 to 6 meter. Shafts with larger diameters are in the development stage but not yet applied. From evaluation of full scale experiences the designers of the process consider a shaft depth of 60 meters as the optimum regarding investment costs, treatment performance and hydraulic flow stability.

In the shaft the activated sludge mixture is circulating by two channels, the "downcomer" with downwards directed liquid flow and the "riser", the channel with upward flow. Internal circulation is maintained by the aeration air transported by a compressor to the downcomer. See figure 1. The air also provides the oxygen for the activated sludge process.

As the downward liquid velocity exceeds the rise velocity of the air bubbles, the bubbles are entrained in the liquid in downward movement. By increasing hydrostatic pressure the oxygen dissolves relatively quickly in the process liquid. More oxygen will be effectively available for the bacteria compared with conventional processes under atmospheric conditions. So a higher oxygen transfer rate is the result. The amount of air for the process and the amount of off-gas (to be treated for environmental reasons) can be reduced. This positive feature has to be offset against the higher energy consumption per m3 of air due to the increased static head. It can be calculated that the net positive effect on aeration efficiency is only minimal.

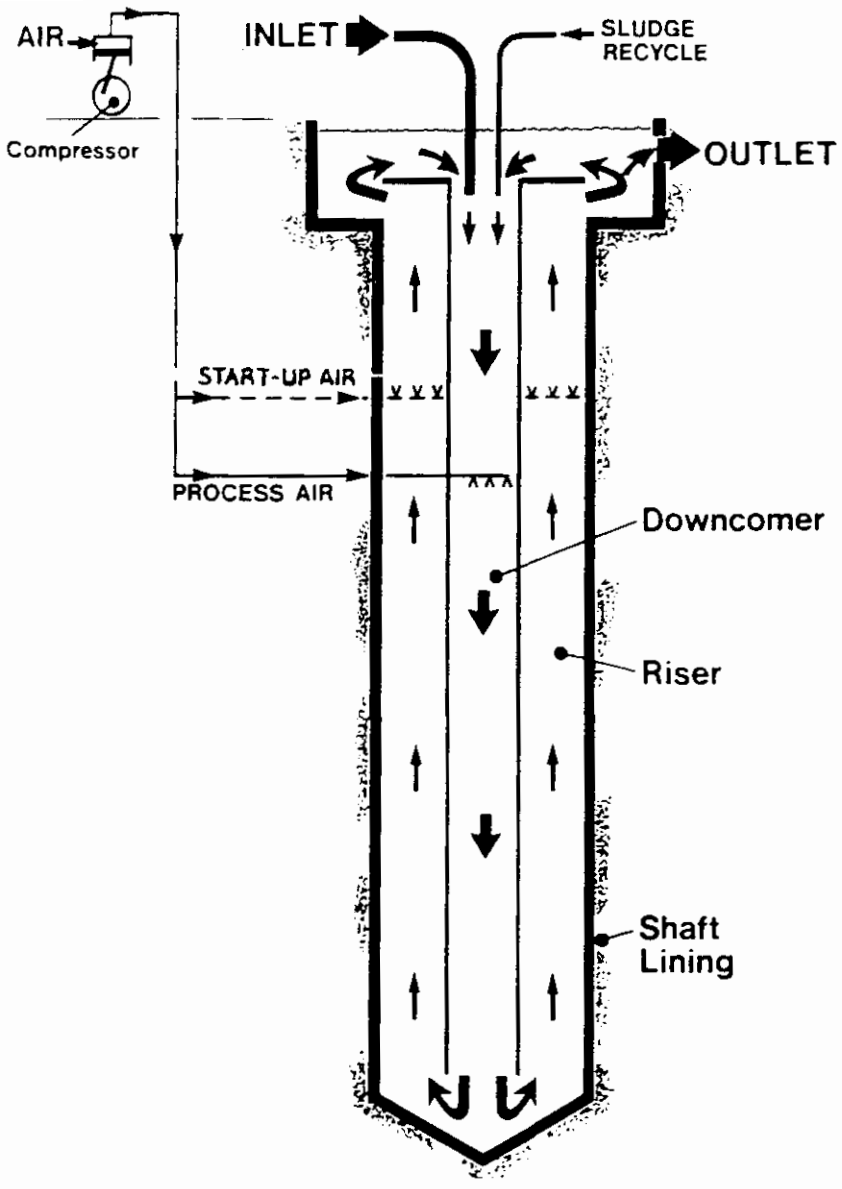


Figure 1. Deep Shaft cross section

The air expands in the riser section of the shaft. So a density difference occurs between the gasfree watercolumn in the downcomer and the corresponding part of the riser section. Liquid circulation is maintained by this density difference. See figure 2.

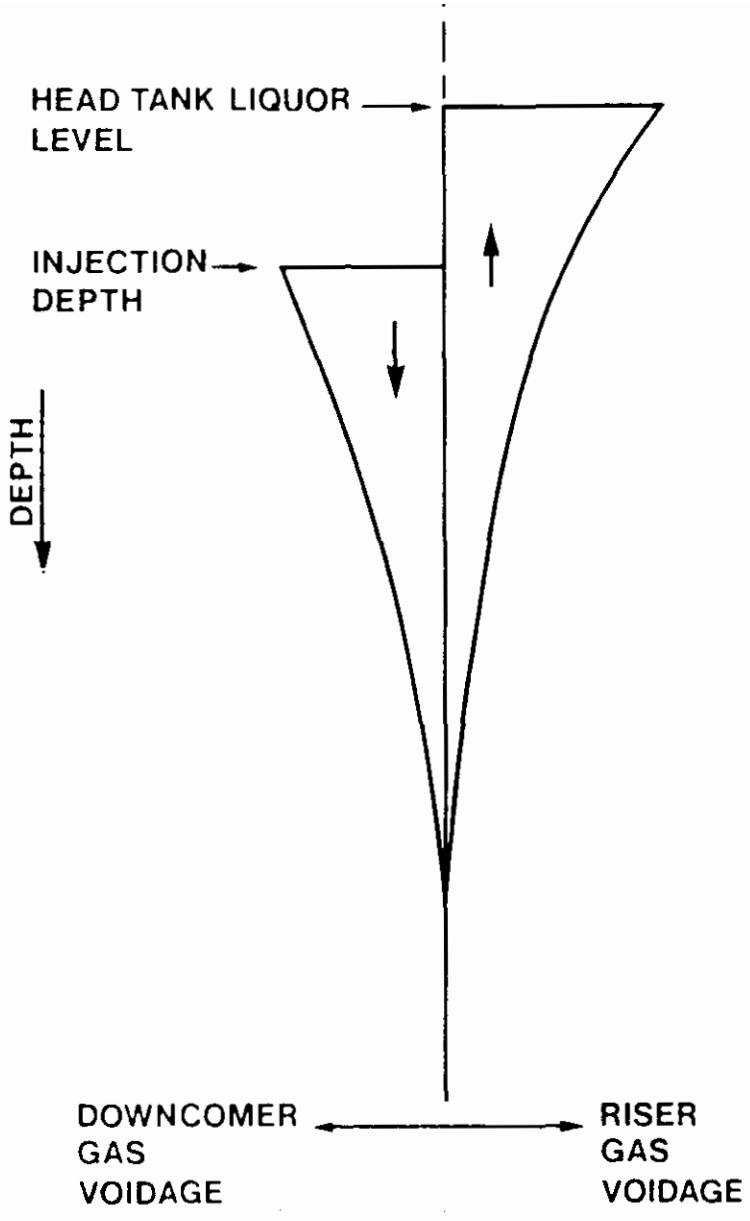


Figure 2. Voidage in downcomer and riser sections of the Deep Shaft.

To start the process initial air has to be supplied to the riser section of the shaft. As soon as the desired liquid velocity is generated, the start up air is shut off and the air suppletion is continued in the downcomer.

In the Shaft a very intensive turbulence occurs to promote a good mass transfer of oxygen to the cells but also of dissolved substrate to the cells. By the rapid transition from hydrostatic pressures of over 10 atm to atmospheric pressure problems can occur with the separation air/sludge/water. To promote separation different methods have been developed such as vacuum degasification, flotation or downstream stripping/second stage activated sludge. See figure 3.

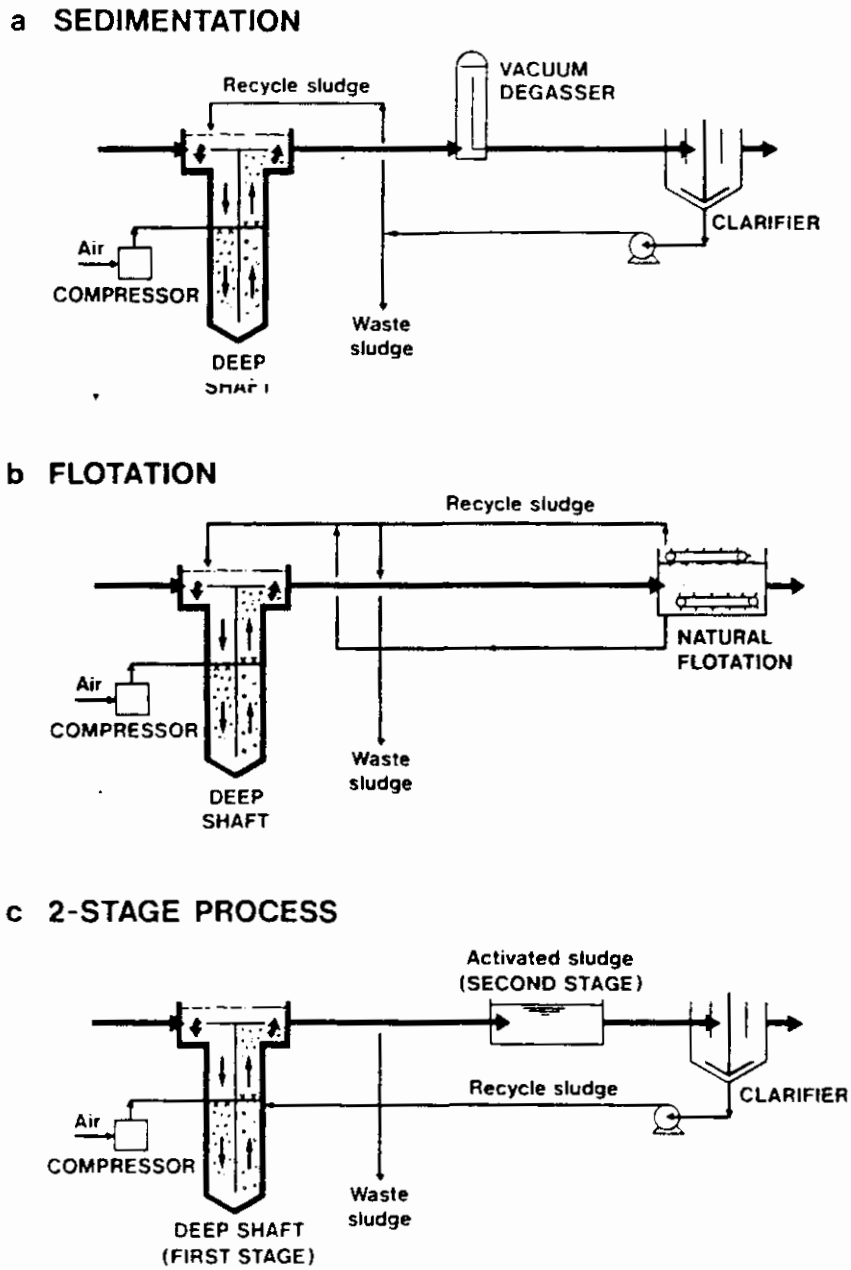
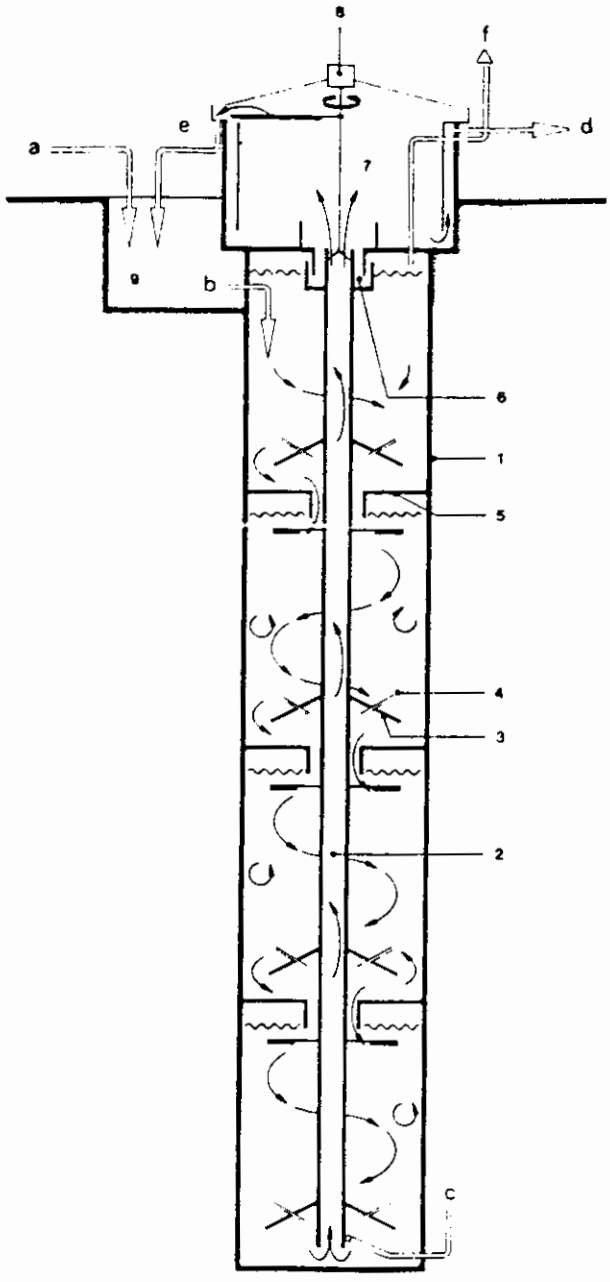


Figure 3. Deep Shaft systems with downstream gas/sludge/water separation

The selection of the separation process depends on the type of wastewater to be treated and on local factors.
The Deep Shaft is a high loaded biological system with a relatively short sludge residence time of only 1 to 2 days. Nitrification is therefore impossible. Denitrification is possible if nitrified effluent is recycled and the functions of circulation and aeration are separated as is the case with the Deep Shafts of the Leer sewage treatment plant in Germany.

Descripion of the Multireactor system

See figure 4. The raw water flows by gravity into the reactor just under the bottom plate of the separator. The riser pipe with the mushroom shaped syphoncarriers takes care of mixing of the influent stream with the contents of the reactor. The influent flows downstream countercurrent with the rising air bubbles, passing through the aeration chambers. At the bottom the sludge/water mixture flows upward through the riser pipe to the topmounted flotation unit separating the sludge from the effluent which is ready for discharge.
The sludge/water mixture is subjected to a decreasing hydrostatic pressure flowing upward to the separator. Therefore the dissolved gas in the mixture is released in the form of small bubbles attaching to the sludge particles. The sludge is then floating in the flotation chamber. To increase the separation efficiency a separate circulation loop is equipped with a flotation cell where air under presure is introduced into the liquid.
The sludge content in the reactor can be as high as 8 to 15 gram MLSS per liter. The sludge content is controlled by the thickness of the flotation layer in the flotator which can be adjusted by automatic control.



1. shaft
2. riser
3. "mushroom"
4. siphon
5. plate
6. waterlock
7. separator
8. E-motor
9. pump pit

a. influent
b. influent + sludge recycle
c. air
d. effluent
e. sludge
f. off-gas

Figure 4. Schematic drawing of the Multireactor

The compact subsurface way of realization of Deep Shaft and Multireactor demonstrates the similarity of the two systems. But there are also a number of remarkable differences:

* the Deep Shaft is a perfectly mixed reactor while the Multireactor shows plug flow behaviour
* the contents of the Deep Shaft circulates many times in the system while the Multireactor essentially is a one pass system
* aeration is for propulsion/mixing and oxygen input in the Deep Shaft.
  Aeration in the Multireactor is only for oxygen input
* opportunities for integrated nitrification/denitrification are greater with the Multireactor

2. EXPERIENCES

According to the reference list on this moment 50 Deep Shaft installations have been realized and are in full operation. See Appendix.
These installations have been distributed as follows:

40 in Japan
1 in the USA
4 in the UK
3 in Canada
2 in Germany

14 of these installations, mainly the Japanese, have been designed for treatment of sewage (see Table 1), 4 for the treatment of mixed waste water and the rest is only for industrial use. The relatively big penetration of the systems in Japan is remarkable.

			BOD		
	capacity [p.e. of 54g]	load [kg/d]	influent [g/m^3]	effluent [g/m^3]	efficiency [%]
Toyonaka	13.000	700	300	20	93
Tilbury	130.000	7.050	800	30	96
Portage	105.000	5.670	500	30	94
Matsushima	1.500	80	60	20	67
Virden	5.000	270	200	30	85
Leer	230.000	12.480	830	80	90
Osaka	2.600	140	620	20	97
Osaka	6.100	329	500	20	96
Osaka	1.300	68	68	5	93
Tokyo	4.600	250	616	20	97
Tilbury	407.000	22.000	600	60	90
Mie	10.600	570	1.580[1)]	170	89
Tokyo	2.500	136	135	8	94
Tokyo	3.100	170	444	20	95
Kanagawa	1.400	75	75	3	60
Homar	12.000	450	160	30	81
Osaka	2.200	117	390	10	97
Osaka	1.500	79	600	20	97

1) nightsoil

Table 1. Deep Shaft references for sewage treatment

From Table 1 it can be concluded that the removal of BOD generally is in the order of 90 %.

Space limitations and other factors in Japan are responsible for this development. Very important is the recycling concept of the Japanese waste water teatment in the larger building and office complexes. See figure 5.

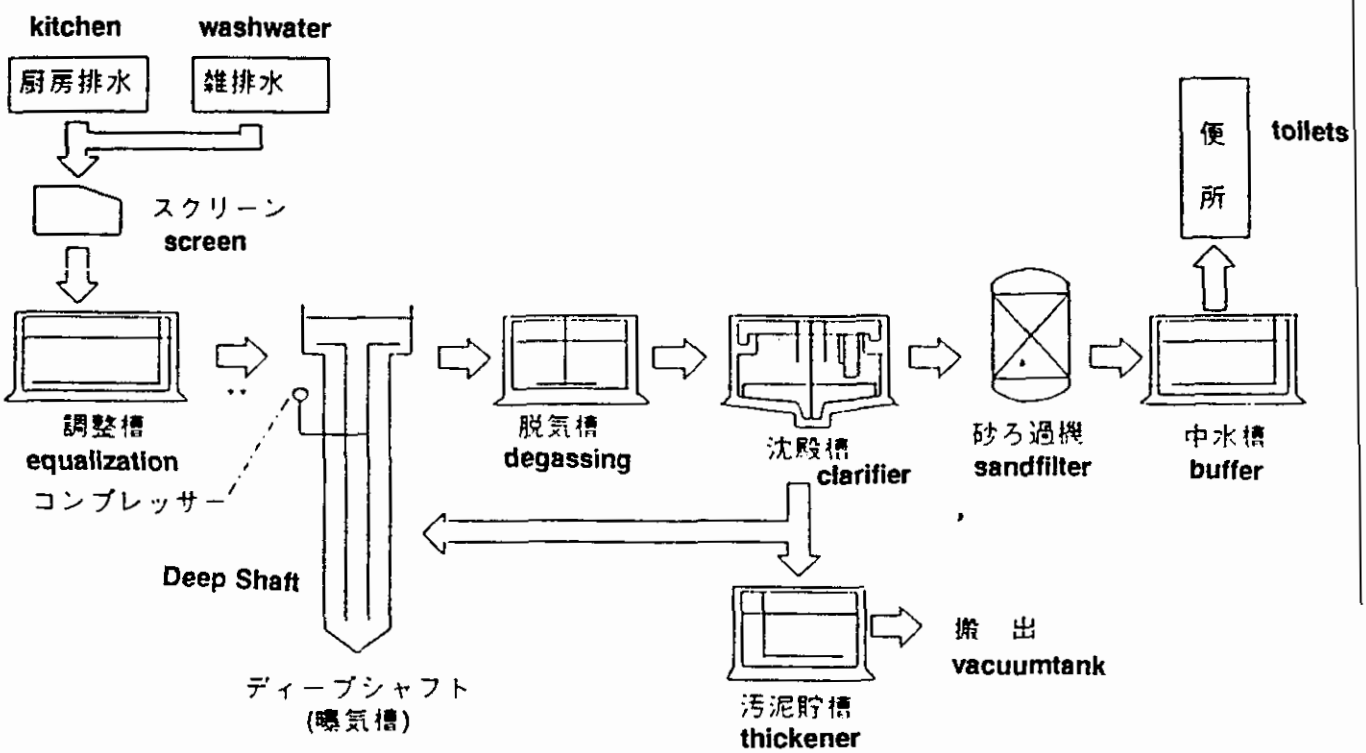


Figure 5. Schematic of water reuse in Japanese building complexes

There is a separate collection system in these buildings for kitchen waste water and toilet water. The kitchen waste water is collected and treated locally by for instance a Deep Shaft system. The Deep Shaft effluent then is used as flush water for the toilet system.

3. EVALUATION

The patent holder, ICI, and the licensee for the UK, formerly PCI, state that the Deep Shaft process offers to the user the following advantages:

* 50 % less space requirements in comparison with conventional processes
* no separate sludge stabilization
* good sludge dewaterability
* reduction of capital costs for larger sewage treatment plants with incoming BOD exceeding 400 mg/l
* no noise and odour emission
* low sludge volume index

These issues are discussed below.

Space requirements

In the ICI and PCI brochures the relative reduction of land requirement is illustrated. See figure 6.

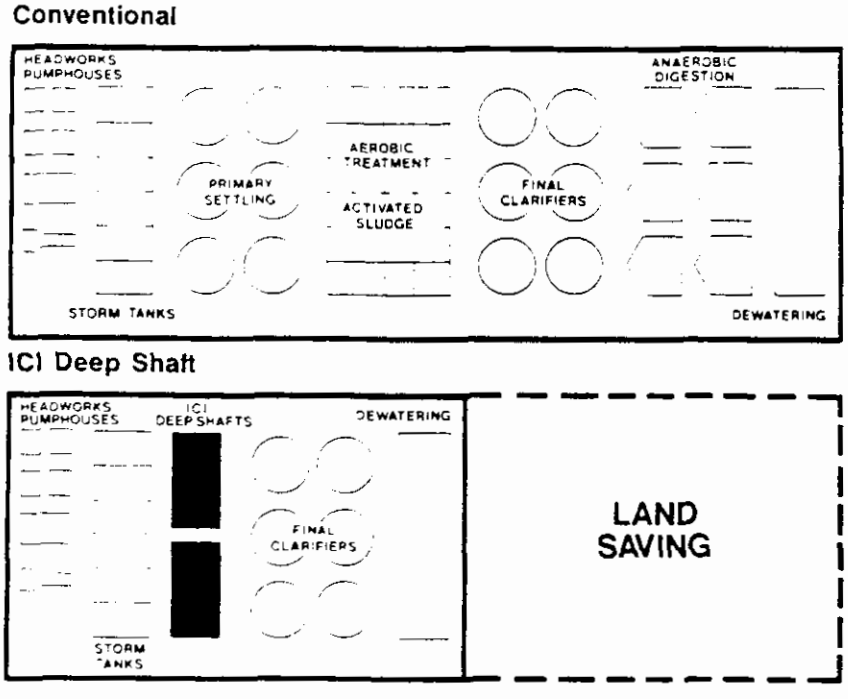


Figure 6. Claimed space reduction by application of Deep Shaft system

The claimed space reduction is brought about by the following factors:

* no pretreatment
* smaller aeration tank surface
* no sludge stabilization

The relative space reduction is dependent on the concentration of the wastewater to be treated. In the literature an example has been described of a waste water with a BOD of 2500 mg/l. The Deep Shaft showed a space reduction of 85 % in comparison with an oxidation ditch. It is not known however if the effluent quality of both systems was comparable.

In Tilbury (UK) the Water Authority did not give much priority to space considerations. It was decided to degas the Deep Shaft mixture by bubble aeration. The reduction of space requirements was only minimal. Another scheme, in Leer (Germany), showed some reduction in area.

In general it can be concluded that a proper comparison of systems will show only minor reductions in area requirements by the application of Deep Shafts systems for the treatment of sewage. Hardly any reduction is to be expected in those cases where strict requirements are to be fulfilled with respect to nitrogen and phosphorous removal. Important reductions are however possible when treatment of waste waters with elevated BOD concentrations is necessary. This can be the case with the (co)treatment of concentrated industrial wastewaters.

Sludge stabilization

The licensor does not require separate sludge stabilization. This decreases of course the capital and operation costs. Moreover the treatment plant is simpler in operation and maintenance. Seen the design sludge age of 1 to 2 days it can not be expected that the sludge despite the higher oxygen tension and higher conversion rates will be fully stabilized. So sludge stabilization measures should be considered for strict environmental control.

Dewaterability

In general non stabilized sludge is difficult to dewater. By lack of reliable data no definite statements can be made on this point.

Reduction of capital costs

ICI states that in Japan in 30 % of the cases an important argument in the clients decision were the lower investment costs. In the literature no indications have been found supporting this remark. Therefore a cost comparison under Dutch conditions will be presented later.

Reduction of environmental emissions

Because of the reduced interface between the aeration tank and the atmosphere the emission of odour from the Deep Shaft will be minor. But if degassing is realized by bubble aeration (as is the case in Tilbury and Leer) the emission will increase again. Furthermore, the treatment of non stabilized sludge will also be a potential odour source, although no literature data could be found to confirm this.

Sludge Volume Index

Although the explanations in the referred literature are not clear the values of the SVI in literature are mainly below 100 ml/g.

Nitrification

Deep Shaft systems can be retrofitted to nitrification in the following two ways (see figure 7):

* nitrifying trickling filter downstream from Deep Shaft
* expansion of downstream activated sludge tank

Also retrofitting an activated sludge system with an upstream Deep Shaft to remove BOD can be an attractive proposition at those (industrial waste treatment) situations where a waste stream with a high concentration of BOD is to be treated and strict nitrification requirements have to be fulfilled.

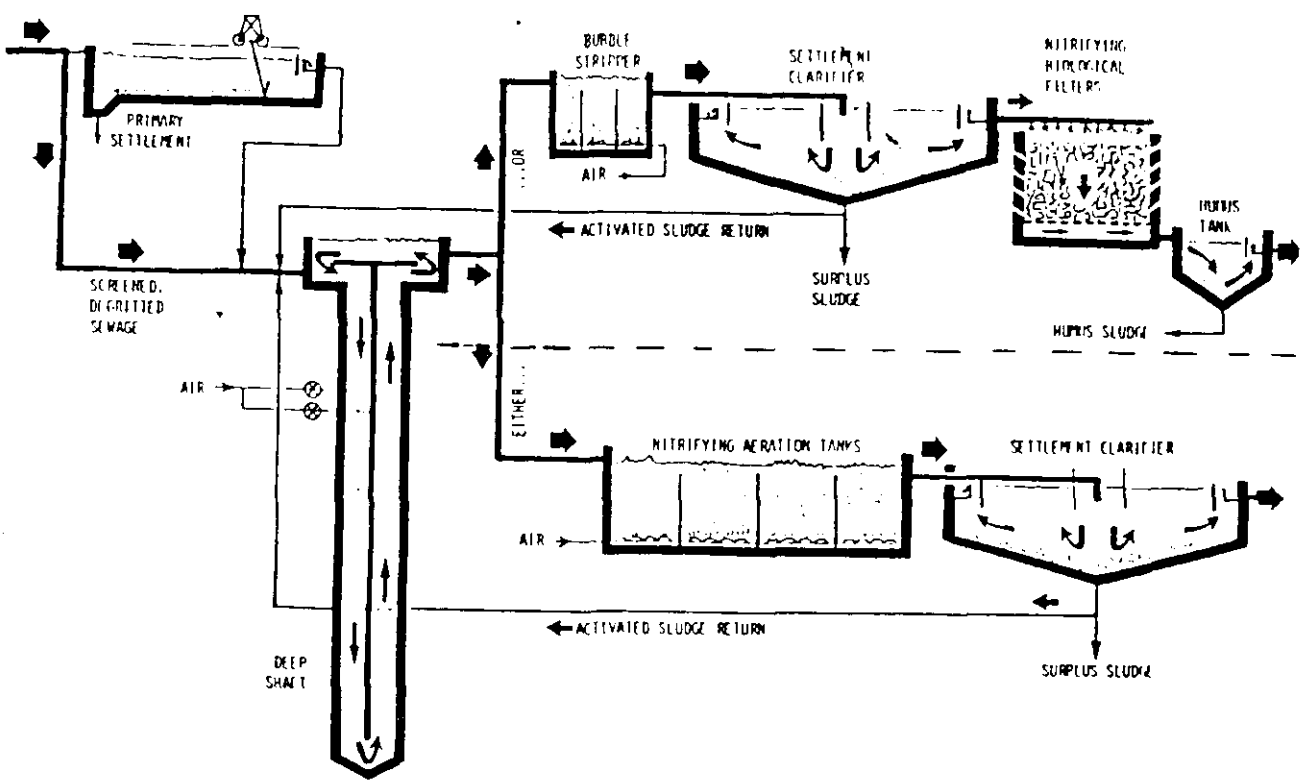


Figure 7. Retrofitting an existing Deep Shaft system for nitrification.

The Multireactor performs well in the food industry where three full scale applications are in operation now in the Netherlands.
But until now no experience has been gained with the effect of diurnal flow variations on the efficiency of the flotation system.
Therefore a pilot plant study is now under way to reveal these effects. Important dimensions and preliminary research results are shown below:

Shaft:	depth	18.5 m
	diameter	1.5 m
	volume	30.5 m^3
Flotation:	surface	6.4 m^2
	volume	13.5 m^3
Influent	COD	500 mg/l
(arg. dry weather)	BOD	275 mg/l
	TKN	70 mg/l
	NH_4-N	60 mg/l

BOD/sludge load	0,15	kg/kgMLSS.d	(arg.)
Flotation DS load	14	$kg/m^2.h$	(max.)
Flotation surface load	4	$m^3/m^2.h$	(max.)

DWF/RWF	1 : 4	
DWF	3,8	m^3/h (max.)
RWF	15	m^3/h (max.)

MLSS in reactor	8	kg/m^3 (arg.)
	13	kg/m^3 (max.)

Treatment efficiency for T > 12°C and SS < 20 mg/l:

COD	89%
BOD	98%
TKN	> 88%
NH_4-N	> 90%
Total N	45%

4. FEASIBILITY IN THE NETHERLANDS

The feasibility will be discussed using the following items:
* effluent quality
* energy consumption
* sludge production and dewaterability
* space requirements
* investment costs

Effluent quality

At a proper design load an average BOD concentration in the effluent of 20 to 25 mg/l BOD is feasible. By P incorporation at sludge growth a phosphorous removal of 30 to 40 % can be predicted.
As there is no nitrification/denitrification possible due to the low sludge residence times, the reduction of nitrogen compounds will only be minimal. Therefore it can be concluded that application of one pass Deep Shaft systems is not attractive in view of the very strict requirements regarding nitrogen and phosphorous. If Deep Shaft systems can be interesting as a first step of a staged system is a matter of cost comparison which will be presented later. The Multireactor shows promise according to the first results, but definite conclusions can be drawn after full evaluation of the total test period.

Comparative energy consumption data of the Multireactor are not available yet.

Energy consumption

The energy utilization efficiency in Tilbury ranges from 0,5 to 2 kg BOD/kWh. That numbers are not better than the data of a conventional sewage treatment system. In Tilbury it is obvious however that the installation is not operated properly and with a better instrumentation and control a higher efficiency is possible. See figure 8.

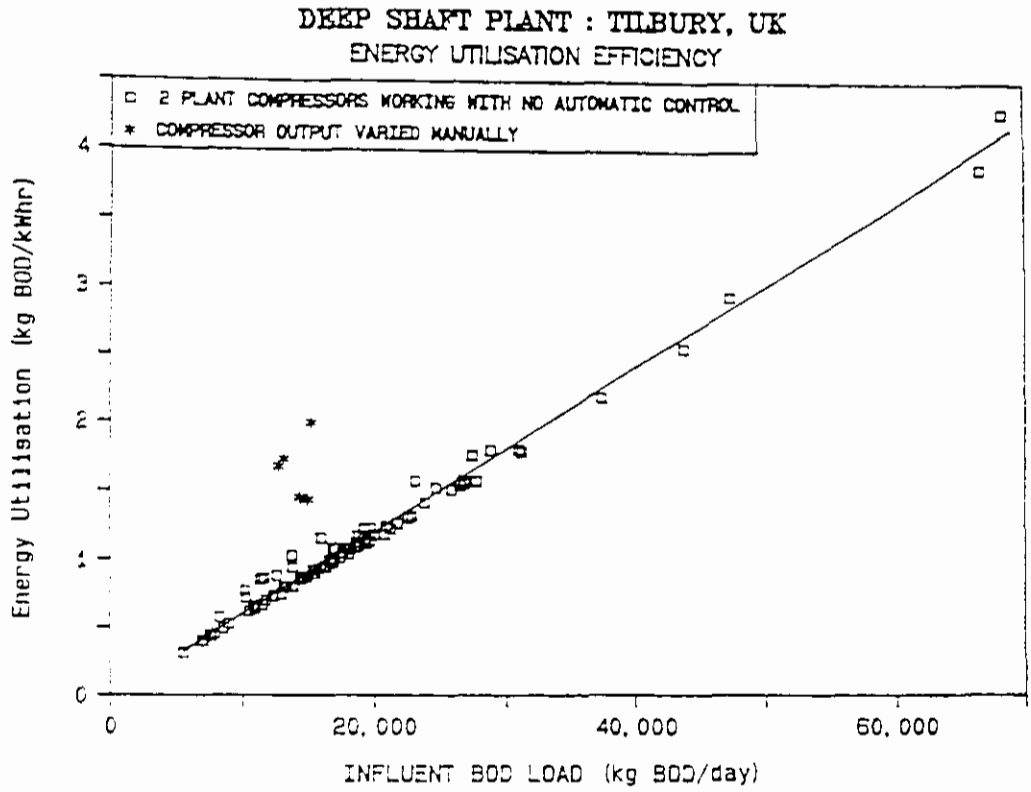


Figure 8. Energy utilization efficiency as a function of load of the STP Tilbury

Sludge production and dewaterability

In the Deep Shaft and Multireactor literature it is reported that the sludge production is less compared with the conventional systems. However these statements are not underligned by operational data, so that no firm conclusions on this issue are possible.
Only two references of CST-data have been found, indicating that dewaterability of Deep Shaft sludge is better than oxydation ditch sludge. These findings should be considered with care as there the CST-value is only indicative of dewaterability. Dewatering characterics of Multireactor domestic surplus sludge is still to be tested.

Space requirements

The original lav-out of the Deep Shaft (with vacuum degasification) and with conventional effluent requirements, only pertaining to BOD-removal, offers a distinct advantage in space requirements over conventional activated-sludge systems.
The relative advantage is proportional with the strength of the waste water to be treated.
Modern requirements, especially with respect to nutrient (N,P) removal have changed the picture dramatically. For the Deep Shaft a staged process scheme is necessary, almost eliminating the relative advantages of the past.
If the sludge separation unit of the Multireactor is able to deal effectively with the fluctuating behaviour of domestic sewage flow, then the Multireactor will show promise as this reactor is better able to nitrify and (partly) denitrify.

Cost comparison

To be able to compare the investment costs of the Deep Shaft and of conventional technology respectively these costs have been estimated on a basic design basis.
Stating point was an effluent value of max. 20 mg/l BOD and full nitrification above 10°C.
The design basis for both systems was:

- load	p.e.	50.000	population equivalents
	hydraulic	500	m^3/h DWF
		1.500	m^3/h RWF
		7.500	m^3/d average
	organic	2.700	kg/d BOD

Both schemes consisted of the following units:
- influent pit
- screen
- influent pumping station
- sand-trap
- aeration:

A. Deep Shaft	depth	70 m
	diameter	3,6 m
	volume	700 m^3
	sludge load	0,5 kg/kg.d

The "Leer"-variant of the Deep Shaft with a screw pump on the head tank, see Figure 9, has been selected for the cost estimation.

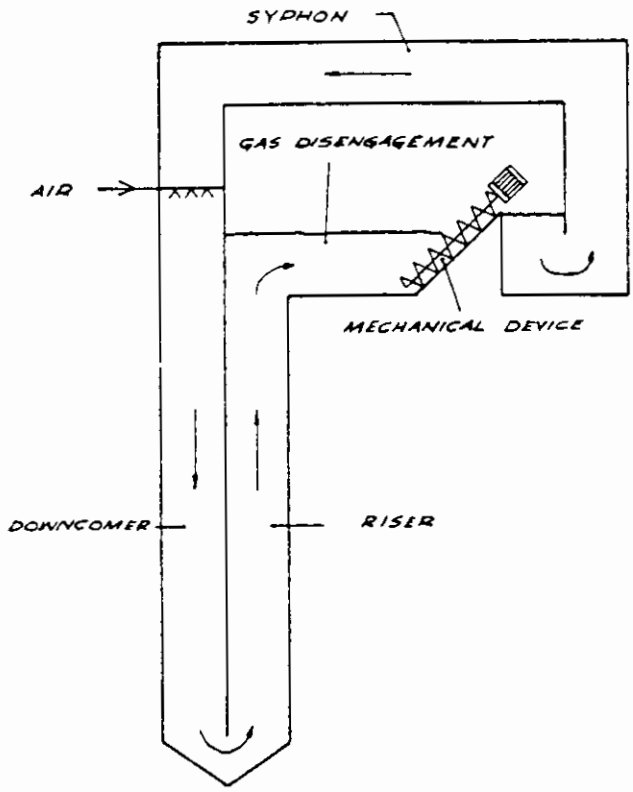


Figure 9. "Leer (Germany)"-variant of the Deep Shaft with separate propulsion by a screw pump.

- 2nd stage aeration/degassing/nitrification (only Deep Shaft)

		volume	1.080 m^3
		BOD load	270 kg/d
		sludge load	0,05 kg/kg.d
	B. Carrousel	volume	13.500 m^3
		sludge load	0,05 kg/kg.d

- final clarifier

	surface	1.500 m
	surface load	1 $m^3/m^2.h$

Sludge treatment was considered comparable and has not been estimated.

With an accuracy of $\pm$ 30% the cost estimations resulted in the following figures (in DFL, price level Feb. 1991).

	Deep Shaft	Carrousel
Civil	$4,35 * 10^6$ [1]	$3,5 * 10^6$
Mechanical	$1,95 * 10^6$	$1,8 * 10^6$
Electrical	$1,05 * 10^6$	$1,05 * 10^6$
Total	$7,35 * 10^6$	$6,35 * 10^6$

1) The separate estimate of the Deep Shaft is DFL $1,1 * 10^6$.

It can be concluded that the investment costs of a Deep Shaft system are DFL $1 * 10^6$ higher than conventional treatment with the stated design capacity and identical strict effluent requirement. The cost difference lies within the accuracy limits of the estimates.

5. CONCLUSIONS

1. Application of the Deep Shaft in the Netherlands for the treatment of domestic waste water with a BOD of 250 mg/l or less is not attractive given the cost figures and minimal space advantages under the present strict environmental requirements.

2. Although definite figures on the sludge production and sludge dewatering fail it is not expected that these factors will make the Deep Shaft more feasible.

3. Under certain condition, e.g. high strength waste water with BOD-values for above 250 mg/l, the Deep Shaft can be an attractive option.

4. The applicability of the Deep Shaft concept has been increased by the system-users as follows:
   - stripping/aeration tank in stead of vacuum degasifier (Tilbury, UK)
   - separate propulsion by a screw pump (Leer, Germany)
   - aeration system

5. The remarkable Japanese interest in the Deep Shaft technology (40 of 50 references) can be explained from specific factors as the immanent urgence of water conservation and recycling, together with very high ground costs. These factors are quite different from the situation in the Netherlands.

6. The Multireactor shows promise as a compact, high-performance system for waste water treatment on the basis of preliminary pilot-plant results. Definite conclusions can be made after finishing the test period and full evaluation of pilot plant results.

6. REFERENCES

1 Anon. (1979) The ICI Deep Shaft Effluent Treatment Process. ICI Produktinformatie.

2 Anon. (1983) Deep Shaft II., World Water, Sept. 1983, p. 50.

3 Anon. (1987) Het ICI-Deepshaft Systeem en de Multireactor, Onderzoeksplan DHV Raadgevend Ingenieursbureau.

4 Anon. (1987) Deep Shaft "Knife Edge" Technology in Tilbury., Surveyor, vol. 168, no. 4953, 16 July 1987, pp. 12, 13.

5 Anon. (1987) World's largest deep shaft. Water Services, Vol. 91, no. 1098, p. 347.

6 Anon. (1987) Deep shaft reaches new heights. WQI No. 3, 1987, pp. 18, 19.

7 Anon. (1988) Deep Shaft Activated SLudge Process for treatment of sewage and industrial wastewater. Produktinformatie PCI 1201 Process.

8 Brignal. W.J. (1982) Latest Developments in the Deep Shaft Wastewater Treatment Process., Public Health Engineer, Vol. 10, No. 3, 159 - 163.

9 Cox, G.C. et al. (1980) Use of the Deen-Shaft Process in Uprating and Extending Existing Sewage-Treatment Work.

10 Daly, P.G. and Shen, C.C. (1988) The Deep Shaft Biological Treatment Process., 43rd Annual Purdue Industrial Waste Conference, May 1988.

11 Fields, P.R. (1988) Sewage Treatment by Deep Shaft in the UK: a Major New Development. Annual Conf. Japan Sew. Works Assoc., Tokyo, May 1988.

12 Fields, P.R., Daly. P., Kobayashi, O and Hashimoto, S. (1989) Industrial Wastewater Treatment with Deep Shaft., Preprint Japan Sewage Works Association Conference, May 1989.

13 Hemming, M.L. (1979) General Biological Aspects of Waste-Water Treatment Including the Deep-Shaft Process. Wat. Poll. Control 78, pp. 312-325.

14 Hines, D.A., Bailey, M., Ousby, J.C. and Roesler, F.C. (1975) The ICI Deep Shaft Aeration Process for Effluent Treatment., Paper presented at the Conference of the Institution of Chemical Engineers, York, England, April 1975.

15 Robinson, A.B. and Cowen, R. (1985) Design and Construction Features of the Second Generation Tilbury Deep-Shaft Plant. Water Poll. Control. Vol. 84, nr. 4 p. 515.

16 Anon. J.C.J. Deep Shaft Design Manuel - Chapter 12 "Shaft construction".

17 Bulte, AWZI Leer - mondelinge informatie 1989.

18 Dunlop E.H., Characteristics of sludge produced by the Deep shaft, Society of Chemical Industry, Conference, 1977

19 STORA, Slibontwatering, delen 2 en 3, Optimalisering van slibontwatering met polyelektrolyt, 1981/82.

20 Persoonlijke waarnemingen - Tilbury, 1989.

21. J.B. Kok, Adjustment of Multireactor for treatment of sewage, Milieutechniek (3), (1990), p. 104 (in Dutch)

22. S. Engwirda, Flotation clarification after high pressure aeration, I, H_2O, 18, (1985), (20), 418 (in Dutch)

23. S. Engwirda, Flotation clarification after high pressure aeration, II, H_2O, 19, (1986), (18), 410 (in Dutch)

MEMBRANE BIOREACTORS FOR WASTEWATER TREATMENT

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INTRODUCTION

Industrial use of synthetic membrane for desalination has been started since asymmetric membrane was developed in 1960. Then, the continuous development of membrane technology including microfiltration and ultrafiltration has expanded its application fields; e.g., ultrapure water production for IC industry, refinery of pharmaceuticals, antibiotic production, concentration and decolorization of products in food industry, solid-liquid separation in bioprocess and so forth. It also gives a new direction in water/wastewater treatment.

Pressure driven crossflow filtration is usually applied in micro/ultra-filtration as well as hyperfiltration. Microfiltration (MF) removes particles down to submicrons and hyperfiltration (reverse osmosis, RO) rejects dissolved ion molecules. Ultrafiltration (UF) is in between and removes macromolecules, such as protein, starch and so forth.

Strathmann(1984) summarized successful applications of ultrafiltration to industrial wastewater treatment, such as the recovery of electrophoretic paints, separation of oil-water emulsion, recovery of proteins and starch in food industry, treatment of pulp and paper industry effluents and so forth. One of the largest treatment plant of pulp processing wastewater with 8,000 m^3/d has been successfully operated in Japan, using polysulfone UF tubular membrane module with molecular cut size of 8,000 (Ikeda, 1990).

In water purification process, it is necessary to remove particulate and colloidal matters. Besides RO, MF/UF can achieve it without chemical addition. Moreover, microfiltration

membrane with a lower pore size can assure bacteria removal and ultrafiltration membrane with a lower molecular cut off size can assure virus removal.

An attempt of use of hollow fiber ultrafiltration has been made in France, demonstrating that, from a pilot scale experiment, the ultrafiltration is a readily available technology for small scale drinking water treatment (Mallevialle et al., 1989).

In Japan, wide pollution of water resources sometimes requires high degree of treatment in water purification plants. The plants are usually suffer from the space for additional treatment processes, such as biological filter, activated carbon, ozonation and so forth. It is, anyhow, advantageous that the membrane process makes the system compact eliminating coagulation/sedimentation tanks and sand filtration units. The membrane filtration might replace the traditional treatment unit processes when the replacement period for existing water purification plants comes in the early next century. However, the long term performance, reliability and economy especially in large scale operation are unknown. So the feasibility studies have been recently started in some laboratories and water purification cites in Japan. Preliminary evaluation of a water purification plant designed with microporous hollow fiber membranes shows that the membrane plant could be constructed from 1/4 to 1/8 in space and from 1/10 to 1/16 in tank volume of a conventional plant if the designed volume flux of 1 to 2 m/d could be achieved (Fujita, 1990).

An activated sludge/ultrafiltration process has been developed in the United States for domestic wastewater treatment in 1960's (Smith et al., 1969, Bemberis et al., 1971). High concentration of microorganisms achieved by membrane separation gives some advantages to the process: compact size and little sludge production. In addition, the process operation does not suffer from sludge bulking and disinfection of the effluent might not be necessary.

Membrane-coupled biological wastewater treatment processes have been used for nonpotable water reclamation system in office buildings, department stores and others in large cities in Japan, Ohkata(1989) reported a successful example: Fig.1 shows the schematic flow diagram of the treatment process in a department store building in Tokyo. The treated water quality of the UF filtrate was quite satisfactory (Table 1). He also showed an estimation of the operating and maintenance cost (Table 2) and compared it with the charges for tap water usage and wastewater discharge to sewerage, as shown in Table 3. This table clearly shows the cost of water consumption in large cities in Japan is very high especially for large users because those administrations have adopted gradual increase systems of water charge. In addition, Tokyo and Hukuoka City administrations make effort to have nonpotable water reuse system in large buildings. These situations give incentive to the membrane applications.

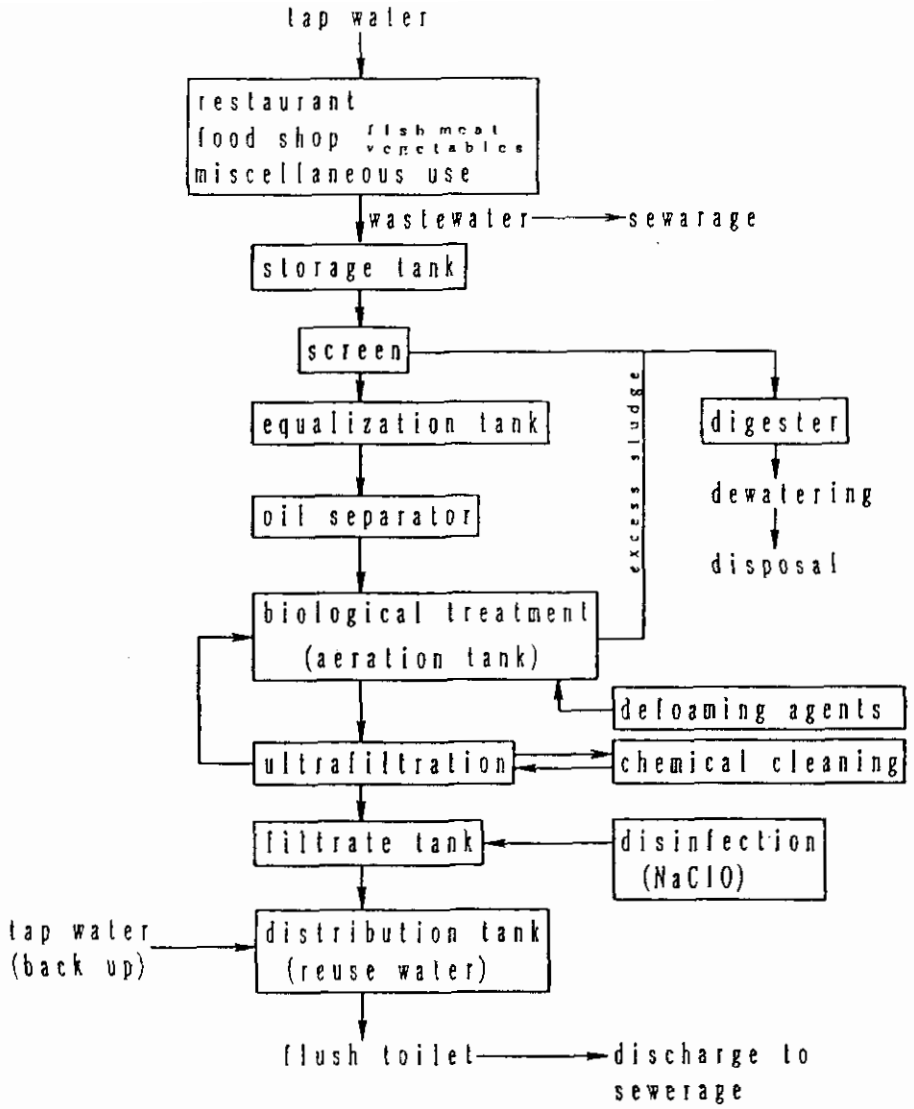

Fig. 1. Schematic flow diagram of UF water reclamation system (after Ohkata, 1989)

Table 1. Performance of UF water reclamation system

(after Ohkata, 1989)

		Influent			Dissolved Liquor in bioreactor			UF effluent		
		max.	min.	av.	max.	min.	av.	max.	min.	av.
pH		6.98	4.90		7.20	6.72		7.30	6.79	
BOD	mg/l	1600	33.4	385	14.5	2.4	7.6	3.2	<1	<1
COD_{Mn}	mg/l	1440	34.2	261	34.9	26.1	30.8	7.1	4.0	5.9
SS	mg/l	1000	90	630				0	0	0
n-hexane extract	mg/l	760	19.2	130				tr	tr	tr
color	deg.							85	53	71
turbidity	deg.							0	0	0

note. bioreactor MLSS 1400-8800 (av. 4600) mg/l
water temperature 18.5- 35,3 C.

Table 2. Operating and maintenance cost of UF water reclamation system (after Ohkata, 1989)

Membrane replacement	108	
Electricity	80	(yen/m^3)
Chemicals	8	
Maintenance	55	
total	251	

Table 3. Water charges for tap water usage and discharge to sewerage in large cities in Japan (based on 200 cubic meter per day of usage) (after Ohkata, 1989)

(yen/m^3)

City	tap water	sewerage	total
Tokyo	382	295	677
Yokohama	325	215	540
Kawasaki	298	125	423
Nagoya	284	203	487
Ohsaka	298	130	428
Hukuoka	453	173	626
Kitakyushu	290	137	427

However, the power consumption of the existing membrane process applied to the nonpotable water reuse system is as high as 3 to 5.5 Kwh/m^3 of water treated (Uchida, 1983). Since the energy consumption is about ten times higher than that of the conventional activated sludge processes, its applications are still limited so far.

Another example of the application of existing ultrafiltration membrane processes is in collected human excreta treatment plants, because high concentrated sludges from biological treatment of night soil create a problem of solid liquid separation in traditional treatment processes. Several intensive researches have been done to obtain rational designs of ultrafiltration used for the human excreta treatment (Magara & Itoh, 1991, Sato & Ishii, 1991).

Anaerobic treatment coupled with membrane separation were also investigated by several researchers (Grethlein, 1978, Li et al., 1984, Okuno et al., 1986). Kimura (1991) summarized Japan's Aqua Renaissance '90 Project which aimed to create a new wastewater treatment and water reuse system, i.e., energy recovery by methane gas production from anaerobic wastewater treatment and its advanced post treatment with membrane separation for water reuse.

Ultra/micro-filtration of raw wastewater is also worthwhile to be considered, although it requires post treatment of dissolved organic pollutants which can pass the membranes.

A shortcoming of existing crossflow filtration is its high

operational cost. The main reason for the high cost is due to the recirculation pump which maintains high cross flow velocity parallel to the membrane surface in order to keep the flux undeclined. The elimination of the recirculation pump will reduce the power consumption remarkably. This gives an idea of direct membrane separation of biological sludges in a bioreactor.

In cross flow ultra/micro-filtration, many attempts have been made to attain high volume flux, which needs a well designed operation and maintenance, such as frequent chemical cleaning, backwashing, etc., and increases operational cost. It is not easy to maintain high flux especially in wastewater treatment, because serious fouling or clogging may occur during the filtration of the wastewater contaminated by unknown materials. Increase of membrane surface area per unit volume is another approach to obtain enough filtrate. Hollow fiber membranes can be used for this purpose.

The following part of this article aims to summarize the series of the author and his colleagues' works on the membrane bioreactors for wastewater treatment.

MEMBRANE SEPARATION CHARACTERISTICS

Figures. 2 and 3 show the results obtained in a laboratory scale experiment where synthetic wastewater was treated in activated sludge process and the cross flow filtration through flat sheet membrane was applied (Fuchigami et al., 1987). As shown in Fig. 2, the volume flux increased with increase in the pore size in a certain range, i.e. between UF10 (nominal molecular weight (n.m.w.) cut off of 10,000) and UF50 (n.m.w. cut off of 50,000). At pore sizes larger than UF50, the volume flux reached a plateau up to a limit pore size, i.e. about 1.0 μm (MF0.1). The flux then decreased again beyond the limit. Chiang (1988) determined the membrane resistance and gel layer resistance in the range of UF and showed that the observed gel layer resistances were almost the same regardless of UF cut off size. The membrane resistance, however, decreases with increase in UF cut off size. In the range from UF10 to UF50, a decrease in membrane resistance contributed a decrease in total resistance resulting an increase in volume flux. On the other hand, gel layer resistance became predominant beyond UF50 so that the flux changes little up to a certain limit. Beyond the limit, the pore size became too large comparing bacterial size and/or particle size of suspended materials, and they were allowed to enter the inner pores of membrane, causing severe clogging inside the membrane at last.

As for the effluent water quality, little difference was observed in different pore sizes of membranes. Actual cut off characteristics, as shown in Fig. 3, were almost similar regardless of nominal m.w. cut off/pore size. This is because separation characteristics is determined not by an intrinsic membrane characteristics but by a dynamic state of membrane (i.e. membrane and gel/polarization layer).

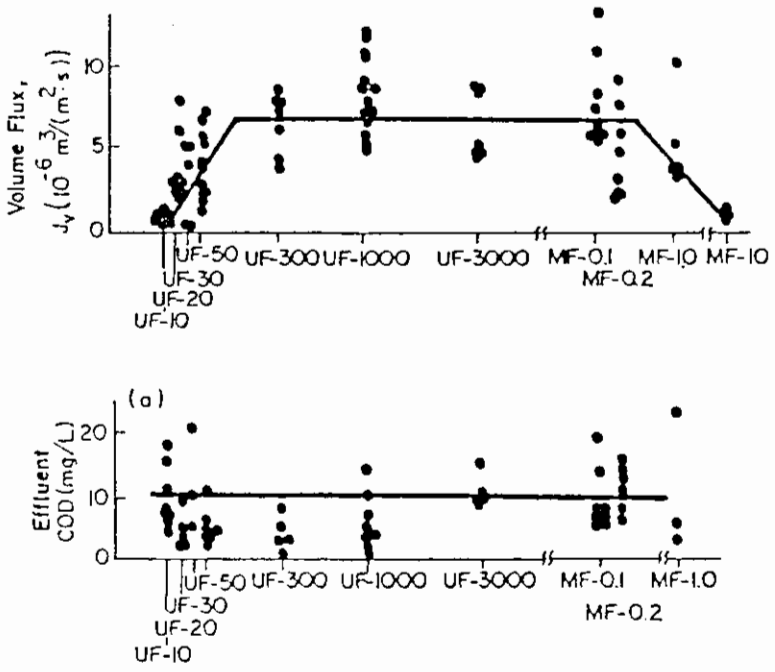

Fig. 2. Effect of membrane pore size on volume flux and effluent COD. UF-x: x indicates nominal molecular weight cut off in thousands. MF-y: y indicates pore size in micro meter. Materials: polysulfone/UF-10, 30, 50, 300, 1000, 3000, MF-0.1, 10/; polyolefine/UF-20/; polytetra-fluoroethylene/MF-0.2, 1.0. (Fuchigami et al., 1987)

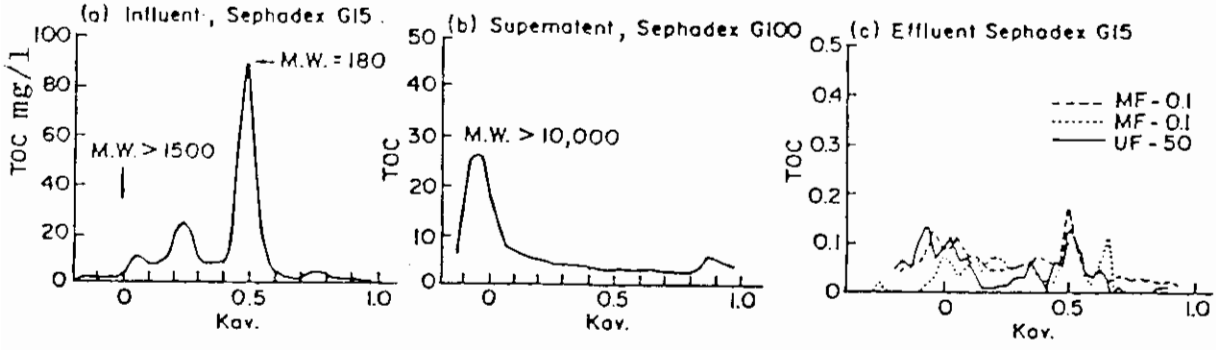

Fig. 3. Gelchromatogram of influent, effluent and supernatant of mixed liquor in aeration tank. (Fuchigami et al., 1987)

There should be an appropriate range of pore sizes for activated sludge separation. In economical sense, microfiltration (MF) membrane is cheaper than UF membrane. Therefore, around 0.1 μm in MF range was chosen as an appropriate pore size in the following studies.

How about virus removal? Urase et al. (1991) investigated the permeability of RNA-F-specific coliphage, Q_β, through MF membrane made of polyvinylidene fluoride with pore size of 0.1 μm during the crossflow filtration of poly-methyl- metaacrylate (PMMA) suspension (average particle size = 0.15 μm) and pond water (SS = 21 mg/l, COD = 30 mg/l). Fig. 4 shows that the flux decreased similarly in both PMMA suspension and pond water filtration, however, the respective rejections of Q_β were quite different. As a nature of PMMA suspension, the cake layer was formed on the membrane which mainly contribute to the total resistance increase but could not reject Q_β effectively. On the other hand, the rejection of Q_β was rapidly increased in the pond water filtration, suggesting that the barrier deposition, as categorized by Fane et al. (1983), took place due to the gel layer formation by dissolved organic matters in the pond water. Similar result was observed in the filtration of activated sludge.

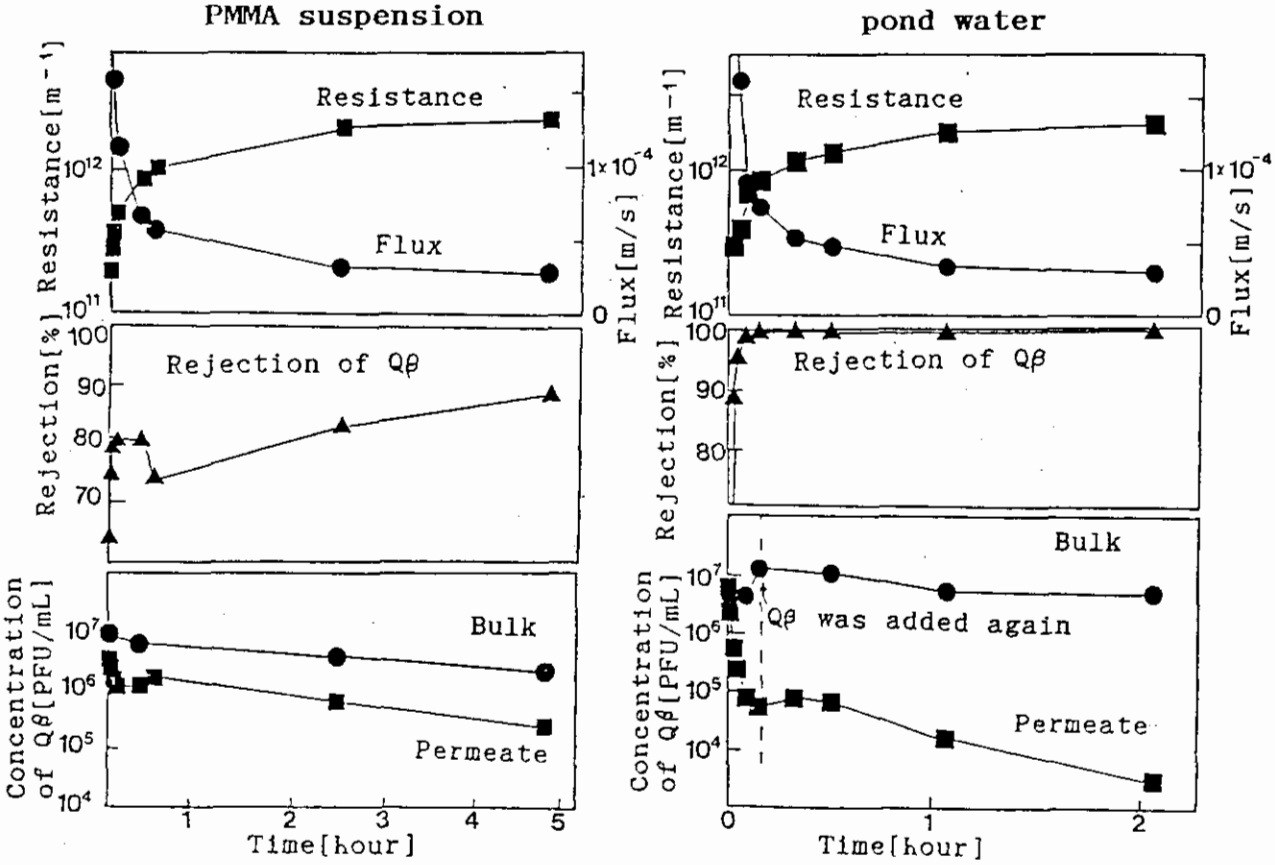

Fig. 4. Crossflow filtration of PMMA suspension and pond water (Urase et al., 1991)

DEVELOPMENT OF HOLLOW FIBER MEMBRANE BIOREACTOR

Direct membrane separation using hollow fiber membranes in activated sludge processes is to be discussed below. Several questions may arise.

How is the clogging problem? It may be serious, if a membrane is directly immersed in high concentration of suspended solids.

Yamamoto et al. (1989) made use of microporous hollow fiber membranes for direct solid-liquid separation in activated sludge aeration tank. Their results gave the conclusion that the key factors for the stable operation are low transmembrane pressure and intermittent filtration. Figure 5 shows transient flux changes from the start of filtration at various initial transmembrane pressures. It is obvious that the flux became insensitive to the pressure in a very short time of operation. The solid lines (a) and (b) are the clean water fluxes through new and used membranes, respectively. The difference between the lines (a) and (b) indicates degree of fouling/clogging inside membrane. On the other hand, the difference between the line (b) and the data points indicates degree of resistance/clogging on the membrane and/or inter fibers: this kind of clogging may result unrecoverablly in dead end of operation but can be prevented by choosing an appropriate pressure applied. In this sense, lower pressure operation, which results in little progress of such kind of clogging, is more preferable.

Figure 6 shows the results of continuous filtration. The fluxes decreased rapidly in a short period of operation. Suction was used in getting filtrate, accordingly transmembrane pressure increased to almost vacuum pressure with the progress of clogging. An intermittent filtration was found to be effective in order to keep flux undeclined. The stable operation (4 h detention time) were kept over an operational period of 4 months at an average net flux of 3×10^{-7} $m^3/(m^2\ s)$ (0.026 m/d).

Since the sludge was not removed from the process, in other words, the process includes aerobic digestion of sludge, organic stabilization and inorganic accumulation in the aeration tank was investigated.

It was concluded that a steady state F/M ratio (on a COD - MLSS basis) approached ca. 0.1 d^{-1}, establishing the corresponding steady state MLSS concentration about ten times as large as the volumetric organic loading and that almost complete oxidation of biodegradable influent organic materials could be achieved. Even though the flux was low enough to get stable operation, there was the critical sludge concentration beyond which severe clogging could happen: it was 30,000 to 40,000 mg/l which was corresponding to the critical volumetric organic loading of 3 to 4 $kgCOD/(m^3 d)$.

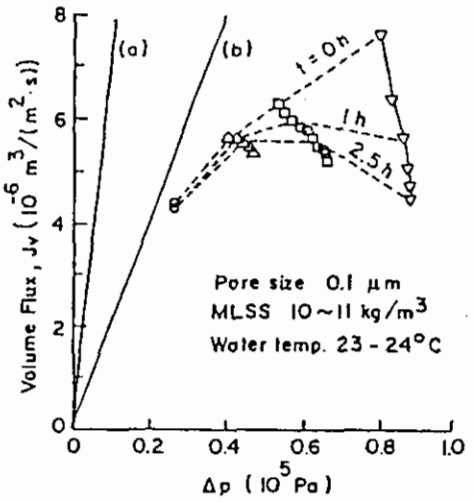

Fig. 5. Transient flux change from the start of hollow fiber filtration. The solid line (a) and (b) shows clean water fluxes through new and used membrane, respectively. (YAMAMOTO et al., 1989)

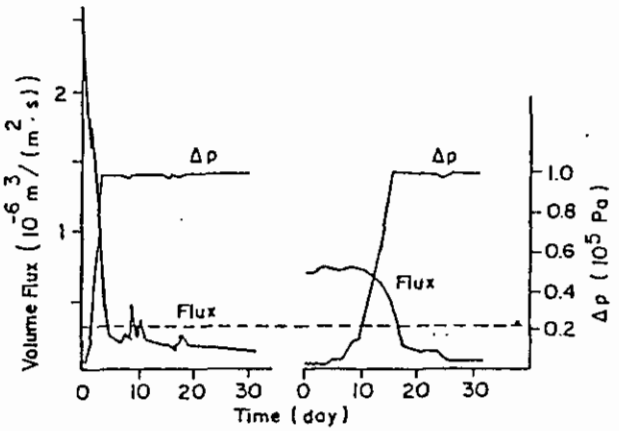

Fig. 6. Flux and transmembrane pressure changes in continuous filtration through hollow fiber membranes. The dotted line express a stable flux obtained in a long term operation by intermittent filtration. (YAMAMOTO et al., 1989)

Treatment of actual wastewater gave similar results as shown in Fig. 7. The effluent COD and turbidity was as low as 10 mg/l and 0.2 NTU, respectively. At a high flux had tendency to give severe clogging, nevertheless it was operated under the critical organic loading mentioned above. No accumulation of non-volatile solids was observed after reaching steady state condition (Yamamoto and Mahmood, 1988).

Hiasa et al. (1990) investigated the operational mode of intermittent filtration to get higher average net flux: 1 min. on/1 min. off intermittent operation was found to give an enough and stable flux of 0.14 m/d for 22 days of operation.

Modification of the process to SBMR (sequencing batch membrane reactor) was tested for the treatment of high strength organic wastewater including heavy metal, chromium (see Fig. 8 and Table 4). A combination of aerobic anaerobic treatment increased the critical organic loading. When the sludge was removed, the critical organic loading increased also, e.g. it would be 10 kgCOD/(m^3d) at SRT of 10 days, as illustrated in Fig. 9 (Yamamoto and Win, 1991).

The application to on-site domestic wastewater treatment was also investigated in both laboratory and pilot scale experiments (see Fig. 10). Results are summarized in Table 5. For small scale treatment, it is not necessary to make the bioreactor too small because fluctuation of inflow needs, anyhow, an eqaulization tank. HRT was, therefore, chosen 0.5 to 1 day so that the bioreactor could equalizing the flow fluctuation. Low MLSS concentrations means the bioreactor was operated at far below the critical organic loading. The average fluxes were about 0.1 m/d for a month- operation (Yamamoto and Sopajaree, 1990).

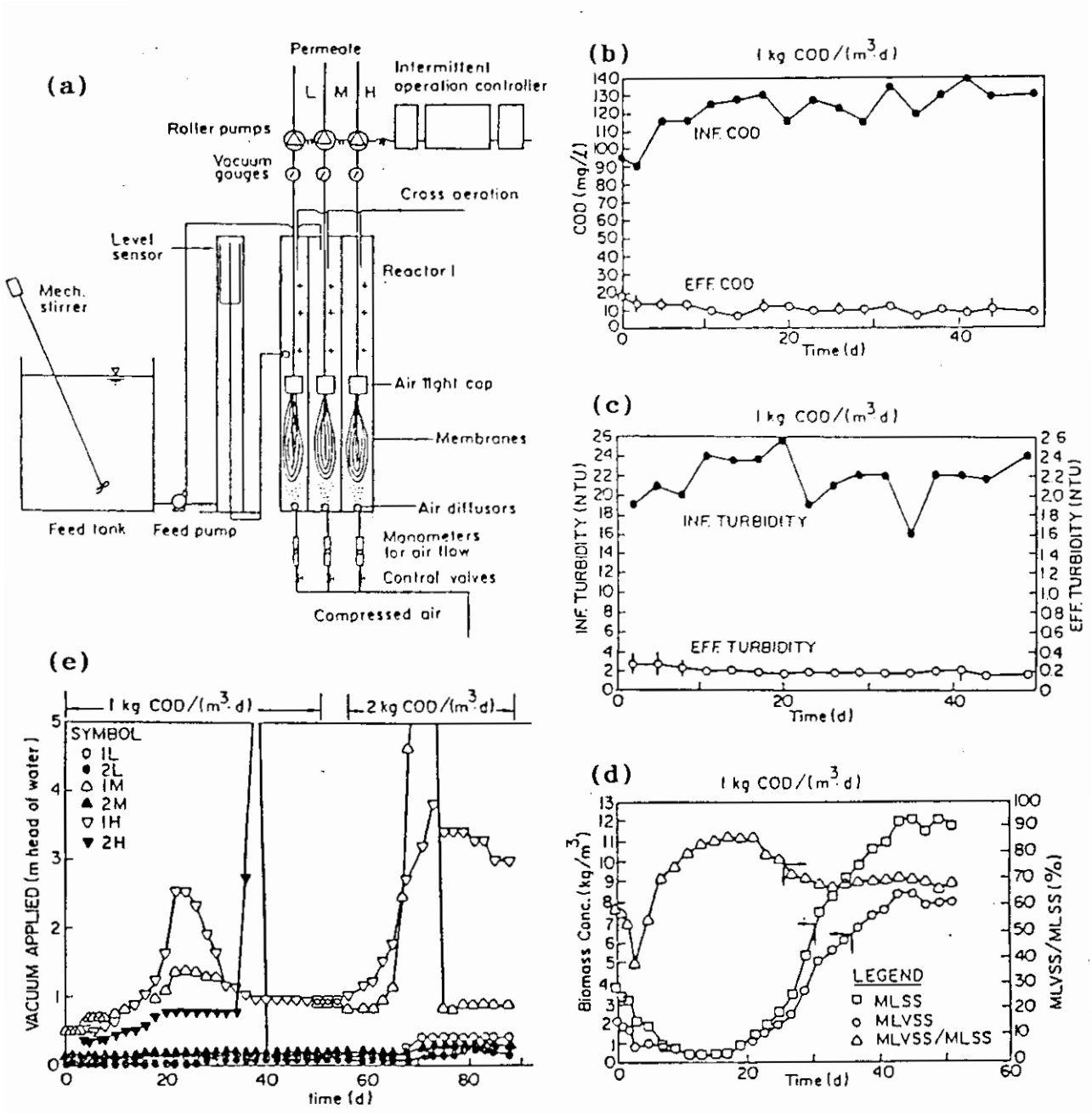

Fig. 7. (a) Experimental apparatus, (b) Influent/effluent COD, (c) Influent/effluent turbidity, (d) MLSS, MLVSS and VSS/SS, (e) Variation of suction pressure (symbols: 1 and 2 indicates 0.03 and 0.15 micro meter pore size, respectively. L, M and H indicates the volume flux of 4.17, 6.94 and 9.72 x 10^{-7} $m^3/(m^2 s)$, respectively. (Yamamoto & Mahmood, 1988)

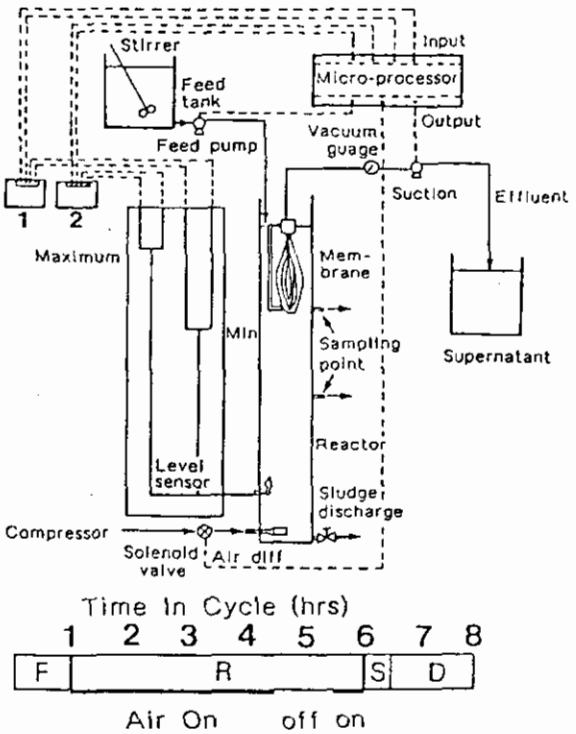

Fig. 8. Laboratory scale SBMR and operating schedule of batch treatment (Yamamoto & Win, 1991).

Table 4. Performance of SBMR (Yamamoto & Win, 1991)

	Influent	Effluent			Influent	Effluent		
Reactor		R1	R2	R3		R1	R2	R3
SRT (day)		550	20	10		550	20	10
VOL* (kgCOD/(m^3d)		3	3	3		5	5	5
pH	9.5	7.6	7.6	7.6	9.6	7.8	7.8	7.8
COD (mg/L)	2960	186	139	161	4980	225	185	218
% removal		93.7	95.3	94.6		95.5	96.3	95.6
NH_3-N (mg/L)	105	45.3	34.1	43.5	155	47.7	37.0	51.7
% removal		56.9	67.6	58.9		69.2	76.1	66.7
Org-N (mg/L)	45	6.5	5.8	5.9	47.2	9.9	7.4	11.6
NO_2-N (mg/L)	0.12	19.0	14.6	18.6	0.13	19.3	13.1	16.4
NO_3-N (mg/L)	3.8	0.14	0.11	0.13	3.8	0.23	0.22	0.34
% TN removal		53.9	64.5	55.9		62.5	71.9	61.2
Cr (mg/L)	25.3	1.08	1.11	1.15	24.6	0.84	0.90	0.99
% Cr removal		95.7	95.5	95.4		96.6	96.3	96.0

	Influent	Effluent		
Reactor		R1	R2	R3
SRT (day)		550	20	10
VOL* (kgCOD/(m^3d)		10	10	10
pH	9.7	7.8	7.8	7.7
COD (mg/L)	9580	357	328	361
% removal		96.3	96.6	96.2
NH_3-N (mg/L)	173	26.3	21.0	23.6
% removal		84.8	87.8	86.3
Org-N (mg/L)	41.7	15.2	13.5	16.4
NO_2-N (mg/L)	0.14	16.4	12.8	12.6
NO_3-N (mg/L)	4.0	2.0	0.57	0.65
% TN removal		72.6	78.1	75.6
Cr (mg/L)	25.1	0.57	0.70	0.85
% Cr removal		97.7	97.2	96.6

*Note VOL: Volumetric Organic Loading

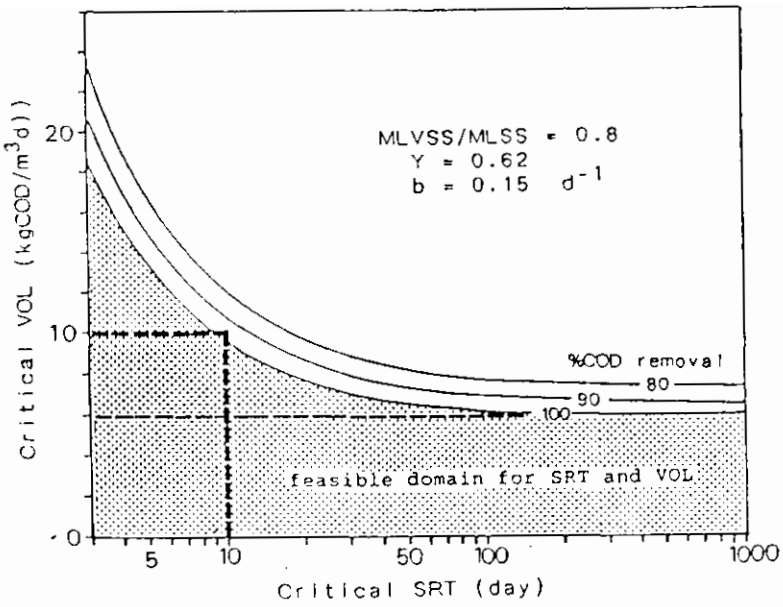

Fig. 9. Design chart for volumetric organic loading (VOL) and sludge retention time (SRT) (Yamamoto & Win, 1991).

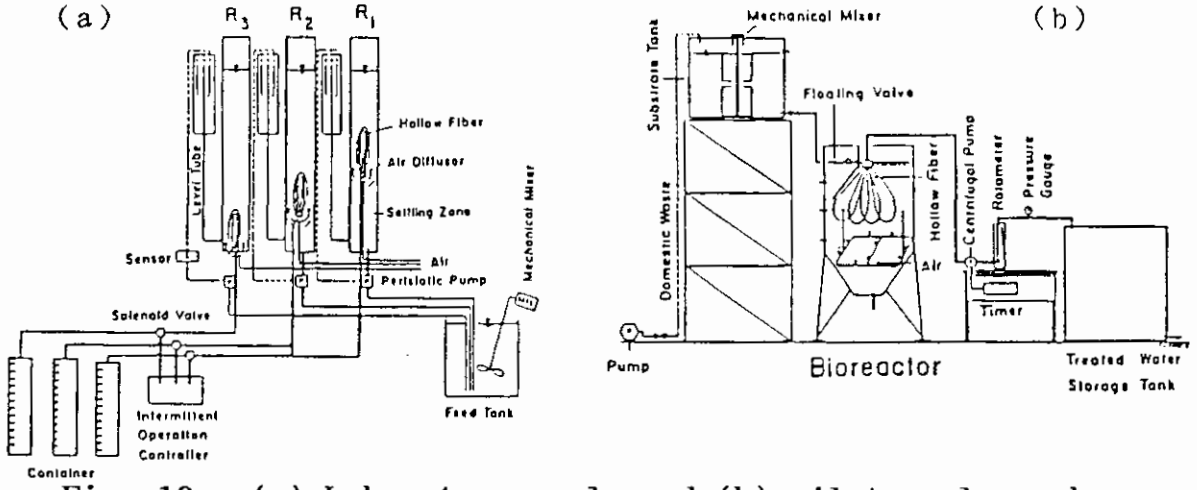

Fig. 10. (a) Laboratory scale and (b) pilot scale membrane bioreactors (Yamamoto & Sopajaree, 1990).

Table 5. Performance of membrane bioreactors (Yamamoto & Sopajaree, 1990).

	Laboratory scale				Pilot scale							
Reactor		R1	R2	R3		R1	R2	R3	without		with partition	
HRT (day)			1				0.5		1			
air flow (L/min)			1.5				1.5		11.5		13.0	
MLSS (mg/L)		2230	2540	2530		3230	3400	3590	1320		1280	
MLVSS/MLSS	(%)	60.9	56.6	58.2		51.7	53.4	57.5	66.2		-	
pressure head	(m)	0.40	0.44	0.46		0.98	1.15	1.18	0.95		-	
	Inf.	Effluent			Inf.	Effluent			Inf.	Eff.	Inf.	Eff.
COD (mg/L)	155	10.5	12.4	13.0	153	10.5	11.8	12.4	228	21.0	255	9.0
%COD removal		93.2	92.0	91.6		93.1	92.3	91.9		90.8		96.5
TN (mg/L)	17.6	13.5	14.1	14.0	15.1	13.0	14.2	9.8	9.1	7.4	18.2	8.0
NOx-N (mg/L)	0.81	12.5	13.4	13.0	0.48	11.6	12.5	8.2	0.53	6.0	1.4	7.5
pH	7.3	8.0	7.9	7.9	7.0	8.0	7.9	8.0	8.0	8.1	7.5	7.8

PERFORMANCE OF CROSS FLOW HOLLOW FIBER FILTRATION OF POLYMER SUSPENSION AND ACTIVATED SLUDGE

Hollow fiber membrane module usually consists of hundreds of fiber threads. It was obtained that intermittent filtration was effective to achieve long-term stable operation of the module as mentioned above. This implies that the filtration was controlled by cake/gel layer on the hollow fiber membrane and that the cyclic release of applied pressure by intermittent filtration recovered compaction of the cake/gel layer to some extent. In addition, mutual influence of neighbor hollow fibers would significantly affect accumulation of sludges in the module.

In order to separate the effect of intermittent filtration through single hollow fiber from that through a bundle of hollow fibers, the performance of single hollow fiber filtration of poly methyl meta acrylate (PMMA) particles and activated sludges was investigated in a well controlled tubular flow channel. The results showed the effect of intermittent filtration on the flux was large when the particle size was much larger than the pore size of the membrane. Little effect was observed, however, when the particle size was almost equivalent to the pore size. This is because the clogging took place due to the particles entrapped inside membrane pores and it was hardly recovered by the intermittent operation. The effect on activated sludge filtration was in between but a little (Wong et al., 1990). Therefore, it is concluded that the intermittent suction (or filtration) mainly contributes to prevent unrecoverable accumulation of sludge in-between fibers, i.e. interfiber clogging.

The mutual influence of hollow fibers was intensively investigated (Wong et al. 1991). They used UF polysulfone hollow fibers with outer skin layer (n.m.w. cut off = 20,000, outer diameter = 1.4 mm, inner diameter= 0.8 mm). Figure 11 shows the experimental apparatus and the results. Pure water flux was unchanged with various numbers of the hollow fibers. On the other hand, the flux changed with changes in packing density of membrane in activated sludge filtration.

Up to a certain packing density, the flux was kept constant at a constant crossflow velocity. In this region, the fibers were dispersed well and acted independently like single fiber. Beyond the region, the flux decreased sharply with increase in the packing density, where sludge accumulation inside the fiber bundle started. Then, the flux increased a little again, where the fully accumulated sludge inside the bundle was observed. This was explained by a theoretical consideration that a relative decrease of accumulated sludge portion to unit membrane surface by increasing number of fibers corresponded a decrease in average thickness of gel layer on the membrane, resulting in increase in flux. When the packing density reached its maximum value, the flux decreased again because the accumulation of sludge took place on the peripheral surface of the fiber bundle.

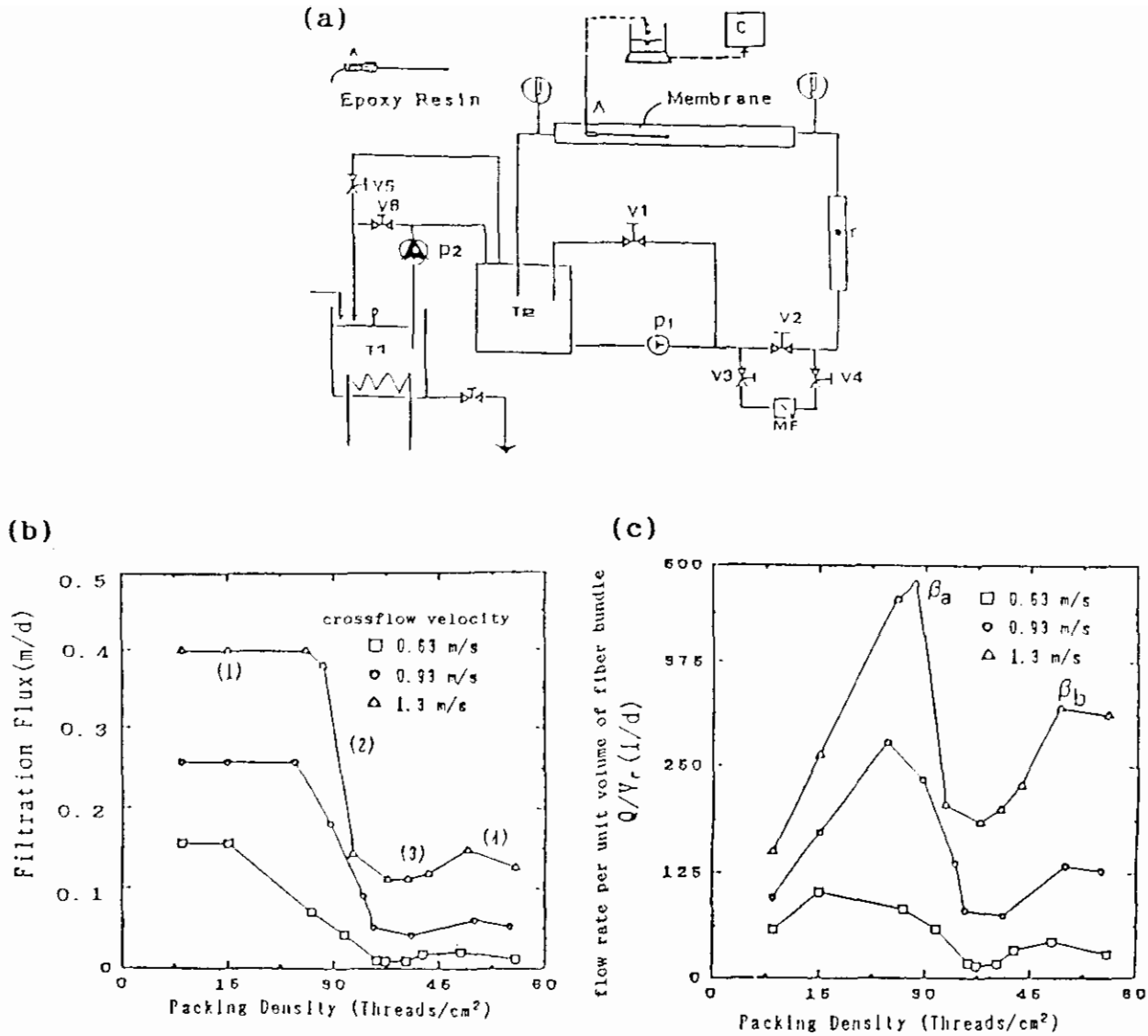

Fig. 11. (a) Experimental set-up, (b) Flux vs. packing density, (c) flow rate per unit volume of fiber bundle vs. packing density (operating conditions: transmembrane pressure difference = 59 kPa, temperature = 25 C), (Wong et al., 1991)

As expected, the maximum flow rate per unit volume of the fiber bundle was obtained at the critical point of packing density, below which the fibers were well dispersed. It is very important to have a well dispersed condition for fibers to get a high flux.

These results suggests there would be the optimum hollow fiber spacing and the importance of the design of hollow fiber modules. With an appropriate design, the flux would become much higher than that presently obtained. This will make hollow fiber microfiltration economically feasible for both water and wastewater treatment.

IMPROVEMENT OF HOLLOW FIBER MEMBRANE BIOREACTOR

Those fundamental studies mentioned above gave an idea to modify the hollow fiber membrane bioreactor. If the hollow fibers are kept in well dispersed condition by modification, we can expect a higher flux. A small mixing tank was used for such purpose. Figure 12 shows the experimental set up. The maximum 4 hollow fiber modules (0.3 m^2 of surface area per module) can be set in the inner small tank (ca. 10 L) with a mixer (ca. 290 rpm). The tank is open to the main reactor tank (ca. 60 L) at the top and the bottom, where mixed liquor comes from the top and returns through the bottom holes. The experiment has been recently started using synthetic wastewater at a controlled temperature of 20 C. Continuous filtration was applied. Rings were tested to separate hollow fibers in a module as shown in Fig. 12. A reverse rotational mode of mixer (30 seconds clockwise and 30 seconds anticlockwise rotation) was also tested. Although the data obtained so far are not enough, Fig. 13 shows a potential in getting higher flux by addition of a small mixing tank in the reactor.

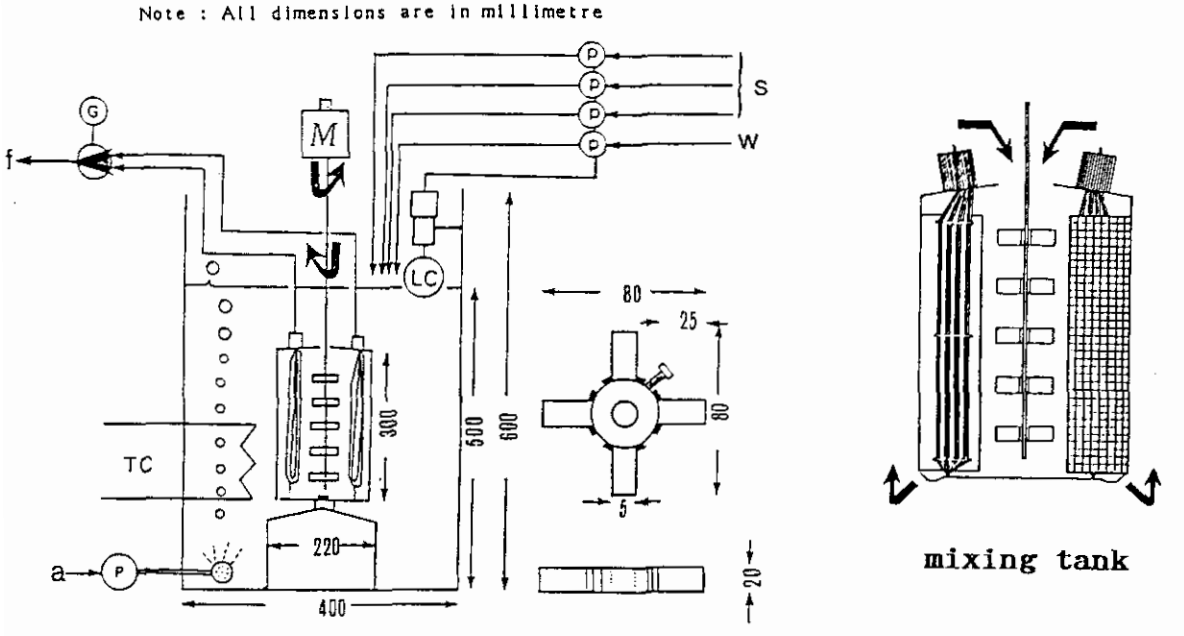

Fig. 12. Membrane bioreactor with hollow fiber module in a small mixing tank.

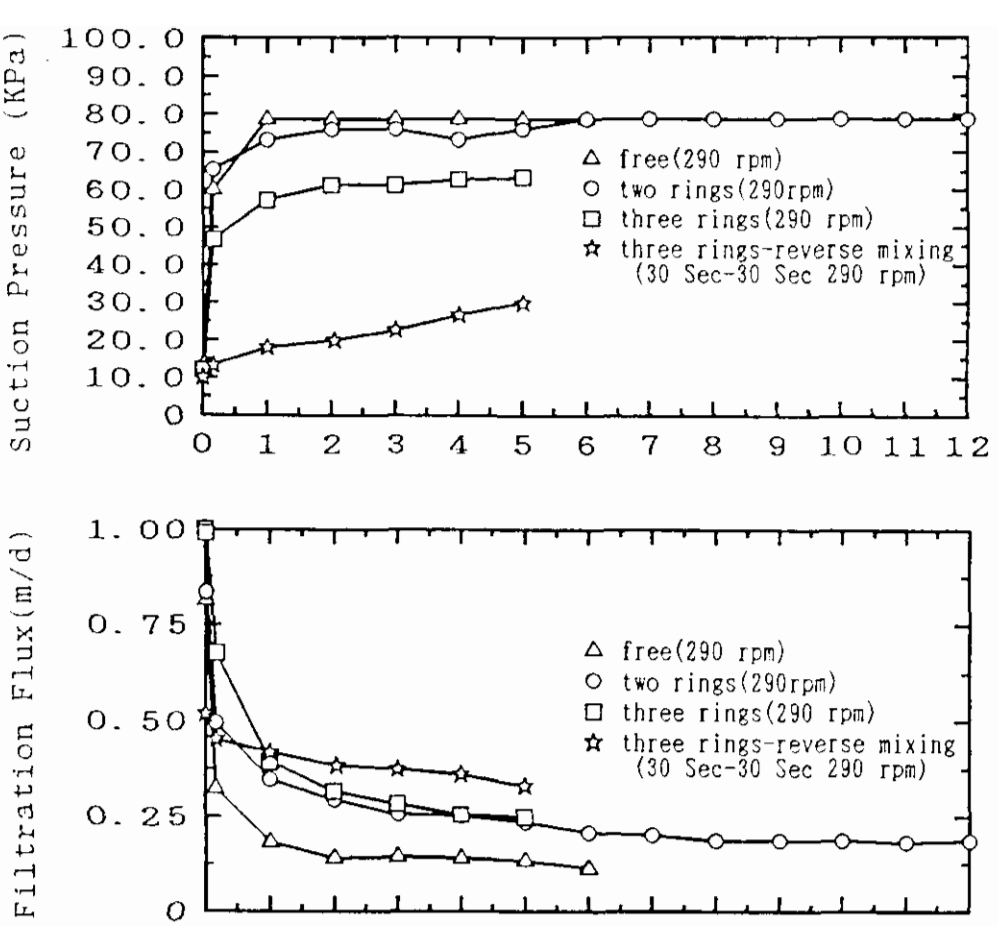

Fig. 13. Flux and suction pressure of hollow fiber module in a mixing tank.

CONCLUDING REMARKS

Series of studies on the development of hollow fiber membrane bioreactors revealed the process feasible for various wastewater treatment in terms of satisfactory and reliable operation. We can expect high degree of treatment in terms of organic removal, bacterial removal, nitrogen removal and so forth, with suspended solid free effluent all the time.

It is important to find an appropriate design of hollow fiber membrane module, with which a high enough flux is obtained to make the process economically more feasible. One promising improvement of the hollow fiber membrane bioreactor would be the enhancement of mixing in membrane separation zone; a small mixing tank, for example, was very effective to get higher flux.

REFERENCES

Bemberis, I., Hubbard, P.J. and Leonard, F.B. (1971). Membrane sewage treatment system - potential for complete wastewater treatment. Presented paper at Winter Meeting, American Soc. of Agricultural Engineers, Illinois, U.S.A.

Chiang, H.C. (1988). Effect of operating parameters in ultrafiltration on the separation of mixed liquor suspended solid. AIT Thesis EV-88-6, Bangkok, Thailand.

Fane, A.G., Fell, C.J.D. and Waters A.G. (1983). Ultrafiltration of protein solutions through partially permeable membranes- the effect of adsorption and solution environment. Journal of Membrane Science, 16, 211-224.

Fuchigami, Y., Yamamoto, K., Asami, K. and Matsuo, T. (1987). On the activated sludge process combined with membrane separation. Proc. of Environ. & Sanitary Eng. Research, 19, 239-249 (in Japanese).

Fujita, K. (1990). Design of waterworks purification plant using micro-filtration membrane. J. Japan Water Works Association, 59, No.4, 10-18 (in Japanese).

Grethlein, H.E. (1978). Anaerobic digestion and membrane separation of domestic wastewater. J. Water Poll. Control Fed., 50, 754-763.

Hiasa, M., Hanaki, K. and Matsuo, T. (1990). Efficient operation of intermittent suction in membrane separation activated sludge process using hollow fiber module. Proc. 45th Annual Meetings, JSCE, 2, 1076-1077 (in Japanese).

Kimura, S. (1991). Japan's Aqua Renaissance '90 Project. Wat. Sci. Tech., 23, Kyoto, 1573-1582.

Li, A., Kotari, D. and Corrado, J.J. (1984). Application of membrane anaerobic reactor system for the treatment of industrial wastewater. Presented paper at 39th Annual Purdue Industrial Waste Conference, Indiana, U.S.A.

Magara, Y. and Itoh, M. (1991). The effect of operational factors on solid/liquid separation by ultra-membrane filtration in a biological denitrification system for collected human excreta treatment plants. Wat. Sci. Tech., 23, Kyoto, 1583-1590.

Mallevialle, J., Bersillon, J.L. and Anselme, C. (1989). Use of hollow fiber ultrafiltration for particulate removal in drinking water. Proc. of Workshop on Clean Technology, Asian Institute of Technology, Bangkok, Thailand, November, 181-193.

Ohkata, M.(1989). Water reclamation system using UF method. Sangyo Kikai, November, 1-4 (in Japanese).

Okuno, N., Ishida, K. and Tanaka, Y. (1986). Application of anaerobic fixed bed process to domestic wastewater treatment followed by membrane separation. Presented paper at 3rd German/ Japanese Workshop on Wastewater and Sludge Treatment, Tsukuba.
Sato, T. and Ishii, Y. (1991). Effect of activated sludge properties on water flux of ultrafiltration membrane used for human excrement treatment. Wat. Sci. Tech., 23, Kyoto, 1601-1608.

Smith, Jr. C.V., Gregorio, D.D. and Talcott, R.M. (1969). The use of ultrafiltration membrane for activated sludge separation. Presented paper at 24th Annual Purdue Industrial Conf.

Strathmann H. (1984). Water and wastewater treatment experience in Europe and Japan using ultrafiltration. in Synthetic Membrane Processes (Belfort, G. ed.), Academic Press Inc., 343-375.

Uchida, S. (1983). The most compact system for nonpotable water. Zosui Gijutu, 9, No.3, 33-37 (in Japanese).

Urase, T., Yamamoto, K. and Ohgaki, S (1991). Effect of membrane surface deposit on permeability of coliphage, Q . Proc. 25th Ann. Conf. of JSWPR (in Japanese).

Wong, Y.K., Yamamoto, K and Ohgaki, S. (1990). Performance of single thread hollow fiber membrane on polymer suspensions and activated sludge. Presented paper at 26th Annual Meetings of Environmental Engineering, JSCE, 71-73.

Wong, Y.K,, Yamamoto, K. and Ohgaki, S. (1991). Factors controlling flux in externally pressurized hollow fiber module for solid/liquid separation. Proc. 25th Ann. Conf. of JSWPR (in Japanese).

Yamamoto, K. and Mahmood, T. (1988). Hollow fiber microfiltration for solid-liquid separation in activated sludge process. Proc. 2nd National Symposium on Environmental technology and Management, Chiang Mai, Thailand, October, 251-259.

Yamamoto, K., Hiasa, M., Mahmood, T and Matsuo, T. (1989). Direct solid-liquid separation using hollow fiber membrane in an activated aeration tank. Wat. Sci. Tech., 21, Brighton, 43-54.

Yamamoto, K. and Sopajaree, K. (1990). Membrane separation activated sludge process for on-site wastewater treatment. Proc. 24th Ann. Conf. of JSWPR, 51-52 (in Japanese).

Yamamoto, K. and Win, K.M. (1991). Tannery wastewater treatment using a sequencing batch membrane reactor. Wat. Sci. Tech., 23, Kyoto, 1639-1648.

SCALE-UP OF THE AEROBIC AIRLIFT SUSPENSION BIOFILM REACTOR

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ABSTRACT

A new aerobic reactor concept has been developed for the aerobic purification of sulfide, COD and NH_3-containing waste water. This reactor is based on biofilm formation on small suspended particles in an air-lift suspension reactor. Aspects to be discussed are design considerations, scale-up of transport phenomena and biological purification performance from 0.35 m^3 to 300 m^3.

INTRODUCTION

Biological treatment of waste waters has been developed since the beginning of this century for the purification of municipal waste-waters. This has resulted in a large variety of systems. Since the early seventies there has been an increasing demand for the purification of industrial waste waters. However the available large scale aerobic technologies of sewage treatment are nog very suitable for application on industrial production sites for reasons indicated in Table 1.

Table 1 Negative aspects of conventional treatment technologies and their origin

Negative aspect	origin
- large reactor volume	: low sludge concentration
- large settler area	: low settling rate biomass
- emissions of noise, odours and aerosols	: large, open vessles
- high energy consumption	: aerobic processes
- low treatment efficiency	: low sludge age
- high surplus sludge production	: low sludge age

This has prompted the development of anaerobic purification technologies, which have the advantage of begin compact, having energy-production and low surplus sludge production (Lettinga).
However an anaerobic pretreatment always needs an aerobic post treatment to purify residual COD, sulfide and ammonia. Furthermore its becomes increasingly difficult for the conventional sewage treatment systems to meet the increased effluent standards of nitrogen. In addition the aspect of surplus sludge production and emissions becomes more important. Due to increased quality standards the application of sewage sludge in agriculture becomes more and more restricted. Also the standards of acceptable emission of noise, odours and aerosols from the treatment plants are being tightened. This leads to costly modification of existing conventional treatment plants

Table 2 Design requirements of an advanced aerobic reactor

- high capacity for COD, NH_4^+ and S^{2-} oxidation
- small area
- completely closed
- low sludge production
- high treatment efficiency
- low energy consumption

Therefore there appears a need (Table 2) for an aerobic treatment system which has a high treatment capacity of COD, sulfide and ammonia, which has a small area and is completely closed, has a low surplus sludge production, a high treatment efficiency, low energy consumption.

In this paper a reactor design will be presented which meets these requirements to a considerable extent.

THE CONCEPT OF THE AIRLIFT SUSPENSION REACTOR

Table 3 lists a number of requirements which are relevant for an advanced reactor type. The question is now which reactor type would best be suited to meet said requirements. In Table 3 a number of arguments and solutions are listed.

Table 3 Reactor design in relation to requirements

Requirement	Design
- small area/completely closed	- tall reactor
- no sulfide inhibition	- ideally mixing by air sparging
- high sludge age : nitrification : low surplus sludge : high efficiency	- growth in biofilms
- high treatment capacity	- high active biomass concentration through high surface area using small carriers

AIRLIFT-SUSPENSION REACTOR

A completely closed reactor with a small area means that a tall reactor is required. This is in line with the concepts of Deep shaft, Biohoch rector, Turmbiologie etc.
The treatment of NH_4^+ in the presence of sulfide poses a problem because sulfide inhibits the nitrification (Ref. 1).
This means that the microbial oxidation of sulfide should be promoted and that the influent waste water should preferably be ideally mixed. This can be achieved in a tall column by air-sparging. Hence, oxygenation with dissolved oxygen is excluded.
The realization of a high treatment efficiency, a low sludge production and nitrification all point to the need for a high sludge age. This can be most directly realized by growing the biomass as a biofilm on a surface in the reactor (Ref. 2).
A high treatment capacity can only be achieved with a high biomass concentration. This means that in the reactor a high surface area is needed.
This area can be provided in many ways, but a particularly efficient method seems to be to use small suspended (sand) particles upon which biofilm formation occurs (like in fluidized bed reactors, Ref. 4)
These design requirements can in principle be met in an airlift suspension reactor (Figure 1).

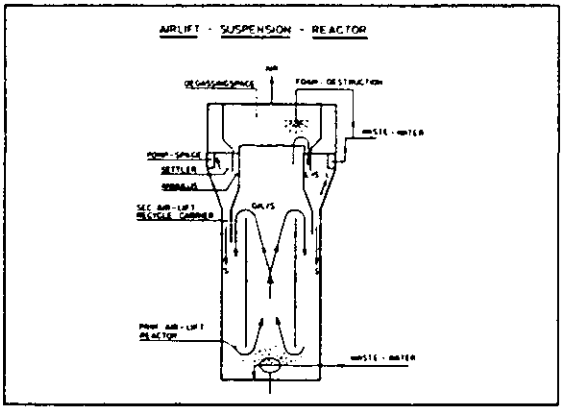

Fig. 1 Geometry of the air-lift suspension reactor

In this reactor the area for growth of biofilms is present as small suspended particles. The integrated reactor contains a lower reaction space and a separator on the top. The reaction space is in fact an internal airlift reactor which provides the O_2-transfer, the ideal mixing and which also keeps the biofilm particles in suspension. An additional advantage is that the shear force on the biofilms is not to high in such a kind of reactor.
In the separator, the 3-phase mixture of air/waste water/carrier material is first degassed in the annulus.
The downward flowing water and carrier mixture then flows into the settler space. Here the biofilm particles do settle, while the water flows upward into the headspace and subsequently leaves the reactor. The settled carriers finally have to return into the reactor; this is performed, not by pumping to avoid shear on the biofilms, but by the action of a secondary air lift.

In relation to the full scale application of this reactor concept the following three aspects will be dealt with
- choice of carrier-particle material and diameter
- comparison of pilot and full scale in relation to gas hold-up, liquid circulation and O_2-transfer and carrier losses
- comparison of waste water purification results of pilot and full scale

CHOICE OF CARRIER DIAMETER AND MATERIAL

The amount of biomass which can be accumulated in the reactor is related to the amount of carrier surface which is available and the biofilm thickness.

With respect to the biofilm thickness one has to consider the fact that in aerobic biofilms the O_2-penetration depth is seldom higher than 100 μm. Therefore, a biofilm thickness of around 100 μm guarantees that all biomass is aerobically active. Thicker biofilms contribute not to the aerobic conversion capacity.

The amount of carrier surface is related to the solids volume fraction in the reactor and the carrier diameter. With respect to the volume fraction of solids it is well known that too high values lead to a decrease in O_2-transfer. Furthermore, it will appear that the airlift circulation velocity drops to zero at too high values of solids. Therefore, a value of maximally 10% (v/v) of carrier material is assumed.

It is then possible to investigate the effect of carrier diameter on the maximal amount of biomass in a biofilm reactor using conventional liquid-solid fluidization theory (Ref. 8,9). The result is shown in Figure 2.

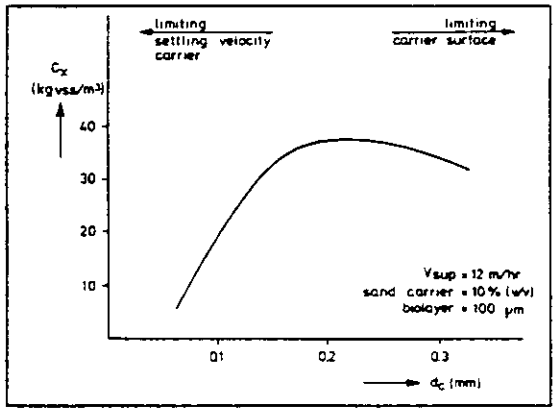

Figure 2 Effect of carrier diameter on biomass concentration in a fluidized bed reactor

It can be seen that, with respect to biomass concentration, there is an optimal carrier diameter around 0.2 mm if one uses sand-like materials (for economic reasons). A smaller diameter gives a lower settling velocity (and hence wash out) and a larger diameter gives less surface area (and hence less biomass). Both effects lead to sub-optimal biomass concentrations below 40 g/l. Although these calculations are based on liquid/solid fluidization theory it is assumed that they are relevant for the airlift reactor, because the reactor biomass content is controlled by the liquid solid settling tank on top of the reactor. Based on these calculations a particle diameter of about 0.2 mm and a volume fraction of about 10% was chosen.

The second question is the choice of the carrier material in relation to the biofilm formation properties. Considerations of cost and sufficient mechanical strength to endure the shear stress in the airlift reactor limits this choice to the natural, mineral, sand-like materials. An extensive investigation (Ref. 10) with respect to biofilm forming properties (in an airlift suspension reactor fed with a COD/NH_4^+/S^{2-} medium) revealed that 3 types of biofilm formation occur on different carriers (Table 4).

Class 1 carriers (lava, basalt, pumice) show extensive biofilm formation which is homogeneous distributed. Nitrification to NO_3^- occurs within 5-10 weeks. Class 2 carriers (quartz, sand) contain much less biomass, which is not homogeneous distributed over the carrier surface. Nitrification only reaches the NO_2^- stage. Class 3 shows very little biofilm formation (Ref. 10).
These results can be rationalized to some extent by the observation that biofilms always start in the surface cavities of the carriers and that class 1 carriers have very rough surfaces with many cavities, while class 2 and 3 carriers are much more smooth. Based on these results basalt of 0.2 mm diameter has been chosen.
Figure 3 shows a picture of all-around biofilms of about 150 μm thickness on lava carrier (0.2 mm)

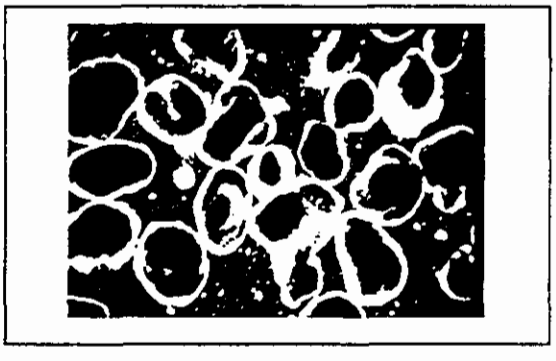

Figure 3 All-around aerobic biofilm on carrier

SCALE-UP OF TRANSPORT PHENOMENA

In an aerobic purification reactor the O_2-transfer is of major importance. Furthermore, for the 3-phase airlift suspension reactor a major requirement is to keep the carrier materials, which are present in a mass concentration of 100-250 g/l, in suspension.
Finally, the 3-phase separator should keep the carrier material inside the reactor; only a very limited washout-loss of carrier solids can be tolerated.
Therefore, these aspects were studied on a pilot reactor (H = 11m, D = 0.2m) and finally compared with a full scale reactor (H = 19m, D = 4.5m) as a function of superficial air-velocity.

Figure 4a shows the gas hold-up in waste water of the pilot airlift suspension reactor. It appears that the gas hold-up compares favourably with the regular bubble column (Ref. 11). Furthermore, it is found that the presence of carrier material (up to 250 g/l) has not a very dramatic impact on the gas hold-up.
Figure 4b shows the gas hold-up in canal water or waste water in the full scale reactor as a function of superficial gas velocity and carrier concentration.
Compared to the pilot reactor nearly the same results are obtained, although, now one observes a small systematic effect of increased carrier concentration. Also one observes that in waste water a higher hold-up is obtained than in canal water. Probably there is less bubble coalescence in waste water, which contains much more salts than canal water.

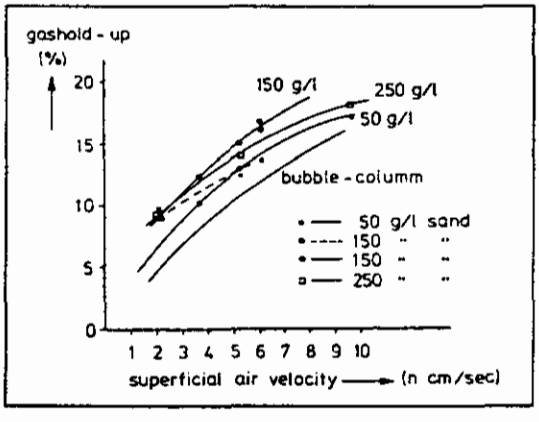

Figure 4a Gas hold-up in a pilot (H = 11m, D = 0.2 m) air lift suspension reactor in waste water

biofilm properties	quality class carrier good 1	moderate 2	bad 3
biofilm coverage: 1*)qualitative	homogeneous	not homogeneous	irregular
2*) quantitative (mg ODM/g carrier)	100-250	20-50	10
ammonium oxidizing activity g NH_4^+-N/g ODM day	0.6-0.2*	0.5	-
nitrite oxidizing activity g NO_2^--N/g ODM day	0.35	0	-
period required for 90% S^{2-} oxidation (weeks)	<1	<1	<1
period required for 90% NH_4^+ conversion (weeks)	2-4	6	-
period required for 90% NO_2^- conversion (weeks)	5-10	-	-
biofilm thickness (μm)	60	-	-

* during the start-up the ammonium oxidizing activity decreases

Table 4 Biofilm formation categories of different mineral carriers

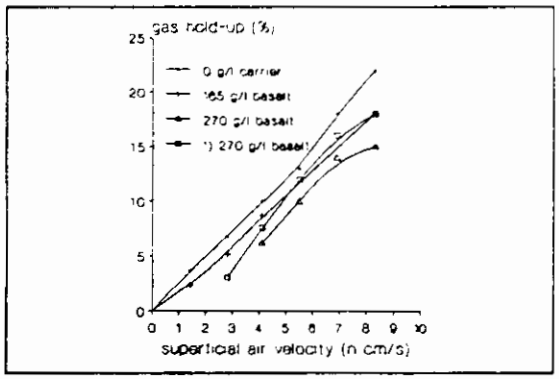

Figure 4b Gas hold-up in a full scale (H = 19 m, D = 4.5m) air lift suspension reactor with canal water.
*) experiment carried out with waste water

The airlift liquid circulation velocity is of major importance to keep the carrier material in suspension. The liquid velocity was measured by an Ultra Sonic measurement device. Figure 5a shows the results on the pilot reactor, where superficial air velocity and carrier concentration was varied. Three important results can be observed.

First it appears that already at low gas velocities the circulation velocity is higher than the single bubble rise velocity (25 cm/sec). This means that the gas most probably circulates into the downcomer. This is in accordance with the finding that the obtained gas hold-up is nearly the same as for a bubble column. Secondly the addition of carrier material leads to a decrease in circulation velocity. More specifically it is found that there exists a critical superficial gas velocity below which the circulation cannot be maintained. This velocity is, however, quite low (0.5 to 1.5 cm/sec). Finally, it should be mentioned that the obtained circulation velocity for the situation without carrier agrees well with the correlations for the liquid velocity in gas-liquid airlifts (Ref. 11), which predict a cube root influence of superficial air velocity and column height.

$V_{circ} = (g\ H\ V_{sup})^{1/3}$

Figure 5b shows the liquid circulation velocity for the full scale reactor. It appears that the observed effects of gas superficial velocity and carrier concentration are similar to the pilot scale. There are, however, two differences, which merit some attention. The liquid circulation velocities are higher on a full scale, in accordance with the mentioned cube root effect of reactor height. Furthermore, the carrier remains in suspension at full scale even at low gas velocity (< 1 cm/sec).

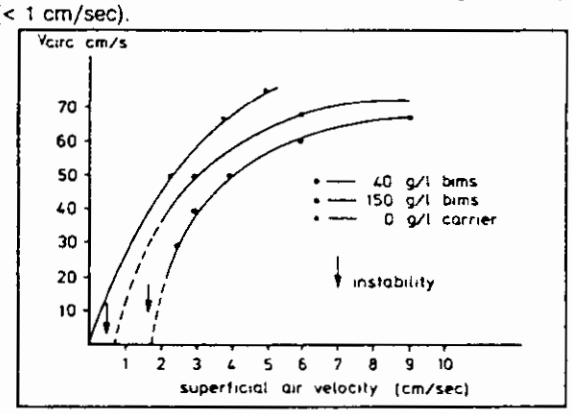

Figure 5a Liquid circulation velocity in a pilot airlift suspension reactor

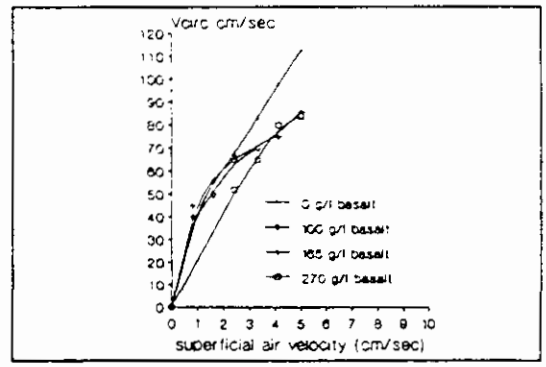

Figure 5b Liquid circulation velocity in a full scale airlift suspension reactor

From these results it appears that the full scale airlift suspension reactor can be considered ideally mixed for liquid and solid. Sampling of reactor contents at different heights has indeed shows that there is no gradient in solids content. For the full scale airlift, operating at 5 cm/sec air velocity with 270 g/l carrier one obtains a solids circulation of about 1.8 ton of carrier per second and a liquid circulation time of about 40 seconds.

The O_2-transfer has been measured (stationary method) on pilot scale. The found k_La-values are shown in Figure 6 for the pilot reactor containing 150 g/l carrier. The effect of superficial gas velocity on k_La compares well to the correlations found for bubble columns (Ref. 11). However, the absolute k_La-values in the airlift suspension reactor are about 20% less then in the bubble column. Probably this is due to the presence of carrier material. Furthermore, it appears that at a gas superficial velocity of 4 cm/sec the maximal O_2-transport rate (at dissolved O_2 is zero) is about 15 kg O_2/m^3 day. For the full scale plant the O_2-transfer data are not available yet. However, based on the gas hold-up data and the biological purification capacity (see below) there appears to be no major discrepancies from the pilot results.

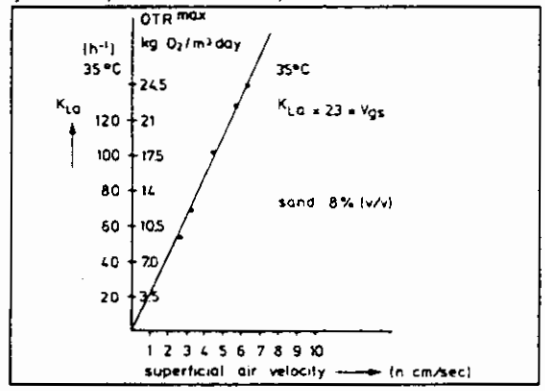

Figure 6 O_2-transfer in a pilot airlift suspension reactor

The performance of the 3-phase separator with respect to carrier wash-out has been quantified on the pilot reactor. Figure 7 shows the solid wash-out as a function of liquid superficial velocity in the settler and of the downward liquid velocity in the annulas. It is found that the wash-out of carriers starts above a liquid superficial

of 5 m/hr and that wash-out rises rapidly if the downward annular liquid velocity (Figure 1) exceeds 4 cm/sec. It has been observed that the small foam like air-bubbles become then entrained through the annulus into the settler, where the carrier settlement becomes disturbed. It should be noted that a solids loss of 100 g/m²hr (Figure 7) still means a half-time of carrier solids in the reactor of 3 years. Measurements of carrier loss on full scale (Figure 9) have shown that the full scale 3-phase separator performs satisfactorily.

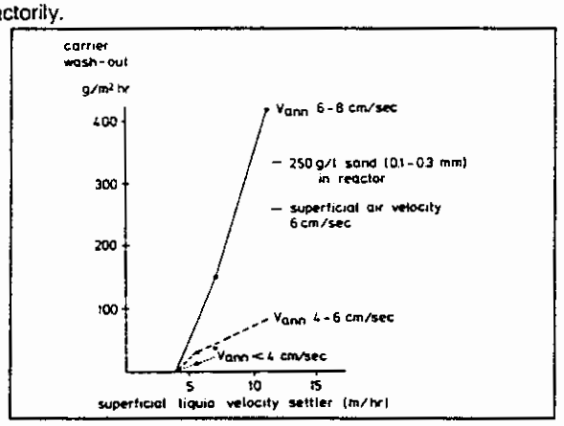

Figure 7 Carrier wash-out at the pilot airlift suspension reactor

In conclusion it appears that the technological scale-up of the airlift suspension reactor has been achieved successfully from the point of view of gas hold-up, liquid circulation, O_2-transfer and solids wash-out.

COMPARISON OF PURIFICATION RESULTS ON PILOT-SCALE AND FULL SCALE

On lab (2 and 2.5 ltr) and pilot scale (0.35m³) the aerobic purification of anaerobic effluent containing sulfide, residual COD and NH_3 was studied. Figure 8 shows a typical example (Ref. 12).
The experimental conditions and overall results are listed in Table 4. The reactor was inoculated with 1% carrier particles with biolayers.
COD and sulfide oxidation occurred within a few days, ammonia oxidation into NO_2^- took about 2 weeks and NO_2^--oxidation into NO_3^- needed about 7 weeks. The total oxidative purification capacity reached about 15 kg O_2/m^3 day. The observed microbial sequence is probably the result of S^{2-} inhibition on NH_4^+ oxidation and of NH_4^+ inhibition on NO_2^- oxidation. Furthermore, it appeared that, despite the relatively high shear rate, biolayer formation posed no problems and a high biomass concentration of 15-25 g/l was achieved. Typical biofilm thicknesses are 50-150 μm. The nitrification capacity of the biomass was 0.1-0.2 g N/gVSS day, which resulted in a high N-removal capacity of about 1.5-2 kg N/m³ day.

Based on the favourable lab and pilot results two full scale airlift suspension reactors were constructed in Delft, The Netherlands and were started-up medio 1987. Table 5 contains the reactor dimensions, the operating conditions and the design removal rates. The reactors are about 300 m³ volume each at a height of 19 m and a diameter of 4.5 m. The carrier material is basalt of 0.23 mm diameter at a concentration of 140 kg/m³.

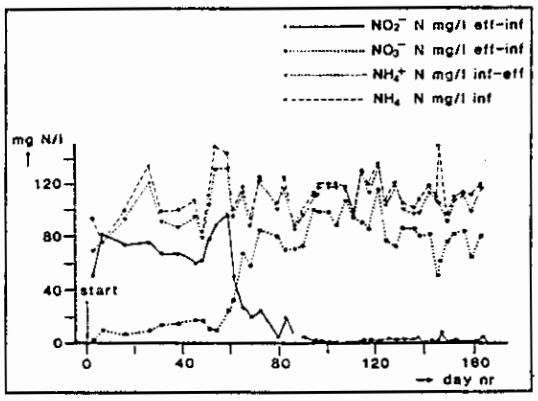

Figure 8 Aerobic purification of anaerobic sulfide and ammonia containing waste water in a pilot airlift suspension reactor (0.025m³)

Table 4 Aerobic purification of anaerobic sulfide and ammonia containing waste water; reactor conditions, purification results and time sequence

CONDITIONS		CARRIER	
reactor scale	25 l	concentr.	200 g/l
Temp./pH	30 C/7.1	density	1600 kg/m³
residence time	2-2.5 hr.	diameter	0.1-0.3 mm
air velocity	5.6 cm/sec		

RESULTS		Efficiency
Nitrification	1.8 kgN/m³day	96-98 %
Sulfide oxidation	2 kgS/m³day	100 %
Fatty acids etc.	1.5 kgCOD/m³day	100 %
Total oxidation	14 kgO_2/m³day	

Biomass	15-25 g/l
Biofilm	50-100 μm
Nitrific. activity	0.1-0.2 gN/gVSS day

TIME SEQUENCE	
Sulfide oxydation:	> 3 days
Ammonium oxydation:	> 2 weeks
Nitrite oxydation:	> 7 weeks

The carriers are kept uniformly in suspension at an air superficial velocity of about 4.2 cm/s. The waste water residence time is 2 hrs. The volumetric design removal rates are 3.8, 1.6 and 0.9 kg/m³ day for carbonaceous COD, nitrogen and sulfide, amounting to a total oxidation rate of 11 kg O_2/m^3 day.

Table 5 Full scale airlift suspension reactor: dimensions, operating conditions, design removal rates

REACTOR DIMENSIONS	
Volume	284 m³
Liquid height	19 m
Diameter	4.5 m

OPERATION	
Temperature	35 C
pH	7.5
Basalt carrier	140 kg/m³ (0.23mm)
Air	2400 Nm³/hr
Superficial air vel.	4.2 cm/sec
Air hold-up	8 % (v/v)
Waterflow	3500 m³/day
Residence time	2 hrs

DESIGN REMOVAL RATES

	VOLUMETRIC (kg/m^3day)	BIOMASS SPECIFIC (g/g day)
COD	3.8	0.085
N-Kj	1.6	0.040
Sulfide	0.9	0.022
Oxygen	11	0.28

The growth of biomass as biofilm on the carrier has been very impressive. Figure 9 shows that within 5 months the biomass concentration reached 40 gVSS/l. The carrier concentration remained nearly constant at a level of about 140 g/l, indicating that the 3-phase separator performed satisfactorily in the prevention of carrier wash-out. The volumetric hold-up of settled particles reached a value of 50%, which is very high. The biofilm formation on the carrier is extensive, leading to 200 mg of biomass per gram of carrier. Usually a biofilm thickness of about 200 μm was observed. However, large fluctuations in film thickness sometimes occur, with rapid recovery (day 110). The reasons for this are not known yet, but this shows the need for a more fundamental understanding of biofilm dynamics in this type of reactor.

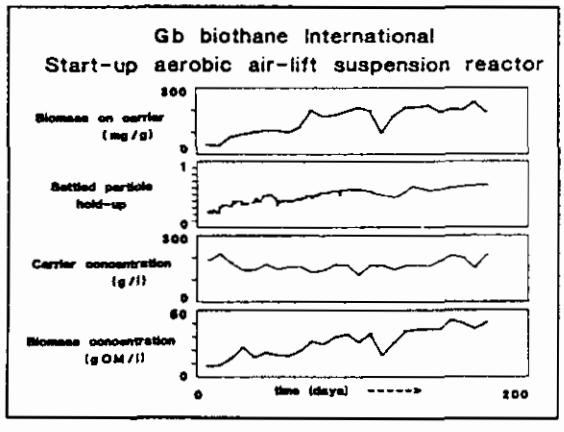

Figure 9 Biomass and biofilm development during start-up of the full scale airlift suspension reactor in Delft

The purification performance has been quite satisfying over the past several years. Typical results are shown in Fig. 10, which covers a 38 day period in 1989. The COD removal amounts to about 700 mg/l (where all fatty acids are oxidized), the ammonia removal is about 70 mg/l which is then only converted into NO_2^-, and the S^{2-}-removal is about 150 mg/l (where all S^{2-} is oxidized into SO_4^{2-}). This reactor performance leads to the following three remarks.

- Despite the plentiful presence of S^{2-} there are no odour problems due to the completely closed reactor concept and the complete S^{2-}-oxidation.
- There is apparently no surplus sludge production of organic solids as can be seen from the in- and out-flowing solids-COD of about 700 mg/l.
- Contrary to the pilot results only partial NH_4^+-oxidation is achieved.
- The biomass concentration on full scale (40 g/l) is much higher than on lab or pilot scale (20 g/l).

Table 6 Purification performance of full scale aerobic air-lift suspension reactor Delft

PARAMETER (mg/l)	ANAEROBIC EFFLUENT	AEROBIC EFFLUENT	EFFICIENCY (%)
COD-total	1960	1266	35
COD-soluble	1173	522	53
COD-solids	787	714	-
N-Kj.-total	246	167	33
NH_4^+-N-soluble	171	98	43
NO_2^--N 0	74		
SO_4^{2-}-S 9	153		

A possible explanation of the last three points follow from a comparison (Table 7) of design (Table 5) and actual removal rates.

Table 7 Comparison of actual and design removal rates for the full scale airlift suspension reactor

VOLUMETRIC REMOVAL RATE (kg/m^3 reactor day)

	ACTUAL	DESIGN
COD	8.5	3.8
N-Kj (to nitrite)	0.9	1.6
S_{2-} (to sulphate)	1.8	0.9
Oxygen consumption	12	11

BIOMASS SPECIFIC REMOVAL RATE (g/g day)

	ACTUAL	DESIGN
COD	0.210	0.085
N-Kj	0.022	0.040
S_{2-}	0.045	0.022
Oxygen consumption	0.300	0.280

It appears that the actual organic COD-load and the S^{2-}-load on the system are about double the design values. This is mainly due to a changed waste water composition. Not surprisingly this leads to a decreased N-removal capacity. Of particular interest is that the actual aerobic purification capacity of 12 kg O_2/m^3 day indeed meets the design value.

The absence of surplus sludge production is probably due to the low sludge load of about 0.2 g/g day in combination with the high temperature of 35°C. It has been shown before (Ref. 2) that such low sludge loads lead to negligible sludge production As such this property of the airlift suspension reactor is very attractive due to the ever increasing problems of sludge disposal. Therefore, the aspect of biomass yield in biofilm systems merits more fundamental understanding.

The higher biomass concentration on full scale compared to the pilot scale probably is due to the much higher organic load on full scale. This promotes a higher biomass accumulation. On the other hand, the possible aspect of less shear on full scale, cannot be excluded.
In conclusion it appears that the biological purification performance on full scale is satisfying. The differences to the pilot scale are probably due to a changed waste water characteristic. Most notably is however, the result of no surplus sludge production.

CONCLUSION

The airlift suspension reactor using biofilms on small suspended particles has been successfully scaled-up from lab to full scale using a difficult waste water. This reactor concept is promising due to its high purification capacity for COD, sulfide, ammonia, small dimensions, and absence of surplus sludge production. A wider application of this process seems possible, especially for difficult waste water. A better understanding of biofilm dynamics and biomass production on small suspended particles is however, desirable.

At this moment the reactor has already been tested on lab scale (0.025 m^3) for the purification of municipal waste water (Ref. 13) and its application to industrial effluents is under way. Especially the results for sewage are promising with maximal nitrification and COD-removal at 1-2 hrs waste water residence time.

References

1. I. Pascik, T. Mann
   The two-step nitrification of ammonia rich waste water
   Wat. Sci. Tech. 16, 215-223 (1984)
2. J.J. Heijnen
   Biological industrial waste water treatment minimizing biomass production and maximizing biomass concentration
   Ph.D. Thesis, Delft University of Technology (1984)
3. G. Lettinga, A.F.M. van Velsen, S.W. Hobma, W. de Zeeuw, A. Klapwijk
   Use of the upflow sludge blanket reactor concept for biological waste water treatment, especially for anaerobic treatment
   Biotech. and Bioeng. 22, 699-734 (1980)
4. J.J. Heijnen, A. Mulder, W.A. Enger, F. Hoeks
   Review on the application of anerobic fluidized bed reactors in waste water treatment
   The Chemical Engineering Journal 41, B37-B50 (1989)
5. J.J. Heijnen, A. Mulder, R. Weltevrede, J. Hols, H.L.J.M. van Leeuwen
   Large-scale anaerobic-aerobic treatment of complex industrial waste water using biofilm reactors
   Wat. Sci. Tech. 23, 1427-36 (1991)
6. D. de Beer
   Micro electrode studies in biofilms and sediments
   Ph.D. Thesis, University of Amsterdam (1990)
7. C.M. Hooijmans
   Diffusion couples with bioconversion in immobilized systems
   Ph.D. Thesis, Delft University of Technologie (1990)
8. D. Kunii, O. Levenspiel
   Fluidization Engineering (1977)
   R.E. Krieger, Publishing Co., New York
9. L.T. Mulcahy, W.K. Shieh
   Fluidization and reactor biomass characteristics of the denitrification fluidized bed biofilm reactor
   Wat. Res. 21, 451-458 (1987)
10. A. Mulder, J.J. Heijnen
    The effect of carrier characteristics on the biofilm development in airlift suspension reactors
    In: Proceedings 2nd Netherlands Biotechnology Congress 1988, ed. Netherlands Biotechnological Society c/o TNO, P.O. Box 108, 3700 AC Zeist, The Netherlands
11. J.J. Heijnen, K. van 't Riet
    Mass transfer, mixing and heat transfer phenomena in low viscous bubble column reactors
    The Chemical Engineering Journal 28, 21-42 (1984)
12. A. Mulder, J.J. Heijnen, J. Hols
    Post treatment of anaerobic sulfide and ammonia containing effluents from methane fermentations in high rate fluidized bed reactors on laboratory and pilot scale
    NVA/EWPCA Water Treatment Conf. "Anaerobic Treatment; a grown up technology", Amsterdam (1986), 413-421
13. D. Eikelboom, R. Kampf, F. van Koorneburg
    Biofilm formation in an airlift reactor for the sewage purification (in Dutch)
    H_2O 20, 388-392 (1987)

POSSIBLE ROLE OF ANAEROBIC DIGESTION IN SEWAGE TREATMENT

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INTRODUCTION.

The application of anaerobic digestion for wastewater treatment is of rather recent date, although there exist already considerable experience with the method in the Netherlands for more than 15 years. As far as the interest in the research and in the practical application of anaerobic treatment concerned the Netherlands can be considered as a pioneer. Research started in the Netherlands in 1970 at the Agricultural University, since 1972 in close cooperation with polluting industries, i.e. the sugar beet industry and potato starch industry, and later on various others and other research institutes and universities as well. Polluting industries in the eraly 70-ties became highly interested in the anaerobic wastewater treatment option, in view of the expectation that this method would reduce the wastewater treatment costs substantially, but also because of other important principle benefits of the anaerobic treatment method over the conventional aerobic treatment systems as well.
The anaerobic digestion technology is in fact mainly effective in removing organic pollutants from wastewaters, slurries and/or solid organic wastes. As a matter of fact it is mineralization process, converting biodegradable organic compounds in methane and carbon dioxide, and leaving in the aqueous solution compounds like ammonia, sulphide, phosphate. Contrary to conventional low loaded aerobic treatment systems anaerobic treatment therefore principly comprises a pretreatment method. However, despite that anaerobic treatment nevertheless offers big potentials. This applies both for treatment of industrial wastewaters and sewage, although unfortunately the potentials of the method for treating sewage and low strength industrial wastewaters in many countries still is hardly recognized and/or accepted. The sometimes obstinate reluctant attitude of many wastewater treatment authorities, including professors and engineers from consultancies and pollution control industries, frequently can be attributed to a basic lack in insight in the principles of the anaerobic digestion process, frequently to a certain prejuidice, but presumably sometimes also to the fact that anaerobic treatment in terms of technology is not really a 'high technology', requiring a lot of advanced expensive instrumentation. Moreover, as far as highly industrialized countries concerned, there sofar exists little need for alternative methods, because well performing conventialaerobic systems have been implemented and applied here for many years. These systems, or modern more sophisticated versions of these systems, frequently can meet the high standards set for effluent discharge. This certainly is impossible when applying solely anaerobic treatment, particularly for moderate climatic regions, which conditions apply for major part of wealthy world.
Despite these problems with respect to the implementation of anaerobic sewage treatment, the method offers big prospects. First of all

this applies for the tropical regions and very likely also for subtropical climates. The following reasons exist herefore:

1. An effective environmental protection can be accomplished at very low costs, i.e.
   - the method can be applied in technically simple and inexpensive reactors, and for its operation it generally doesn't need high grade energy, like is the case for most of the conventional aerobic systems. Its application therefore does not depend on the supply of electricity or any other high grade energy source. Instead it produces an useful energy carrier in the form of biogas from the biodegradable organic pollutants.
   - the method is applicable to a large variety of wastewaters.
   - the method can be applied at practically any place and at any scale, which will lead to very significant savings in the construction costs, the use and the maintenance of expensive sewer systems.
   - the production of excess sludge (generally well stabilized) is low.
   - anaerobic sludge can be preserved unfed for long periods of time without any serious detoriation in quality. This makes anaerobic treatment quite attractive for application in campaign industries.
2. The method may result in the development and the application of integrated environmental protection systems, e.g. the application of fish ponds for post treatment (i.e. combined treatment and fish cultivation), reuse of effluents for irrigation and fertilization, reuse of sludge for soil conditioning, recovery of bulk products like ammonia, sulfur. Natural resources therefore can be recovered and/or preserved.

At the present stage of knowledge and technological development little of the presumed previous drawbaks remain, such as the relatively low rate of first start-up, and high susceptibility. Although the methanogens are quite suscebtible for a large number of xenobiotics, the situation is significantly less serious than thought previously.

II. ANAEROBIC WASTEWATER TREATMENT.

Anaerobic wastewater treatment systems.
Unlike aerobic treatment systems, the loading rates of anaerobic reactors are not limited by the supply of any reagent, e.g. like oxygen in aerobic systems. The more sludge retained within the reactor system under operational conditions, the higher are the potential loading rates of the system, provided a sufficient contact between sludge and waste water can be achieved and maintained. These two conditions have to be met. In addition there should not exist any serious transport limitations of the substrate and endproducts in the bacterial biomass.

The required high sludge retention of the modern high rate anaerobic reactors is based on:

1. Bacterial sludge entrapment, in the interstices between support material present in the reactor and bacterial attach-

ment on the external surfaces of the packing material. The wellknown upflow Anaerobic Filter is based on these ideas.

2. Bacterial immobilization by an attachment mechanism to fixed support material, i.e. the well known down flow stationary fixed film system (AFF) as developed by van der Berg and co-workers or to mobile particulate surfaces, such as the Anaerobic Attached Film Expanded Bed (AFFEB) process and fluidized bed systems (FB).

3. Sludge bed (blanket) reactors, such as the Upflow Anaerobic Sludge Bed (Blanket) process

All the modern concepts can be designated as high rate systems, although there exist considerable differences in the maximum achievable (organic and hydraulic) loading potentials of the various systems, as well as toward their applicability for treating partial soluble waste waters. The reasons for the different loading potentials have to be found in differences in the maximal sludge hold-up and/or the amount of contact that can be achieved between sludge and incoming waste water.

The research in the Netherlands started in 1970 using upflow anaerobic filter systems. Although rather satisfactory results were obtained at laboratory scale in treating potato starch wastewater, the further development of AF system was abandonned for the following reasons:

- maximum achievable space loading rates exceeding 15 kg/m^3 .day $^{-1}$ were considered impossible, unlesss a high treatment efficiency is not the main objective.
- severe clogging problems were expected upon scaling up the system.
- distinct indications were obtained, that a more proper system could be developed.

By far the most research in the Netherlands has been carried out since 1971 on the Upflow Anaerobic Sludge Bed (UASB) reactor concept) and - since more recently - also on Fluidized Bed systems (Heynen) and Expanded Granular Sludge Bed (EGSB) reactors (Lettinga et al.). The UASB- (and EGSB-) concept is based on the following ideas:

1. Anaerobic sludge inherently has good settling properties, provided the sludge is not exposed to heavy mechanical agitation. For this reason mechanical mixing generally is omitted in UASB-reactors, and if not, only intermittent and/or gentle mechanical mixing is employed.
2. For achieving the required sufficient contact between sludge and waste water, the system relies on
   - the agitation brought forth by the natural gas production.
   - an even feed inlet distribution over the bottom of the reactor.
3. Well settling sludge aggregates dispersed under the influence of the biogas production (which is particularly quite heavy at higher organic space loads and in tall reactors) are effectively retained by separating (collecting) the biogas in a gas collector system placed in the upper part of the reactor. The biogas is released from the reactor via this device. By separating the biogas in this way, a settler is created in

the upper most part of the reactor. Sludge particles can coalesce and settle out here.

4. Sludge aggregates settled out in the settler compartment are enabled to slide back into the digester compartment, situated beneath the Gas-Solid-Separator (GSS-) device.
5. The wash-out of a scum layer at the liquid interface in the settler compartment, e.g. consisting of floating active anaerobic sludge, can be substantially reduced, by installing a baffle in front of the effluent weir.

The GSS-device represents an essential accessory of a UASB-reactor and in specific modifications of this system as well. Although some researchers suggest to replace the GSS-device by a packed bed in the upper part of the reactor, this idea to our opinion doesn't represent a proper alternative for a GSS-device, because it is much less effective in retaining viable - less well settling - sludge aggregates. Moreover, neither there exist economical nor technical reasons to look for alternatives. Very effective designs for the GSS-device are available, and - after all - these devices doen't increase the investment costs substantially.

The UASB-concept is by far the most widely applied high rate system. Although originally developed for mainly soluble and medium strength types of wastewaters its application to more complex (partially soluble), to cold (i.e. sub-mesophilic conditions) and to very low strength waste waters, such as sewage, certainly is quite well possible.

The experience obtained sofar with the full scale application of the UASB-system with a large variety of different types of wastewaters, including also domestic sewage, is quite satisfactory.

EGSB-systems use granular sludge and operated at high superficial liquid velocities (> 6 m/hr) in order to improve the contact between sludge and wastewater by expanding (at least partially) the sludge bed. These systems look quite attractive for various types of wastewaters, but sofar a 'true' EGSB-reactor (i.e. in which granular seed sludge is used) has not been implemented at full scale. However a specific kind of expanded granular sludge bed reactor, the Internal Circulation-UASB system, has recently been installed for treating brewery wastewater. This IC-UASB in fact is an EGSB reactor in which an internal sludge and liquid recirculation is accomplished by collecting the biogas halfway the reactor height using an in-built gascollector. (Vellinga, 1986: Voorter, 1989). In fact the lifting forces of the separated biogas are employed in this system to recirculate granular sludge via the lower part of the reactor.

A presumed interesting development in the anaerobic reactortechnology concerns the socalled **attached-film Fluidized Bed concept.** As far as reactor-system concerned it resembles closely the UASB-concept, except that relatively very tall reactors are employed, e.g. as proposed for the EGSB-system. In this FB-concept one completely relies on the existence of consistent and more or less uniform attached films. An complete and uniform fluidization otherwise would be impossible. It is obvious that the control of the very complex process of film build-up (and de-attachment or detoriation) represents the big practical problem. In the case **complete fluidization** of the bed would be pursued, which in fact was considered to be the prerequisite for **anaerobic FB-systems**, the lighter (more flocculent) aggregates will continuously rinse out from the system.

In the Netherlands considerable emphasis has been put on the development of anaerobic, but also of acidogenic, anoxic and aerobic FB-systems (Heynen). Although results achieved at pilot plant scale

looked promising, the performance of full scale instalations in fact didn't meet completely the high expectations. Presently the installed reactors are no longer operated in a complete fluididized bed mode, but instead in an expanded bed mode. As a matter of fact the FB-concept has been converted in an EGSB-reactor. This mode of operation provides in an excellent performance, e.g. for treating yeast wastewater.

III.THE APPLICATION OF ANAEROBIC TREATMENT TO SEWAGE.

Based on the laboratory and pilot plant experiences with industrial wastewaters, cmprehensive investigations on the anaerobic treatment of raw sewage at the Agricultural University commenced in 1976. The first main objectives of these investigations were to assess the feasibility of flocculent sludge UASB-systems at temperatures exceeding 20 ^{o}C, under tropical conditions, viz.:

- for removing biodegradable soluble COD,
- for removing and stabilizing dispersed solids present in the sewage.

After the promising results obtained under the higher ambient temperature conditions (viz. approximately 20 ^{o}C), since 1978 experiments were started at lower temperatures, viz. ranging from 8 to 20 ^{o}C, characteristic for Dutch conditions.
The reasons for starting these investigations are to be found in the principle advantages of the anaerobic sewage treatment method over conventional methods. Table 1 lists these potential advantages, while Table 2 summarizes the drawbacks of this approach.

TABLE 2. Benefits of anaerobic (pre)treatment of domestic sewage over conventional aerobic methods.

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1. The method is simple in construction and operation, consequently inexpensive.

2. The method generally doesn't require the supply of electricity.

3. The method can be applied at very small and at very big scale, enabling a decentralized application.

4. Application of anaerobic wastewater treatment, when applied in a decentralized mode, will lead to very substantial savings in the investment costs of sewerage systems.

5 The excess sludge production is low, particularly when expressed in volume, because the thickening characteristics of the sludge are very good.

6 The sludge is well stabilized.

TABLE 3. Drawbacks of anaerobic treatment of domestic sewage

1. The method can not accomplish a complete treatment, because in fact merely organic compounds and TSS will be removed.

2. The method is fairly susceptible to the presence of large number of chemical compounds. However generally toxicity problems will not occur for domestic sewage.

3. The application of the method is quite sensitive for lower ambient temperatures.

III.1. Applicability of anaerobic treatment under tropical conditions.

Although for the still very few insiders there don't exist any serious doubts about the big potentials of anaerobic sewage **pretreatment** under tropical conditions, unfortunately the big majority of wastewater engineers very likely has a very different opinion, mainly due to a lack of reliable information. Until sofar only very few people have been active in the research in this field, while the number of people involved with the implementation presumably is even smaller. Fortunately the situation is slowly improving, gradually more engineers, university professors, pollution control authorities are becoming informed about and interested in the method.
The research in the Netherlands on anaerobic treatment started with experiments carried out at the Agricultural university at 20 ^{o}C and conducted in 0.03, 012 and 6 m^3 pilot plant scale.
Based on the promising results obtained, a proto-type UASB-reactor was designed for domestic sewage treatment. A schematic diagram is shown in Fig. 1.

A pilot plant of 64 m^3 volume (dimensions 4 x 4 x 4 m) was built in Cali (Colombia) according to this design. The investigations with this plant were started in the beginning of 1983. The experiments were carried out by the Uiversidad del Valle, the Agricultural University and HASKONING consultancies, Empresas Municipales de Cali. One of the major reasons to start the research in Cali, was the presumed urgent need for inexpensive waste water treatment systems in many developing countries. The execution of this project has been made possible by grants of the Dutch government. The first phase of these fairly extensive investigations were concluded in 1986 and the second phase early 1990.
The results of these investigations are summarized in Table 4.

The UASB-system for sewage treatment can be employed at very small scale (house on-site or community on-site), but also at very big scale. Moreover the process can be applied for unsettled and for settled wastewater. Most experience sofar exists for raw sewage and raw domestic wastewater. In latter case we in fact are dealing with combined primary and secondary treatment.

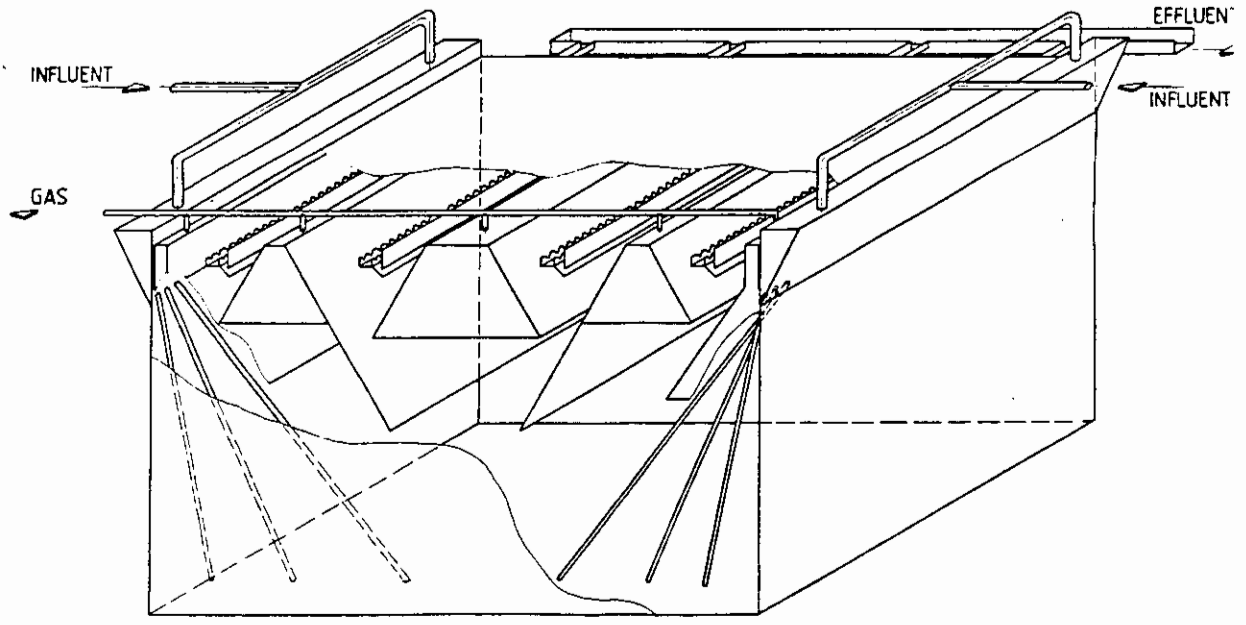

Figure 1. Schematic diagram of the 64 m^3 pilot plant investigated in Cali, Colombia. Basically the same appraoch was choosen for various full scale installations installed in Colomba, Brazil and India.

In the meantime results very similar to those in the Cali-plant have been obtained at full scale, viz. a 1200 m^3 reactor in Kanpur, India, and various installations in Colombia and in Brazil (Gomez, 1985.

Evidence has been obtained that in UASB-type reactors at temperatures in the range 20 - 24 ^oC very similar results can be achieved compared to those in Cali at temperatures exceeding 24 ^oC at the same liquid detention times.

Table 4. Summary of the results obtained in the 64 m^3 pilot plant in Cali treating domestic raw sewage sewage.

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1. A UASB-plant treating raw domestic sewage can be started up at a HRT of appr. 6 hrs within a period of 6 - 12 weeks without supplying seed sludge. Immediately after starting feeding of the plant at least 50 % COD-removal efficiency will be obtained with types of sewages similar to those of Cali.

2. Treatment efficiences (at HRT 4 - 6 hrs):

COD (total/total)	:	50 - 75 % (65%)
COD (total/ filtered)	:	70 - 90 %(80%)
BOD (total/total)	:	70 - 90 %(80%)
COD (filt/filt)	:	up to 60 %
TSS	:	60 - 85 %(70%)

   Treatment efficiency can be improved by simple post-treatment facilities.

   Under conditions of low night-time and high day-time flow even higher treatment efficiencies will be obtained.

3. Gas production and conversion of COD in methane:
   methane-gasproduction:
   0.19 Nm^3/kg $COD_{removed}$
   0.33 CH_4-COD/kg $COD_{removed}$
   56 - 63% of the methane leaves the reactor with the effluent solution.

4. Sludge production:
   generally : 0.1 kg COD/kg COD_{in}
   max. value found: 0.25 kg COD/kg COD_{in}
   0.4 - 0.6 kg TSS/kg TSS_{in} (= 0.06 - 0.1 kg TSS/m^3)

5. Sludge retention of the reactor and sludge age.
   sludge retention:
   31 - 37.5 kg TSS/m^3
   9.4 - 12.5 kg VSS/m^3
   sludge age:
   35 - 100 days (when reactor is full)

6. Sludge characteristics:
   ash content : 55 - 65 %
   spec. meth. act.: > 0.1 kg COD/kg VSS.day
   stability : 20 - 50 l CH_4/ kg sludge
   drying characteristics: 20 kg/m^2 in 7 days results up to 40 % DS.

III.2. Applicability of anaerobic treatment of sewage in moderate climates.

III.2.1. Introduction.

The big challenge obviously is to demonstrate the feasibility of the anaerobic treatment of sewage in moderate climates.
Since many years we conducted investigations in this field at the Agricultural University, initially on the basis of own funds, later increasingly on the basis of grants provided by the Dutch government.
The experiments were started in 1978 using 0.06 -to 0.24 m^3 UASB-reactors, since 1979 we with a 6 m^3 and 3 m tall pilot plant and since 1985 with a 20 m^3 and 6 m tall UASB-reactor. The initial experiments were conducted with raw sewage and with flocculent sludge, from 1979 onwards also granular seed sludges, i.e. cultivated on industrial wastewaters, were used.
In 1986 a research project was started dealing with EGSB- and FB-systems and settled sewage.
All pilot plant experiments were complemented with a variety of bench scale experiments.

III.2.2. Assessment studies of potentials of anaerobic treatment.

A large number of laboratory treatability assessment studies have been made using conventional batch digestion experiments and UASB- and/or EGSB- recirculation experiments using granular sludge columns and a batch of wastewater. Particularly the latter type of experiments provide reliable information about the maximum achievable treatment efficiency at different ambient temperatures, types of sludge used, hydraulic retention times etc., towards various distinguished pollutants present in the wastewater.

Batch recirculation trials under UASB-conditions (superficial velocity v_f = 1 m/hr) conducted with **raw sewage** showed that an almost complete removal of SS_{coarse}-COD can be accomplished, whil 50 - 78 % $SS_{colloidal}$ and 73- 85 % $COD_{soluble}$ can be removed in the temperature range 10 - 20 ^oC.

Conventional batch experiments conducted with **settled sewage** and with sewage adapted granular anaerobic sludge revealed that at 20 and 30 ^oC up to 53 % of the pollutants present in settled sewage can be converted in either methane or sulfide (depending on the sulfate content of the wastewater). At 8 ^oC this is only appr. 20 %. With a sugar beet wastewater cultivated sludge the conversion in methane is 5 % higher, which at the same % of sulfate reduction would result in appr. 58 % efficiency.

The results of the batch recirculation experiments conducted under EGSB-conditions gave maximum removal efficiencies for settled sewage (with verage fractional composition: 41 % soluble, 28 % Collidal, 31 % coarse SS) as summarized in Table 5.

When operating the column under UASB-conditions, viz. at a superficial velocity v_f= 1 m/hr (instead of at 6 m/hr), the coarse SS-COD is removed for >84 %, the removal of soluble COD drops to 44 % and the removal of colloidal matter remains practically unchanged, resulting in an overall removal efficiency of 65 %.

Table 5. Results of batch recirculation experiments conducted under EGSB-conditions.

Temperature range	12- 20 °C	8 °C
coarse SS	0 %	0 %
colloidal SS	60-70 %	60 %
soluble	53-56 %	33 %
overall	40 %	30 %

Clear evidence was obtained that it is also possible to remove almost completely the colloidal fraction by modifying the design of an EGSB-system in such a way that in the upper part of the reactor a layer of flocculent sludge is maintained. As it is relatively simple to eliminate well settleable coarse suspended matter from the effluent, the maximum achievable treatment efficiency in a well designed and properly operated combined EGSB-clarifier system would approach approximately 82.5 % (when 58 % of the soluble COD is removed). Assuming that in the primary treatment step 33 % of the COD of the raw sewage will be removed, the **absolute maximal overall COD-treatment efficiency** of total system would amount to appr. 88%.

III. 2.3. UASB-systems.

One-step flocculent sludge bed reactors.
The experiments were conducted with raw sewage using reactors varying in size from 0.03 to 6.0 m^3 (in height from 1 m to 3 m). In the smaller reactors 1 static feed inlet point was used, while in the 6 m^3 reactor it was possible to introduce the feed via a rotating manifold.
The results obtained at 18-20 °C, at HRT= 8 hrs and higher, both at 0.12 and 6.0 m^3 scale were fairly promising in terms of treatment efficiency (E), applicable space load (Q) and sludge stabilization. On the basis of unfiltered effluent and influent samples, a COD-treatment efficiency ($E_{t/t}$) ^{u}p to 65 % $_{c}$an be achieved under dry wheather conditions (dwc), i.e. at influent COD-values exceeding 300 mg/l. However $E_{t/t}$ drops to 20 - 40 % when influentCOD-values become significantly lower than 300 mg/l. Based on paper filtered effluent samples, the treatment efficiency ($E_{f/t}$) amount to 70-80 % for dwc-conditions and to appr. 45- 65 % for rwc-conditions. The investigated HRT ranged from 8 - 12 hrs. The conversion of $COD_{supplied}$ into CH_4-COD amounted to 50 - 70 %.
At temperatures ranging from 18 - 20 °C, the removal of SS represents the main limiting factor with respect to the performance of the system. Under these temperature and losding conditions a fairly satisfactory sludge stabilization can be achieved.
At lower temperatures the removal of SS still is the main bottleneck, but then also the removal of soluble COD becomes poorer. Clear evidence has obtained that for improving the removal of soluble compounds, the sludge water contact in the system needs to be improved in some way or another. However whatever method will be choosen for that, it should be accomplished in a mode that the removal of SS (or washout of sludge) is not detrimentally affected.

As far as the as the use of flocculent sludge concerned, it can be concluded that certainly is a proper material.

One-step granular sludge UASB-reactors.
The use of granular seed sludge for treating sewage was investigated in UASB-reactors ranging in size from 0.030 to 20 m^3, in height from 1 m to 6 m, and in diameter from 0.18 to 2.1 m. The bigger reactors were equiped with a rotating feed inlet system. The investigations in the 20 m^3 reactor was partially also granted by Paques B.V.
The 0.12 m^3 granular sludge experiments with Bennekom sewage gave $E_{t/t}$ values under dwc-conditions varying from 50 -60% and $E_{f/t}$-values ranging from 75 - 85 % at HRT= 7hrs and temperatures exceeding 12 ^oC. At temperatures below 12 ^oC the HRT has to be increased to 9 -14 hrs for achieving these values for $E_{t/t}$. At loading rates below 1.5 kg COD/m^3.day 40 - 80 % of the $COD_{removed}$ is converted in CH_4.
The average excess sludge production over the year amounts to 10 kg DS/p.e.year. Under rwc-conditions $E_{t/t}$ drops to 30 %, but the $E_{f/t}$ remains in the range 50 - 70 %.
Parallel experiments conducted in reactors of different heights, but further under the same conditions in terms of temperature, HRT and wastewater compositions, clearly demonstrated that the treatment efficiency drops down at increasing reactor height. When applying superficial velocities exceeding 0.5 m/hr the sludge retention of one step granular sludge UASB-systems becomes distinctly poorer.
On the other hand experiments conducted with EGSB-systems revealed a better soluble COD-reduction compared to conventional UASB-systems, which mainly can be attributed to the significantly better contact between sludge and wastewater in EGSB-systems.

The investigations in the 20 m^3 reactor were made at three locations, viz. successively at the pilot plant site of the Agricultural University treating domestic wastewater of the village Bennekom, the municipal sewage treatment sites of Bergambacht and of Lelystad. In latter case the experiments were carried out with sewage of a separated sewer system.
As far as sewage from combined sewers concerned the results show that 60 % COD-reduction ($E_{t/t}$) only can be achieved for Bennekom sewage at temperatures below 13 ^oC in an one step granular sludge UASB-system under dwa-conditions (COD_i > 300 mg/l, provided the influent is distributed as evenly as possible over the bottom part of the reactor.
For the Bergambacht sewage (BOD/COD= 3.5), which is more septic compared to the Bennekom sewage, $E_{t/t}$-values varied from 44 - 54 % at dwa-conditions for temperatures in the range 13 - 17 ^oC and HRT = 8.7 - 15 hrs. When operated at high hydraulic loads under rwa-conditions (HRT =2.8), the system suffered from a considerable wash-out of accumulated flocculent sludge present in the reactor (i.e. entrapped during the period of lower hydraulic loading rates).
The experiments conducted with sewage from the separated sewer in Lelystad revealed a dramatic decline in the specific activity of the granular seed sludge EB (cultivated on paper wastewater at the Eerbeek industrial site, EB) during the winter season, viz. from app. 0.33 to less than 0.02 kg COD/kg .day at 30 ^oC. Such a decline didn't occur with the sewage from a combined sewer. Moreover any recovery of the methanogenic activity was not observed during the summer period.
The reactor in fact mainly acted as an acidifying system, i.e. VFA-concentrations up to 200 mg COD/l were found in the effluent. Conse-

quently the system can better be used under these conditions as VFA-generating system, e.g. for promoting the biological phosphate removal in an aerobic treatment system. On the other hand by placing a second granular sludge UASB-reactor in series with acidifying reactor, the VFA-COD can be removed satisfactorily, whereas a serious drop in specific methanogenic activity of the granular sludge doesn't occur in that case. The main reason for the deterioration in the methanogenic activity of the sludge presumably has to be found in the sorption of colloidal solids at the surface of the granules. As a result, the active organismens will become entrapped in a layer of solid substrate ingredients through which the supply of substrate to the bacteria from the bulk of the solution is seriously hampered. This particularly is the case at lower temperatures, because the rate of degradation (hydrolysis) of these ingredients - at least as far as biodegradable - will drop to very low values. This phenomenon has thoroughfully been investigated in experiments with slaughterhouse wastewater (Sayed,Thesis, Agricultural University, 1988). On the other hand in parallel experiments conducted in the laboratory it was shown that the granular sludge used in the 20 m^3 was of poor quality. Significantly better results were obtained with granular sludges cultivated on other wastewaters (investigatioins conducted by G.Rijs, 1989).

Like was found earlier with sewage from a combined sewer, the 20 m^3 reactor suffered from a poor water sludge contact. From this, but also from other results obtained with one step granular sludge UASB-systems it is clear that the design of the concept needs distinct improvements in order to achieve a satisfactory COD-reduction for raw sewage. S it was found that:

- the height should be below 3 - 4 m,
- the feed inlet distribution should be as even as possible; at lower temperatures (< 12 ^oC) the number of feed inlet points should be at least 1 per m^2.
- it looks advantageous to subdivide the digester compartment (see Figure 2),
- presumably the GSS-device can be improved.

Although some of these points already were known at the time the 20 m^3 pilot plant experiments were programmed for treating the sewage from the separate system in Lelystad, it was decided to continue the experiments with the original - consequently in fact inadequate - reactor system.

Two and three step reactor configurations.

On theoretical grounds it can be shown that an one-step reactor configuration in terms of performance is less effective than a two or more step configuration, operated at the same HRT. In latter case plug flow conditions, which are beneficial for achieving a higher treatment efficiency, will be approached somewhat more closely. This is true for treating medium strength soluble wastewaters under higher loading conditions (in which case the separate reactor modules behave more closely as completely mixed systems), but also for treating low strength, partially soluble wastewaters like raw sewage, although there then exist some additional reasons for this.

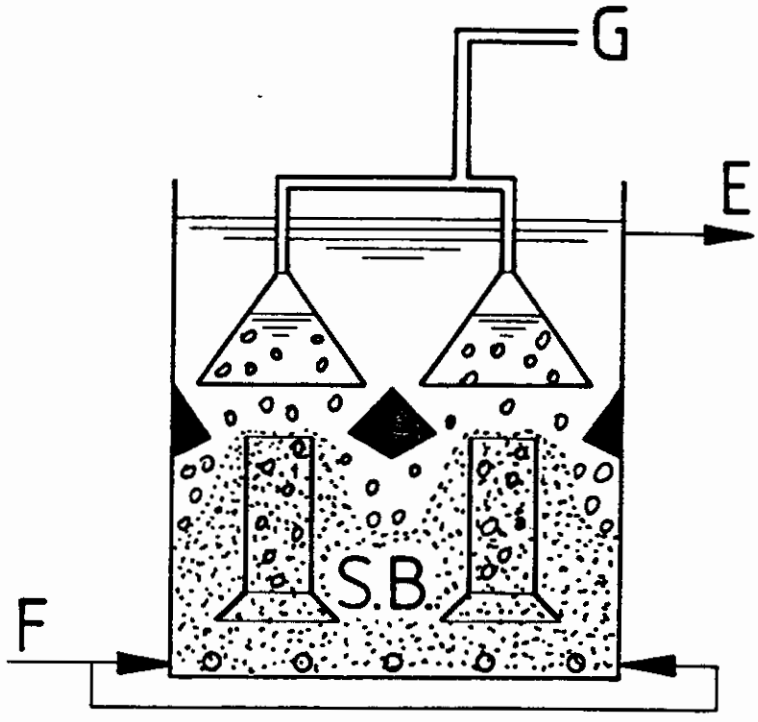

Figure 2. A UASB-reactor design with a subdivided digester compartment for improving the contact betwen sludge and wastewater.

Indeed significant better results were obtained in a two-step and three-step reactor systems. The first reactor in that case serves for removing
major part of the SS from the raw sewage, and also as a liquefying + acidification system. The second reactor (and third reactor) serves for the removal of soluble and/or colloidal compounds. The results obtained with such a multi-step configuration are significantly better than with an one-step reactor operated with the same wastewater, at the same HRT.
There exists no doubt that this multistep configuration offers interesting prospects, the more so because the design and mode of operation of the separate modules can be optimized, while the different modules also can be integrated in one reactor unit. The second and/or the third module could be designed and operated as an EGSB-system. Latter systems, provided they are well designed and operated, are superior over conventional UASB-reactors for removing soluble matter, but sometimes also for the removal of finely suspended matter from wastewater, as was demonstrated by Rinzema (1988) in experiments with edible oil wastewater.

III. 2.4. EGSB- and FB-systems.

As follow-up of the UASB-experiments and on the basis of promising results obtained in preliminary small scale laboratory experiments conducted with soluble substrates, extensive investigations were started with EGSB-systems in 1986 in order to assess the use of these systems for treating settled sewage. Simultaneously also FB-systems of the concept developed by Heynen et al were investigated. The reason to include these systems in the research were the rather promising results obtained in comprehensive pilot plant experiments conducted with an medium strength industrial wastewater. It was expected that the concerning FB-concept might represent an attractive alternative for the treatment of settled domestic sewage. This particular part of the research was partially granted by the Gist Brocades Company.

One step systems.
In the various experiments conducted different reactor sizes have been used, viz. varying in height from 2 m to 5m. The applied superficial velocities (v_f) in EGSB-systems always amounted to appr. 6 m/hr and in the FB-systems up to 12-24 m/hr at the start and 10-12 m/hr after completion of the start-up. These V_f-values were adjusted by applying the appropiate effluent recycle ratio at the imposed HRT.

FB-systems.
The results obtained with FB-systems started at HRT= 2.6 and at 0.67 hrs (V_f= 24 m/hr, later 10-12 m/hr) and at a temperature of 10 - 13 ^{o}C (spring time) using sand as carrier, were quite clear as far as their applicability to settled sewage treatment concerned. For COD-reduction they don't offer any prospect, i.e. less than 7 %. As a matter of fact, after a kind of biofilm has been developed on the carrier material, they merely act as a pre-acidification reactor. Under dry wheather conditions and at HRT 2.6 and 0.67 hr approximately 59 % respectively 46 % of the amount of VFA formed in a long term batch experiment can be produced. Assuming that the coarse suspended solids will not be acidified in these reactor types considering the poor removal of these pollutants in FB-systems, the extent of acidification achieved in fact amounts to 67 % and 52 % respectively of that obtained in a long batch experiment.
Somewhat better results were obtained at HRT= 6 hrs and at a temperature of 18-21 ^{o}C (and at v_f=12-13 m/hr), particularly when using basalt as carrier particles, because up to 33 % $COD_{soluble}$ can be removed 3 month after the start of the system, while with sand as carrier material this still was only 7 %.
Experiments carried out with basalt + precultivated attached biofilm (spec. act. 0.3 kg COD/kg VSS.day at 30 ^{o}C) in a 205 L reactor at ambient temperatures 10 - 14 ^{o}C, HRT= 2.0 - 2.7 hrs gave very poor results. The system was started at 10 ^{o}C and the first 90 days of operation the avaerage COD amounted anly to 250 mg/l due to an extraordinary rainfall.
At higher temperatures (appr. 20 ^{o}C) using a 15 L reactor approximately 31 % of the soluble COD could be eliminated at HRT=1.5 hrs, which is 15 % lower than obtained in an EGSB-reactor under comparable conditions.

EGSB-systems.
EGSB-systems perform significantly better than FB-systems. In reactors using paper wastewater cultivated granular sludge, viz. EB-sludge (also used in the 20 m^3 UASB-reactor) and BT-sludge up to 46 % of the soluble COD-fraction could be eliminated under dwa-conditions at temperatures exceeding 13 ^oC and at HRT = 2 hrs. The efficiency increases only slightly upon increasing the HRT from 2 to 7 hrs. During winter time (average temperature 9.4 ^oC) the efficiency drops to appr 20 %. The efficiencies obtained with the different types of paper wastewater cultivated granular sludges were fairly similar.
The acetoclastic activity of the BT-granular sludge improves during the experiment from 0.17 to 0.25 kg COD/kg VSS.day at 30 ^oC.

When using a higher quality granular sludge, viz. a **granular sludge cultivated on settled sewage**, up to 43% of the soluble fraction can be removed at HRT as low as 0.8 hrs under dwc-conditions and temperatures exeeding 13 ^oC. The $E_{t/t}$ amounts to 30- 35 % under dwc-conditions at HRT = appr 2 hrs. Under rwc-condtions 18 -23 % of the soluble COD-fraction is removed.
A very interesting finding of the experiments with the granular sludge cultivated on settled sewage undoubtedly is the observation that this sludge exerts a relatively very high specific acetoclastic activity, viz. amounting to 0.31 kg COD/kg VSS.day, consequently higher than the acetoclastic activity of granular seed sludge after it has been exposed for a prolonged period to sewage. The phenomenon of sludge granulation as it proceeds in the FB-EGSB reactorsystems is of particular practical interest.

Two step configurations.
The investigations with a FB-FB combination gave poor treatment results when operated at a total HRT =4 - 5 hrs. A two step FB-system doesn't offer any promise for practice. However, a two-step EGSB-configuration may represent an attractive solution as pretreatment step. The average $COD_{soluble}$-reduction in that case amounts to 51-53 % under dwa-conditions, at HRT_{total}= 4.2. hrs (1.0 hr in the first reactor) and in the temperature range 13 - 20 ^oC. The values found for $E_{r/r}$ ranged from 43 to 55 %.

III.2.5. Formation of active granular sludge.
Although FB-systems performed quite disappointing in terms of treatment efficiency, the experiments with inert carriers (sand and basalt) were very informative with respect to the cultivation of an active granular sludge on settled sewage. The experiments clearly reveal that in the operation of a system using inert carrier materials, the attention should be focussed on the retention of biomass aggregates and not on accomplishing a complete fluidization of the bed, which in fact is an illusion regarding the inhomogenity and non-uniformity of the particles/aggregates present. A sufficient sludge bed expansion suffices for achieving the required good contact between sludge and wastewater.
As a matter of fact the granulation process as it proceeds in an expanded (or fluidized) inert carrier system, doesn't deviate from that in a UASB-reactor. The conditions for granulation in such a system become favourable when it is operated at v_f= up to 5 -7 m/hr, consequently as applied under EGSB-conditions. It was also

shown that granulation proceeds well at appr. 20 ^{o}C using digested sewage as seed, provided the reactor is operated at HRT= 5 hr and V_f = 8 m/hr. After 1 month small granules were alreadyobserved in both reactors investigated, and after two months the reactors contain granules with a diameter of approximately 1 mm. Like observed in the granulation in UASB-reactors the first generations of granules are mechanically rather fragile, but gradually they mature to more rigid aggregates. The VSS-content of the aggregates gradually increases up to appr 70 -80 %, and the methanogenc activity raeches values as high as 0.31 kg COD/kg VSS.day.

IV.Conclusions.

From the information available sofar it can be concluded that anaerobic treatment of sewage indeed may offer some potentials for the Dutch situation as pretreatment step in specifiuc cases, although the design of the UASB- and/or EGSB-system needs distinct improvements. From the investigations adequate data was obtained about how these improvements can be accomplished.

The prospects of anaerobic sewage pretreatment using UASB-or EGSB-reactors looks particularly promising for regions were sewage temperatures don't drop below approximately 10 - 12 ^{o}C. Such conditions may prevail in sub-tropical regions, e.g. in the Mediterranean regions.

Of particular interest is the observation of the formation of a granular sludge of a relatively very high specific activity on settled sewage. Together with the information obtained from previous comprehensive investigations, sufficient insight is available in the mechanism of the granulation process.

Literature.

Grin, P., R.E.Roersma, G.Lettinga. Anaerobic treatment of raw sewage at lower temperatures. Proceedings European Symposium on Waste Water Treatment, Noordwijkerhout 23- 25 November, 1983, 335-347.

Grin, P.R., R.Roersma, G.Lettinga. Anaerobic treatment of domestic sewage at temperatures from 9 - 20 ^{o}C. Proceedings of a seminar workshop 'Anaerobic Treatment of Sewage', M.S.Switzenbaum, editor, 109-124, Amherst, Massachusetts, 27-28 June, 1985

Heynen J.J., Biological Industrial Wastewater treatment minimizing biomass production and maximizing biomass concentration, Thesis, delft Technical University, 1984.

Heijnen, J.J., A. Mulder, W. Enger and F. Hoeks, Proc. Aquatech'86 Conference, 15/19 Sept 1986, A'dam,1986, 159-174. Review on the application of anaerobic fluidized bed reactors in waste-water treatment.

Heijnen, J.J., Reprints Verfahrenstechnik Abwasserreiningung, GVC-Diskussionstagung, 17-19 Oktober, Baden Baden,1988, 203-218. Large scale anaerobic-aerobic treatment of complex industrial wastewater using immobilized biomass in fluid bed and air-lift suspension reactors.

Hulshoff Pol, L.W. and G. Lettinga, 1986. New technologies for anaerobic wastewater treatment. Water Science and Technology, 18, 12, 41-53.

Hulshoff Pol, L.W., K. Heynekamp, G.Lettinga, The selection pressure as a driving force behind the granulation of granular sludge, in

"Granular Anaerobi Sludge; Microbiology and Technology", Proceedings of GASMAT-workshop, 25-27, October, 1987, Lunteren, 146-153.
Last, A. van der, G.Lettinga. Pilot plant experiments with settled domestic sewage using EGSB and FB reactors. (non-published results)
Lettinga, G. A.F.M. van Velsen, S.W. Hobma, W.J. de Zeeuw and A. Klapwijk, 1980. Use of the Upflow Sludge Blanket (USB) reactor concept for biological wastewater treatment. Biotechnology and Bioengineering, 22, 699-734.
Lettinga, G., S.W. Hobma, L.W. Hulshoff Pol, W.J. de Zeeuw, P. de Jong, P. Grin and R. Roersma, 1983. Design, operation and economy of anaerobic treatment. Water Science and Technology, vol 15, pp 177-195.
Lettinga, G., Roersma,R., Grin, P. Anaerobic treatment of domestic sewage using a granular sludge bed UASB-reactor. Biotechnology and Bioengineering, XXV, 1701-1723, 1983.
Lettinga, G. L.W. Hulshoff Pol, I.W. Koster, W.M. Wiegant, W.J.de Zeeuw, A. Rinzema, P.C. Grin, R.E. Roersma and S.W. Hobma, 1984.High-rate anaerobic wastewater treatment using the UASB-reactor under a wide range of temperature conditions. Biotechnology and Engineering reviews, vol 2, 253-284.
Lettinga, G. and L.W. Hulshoff Pol, 1986. Advanced reactor design, operation and economy. Water Science and Technology, 18, 12, 99-108.
Lettinga, G., Hulshoff Pol,L.W., Reprints Verfahrenstechnik Abwasserreinigung, GVC-Diskussionstagung, 17-19 Oktober, Baden Baden,-1988, 187-202. Die Anwendung von Schlammbettreaktoren.
Man A.W.de, A. van der Last, G.Lettinga, Proc. 5th Int. Conf.An. Digestion, Bologna, 1988, 197-210. The use of EGSB and UASB anaerobic systems for low strength soluble and complex wastewaters at temperatures ranging from 8 - 30 ^{o}C.
Schellinkhout, A., Lettinga, G., Van Velsen A.F.M.. Louwe Kooijmans, J., Rodriguez, G., The application of the UASB-reactor for the direct anaerobic treatment of domestic waste water under tropical conditions, Proceedings of seminar/workshop 'Anaerobic Treatment of Sewage", M.S.Switzenbaum, 27-28, June, 1985, Amherst, USA, 259-276.
S.H.J.Vellinga, P.J.F.M.Hack, A.J. van der Vlugt. Proc. Aquatech'86 Conference, 15/19 Sept 1986, A'dam,1986, 547-562." New type 'High rate' anaerobic reactor.

THE INFLUENCE OF RETENTION TIME ON REACTOR PERFORMANCE AND BACTERIAL TROPHIC POPULATIONS IN ANAEROBIC DIGESTION PROCESS

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THE INFLUENCE OF RETENTION TIME ON REACTOR PERFORMANCE AND BACTERIAL TROPHIC POPULATIONS IN ANAEROBIC DIGESTION PROCESS

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ABSTRACT

The influence of retention time(RT) on reactor performance and bacterial trophic populations has been investigated by using a starch-processing chemostat reactor. RT has a considerable effect on the population levels of H_2-producing acetogens, methanogens and hydrogenotrophs and on the composition of fermentative products, but it has little effect on the population levels of fermentative bacteria and the practical acidification. The population levels of acetate-utilizing methanogens and CH_3OH-utilizing one decreased as RT decreased, and they were washed out at RT 12 h and 4.2 h , respectively. The populations of H_2-utilizing methanogens and homoacetogens increased with the decrease of RT, and the maximum levels of these bacteria were 7.9×10^9, and 3.5×10^{10} (MPN/ml), respectively, at RT 6 h and 12 h, respectively. Even at RT 1.5 h, methane was still produced in the acidogenesis, which meant that it was impossible to separate completely methanogens from acidogens using the method of kinetic control. The population of fermentative bacteria was always as high as 10^8 to 10^9MPN/ml when RT were between 244.8 h and 1.5 h. The population of H_2-producing acetogens was 10^8 to 10^{11} MPN/ml when RT was longer than 3.12 h. The reasonable proportion of H_2-utilizing bacteria and H_2-producing bacteria maintained the fermentation mainly to acetate even under the condition of high concentration hydrogen in headspace of the digester. The best time for phase separation is at the RT of about 12 h.

KEY WORDS

Anaerobic digestion process, retention time, MPN method, bacterial population levels, fermentative bacteria, H_2-producing acetogens, homoacetogens, methanogens, phase separation, fermentative products.

INTRODUCTION

Much intensive research has been carried out by investigating on both abiotic and biotic components and their interactions in order to elucidate the mechanism of anaerobic digestion process and to improve the practical applications of the process. Since anaerobic digestion process is a relatively slow and complex process, the studies are very important on shortening the retention time (RT) for various kinds of waste in digesters and on obtaining the combined and coordinated metabolic activity of the digester population. When RT was reduced, the changes in the composition of the methanogenic population were occurred(Lawrence et al..1969; Noike et al .. 1985), furthermore, the so-called rate-limiting step of the methanogenesis could be speeded up in digesters fed with a mixture of VFA because the volumetric acetate (HAc) degradation rate in a digester fed with a mixture of VFA was about three times faster than that in a digester fed with a single component of VFA (Lin et al.. 1989). Since the rate of methanogen growth depends on the substrate catabolized, Zeikus (1980) pointed out that it should now be recognized that methanogens were not slow growing per se but possess a most efficient metablism. Previous studies on two-phase anaerobic digestion process showed that methanogens would be washed out of the first phase reactor when RT time was at 12.5 h (Ghosh et al.. 1974; Endo et al. 1982), while Chartrain et al.(1987) demonstrated that gradually decreases in the chemostat RT showed excellent biomethanation performence at RT as low as 25 h and caused the levels of important carbon-degrading populations to fluctuate and decreased. Studies on the characteristics of substrate degradation in anaerobic digestion process indicated that the effect of RT on the distribution of fermentative products was very great but very complicated. The investigation of Hanaki et al. (1987) using a infant milk processing chemostat demonstrated that with the increase of RT, propionate (HPr) increased markedly, HAc increased moderately and n-butyrate (n-HBu) decreased, while the experiment of Chartrain et al. (1987) using a whey-processing chemostat indicated that the intermediary carbon metabolites accumulated with the decrease of RT in the following order: HAc, HBu, HPr, lactate, ethanol, and lactose and the variation pattern of HAc in their experiment was the same as that in the studies of Ghosh et al. (1974) and Noike et al.(1985) employing glucose and starch processing chemostats. In addition, it was reported that longer RT resulted in increased HAc but no change in HPr (Dinopoulou et al.. 1987). All of these experiments show that it is very important to study the effect of RT on anaerobic digestion process, but sufficient data are not available to reveale a comprehensive integration of knowledge to clarify the effect of RT on reactor performance and the bacterial population levels in anaerobic

digestion, to say nothing of the effect of RT on phase separation in two-phase anaerobic digestion process.

The main purpose of the present study is twofold: first, to investigate the influence of RT on the performance of a starch-processing chemostat ecosystem and on the bacterial trophic populations in the same degester; second, to interpretate the correlations among the bacterial trophic populations in the reactors, the metabolic characteristics of bacteria and the distribution of fermentative products of the reactors.

MATERIAL AND METHODS

Reactors and Substrate.

A schematic drawing of the apparatus utilized in this study is presented in Figure 1. The chemostats had 9-liter total capacity vessels with a 6-liter working volume for the RTs of 244.8, 120, 60, 48, 42, 33.6 and a 1.4-lite total capacity vessels with a 0.6-liter working volume for the RTs of 48, 24, 12, 8.4, 6, 4.2, 3.12, 2.5, 1.5 (h). Table 1 shows the chemical composition of synthetic substrate, in which the soluble starch was used as the sole source of energy and carbon. Substrate stored in a refrigerated reservoir at about 5℃ was continuously fed to the reactors and completely mixed by gas circulation. The temperature in the digesters was controlled to 35 $\pm$ 1 ℃ and no external pH control was exerted. The seed sludge, obtained from a mesophilic night-soil digester in the Minamigamo Sewage Treatment Plant of Sendai, Japan, had been acclimatized to the synthetic substrate for more than 3 months by gradually decreasing RT from 480 h to 244.8 h.

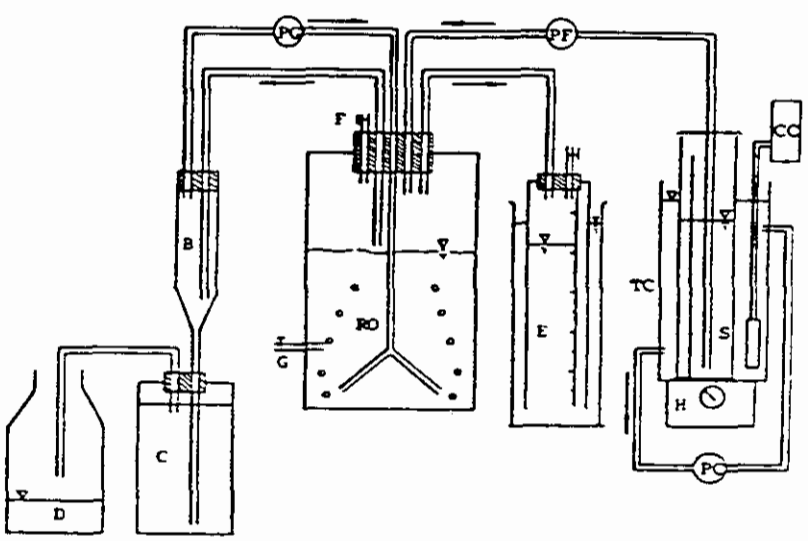
Experimental Design and Analytial Methods.

The experimental work consisted of a number of steady-state, continuous-flow runs with RT ranging from 1.5 h to 244.8 h. In order to achieve a steady state, the digesters were operated over 6 to 8 RT or three weaks, whichever was longer, after which the analyses of the performance of the reactors were repeated 5 or 7 times for 2 to 3 weeks under the steady state condition and the average of the analytic results was used as the steady state data.

The mixed liquor volatile suspended solids (MLVSS), COD_{cr} and pH were measured using the Standard Methods. Volatile fatty acid (VFA) were analyzed with a gas chromatograph having a flame ionization detector. Gas composition analyses were carried out with a gas chromatograph equipped with a thermal conductivity detector. Total and soluble saccharides were measured with the Anthrone method. Total and soluble proteins were analyzed by the

Table 1 Composition of Substrate

Component	Content (mg/l)
Starch	10500
NH_4HCO_3	4740
$NaHCO_3$	2000
K_2HPO_4	125
$MgCL_2 \cdot 6H_2O$	100
$MnSO_4 \cdot 5H_2O$	15
$CuSO_4 \cdot 5H_2O$	5
$CoCL_2 \cdot 6H_2O$	0.125
$FeSO_4 \cdot 7H_2O$	25



RO: One-phage reator G: Mixed liquor sampling port
B.C.D: Mixed liquor overflow system PF: Feed pump
E: Gas collection system F: Gas sampling port
PG: Gas recirculatio pump S: Substate tank
TC.PC.CC: Cool dip system

Fig. 1 Schematic of Experimental Apparatus

Lowry's method.

Procedures for Bacterial Counts

For the enumeration of bacteria, the procedures developed by Hungate(1950) and its modification (Chartrain et al., 1986) were utilized in this study. The basal media shown in Table 2 for the most probable number (MPN) determination and the anaerobic dilution solution were made up based on the references (Braun et al., 1979; Li et al, 1989b). Straight-sided culture tubes (12 by 280 mm) that were screw-capped and sealed with flanged butyl stoppers were used in this study. Gases that entered the tubes through the gassing manifold were always scrubbed free of oxygen traces by passing through a 350 ℃ heated copper column. 10ml of digester sample withdrawn from each reactor was immediately injected into a 120 ml pressure vial containing 90 ml of anaerobic dilution solution, and then the vial was place on a Vortex mixer for 2 min. for the disruption of the bacteria flocs. After diluted by serial 10-fold dilution, 1 ml of the appropriate dilution was used to inoculate different media with serial syringes as transfers. The enumerations were performed by the MPN technique with five tubes per dilution. The tubes were incubated for 40 to 60 days at 37 ℃, and the results were interpreted by MPN according to the analytical methods for MPN determination shwon in Table 3. Morphological examination and the counting of the total number of bacteria were made with the aid of a phase-contrast microscope.

Table 3 Analytical Methods for MPN Determination

Metabolc Groups	Analytical Methods
Fermentative Bacteria	Volatile Fatty Acid
H_2-producing Acetogens	HAC and H_2 Gas
Homoacetogenic Bacteria	HAC and H_2, CO_2, CH_4 Gas
H_2-utilizing Methanogens	CH_4 Gas
HAC-utilizing Methanogens	CH_4 Gas
HCOOH-utilizing Methanogens	CH_4 Gas
CH_3OH-utilizing Methanogens	CH_4 Gas

RESULTS AND DISCUSSION

Effect of Incubation Time on the Enumeration of Methanogens

The optimum time of incubation at 36± 1 ℃ was determined using the medium containing different carbon source for enumerating different trophic-grops methanogenic bacteria. Figure 2 shows the effect of incubation time on bacterial counts by using the MPN technique. Siebert and Hattingh (1967) have suggested that the optimum time of incubation at 38℃ was 8 days.

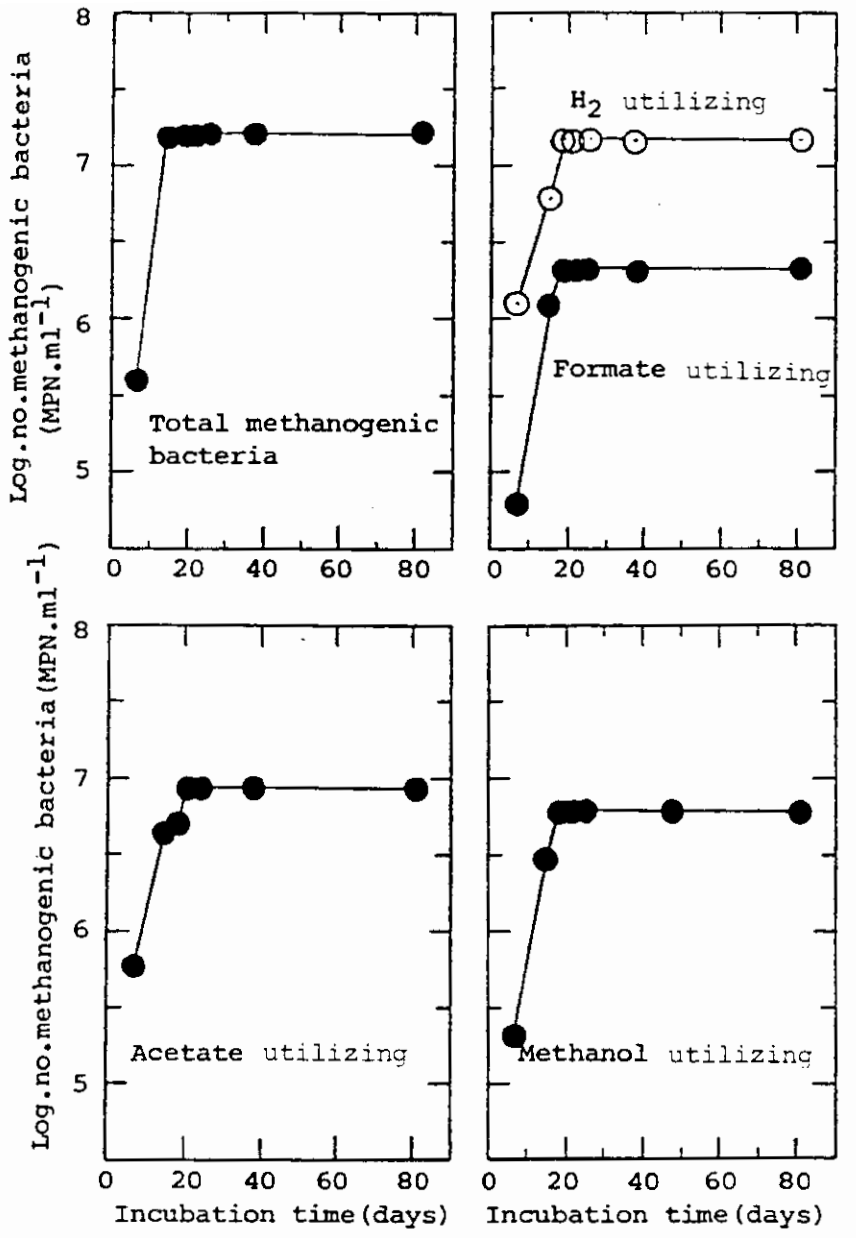


Fig. 2 Effect of incubation time on enumeration of different groups of methanogenic bacteria.

according to their experiments with the incubation period of 13 days. In this investigation, the incubation time ranged from 7 days to 82 days as shown in Fig. 2. It was possible to yield visible methane gas production in the tube inoculating with a 10^{-5} to 10^{-6} dilution for 7 days after the inoculation, but methane gas production was not observed in the test tube which was inoculated by further higher dilution. The incubation time of 25 to 30 days is necessary to obtain the most stable experimental results, and significant change in the count was not observed at the incubation time of over 30 days. These results agree with incubation time reported by other researchers (Chartrain et al. 1986; Makie et al. 1981; Qian et al. 1985). Therefore, The incubation time of one month was adopted in this study.

Influnce of RT on the Performance of Anaerobic Reactor.

Figure 3 shows the steady-state data on gas production rate and gas composition. When RT was longer than 24 h, gas prodction rate increased slightly with the decrease of RTs, but when RTs were between 24 h and 3.12 h , the gas production rate increased dramatically until the maximum 28460 ml/l/d being reached. After 3.12 h, the gas production rate decreased, which meant that the fermentation retarded. The major gas products was carbon dioxide. The amount of methane produced decreased as RTs decreased from 244.8 h to 12 h, and increased sligtly beyond that point until it dropped again after 3.12 h. The production of methane in acidogenesis has been reported by many workers. Cohen et al (1979) reported that *no methan* could bedetected in the reactor at RT 10 h, pH 6.0 and using glucose as subtrate. Others reported that the production of methane ceased at RT 0.93 h (Ghosh et al.,1974),and 7.0 h (Noike et al..1985). The variation pattern of methane in this study, although different from the results reported by previous workers, showed that the methanogenic process did exist in acidogenesis even though RT was as short as 1.5 h. Like previous studies (Hanaki et al.. 1987), hydrogen gas was produced vigorously at shorter RTs. The amount of hydrogengas were 12%, 21%, 26%,33%, at RT 12, 8.4, 6, 4.2 (h), respactively. When RT was shorter than 4.2 h, the hydrogen produced decreased with the decrease of RT. This variation pattern of gas composition is due to the transition of anaerobic bacteria, especialy H_2-producing and H_2-utilizting bacteria, in chemostat in response to the changes of RT.

The composition of the individual VFA in the effluent of the reactors, MLVSS and pH are shown in Figure 4. HAc, HPr and n-HBu were always the main products. The concentration of HAc increased as the RT decreased from 244.8 h to 60 h, after that it decreased as the RT decreased to 24 h, and then it increased again with the decrease of RT until it reached its maximum concentration of 3261 mg/l at the RT of ca. 12 h, after that, the variation pattern of HAc showed a general tendency to vary proportionally with RT. The

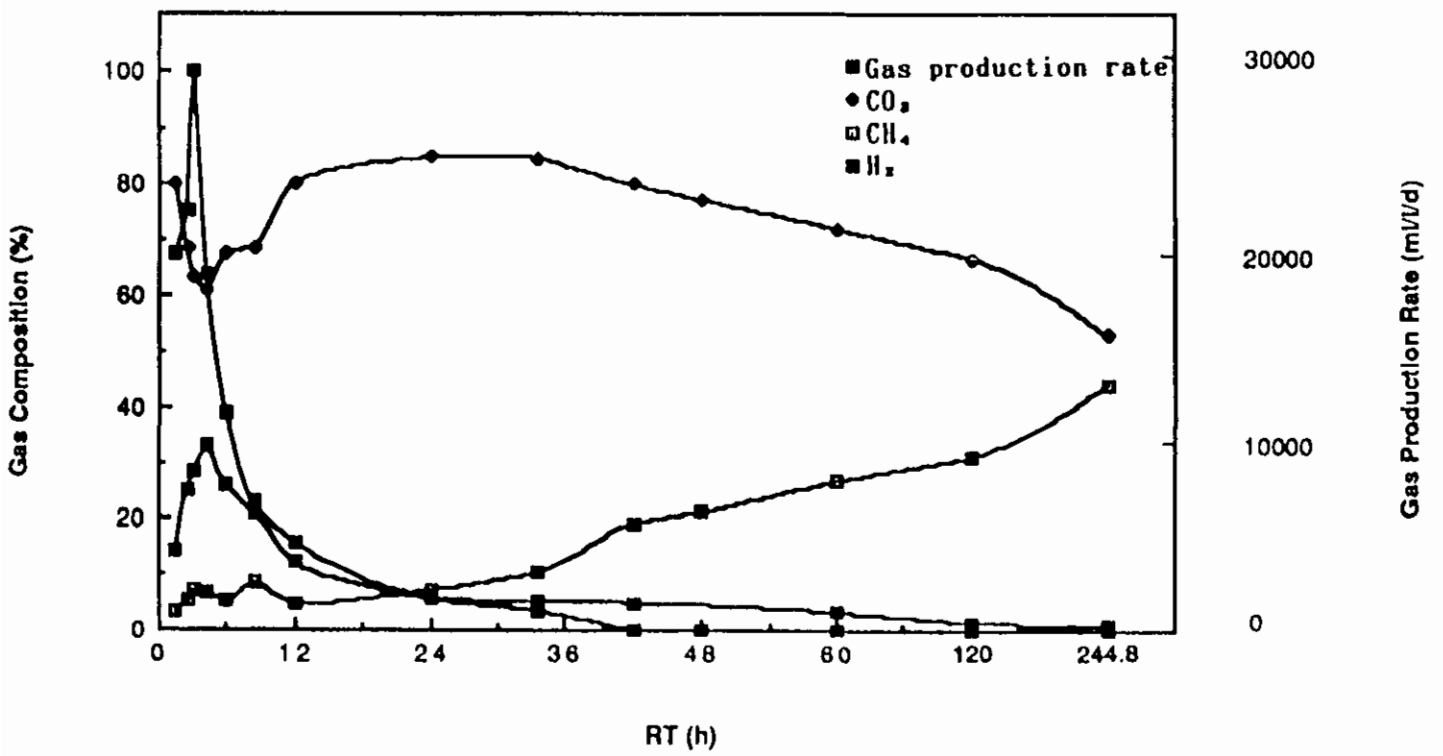


Fig. 3 Effect of RT on gas composition and gas production rate

accumulation of HPr was found as the RT decreased from 244.8 h to 42 h, and between the RT of 36 h and 12 h, the ratio of HPr to the total VFA became very small, therefore there was no obvious evidence showing that the accumulation of HPr occurred duing this period. So did the variation pattern of n-HBu. When RT was shorter then 8.4 h, the accumulation of HPr and n-HBu occurred again. It was found that, although at the RT of 244.8 h the VFA concentrations were relatively low, they showed the dramatic increase with the decrease of RT, which meant that the function of reactor was changed from methanogenesis to methanogenesis plusing acidogenesis and finally to acidogenesis as the RT decreased from 244.8 h to 1.5 h. It should be noticed that the composition of VFA at the RT of 12 h seemed to be more reasonable than those at other RTs if the effluent of the reactor at this RT was used as the effluent of acidogenesis, the first-phase of two-phase anaerobic digestion process. This is because that HPr and n-HBu with low concentrations, as well as HAc with high concentration is more sutiable for methane production in methanogenesis, the second-phase of two-phase anaerobic digestion process.

In order to investigate the shifting of methanogenesis to acidogenesis, and to understand the capacity of acid prodution of acidogenesis, the total practical acid production rate and practical acidifiction were calculated employing the following equations based on the two-stage theory of anaerobic degestion:

$$V_{VFA} = Q/V*(C_{Ae}-C_{Ai})+M_D/Y+H_D \qquad (1)$$

where: V_{VFA}: total practical acid production rate (mg-COD/l·day),
C_{Ai}: total acid concentration in influnt (mg-COD/l),
C_{Ae}: total acid concentration in efflunt (mg-COD/l),
Q: loading rate of influnt (l/day),
V: working volume of reactor (l),
M_D: methane production rate (mg-COD/l/d),
H_D: hydrogen production rate (mg-COD/l/d),
Y: methane yielding coefficient of acid (Y=0.96(Hanaki et al.,1980))
(mg-COD/mg-COD).

$$\text{practical acidification} = \frac{\text{quantities of fermentative products}}{\text{initial substrate concentration}} \qquad (2)$$

Fig.5 shows the calculating results of total practical acid production rate, COD loading rate and the practical acidificaton in relation to the changes of RTs. The degree of practical acidification varied between 60 and 78% of the initial COD concentration, showing no dramatic change with RT when RT

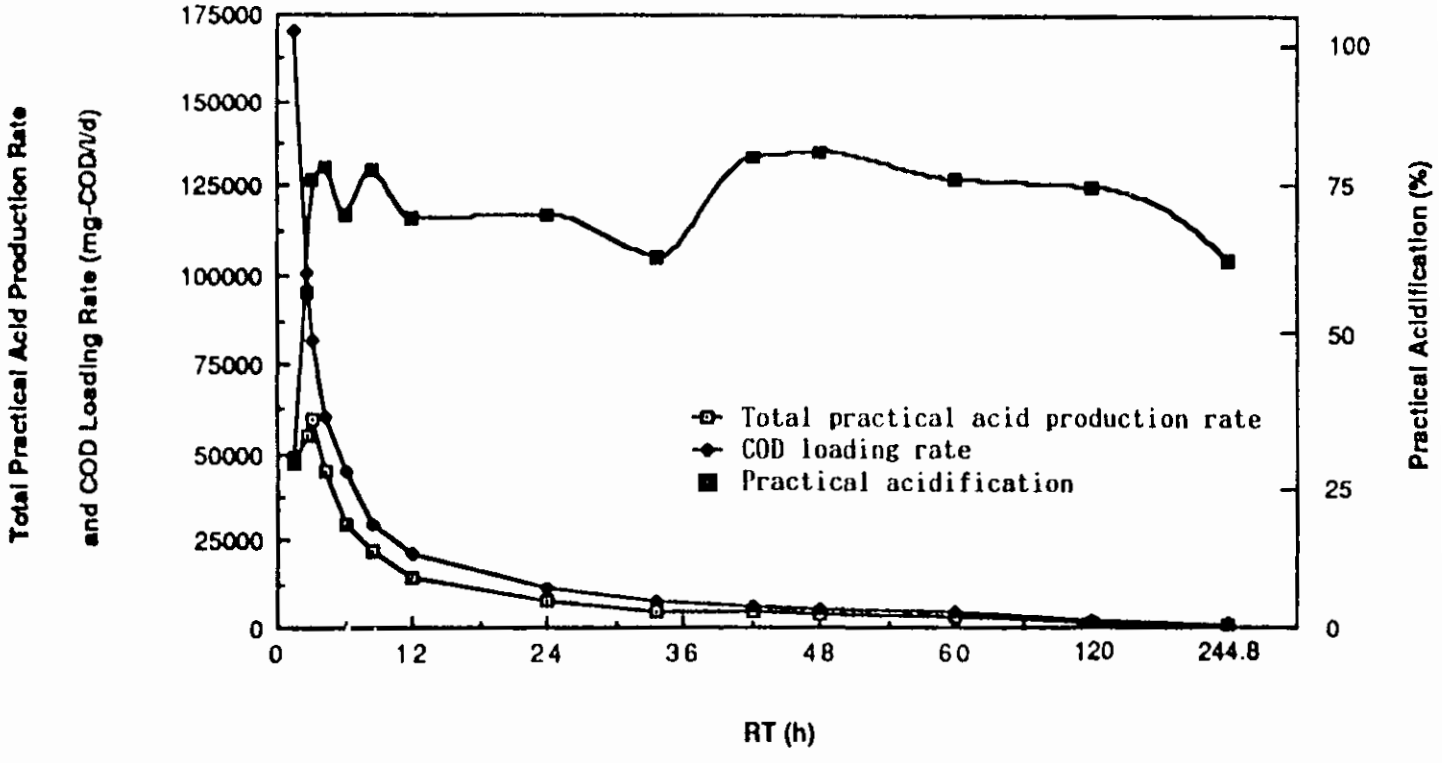
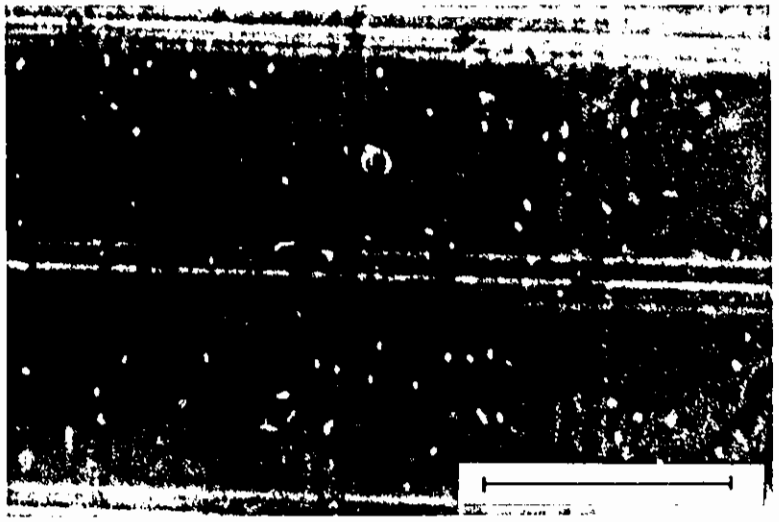


Fig. 5. Effect of RT on total practical acid production rate and practical acidification

was longer than 3.12 h. but it dropped sharply at the RT shorter than 3.12 h . These results meant that the practical acidification of a conventional anaerobic digester remained almost constant regardless of the digester being methanogenesis or being acidogenesis. that is to say. the practical acidification did not affected greatly by RT as long as the RT did not short enough to make a large number of anaerobic bacteria been washed out of the reactor. Even if RT affected the practical acidification. the degree of the effect did not excessed 15 %. furthermore. the COD loading rate did not affected drastically the practical acidification. These results corresponded with the results reported by Dinopoulou et al. (1987). From Fig. 5. it can also seen that. although the total practical acid production rate was not greater than 584 mg-COD/l·day at the RT of 244.8 h. it increaced exponentially as RT decreased from 244.8 h to 3.12 h. in other words. it increaced dramaticlly with the increase of COD loading rate. showing the great acidogenic capacity of the digester without any overloading phenomenon occured. In the light of the above statement. in addition to the fact that the degradation of starch in the reactors was as high as 95 to 98%(see Figure 4) even that RT was as short as 4.2 h. it can be concluded that the hydrolysis of starch and acid forming would not be rate-limiting stages. furthermore. the tremendous acid production capacity of anaerobic digestion process can be exploited by introducing acidogenesis of the two-phase digestion proces to its maximum limitation without any sacrificing of the practical acidification.

Influnce of RT on the Population levels of Anaerobic Bacteria.

Microbial populations in anaerobic digestion has been reported by many investigators. It has been found that RT was a significant factor in selecting the predominant microbial species(Lawrence et al., 1969; Ghosh et al.,1974.; Cohen et al.,1980; Chang et al., 1982; Chartrain et al., 1987; Li et al., 1989). One of the objectives of the present study is to obtain direct experimental evidence for the influnce of RT on the population levels of anaerobic bacteria. and therefore we can use this information to study the ecophysiological features of the anaerobic digestion process. Figure 6 shows the shifting of the microscopic appearances in the steady-state chemostats with the decrease of RT. When RTs were longer than 48 d. the coccus-like bacteria was dominant with very small quiantity of short bacilli ones. both of them being free-living. When RTs were between 40 to 15 h. the rod-shape bacteria increased rapidly. When RTs were between 12 to 3 h the rod-shape bacteria. most of them being straight. but some of them slightly curived. became dominant and a large number of long filaments were observed. and they existed mainly in the form of the skeleton of flocs with considerable quantities. During these periods. the bacteria reached their



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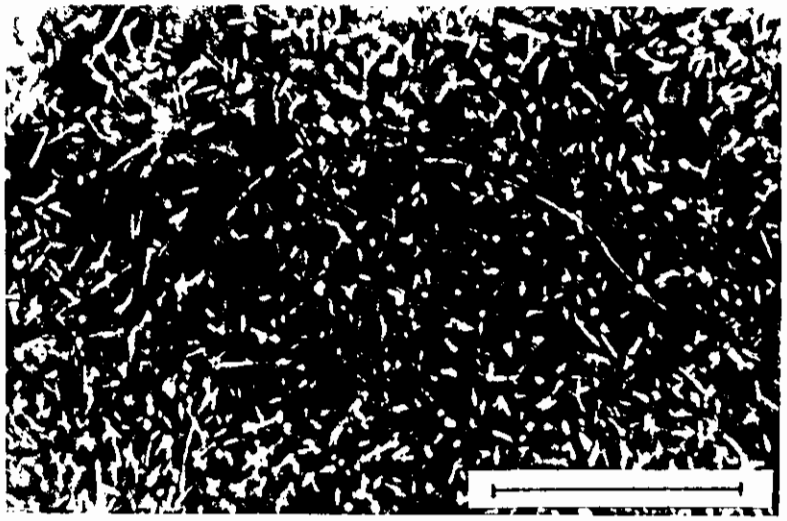

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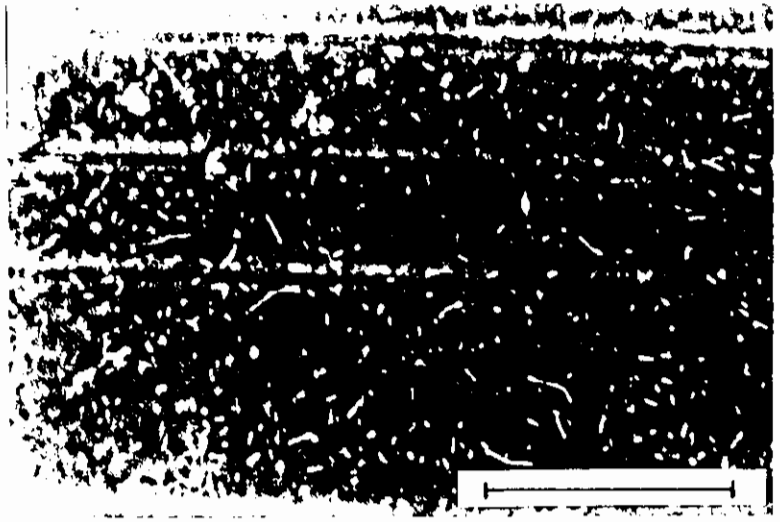

B

D

Fig.6 Phase-contrast photomicrographs of the starch-processing chemostat bacterial population. The bar represents 50 μm. (A) SRT>2 d; (B) SRT between 15~40 h; (C) SRT between 12~3 h;and (D) SRT<2 h.

maximum quantities as shown in Fig. 7. After RT 2 h, the long filaments disappeared completly, and the dominant bacteria was strightly rod-shape bacteria. Although flocs still existed, their quantities were very small and their skeletons consisted of the same bicilli-form bacteria as the free-living cell. It is interesting to notice that the time for maximum MLVSS to appear was different from the time for maximum population levels to appear (see Table 4), which, in fact, meant the shifting of bacteria.

Table 4 shows the culture counts and direct counts of different trophic group population levels on steady state condition. From these results, we can get Figure 7 which shows the influnce of RT on the bacterial population levels. Recently, fermentative bacteria in anaerobic digester have been enumerated by several workers who have found that the total number of fermentative bacteria, the number of lactose degrading bacteria, the number of cellulose degrading bacteria, the number of protein degrading bacteria and the number of starch degrading bacteria are 10^8 to 10^{11} (Mah et al., 1968; Toerien et al., 1967; Kotze et al., 1968; Kirsch, 1969; Chen, 1987), 10^8 to 10^{11} (Chartrain et al.,1987), 10^4 to 10^7 (Toerien et al.,1967; Chen, 1987), 10^4 to 10^8 (Siebert et al.,1969; Chen, 1987), and 10^7 to 10^8 MPN/ml (Chen,1987), respectively. The result of the enumerated fermentative bacteria in this study is in accordance with these studies. The number of fermentative bacteria increased slowly with the decrease of RT when RT was longer than 12 h. When RTs were between 12 h and 3.12 h, the population levels of the fermentative bacteria was ca. 3.3×10^9 MPN/ml, the maximum number of this kind of bacteria in this study. When RT was shorter than 3.12 h, the levels of these bacteria began to decrease, but even at RT 1.5 h, the levels of the bacteria was still as high as 3.3×10^8 MPN/ml. These results meant that RT had little effect on the population levels of fermentative bacteria, rather the organic loading rate might be very important for fermentative bacteria to grow as long as RTs was longer than the minimum generation time of the fermentative bacteria. In other words, the fermentative bacteria can grow very well within large range of RTs, and therefore make it sure for the first step of anaerobic digestion to advance smoothly. Table 5 shows the results of MPN counts using other substrates such as starch, peptone, meat extract, etc, as the source of energy and carbon. From Table 5, it can be seen that, although the substrate of the digester was starch, there existed a large number of fermentative bacteria in the reactors who can degrade protein and meat extract even though amoung those protein and meat degrading bacteria, there might be possible for some of starch degrading bacteria, who could degrade protein and meat extract as well, to be existencn. On the contrary, celluose degrading bacteria was found to be 4.7×10^5 MPN/ml in the reactor.

Fig. 7 shows that the population levels of H_2-producing acetogenic

Table 4 Trophic Group Populations on Stead State Condition (MPN Cell/ml)*

Bacterial groupe	HRT (h) 244.8	120	60	24	12	6.0	4.2	3.12	2.5	1.5
(Fermentative) (H_2-producting acetogenic)	1.8×10^{8}	2.3×10^{8}	7.0×10^{8}	9.4×10^{8}	3.3×10^{9}	3.3×10^{9}	1.1×10^{9}	3.3×10^{9}	4.6×10^{8}	3.3×10^{8}
Total	2.8×10^{8}	5.6×10^{8}	7.9×10^{8}	2.3×10^{9}	5.4×10^{10}	1.6×10^{11}	7.9×10^{8}	1.3×10^{8}	7.9×10^{7}	4.9×10^{7}
HPr-utilizing	1.1×10^{8}	2.4×10^{8}	3.2×10^{8}	3.5×10^{8}	2.4×10^{9}	2.8×10^{8}	3.3×10^{7}	1.3×10^{8}	4.9×10^{7}	2.4×10^{7}
HBr-utilizing	2.8×10^{8}	3.3×10^{8}	4.9×10^{8}	5.4×10^{8}	9.2×10^{9}	$>1.6\times10^{8}$	7.9×10^{7}	4.9×10^{7}	3.3×10^{7}	2.4×10^{7}
(Homoacetogenic) (Methanogenic)	1.3×10^{7}	4.6×10^{7}	2.4×10^{8}	7.9×10^{8}	3.5×10^{10}	6.4×10^{9}	7.0×10^{8}	1.1×10^{7}	2.4×10^{7}	7.9×10^{6}
Total	1.4×10^{7}	7.9×10^{7}	1.7×10^{8}	1.4×10^{8}	1.1×10^{9}	5.4×10^{9}	1.1×10^{8}	ND	ND	ND
H_2-utilizing	1.25×10^{8}	7.9×10^{7}	1.1×10^{8}	1.3×10^{8}	1.1×10^{9}	7.9×10^{9}	1.2×10^{9}	1.4×10^{7}	4.6×10^{6}	4.9×10^{5}
HAc-utilizing	1.9×10^{6}	1.7×10^{6}	1.1×10^{6}	1.7×10^{5}	8.0×10^{3}	4.9×10^{2}	2.4×10^{2}	2.3×10^{2}	2.3×10^{1}	1.3×10^{1}
HCOOH-utilizing	9.4×10^{5}	3.9×10^{7}	4.9×10^{6}	5.4×10^{5}	5.0×10^{3}	1.1×10^{5}	$>5.4\times10^{5}$	ND	ND	ND
CH_3OH-utilizing	1.2×10^{7}	1.7×10^{7}	1.1×10^{7}	1.6×10^{7}	1.3×10^{6}	4.9×10^{5}	7.9×10^{8}	ND	ND	ND
SRB	ND**	ND	ND	$>1.6\times10^{8}$	7.9×10^{7}	4.9×10^{7}	3.5×10^{7}	4.9×10^{6}	4.9×10^{5}	2.4×10^{5}
MLVSS (mg/l)	484.3	493.5	529.7	1183.7	1254.0	1307.7	1510.1	1742.6	1563.1	628.4

*: 5 tubes per dilution. Only one enumeration was performed on each steady state condiction.
**: No determination.

Table 5 the fermentative bacteria utilizing different substrates (HRT= 4.2 h)

Substrate	Cellulose	Peptone	Starch	Meat extract	Glucose	Total*
Population (MPN/ml)	4.7×10^{5}	1.7×10^{7}	1.1×10^{9}	7.9×10^{7}	1.1×10^{9}	1.1×10^{9}

*: Total=Glucose + Peptone + Meat extract.

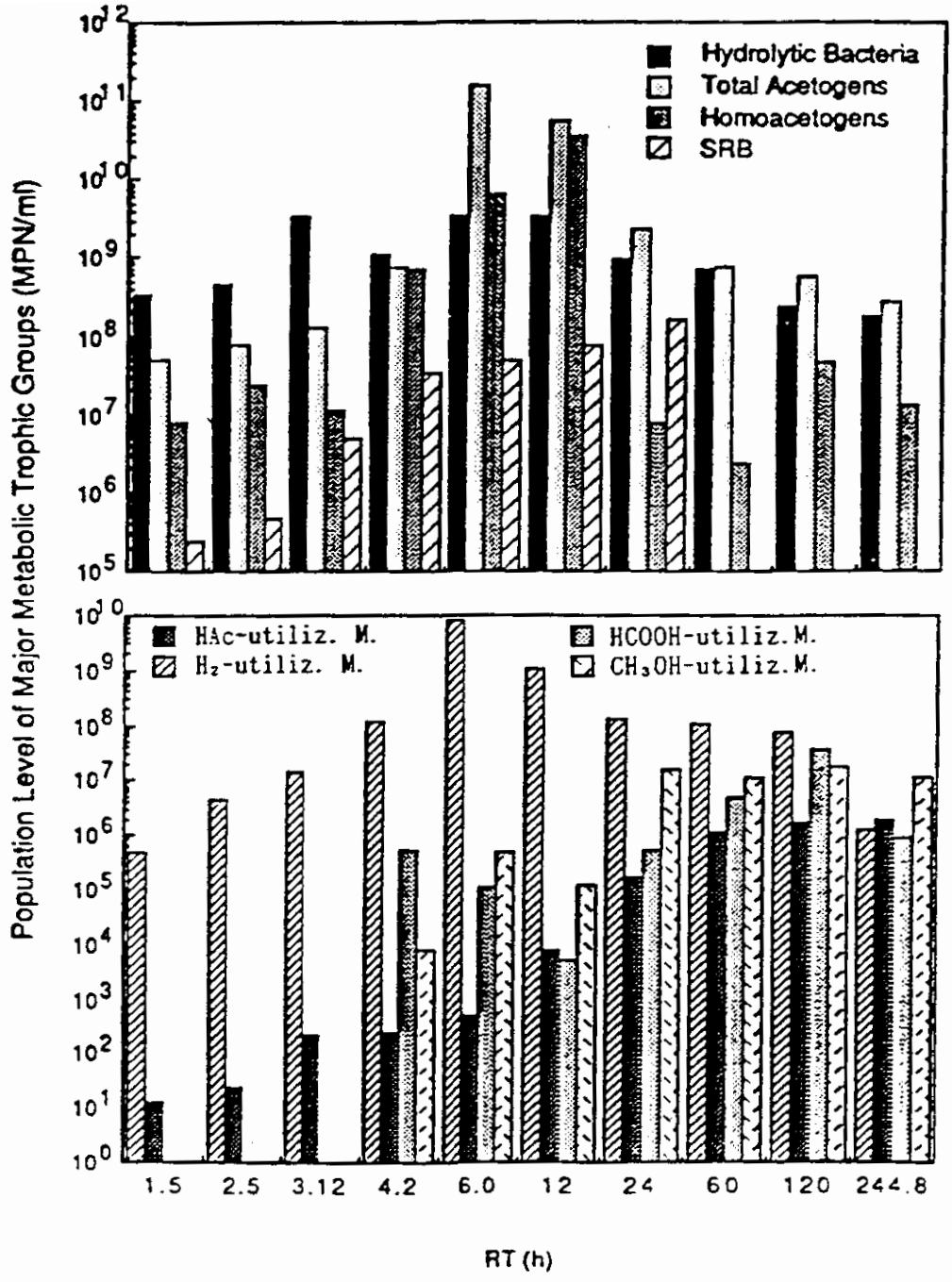

Fig. 7. Effect of RT on the trophic group population levels in the reactor

bacteria increased greatly with the decrease of RT when RT was longer than 6 h, and after that , it decreased with the decrease of RT. It is very interesting to notice that the increase of the levels of H_2-producing acetogens with the decrease of RT was usually greater than that of fermentative bacteria with the same decrease of RT. This, in fact, implied that the specific substrate utilization rate of the fermentative bacteria was greater than that of H_2-producting acetogens so that H_2-producing acetogens could have more abundant substrate, such as VFA, for them to utilize, and consequently, to grow. The levels of HBu-utilizing acetogens was usually greater than that of HPr-utilizing one, which means that HBu is easer to be utilized by acetogens than HPr. Comparing the results of this study with those of other studies, it can be found that the population levels of H_2-producing acetogens in this is 10 or 100 times higher than the levels of 10^5 to 10^7 MPN/ml, reported by the previous studies (Mackie et al., 1981; Zinder et al., 1984; Li et al., 1989a), but compared favourably to the levels of 10^8 to 10^{10} MPN/ml (Chartrain et al., 1986a, 1987), and 10^8 to 10^{12}MPN/ml (Zhao et al., 1987). Since the determination of the population levels of H_2-producting acetogens using MPN method is very difficit unless these bacteria are in syntrophic association with H_2-utilizing bacteria such as methanogens and sulfate reducer. For this reason, some researchers inoculated H_2-utilizing bacteria into the tubes for MPN count of these bacteria. In this study, limited by the experimental condition, we did not use this method. When the VFA and gas composition in the tubes for MPN count of these bacteria were checked, it was found that the concentrations of HAc and H_2 were very high when the tubes with low dilution(i.e. 10^5 to 10^7) were checked (for the reason of hydrogenotrophs existing in these tubes) and therefore, in this situation, the determination of the bacteria was not difficult; while the concentrations of HAc and H_2 were relatively low in the tubes with high dilution (i.e. higher than 10^8), in this situation, we compared the concentration of HAc and H_2 in the tubes for MPN count with those in the tubes for background, and obtained the results showen in Table 4. Because the method for the determination of these bacteria (checking both gas and VFA compositions) was very clear and the addition of yeast extract as well as the digester supernatant has no noticable effluence to the compositions of gas and VFA in this study (data not showed), moreover, the incubation time was very long, we concluded that the results presented in Table 4 reflected the real population levels of H_2-producing acetogens in the reactors used in this study. Further, since the experimental conditions are usually different from each other, it shoule be cautious in comparing the experimental results with each other.

From Fig. 7, it is very interesting to find that a relative high population levels of sulfate-reducing bacteria(SRB) was also in present in

the sulfate-limiting environment in the digester emploied in this study. The reasons might be twofold: first many SRBs are able to utilize a vast veriaty of the fermentative end products produced by other anaerobes as electron donors while sulfate, though very little in the reacter, been utilized as electron acceptor (Ueki et al., 1986). Even if the sulfate is not available , interspecies hydrogen transfer can allow the growth of the sulfur reducer by substrate-level phosphorylation (Bryant et al.,1977; McInerney et al., 1981; Chartrain et al.,1986b), and second, other bacteria such as acetogens, enumeated for a long time in sulfur-rich culture for MPN count of SRB, might transform their genetic factor to be able to utilize sulfur as electron. It should be pointed out that the color of the digester fluid was white in stead of black. This was because the concentration of Fe^{3+} was very low so that the sediment of FeS was not rich enough to make the digester fluid black. Recently, besides the isolation of various species of SRB, the anaerobic digestion of various intermediates such as lower fatty acids, alcohols and H_2 by SRB has been investigated experimentally, in addition, the relationship between SRB and methanogens has been studied, especially in relation to the competiton between SRB and methanogens for substrate such as HAc and H_2. Some workers used SRB and other bacteria to investigate the mechanism of interspecies H_2 transformation. In this study, SRB not only competed with methanogens and homoacetogens or even H_2-producing acetogens for substrates such as HAc, H_2 and lower fatty acids but also showed the HPr-producing ability (data not showed). All of these facts,in addition to the relatively large number of SRB (10^7 to 10^8MPN/ml) exsiting in the sulfate-limiting environment in the digester employed in this study even at very short RT, make us conclude that it is necessary to make further study on the characteristis of this bacteria.

The variation pattern of the methanogens with the change of RT was very comples but full of interesting. Figure 7 shows that the HAc-utilizing methanogens decreased slowly with the decrease of RT when RT was longer than 12 h, after that, it dropped sharply to the insignificant levels of 10^1 to 10^2 MPN/ml, which meant that the HAc-utilizing methanogens were unable to exist at short RT, i.e. RT short than 12 h. Of all kinds of methanogens, only Methanosarcina and Methanothrix can utilize HAc as substrate(Smith et al., 1978; Zinder et al., 1979; Huser et al.,1982; Zehnder et al.,1980). Since the standard free energy ofthe reaction for acetoclastic methanogens to cleave the acetate molecule(-31KJ/mol of methane) is nearly equal to that required for synthesis of a molecule of ATP from ADP and inorganic phosphate (+31.8KJ/mol), the specificgrowth rates of acetoclastic methanogens are very low(Thauer et al., 1977). When methanogens are cultivated with pure cultures, the doubling times of M. soehngenii and Methanosarcina in mesophilic condition are 6.3 d (Zehnder et al.,1980), 23 to 37.8 h (Smith et

al..1978; Sowers et al.,1984). respectively. but when other substrates are used. the doubling time of them may change. (i.e. Methanosarcina acetivorans C2A have doubling times of 24.1.5.2. 6.7. 7.8. and 7.3 (h). respectively. with respone to the substrates of sodium acetate. methanol. methylamine. dimethylamine. and trimethymine. respectively (Sowers et al..1984)). While in cocultures. the acetoclatic methanogens' doubling times are 2.32 d (Chang et al.. 1982). 2.05 d (Lawrence et al..1969). 15.94 h (Chartrain et al..1987). 4.8 h (Ghosh et al..1974). The result of this study is in accordance with the studies reported by Chartrainet al. and Ghosh et al.

In contrast to HAc-utilizing methanogens. the levels of H_2-utilizing methanogens increased with the decrease of RT. especially whan RT was lowered below 24 h. It has been reported that there were 10^8 to 10^{10}cell/ml H_2-utilizing methanogens in the Chemostat (Zinder et al..1984; Chartrain et al.. 1986a). and once the partial pressure of H_2 in the digestor was enhanced the rates of methanogenesis would enhanced greatly. these results implied that H_2-utilizing methanogens were quite far from saturation for H_2. In fact. of 50 species of methanogens discovered up to now. 38 species of methanogens can utilize H_2 as their substrate(Vogels et al..1988). Researches show that the minimum generation time of these methanogens (in mesophilic and pure culture) can be as short as 4 to 11 h (Vogels et al.. 1988). these rapid multiplicative characteristis make itpossible for H_2-utilizing methanogens to increase with the decreasing of RT unless RT is shorter than the minimum generation time of them. In the reactors employed in this study. the population levels of fermentative bacteria and H_2-producting acetogens increased when RT became shorter and hydrogen gas was produced vigorously at shorter RTs. therefore. it is reasonable that. in contrast to the washout of acetate-utilizing methanogen at RT shorter than 12 h. H_2-utilizing methanogen increased with the decreaseof RT because they could obtain a larger quantity of substrate for them to grow. This result provides the first evidence that H_2-utilizing methanogens increase with the decrease of RT as long as RT is longer than the minimum generation times of these methanogensese.

Methanogens utilizing HCOOH as their substrates showed irregulate variation pattern with the change of RT. Since half of methanogens who can utilize H_2 as their substrate can usually utilize HCOOH for the same thing (Vogels et al..1988). the increase of H_2-utilizing methanogens made it possible for these methanogenes utilize HCOOH as their substrates for their growth and for methane production at the same time. Although the specific substrate utilization rates would determine whichever substrates were utilized by these H_2/HCOOH utilizing methanogens in the reactors in this study. they did emerge in bacterial counts as long as the substrates in the incubation tube could be degraded by them. Unlike HAc-utilizing

methanogens who showed dramatic decline after 12 h, the CH_3OH-utilizing methanogens decreased slowly with the decrease of RT. Of 10 species CH_3OH-utilizing methanogens, there are 5 species who can utilize H_2 as well (Vogels et al.,1988), which may be the reason of the veriation pattern of the CH_3OH-utilizing methanogens.

The variation pattern of homoacetogenic bacteria in Figure 7 is the most complex one. With the decrease of RT, the homoacetogens decreased at the RT longer than 48 h. After RT 24 h, these bacteria increased tremendously until the maximum number 3.5×10^{10} MPN/ml been reached at RT 12 h. When RT was shorter than 12 h, the levels of these bacteria declined again with the decrease of RT, but even at RT 1.5 h, there was 7.9×10^{7} MPN/ml homoacetogens still existing in the reactor. Up to now, the population levels of homoacetogens been reported are usually 10^{5} to 10^{7} cell/ml (Ohwaki et al.,1977; Braun et al.,1979; Chartrain et al.,1986a; Li et al., 1989b), but it should be noticed that all of these results were obtained using the samples of fresh water sediments or anaerobic digesters with biological solid retention time (SRT) longer than 1 d. The results of this study at RTs being longer than 24 h comfored to the previous studies. According to the recent studies, when SRTis longer than 1 d, as H_2 scavengers, homoacetogens are out competed by SRB and methanogens since homoacetogens have much higher H_2 threshold levels (520 to 950 ppm) than SRB (5 to 9 ppm) and methanogens (28 to 100 ppm) (Ralf et al.,1988). For the reasons of the lack of H_2 and the competition betweenhomoacetogens and other fermentative bacteria for the substrates, the rapid growth of homoacetogens is limited seriously, therefore, homoacetogens is usually consided as a kind of non-important or low-level bacteria. In this study, RT was shortened to such a degree that the accumulating of H_2 in the reactor made it possible for homoacetogens to break through their H_2 shreshold levels so that they could not only utilize H_2/CO_2 but also the complax substrats for HAc prodution and their growth. In the light of above statement, in addition to the fact that the doubling time of homoactogens are between 1.75 h and sevrel hours (the longest one is only 29 h) (Noike et al., 1989), it is reasonable for homoacetogens to grow rapidly until the populations reach the numbers of 10^{9} to 10^{10} MPN/ml. With such large number of the population, homoacetogens play at least three important roles in anaerobic digestion, that is,ⓐ by using H_2 to produce acetic acid (in COD banlance of anaerobic digestion, the amount of acetic acid produced by homoacetogens from CO_2+H_2 is about 1.3 to 5.3% (Mackie et al.,1981)), they contribute to the interspecies hydrogen transfer (IHT), and therefore, make it possible for syntropic bacteria to maintain high substrate turnover rates. This is of very importance, especially under digester overload conditions, in which the syntrophic reactions become rate limiting(Zeikus,1983); ⓑ fermating

intermidiate organic materials such as lactat, methanol, formate, pyruvic acid etc. into acetic acid, and therefore, promoting the recovery of acetic acid (Yang et al.,1987); and, even more pimportant, © promoting the fermantative stage of anaerobic digestion by converting complex compounds such as carbohydrate, amino acid, etc. directly to acetic acid (Zeikus,1980; Ruyet et al.,1984; Baronofsky et al.,1984;). This characteristic has been reported by some workers (Noike et al, 1989; Li et al,1989b). In this study, when using $CHCl_3$ as inhibiter, it was found that, of total acetic acid produced in the reactor, at least 20 to30% was produced directly by homoacetogens(Zhang et al.,1989b). Based on the above discussion, it is necessary that the important role of homoacetogens in anaerobic digestion needs to be investigated further.

The Relationship Between Anaerobic Bacteria and Their Fermentative Products.

Recently, a general perspective of substrate conversion rates and nutritional requirements of major groups of anaerobic bacteria are explored in more detail, focused particularly on the role of hydrogen in selecting populations and directing the nature of metabolic intermediates (McInerney et al.,1981; McCarty et al.,1986; Harper et al.,1986; Thiele et al.,1988 a&b ; Mosey et al.1989). It has been reported that the increase of H_2 presure in digester will usually cause the change of VFA distribution (i.e. the accumulation of HPr(Bryant et al.,1967; Rufener et al.,1968; Boone et al., 1987)) or even the type of fermentation (Jouner et al.,1966; Miller et al., 1973; Thauer et al.,1977; Cohen et al., 1982; Harper et al.,1986), but not inhibit the syntrophic methanogenic reactions in microbial aggregates (i.e. floc) (Conrad et al., 1985; Thiele et al.,1988 a&b). In this study, although the concentration of VFA was raletively low at RT 244.8 h, it showed the increase between RT 244.8 h and 48 h. The reasons may be twofold: first, the decrease of RT (which meant the increase of COD loading rate in this study) made it possible for fermentative bacteria with shorter doubling time to increase rapidly second, since the population levels of H_2-utilizing methanogens, homoactogenic bacteria as well as SRB were 100-fold lower than those of fermentative and acetogenic bacteria (Fig. 7) the removing of hydrogen and/or the IHT were not perfact, caursing the accumulations of hydrogen and the inhibition of the convensions of HPr.HBu(and presumably other VFA) to HAc. On the other hand, in spite of the vigorous increase of hydrogen gas in headspace phase (see Fig.2) at the RT shorter than 24 h, the concentrations of HPr and HBu showed the tendency of decrease to be varied proportionally with RT between 24 h and 12 h(see Figure 4). To understand these results, it is important to notice that the H_2-utilizing methanogens and homoacetogens increased rapidly with the decrease of RT during the same period (see Fig. 7). The larger quantities of these hydrogenotrophs made it

possible for them to grow together with syntrophic bacterias by species juxtapositioning. This was true when we saw the phase-contrast photomicrographs which showed that when RT was shorter than 24 h, the number of large flocs, within which the bacteria immobilized, increaced. These results support the general concept that cell size increases, and slime formation and intracellular reserve material accumulation account for the increase of biomass when RT are shortened. The dramatic increase of hydrogen in the reactor (Fig.3.) seemed to show that the regeneration of NADH was accomplished predominantly by the reduction of proton to form hydrogen gas , instead of by the fermentation of pyruvate to HPr, etc. and/or that of acetyl-CoA to HBu, which resulted in the decrease of the production of HPr and HBu. According to the above statement, it seems that the enough population of hydrogenotrophs (H_2-utilizing methanogens,homoacetogens and SRB),who can integrat with syntrophic acetogenic bacteria to form microbial aggregate, is the most important factor for the acidogensis of two-phase digestion. In other words, syntrophic acetogens and hydrogenotrophs were predominantly located in an ecological compartment (i.e..floc), in which the H_2 produced by fermentative and acetogenic bacteria was then to be used as protomotive force by SRB, the formation of acetic acid by homoacetogens and the formation of CH_4 by methanogens so that the overall conversion of VFA, such as HPr and HBu was maintained without the potential inhibition by high H_2 concentrations in the bulk aqueous phase(Thiele et al..1988a).

The veriation pattern of HAc is the same as that of other studies(Endo et al., 1982: Chartrain et al..1987), that is, the decreased RT caused the level of HAc to increase until the concentration of HAc reaches its maximum at the RT 12 h (although the time for maximum concentration may be different from one study to another), after that, the concentration of HAc decreases with the decrease of RT. Mosey has pointed out that scavenging of hydrogen by lithotropic methanogens enables the anaerobic fermentation of sugars to proceed directly to acetate, by passing both the formation and subsequent degradation of higher acids(Mosey et al..1989), but we would like to think that the following may acount for the accumulation pattern of HAc: first, the increase of the number of acidogenic bacteria (here homoacetogens is not included though they migth be as well to be considered as a kind of acidogenic bacteria) may increase the production of acetate straightly ; second, the increase of the metabolic efficiency and the growth yield of the syntrophic acetogenic bacteria make it possible that transformation from butyrate and propionate acids to acetate been enhanced: third, what we want to emphasize is that the contribution of homoacetogenic bacteria to the accumulation of acetate: and finally, the washout of acetate-utilizing methanogens make acetate accumulation.

When RT was between 8.4 and 3.12 h, the variation of VFA showed complex

pattern, but the tendancy of it was that the decrease of HAc, the increase and then the decrease of HPr and HBu, which resulted from the change of the distribution of the bacteria in the reactor. The dramatic decrease of the H_2-producing acetogens and the homoacetogens during RTs between 8.4 and 3.12 h may be one of the reasons for the decrease of HAc, and the increase of HBu . When RT was shorter than 3.12 h,the quantity of total practical VFA producing rate, gas producing rate began to drop sharply, which showed the overloading phenomenon of the reacter. From the results showen in Fig.7, it can be understood that the washout of the bacteria was the very reason of the overloading phenomenon.

Washout Phenomena and Phase Separation

The washout phenomena and the mechanisms of two-phase digestion process as well as phase separationn have been discussed for nearly 20 years. Since the influence of RT on the distribution of anaerobic bacteria, especially on the acetogens and methanogens, is not known very clearly, it is very difficult for researchers to anwser many basic questions such as the washout phenomena of methanogens in acidogenesis, whether methanogens, especially H_2-utilizing ones, really do not exist in acidogenesis after the washout of HAc-utiliting methanogens, the function of H_2-producing acetogens in first and second phase, even that H_2-producing acetogens belong to the first phase or the second one was not fully understood. The results of this experiment show that the washout phenomena are directly in relation to the change of RT From Fig.7, it can be found that instead of the washout of all of the methanogens, only HAc-utilizing methanogens and CH_3OH-utilizing methanogens decreased rapidly with the decrease of RT when RT was shorter than 24 h and 6 h, respectely, which means the washout phenomena of these bacteria did occured. Since the number of HAc-utilizing methanogens and CH_3OH-utilizing ones at RT of 12 h and 4.2 h, respectly , were only 8.0×10^3, 7.9×10^3MPN/ml, respertly, it can be concluded that the washout of HAc-utilizing methanogens and CH_3OH-utilizing ones were completed at RT of 12 h and 4.2 h, respectly. From Figure 8, it is known that the washout of HCOOH-utilizing methanogens occured when RT was shorter than 24 h, but when RT wasshorter than 12 hr., the population levels of these bacteria showed the tendency to increase. On the other hand, the H_2-producing acetogens, the homoacetogens and most importantly, the H_2-utilizing methanogens showed not only no washout in the range of RT from 244.8 h to 6 h took place but also the increase tendency in this certant RTs, and even at RT 1.5 h, there was no sufficient evidence to show the completion of the washout of these bacteria.

It should be noticed that at long RT (i. e. 244.8 h), the concentration of VFA in the effluent of the reactor was relatively low, but the distribution of anaerobic bactiera was not reasonable because the levels of

fermentative bacteria and H_2-producing acetogens were 10 to 100 times higher than those of methanogens and homoacetogens. These results implyed the limitation of conventional anaerobic digester. On the other hand, at short RT (i.e. 12 h), the distributions of both VFA and the anaerobic bacteira were reasonable. By shortening RT to the best time for phase separation, a larger quantity of fermentative bactaeria can be obtained, and what is more, the same large quantities of H_2-producing acetogens and hydrogenotrophs with a reasonable proportion can be obtained, which not only brings the potential ability of fermentative bacteria into full play but also intersifies the IHT so that a reasonable and constant production of major intermediates such as VFA can be maintained in the acidogenesis. If the effluent of this acidogenesis is used as the influent of a methanogenesis, it will result in a efficient second phase because it is easy for the bacteria in second phase , especially the H_2-producing acetogens and hydrogenotraphs, to establish a harmonious ecosystem. Therefore, these results demostrated that the phase separation by high dilution rate would strengthen the ecological relationship among anaerobic trophic groups rather than weaken or destroy this relationship.

Now, the problem is that what the best time for phase separation is. Some workers pointed out at RT 12.5 h methanogens would be washed out of the first phase reactor using kinetic controls (Ghosh et al.,1974; Endo et al., 1982). It shoud be noticed that the time for maximum MLVSS to appear (at RT 3.12 h)was different from the time for maximum MPN to appear(12 to 6 h), and that the maximum practical acidification (77 to 78%) appearedat RT 42 to 48 h, but another summit of practical acidification (74%) occurred at RT 8.4 to 4.2 h. These results correspond to that reported by previous resechers (Ghosh et al.,1974; Endo et al., 1980; Chartrain et al.,1978). The phenomenon that the time for acidogens to present in maximum levels is different from the time for maximum acidification to appear is called non-linking kinetics fermentation. Therefore, the best RT for phase separation is different when the object of achieving the maximum MLVSS or maximum fermentation efficiencyis considered. Since the object of anaerobic digestion is to achieve the maximum degradation of pollutant and maximum recovery of methane, it is necessary to engineer the operation of acidogenesis towards those acids which are suitable substrates for methanogens. From the results of our study, at RT 42 to 48 h, the rate of practical acids production was too slow to guarantee the treatment efficiency, while at RT 3.12 h, the fermentative products was not reasonable for the second phase, on the contrary, at RT 12 h or so because the raletively high practical acidification (74%) and the rate of practical acids production apperaed and the fermention of substrate was proceeded mainly to acetate, the best time for phase separation was at RT 12h or so.

Here,we want to point out, based of the above statements, it is unnessary to worry about that the vigerous production of hydrogen will lead to the accumulation of propionic acids or other acids and to the waste of H_2/CO_2. Since hydrogen will be one of the excellent energy resources in the near future,why not we engineer two-phase anaerobic digestion toward the maximum production of hydrogen and VFA with reasonble proportion in the first phase as well as the maximum production of methane in the second one so that the two-phase anaerobic digestion may be full of more attractive than ever before.

CONCLUSIONS

In particular, the following specific conclusions may be drawn from the work presented herein:

1. RT has a considerable effect on the population levels of anaerobic bacteria and their fermantative products, but it has little effect on the population levels of fermentative bacteria and the practical acidification.
2. By decreasing RT, the washout of acetate-utilizing methanogens and CH_3OH-utilizing ones were accomplished at RT 12 h, 4.2 h or so, respectly.
3. When RT were shorter than 1 d, about 10 to 30% H_2 was produced, which resulted in the rapid propagations of hydrogenotrophs. The levels of H_2-utilizing methanogens were 4.9×10^8 to 7.9×10^9 MPN/ml, with 7.9×10^9MPN/ml being the maxmum number at 6 h, and therefore, the methane production was still proceeded even when RT was as short as 3.12 h; while the levels of homoacetogens were 7.9×10^6 to 3.5×10^{10}MPN/ml, with 3.5×10^{10}MPN/ml being the maxmum number at SRT 12 h, the dramaticly propagated homoacetogens played an important role in the respects of fermentating complex compounds as well as a broad spectrum of intermidiaters into acetate and the interspecies hydrogen transfer.
4. The principle of phase separation is the achievement of a large quantity of fermentative bacteria, the establishment of healthy populations of syntrophic acetogens and hydrogenotrophs with a reasonable propotion and the production of constant and reasonable distribution of major intermediates instead of orienting the object of the simple and artifical seperation between acidogens and methanogens. In fact, it is impossible, and it is unnecessary to separate completely enrichment cultures of acidogens and methanogens in phase by kinetic control involving manipulation of RT.
5. The best time for phase separation may be at SRT of 12 h.

References

1. Boone, D. R. and L. Xun. (1987). Effects of pH, temperature, and nutrients on propionate degradation by a methanogenic enrichment culture. Appl. Environ. Microbiol., 53, 1589-1592.
2. Baronofsky, J. J., W. J. A. Schreurs, and E. R. Kashket. (1984). Uncoupling by acetic limits growth of and acetogenesis by clostridium thermoaceticum. Appl. Environ. Microbiol. 48, 1134-1139.
3. Braun, M., S. Schoerth, and G. Gottschalk. (1979). Enumeration of bacteria forming acetate from H_2 and CO_2 in anaerobic habitats. Arch Microbiol., 120, 201-204.
4. Bryant, M. P., E. A. Wolin, M. J. Wolin and R. S. Wolfe. (1967). Methanobacillus omelianskii, a symbiotic association of two species of bacteria. Arch. Microbiol., 59, 20-31.
5. Bryant, M. P., L. L. Campbell, C. A. Reddy, and M. R. Crabill. (1977). Growth of Desulfovibrio in lactate or ethanol media low in sulfate in association with H_2-utilizing methanogenic bacteria. Appl. Environ. Microbiol. 33, 1162-1169.
6. Chang, J. E., T. Noike, J. Matsumoto. (1982). Effect of retention time and feed substrate concentration on methanogenesis in anaerobic digestion. Proc. of JSCE No. 320, 67-76. (in Japanese)
7. Chartrain, M., and J. G. Zeikus. (1986a). Microbial ecophysiology of whey biomethanation: Characterization of bacterial trophic populations and prevalent species in Continuous culture. Appl. Environ. Microbiol. 51, 188-186.
8. Chartrain, M., and J. G. Zeikus. (1986b). Microbial ecophysiology of whey biomethanation: Intermediary metabolism of lactose degradation in continuous culture. Appl. Environ. Microbiol. 51, 180-187.
9. Chartrain, M., L. Bhatnagar, and J. G. Zeikus. (1987). Microbial ecophysiology of Whey biomethanation: comparison of carbon tranaformation parameters, speciescomposition, and starter culture performance in continuous culture. Appl. Environ. Microbiol., 53, 1147-1156.
10. Chen, M. (1987). Difference in sporogenous bacterial populations in *Thermophilic* (55℃) *and mesophilic* (35 ℃) anaerobic sewage digestion Appl. Environ. Microbiol., 53, 2414-2419.
11. Cohen, A., R. J. Zoetemeyer, A. Van Deursen, and J. G. van Andel. (1979). *Anaerobic digestion of glucose with separated acid production and* methane formation. Water Res. 13, 571-580.
12. Cohen, A., A. M. Breure, J. G. van Andel and A. van Deursen. (1980). Influence of phase separation on the anaerobic digestion of glucose — I *Maximum* COD -turnover rate during continuous operation. Water Res. 14, 1439-1448.
13. Cohen, A., A. M. Breure, J. G. van Andel and A. van Deursen. (1982). Influence of

phase separation on the anaerobic digestion of glucose — II stability, and kinetic responses to shock loadings. Water Res. 16,449-455.

14. Conrad,R., T.J.Phelps,and J.G.Zeikus.(1985).Gas metabolism in suppport of juxtapositioning of hydrogen-producing and methanogenic bacteria in sewage sludge and lake sediments.Appl.Environ.Microbiol.50,595-601.
15. Dinopoulou,G.,T.Rudd and J.N.Lester. (1987). Anaerobic acidogenesis of a comples wastewater: I .the influence of operational paraneters on reactor performance. Biotechnology and Bioengineering. 31,958-968.
16. Endo, G., T. Noike and J. Matusmoto. (1982). Characteriatics of cellulose and glucose decomposition in acidogenic phase of anaerobic digestion. Proc. of JSCE No.325,61-68. (in Japanese)
17. Ghosh,S.,and F.G.Pohland. (1974). Kinetics of substrate assimilation and product formation in anaerobic digestion. J.Water Pollut.Control Fed.,46, 748-759.
18. Hanaki,k.,T.Matsuo. (1980). Degradation processes of substrates in anaerobic digestion(I). J. Japan Sewage Works Association.17,50-59. (in Japanese)
19. Hanaki, K., T. Matsuo, M. Nagase, and Y. Tabata. (1987). Evaluation of effectiveness of two-phase anaerobic digestion process degrading complex substrate. Wat. Sci. Tech., 19, 311 322.
20. Harper,S.R., and F.G.Pohland. (1986). Recent developments in hydrogen management during anaerobic biological wastewater treatment. Biotechnology and Bioengineering. 28, 585-602.
21. Huser,B.A., K.Wuhrmann, and A.J.B.Zehnder. (1982). Methanothrix soehngenii gen. nov.sp.nov., a new acetotrophic non-hydrogen-oxidizing methane bacterium. Arch.Microbiol.132,1-9.
22. Jouner,A.E.& R.L.Baldwin. (1966). Enzymatic studies of pure cultures of rumen microorganisms. J.Bact.,92,1321-1330.
23. Kirsch,E.J. (1969). Studies of the enumeration and isolation of obligate aerobic bacterial from sewage sludge. Dev.Ind.Microbiol.,10,170-176.
24. Kotze',J.P.,P.G.Thiel,D.F.Toerien,W.H.Hattingh, and M.L.Siebert.(1968). A biological and chemical study of several anaerobic digesters. Wat.Res., 2,195-213.
25. Lawrence,A.W.,and P.C.McCarty. (1969).Kinetics of methane fermentation in anaerobic treatment. J.Wat. Pollut. Control. Fed.42,Res.Suppl.R1-R17.
26. Li,Y.Y. and T.Noike. (1989). Metabolic Characteristics and distribution of fatty acid-utilizing bacteria in anaerobic digester. Proceeding of the 23st Annual Conference of the Japan Society on Water Pollution Research. pp.445-446. (in Japanese)
27. Li,Y.Y. and T.Noike. (1989). Characteristics of bacterial population and organic matter degradation in anaerobic sludge digestion -- on

methanogenic bacteria and homoacetogenic bacteria. Japan J. of Wat. Pollu. Res., 12, 771-780. (in Japanese)

28. Lin, C.Y., T.Noike, H.Furumai and J.Matrumoto. (1989). A dinetic study on the methanogenesis prociss in anaerobic digestion. Wat.Sci.Tech. 21,175-186.

29. Mackie, R.I., and M.P.Bryant. (1981). Metabolic activity of fatty acid-oxidizing bacteria and the contribution of acetate, propionate, butyrate and CO_2 to methanogenesis in cattle waste at 40 and 60 ℃ . Appl. Environ. Microbiol. 41,1363-1373.

30. Mah, R.A. and L.Sussman. (1968). Microbiology of anaerobic sludge fermentation. I. Enumeration of the nonmethanogenic anaerobic bacteria. Appl. Microbiol., 16,358-361.

31. McCarty, P.L., and D.P.Smith. (1986). Anaerobic wastewater treatment. Environ. Sci.Technol., 20,1200-1206.

32. McInerney, M.J., and M.P.Bryant. (1981). Anaerobic degradation of lactate by syntrophic associations of Methanosarcina barkeri and Desulfovibrio species and effect of H_2 on acetate degradation. Appl.Environ.Microbiol. 41,346-351.

33. Miller, T.L.& M.J.Wolin. (1973). Formation of hydrogen and formate by Ruminococcus albus. J. Bact., 116,836-846.

34. Mosey, F.E., and X.A.Fernandes. (1989). Patterns of hydrogen in bogas from the anaerobic digestion of milk-sugars. Wat. Sci.Tech., 21,187-196.

35. Noike, T., G.Endo, J.E.Cheng, J.I.Yaguchi and J.I.Matsumoto. (1985). Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. Biotechnology and Bioengineering. 27,1482-1489.

36. Noike, T., and Y.Y.Li. (1989). State of the art on anaerobic bacteria for wastewater treatment 2. Acid-producing bacteria. pp.126-145. Study on Anaerobic Wastewater Treatment. J.Matsumoto (Ed.). Japan Society of Civil Engineers. (in Japanese)

37. Ohwaki, K. and R.E.Hungate. (1977). Hydrogen utilization by Clostridia in sewage sludge. Appl.Environ.Microbiol. 33,1270-1274.

38. Qian, Z.S., M.C.Chen and Z.Y.Chen. (1985) Estimation of various groups of bacteria in the digesting slurry of cow manure. China Biogas. No.10,6-11. (in Chinese)

39. Ralf, C.R., H.J.Seitz, and R.Conrad. (1988). The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Arch Microbiol. 149,350-357.

40. Rufener, W.H. and M.J.Wolin, (1968). Effect of CCl_4 on CH_4 and volatile acid production in continuous culture of rumen organisms and in sheep rumen. Appl.Microbiol.16,1955-1956.

41. Ruyet, P.L., H.C.Dubourguier, and G.Albagnac. (1984). Homoacetogenic

fermentation of cellulose by a coculture of Clostridium thermocellum and Acetogenium kivui. Appl.Environ.Microbiol.48,893-894

42. Siebert,M.L. and W.J.Hattingh (1967). Estimation of Methane-producing bacteria numbers by the most probable number (MPN) technique. Wat. Res., 1, 13-19.

43. Siebert,M.L. and D.F.Toerien.(1969). The proteolytic bacteria present in the anaerobic digestion of raw sewage sludge. Wat. Res.,3,241-250.

44. Smith,M.R., and R.A.Mah. (1978). Growth and methanogenesis by Methanosarcina strain 227 on acetate and methanol. Appl.Environ. Microbiol.7,156-161.

45. Sowers,K.R.,S.F.Baron, and J.G.Ferry. (1984). Methanosarcina acetivorans sp.nov.,and acetotrophic methane-producing bacterium isolate from marine sediments.Appl. Environ.Microbiol. 47,971-978.

46. Thauer,R.K.,K.Jungermann, and K.Decder.(1977).Energy conservation in chemotrophic anaerobic bacteria. Bact.Rev.41,100-180.

47. Thiele,J.H.,M.Chartrain, and J.G.Zeikus.(1988a).Control of interspecies electron flow during anaerobic digestion: role of floc fornation in syntrophic methanogenesis. Appl. Environ. Microbiol. 54,10-19.

48. Thiele,J.H.,and J.G.Zeikus. (1988b).Control of interspecies electron flow during anaerobic digestion: Significance of formate versus hydrogen transfer during syntrophic methanogenesis in flocs. Appl. Environ. Microbiol. 54,20-29.

49. Toerien,D.F.,M.L.Siebert,and W.H.J.Hattingh. (1967). The bacterial nature of the acid forming phase of anaerobic digestion. Water Res.1,497-507.

50. Ueki,A.,k.Matsuda,and C.Ohtsuki. (1986). Sulfate-reduction in the anaerobic digestion od animal waste. J.Gen.Appl.Microbiol.,32,111-123.

51. Vogels, G. D., J. T. Keltjens, and C.ven der Drift. (1988). Biochemistry of methane production. Biology of Anaerobic Microorganisms. A. J. B. Zehnder (Ed.). Wiley-Interscience. pp 61-90.

52. Yang,S.T.,I.C.Tang,and M.R.Okos. (1987). Kinetics of homoacetic fermentation of lactate by Clostridium formiciaceticum. Appl.Environ. Microbiol.53,823-827.

53. Zehnder,A.J.B.,B.A.Huser, T.D.Brock, and K.Wuhrmann. (1980). Characterization of an acetate-decarboxylating,non-hydrogen-oxidizing methane bacterium. Arch Microbiol.124,1-11.

54. Zeikus,J.G., (1980). Microbial populations in digestors,p.61-90. In D.A. Stafford,B.I.Wheatley,and D.E.Hughes(ed.),Anaerobic digestion.Applied Science Publishers,Ltd.,London.

55. Zeikus,J.G., (1983). Metabolism of one-carbon compounds by chemotrophic anaerobes. Advances in Microbial Physiology.24,215-299.

56. Zhang, T. C., and T. Noike.(1989a). Comparison of one-phase and two-phase anaerobic digestion in characteristics of substrate degradation and

bacterial population levels. (presented to IAWPRC 15th Biennial Internatioal conference. Kyoto, Japan, 1990)

57. Zhang, T.C., and T. Noike. (1989b). The function of homoacetogenic bacteria in anaerobic digestion process. Proceedings of Annual Conference of Japan Society of Civil Engineers. Nagoya, Japan. II-474 pp.1018-1019. (in Japanese)

58. Zhao, Y., and Z. Qian. (1987). Studies on the microbial ecology in anaerobic filter with the slaughter-house wastewater. China Biogas. 5, No.2, pp 6-14. (in Chinese)

59. Zinder, S.H., and R.A. Mah. (1979). Isolation and characterization of a thermophilic strain of Methanosarcinaunable to use H_2-CO_2 for methanogemesis. Appl. Environ. Microbiol. 38, 996-1008.

60. Zinder, S. H., S. C. Cardwell, T. Anguish, M. Lee, and M. Koch. (1984) Methanogenesis in a thermophilic (58 ℃) anaerobic digester: Methanothrix sp. as an important aceticlastic methanogen. Appl. Environ. Microbiol. 47, 796-807.

A PHYSIOLOGICAL APPROACH TO WASTE WATER TREATMENT WITH COMPLETE SLUDGE RETENTION

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INTRODUCTION

The conventional aerobic treatment of domestic wastewater cannot meet modern standards. For example, the conversion rates per volume of wastewater components are too low, nitrogen removal is insufficient and sludge production is too high. These problems are caused by a non optimal performance of treatment plants, i.e., the removal rate of sludge from the plant has to be too high. This results in a low biomass concentration, a high biomass production and the wash out of slowly growing bacteria, e.g., autotrophic nitrifiers, and organisms such as ciliates, rotifers and nematodes. The basis for this behaviour of activated sludge is easily explained by physiological considerations. Bacteria need energy to remain viable and they use energy for the formation of biomass. The energy needed to remain in a viable state is called the maintenance demand; the maintenance of intracellular ion concentrations and the right intracellular pH, and the continuous replacement of enzymes and RNA are among the processes contributing to the maintenance energy demand. If the energy supply is a carbon source, for most bacteria the only source of energy, maintenance processes result in carbon dioxide production. On the other hand, during growth the carbon source is converted to biomass. It is obvious that energy expenditure to remain viable has priority over expenditure for growth. So, bacteria will convert substrate to carbon dioxide for maintenance purposes and they will use substrate provided in excess for growth and multiplication. In a conventional wastewater treatment plant: the sludge concentration is low, thus the energy content of the influent is divided over a limited number of bacteria. This leads to a relatively high biomass increase. If the biomass produced is continuously removed from the plant, the remainder will continue to produce biomass. Furthermore organisms such as nitrifiers,

protozoa and metazoa, which are unable to adjust growth to the removal rate, are bound to become extinct.

A simple solution seems to be the deletion of sludge removal. Biomass concentration will then increase and the energy supply per bacterium will decrease until the supply equals the maintenance demand. Intuitively one would expect that, at a maximal biomass concentration, all the carbon sources in the influent are converted to carbon dioxide, i.e., the carbon sources are fully mineralized (as explained in the next paragraph, this is a simplification). Unfortunately, this solution is not practicable for technical reasons. As sludge would accumulate, the separation of activated sludge and effluent would become impossible. Therefore we need a new generation of plants, in which sludge and effluent are not separated by a conventional settling tank.

We can think of two reactor modes in which biomass is completely retained: one in which the biomass is attached to sand, basalt, plastic, etc, particles and one in which the biomass is retained by a filter with a diameter less then one micrometer (recycling fermentor). The latter mode turned out to be too expensive for practical purposes, but it is the most suitable one for research purposes, because it has fewer variables due to spatial uniformity. So, the recycling fermentor is the best option for physiological studies.

OUTLINE

We don't expect that biomass retention will lead to a complete mineralisation of carbon sources, because the composition of activated sludge is complex. It consists of many different species of bacteria and various species of protozoa and metazoa may be present. In this complex system many ecological interactions occur. Bacteria compete for various substrates, and protozoa and metazoa predate on bacteria and in some cases on each other. So, it is unlikely that the total sludge concentration will gradually increase at a continuous slower rate till a maximum is reached. Changes in the cellular physiology also affect the validity of the simplistic expectation. If bacteria are shifted to a low specific growth rate, regulatory mechanisms are triggered. This is called the stringent response. During this response the protein content of cell increases (in the formation of biomass protein synthesis demands the most energy). The maintenance will also increase (Stouthamer *et al.*;1990). Finally, the ever changing quantity and quality of the influent of a wastewater treatment plant is an important factor. At a sudden decrease of the energy content of the influent the population is faced with starvation. If part of the population dies, the performance of the plant might be reduced.

If we want to predict the increase in sludge production and carbon dioxide evolution in a treatment plant, we need to cover many physiological and ecological phenomena. In view of all this our research strategy is as follows:

1 Investigation of the degree of mineralisation in a recycling fermentor. For that purpose activated sludge from an oxidation ditch has been cultured on both synthetic medium and wastewater. Some results will be discussed in the next paragraph.

2 Use of mass balances (of nitrogen, oxygen and carbon) to describe the behaviour of subpopulations. These are used to estimate the growth of autotrophic nitrifiers in a recycling fermentor.

3 Determination of the physiological status of the whole population in the recycling fermentor. This will be done to explain the change in energy expenditure.

4 Investigation of the of protozoa and metazoa. Predation by these organisms introduces an extra step of mineralisation. Moreover this prevents the activated sludge from aging and increases the viability. This is part of a co-project and will be dealt with by C.H. Ratsak and S.A.L.M. Kooijman.

5 Interpretation of the results by a mathematical model. The model was developed and tested for a variety of higher organisms by Kooijman (1986). The model is based on the energy budgets of individual organisms. Last year it was further developed for bacterial monocultures under chemostat like conditions (S.A.L.M. Kooijman *et al.*; submitted). The investigations will be extended to monocultures in dynamic systems. The implementation of the model in an ecological context is subject of future research.

SOME EXPERIMENTAL RESULTS AND DISCUSSION

Activated sludge from an oxidation ditch, in which domestic wastewater is treated, was cultured in a recycling fermentor on both synthetic and natural wastewater. The biomass was retained by an internal or external membrane unit with 0.22 μm pore size. The pH was kept constant between 6.8 and 7.0. The carbon and nitrogen content of the synthetic medium was 3 mM glucose, 3 mM acetate, and 5 mM ammonium chloride. The provision rates were varied; the hydraulic loads are less than one day and the food to microorganism ratio's are comparable to those of oxidation ditches. The real wastewater varied in quality: 100 to 500 mg carbon per litre, and 1 to 5 mM ammonium. The carbon source was mainly acetate, while the nitrogen supply was mainly ammonium. The carbon concentration in the influent expressed as acetate equivalents varied from 4 to 20 mM. Samples were regularly drawn to determine biomass concentration, nitrate concentration and effluent quality. The gas exchange was continuously measured by mass spectrometric analysis.

In all experiments the increases in biomass concentration, the rate of carbon dioxide production and oxygen consumption and nitrate formation followed the same pattern. During the first ten days the biomass concentration increased, while the supplied ammonium was not completely converted to nitrate. After this period of adaptation, biomass concentration increased slowly, while ammonium that was not incorporated in biomass and excreted proteins, was fully nitrified. After that, 70-80% of the supplied carbon was mineralized to carbon dioxide (see Table 1 and Fig. 1).

At times, part of the microbial population lysed. This resulted in an immediate increase of carbon dioxide production, oxygen consumption and nitrate formation. This oxidation of biomass is called cryptic growth. This

phenomenon indicates prevention of aging and loss of viability by turnover of biomass. See figure 1 for a typical example. After day 15 part of the biomass lysed. The dead biomass is immediately used by the remainder for mineralisation processes (see the peaks in oxygen consumption, carbon dioxide production and nitrate formation; the latter even exceeds 3 times the concentration of the supplied ammonium). In reactors with internal recycling filters the abundance of protozoa and metazoa increased. Especially rotifers, thecamoebae and ciliates increased in number. This was not observed in fermentors with external recycling filters. The shear in those fermentors is considerable higher. Shear is known to damage these organisms. In both modes the number of flagellates increased. These increments also indicate that aging of bacterial biomass is prevented.

In one experiment the carbon to nitrogen ratio (C/N) of the influent was 4 times increased. After 39 days the glucose and acetate concentrations were increased while the ammonium supply remained the same. Before the shift-up the culture behaved similarly to the previous ones. After that, the heterotrophic biomass concentration rapidly increased. The nitrate concentration in the culture felt to zero, because it was all built in biomass (see Fig. 2). For that reason the heterotrophs also used all ammonium. The autotrophs disappeared, probably because they were starved or predated. After one week the growth of the heterotrophs stabilized. The culture lost the ability to nitrify and therefore the ammonium concentration increased (see Fig. 2). This shows that nitrification heavily depends on the carbon to nitrogen ratio.

We determined the production and consumption rates of components containing carbon, nitrogen and oxygen. We were thus able to construct mass balances. Therefore, the accuracy of the measurements can be determined by calculating the recovery of each element. This procedure is indispensable for scientific reasons, but, as far as we know, it has not been

used before in wastewater research. Mass balances allow one to predict the magnitude of one missing component. We calculated the growth behaviour of autotrophic nitrifiers, because the carbon dioxide fixation of these bacteria can be made explicit. This method showed that at the end of the experiment the nitrifying biomass was a considerable part of the total biomass.

Twenty years ago Gaudy *et al.* (1970) investigated the growth behaviour of activated sludge and the stability of purification of synthetic wastewater in a reactor with complete sludge retention. They showed that biomass retention results in stabilization of sludge production (i.e., increases in biomass concentration were followed by decreases accompanied with sludge oxidation). During this experiment, which lasted for two years, the performance of the reactor didn't change. Unfortunately, they used glucose as sole carbon source. Sugars have a high energy content and do not occur in domestic wastewater. Moreover, the substrate was supplied intermittently, while the bacteria had to survive an anaerobic phase. This procedure could have selected a population which is able to grow anaerobically and is able to take up substrate rapidly. More recently Chaize and Huyard (1991) treated wastewater in a reactor with membrane retention. Their results were principally the same: sludge production is stabilized, while the effluent had a low carbon content.

The results of Gaudy *et al.* and Chaize and Huyard are consistent with our conclusion that, from a biological point of view, biomass retention is a feasible alternative to the conventional treatment. However, much physiological research remains to be done.

LITERATURE

Chaize S. & Huyard, A. (1991): Membrane bioreactor on domestic wastewater treatment sludge production and modelling approach; Wat. Sci. Technol. 23:1591-1600

Gaudy, A.F., Ramanathan, M. Yang, P.Y. & DeGeare, T.V. (1970): Studies on the operational stability of the extended aeration process; J. Wat. Poll. Contr. Fed. 42:165-179.

Kooijman, S.A.L.M. (1986): Population dynamics on basis of budgets. In: The dynamics physiologically structured populations (eds. J.A.J. Metz & O. Diekman) Lecture notes in biomathematics, Springer Verlag, Heidelberg. p 266-297

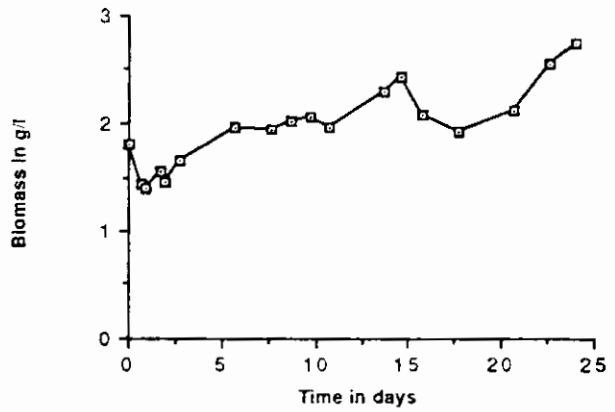
Kooijman, S.A.L.M., Muller, E.B. & Stouthamer, A.H.: Microbial growth dynamics on the basis of individual budgets; submitted

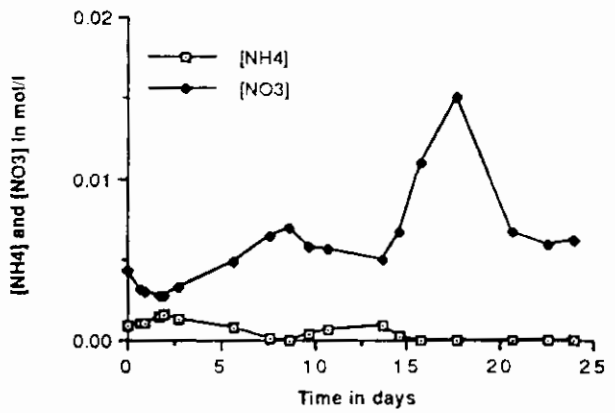
Stouthamer, A.H., Bulthuis, B.A. & Verseveld, H.W. van (1990): Energetics of growth at low growth rates and its relevance for the maintenance concept. In: Microbial growth dynamics (eds. R.K. Poole, M.J. Bazin & C.W. Keevil). SGM Special publications IRL Press, p85-102.

Experiment	SYNTHETIC WASTEWATER A	SYNTHETIC WASTEWATER B	REAL WASTE-WATER
Duration of experiment (days)	40	38	60
Substrate provision rate (mmol per liter culture per day)			
- glucose	3.0	6.2	
- acetate	3.0	6.2	4-20[1]
- ammonium	5.0	10.4	
Maximal oxygen demand (gram per liter culture per day)	0.75	1.6	
with nitrification	1.0	2.3	
% carbon recovery	98.4	97.8	90[2]
% carbon to biomass	9.3	4.5	7
% carbon to carbon dioxide	78.0	79.2	72
% carbon to effluent	9.0	13.3	7
% carbon lost by sampling & technical failure			5
% nitrogen recovery		97.2	.2
% nitrogen to biomass		8.9	
% nitrogen to nitrate		67.1	
% nitrogen not (completely) nitrified		0.9	
% nitrogen to protein in effluent		18.3	

Table 1: Allocation of nitrogen and carbon in influent to biomass, carbon dioxide and components in effluent at two different hydrolic loads with synthetic waste water and one with real waste water of varying quality. [1] the carbon content of the waste water is expressed as acetate equivalents; see text for explanation. [2] The calculations with data from the experiment with real waste water are only prelimenary, because not all components are yet determined.

Figure 1: Biomass concentration, ammonium and nitrate concentration, and gasevolution during the first 25 days of the experiment denoted as A in table 1.





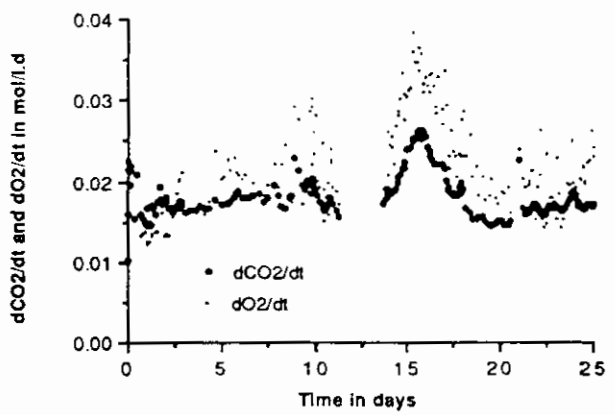
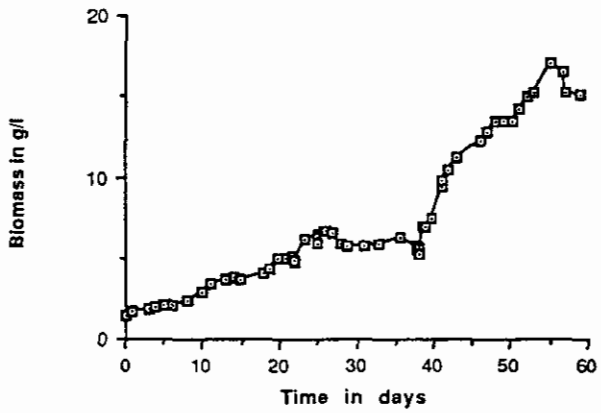
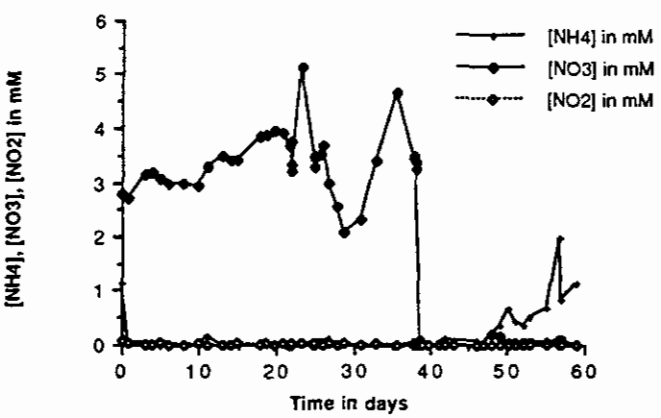


Figure 2: Increase of biomass concentration and change of the nitrate and ammonium concentration after a shift-up of the glucose and acetate but not the ammonium concentration at day 39. Conditions and results of the first 39 days are summarized in Table 1 under experiment B





CONTROL AND AUTOMATION ASPECTS IN BIOLOGICAL DENITRIFICATION PROCESSES IN JAPAN

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CONTROL AND AUTOMATION ASPECTS IN BIOLOGICAL DENITRIFICATION PROCESSES IN JAPAN

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ABSTRACT

We tried to monitor directly NH4-N and NOx-N in a mixed liquid of biological denitrification reactor by using UF unit.

The problem of clogging in the automated analyzer was solved by the UF treatment prior to the determination. New automated NH4-N analyzer consists of a gas separation unit and a deteciton unit. For more than 6 months, the NH4-N monitoring system has worked continuously.

For determination of NOx-N in a mixed-liquid of bioreactors, we developed UV 2 wavelengths (220nm & 250nm) absorption method to eliminate color interference. This is a simple and fast method, and applicable to any biological denitrification reactor(municipal wastewater and collective human excreta etc.)

Also we tried to control the feed air ratio in a biological denitrification reactor by NH4-N itself. Direct AmmoniUm Control system [DUC system] using fuzzy inference enabled quick response and high nitrogen removal.

KEYWORDS

biological denitrification; control; NH4-N; NOx-N; UV absorption; ultra-membrane-filtration; fuzzy inference; human excreta; automated analyzer;

INTRODUCTION

With the remarkable development of micro-processing technology, in many wastewater treatment plants, automation of data logging and process monitoring have made possible. Control technologies have been also popularized, but in biological treatment processes (aeration reactor etc.) popularizations of control technology are not so fast due to restriction of development of water-quality sensors.

Application in plant use, water-quality electrodes (DO, ORP, pH, NH4-N etc.) have some problems such as reliability, reproducibility and response speed.

Recently, magnetic-stirrer-type electrodes have been developed to solve these problems.

In this report, we studied as an application of this type automated NH4-N analyzer in a biological denitrification reactor.

In recent years, the biological denitrification technology has considerably

been improved especially in collective human excreta treatment plants in Japan. In order to cope with the stringent effluent quality regulations set for human excreta treatment plants, the non-dilution and high-load biological denitrification process with ultra-membrane-filtration for solid/liquid separation has been developed.

In this study, a typical example of this process is reported. Also we studied an application of direct NH4-N control system using fuzzy inference to a non-dilution high-load plant.

1. MAGNETIC-STIRRER-TYPE CONTINUOUS POLISHING SYSTEM FOR ELECTRODES AND IT'S APPLICATION TO AUTOMATED ANALYZERS.

Magnetic-stirrer-type continuous polishing system for electrodes was developed for automated analyzers. The surface of electrode membranes is polished by an abrasive or a plastic coated magnet bar which is driven by a motor to eliminate adsorbing substances and reaction products brought in measurements(Fig.2). The reproducible Nernstian responses were obtained for the ion-selective ceramic and metal electrodes for a long term.

The system could be efficiently applied to automated analyzers such as for total cyanide, ammonia, ORP and pH.

FIG-1 shows appearance of this type of pH detector. The detector is set in a sample solution and both pH glass electrode and reference electrode are continuously polished. The detector is calibrated by injecting pH7 and pH4 standard solutions through compressed air (carrier) tube to pH glass electrode without pulling up.

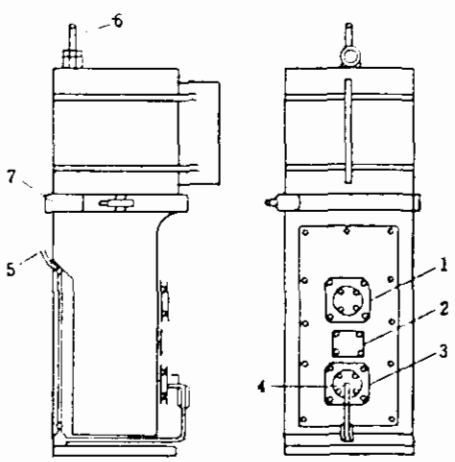


Fig.1. Appearance of the magnetic-stirrer-type continuous polishing system for pH detector.
1: reference electrode; 2: temperature-compensating electrode; 3: pH glass electrode; 4: nozzle; 5: compressed air & standard solution tube; 6: signal cable; 7: coupling band

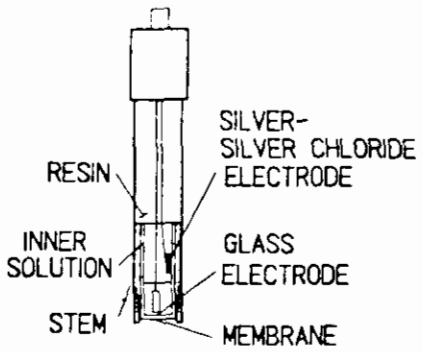
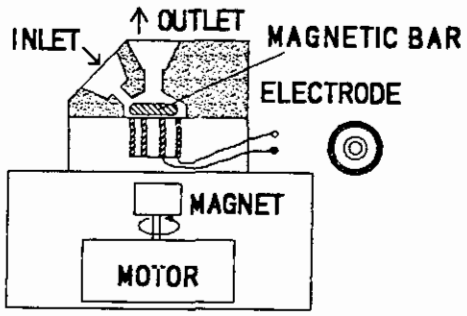


Fig.3. Sectional view of a conventional type gas permeable-membrane electrode.

FIG-2 shows sectional view of a magnetic-stirrer-type continuous polishing conductivity cell of NH_4-N automated analyzer. FIG-4 is schematic diagram of this analyzer. FIG-3 shows a conventional type gas permeable-membrane electrode. In this new automated NH_4-N analyzer, the gas sepatation unit having a gas-permeable membrane is separated from the detector unit measuring electric conductivity and both units are set in thermo-controled boxs. NaOH injection into a sample turns ammonium ion to ammonia gas. The gas separation unit collects only an ammonia gas and again turns to ammonium ion into the carrier solution (dil H_2SO_4) which reaches the electric conductivity cell.



SECTIONAL VIEW OF CONDUCTIVITY CELL

Fig.2. Sectional view of magnetic-stirrer type continuous polishing conductivity cell of NH4-N automated analyzer.

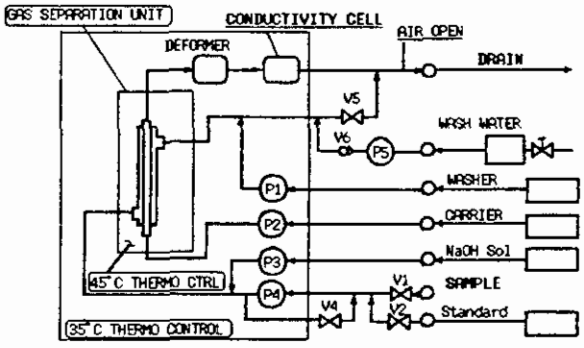


Fig.4. Schematic diagram of automated analyzer for ammonia.

Table-1 shows the features of the improved analyzer. Rate determining step of a conventional electrode is the diffusion process (reaction speed several 10sec-several 10minutes). On the other hand, the improved analyzer can minimize the thickness of diffusion layer by strong turbulence of stirrer, then response speed can be minimized.

TABLE 1 Comparison of magnetic-stirrer type conductivity method with conventional type electrode.

I T E M	Conductivity method	Conventional method
Measureing method	concentration with membrane tube and measurement by coductivity	gas permeable membrane electrode
Measurement range	0 - 0.1 mg/l N to 0 - 100 mg/l N variable	0 - 20 mg/l N
Output charactaristic	linear to NH4-N concentration	logarithmic to NH4-N concentration
Effect of temperature	small (separation unit and detector unit are set in thermo-controlled box)	large (electrode is directly dipped in a sample solution)
Response speed	quick	slow
Sensitivity	min 0.002mg/l N	min 0.04mg/l N
Washing period	every time after detection	periodically

2. REAL-TIME MONITORING OF AMMONIUM NITROGEN IN A BIOLOGICAL DENITRIFICATION TANK USING MAGNETIC-STIRRER TYPE AUTOMATED ANALYZER PRETREATED WITH UF UNIT.

MONITORING SYSTEM

FIG-5 shows the flow of the real-time monitoring system. The system is placed in a non-dilution and high-load biological denitrification pilot plant treating collected human excreta(10kl/day).

UF unit consists of a tubular UF(made of poly-olefin 11.5ø) and a recirculation pump. The MLSS of the reactor is as high as 15,000-18,000mg/l, but SS in permeate is 0mg/l to prevent clogging of sampling tube. FIG-6 shows the specification of the automated NH_4-N analyzer.

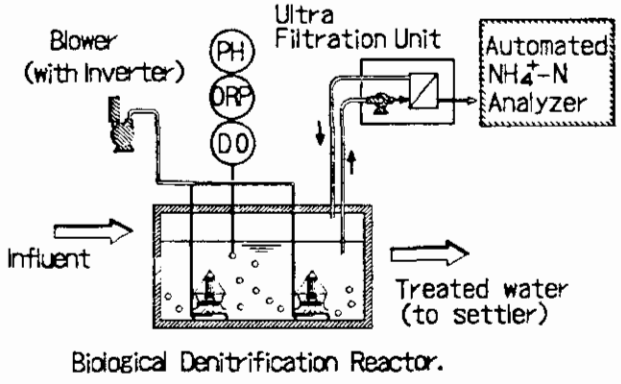


Fig.5. Flow diagram of real-time monitoring system.

Specification of the NH4+ N AUTOANALYZER

Measurement	Information
Method	Concentraiton by Membrane and Conductivity
Range	0-10 mg/l N(Standard) 0-0.1mg/l to 0-100mg/l N (Option)
One Cycle	30min or 60min
Accuracy	±5% of full Scale
Size(mm)	W500 x D570 x H700
Weight(kg)	60
Supplier	KIMOTO ELECTRIC CO.,LTD.
Model	VS-3820

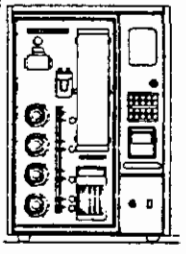


Fig.6. Specification of NH4-N automated analyzer.

RESULT AND DISCUSSION

FIG-7 and FIG-8 show NH4-N values measured once an hour. For more than 6 months this system has worked continuously in the plant only with supply of reagents and chart paper. This system can be maintained very easily.

TABLE-2 and FIG-9 show the analytical data of NH4-N in the bioreactor by this system and by distillation-titlation method. As shown in FIG-9, the results of parallel analysis of 47 samples obtained by both methods agreed well throughout the analytical range. The statistical data demonstrating the agreement was as follows: r=0.992; y=0.98x+1.88; n=47.

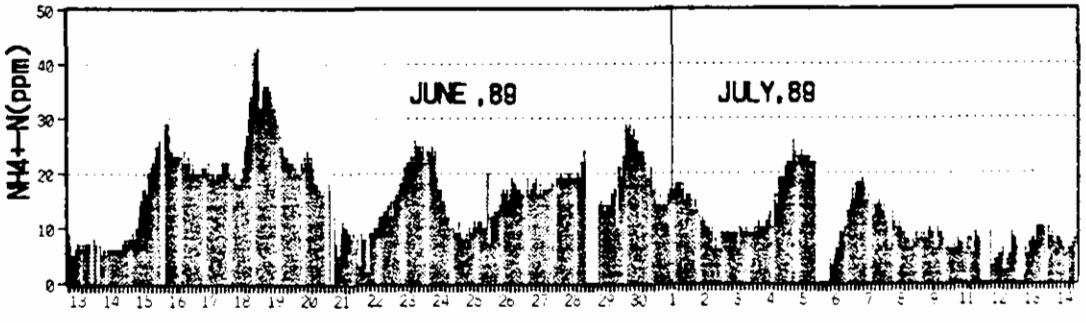


Fig.7. Values of NH4-N monitored once an hour.

TABLE-2 Comparison of NH_4-N values(mg/ℓ)

date	NH_4-N values(mg/ℓ)	
1989	Automated analyzer	Distillation titlation method
5/18	0	1.65
23	1	0.91
24	22	29.2
25	1	1.68
26	0	1.33
28	4	5.46
29	3	4.34
30	3	4.13
31	3	3.92
6/1	4	3.57
2	3	4.73
3	2	3.71
4	2	3.57
5	0	3.71
6	11	12.2
7	11	4.1
8	15	11.6
9	38	37.3
10	76	80
11	57	60.9
12	23	28
13	7	7
14	7	8.4
15	25	26.4
16	22	24.6
17	22	25.5
18	42	43.8
19	22	27.7
20	16	22.7
21	6	10
22	14	15.3
23	24	27.2
24	10	11.6
25	10	12.9
26	17	18.8
27	18	18.5
28	22	23.1
29	21	21
30	21	24.5

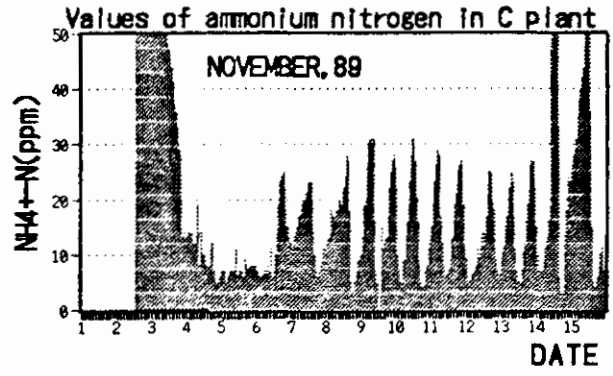


Fig.8. Values of NH4-N monitored once an hour.

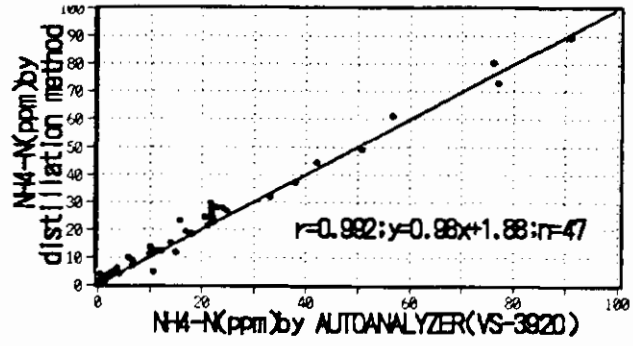


Fig.9 Relationship between NH4-N values found by the distillation/titlation method and by the automated analyzer.

3. MEASUREMENT OF OXIDIZED NITROGEN BY UV ABSORPTION METHOD IN A BIOLOGICAL DENITRIFICATION TANK.

To know the trend of inorganic nitrogen in a biological denitrification reactor, a simple rapid and reliable method to determine NOx-N is necessary in addition to rapid detection of NH4-N .

It is well known that NO2-N and NO3-N have strong absorbancy in the range (200nm-250nm) of UV spectra. But in this range, many substances have strong absorbancies, so determination of NOx-N by UV absorption is limited to clean water such as field investigation (sea, lake or river).

Effluents from collected human excreta treatment plant are colored brown. : elimination of color interference is very important.

We developed UV 2 wave-lengths absorption method to eliminate color interference.

ANALYTICAL METHOD AND SAMPLING PLANTS.

FIG-10 and FIG-11 show absorption spectra of NO2-N and NO3-N standard solution (1-25mg/l) respectively.

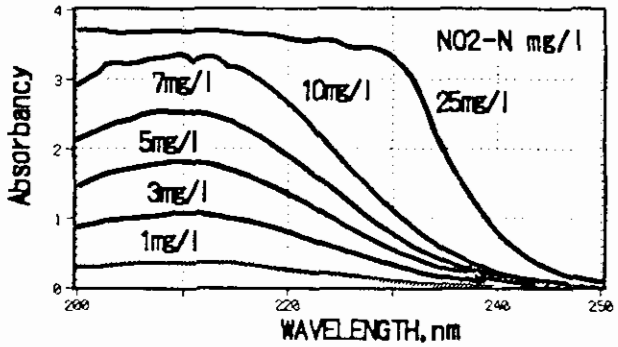


Fig.10. Absorption spectra of NO2-N standard solution.

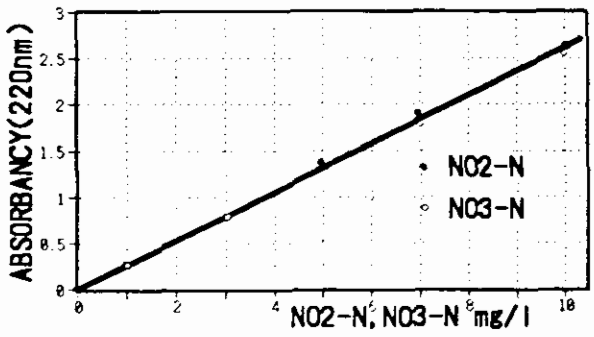


Fig.12. Calibration curve of NO2-N and NO3-N at wavelength 220nm.

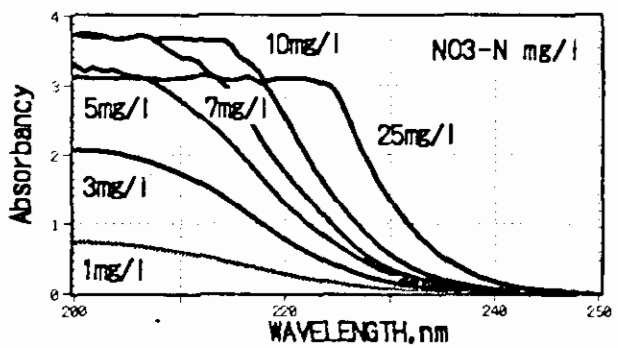


Fig.11. Absorption spectra of NO3-N standard solution.

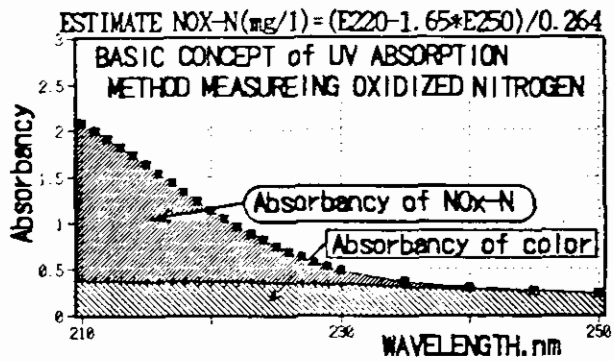


Fig.13. Basic concept of UV absorption method measuring NOx-N.

TABLE-3 NO_2-N, NO_3-N Concentration by Technicon analyzer and UV method in different plants.

PLANTS			Technicon Method N(mg/l)			UV Method N(mg/l)		sampling period
NAME	PROCESS	n	NO_2-N	NO_3-N	NO_x-N (σ)	NOx-N (σ)	E220'/E250(σ)	
A	D-N(2STAGE)	22	0.09	2.14	2.23(0.260)	2.24(0.335)	1.641(0.046)	90-9/25-10/8
B	D-N(2STAGE)	28	0.19	0.49	0.68(1.082)	0.68(0.976)	1.628(0.019)	90-9/24-10/7
C	AEROBIC DIG	24	0.05	0.03	0.08(0.041)	0.09(0.491)	1.825(0.032)	90-9/27-10/11
D	D-N(1STAGE)	10	0.06	12.48	12.72(1.657)	12.71(1.254)	1.241(0.156)	90-10/1-10/13

【ANNOTATION】(σ) :STANDARD DEVIATION.
E220' :ABSORBANCY eliminated that of NOx-N.
D-N :DeNitrification process.
AEROBIC DIG :AEROBIC DIGESTION process.

Absorbancies of NO2-N and NO3-N are almost zero at 250 nm or more.
FIG-12 is a calibration curve of NO2-N and NO3-N. As NO2-N and NO3-N have equal sensitivities at UV 220 nm , and absorbancy at 220 nm shows total NOx-N absorbancy. The UV spectrophotometer used in these experiments is a HITACHI U-2000 double-beam spectrophotometer with flow cell.
FIG-13 shows the basic concept of UV absorption method measuring NOx-N.
NO2-N and NO3-N concentration by the Technicon analyzer and UV method in four plants are shown in TABLE-3. Sample liquids from treatment plants were filtered by No.5C paper before measurement.

FIG-14 shows typical UV absorption spectra in different plants(dilution ratio=10). FIG-15 shows revised UV absorption spectra by eliminating absorbancy of NOx-N. This spectra is supposed to show absorption of color. These spectra from each treatment plants resembles very well and E220(absorbancy at 220nm)/E250 ratios are almost constant in these plants.

RESULT AND DISCUSSION

ORGANIC INTERFERENCE

According to previous studies, organic materials which have absorbancies in the UV range interfere NOx-N determination. However, data show no interference in this test because of decomposition of interfering materials.

COLOR INTERFERENCE

Sample solutions of each plants treating collective human excreta are colored brown. This color has absorbancy in UV range. It is obvious in this test that E220 (without NOx-N adsorption) has constant ratio to E250 in each plants. In this way interference by color can be eliminated by calculation.

COMPARISON OF METHODS

As shown in FIG-16, the results of parallel analysis of 60 samples obtained by both methods agreed well. The statistical data indicating the agreement was as follows.

r=0.998; y=0.990x + 0.03; n=60

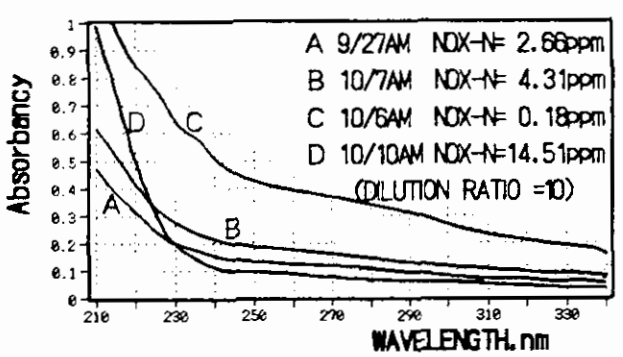


Fig.14. UV absorption spectra in each plants treating collective human excreta.

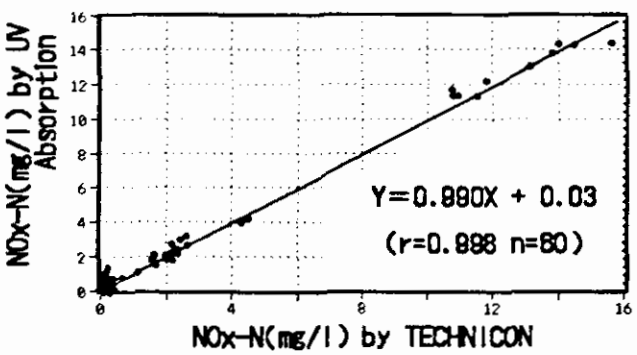


Fig.16. Comparison of NOx-N between UV method and TECHINCON method.

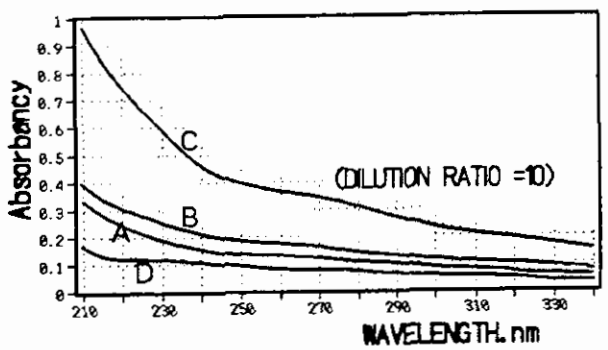


Fig.15. Revised UV absorption spectra in each plants by elimiating absorbancy of NOx-N.

4. TYPICAL CONTROL SYSTEM IN A NON-DILUTION AND HIGH-LOAD BIOLOGICAL DENITRIFICATION PROCESS IN JAPAN.

The treatment technology of collected human excreta has rapidly changed by the development of biological denitrification process in Japan.

And recently non-dilution high-load biological denitrification systems with high performance aeration devices have been operated. In these systems, Total-N removal ratio was increased and caustic soda to adjust pH and methanol for carbon source are disused.

Ultra-membrane filtration process is now applied for the solid/liquid separation step.

In this section, we study KUBOTA U-tube process as a typical excellent example applied in Japan.

GENERAL VIEW OF U-TUBE PROCESS.

Fig-17 shows a structure of U-tube deep reactor system. Standard composition of screened collected human excreta are as follows: BOD 12,000mg/l SS 18,000mg/l T-N 4,500mg/l PO_4^{3-} 1,000mg/l. This process is fed intermittently with pretreated human excreta and air, and the pH, DO, ORP are monitored.

Mixed liquid is fed to the top of air-liquid mixing pipe, where air is aspirated through the ejector. Efficiency of oxigen uptake rate is as high as about 50%.

In the U-tube reactor DO level is controlled about 0.5mg/l, nitrification and denitrification proceed simultaneously. Then BOD and Total-N are removed in the single reaction tank. Fig-18 shows vertical variation of DO levels in this reactor.

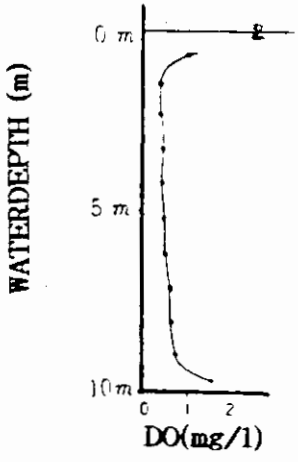


Fig.18 Vertical variation of DO levels in the reactor.

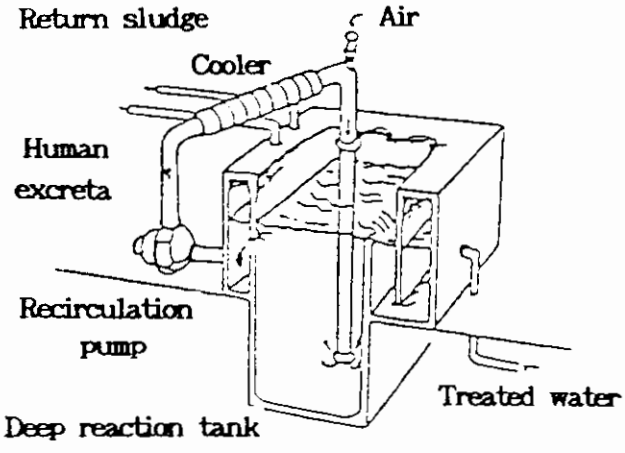


Fig.17 Structure of U-tube deep reactor system.

OPERATION AND CONTROL
OF U-TUBE PROCESS.

Fig.19 shows a operation control mode of U-tube process. Screened human excreta is fed intermittently once in 3 - 4 hours. Air flow rate is increased stepwise to follow the preset DO pattern by computer control.

Rapid increase of DO level in the final part of the cycle means ending of nitrification and denitrification. That is to say, rapid increase of DO level shows appropriate air volume was fed in this cycle.

The computer inputs total air volume fed in the cycle and average DO level at the final part(in 5 - 10min), then outputs air feed volume to next cycle.

In this way, DO levels in the U-tube reactor are automatically controlled in the range about 0.5 mg/l.

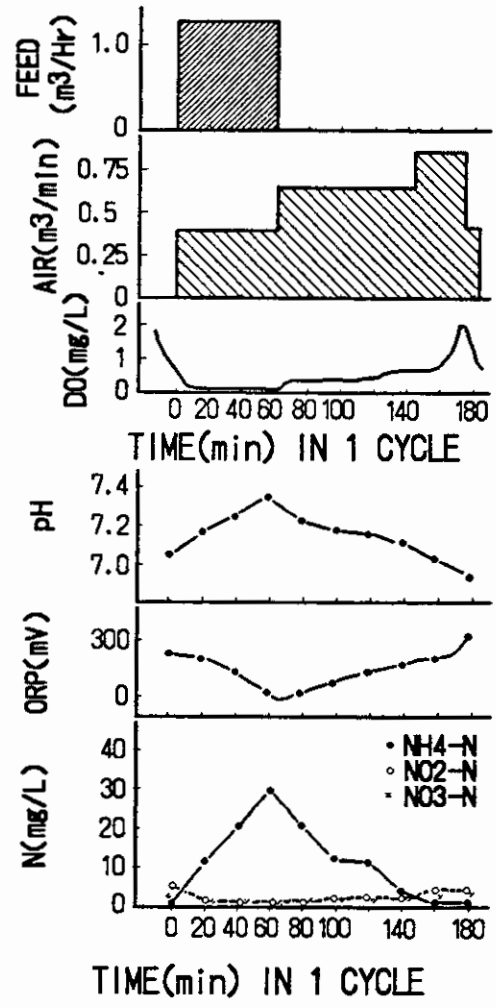


Fig.19 Operation control mode of U-tube process.

5. NEWLY DEVELOPED DIRECT AMMONIUM CONTROL SYSTEM USING FUZZY INFERENCE.

The U-tube process just mentioned is a very excellent control system and T-N removal rate exceeds 99% or more. But this system utilizes DO level and DO trend pattern, intermittent feed is necessary and 1 cycle consumes 3-4 hours.

NH4-N is one of the most difficult substance to be removed in a biological denitrification process. NH4-N had been controlled so far by indirect indexes such as DO, ORP, pH etc., because NH4-N is invisible and no automated analyzer had been served due to less stability and disturbance of SS compornents.

SEQUENCIAL PROGRAM OF DIRECT AMMONIUM CONTROL.

We tried a direct ammonium control system by using the improved NH4-N automated analyzer with UF unit. Fig.20 shows the flow diagram of this pilot plant. Volume of the biological reactor is 38m³ (2.2mϕ × 10mD), and highly efficient submerged axial flow aerator and air blower with inverter-motor are used for aeration devices. Screened human excreta was fed continuously (10kl/d), and aeration of this reactor was intermittent(30 min / cycle).

Fig.21 shows an example of sequential control program. Fig.22 shows a daily change of NH4-N value. As the result, NH4-N value varied up and down periodically because of time lag in the control program. This control program was not proper in this case. But it it obvious that NH4-N can be controlled by feeding appropriate air.

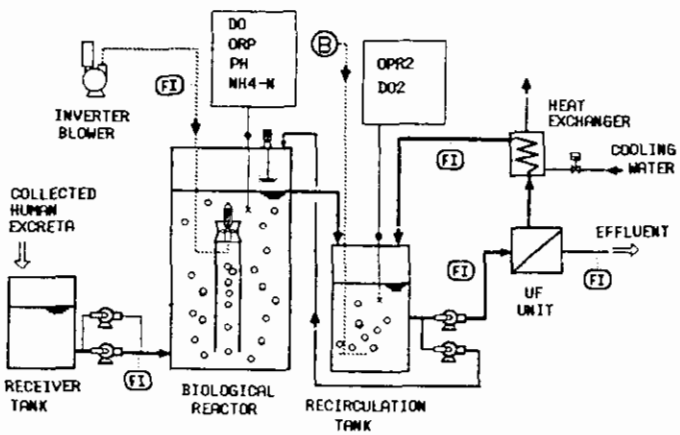


Fig.20 Flow diagram of pilot plant.

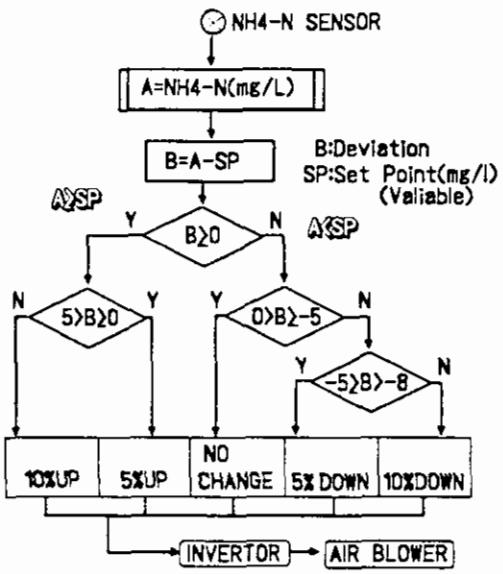


Fig.21 An example of sequential control program using ammonium analyzer.

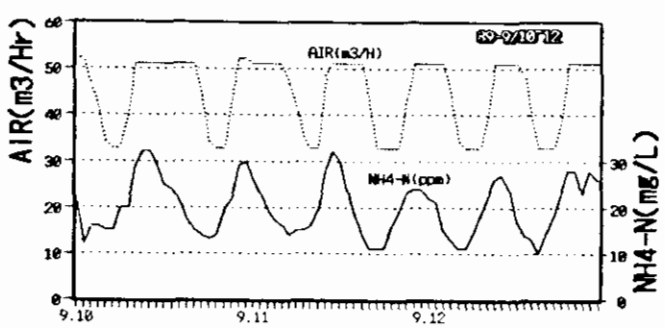


Fig.22 Daily changes of NH4-N value.

DIRECT AMMONIUM CONTROL USING FUZZY INFERENCE.

Biological treatment such as denitrification is a complex reaction of many unit operation and biological reaction. In such treatment, using single index to control has limitation. Fuzzy control is a multi-index control, we used fuzzy inference combined with direct ammonium control system.

Human excreta was fed continuously and aeration of this reactor was intermittent(30min/cycle).

Fig.23 shows the control system using fuzzy inference. Indexes used are DO, ORP, pH and NH4-N, output is air flow rate. As this fuzzy control system changes output every 5 minutes, this system is stable and performs quick response.

Fig.24 shows trend pattern of each indexes. ←values show set point of each indexes. Fuzzy controller takes each deviations and adequate air flow rate is delivered to inverter.

In Fig.24, feed of human excreta was rapidly increased 2.4times and continued 2 hours. Fuzzy controller increased air flow rate rapidly and within 2 hours, NH4-N concentration recovered.

Very flexible control can be done using direct ammonium control system with fuzzy inference.

In this test, we used ORPX-system (fuzzy control software of OC Engineering.) and 16bit personal computer (i80286 + i80287) set.

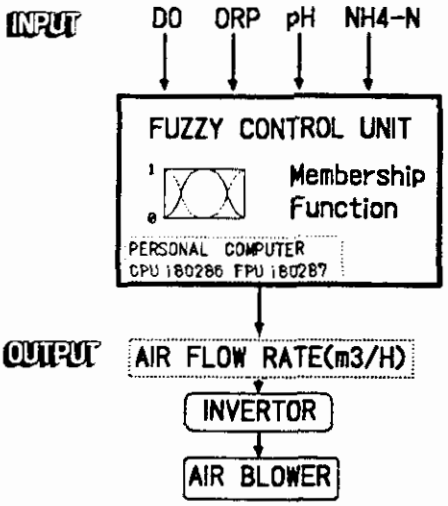


Fig.23 Basic concept of fuzzy control system.[FUZZY-DUC]

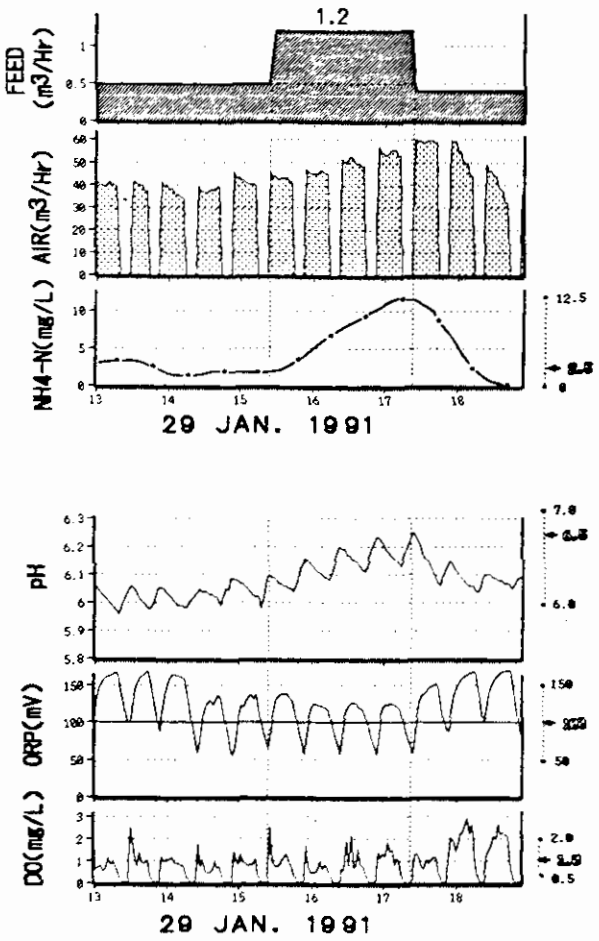


Fig.24 Operation data of FUZZY DUC system.

REFERENCES

Aoi,T.,Hirata,H.,Okaniwa,Y.,Motomura,K.,Toyazaki,Y.,Miura,A.,(1990): Water pollution research and control, Kyoto, Jly29-Agst3, pp5-8

Aoi,T.,Okaniwa,Y.,Iida,M.,Motomura,K.,(1990): Water pollution research and control, Kyoto, Jly29-Agst3, pp1-4

Magara,Y.,Itoh,M.,(1990): Wat.Sci.Tech. Vol.23, Kyoto, pp1583-1590

Sato,T.,Ishii,Y.,(1990): Wat.Sci.Tech. Vol.23, Kyoto, pp1601-1608

Ishida,K.,Izumi,K.,(1987): MIZUSYORI GIJUTU, pp579-590

Hirata,H.,(1990): BUNSEKI KAGAKU, ppT157-T161

Pederson,K.M.,Kümmel,M.,Søeberg,H.,(1990): ICA, Proceedings of the 5th IAWPRC Workshop, pp171-178

Teichgräber,B.,(1990): ICA, Proceedings of the 5th IAWPRC Workshop, pp179-185

EFFLUENT 2000 JAPAN

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Effluent 2000 Japan
(Water Pollution and Eutrophication Measurement for Enclosed Water Areas in Japan)

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Introduction

Eutrophication preblems in long and narrow islands surrounded with great oceans such as Japan where is surrounded with the pacific ocean and the Japan sea might be different from them in continental countries where have many inner lakes. Because the carrying capacity for N and P in a great ocean is very larger than that in inner lakes.

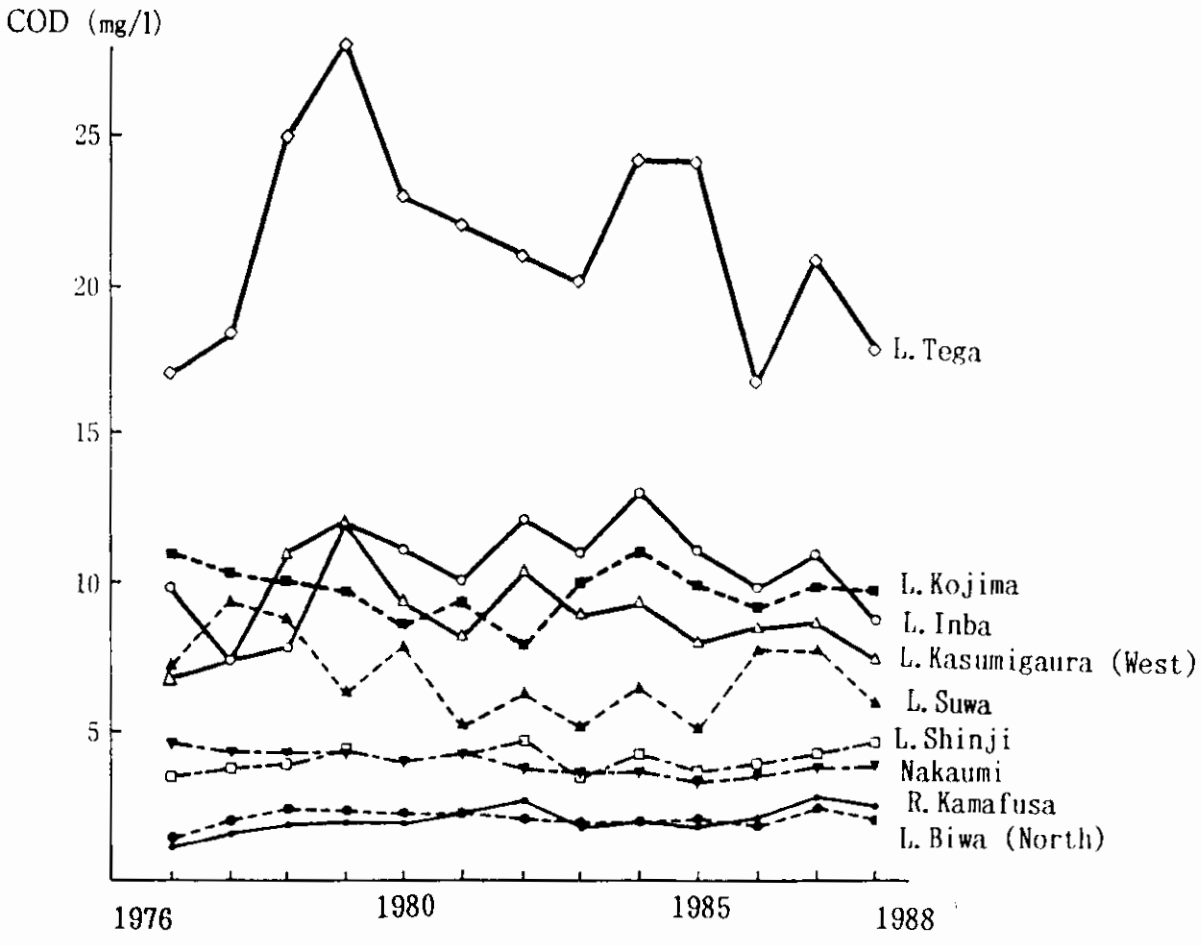
In this paper, we report about 1) An outline of water pollution in the enclosed water areas of Japan, 2) Strengthening of area wide total pollutant load control system for COD, 3) Environmental quality standards for N and P in lakes and reservoirs, 4) Approach to enactment of environmental quality standards for N and P in enclosed coastal seas, 5) Future and recent progress in wastewater treatent techniques and 6) Fundamental strategies for reduction of pollution load for COD, N and P discharged into the enclosed water areas in Japan.

Water Pollution in the Enclosed Water Areas of Japan

In the enclosed or semi-closed water areas where little exchange of water take place and pollutants contained in the water are prone to accumlate, above all population and industries concentrate, water pollution is a very serious problem. The environmental quality standards (EQS) for COD_{Mn} have not met in the representative lakes in Japan such as lake Biwa, lake Kasumigaura, or lake Suwa. The transition of COD_{Mn} in the representative lakes is shown in Fig. 1.

Water pollution in enclosed sea areas is actualized in the compliance ratio

Figure 1. The Transition of COD_{Mn} in the Representative Lakes in Japan.



Source: Japan Environmental Agency

with EQS or outbreaks of red tide. The transition of compliance ratio with EQS for COD_{Mn} in the representative enclosed sea areas such as Tokyo bay, Ise bay and Seto Inland Sea is shown in Fig. 2. Yearly changes in the number of confirmed outbreaks of read tide in the Seto Inland Sea is shown in Fig. 3.

Strengthening of Area Wide Total Pollutant Load Control System for COD

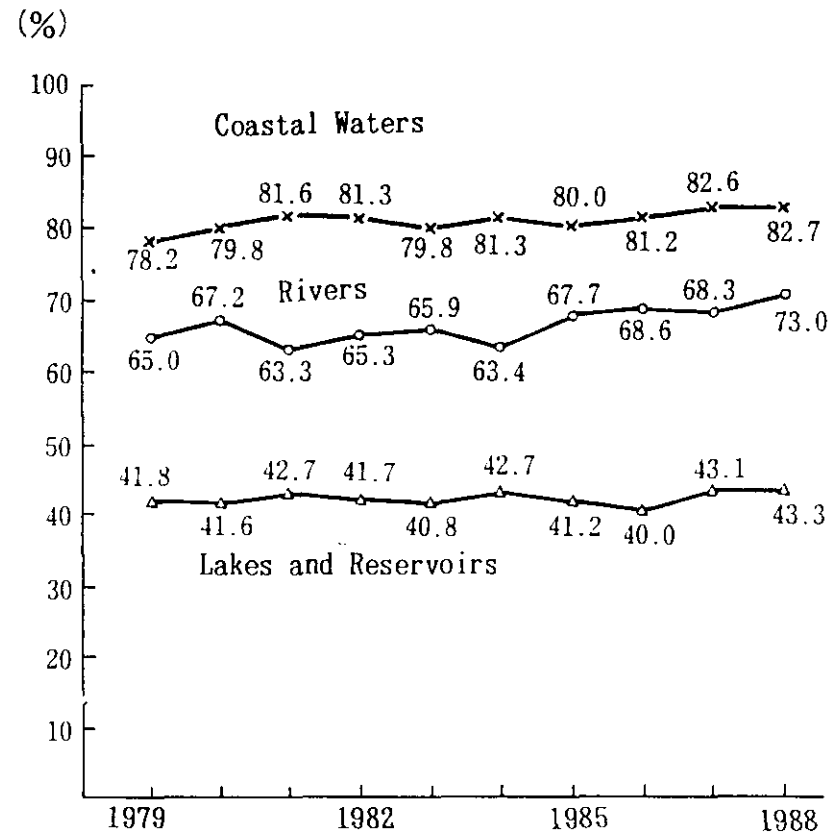
Since 1979, the Area Wide Total Pollutant Load Control System for COD_{Mn} has introduced to Tokyo bay, Ise bay and Seto Inland Sea as shown in Table 1. With this system, the following measures have promoted strongly with the first control from 1979 and the second control from 1987.

1) Determinating the control standards for the total COD load discharging into the area.
2) Promoting the measures concerning municipal and domestic wastewater including expansion of areas coverd with the sewage treatment system.
3) Determinating the control level of COD load that is discharging from each industrial plant.

As organic water pollution in these 3 sea areas, however, has not dissolved yet and population or industrial products have a tendency to increase still, the third control is being planned to further reduce the total COD load as follows.

1) Promoting construction of domestic wastewater treatment facilities.
2) Promoting introduction of advanced treatment in domestic wastewater treatment facilities.
3) Promoting practical activity to make reduce domestic pollutant load in the genaration stage.
4) Strengthening of control measure to industrial plants that are discharging high COD load in considering impartiality of control measure.
5) Strengthening of control measure to industrial plants that are regulated already.
6) Application of harder control measure to new constracted industrial plants.
7) Enlargement of control measure to small scale plants where their

Figure 2. The Transition of Compliance Ratio with Enviromental Quality Standards for COD_{Mn}.



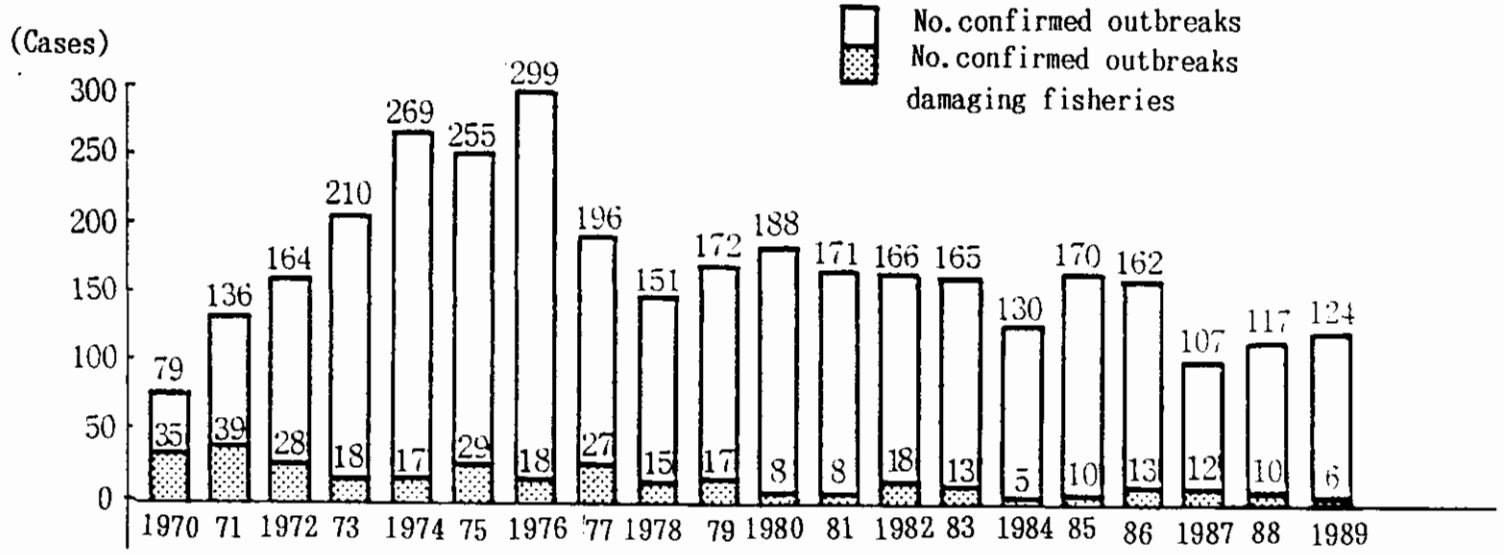
Notes: 1. "National average" denotes the mean value of sea areas except semi-closed water areas (The Seto Inland Sea, Tokyo Bay and Ise Bay).

2. Compliance ratio with EQS

$$= \frac{\text{No. of water areas complying with EQS}}{\text{No. of water areas to which EQS is applied}} \times 100$$

Source: Japan Environmental Agency

Figure 3. Yealy Changes in the Number of Confirmed Outbreaks of Red Tide in the Seto Inland Sea.



Source: Fishery Agency

Table 1. Area Wide Total Pollutant Load Control for COD_{Mn} applied to in the Tokyo bay, the Ise bay and the Seto Inland Sea.

	Actual COD Loads in Generation Stage (ton/day)			Goal of COD Reduction (ton/day)	Ratio of Reduction (%)	
	1979	1984	1988	1989	89/84	89/79
Tokyo bay						
Domestic Sewage	324	290	255	249	86	77
Indust. Wastewater	115	83	80	78	94	68
Others	38	40	38	38	95	100
Total	477	413	373	365	88	77
Ise bay						
Domestic Sewage	151	150	145	140	93	93
Indust. Wastewater	119	101	99	98	97	82
Others	37	35	34	34	97	92
Total	307	286	278	272	95	89
Seto Inland Sea						
Domestic Sewage	486	443	416	402	91	83
Indust. Wastewater	429	367	358	355	97	83
Others	95	89	88	87	98	92
Total	1010	899	862	844	94	84
3 Sea Areas						
Domestic Sewage	961	883	816	791	90	82
Indust. Wastewater	663	551	537	531	96	80
Others	170	164	160	159	97	94
Total	1794	1598	1513	1481	93	83

COD load from Pulp and Paper industries occupies 27 % of the total COD load from all industrial wastewaters within these 3 sea areas.

wastewaters have not regulated.

8) Taking measures for livestock wastewater.

9) Taking measures for aquarculture wastewater.

10) Taking measures for the removal of bottom sediments.

For reduction of COD load from industrial plants, severer effluent standards for COD than national effluent standards are applied to the designated industrial plants where the Area Wide Total Pollutant Load Control System is applied.

Environmental Quality Standards for N and P in Lakes and Reservoirs

According to progress of eutrophication, many lakes and reservoirs in Japan had been damaged by outbreaks of green or red algae for their water use such as drinking water or fishing. In order to control eutrophication of lakes and reservoirs, Environmental Quality Standards and Effluent Quality Standards for N and P in Lakes and Reservoirs as shown in Table 2 and 3 were established in 1985.

The effluent quality standards for P are applied to all eutrophicated lakes and reservoirs. That for N of are applied to lakes and reservoirs where nitrogen is considered to be a limiting factor for algal growth on account that the N/P ratio is less than 20 on the weight base, P concentration is over 0.02 mg/l, AGP test results and so on.

The categories of environmental quality standards for N and P have been designated in 44 water areas in 40 lakes. And the effluent quality standards have been applied to 1,066 lakes for P and 78 lakes for N respectively.

Approach to Enactment of Environmental Quality Standards for N and P in Enclosed Coastal Seas

In order to establish the environmental quality standards for N and P in Enclosed Marine Coasts, the investigation is continued by a working group of Japan Environmental Agency. A result of this investigation for 3 years from 1987 till 1989 was demonstrated as a interim report. This outline is as follows.

1) Analysis of correlation among water quality items

Table 2. Environmental Quality Standards for N and P in Lakes and Reservoirs.

Category	Purpose of water use	Standard Values	
		Total-Nitrogen	Total-Phosphorus
I	Conservation of Natural Environment, Uses listed in II, III, IV, V.	0.1 mg/l or less	0.005 mg/l or less
II	Water supply class 1, 2, 3, Fishery class 1, Bathing, Uses listed in III, IV, V.	0.2 mg/l or less	0.01 mg/l or less
III	Water supply class 3, Uses listed in IV, V.	0.4 mg/l or less	0.03 mg/l or less
IV	Fishery class 2 Uses listed in V	0.6 mg/l or less	0.05 mg/l or less
V	Fishery class 3, Industrial water, Agricultural water, Conservation of Environment.	1.0 mg/l or less	0.1 mg/l or less

Conservation of natural environment : Conservation of scenic points and other natural resources.

Conservation of : Up to the limits at which no unplesantness is caused to the people in their daily lives including a walk along the shore.

Table 3. Effluent Quality Standards for N and P into Lakes and Reservoirs.

Standard Values					
Total-Nitrogen	:	Maximum	120 mg/l,	Daily Average	60 mg/l or less
Total-Phosphorus	:	Maximum	16 mg/l,	Daily Average	8 mg/l or less

Effluent Quality Standards for P are adopted to all eutrophic lakes and reservoirs.

Effluent Quality Standards for N are adopted to the eutrophic lakes and reservoirs where N is understood as a limiting factor for phytoplankton growth.

Provisional standards within limited 5 years are adopted to the industrial wastewater that it is difficult to adopt the standards immediately.

From analysises of many water quality data in 14 japanese marine coastal areas such as Tokyo bay, Osaka bay, Ise bay, Mikawa bay, Hiroshima bay, Hakata bay, Harimanada, Kagoshima bay, Saroma lake and Sagami bay, the relationships between 2 kinds of water quality items are shown as Table 4.

2) Algal growth potential test in each water area

In order to confirm the limiting factor for phytoplankton production, the algal growth potential (AGP) test was adopted to water samples in these areas. From the AGP test of 469 samples, the limiting factor for phytoplankton production was proved as shown in Table 5.

In 469 tested samples, the limiting factor was N for 173 samples (37 %), P for 150 samples (32 %), both N and P for 48 samples (10 %), other matters such as vitamin B_{12}, F_e etc. for 84 samples (18 %), unknown for 14 samples (3 %) respectively. Therefore, N and P occupied about 80 % as the limiting factor in these water areas.

As the limiting factor for each phytoplankton class, N and P occupied about 90 % in Mixed classes, 80 % in Bacillariopkyceae and Rhaphidophyceae, about 60 % in Eugenophyceae, about 37 % in Dinophyceae respectively.

3) Water Quality Troubles in Japan Coastal Seas

Water quality troubles caused by eutrophication or organic pollution occured in Japan coastal seas are as follows.

(1) Water quality troubles caused by eutrophication

As water quality troubles caused by eutrophication, the troubles such as fishery damage, swimming troubles and troubles to industrial water supply caused by outbreaks of red tide, odour troubles and scenic troubles caused by outbreaks of red tide or seaweeds have been confirmed in Japan coastal seas.

(2) Water quality troubles caused by organic pollution

As water quality troubles caused by organic pollution, fishery damage and decrease of aquatic products, odour troubles and scenic troubles caused by formation of DO deficit water area or anaerobic sediment areas have been confirmed.

4) Desirable N and P levels for marine utilization

As each desirable N and P level for each marine utilization from the relationships between the occurrence of water quality trouble for water use and

Table 4. The Relationships between 2 kinds of Water Quality Items.

Items	Regression Line	Regression Line (Logarithm)
Chlorophyll-a : T-N, T-P (Summer) (Year)	Chl-a(S)= 34.3 T-N−1.84 r=0.875 n=30 Chl-a(S)= 453 T-P−4.52 r=0.942 n=30	log Chl-a(S)= 1.45log T-N+1.53 r=0.808 n=30 log Chl-a(S)= 1.64log T-P+3.31 r=0.824 n=30
Transparency :Chlorophyll-a (Year) (Summer)	Transp=−0.113 Chl-a(S)+7.06 r=−0.661 n=30	log Transp=−0.327log Chl-a(S)+0.984 r=−0.833 n=30
C O D : Chlorophyll-a (Year) (Summer)	COD= 0.0627 Chl-a(S)+1.47 r=0.918 n=30	log COD= 0.298log Chl-a(S)+0.0707 r=0.881 n=30
D O : C O D (Summer, Bottom) (Year)	DO=−12.6 COD+101 r=−0.762 n=24	log DO=−0.567log COD+2.03 r=−0.774 n=24
Transparency : C O D (Year) (Year)	Transp=−1.84 COD+9.79 r=−0.732 n=30	log Transp=−0.993log COD+1.03 r=−0.856 n=30
C O D : Chlorophyll-a (Year) (Year)	COD= 0.111 Chl-a(Y)+1.34 r=0.953 n=30	log COD= 0.367log Chl-a(Y)+0.0565 r=0.918 n=30
Chlorophyll-a :T-N, T-P (Year) (Year)	Chl-a(Y)= 20.0 T-N−0.141 r=0.869 n=30 Chl-a(Y)= 266 T-P−1.79 r=0.944 n=30	log Chl-a(Y)= 1.27log T-N+1.32 r=0.833 n=30 log Chl-a(Y)= 1.44log T-P+2.89 r=0.854 n=30
Chlorophyll-a:Chlorophyll-a (Summer) (Year)	Chl-a(S)= 1.67 Chl-a(Y)−1.15 r=0.976 n=30	log Chl-a(S)= 1.15log Chl-a(Y)+0.0120 r=0.974 n=30
T-N, T-P :Transparency (Year) (Year)	T-N=−0.0767 Transp+0.868 r=−0.517 n=30 T-P=−0.0073 Transp+0.0801 r=−0.605 n=30	log T-N=−0.954log Transp+0.213 r=−0.672 n=30 log T-P=−1.011log Transp−0.783 r=−0.787 n=30
T-N, T-P : D O (Year) (Summer, Bottom)	T-N=−0.0182 DO+1.75 r=−0.771 n=24 T-P=−0.0014 DO+0.144 r=−0.747 n=24	log T-N=−1.811log DO+2.91 r=−0.814 n=24 log T-P=−1.66log DO+1.59 r=−0.776 n=24

Chlorophyll-a :(mg/m^3), T-N :(mg/l), T-P :(mg/l), Transparency : (m) ,
C O D : (mg/l), D O : (%).

the N or P concentration level at that place and on that time, the following levels as shown in Table 6 are proposed by the working group.

(1) Conservation of the natural environment

As desirable N and P levels for conservation of the natural environment, the N and P level in marine park areas are selected. Trancparency in many marine park areas of Japan coasts is over 10 m. The N and P level where trancparency is over 10 m corresponds to below 0.2 mg/l and below 0.02 mg/l respectively.

(2) Sea bathing and swimming

The 75 % value of N and P levels in marine resort areas of Japan coasts is0. 40 mg/l and 0.03 mg/l respectively. As the N and P levels where water quality troubles have occured 0.30-0.90 mg/l and 0.03-0.14 mg/l respectively, As desirable N and P levels for sea bathing and swimming, Below 0.3-0.4 mg/l for N and below 0.03 mg/l for P are proposed respectively.

(3) The minimum level for the environmental conservation

As the minimum eutrophic level for the environmental conservation, the minimum DO level for existance of benthos in the bottom layer in a summer season is selected. From many data, it is estimated that the minimum DO level requested is to be 2-3 ml/l and its DO level corresponds to below 0.6-1.0 mg/l for N and 0.05-0.09 mg/l for P respectively.

Table 5. The Limiting Factor for phytoplankton Growth in Marine Coast of Japan.

Phytoplankton Class	N	P	N P	V.B_{12}	Fe	Mn	Other	Unknown	Total
Cryptophyceae							1		1
Dinophyceae	1	5		4	2			2	16
Bacillariophyceae	67	40	24	5	11		4	13	164
Rhodophyceae	57	82	17	2	18	3	1	16	196
Eugelenophyta	1		6	2			2		11
Chlorophyceae	18							3	21
Mixed Classes	29	23	1		2		1	4	60
Total	173	150	48	13	33	3	11	38	469

Table 6. Performances in biological treatment plants

An example of an industrual waste water treatment plant(fermentation industry)

	Raw water mg/l	Treated water mg/l	Removal ratio %	
COD	3000	900	70	Activated sludge process. Treated
T-P	40	12	70	water is discharged after being
T-N	1200	480	60	diluted with 12 times cooling water.
	Raw water	Treated water	Removal ratio	
COD	170	54	68	Activated sludge method.
T-P	3	1	66	
T-N	140	120	14	

An example of a Night soil treatment plant (1975)

	Raw water mg/l	Treated water mg/l	Removal ratio %	
COD	7000	2100	70	Activated sludge process. Treated
T-P	340	240	30	water is discharged after being
T-N	5000	3500	30	diluted with 19 times fresh water.
BOD	13500	600	95	

An example of a Night soil treatment plant (1984)

	Raw water mg/l	Treated water mg/l	Removal ratio %	
COD	7000	60	> 99	High loading activated sludge process
T-P	340	2	> 99	with UF membrane for separation.
T-N	5000	40	> 99	Treated water is discharged after
BOD	13500	20	> 99	being diluted with 1 times fresh water.

An example of a sewage treatment plant

	Raw water mg/l	Treated water mg/l	Removal ratio %	
COD	76	5	93	Biological nitrogen removal process.
T-P	3.6	0.1	97	
T-N	29	6	79	
BOD	200	1 >	99	

(4) Fishing industries

The relationships among the amount of fish catch, the primary product or the N and P load per sea area were investigated. Desirable N and P levels for fishing industries are between the level for conservation of the natural environment and the minimun level for the environmental conservation. However, that levels have not concreted yet. On this subject, the investigation is continued now.

Future and Recent Progress in Wastewater Treatent Techniques

In order to pass the national effluent standards based on the water pollution control law, the more stringent effluent standards regulated by the local governments or the most stringent effluent standards that promised to the regional inhabitants, the treatent techniques for industrial and municipal wastewater have progressed.

Remarkable progress is recognized in newly constructed night soil treatment plants or sewage treatment plants as shown in an example of table 6. As almost of night soil in Japan has been treated with some methods already, in order to reduce further the pollution loads from municipal and domestic wastewaters, it is requested to raise the ratio of treatment of municipal and domestic grey wastewaters (excluding night soil) so far as about 100 % from present about 45 % and to raise the removal efficiency in all treatment plants.

Most of industrial wastewater treatment plants were constructed in 1970's. Remarkable progress for the industrial wastewater treatment had been recognized on that time too. In the factories where high pollution loads are discharged, however, the margins for improvement of industrial wastewater treatment techniques are still remained. Therefore, the further improvement of industrial wastewater treatment is requested to these factories.

Fundamental Strategies for Eutrophication Control

Pollution loads discharged into the marine coast of Japan are summarizes in Taable 7. For the reduction of pollution loads for COD, N and P, the following strategies are proposed.

(1) The movement of N and P due to food relating matters has to be paid attention to and the management strategies to reduce their movement has to be taken seriously.

(2) The most fundamental strategy for reduction of COD, N and P load discharged into water areas is to reduce in each origin of their loads. This

Table 7. Pollution loads discharged into the marine coasts of Japan

(Nakanishi, Ukita, et al.)

	1970 (10^3t/Y)		1984 (10^3t/Y)		note
Origin	N	P	N	P	
Residential	281(32.9)	43.7(46.5)	315(38.7)	32.4(43.3)	reaching-ratio
Food-processing	55(6.4)	9.7(10.3)	44(5.4)	8.0(10.7)	N:83%
Chemical fertilizer production	128(15.0)	22.0(23.4)	77(9.5)	16.8(22.4)	P:81%
Stockbreeding	32(3.7)	8.6(9.5)	26(3.2)	8.0(10.7)	(reaching-ratio for industries that located at coastal area,
Agricultural land	213(24.9)	6.5(6.9)	213(26.2)	6.5(8.7)	such as Chemical fertilizer,
Coal, petrochemical	33(3.9)	-	26(3.2)	-	Coal, petrochemical, and
Other natural organic matter	12(1.4)	3.2(3.4)	12(1.5)	3.2(4.3)	Precipitation is set to 1.0)
Precipitation	100(11.7)	-	100(12.3)	-	
Total	854(100%)	94.0(100%)	813(100%)	74.9(100%)	

strategy has to be promoted strongly. Reduction of P content in synthetic detergent, treatment of garbage not as sewage but as solid waste, reuse of human and livestock waste as fertilizer and so on are good example for this purpose.

(3) Adjustment of sewage treatment system has to be promoted strongly. In Japan, 96 % of night soil and 45 % of municipal and domestic grey wastewaters are treated in sewage treatment plants, Jokasou (the treatment facilities for flush toilet) and night soil treatment plants (the treatment facilities for storage tank toilet) and so on in 1990. Therefore, treatment of the rest of 55 % of grey wastewaters is urged.

Remarkable good results in performances of night soil treatment plants or sewage treatment plants are obtained as shown Table 6.

(4) Industrial wastewater had beem improved strinkingly for several yeara of 1970's. However, as the remarkable improvement thereafter has not recognized, more effort for reduction of pollution loads from industrial wastewater is should be requested.

(5) Judging from the eutrophic levels and the carrying capacities of pollutants that are not toxic substances and nutrient matters as COD, N, P, selection ofwater areas to receive these pollution loads from is a very effective strategy for eutrophication control. Diversion after treatment of the nutrient loads discharged from highly eutrophicated water areas such as lake Kasumi, lake Suwa, Tokyo bay, Osaka bay into the adjacent sea areas of Japan where have larger carrying capacities might be recommend.

References

1) Japan Environmental Agency: 1990, The Annual Report on Environmental Pollution in 1989.
2) Japan Environmental Agency: 1982, Environmental Quality Standard for N and P in lakes and Reservoirs.
3) Japan Environmental Agency: 1990, An Interim Report on A Goal of Water Quality for N and P in Marine water areas.
4) Japan Environmental Agency: 1990, Fundamental Concepts for Application of the third Area Wide Total Pollutant Load Control to the Tokyo bay, the Ise bay and the Seto Inland Sea.

NEEDS FOR RESEARCH IN THE FUTURE

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Introduction

The state of the surface water is not just determined by the effluent from the treatment plant. Other sources also have a part to play. For the situation in the Netherlands these might include the intensive cattle farming and the non point sources. Other mayor points are the contamination on the surface water and the water sediments. The target for the future has to be the reduction of this contamination.
From this it must be clear that the treatment of waste water cannot be seen as an isolated activity.

In the past research was planned at the moment when stricter effluent requirements appeared. For getting an idea of the research in the future, you have to predict the development in the effluent requirements. On this basis appropriate measures have to be taken.
These appropriate measures generally concern research with the purpose of identifying the technological tools that will be needed to reach the new requirements. In a number of cases it will be possible to meet tighter requirements by applying existing technologies. In other cases the new requirements are almost impossible to fulfil with the means now available. Research will then have to lead to equipment and methods that can meet the future standards.

The question that arises -- and it is quite a legitimate one -- is: can we predict what requirements will be current in the year 2000 -- that is, 9 years ahead. The answer to this is clear enough: no, we can't. Nor have we ever been able to. History makes

this clear. If we look at the recent past, for instance the requirements related to nitrogen and phosphate, we see that very few people, including the policymakers, were able to foresee that already in 1990 many water quality boards would be so intensely occupied with the removal of these substances. Our failure to foresee this is no disgrace. We are not in the business of forecasting. The only thing expected of us here is an understanding of waste-water treatment in its broadest sense.

1. Present standards

This introduction makes clear that talking about effluent 2000 can only be a discussion of scenarios. Scenarios and their consequences for the treatment plant. We will therefore look at the possible scenarios and derive from them measures that will have to be taken to meet the requirements imposed. In a number of cases these requirements cannot be met with available technology and so the need for research is obvious.
At present the requirements that treatment plants in the Netherlands must meet are roughly:

BOD	20 mg/l
Suspended solids	30 mg/l
Total phosphate	1-2 mg/l
Total nitrogen	10-15 mg/l

In recent years the requirements have been tightened, particularly for the nutrients nitrogen and phosphate. In view of the seriousness of the eutrophication problem, it may be expected that the requirements for these substances will become even tighter. Values of 1 to 2 mg/l total phosphorous are now frequently mentioned. These levels can be attained with the present technology. With chemical dephosphatation the effluent contents are of this order of magnitude. With advanced post-treatment systems, such as the pellet reactor or magnetic separation even lower levels can be obtained. This holds also for biological

dephosphatation by the phostrip process. These techniques will still perform adequately even for requirements of 1 mg/l or less. Requirements of 0.5 mg/l or lower will only be attainable with the combination of post-treatment sand filters and chemical dosing, or with more closely optimized pellet reactors or magnetic separation units. Well-optimized sand filters can possibly reach about 0.3 mg total P/l. If a still lower figure is required only membrane filtration has to be taken into consideration.

For nitrogen removal the situation will be difficult. To remove nitrogen we have to rely entirely on biological methods -- in fact only one biological method. First nitrification, then denitrification. This is in strong contrast to phosphate removal, where there is a choice of three or four techniques that should all be able to meet the most likely future requirements. With the current techniques, and the nitrogen contents that apply for Dutch waste water, a requirement like an annual average of 10 mg total nitrogen/l is attainable. A much lower value can not be achieved because of the prevailing water temperatures. At the winter temperatures of 10 °C or lower the nitrification activity decreases strongly. The incomplete nitrification means that the concentration of nitrogen compounds remaining in the water is too high, even after the subsequent denitrification. Requirements that exceed the 10 mg/l mentioned of total nitrogen/l are only attainable with new treatment systems, that should be less sensitive to low temperatures (for example by covering) or systems that maintain sufficient activity to oxidize the nitrogen. At the end we can think of systems in which the nitrifier content is that high that even at very low temperatures the total activity is still sufficient. The individual activity per bacterium decreases, but this is amply compensated by the large amount of biomass. Something similar could also be achieved by the continuous addition of

nitrifying organisms. These organisms have to be produced in a separate process.
Research into organisms that nitrify at very low temperatures can be very interesting here.

Heavy metals and organic micropollutants are also present in raw sewage. In view of the effluent standards for municipal waste water treatment plants, these components have not been discussed so far. We may expect that this may change in the very near future.
Developments in analytical chemistry have enlarged the possibilities to determine very low contents of these components in water.
Another item is the extensive range of problems caused by the sediments of the surface waters. In the Netherlands the sediments of almost all the larger surface waters are found to be seriously polluted. A number of causes for this can be identified. Two important sources are industrial discharges and the inflow of pollutants from abroad through the big rivers. The latter in particular is of great consequence for the waters that are strongly affected by the international rivers. For smaller regional waters the effluent from treatment plants also plays a part. Heavy metals and organic micropollutants are xenobiotics that ought not to be tolerated in surface water, not even in low concentrations. Many opinions on this have been expressed in recent years. The possibility of such substances ending up in the food chain is generally admitted to be very real. Because of this point in particular it can be assumed that the effluent requirements will be made much more strict. Techniques are already available that will largely meet such standards. The substances mentioned in the treated waste water are largely bound to the suspended matter still present in the effluent. Removal of the suspended matter is generally sufficient to meet the requirements imposed. Sand filtration with coagulation filtration, which will also take care of the lower phosphate

contents, seems the obvious method. If much lower contents are required, and here we are usually already looking below the detection limit, then even more advanced forms of filtration come into the picture.

Disinfection of waste water is hardly applied in the Netherlands. Effluent is only treated in a limited number of cases, in particular by chlorination. At the moment it can be stated that treatments like UV or chlorination followed by a filtration phase is also sufficient for future requirements to be met.
After the attention payed to nitrogen and phosphate in recent years, I expect that sulphate will be the object of attention in the near future. It is not easy to remove sulphur compounds from water. In aerobic treatment these compounds are converted into sulphate, which makes removal very difficult. In the Netherlands there is a system for removing sulphur compounds from industrial effluent; in this method an anaerobic reactor has to be used as the first stage. This will not be a practical option for domestic wastes.
The conclusion is that we have to tackle the problem at its source, in this case, for example, by not adding sulphur containing compounds to detergents.

The most important constituent of the raw sewage is organic matter. Requirements for removal have been set, by the requirements for biological and chemical oxygen demand. The standards vary according to the capacity of the receiving surface water. For the big rivers the annual average is about 20 mg/l, for smaller waters about 5-10 mg/l. These requirements turned out to be attainable. The question is, can stricter requirements be fulfilled too. Probably they can, and possibly without many problems because of the strict requirements related to the nitrogen removal. If the annual average content has to be lower than 10 mg of total nitrogen/l the organic matter has also to be decomposed extensively. In general the biological

oxygen demand will then already be below 10 mg/l. With the tightening of phosphate requirements and the installation of effluent filtration automatically a sharp decrease in the content of organic matter will be achieved.

2. Future policy

The examples for the various substances actually show that tightening the effluent requirements will eventually lead to a form of effluent filtration. Except for those components for which a biological method can be used, of course. It appears to be a good idea to approach the matter in another way. This approach is based on various scenarios. The scenarios tie in with the quality requirements for the surface water.

In recent years a number of policy documents have been published, all with the objective of improving the quality of the environment. Aims for the development of surface water quality are indicated in these documents. The most recent one, the Third Policy Document on Water Management, talks about the General Environmental Quality (GEQ) for the year 2000. GEQ is based on strict requirements with respect to heavy metals and organic micropollutants. A prediction with respect to the quality requirements for the effluent could for example be the GEQ or a multiple of the GEQ. Other scenarios are even more strict. For example, the effluent must be suitable as the raw material for the production of drinking water or it must be possible to discharge it into surface water intended ambience for fish of the salmon type. It will be clear that the last scenario in particular will lead to far-reaching technological intervention into the treatment plant. Filtration seems likely here. From all the programs and scenarios it emerges that many of the requirements can be achieved in much the same way. A very low loaded biological treatment plant, a phosphate removal and effluent

filtration. Here we are thinking about techniques that certainly cost a lot of money, but are already available now. The nitrogen removal should result in effluent contents of 5 mg of total nitrogen/l. To achieve this with reliable technologies new techniques should be developed. This will be one of the objectives of research in the years ahead. Effluent filtration, through the removal of suspended matter, causes low contents of heavy metals and organic micropollutants. So far membrane filtration has not been taken into consideration for the post-treatment of sewage. However, since membrane technology is developing very fast, research aimed at the application of membranes for the filtration of treated effluents should be started without delay.

If the requirements imposed on the effluents have to be satisfied, there will be a moment that the deciding factor is not longer the method of treatment, but the quality of the waste water supplied. At this point we have to go back to the source. In a number of cases the source will be industrial, with a discharge of effluent into the sewerage system. Sometimes exclusively domestic discharges are concerned. Certain products can be the reason for certain effluent requirements not being met. The only remaining possibility in those cases is to ban the product or to replace it by other means. A recent example is the introduction of phosphate-free detergents. At this moment consideration has to be given to the full integration of effluent treatment and controlling pollution at the source.

3. Research in future

From the preceding research programmes (like programme RWZI 2000) on the treatment of waste water a number of unsolved items will remain.

The space required, sludge production, nuisance to the environment, and the high costs are aspects that will

also continue to play a part in the future. For these reasons these questions will also have to be addressed to in a future programme. The space required and the strongly related nuisance to the environment will only increase. Built-up space in countries like the Netherlands and Japan will continue to grow, and people are growing more sensitive to nuisance like foul odours and noise. This means that the need for treatment plants with a good answer to these problems becomes even larger. The disposal of the sewage sludge produced in itself remains a problem. Many methods for sludge processing are in operation. The processing of sewage sludge to yield a useful product is in fact an option of the past. The main objective of the methods employed in sludge processing therefore is reduction of the problem by reducing the volume. What generally happens during the processing of sludge is still largely unknown. Research in this field should proceed without delay. The same applies for the research that might lead to a reduction in sludge production. It will never be possible to develop a treatment plant that does not produce any sludge. In any case a quantity of primary sludge will always be generated. Nevertheless a reduction in the production of secondary sludge does seem possible. Every reduction is important here, and helps to reduce the problem. The costs of the treatment of waste water have become very high, largely because of the many problems that also demand a solution. Research with reduction of the costs as its primary objective therefore continues to be necessary.

A future research program will therefore deal less with meeting new effluent requirements in the strict sense, but will rather take the treatment plant as a whole as the object of research. This is the only way of giving a balanced interpretation to a future project effluent 2000. In scheming such a programme it is important to take the lessons of the past to heart. Up to now, research is planned at the moment when the problem is

identified. The short term was considered far more important than the rather longer term. The long-term work was usually dismissed as being too fundamental. The philosophy is partly altered because of the project RWZI 2000. In this project the short-term work has been included but so did a number of other factors that come into play in the long term. For the first time the waterboards appear to be prepared to support work that would not lead to the solution of a problem in the short term. The long-term research on the reduction of sludge production as well as the research into the mechanisms underlying the dewatering of sewage sludge have been good examples of this. This kind of approach should continue in the future. On the one hand research that will lead to useful results in the short term, on the other hand research with results that will be useful in 5 or 10 years' time. This will inevitably be the only approach that will enable anticipation of new and future effluent standards in good time.

To have the needed technology available at the right time in the next decade is a challenge for all people who are involved with waste water treatment but also for the politicians who will have to supply money.

MUNICIPAL WASTE WATER TREATMENT SYSTEM COMBINING PHYSICO-CHEMICAL AND BIOLOGICAL PROCESSES

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MUNICIPAL WASTEWATER TREATMENT SYSTEM COMBINING PHYSICO-CHEMICAL AND BIOLOGICAL PROCESSES

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INTRODUCTION

Municipal wastewater treatment is normally carried out in steps, traditionally with primary settling as the first step, biological treatment as the second step and sometimes chemical treatment as the third step. The most popular municipal wastewater treatment system has been the activated sludge processes consisting of the primary clarifier, aeration tank and secondary clarifier. Recently, the biofilm processes such as the rotating biological contactors and the submerged biofilters have become popular, especially in the small scale municipal wastewater treatment plants. However, the system itself is the same, except that the aeration tank is replaced by the biofilm reactor.

It has been reported that the major part of the contaminants in raw municipal wastewater is associated with particles and the effluent from the primary clarifier contains many organic particles with the size of less than 0.1 mm. In the conventional municipal wastewater treatment processes such as the activated sludge or biofilm processes, such organic particles are biologically dissolved then oxidized in the aeration tank or biofilm reactor.

Chemical treatment has been used to remove the phosphorous from the wastewaters. According to the Scandinavian experience (Ødegaard, 1988), the direct chemical treatment of municipal wastewater can produce an effluent with a very low concentration of suspended solids and phosphorous. However an additional biological treatment is usually required, because the effluent still contains rather high concentration of soluble organics and ammonia nitrogen.

In order to meet more strict effluent standard, it would be required to develop the combined physico-chemical and biological municipal wastewater treatment systems. The principal objectives of this paper are, (1) to present the experimental results on the chemical treatment of municipal wastewater by the Jet Mixed Separator (JMS) which can be used as a unit process for the physico-chemical treatment, (2) to demonstrate the performance of an upgraded Rotating Biological Contactor (RBC) which can be used as a unit process for the biological treatment, and (3) to show the experimental results on combined municipal wastewater treatment system of the JMS and upgraded RBC.

CLASSIFICATION OF CONTAMINANTS IN MUNICIPAL WASTEWATER

Contaminants of interest in municipal wastewater range in size from less than 0.001 μm to well over 100 μm. The size ranges of typical organic contaminants characteristics of settled municipal wastewater are presented in Fig.1(Levine, et. al, 1985). Imhoff(1939) presented in his classical pocket book, table showing the

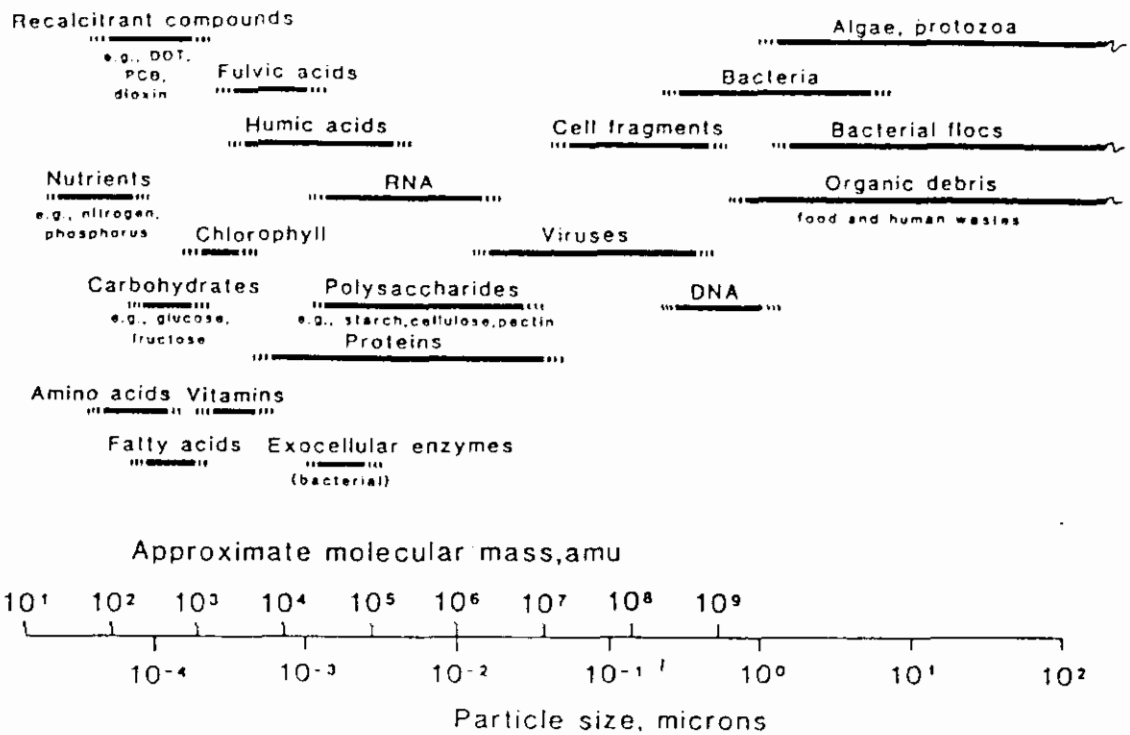

Fig.1 Typical organic constituents in settled wastewater.

distribution of settleable and non-settleable suspended matter in wastewater, demonstrating that half of the organic matter was associated with the suspended solids. In early Americal studies, the organic contaminants in wastewater were separated into four size fractions by successive sedimentation, centrifigation and filtration. The fractions were classified by size range as settleable, supra-colloidal, colloidal and soluble. A later study by Munck et al. (1980) confirmed the results of the earlier investigations, even though Munck used a slightly different definition of the size ranges and used only filtration to separate the fractions. As shown in Table 1, Munck et al. also investigated the distribution of total phosphorous and organic nitrogen. Even if most of phosphorous in wastewater appeared as soluble phosphorous, the amount of phosphorous associated with particles was found to be significant, and even more so was the amount of organic nitrogen. The primary effluent still contains a lot of particles with the size of more than 0.1 m, as shown in Fig.2, which demonstrates that 60 - 70 % of the organic material measured by the TOC test was associated with particles larger than 0.1 m, while virtually no organic matter was detected in the size range

Table 1 Classification of contaminants in wastewater.

Size Range	Classification			
	Soluble	Colloidal	Supra-colloidal	Settleable
	<0.025 μm	0.025-3 μm	3-106 μm	>106 μm
BOD_5 (% of total)	17	16	46	21
COD (% of total)	12	15	30	43
TOC (% of total)	22	6	36	36
Tot P (% of total)	63	3	12	22
Org. N (% of total)	27	15	38	20

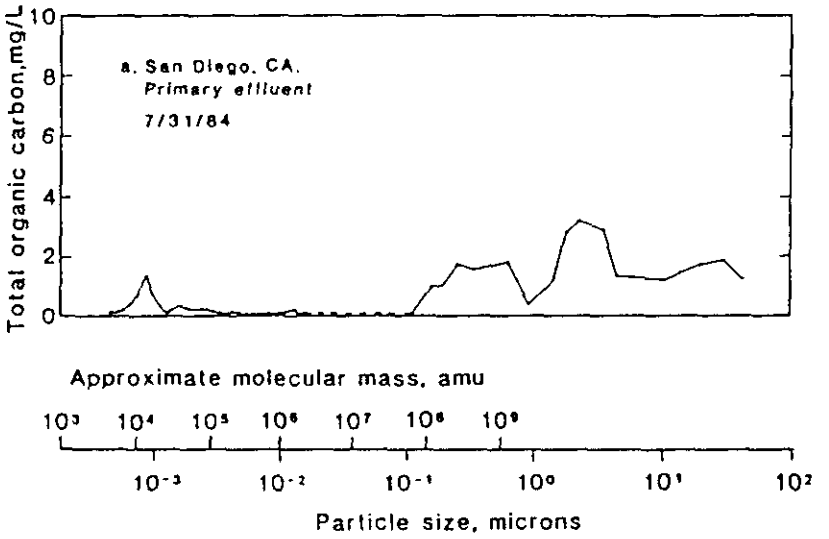

Fig.2 Size distribution of the organic constituents in primary effluent.

Table 2 Percentage of total metal associated with suspended solids in raw wastewater.

Metal	Zn	Cu	Ni	Cr	Pb	Cd
%-suspended	51	48	13	71	71	82

between 0.001 - 0.1 μm. Table 2 demonstrates that the percentage of total metal associated with suspended matter in the raw wastewater of a Swedish wastewater treatment plant was in the range of 50 - 75 %, with the exception of nickel.

PHYSICO-CHEMICAL MUNICIPAL WASTEWATER TREATMENT

It is generally known that trivalent salts are good at removing suspended solids from a turbid water. In the process used to treat surface water, to produce potable water, aluminum salts are often used to destabilize and flocculate the pollutants. Sometimes, highly charged metal ion complexes such as polymerized aluminum chloride(PAC) are used because they are more effective in this process than a salt with a lower valency or charge. Also the combination of an inorganic salt and an organic compound can be used.

The same types of chemicals are used for wastewater treatment, both for direct coagulation and flocculation, pre-coagulation and flocculation, and for post-coagulation and flocculation. Physico-chemical treatment may be applied to raw wastewater as shown in Fig. 3. A good example of the physico-chemical treatment plant is the VEAS treatment plant outside Oslo which has been designed to handle about 400,000 m^3/day. In Norway there are over 100 such treatment plants. Table 3 shows

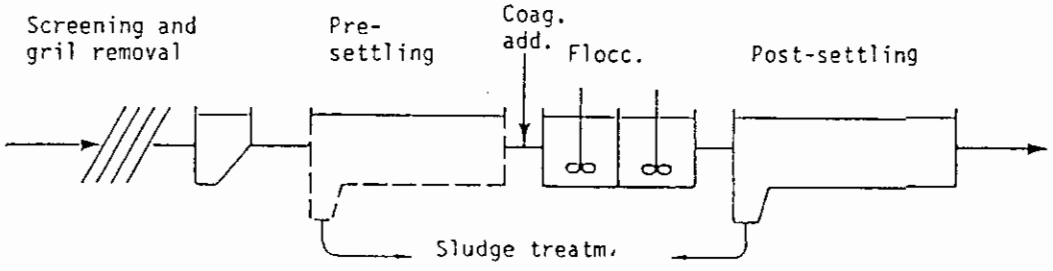

Fig.3 Schema of non-biological and chemical treatment plants.

Table 3 1985 yearly averaged treatment results in 56 chemical treatment plants in Norway.

Process	Number of plants	SS			BOD			COD			TOT.P		
		In	out	%	In	out	%	in	out	%	In	out	%
PP	23	172	27	84.3	216	42	80.6	313	92	70.6	5.5	0.54	90.2
SP	33	218	22	89.9	238	36	84.9	404	74	81.6	7.1	0.50	93.0
PP & SP	56	387	24	87.6	229	39	83.0	360	83	76.9	6.2	0.52	91.6

PP:primary precipitation, SP:secondary precipitation.

the 1985 year average treatment results obtained in 56 randomly selected physico-chemical treatment plants in Norway.

The Scandinavian experience clearly shows that the physico-chemical treatment produce an effluent with a very low concentration of suspended solid and phosphorous, but rather high concentration of soluble organic matter and ammonia nitrogen. Therefore, the additional biological treatment is required to achieve a higher removal efficiency of organic matter and ammonia nitrogen.

Physico-chemical Treatment by Jet Mixed Separator

The authors(1990) invented a solid-liquid separator with porous plates inserted vertically in the channel perpendicular to the flow. On the one-half of the plate, alternating right and left side, there are many small holes. The water passes through holes in the plates, thus creating jets which gently mix the water on itself. If the coagulant is added, simultaneous flocculation of suspended particles and sedimentation of grown-flocs occur in the channel, which is called the Jet Mixed Separator(JMS).

In the previous paper(Watanabe et. al, 1990), the followings have been described: (1) the development of the theoretical basis of the JMS; (2) a description of the hydraulics of the JMS; (3) a picture of the solid-liquid separation efficiency. The JMS has been applied to the chemical treatment of municipal wastewater.

The length, width and depth are 200 cm, 30 cm and 85 cm, respectively. The porous plate, which has 48 holes with the diameter of 0.8 cm on the one-half side, was arranged at a constant distance of 10 cm or 20 cm. Settled municipal wastewater was fed into the JMS. The hydraulic retention time was 45 or 90 min. Polymerized aluminium chloride(PAC) was used as the coagulant with the addition of 5 or 10 ppm. Fig. 4 shows the removal efficiency of the suspended solids at the plate distance of 10 cm. As the colloidal particles was removed, turbidity decreased as shown in Fig. 5. TOC concentration decreased as the result of the removal of organic particles (Fig. 6). However, as the removal of soluble organics by the chemical coagulation was almost negligible, the removal efficiency of TOC was not high. Fig. 7 shows the size distribution of TOC in each stage of the JMS. Figs. 8 and 9 show the reduction of soluble and total phosphorous (as PO_4^{3-}). As seen in Fig. 10, which show the size distribution of phosphorous in each stage of the JMS, soluble phosphorous were effectively precipitated, and removal efficiency of the precipitated phosphorous were very high. Fig. 11 shows the effect of plate distance on the performance of the JMS as the simultaneous coagulation-sedimentation process of municipal wastewater.

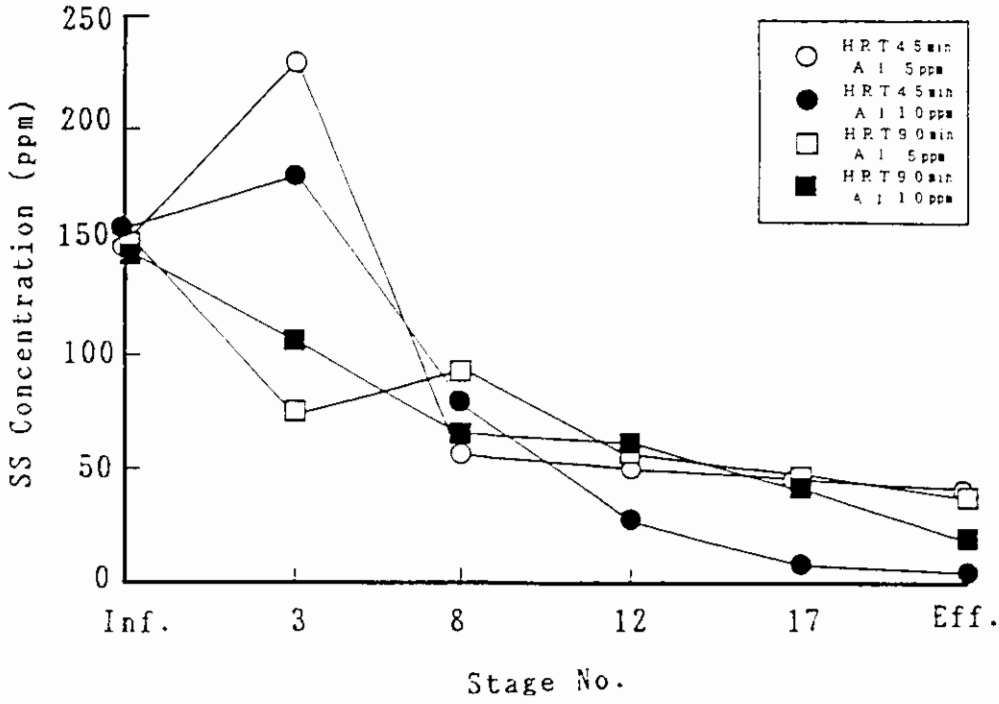

Fig. 4 Removal efficiency of suspended solids in JMS
(Plate distance= 10 cm)

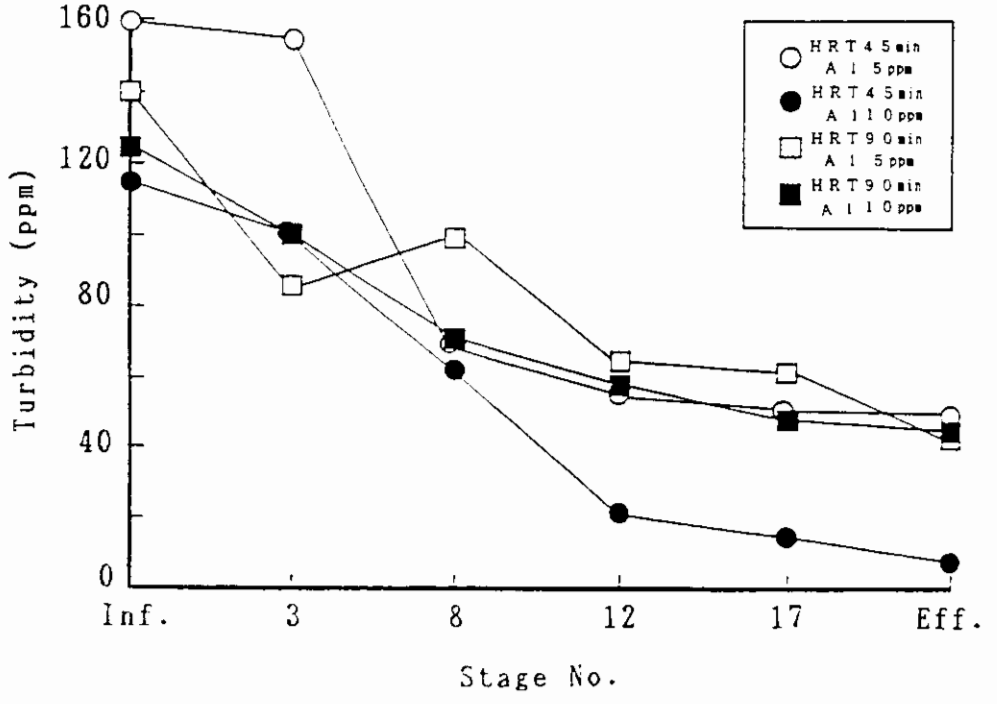

Fig. 5 Removal efficiency of turbidity in JMS
(Plate distance= 10 cm)

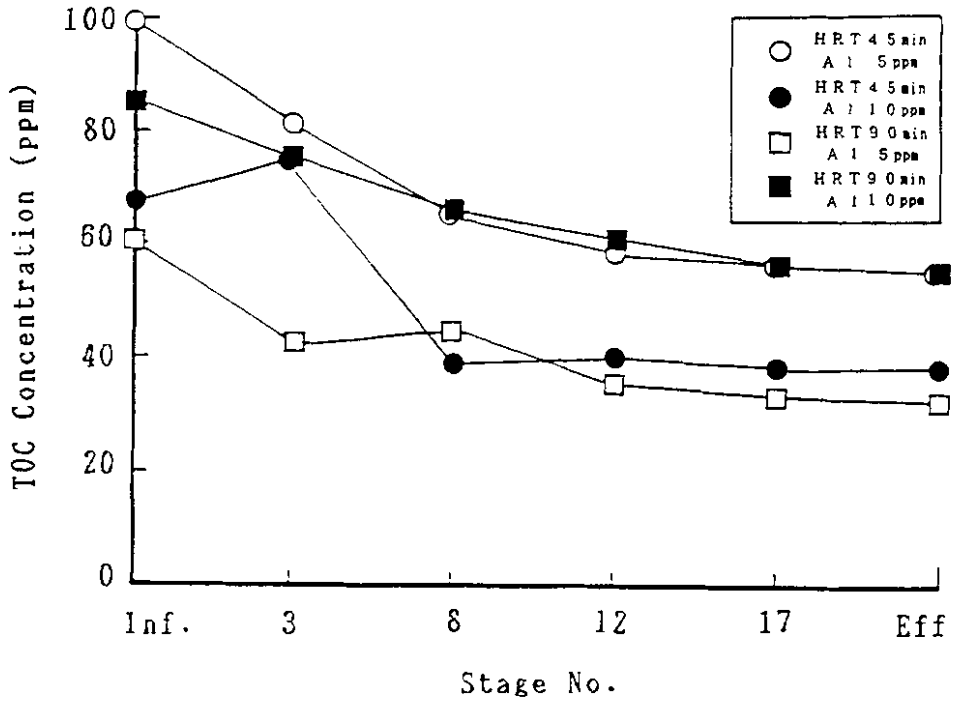

Fig.6 Removal efficiency of TOC in JMS
(Plate distance= 10 cm)

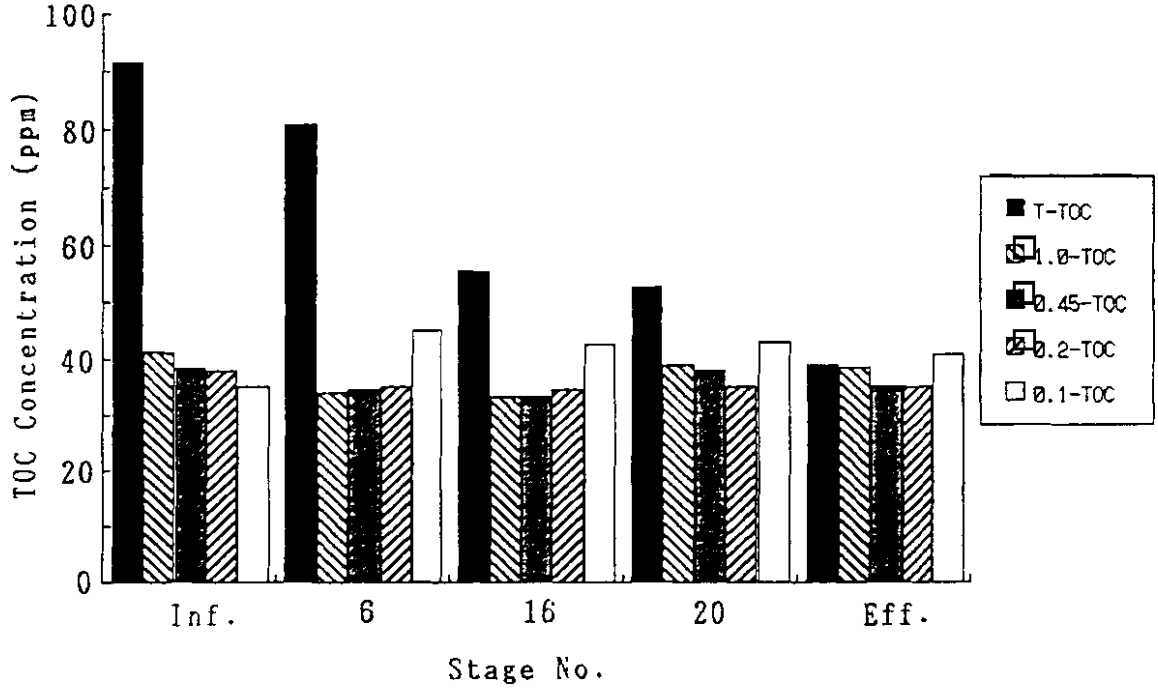

Fig.7 Size distribution of TOC in JMS
(Plate distance= 20 cm)

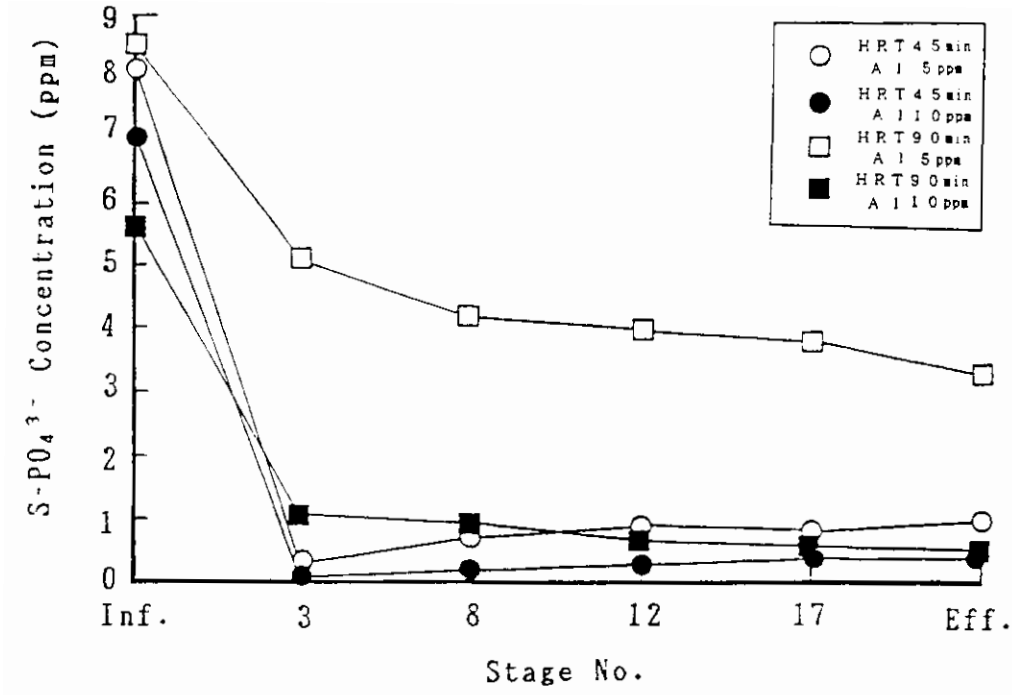

Fig. 8 Removal efficiency of soluble phosphorous
(Plate distance=10 cm)

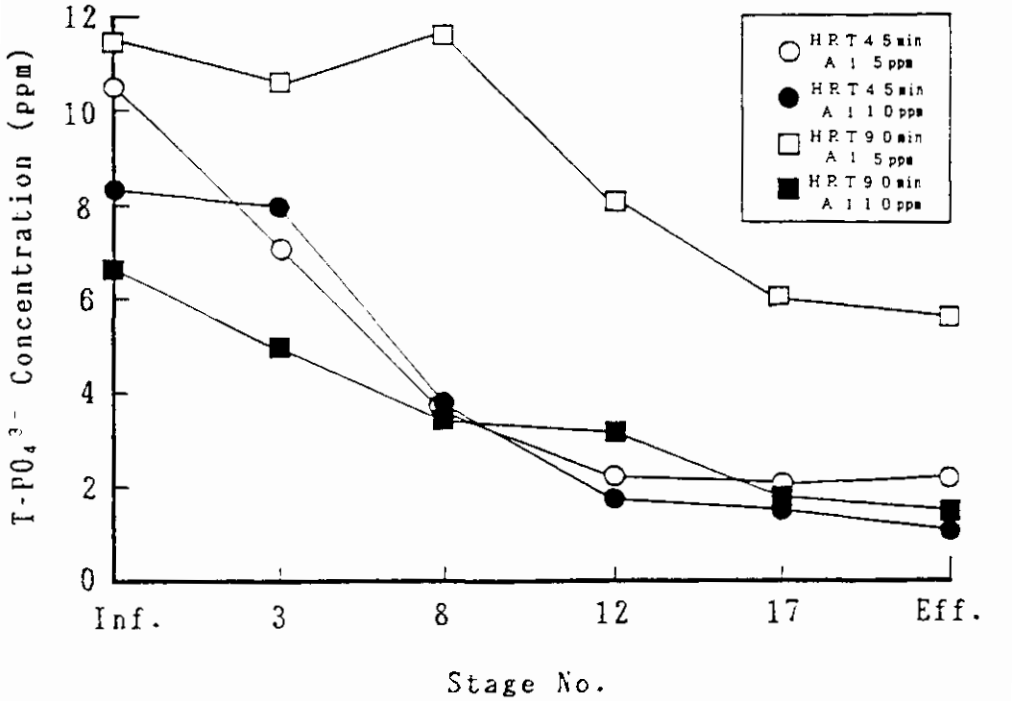

Fig. 9 Removal efficiency of total phosphorous
(Plate distance=10 cm)

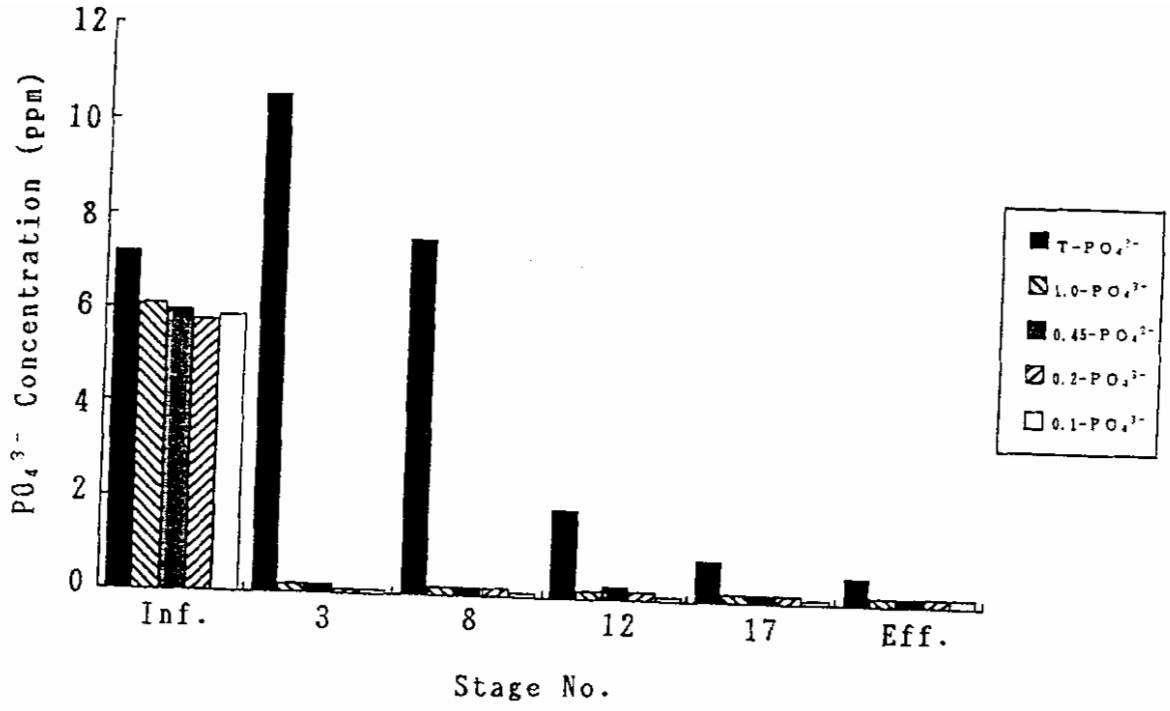

Fig.10 Size distribution of phosphorous in JMS
(Plate distance= 10 cm)

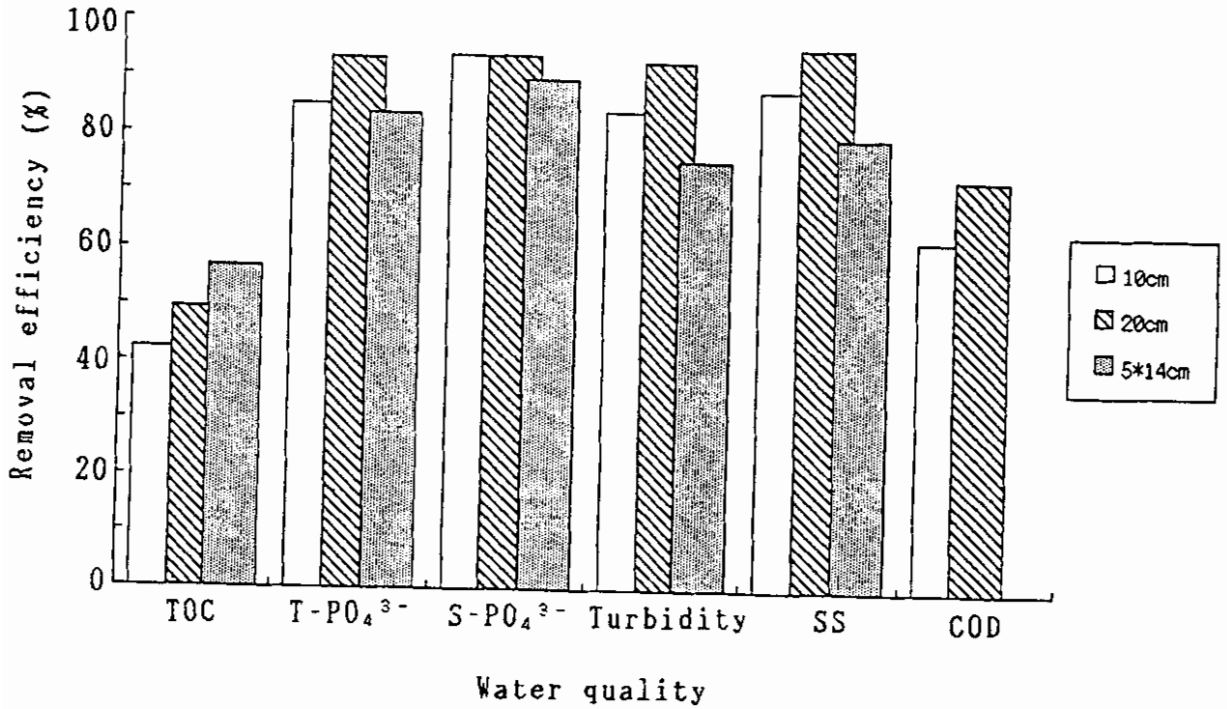

Fig.11 Effect of plate distance on JMS performance
(HRT=45 min., Al dosage=10 ppm)

PERFORMANCE OF AN UPGRADED ROTATING BIOLOGICAL CONTACTOR

The Rotating Biological Contactor (RBC) is an aerobic biofilm reactor for treating demestic and industrial wastewaters. It consists of series of large-diameter mediae, mounted on a horizontal shaft and placed in a trough. The biofilm consists of various bacteria such as heterotrophs and autotrophs that naturally develope on the media surface. Dominant species and properties of biofilm depend on the wastewater characteristics and the reactor operating conditions. The mediae slowly rotate with approximately 40 to 50 % of the surface area submerged in the wastewater to be treated. The rotation of the media plays an important role on the following aspects:

(1)Aeration ;it provides an alternative exposure of the biofilm to air and water. During the rotation of the biofilm in air ,oxygen is supplied to the biofilm microorganisms ,while during the rotation in the water, substrates diffuse into the biofilm, and (2) Mass transfer;far from the rotating media, the liquid moves toward the media surface and ,in a thin layer immediately adjacent to its surface the liquid acquires a rotating motion. The liquid also acquires a radial velocity under the influence of centrifugal force. Therefore ,the rotating action of media hastens the mass transfer to the biofilm.

The goal of the mathematical modelling of biofilm reaction is to express the substrate flux as a direct function of the bulk substrate concentration which is readily measurable and the concentration of interest. Watanabe et al. (1980, 1982, 1984) presented the RBC kinetics based on a heterogeneous biofilm model and analyzed the experimental data of the denitrification, nitrification, and simultaneous organic oxidation, nitrification and denitrification by the simulation and steady-state approximation of the kinetics. In this paper, the proposed biofilm kinetics is applied to predict the relationship between the ammonia flux and bulk ammonia concentration at various sizes and rotation speeds of media. Experimental verification of the calculated relationship is also carried out. The effect of bulk organic concentration on the ammonia flux is experimentally examined. In order to promote the external diffusion of soluble substrates and to reduce the media weight, a new media, i.e., reticulated media with the surface protrusions is proposed, and the performance of the nitrification RBC with new media is analized by the proposed biofilm kinetics.

In a conventional RBC system, the final clarifier has been used to separate the treated wastewater and detached biomass. The detached biomass is exposed to the turbulent shear in the trough during the transport to the final clarifier. Due to such an exposure ,the detached biomass is broken down into smaller particles which can not settle in the clarifier. As a result, it has been reported that the effluent from the RBC system contains a lot of small organic particles.

In this paper , the solid-liquid separation efficiency of a two-story RBC is investigated to demonstrate that it has a high removal efficiency of the detached biomass . It means that the final clarifier may not be necessary in the two-story RBC.

Based on the above fundamental reseaeches , the author(1989) innovated an upgraded two-story RBC consisting of the reticulated media with surface protrusions. This paper includes the experimental results on the performance of the upgraded RBC.

Fundamental Basis of Upgraded RBC

(a) Significance of External Mass Transfer in RBC

The biofilm attached to a partially submerged RBC rotates alternately into air and water. During the rotation in the air phase , oxygen is supplied to the microorganisms inhabiting the biofilm, while the substrates diffuse into the biofilm during its rotation in the water phase. The following assumptions are made to formulate the biofilm kinetics: (1) The substratum is assumed to consist of flat and impermeable media, (2) The portion of the media exposed to air contains a liquid film of thickness, L_w, (3) A liquid boundary layer of thickness, L_d exists at the biofilm/liquid interface , and (4) The wastewater in each RBC stage is assumed to be completely mixed.

Using the derived RBC kinetics , the computer simulation of nitrification was carried out to estimate the concentration profles of oxygen and ammonia in the biofilm at various sectors of biofilm during rotation in air and water phases. According to the simulation results , it has been clarified that under oxygen limitation, the dissolved oxygen concentration profile in the biofilm system approaches to its steady-state profile in a very short time after the biofilm sector contacts the air phase, and under ammonia limitation , the ammonia concentration profile in the biofilm approaches to its steady-state profile in a very short time after the biofilm enters the water phase. Based on this evidence , the RBC kinetics can be simplified to steady-state model if the zero-order intrinsic reaction rate is applicable , and Watanabe(1985) formulated an RBC kinetics which is available to calculate the flux of rate-limiting substrate at a given bulk substrate concentration. With the steady-state assumption , the substrate flux through the liquid boundary layer, J_{zi}, is equal to the substrate flux at the biofilm surface, J_{fi}, if D_{fi} is a constant fraction of D_{wi} ($D_{fi} = \alpha D_{wi}$, where α is a constant less than or equal to unity), the following expression is obtained:

$$J_{zi} = (D_{wi}/L_d)(C_{li}^b - C_{li}^s) = (2 \alpha D_{wi} r_i^0 C_{li}^s)^{1/2} \qquad (1)$$

The unknown value of C_{1i} is calculated from the above equation as follows:

$$C^s_{1i} = C^b_{1i} + \lambda - (\lambda^2 + 2\lambda C^b_{1i})^{1/2} \quad (2)$$

$$\lambda = \alpha\, r^0_i\, L_d/D_{wi} \quad (3)$$

If the RBC is operated under oxygen limitation and 50 % of the media area is submerged, the oxidizing substrate flux is given by Eq. 4.

$$J_{zi} = (r^0_i/r^0_o)(J_{oa} + J_{ow}) \quad (4)$$

From the similar procedure used for deriving Eq.1, the oxygen flux to the biofilm rotating in the air phase(J_{oa}) and that in the water phase(J_{ow}) are expressed as:

$$J_{oa} = (D_{wo}/L_w)\ (C^*_o - C^a_{1o}) = (2\,\alpha\, D_{wo}\, r^0_o\, C^a_{1o})^{1/2} \quad (5)$$

$$J_{ow} = (D_{w0}/L_d)\ (C^b_{1o} - C^w_{1o}) = (2\,\alpha\, D_{wo}\, r^0_o\, C^w_{1o})^{1/2} \quad (6)$$

The above mentioned RBC kinetics contains two physical parameters: L_w and L_d. They express the diffusional resistance of oxygen and substrate to the biofilm during its rotation through air and water.

Liquid boundary layer thickness. The liquid boundary layer thickness on the clean surface of a submerged disk is described by Eq.7 (Levich, 1962).

$$L_d = 1.16(D_{wi}/\nu)^{1/3}(\nu/2\pi N_r)^{1/2} \quad (7)$$

Watanabe and Nishidome(1989) have evaluated the effect of the submerged ratio, rotating speed, surface roughness, diameter of media on the liquid boundary layer thickness of the RBC attached by the nitrifying bacteria. As predicted by Eq.7, the liquid boundary layer thickness is not a function of media size if the flow near the media surface is laminar, which is the case of the usual operation of the RBC. It is, however, a strong function of the media submerged ratio, since the hydrodynamic boundary layer may not completely develope in a partially submerged media because of its alternate rotation into air and water. According to Levich(1962), a rough surface changes the nature of the flow past that surface very materially and, consequently, changes the size of the diffusional flow to the surface. In the case of laminar flow, assuming that the height of the protrusion is large compared with the hydrodynamic boundary layer thickness, these protrusions rise above the boundary layer and the flow past them will be the main flow, unrestricted by proximity to the surface. If $Re_{pro.} = U(h)\ h/\nu$ (h= height of the protrusion, U(h)=flow velocity at the top of the protrusion and ν =dynamic viscosity of water) exceeds 20 to 50 the flow past the protrusion, as in the case of any other non-streamlined body, will be accompanied by separation of turbulence. On the downstream, turbulence

appears , while the upstream side is subjected to laminar flow. Watanabe and Okabe (1986) evaluated the effect of surface protrusion on the liquid boundary layer thickness. The height and location of protrusions on the media surface are shown in Fig. 12. They observed the appearance of turbulence behind the protrusion. Fig. 13 shows the batch experimental result for various numbers of protrusion at a fixed rotating speed of 5 rpm. Using the biofilm kinetics , many batch experimental data were analyzed to determine the liquid boundary layer thickness (Watanabe and Nishidome, 1989). Fig. 14 summarizes the rerationship between the liquid boundary layer thickness and operating parameters such as media rotating speed , media submerged ratio and protrusion number.

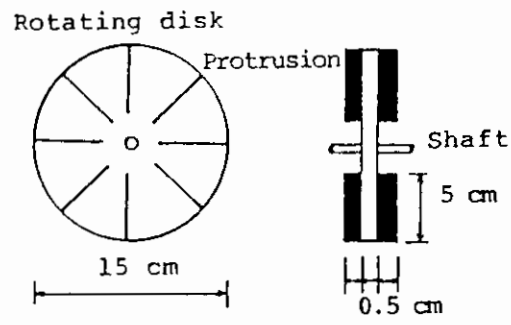

Fig. 12 Media with surface protrusions

Liquid film thickness . There are two expressions available to estimate the thickness of the liquid film. Levich(1962) studied the film thickness entrained on a flat plate vertically withdrawn from a quiescent liquid , and deduced the following equation for the film thickness, L_w:

$$L_w = 0.944(\mu v)^{2/3} \sigma^{1/6} (\rho g)^{1/2} \quad (8)$$

If the physical properties of the wastewater are similar to pure water at 20 C, Eq. 9 is simplified to (Famularo et al, 1978):

$$L_w = 6.85 \nu^{2/3} \quad (9)$$

An alternate expression for the rotating media is given by Bintanja et al(1976):

$$L_w = 0.93 (\nu N_r R/g)^{1/2} \quad (10)$$

Because of irregularity in the biofilm surface , the retained liquid film will be thicker than that obtained in a flat plate. Values reported in the literaure range between 40 and 200 m (Hartmann, 1960; Grieves, 1972; Hansford et al, 1978; Famularo et al, 1978). For the nitrification RBC, Watanabe et al. (1982) obtained a liquid film thickness of 50 m which resulted to a good agreement between simulated and measured ammonia fluxes. Eq. 10 yielded a thickness of about 40 m for the same system.

Intrinsic ammonia oxidation rate The zero-order intrinsic ammonia oxidation rate has been determined by analyzing a batch experimental result of ammonia oxidation (Watanabe and Nishidome, 1989). All batch experimental data used for determinining the liquid boundary layer thickness were analyzed to calculate the zero-order intrinsic ammonia oxidation rate at various water temperatures.

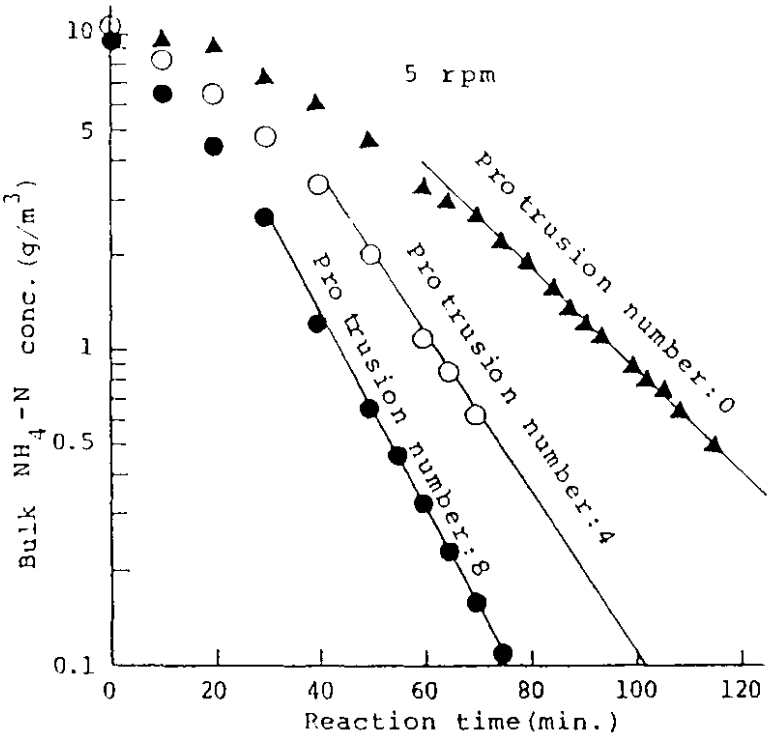

Fig. 13 Effect of protrusions on NH_4-N oxidation rate

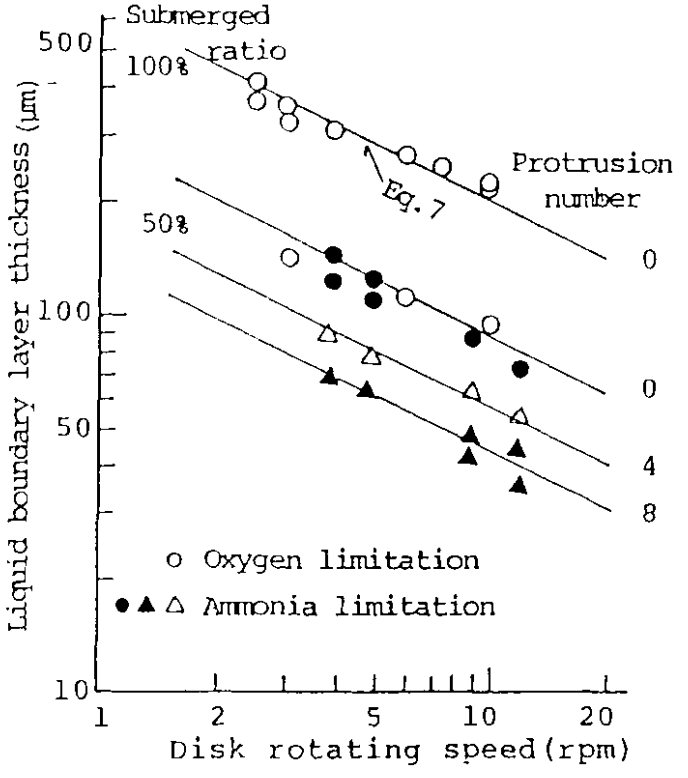

Fig. 14 Relationship between L_d and disk rotating speed

Saturation and bulk concentrations of dissolved oxygen. The saturation concentration of dissolved oxygen is readily known in a given water temperature. As far as the bulk dissolved oxygen concentration is concerned, Watanabe et al. (1980) found that it was kept at about 4.0 ppm in the nitrification RBC operated under oxygen limitation. Experimental verification of the proposed kinetics was made by comparing the calculated and measured ammonia fluxes in various water temperatures and media rotating speeds.

A small scale RBC with the media diameter of 30 cm was used and the steady-state ammonia flux was measured at a given experimental condition. The liquid boundary layer thickness and intrinsic ammonia oxidation rate were obtained from Fig. 15 and our previous results, respectively. The liquid film thickness was assumed to be 10 μm thicker than that calculated by Eq. 10. Fig. 15 shows the comparison of calculated and measured NH_4-N fluxes in the media rotating speed of 2 and 8 rpm.

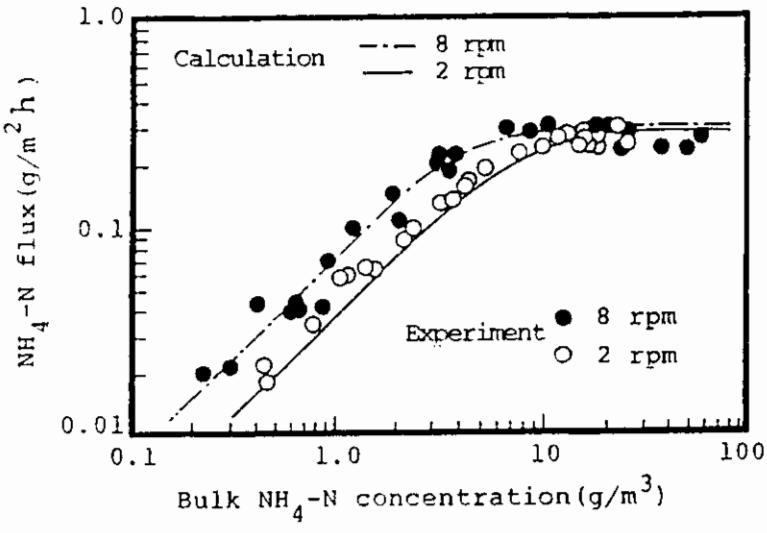

Fig. 15 Measured and calculated NH_4-N fluxes

Basd on the above fundamental researches, the authors (Watanabe et al., 1988) have innovated a reticulated media with protrusions in order to reduce the media weight and the liquid boundary layer thickness. The stainless net of 60 mesh was used as reticulated media. The height and location of 8 protrusions are the same as Fig. 13. The performance of the RBC with the new media was examined by measuring the ammonia flux. Figs. 16 and 17 show the experimental results. At a rotation speed of 10 rpm, half-order biofilm kinetics seems to be realized, which means no liquid boundary layer near the biofilm surface. Masuda, Watanabe and Ishiguro (1987) demonstrated that the heterotrophic, nitrifyning and denitrifying bacteria inhibit the biofilm treating domestic sewage. These microorganisms change their population and activity, depending on the environmental conditions within the biofilm. In aerobic treatment of wastewater with organics and ammonia, competition between heterotrophs and nitrifiers occurs. In a staged RBC, the activity of nitrifying bacteria becomes higher in the later stages where the residual organic concentration is low. Fig. 18 shows the effect of bulk soluble TOC concentration on the NH_4-N flux under oxygen limitation. These experimental data were obtained in the experiment by the RBC unit described in Fig. 12. The ammonia flux is strongly affected by the bulk soluble TOC concentration (SOC). The proposed kinetics can not be applicable to predict the

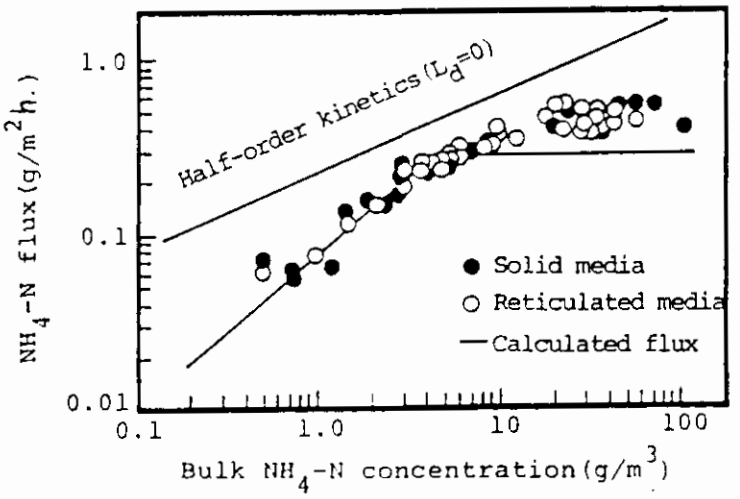

Fig. 16 Effect of protrusions on NH_4-N flux
(Rotating speed= 3 rpm)

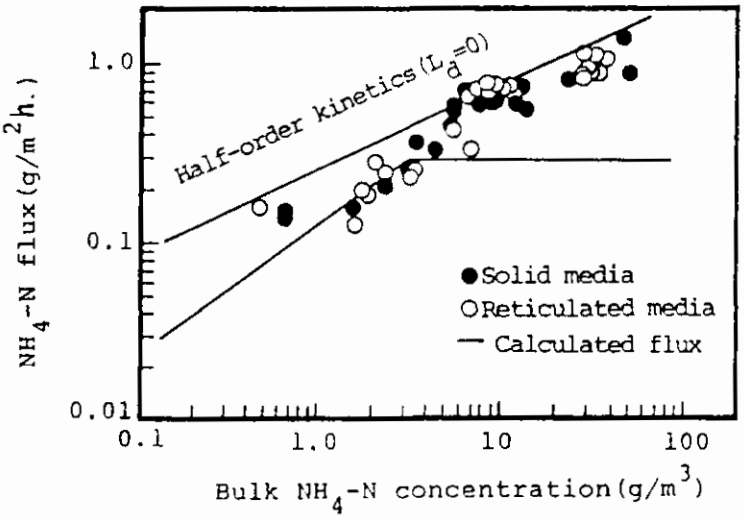

Fig. 17 Effect of protrusions on NH_4-N flux
(Rotating speed= 10 rpm)

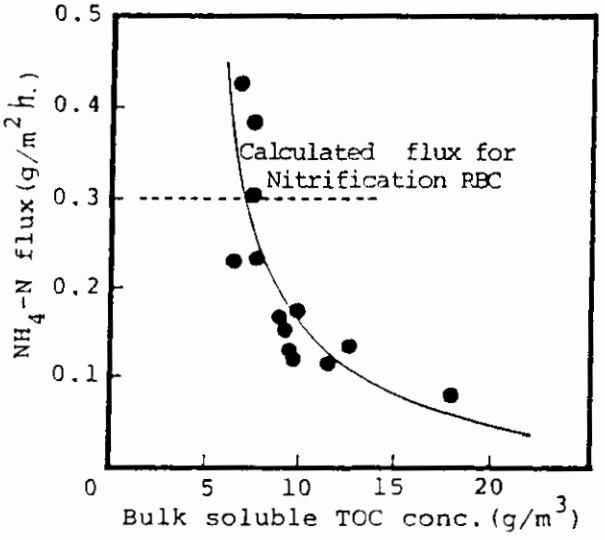

Fig. 18 Effect of soluble TOC on NH_4-N flux

bulk SOC concentration effect on ammonia flux , but the measured ammonia flux approapches the calculated ammonia flux for the nitrification RBC if the SOC concentration is below about 7 ppm.

(b) Simultaneous Removal of Detached Biomass

In a conventional RBC the detached biomass is transported through troughs , then removed in the secondary clarifier. During the transportation the detached biomass is exposed under the mixing produced by the rotation of mediae. It is sometimes broken into smaller non-settleable debris. Two-story RBC was designed to achieve the simultaneous removal of detached biomass in the trough. Its upper and lower parts function as the RBC trough and storage space of settled biomass , respectively. Experimental unit was a single-stage unit (Fig. 19) which was used to make a mass balance of suspended solids in a two-story RBC and to measure the settling velocity of the detached biomass. The settled wastewater from a dormitory was continuously fed into the unit. The average soluble TOC , SS and ammonia concentrations of the wastewater were about 70, 130 and 60 ppm, respectively. Detention time and media rotating speed were fixed at 70 min. and 10 rpm , respectively. Suspended solid concentration in the influent and effluent and the settled sludge volume were measured every day during a week. The same experiment was conducted two times. Table 4 summarizes the average data of mass balance in both experimental runs.

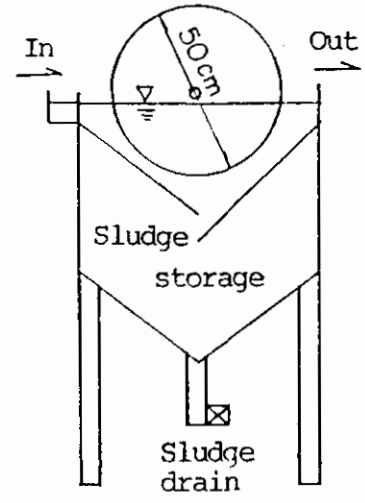

Fig. 19 Two-story RBC

Table 4 Mass balance of SS in two-story RBC

Item	Run 1	Run 2
Water temp. (°C)	18	12
Flow rate (m^3/d)	2.6	2.6
Detention time (min.)	72	66
Influent SS conc. (ppm)	136	135
Effluent SS conc. (ppm)	27	47
Weight of effluent sludge (g/d)	73	130
Weight of settled sludge (g/d)	193	195
Weight of detached biomass (g/d)	266	325
Detached biomass conc. (ppm)	104	120
Removal efficiency (%)	76	66

It is demonstrated that about 70 % of detached biomass was removed into the lower part of the two-story RBC even in a single-stage operation. Fig. 20 shows the settling velocity distribution of influent and effluent suspended solids along with the data for the influent to the secondary clarifier in the four-staged RBC. The opening between the upper and lower parts was closed in the experiment of the settling velocity measurement of detached biomass. Fig. 20 shows that the settling velocity of most of the detached biomass is more than 1 cm/min.

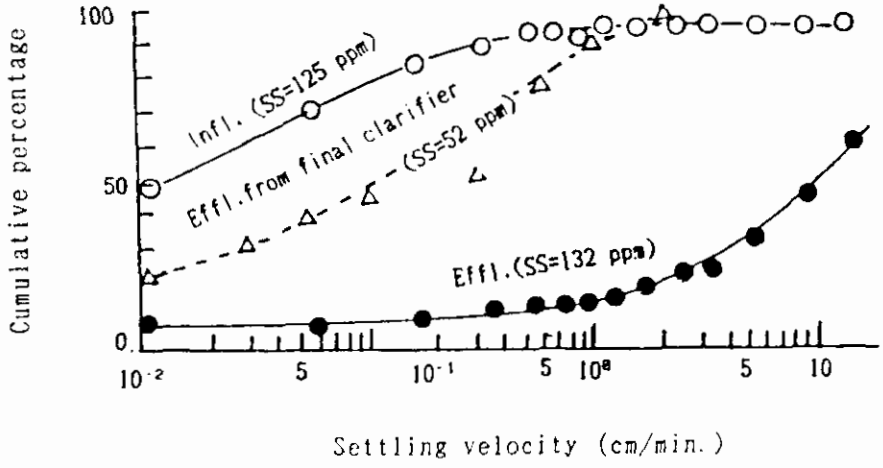

Fig. 20 Cumulative settling velocity distribution of detached biomass

Application to Municipal Wastewater Treatment

(a) Experimental Procedures

Photo. 1 shows the two-story RBC operated in a four-stage. The stainless mesh media with the 8 protrusions was used. Each stage has 14 mediae with the total surface area of 1.98 m^2. The media submerged ratio was 40 %. The media rotating speed was 9.5 rpm. The unit was set in a municipal sewage treatment plant and the effluent from the primary settler was fed into it.

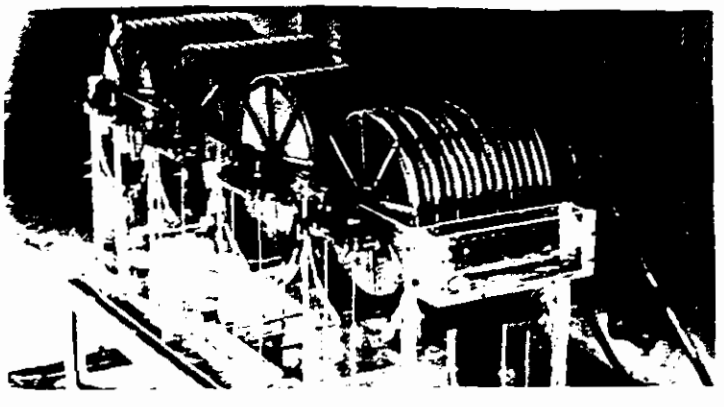

Photo. 1 Upgraded RBC unit

(b) Experimental Results

Fig. 21 shows the influent and effluent concentrations of TOC(suspended+soluble), suspended solids and NH_4-N during four months' operation of the RBC. The water quality was measured in the grab sample. This demonstrates that SOC (soluble TOC) and TOC concentrations in the effluent were about 5 and 10 ppm, respectively, and

the activity of nitrifiers was quite high when the surface loading was between 100 and 50 $\ell/m^2/day$.

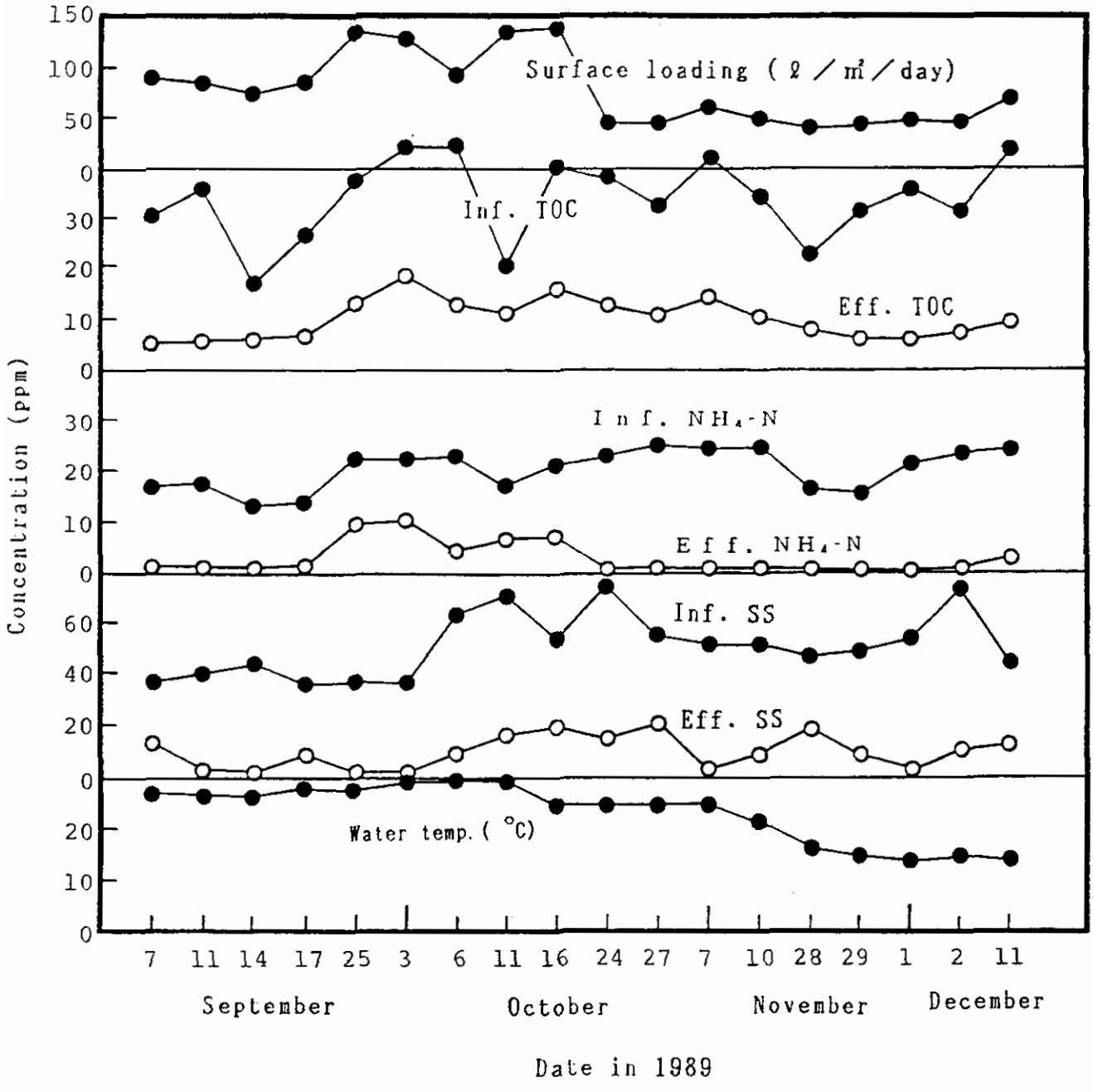

Fig. 21 Operation results of upgraded RBC

Fig. 22 shows the recent experimental results when the RBC was operated in a three-stage unit. The operation of RBC started on 9 October 1990. From the data shown in Fig. 22, it is understood that the biofilm development on the mesh media is very fast and the performance of the upgraded RBC was excellent. Fig. 26 shows the water quality in each stage on 28th October. Figs. 23 to 25 demonstrats the amount of attached biomass and sedimentation rate of detached biomass in each stage which were after one month operation.

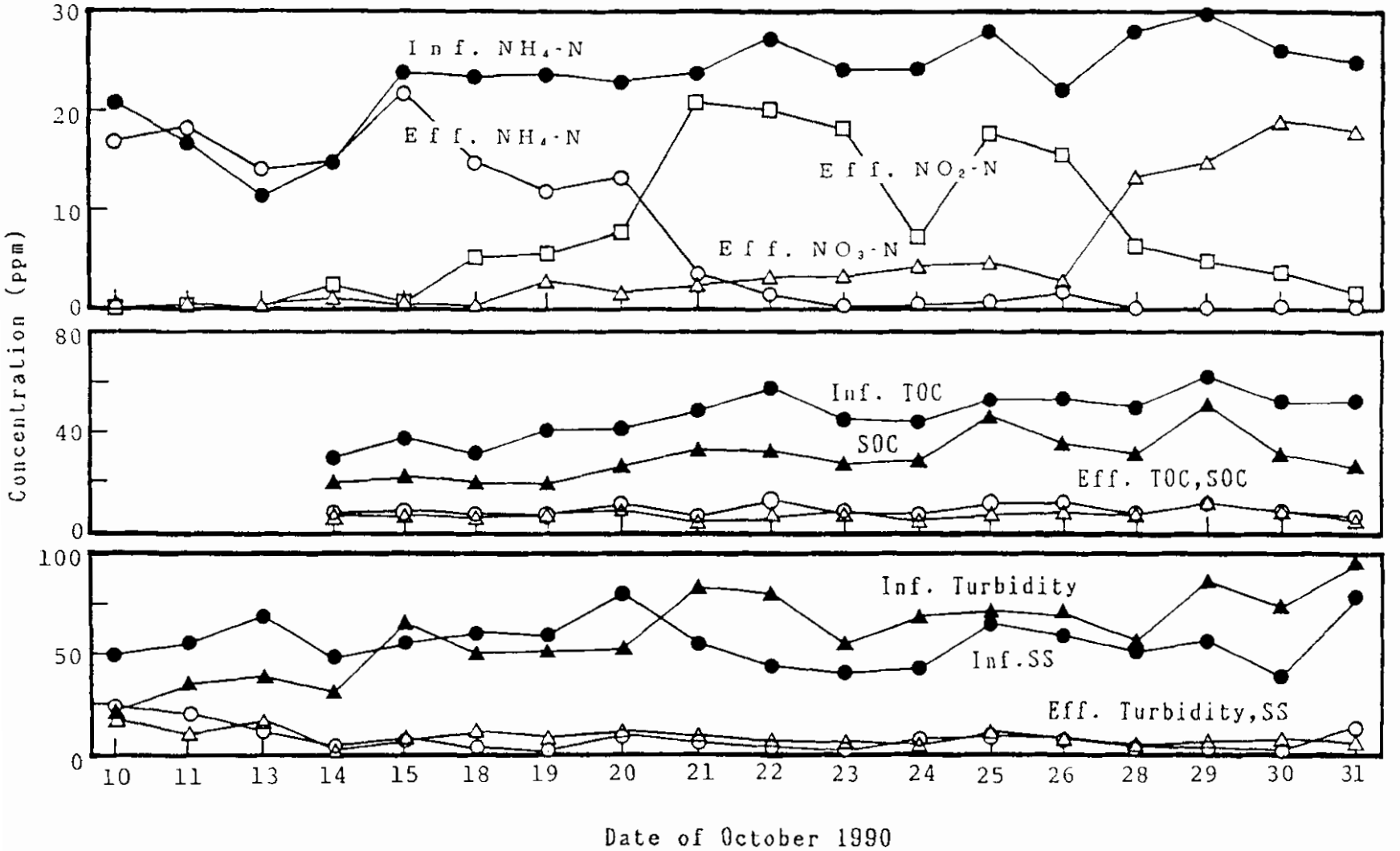

Fig. 22 Operation results of upgraded RBC

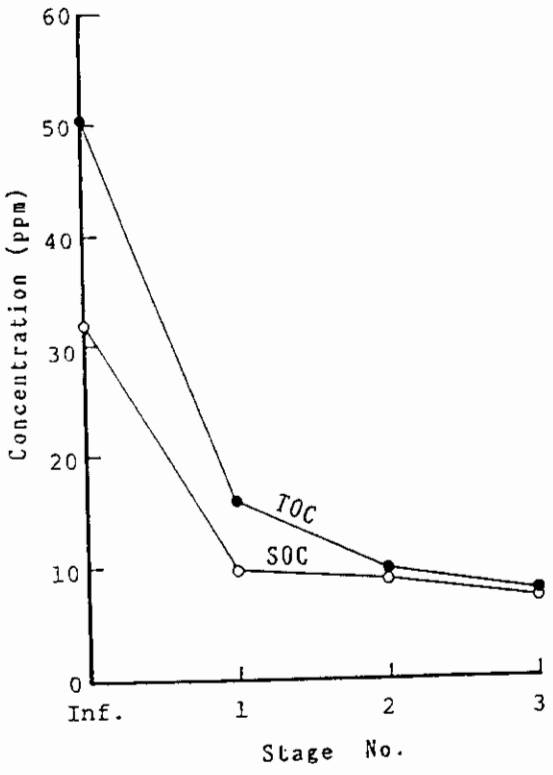

Fig. 23 TOC and SOC concentrations in each stage

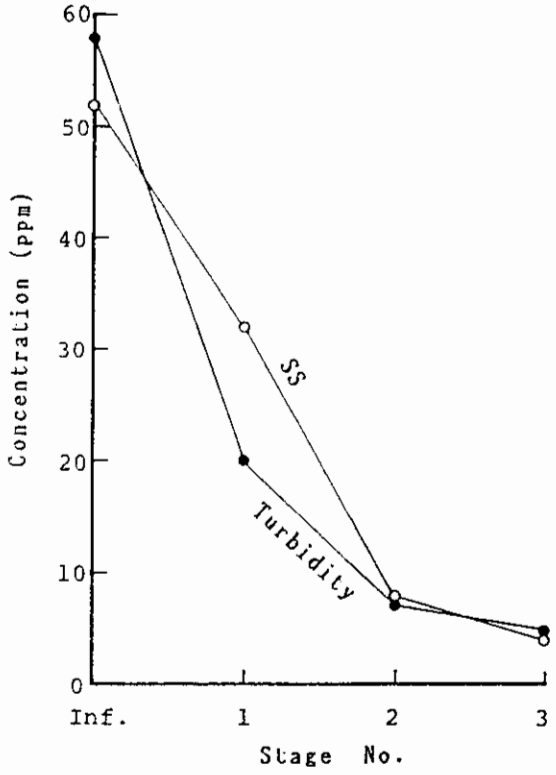

Fig. 24 SS concentration and turbidity in each stage

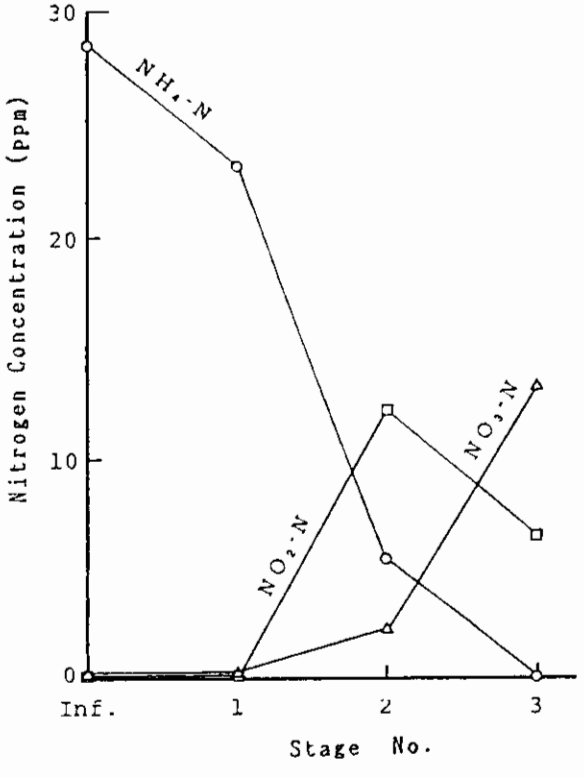

Fig. 25 Inorganic Nitrogen concentrations in each stage

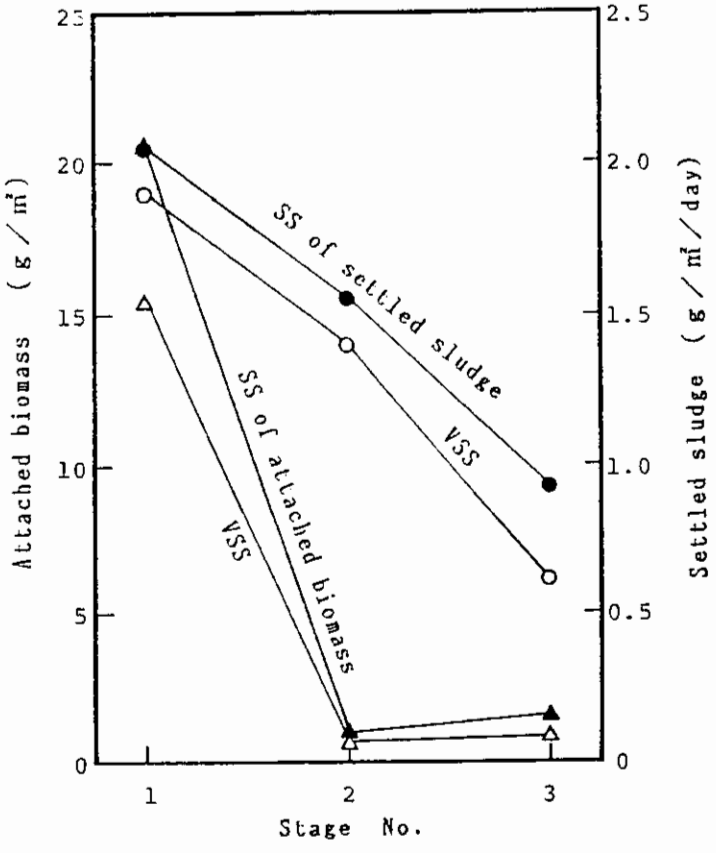

Fig. 26 Amount of attached and detached biomass

(c) Performance of Combined System of JMS and RBC

Figs. 27, 28 and 29 show the experimental results of the combined system of the JMS and RBC. A part of the effluent from the JMS was fed into the RBC. HRT in the JMS and RBC was 45 min. and 3 hours, respectively. Aluminum dosage to the JMS was 5 or 10 ppm. Water temperature was around 15 C. As seen in these figures, the water quality of the RBC effluent was very good.

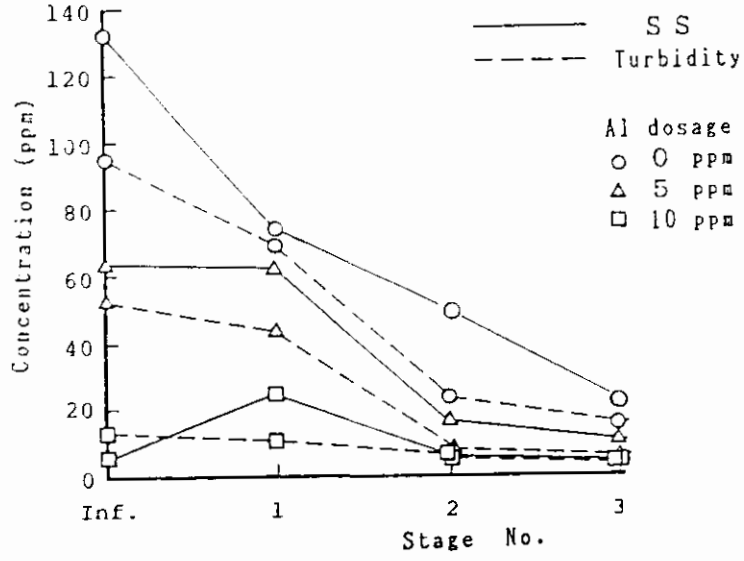

Fig. 27 Effect of Al dosage on removal of SS and turbidity in RBC

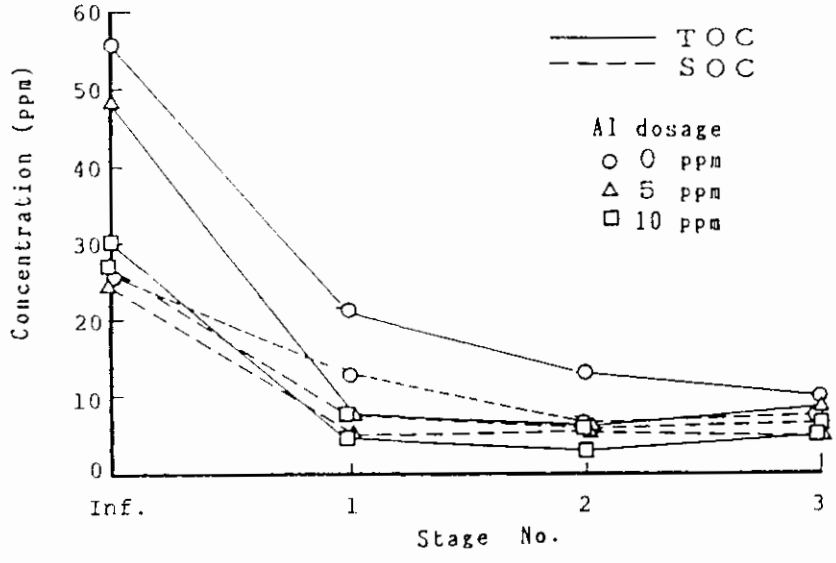

Fig. 28 Effect of Al dosage on removal of TOC and SOC in RBC

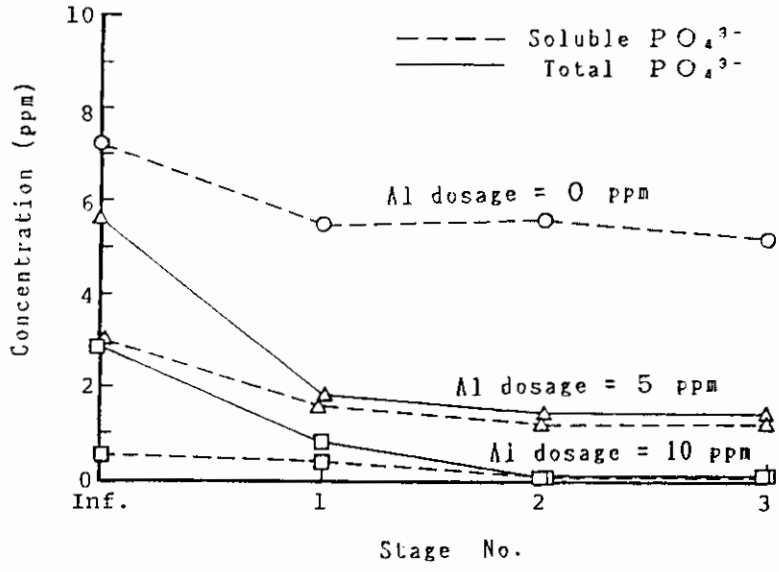

Fig. 29 Effect of Al dosage on removal of phosphorous in RBC

SUMMARY AND COLCLUSIONS

Since the major part of the contaminants in municipal wastewater is associated with particles, direct particle separation is an effective way of lowering the wastewater contaminant level. However, an additional biological oxidation is required to remove the residual soluble organics and ammonia nitrogen. Based on the above point of view, we need to develop the municipal wastewater treatment system composed of physico-chemical and biological methods to meet more strict effluent standard.

In this paper, the experimental data was presented on the chemical treatment of municipal wastewater by the Jet Mixed Separator (JMS) which can be used as a unit process for physico-chemical treatment. The JMS produced an effluent with low phosphorous and suspended solid contents in the hydraulic retention time of less than 1 hour. Removal efficiency of TOC associated with particles was also high. However, the effluent from the JMS is required to be treated biologically for the oxidation of soluble TOC and ammonia nitrogen.

It was also demonstrated the performance of an upgraded RBC which can be used as a unit process for the biological treatment. It is a two story RBC consisted of the stainless mesh media with surface protrusions, whose upper and lower parts function as the RBC trough and storage space of the detached biomass. The upgraded RBC produce an effluent with low TOC, ammonia nitrogen and suspended solid contents in a rather shorter hydraulic retention time.

The perfomance of the combined system of the JMS and upgraded RBC was very high to produce the effluent with very low concentration of TOC, phosphorous and suspended solids in a rather short hydraulic detention time.

NOMENCLATURE

C_{l1}^{b} - bulk substrate concentration (ML^{-3})

C_{l1}^{0} - influent substrate concentration (ML^{-3})

C_{l1}^{s} - substrate concentration at biofilm surface (ML^{-3})

C_{lo}^{a} - DO concentration at biofilm surface during air phase (ML^{-3})

C_{lo}^{w} - DO concentration at biofilm surface during water phase (ML^{-3})

C_{o}^{*} - DO saturation concentration (ML^{-3})

D_f - molecular diffusion coefficient in biofilm (L^2T^{-1})

D_w - molecular diffusion coefficient in water (L^2T^{-1})

D_{wo} - molecular diffusion coefficient of dissolved oxygen (L^2T^{-1})

L_a - effective biofilm depth (L)

L_d - liquid boundary layer thickness

L_w - liquid film thickness (L)

r_1^0 - zero-order intrinsic reaction rate of biofilm ($ML^{-3}T^{-1}$)

r_o^0 - zero-order intrinsic oxygen utilization rate of biofilm ($ML^{-3}T^{-1}$)

N_r = Disk rotating speed (T^{-1})

R = Disk radius (L)

REFERENCES

Bintanja, H. H. J. et al. (1975): Oxygen Transfer in a Rotating Biological Disk Plant, Water Research, Vol. 9

Famularo, J. et al. (1978): Application of Mass Transfer to Rotating Biological Contactor, J. WPCF, Vol. 50

Imhoff, K (1939): Taschenbuch der Stadtenwasserung. 8. Auflage Oldenborg, Munich

Levich, V. G. (1962): Physicochemical Hydrodynamics, Prentice Hall Inc.

Levine, A. D. et al. (1985): Characterization of the Size Distribution of Contaminants in Wastewater, Treatment and Reuse Implication, J. WPCF, Vol. 67

Masuda, S. et al. (1987): Biofilm Microorganisms in Rotating Biological Contactors J. of Japan Sewage Works Association, Vol. 24

Munch, R. et al. (1980): Wastewater Fractions Add to Total Treatment Picture, Water& Sewage Works, Dec.

Ødegaard, H. (1988): Coagulation as the First Step in Wastewater Treatment, Proc. of the 3rd Gothenburg Symposium

Watanabe, Y. et al. (1980): Nitrification Kinetics in a Rotating Disk Reactor, Water Sci. & Tech., Vol. 12

Watanabe, Y. et al. (1982): Simulation of Nitrification and Its Dynamics in a Rotating Biological Contactor, Water Sci. & Tech., Vol. 14

Watanabe, Y. et al. (1984): Mathematical Model of Simultaneous Organic Oxidation, Nitrification and Denitrification in Rotating Biological Contactors, Water Sci. & Tech., Vol. 17

Watanabe, Y. et al. (1986): Mass Transfer in Rotating Biological Contactor, Proc. of the Annual Conference, Japan Society of Civil Engineers

Watanabe, Y. et al. (1990): Nitrification Kinetics and Simultaneous Removal of Biomass In Rotating Biological Contactors, Water Sci. & Tech., Vol. 22

Watanabe, Y. et al. (1990): Theory and Performance of a Jet-Mixed Separator, J. Water SRT-Aqua, Vol. 39, No. 6

BACTIVOROUS GRAZERS: A WAY TO REDUCE SLUDGE PRODUCTION

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Introduction

The present sewage treatment plants have several disadvantages. The most important drawbacks are:

- low volumetric capacity
- large area in use
- high sludge production
- high energy costs
- emission of noise and stench
- limited sludge age failing to support growth of slowly growing organisms (nitrifiers and protozoa)
- low effluent quality

Aim of this project

One way to reduce sludge-production is to select micro-organisms with a high maintenance energy requirement. Lowering the substrate/biomass ratio is a possible selection mechanism. Under these circumstances the bacterial growth rate is low and most utilized substrate is spent on maintenance processes. As a result biomass production is low. This can be realized in reactors which permit biomass retention either by the use of carrier material or by recycling.

Another option is to introduce organisms which predate on bacteria. Through predation the sludge will be converted into energy, water and carbon dioxide. Under optimal conditions the total loss of energy will be maximal and the biomass production minimal.

Biological sewage treatment systems: artificial ecosystems

Besides bacteria, many other (predator) organisms occur in biological treatment plants, for example, ciliates, rotifers nematodes and oligocheates (Curds, 1982, Eikelboom, 1988). Little is known about the relationships between these organisms in a plant, and of the quantitative effect of predation on the bacterial populations.

From an ecological point of view, the activated sludge can be represented as a foodweb. Processes like competition, inhibition, predation, lysis, and cryptic growth are important (Fredrickson and Stephanopoulos, 1981). To study the relationship of predator and prey organisms, several experiments will be performed with single and mixed cultures.

Oligochaetes and nematodes are frequently found in oxidation discs and activated sludge systems. Metazoa ingest 5000 and ciliates 10 cells per minute. Thus these organisms can possibly be a tool for reducing sludge. Experiments will be set up to quantify feeding, maintenance and production rates, on the basis of a dynamic energy budget model (D.E.B.) developed for bacteria and metazoans (Kooijman, 1986).

These experiments aim at a better understanding of predation which may help to realize an extra "predation trap" in the water-purification proces. The positive impact of predation on mineralization and nitrification in sewage treatment plants will also be studied.

The implications of the D.E.B. model for the population dynamics in the reactor will be evaluated. This will facilitate optimization of the process.

Preliminary Results

Ciliates isolated from activated sludge were shown to eliminate bacteria in batch cultures within one day. However, ciliates obtained from a culture collection did not survive on the same bacteria.

Nais elinguis (an oligochaet, length < 12 mm, volume 212 μm^3, volume pharynx 2.64 x 10^6 μm^3) is able to consume as much as 36 million bacteria per day per individual. For a Nais population in river sediment, this would amount to 5-50% of the total population of bacteria per day. In natural habitats 30-40 worms per ml exist (Pfannkuche, 1977).

A ciliate (length 40 μm, volume 2 x 10^4 μm^3) consumes 15,000 bacteria per day (Curds, 1982).

Especially when the sludge loading factor (F/M ratio) is low, that is about 0.1 kg BOD per kg sludge per day, blooms of oligochaetes and nematodes occur. In activated sludge systems 25-50 oligochaetes per ml sludge, and in oxidation ditches 100-1,000 nematodes per ml film are found (Eikelboom, 1988). Hence grazing seems to offer prospects to reduce sludge production.

Experiments to measure the uptake and allocation of energy in isolated individuals are now in progress.

References:

Curds, C.R. (1982)
The ecology and role of protozoa in aerobic sewage treatment processes. Ann. Rev. Microbiol., 36, pp. 27-46.

Eikelboom, D.H. (1988)
Extra toepassingsmogelijkheden voor Protozoa en Metazoa bij de zuivering van afvalwater.
MT-TNO, Rapport nr. R 88/286

Fredrickson, A.G. and Stephanopoulos, G. (1981)
Microbial Competition. Science, Vol. 213, pp. 972-979.

Kooijman, S.A.L.M. (1986a)
Population dynamics on basis of budgets. In "The dynamics of physiologically structured populations" (ed. O. Diekmann & J.A.J. Metz) pp. 266- 297. Lecture Notes in Biomathematics. Springer Verlag. Berlin.

Pfannkuche, O. (1977)
Ökologische und systematische Untersuchungen an naidomorphen Oligochaeten brackiger und limnischer Biotope.
Dissertation, Hamburg.

BIODEGRADATION OF XENOBIOTICS BY SPECIFIC BACTERIA: RESEARCH AND APPLICATIONS

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Introduction

Biodegradation is crucial in the breakdown and mineralization of organic chemicals in soil and water, and as such it is an indispensable link in the carbon, nitrogen and oxygen cycles of our biosphere.

The exponential increase in the production of new synthetic organic compounds during the past century has strained the scope and capacity of these natural cycles. Consequently, there is a need for more information on the detailed operation of the various natural cycles, so that technical and regulatory options can be developed, evaluated, and implemented.

The environment is usually taken to consist of three compartments: soil, (ground)water, and air. For soil and groundwater, biodegradation refers to the removal of contaminants introduced in the past. For wastewater and air, biodegradation refers to the removal of pollutants at the source, to prevent contamination of surface and groundwaters and the atmosphere.

Each compartment requires specific biodegradation technologies. For soil, these may range from land-farming to the application of soil slurry bioreactors. For groundwater, recirculation coupled to water-treatment may be the system of choice, while for wastewater a variety of open and closed bioreactor systems has been developed. Industrial waste air or gas streams may be treated in simple or sophisticated biofiltration systems.

While the technologies differ in appearance, they have major features in common.

First, the contaminants, true to their name, form only a very minor portion of the relevant phase. As a result, large installations and high fluxes are necessary to process small amounts of pollutants.

Second, biodegradation depends on the presence of microorganisms in the relevant installation. They must either be present, remain in, grow in, or be added to the installation.

Third, microorganisms require a balanced supply of nutrients and appropriate environmental conditions (pH, temperature, moisture, electron donors, electron acceptors). These conditions must be met and maintained in the installation.

Fourth, microorganisms are often fairly specific and sometimes quite fastidious with respect to the substrates which they utilize. It may therefore be necessary to tailor the microbial flora in the installation to the composition of the pollutant stream to be treated.

Since the above four features are important for every biodegradation system, it is clear that investigations of the enzymology, genetics and physiology of the bacterial degradation of xenobiotics should contribute significantly to the development of practical biotreatment technologies.

Potential applications of bacteria in biodegradation

A traditional viewpoint among many microbiologists has been that the entire range of microbial life is represented throughout the biosphere, as long as the environmental conditions meet the requirements of the microorganisms in question. In other words, if a pollutant is released at an arbitrary location, and if microorganisms exist which can degrade said pollutant, a population of these organisms will develop locally and consume the pollutant. Conversely, if the pollutant is not degraded, it is considered evident that there are no organisms to degrade it: the pollutant is "recalcitrant" because evolution has not yet selected strains which can dispose of it.

We do not share this viewpoint. First, the utilization of a given pollutant by an organism which does in fact have the necessary genetic information to express relevant enzymes may be limited by local environmental conditions. Second, it is unlikely that every representative of the microbial world is indeed present in every geographic location on this planet. Thus, it is to be expected that a considerable number of "recalcitrant" compounds is in fact biodegradable.

In support of the above viewpoint we have developed a databank, in which microorganisms described in the literature have been matched with the compounds which they can utilize or co-metabolize. A comprehensive literature study was carried out to describe the biodegradability of the compounds that have been designated as priority or black list pollutants in The Netherlands, and to indicate the possibilities and limitations of biological treatment techniques for these compounds (1).

Biodegradation of priority and black list pollutants

The Dutch priority and black lists contain approximately 140 different (groups) of organic compounds including:

- aliphatic hydrocarbons
- halogenated aliphatic hydrocarbons
- polycyclic aromatic hydrocarbons (PAH's)
- (chloro)aromatics
- organotin compounds
- organochlorine pesticides
- organophosphorus pesticides

The literature study showed that:

- complete or partical microbial degradation under <u>aerobic</u> conditions has at present been described for approximately 75% of the priority and black list pollutants.
- <u>Anaerobic</u> degradation has been described for approximately 30% of the priority and black list compounds.
- Relatively many recalcitrant compounds are found among the halogenated aliphatic hydrocarbons: 50% are refractory to biodegradation under aerobic conditions as shown in Table 1.

The database on which these conclusions are based contains literature information on the biodegradation of each of a large number of environmental pollutants. The database is updated four times per year and specific information can be retrieved easily*.

* The database is accessible via Bioclear Environmental Biotechnology, Zernike Park 2, 9747 AN Groningen, The Netherlands.

Table 1. Biodegradability of halogenated aliphatic hydrocarbons under aerobic and anaerobic conditions.

compounds	aerobic	anaerobic
bis (2-chloroisopropyl)ether	-	-
2-chloroethanol	++ P	-
chlorofluoromethane	-	-
3-chloropropene	-	-
chloralhydrate	-	-
chloroprene	-	-
1,2-dibromoethane	+ P	+
1,2-dichloroethane	++ P	++
1,1-dichloroethene	+	+
1,2-dichloroethene	+ P	+
dichloromethane	++ P	-
1,2-dichloropropane	-	-
1,2-dichloro-2-hydroxypropane	++ P	-
1,3-dichloropropene	++ P	-
epichlorohydrin	++ P	-
hexachlorobutadiene	-	-
hexachloroethane	-	-
methylbromide	+ P	-
monochloroacetic aced	++ P	-
1,1,1,2-tetrachloroethane	-	-
1,1,2,2,-tetrachloroethane	-	+
tetrachloroethene	-	+
tetrachloromethane	-	++
1,1,1-trichloroethane	-	+
1,1,2-trichloroethane	-	-
trichloroethene	+ P	+
trichloromethane	+ P	++
vinyl chloride	++ P	-

++ mineralization
+ partial degradation
P Pure culture

Thus, at least 75% of the top pollutants on the Dutch lists, which closely resemble the EPA priority list, can be degraded by one or more microorganisms. As more information accumulates, this percentage will increase. Selection procedures and genetic engineering experiments may result in additional strains capable of degrading some of the remaining "recalcitrant" compounds.

Biodegradation of halogenated hydrocarbons

Chlorinated aliphatic and aromatic compounds have been considered recalcitrant to varying degrees. An investigation of the relative prevalence vs. recalcitrance of a number of these compounds led us to conclude that it would be worthwhile to search for bacteria which degrade short haloaliphatics.

Chlorinated aliphatic hydrocarbons are produced industrially in large amounts for use as solvents, cleaning agents, intermediates for further chemical synthesis, pesticides, etc. (Table 2). Many cases of pollution of aquifers, surface waters, and soils have been reported (2). Furthermore, applications of these chemicals are a cause of increased concentrations of volatile halogenated compounds in some industrial areas, partly due to emission of exhaust gases. Contamination of drinking water by chlorinated methanes, ethylenes, and propanes has also occurred.

Table 2. Production and use of some chlorinated aliphatic hydrocarbons.

compound	production (10^6 tonnes/yr)	use
1,2-dichloroethane	13	vinylchloride, gasoline
vinylchloride	12.0	polyvinylchloride
perchloroethylene	1.1	solvent
trichloroethylene	1.0	solvent
carbon tetrachloride	1.0	solvent, CHC
1,1,1-trichloroethane	0.45	solvent
methylene chloride	0.4	solvent
methylchloride	0.35	solvent, blowing agent
2-chlorobutadiene	0.3	polymers
chloroform	0.24	solvent, CHC
1,1-dichloroethylene	0.1	solvents, polymers

1,2-dichloroethane is a particularly interesting compound. At a production rate of 13 million tons per year it is one of the top man-made products in volume. Until the early 1980's, no organisms were known to degrade this compound. Since then, however, a strain (Xanthobacter autotrophicus GJ10) has been isolated in our laboratory which can grow exclusively on 1,2-dichloroethane (3), and various other strains have been isolated with specificities for a number of other haloaliphatics (Table 3).

When a series of inocula is tested, often only a few enrichments are positive, dependent on the compound tested and the nature of the inoculum. Not all chloroaliphatics are difficult to degrade, however. Chloroacetate utilizing bacteria, for example, can easily be isolated from samples of soil with no history of chemical pollution. Dichloromethane degraders seem to be rare. The distribution of 1,2-dichloroethane degrading bacteria is even more limited, since we observed that most enrichments with non-polluted inocula do not give growth (4).

Table 3. Pure bacterial cultures that use haloaliphatics for growth.

strain	isolated on	growth rate (h^{-1})	reference
Hyphomicrobium	methylchloride	0.09	5
Pseudomonas DM1	dichloromethane	0.11	50
Hyphomicrobium DM2	dichloromethane	0.07	51
Methylobacterium DM4	dichloromethane	0.22	12
Xanthobacter GJ10	1,2-dichloroethane	0.12	3
Pseudomonas CE1	2-chloroethanol	0.09	13
Arthrobacter HA1	1-chlorohexane	0.14	8
Mycobacterium m15-3	1-chlorobutane	-	9
Pseudomonas strain GJ70	1,6-dichlorohexane	-	7
	1,6-dichlorohexane	-	6
Mycobacterium L1	vinylchloride	0.05	14
Pseudomonas AD1	epichlorohydrin	0.20	52

All 1-monochloro-n-alkanes (C1-C12) are degradable by pure bacterial cultures that use these chemicals as a carbon and energy source for growth (3, 5). In general the generation times of these bacteria are in the order of 5-10 h (Table 3). There is quantitative dechlorination, which points to complete detoxification. Pure cultures have also been obtained for dichloromethane (10-12) and the α, w-dihaloalkanes (C2, C3, C4, C6, C9) (3,6,13; Table 3). Terminally halogenated alkanes are in general easier to degrade than secondary alkylchlorides (4). The presence of two or three chlorines bound to the same carbon atom seems to prevent aerobic degradation, since attempts to isolate cultures utilizing 1,1-dichloroethane, 1,1,1-trichloroethane, and 1,1,2-trichloroethane were unsuccessful (4). The same was found with trihalomethanes and carbon tetrachloride. Of the chlorinated ethylenes, only the isolation of a vinylchloride utilizing Mycobacterium strain has been reported (14). In general, the lower the number of halogen substituents, the better the chances of degradation by organisms utilizing the compound for growth under aerobic conditions.

Under anaerobic conditions, several conversions of highly chlorinated aliphatic hydrocarbons to lesser chlorinated compounds that may serve as a growth substrate for aerobic organisms have been found (15), but these will not be further discussed here.

A critical step in the microbial metabolism of chlorinated compounds is the dehalogenation. The enzymes that catalyze dehalogenation act on carbon-halogen bonds in compounds which are absent or occur at very low concentrations in natural environments. As an alternative, dehalogenation may be caused by enzymes that do not directly cleave carbon-halogen bonds but produce unstable intermediates by incorporation of oxygen atoms. During dehalogenation the organochlorine character of the compounds, which is the actual cause of toxicity, is lost. This makes dehalogenation a key step in the bacterial metabolism of chlorinated compounds.

Five dehalogenation mechanisms have been observed in aerobic bacteria. Not all mechanisms have been characterized in detail, but in some cases the enzymes have been purified and studied. Based on the likely degradation mechanisms, their substrate range, the products formed, and the properties of the enzymes, it is possible to understand why some compounds are recalcitrant and what remedies could lead to improved degradation.

Hydrolytic dehalogenases

Hydrolytic dehalogenation has been well documented for the conversion of 2-halocarboxylic acids by bacteria that utilize chloropropionate or chloroacetate for growth. Most of these dehalogenases are small proteins, with molecular weights in the range of 17 to 68 kDa (16,17). The dehalogenases seem to form a rather heterogeneous class of enzymes, since they show different dehalogenation mechanisms and are immunologically distinguishable.

Direct hydrolytic dehalogenation of a chlorinated hydrocarbon was first found with the haloalkane dehalogenase from Xanthobacter autotrophicus GJ10, an organism obtained in our laboratory using enrichment with 1,2-dichloroethane as sole carbon source (3,18). Other 1,2-dichloroethane degrading bacteria have been isolated by us and other groups (4,53). Most of these are facultative methylotrophs, which can partly be explained by the involvement of the quinoprotein methanol dehydrogenase in chloroethanol oxidation. Some chloroethanol-utilizing Pseudomonas strains also produce an inducible PMS-coupled alcohol dehydrogenase for chloroethanol oxidation.

The hydrolytic dehalogenase from X. autotrophicus GJ10 has been purified, characterized (19), and crystallized (20). The three dimensional structure of the protein is currently being resolved (20). Furthermore, we have cloned and sequenced the gene encoding this enzyme. The gene encodes a 310 amino acid polypeptide of molecular weight 35,143 Da and is preceded by two E. coli consensus promotor sequences, explaining the high expression observed in Xanthobacter and other Gram-negative bacteria.

Three other dehalogenases that convert haloalkanes to their corresponding alcohols have been found in Gram-positive bacteria (21-23; Table 4). These proteins have an activity toward the environmentally less important long chain chloroalkanes, but do not convert 1,2-dichloroethane. All enzymes found are composed of a single polypeptide chain of molecular weight 28-36 kDa. The haloalkane dehalogenases seem to form a distinct class of enzymes since no overlap in substrate range with other hydrolytic dehalogenases has been found and the enzymes are not immunologically cross-reactive with the halocarboxylic acid dehalogenases tested so far. We found that antiserum against the haloalkane dehalogenase from X. autotrophicus GJ10 does not react with the halocarboxylic acid dehalogenase from a 2-chloroethanol utilizing Pseudomonas, Pseudomonas 113, Moraxella, or haloalkane dehalogenases from other organisms (4,17).

Table 4. Characteristics of haloalkane dehalogenases.

organism (ref.)	mol. wght.	substrates
X. autotrophicus (4,19)	35,143 Da	C1-C4 1-chloroalkanes C1-C12 1-bromoalkanes epichlorohydrin
strain GJ70 (22)	28 kDa	C3-C6 1-chloroalkanes C4-C9 α,w-dichloroalkanes bis(2-chloroethyl)ether
Arthrobacter HA1 (21)	36 kDa	C2-C9 1-chloroalkanes C3-C6 dichloroalkanes
Corynebacterium m15-3 (23)	36 kDa	C3-C9 1-chloroalkanes C3-C9 α,w-dichloroalkanes C3-C4 α-chloro-w-alkanols

Hydrolytic dehalogenation is particularly attractive for the conversion of chlorinated aliphatics since the enzymes do not need oxygen or cofactors for activity and catalyze simple dechlorination with water as the nucleophile, yielding alcohols as products. A limitation of the dehalogenases is that they are not active with halogens bound to unsaturated carbon atoms, since vinylic halogens are very resistant to nucleophilic displacement reactions. Other compounds that are not converted in this manner are trihalomethanes and alkanes with more than one chlorine bound to the same carbon atom. Furthermore, the enzymes have a relatively low affinity for their substrates. It is not yet clear whether this is directly related to the affinity of the microorganisms for their substrates, causing high Monod constants.

Oxidative conversions

Compounds with vinylic halogens, trihalomethanes, and several other highly chlorinated aliphatics may be degraded by oxidative conversion mediated by monooxygenases produced by methanotrophic bacteria (24-30). Monooxygenases require a reduced cofactor or cytochrome for activity and incorporate one oxygen atom of molecular oxygen in the substrate, while the second oxygen atom is reduced to water (31). Monooxygenase reactions are electrophilic in nature instead of nucleophilic and therefore oxidation provides an alternative for degradation of various compounds that are structurally insensitive to nucleophilic substitution reactions.

The methane monooxygenases are very aspecific (31,32) and have been proposed to oxidize halogenated methanes, ethanes, and ethylenes (24-26). Oxidation can lead to dehalogenation as a result of the formation of chemically labile products, such as gem- halohydrins or chloroepoxides. Typical half lifes of chlorinated epoxides in neutral buffers are 30 h (trans-1,2-dichloroethylene oxide), 5 h (trans-1,3-dichloropropene oxide), 1.5 min (vinyl chloride oxide), and 12 s (trichloroethylene oxide) (26,33,34).

Initial observations on trichloroethylene degradation were made with soil columns (30) and with mixed enrichments of methanotrophs (24). Pure cultures also showed degradation of trihalomethanes and chlorinated ethylenes (4,26,27,35).

Chloroform and chloroethylenes have been found to be converted in soil exposed to methane (4,25,29).

Methanotrophs can, for example, efficiently degrade trans-1,2-dichloroethylene (26). This cometabolic process required the presence of 16 moles of methane per mole of dichloroethylene and produced trans- 1,2-dichloroethylene oxide as a chemically unstable intermediate. The metabolism of other chlorinated ethylenes by a strain of Methylomonas was found to be hindered by the toxicity of primary products of the methane monooxygenase. Conceivably, the unstable gem-halohydrins, epoxides, or reactive aldehydes that are produced from the substrates are toxic to the cells or inhibit methane monooxygenase. As a result, compounds such as chloroform and trichloroethylene, which are relatively inert and of low toxicity to cultures that grow with methanol, caused inhibition of growth at concentrations above 100 μM when methane was supplied as carbon source (26).

We have found that very efficient degradation of trichloroethylene and several other chloroaliphatics can be achieved by a culture of Methylosinus trichosporium, which can produce a soluble type of methane monooxygenase (36) that converts several chlorinated compounds (35; Table 5). Only when cultivated under copper limitation, which caused expression of the soluble type methane monooxygenase, were cells able to degrade trichloroethylene. Dechlorination of the compound was achieved, with only traces of 2,2,2-trichloroethanol and trichloroacetaldehyde being formed. The methane monooxygenase seems to have a low affinity for trichloroethylene since degradation proceeded according to first order kinetics at concentrations below 0.1 mM, with a rate constant of 2 $nmol.min^{-1}.(mg\ cells)^{-1}$. Higher concentrations were toxic and inhibited degradation. In these experiments, formate was used as the electron-donating agent. Besides trichloroethylene, other chloroaliphatics were degraded by M. trichosporium, including dichloromethane, chloroform, dichloroethanes, 1,1,1-trichloroethane, and dichloroethylenes, but formation of chlorinated organic products occurred in some cases (Table 5). The perchlorinated compounds tested, carbon tetrachloride and tetrachloroethylene, were not converted.

The biochemical characteristics of soluble methane monooxygenase from M. trichosporium OB3b and Methylococcus capsulatus (Bath) are very similar and have been studied in detail (31,37). The enzyme is a three component complex. The hydroxylase component A has a molecular weight of 210 kDa containing 2.3 non-haem iron atoms/molecule (37).

The rates that we have observed with cells of M. trichosporium expressing soluble monooxygenase are much higher than the rates found with the same organism expressing particulate monooxygenase (35), and with a type I methanotroph that was grown in the presence of copper, and thus presumably also expressed a particulate type of enzyme (27).

Catabolism of trichloroethylene and dichloroethylenes has also been found with toluene utilizing strains of Pseudomonas putida (38,39). Degradation was concluded to be mediated by toluene dioxygenase encoded by the todC gene (39), and induction of the degradative activity with aromatic compounds was necessary. First order kinetics with a rate of 1.8 $nmol.min^{-1}$ $(mg\ protein)^{-1}$ at 80 μM trichloroethylene was found. These data indicate that trichloroethylene conversion by P. putida proceeds faster than that shown by methanotrophs

producing particulate monooxygenase, but about 100 times slower than the rate that can be obtained by M. trichosporium expressing the soluble enzyme (35).

Table 5. Degradation of chloroaliphatics by M. trichosporium OB3b (35).

compound	chlorinated product(s)[a]
dichloromethane	chloride
chloroform	chloride
carbon tetrachloride	no conversion
1,1-dichloroethane	chloride
1,2-dichloroethane	chloride
1,1,1-trichloroethane	2,2,2-trichloroethanol
trans-1,2-dichloroethylene	chloride, epoxide
cis-1,2-dichloroethylene	chloride, epoxide
trichloroethylene	chloride, 2,2,2-trichloroethanol
tetrachloroethylene	no conversion
1,2-dichloropropane	1,2-dichloro-3-propanol

[a] Incubations were done at 30 °C with resting cells from chemostat cultures grown in medium containing no added copper. Compounds were added at 0.1 mM and formate was used as electron donor.

Recently, a DNA fragment that harbors genes for toluene oxidation in Pseudomonas mendocina was expressed in E. coli and found to enable this organism to degrade toluene at a rate of 1-2 $nmol.min^{-1}.(mg\ protein)^{-1}$ (40). Although this rate is rather low when compared to the velocity of trichloroethylene catabolism in M. trichosporium, degradation was found not to require the presence of an inducer, which is, in case of toluene, an important advantage compared to the P. mendocina strain when practical application is considered.

Oxidative conversions seem of primary importance in organisms that convert chloroaliphatics cometabolically, i.e. the organisms do not use these compounds as a sole carbon source and need a second oxidizable electron donor for growth and for supplying reducing equivalents. The involvement of oxidative conversion as the mechanism for dehalogenation in organisms growing with chloroalkanes, such as methylchloride, 1,2-dichloroethane, and 1-chlorobutane has been suggested (Table 3), but no biochemical experiments with cell free extracts to support this have yet been reported.

Causes of recalcitrance

For some chlorinated aliphatics, attempts to isolate pure bacterial cultures that use these compounds as a growth substrate have repeatedly been unsuccessful. Thus, no cultures have been isolated that use 1,1,1- or 1,1,2-trichloroethane, trichloroethylene, 1,2-dichloropropane and tri- or tetrahalomethanes for growth.

Highly chlorinated aliphatics obviously do not serve as an energy source under aerobic conditions since oxidation does not provide a net yield of reducing equivalents and hydrolysis directly produces carbon dioxide. This holds for

compounds such as chloroform, carbon tetrachloride, and tri- and perchloroethylene.

In other cases, there seems not to be a biochemical factor that makes utilization as a carbon and energy source impossible. Compounds such as 1,1,1-trichloroethane and 1,2-dichloropropane could theoretically be converted by hydrolysis to acetic acid and 1-chloro-2-propanol, respectively, which support growth of pure cultures. Hydrolytic dehalogenases that carry out these conversions have never been found, however. Recalcitrant behavior is therefore probably due to a lack of enzymes that carry out hydrolytic dehalogenations with these substrates. It is possible that enzymes that can hydrolyze these compounds will be found in the future or can be selected in the laboratory.

The same compounds can be converted to, respectively, trichloroethanol and 1,2-dichloro-3-propanol by oxidation, but the methanotrophs that carry out these reactions are unable to derive carbon and energy from the products. Recalcitrance is thus caused in part by the absence of the appropriate combination of a set of catabolic enzymes. A similar situation has been described with bacteria that degrade aromatic compounds, and assembly of new catabolic routes for chlorinated aromatics metabolism has been proposed (41).

Accumulation of toxic intermediates also appeared to be a cause of poor degradation of some haloaliphatics. The soil fumigant 1,2-dibromoethane, for example, can be converted by dehalogenase to 2-bromoethanol and ethylene glycol (6). Furthermore, 2-bromoethanol is a substrate for the halohydrin dehalogenase of another organism, producing ethylene oxide. Although several reactions are possible, 1,2-dibromoethane was found to be very resistant to degradation. We propose that this is caused by the accumulation of toxic bromoacetaldehyde, formed by alcohol dehydrogenase activity and lethal to organisms that carry out the initial dehalogenation. Mutant strains lacking alcohol dehydrogenase still do not utilize 1,2-dibromoethane due to the fact that they could not grow with ethylene glycol. X. autotrophicus GJ10 was also very sensitive to the toxic effects of 1,2-dibromoethane (2 μM), possibly due to accumulation of aldehyde. Thus, recalcitrance was again caused by the absence of the right combination of metabolic activities in a single organism.

Assembly of new catabolic routes using recombinant DNA techniques for combining catabolic genes from different organisms, as suggested for the degradation of substituted aromatic compounds (41), could become feasible for the construction of organisms with enhanced catabolic potential (40). Hydrolytic dehalogenases and monooxygenases would be good targets for such an approach since the substrate ranges of these enzymes generally includes several environmentally important compounds that do not support growth of the organisms that produce the enzymes.

Prospects for the biological treatment of chlorinated hydrocarbons

Specialized cultures, as described here, will become increasingly important for the application of biological techniques for environmental protection and cleanup. Main fields of application include: treatment of industrial waste gases, soil and aquifer decontamination, and elimination of chloroaliphatics from waste-

and groundwater. For slowly growing organisms, immobilization can help to obtain a high steady state level of biomass in the reactor used. With compounds that support growth of microorganisms that perform catabolic reactions, the approach seems straightforward. Only problems related to volatization are expected. Fluidized bed reactors (42) and trickling filters (43) have been investigated for the elimination of, respectively, dichloromethane and 1,2-dichloroethane from contaminated water. Furthermore, the development of treatment systems for removing chlorinated hydrocarbons from waste gas is under way (44,45).

An important goal will be to achieve biological removal of chlorinated hydrocarbons by microorganisms that rely on cometabolic conversion. The applicability of methanotrophs for the removal of trichloroethylene and related compounds will be complicated by three factors. First, degradation is cometabolic and requires the availability of another carbon source for stimulating growth of the desired population. Selective growth of the organisms that exhibit the desired catabolic feat can not always be achieved simply by adding a growth supporting substrate such as methane or toluene, since this often will not be selective for the required species. This is caused by the fact that cometabolism is very strain specific (36,38,39). Thus, only specific cultures of methanotrophs and only certain toluene catabolizing bacteria rapidly degrade trichloroethylene. For the application of such cultures a detailed insight into the ecophysiology of the organisms will be necessary. The development of recombinant DNA techniques as a tool for studying the distribution of catabolic traits may be very useful to tackle this problem.

A second factor is that oxidative cometabolic conversion requires the presence of a second substrate for supplying reducing equivalents needed by the monooxygenase system. Methane may not be optimal for this purpose because of competition. Finally, the first order kinetics, as observed with trichloroethylene, may cause degradation to proceed to sufficiently low levels only after long residence times.

Applicability of methanotrophs has now been studied for aquifer decontamination in the field (46) and with lab scale simulation systems for in situ biorestoration (47). In general, the observations made indicate that trans-1,2-dichloroethylene conversion proceeds more rapidly than trichloroethylene removal. It is possible that the population of methanotrophs used in these systems was far from optimal in the sense that mainly organisms that produce the less efficient particulate methane monooxygenase were present.

So far, no aerobic transformation has been found with perchlorinated compounds, although there seems to be no reason why this is impossible per se. Anaerobic conversions that cause dechlorination have been described for several highly chlorinated compounds, such as carbon tetrachloride, perchloroethylene, and 1,1,1-trichloroethane. These conversions could become very important for removing chlorinated aliphatics at low redox potential, e.g. in anaerobic subsurface environments. The rates are generally in the order of 0.1-10 $nmol.min^{-1}(mg\ protein)^{-1}$ (see reference 49 and publications cited there), which is rather low compared to what can be achieved with cometabolic trichloroethylene oxidation or with aerobic organisms that use chloroaliphatics as a carbon source.

An interesting option might be to combine anaerobic treatment steps for initial dechlorination of highly chlorinated compounds, such as perchloroethylene to tri- and dichloroethylenes, with oxidative treatment to give complete mineralization.

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Literature

1. Keuning, S., and D.B. Janssen, 1987. Microbiologische afbraak van zwarte en prioritaire stoffen voor het milieubeleid. Published by the Dutch Department of Public and Environmental Health, VROM 80007/1-88 (in Dutch).
2. Westerick, J.J., J.W. Mello, and R.F. Thomas. 1984. The groundwater supply survey. J. Am. Water Works Assoc. 76:52-59.
3. Janssen, D.B., A. Scheper, L. Dijkhuizen, and B. Witholt. 1985. Degradation of halogenated aliphatic compounds by Xanthobacter autotrophicus GJ10. Appl. Environ. Microbiol. 163: 635-639.
4. Janssen, D.B., R. Oldenhuis, A.J. van den Wijngaard, and S. Keuning, unpublished experiments.
5. Hartmans, S., A. Schmuckle, A.M. Cook, and T. Leisinger. 1986. Methyl chloride: naturally occurring toxicant and C-1 growth substrate. J. Gen. Microbiol. 132: 1139-1142.
6. Janssen, D.B., D. Jager, and B. Witholt. 1987. Degradation of n- haloalkanes and α,w-dihaloalkanes by wild-type and mutants of Acinetobacter sp. strain GJ70. Appl. Environ. Microbiol. 53: 561-566.
7. Omori, T., and M. Alexander. 1978. Bacterial dehalogenation of halogenated alkanes and fatty acids. Appl. Environ. Microbiol. 35:867-871.
8. Scholtz, R., A. Schmuckle, A.M. Cook, and T. Leisinger. 1987. Degradation of eighteen 1-monohaloalkanes by Arthrobacter sp. strain HA1. J. Gen. Microbiol. 133:267-274.
9. Yokota, T., H. Fuse, T. Omori, and Y. Minoda. 1986. Microbial dehalogenation of haloalkanes mediated by oxygenase or halidohydrolase. Agric. Biol. Chem. 50:453-460.
10. Kohler-Staub, D, and T. Leisinger. 1985. Dichloromethane dehalogenase of Hyphomicrobium sp. strain DM2. J. Bacteriol. 162:676- 681.
11. Kohler-Staub, D., S. Hartmans, R. Gälli, F. Suter, and T. Leisinger. 1986. Evidence for identical dichloromethane dehalogenases in different methylotrophic bacteria. J. Gen. Microbiol. 132:2837- 2843.
12. Scholtz, R., L.P. Wackett, C. Egli, A.M. Cook, and T. Leisinger. 1988. Dichloromethane dehalogenase with improved catalytic activity isolated form a fast-growing dichloromethane-utilizing bacterium. J. Bacteriol. 170:5698-5704.
13. Stucki, G., and T. Leisinger. 1983. Bacterial degradation of 2-chloroethanol proceeds via 2-chloroacetic acid. FEMS Microbiol. Lett. 16:123-126.

14. Hartmans, S., J.A.M. de Bont, J. Tramper, and K.Ch.A.M. Luyben. 1985. Bacterial degradation of vinylchloride. Biotechnol. Lett. 7:383-388.
15. Vogel, T. M., C. S. Criddle, P. L. and McCarty, 1987. Transformations of halogenated aliphatic compounds. Environ. Sci. Technol. 21:722-736.
16. Hardman, D.J., and J.H. Slater. 1981. Dehalogenases in soil bacteria. J. Gen. Microbiol. 123:117-128.
17. Motosugi, K., and K. Soda. 1983. Microbial degradation of synthetic organochlorine compounds. Experientia 39:1214-1220.
18. Janssen, D.B., S. Keuning, and B. Witholt. 1987. Involvement of a quinoprotein alcohol dehydrogenase and an NAD-dependent aldehyde dehydrogenase in 2-chloroethanol metabolism in Xanthobacter autotrophicus GJ10. J. Gen. Microbiol. 133:85-92.
19. Keuning, S., D.B. Janssen, and B. Witholt. 1985. Purification and characterization of hydrolytic haloalkane dehalogenase from Xanthobacter autotrophicus GJ10. J. Bacteriol. 163: 635-639.
20. Rozeboom, H.J., J. Kingma, D.B. Janssen, and B. Dijkstra. 1988. Crystallization of haloalkane dehalogenase from Xanthobacter autotrophicus GJ10. J. Mol. Biol. 200:611-612.
21. Scholtz, R., T. Leisinger, F. Suter, and A.M. Cook. 1987. Characterization of 1-chlorohexane halidohydrolase, a dehalogenase of wide substrate range from an Arthrobacter sp. J. Bacteriol. 169:5016-5021.
22. Janssen, D.B., J. Gerritse, J. Brackman, C. Kalk, D. Jager, and B. Witholt. 1988. Purification and characterization of a bacterial dehalogenase with activity toward halogenated alkanes, alcohols, and ethers. Eur. J. Biochem. 171:67-72.
23. Yokota, T., T. Omori, and T. Kodama. 1987. Purification and properties of haloalkane dehalogenase fron Corynebacterium sp. strain m15-3. J. Bacteriol. 169:4049-4054.
24. Fogel, M. M., A. R. Taddeo, and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Environ. Microbiol. 51:720-724.
25. Henson, J.M., M.V. Yates, J.W. Cochran, and D.L. Shakleford. Microbial removal of halogenated methanes, ethanes, and ethylenes in an aerobic soil exposed to methane. FEMS Microbiol. Ecol. 53:193- 201.
26. Janssen, D.B., G. Grobben, R. Hoekstra, R. Oldenhuis, and B. Witholt. 1988. Degradation of trans-1,2-dichloroethene by mixed and pure cultures of methanotrophic bacteria. Appl. Microbiol. Biotechnol. 29:392-399.
27. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Lidstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54:951-956.
28. Arciero, D., T. Vannelli, M. Logan, and A.B. Hooper. 1989. Degradation of trichloroethylene by the ammonia-oxidizing bacterium Nitrosomonas europaea. Biochem. Biophys. Res. Commun. 159:640-643.
29. Strand, S.E., and L. Shippert. 1986. Oxidation of chloroform in an aerobic soil exposed to natural gas. Appl. Environ. Microbiol. 52:203-205.
30. Wilson, J.T., and B.H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49:242-243.
31. Anthony, C. 1986. Bacterial oxidation of methane and methanol. Adv. Microbiol. Physiol. 27:113-210.
32. Colby, J., D.I. Stirling, and H. Dalton. 1977. The soluble methane monooxygenase of Methylococcus capsulatus (Bath). Its ability to oxygenate n-alkanes, n-alkenes, ethers and alicylic, aromatic and heterocyclic compounds. Biochem. J. 165:395-402.

33. Kline, S.A., and B.L. Van Duuren. 1978. Synthesis and reactions of chloroalkene epoxides. J. Org. Chem. 43:3597-3600.
34. Miller, R.E., and F.P. Guengerich. 1982. Oxidation of trichloroethylene by liver microsomal cytochrome P-450: evidence for chlorine migration in a transition state not involving trichloroethylene oxide. Biochemistry 21:1090-1097.
35. Oldenhuis, R., R.L.J.M. Vink, D.B. Janssen, and B. Witholt. Degradation of chlorinated aliphatic hydrocarbons by Methylosinus trichosporium OB3b expressing soluble monooxygenase. Appl. Env. Micronbiol., in press.
36. Burrows, K.J., A. Cornish, D. Scott, and I. J. Higgins. 1984. Substrate specificities of the soluble and particulate methane monooxygenases of Methylosinus trichosporium OB3b. J. Gen. Microbiol. 130:3327-3333.
37. Fox, B.G., and J.D. Lipscomb. 1988. Purification of a high specific activity methane monooxygenase hydroxylase component from a type II methanotroph. Biochem. Biophys. Res. Commun. 154:165-170.
38. Nelson, M.J.K., S.O. Montgomery, W.R. Mahaffey, and P.H. Pritchard. 1987. Biodegradation of trichloroethylene and involvement of an aromatic biodegradative pathway. Appl. Environ. Microbiol. 52:383-384.
39. Wackett, L. P., and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with Pseudomonas putida F1. Appl. Environ. Microbiol. 54:1703-1708.
40. Winter, R.B., K.-M. Yen, and B.D. Ensley. 1989. Efficient degradation of trichloroethylene by a recombinant Escherichia coli. Biotechnology 7:282-285.
41. Rojo, F., D.H. Pieper, K.-H. Engesser, H.-J. Knackmuss, and K.N. Timmis. 1987. Assemblage of ortho-cleavage route for simultaneous degradation of chloro- and methylaromatics. Science 238:1395-1398.
42. Gälli, R. 1987. Biodegradation of dichloromethane in waste water using a fluidized bed bioreactor. Appl. Microbiol. Biotechnol. 27:206-213.
43. Friday, D.D., and R.J. Portier. 1989. Evaluation of a packed bed immobilized microbe bioreactor for the continuous biodegradation of halocarbon-contaminated groundwater. AWMA/EPA International Symposium on Biosystems for Pollution Control, Cincinatti.
44. Ottengraf, S.P.P. 1987. Biological systems for waste gas elimination. Trends in Biotechn. 5:132-136.
45. Ottengraf, S.P.P., J.J.P. Meesters, A.H.C. van den Oever, and H.R. Rozema. 1986. Biological elimination of volatile xenobiotic compounds in biofilters. Bioproc. Engineering 1:61-69.
46. McCarty, P.L., L. Semprini, and P.V. Roberts. 1989. Methodologies for evaluating the feasibility of in-situ biodegradation of halogenated aliphatic groundwater contaminants by methanotrophs. Proc. AWMA/EPA International Symposium on Biosystems for Pollution Control, Cincinatti.
47. Moore, A.T., A. Vira, and S. Fogel. 1989. Biodegradation of trans-1,2-dichloroethylene by methane-utilizing bacteria in an aquifer simulator. Environ. Sci. Technol. 23:403-406.
48. Stucki, G., U. Krebser, and T. Leisinger. 1983. Bacterial growth on 1,2-dichloroethane. Experientia 39:1271-1273.
49. Gälli, R., and P.L. McCarty. 1989. Kinetics of biotransformation of 1,1,1-trichloroethane by Clostridium sp. strain TCAIIB. Appl. Environ. Microbiol. 55:845-851.
50. Brunner, W., D. Staub, and T. Leisinger. 1980. Bacterial degradation of dichloromethane. Appl. Environ. Microbiol. 40:950-958.

51. Stucki, G., R. Gälli, H.-R. Ebersold, and T. Leisinger. 1981. Dehalogenation of dichloromethane by cell extracts of Hyphomicrobium DM2. Arch. Microbiol. 130:366-371.
52. van den Wijngaard, A., D.B. Janssen, and B. Witholt. 1989. Degradation of epichlorohydrin and halohydrins by three bacterial cultures isolated from freshwater sediment. J. Gen. Microbiol. 135:2199-2208.

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