Modelling of Phosphorus Release and Substrate Uptake by Activated Sludge under Anaerobic Conditions

by

Dongkeun PARK*, Yutaka TERASHIMA*, and Baejung LEE*

(Received December 26, 1987)

Abstract

In order to obtain information about the effects of several factors on phosphorus release and substrate uptake under anaerobic conditions, and also to develop the kinetic model for high polymeric substrate, batch experiments were undertaken using the sludge acclimated in sequencing batch reactors. The main results are as follows: (1) The specific rates of phosphorus release (SRP) and substrate uptake (SRS) in the case using soluble starch were larger than those of polypeptone. (2) The unuptakeable COD of soluble starch was smaller than that of polypeptone under anaerobic conditions. (3) In the case using high polymeric substrate, the $\Delta TP/\Delta COD$ ratio increased with time. The increase rate of $\Delta TP/\Delta COD$ ratio of polypeptone was larger than that of soluble starch. (4) The higher the added substrate concentration was, the larger SRP and SRS of the initial stage were. (5) The content of unreleasable phosphorus of sludge A and B, C, and D were 24, 19, and 22 mgP/gSS, respectively. (6) A model system to express the phosphorus release and substrate uptake was presented based on these results. In this model system, the SRS was expressed by an unsaturation degree of accumulated substrate, uptakeable COD, and an amount of releasable phosphorus in sludge floc. The SRP was expressed by an amount of accumulated substrate and the content of releasable phosphorus.

1. Introduction

In order to protect receiving waters from eutrophication, it is very important to eliminate phosphorus in municipal and industrial wastewaters before discharge. The most widely used process to accomplish the phosphorus removal is chemical precipitation using either lime, alum, or ferric chloride. This method has, however, disadvantages such as a large production of the sludge to be treated and the high chemical cost. On the other hand, the biological excess phosphorus removal process as an alternative has received increased attention in

* Dept. Environ. and Sanitary Eng., Kyoto University.

the last decade.

Phosphorus release under non-aerated (anaerobic) conditions has been recognized as a prerequisite to subsequent aerobic phosphorus uptake in the biological excess phosphorus removal process (Barnard,[1]; Fuhs and Chen,[2]; Nicholls and Osborn,[3]) Nicholls and Osborn[3] fiirst suggested that, under anaerobic conditions, polyphosphorus served as a reservoir for the energy needed to store stable organic compounds like poly-β-hydroxybutyrate (PHB). Comeau *et al.*[4] proposed an integrated biochemical model for phosphorus release and uptake. They stated that the role of polyphosphorus under anaerobic conditions was suggested to be as an energy source for both the reestablishment of the proton motive force, which would be consumed by substrate transport, and substrate storage.

The fate of the removed substrate under anaerobic conditions was discussed by Fukase *et al.*[5], Matsuo and Miya[6], and Comeau *et al.*[7]. Fukase *et al.*,[5] stated that removed glucose and acetate were stored in sludge as glycogen and PHB, respectively. However, Matsuo and Miya[6] suggested that the single removed organic substrate was able to produce not only PHB but also other stored compounds. Also, they said that the main species of stored compounds depended on that of the substrate used. Comeau *et al.*[7] stated that the increase rate of carbon storage as poly-β-hydroxyvalerate (PHV) exceeded that of storage as PHB when short chain fatty acids (SCFA's) containing an odd number of carbon atoms were added to the sludge. On the other hand, more PHB was accumulated than PHV, the addition of SCFA's containing an even number of carbon atoms. Storage of PHV was also favored by the combined addition of acetate and propionate.

The reports on the kinetic model for the biological excess phosphorus removal process are few, because of the unclearness of its biochimical mechanism. Tsuno *et al.*[8], however, developed a kinetic model for the phosphorus removal in an alternating anaerobic-aerobic environment. The characteristic of this model is to incorporate intracellular carbohydrate, PHB, and phosphorus pool into the model.

The purpose of this paper is to discuss the effects of substrate species, substrate concentrations, and sorts of sludge acclimated on phosphorus release and substrate uptake. Also, it is to develop a kinetic model system for the biological excess phosphorus removal of high molecular weight substrates such as soluble starch and polypeptone under anaerobic conditions.

2. Methods and Materials

2. 1 Batch experiments

All experiments were carried out in a thermostatic chamber of 20 ± 2° C. Four bench-scale sequencing batch reactors (SBR) were operated in parallel for sludge acclimation, using a mixture of soluble starch, polypeptone, and meat extract, namely a mixed substrate (SPM). Batch experiments were undertaken using the sludge acclimated (sludge A, B, C and D). Soluble starch, polypeptone, and SPM were used as substrate.

The experimental apparatus is shown in Fig. 1. The anaerobic batch experimental apparatus was equipped with a magnetic stiller, a nitrogen gas supply unit, an off-gas capture trap, and a sampling siphon. Also, a polystyrene sheet was used to prevent heat transfer from the magnetic stiller.

The initial conditions of the anaerobic batch experiments are shown in Table 1. Each of the contents of cellular carbohydrate (CHs: extracellular carbohydrate and intracellular carbohydrate) and sludge volume indexes (SVI's) is shown as an average value of several ones measured after the acclimation was completed. Each character in RUN number X-Y-Z indicates, respectively, the sort of sludge acclimated to each of the different operating patterns of SBR's, substrate

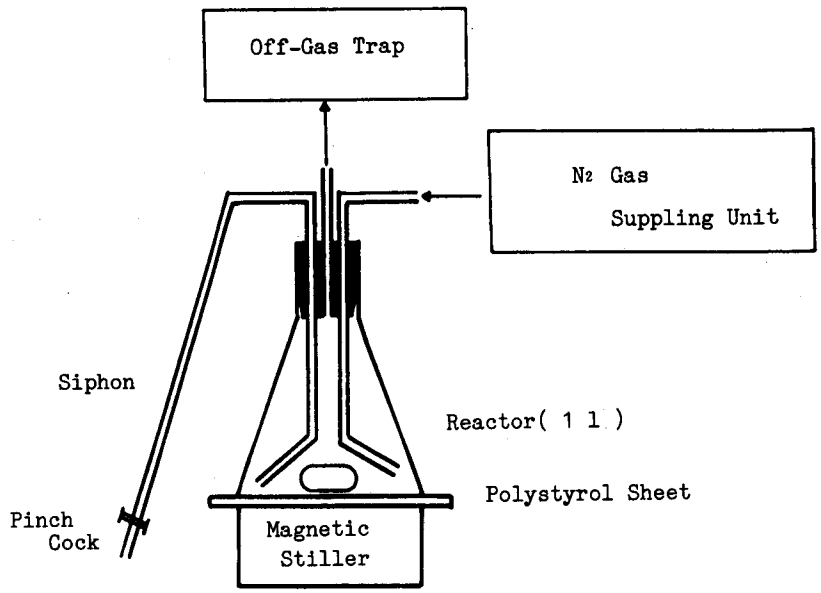

Fig. 1. Experimental apparatus for anaerobic batch studies.

Table 1 Initial Conditions of Anaerobic Batch Experiments

RUN (X-Y-Z)	MLSS (mg/l)	COD (mg/l)	CH (mg/l)	Sludge Characteristics		
				TPs (mgP/gSS)	CHs (mgCH/gSS)	SVI
B-S*-1	993	368	390	64.1	89	48
2	877	373	375	63.5		
3	800	263	265	61.9		
4	877	189	180	63.5		
5	980	66.5	62.9	62.5		
6	980	40.4	38.0	62.5		
B-P**-1	993	378		64.1	89	48
2	800	290		61.9		
3	1000	270		63.9		
4	1000	129		63.9		
5	938	67.2		64.1		
6	938	35.2		64.1		
A-SPM***-1	1046	394	160	63.2	80	50
2	1046	145	66.8	63.2		
B-SPM-1	958	385	185	60.5	89	48
2	909	270	127	60.5		
3	958	135	70.2	60.5		
C-SPM-1	907	421	181	31.4	70	89
2	907	148	68.1	31.4		
D-SPM-1	967	413	194	36.1	72	65
2	967	156	76.4	36.1		

* S: Soluble Starch
** P: Polypeptone
*** SPM: the mixture of Soluble Starch, Polypeptone, and Meat extrat

species, and serial number.

2. 2 **Analytical Methods**

The sludge volume index (SVI) and phosphorus compounds were measured based on standard methods[9]. Mixed liquor suspended solid (MLSS) was determined gravimetrically using a centrifuge. Dicromate COD was analysed colorimetrically by an auto-analyzer. The extraction of extracellular carbohydrate (CH) was performed by the steam extraction method proposed by Brown *et al.*[10]. Intracellular CH was extracted with a solution of 30% potassium hydroxide, and

was determined according to the anthrone method proposed by Tai[11].

3. Results and Discussion

3. 1 Effect of substrate species

In order to compare the effects of the difference of substrate species on phosphorus release and substrate uptake under anaerobic conditions, the time profiles of the increased total phosphorus and uptaked COD in bulk liquid phase (TP*l* and COD*l*) are shown in Fig. 2. As initial conditions, the sort of the sludge used, the value of MLSS, and the ratio of F/M were almost the same in each of the runs.

Each amount of the increased TP*l* and the uptaked COD*l* in RUN B-P-2 in which polypeptone was used as substrate was less, at any time from starting, than each of those in RUN B-S-3 using soluble starch and RUN B-SPM-2

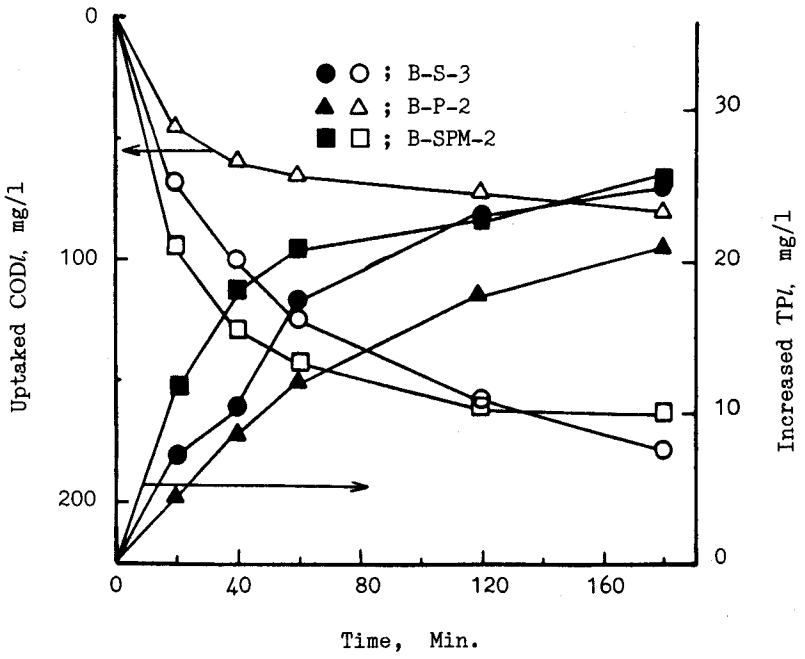

Fig. 2. Effects of substrate species on phosphorus release and substrate uptake.

using a mixture of soluble starch, polypeptone, and meat extract. From this result, it can be estimated that the rates of phosphorus release and substrate uptake in the case using soluble starch are larger than those in the case using polypeptone. The phosphorus release and substrate uptake patterns in the case of RUN B-SPM-2 were similar to those in RUN B-S-3, during 40 minutes at the beginning. After 40 minutes, they were similar to those in RUN B-P-2. Also, it was noticeable that the rates of phosphorus release and substrate uptake in the case of the mixed substrate (RUN B-SPM-2) were a little larger than the case of soluble starch (RUN B-S-3) in the early stage of reaction. This was despite the fact that the mixed substrate contained not a little polypeptone.

Fig. 3 shows the transition of the ratio of increased total phosphorus to uptaked COD in bulk liquid phase, $\Delta TP_l/\Delta COD_l$, with the time of anaerobic reaction. The ratio of $\Delta TP_l/\Delta COD_l$ increased with time from 0.10 to 0.16 in both cases using soluble starch (RUN B-S-3) and the mixed substrate (RUN B-SPM-2), and rapidly increased from 0.098 to 0.27 in the case using polypeptone (RUN B-P-2). From these conditions, it is obvious that the relationship between the phosphorus release and the substrate uptake is not linear in the case using high polymeric substrates such as these in this study. On the other hand, using glucose or acetate of low molecule as substrate, Fukase *et al.*[5] showed that the ratio of $\Delta TP_l/\Delta COD_l$ depended on substrate species, but was constant during the time of anaerobic reaction. The difference between the results of this study and those can be attributed to the differences between the uptaking processes of high polymeric and low molecular substrates. The following are estimated: The

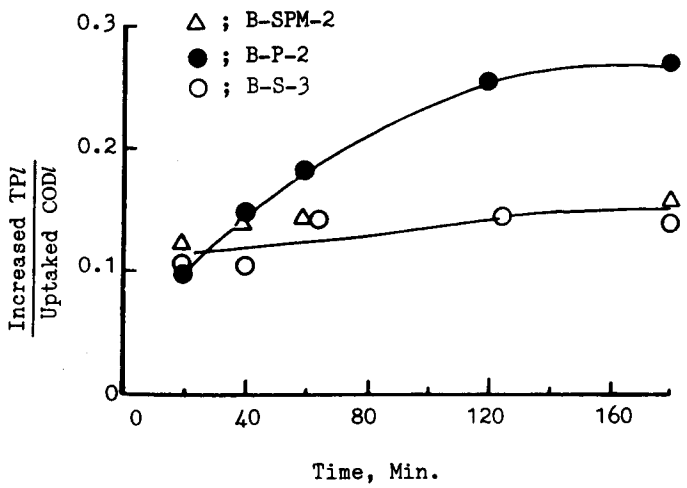

Fig. 3. Effects of substrate species on$\Delta TP_l/\Delta COD_l$.

high polymeric substrates such as soluble starch and polypeptone in bulk liquid phase are adsorbed, at first, on sludge floc without phosphorus release. Then, the adsorbed substrates are hydrolyzed and transported into the cell of microorganism in the sludge floc. Finally, the transported substrates are synthesized to produce stable stored compounds such as PHB and others.

Low molecular substrates such as glucose and acetate are adsorbed and/or transported into the cell without being hydrolyzed, and then are synthesized. Some forms of low molecular phosphorus compounds are released from the cell through the degradation of polyphosphorus compounds to generate the energy for the hydrolysis of high polymeric substrates, and for the transportation and synthesis of low molecular and high polymeric substrates. Some factors influencing the mechanisms of substrates uptake and phosphorus release and/or the strength of the influences are different between high polymeric and low molecular substrates.

Fig. 4 shows the relationships between the specific rates of substrate uptake (SRS) and the substrate concentration in bulk liquid phase (COD*l*), using soluble starch (Fig. 4 (A)) and polypeptone (Fig. 4 (B)). It is obvious in Fig. 4 and also Fig. 2 that the rates of phosphorus release and substrate uptake in the case of soluble starch were larger than those in the case of polypeptone at the same COD*l*. From this, the following are possible: The saturating accumulation capacity for soluble starch is larger than that for polypeptone. Fig. 4 (B) indicates also that, under the condition of different initial COD*l*, each of SRS's decreases, with the decrease of COD*l*, to zero, although the substrate remains

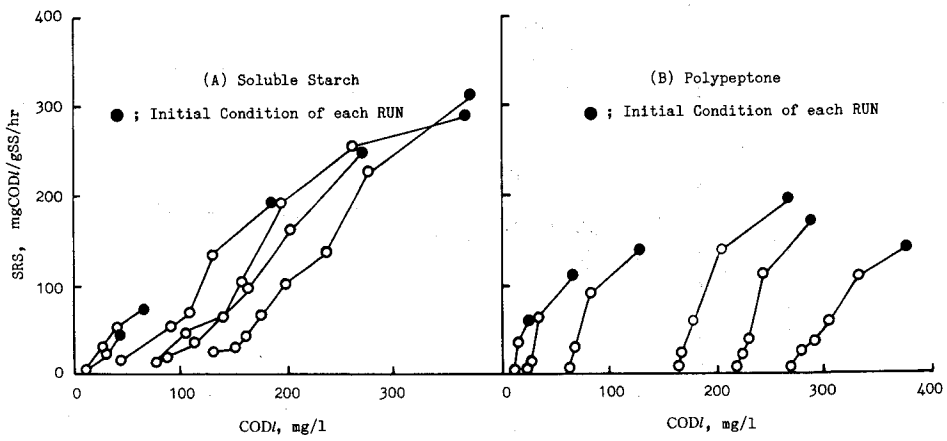

Fig. 4. Relationship between specific rate of substrate uptake (SRS) and COD*l*.

sufficiently in bulk liquid phase. Based on this, the following are possible: At a zero specific rate, the substrate accumulated in microorganism is in saturation, or the releasable phoshorus in microorganism has been dipleted, or a portion of high polymeric substrate is unuptakeable under anaerobic conditions. In the last case, the unuptakeable fraction of polypeptone is larger than that of soluble starch.

Fig. 5 shows the relations of each of the specific rates of phosphorus release (SRP) and substrate uptake (SRS) and the liquid phase concentration of the substrate (COD*l*) in the case using polypeptone to the content of releasable phosphorus (PR). It is obvious that SRS decreases to zero, althogh the substrate remains sufficiently in the bulk liquid and the phosphorus release is still occuring. From this and the above results, it is judged that the high polymeric substrate such as polypeptone has an unuptakeable fraction under anaerobic conditions, and that the unuptakeable fraction of soluble starch is smaller than

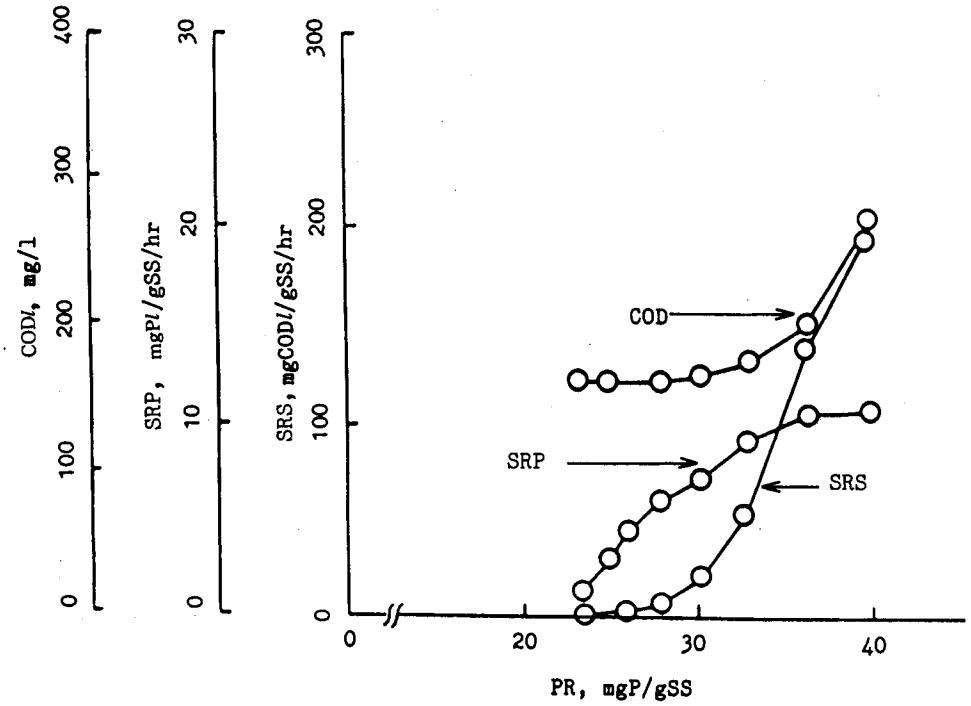

Fig. 5. Interactions of releasable phosphorus, SRS. SRP, and COD*l*.

that of polypeptone.

3. 2 **Effect of initial F/M ratio (substrate concentration)**

Batch experiments were performed taking the initial F/M ratios of 0.04, 0.22, and 0.43 gCOD/gSS in the case using soluble starch, and 0.04, 0.07, and 0.3 gCOD /gSS in the case using polypeptone. The results are shown in Fig. 6. The rates of the phosphorus release and the substrate uptake under anaerobic conditions seem to be on the whole in proportion to the initial F/M ratio. These results indicate that the substrate concentration is one of the very important factors for each of the phosphorus release and substrate uptake under anaerobic conditions. In both cases of soluble starch and polypeptone, the phosphorus release and substrate uptake proceeded rapidly at the beginning, and these rates decreased with the increase of reaction time. It is considered that these phenomena are owing to the decrease of the substrate in bulk liquid phase, the increase of the accumulated substrate in sludge floc, and the decrease of phosphorus in them.

Fig. 7 shows the changes of the averaged specific rates of substrate uptake

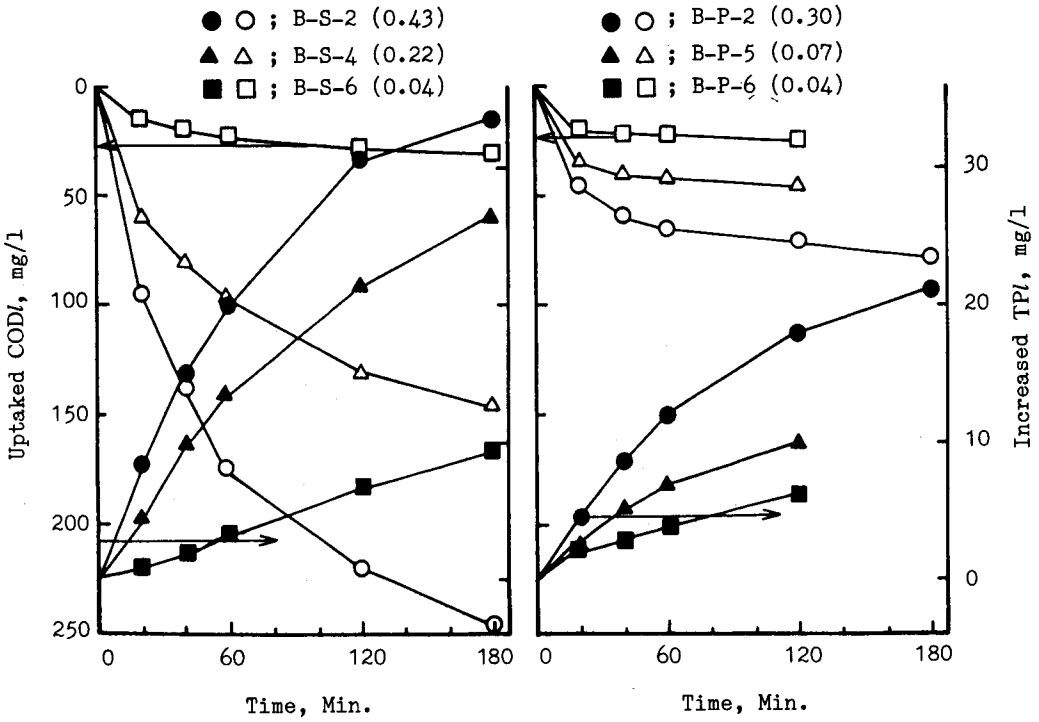

Fig. 6. Effect of substrate concentration on phosphorus release and substrate uptake.

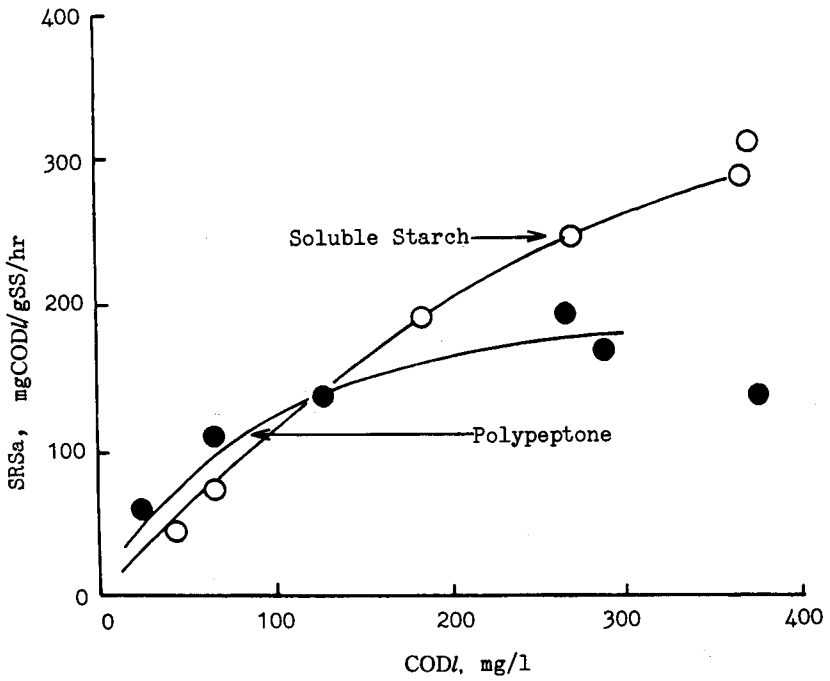

Fig. 7. Relationship between SRS and COD*l* at initial stage.

(SRSa) during the initial 20 minutes with the change of initial substrate concentration in the bulk liquid phase (initial COD*l*), in the cases using soluble starch and polypeptone under anaerobic conditions. SRSa in the case using soluble starch, showed a nearly proportional change to the initial COD*l*. On the other hand, that in the case using polypeptone showed a Monod-type change. Fig. 4 indicates, however, that the higher each of the initial COD*l*'s of soluble starch and polypeptone was, the smaller each of their SRSa's was at any COD*l* after the COD-uptake started. From these, it is estimated that under the condition of high initial substrate concentration in liquid phase, substrate uptake is limited due to a high saturation degree of accumulated substrate.

The transitions of $\Delta TPl/\Delta COD l$ ratio are shown in Fig. 8, taking the initial F/M ratio as a parameter. In the cases using soluble starch, the lesser the initial F/M ratio was, the larger the increase rate of the $\Delta TPl/\Delta COD l$ ratio became, and the less and the more the values of $\Delta TPl/\Delta COD l$ were until and after about 60 minutes, respectively. From the above results, it is probable that the rate of the phosphorus release is apt to be limited more than that of the substrate uptake when both of the saturation degrees of the accumulated substrate and initial F/

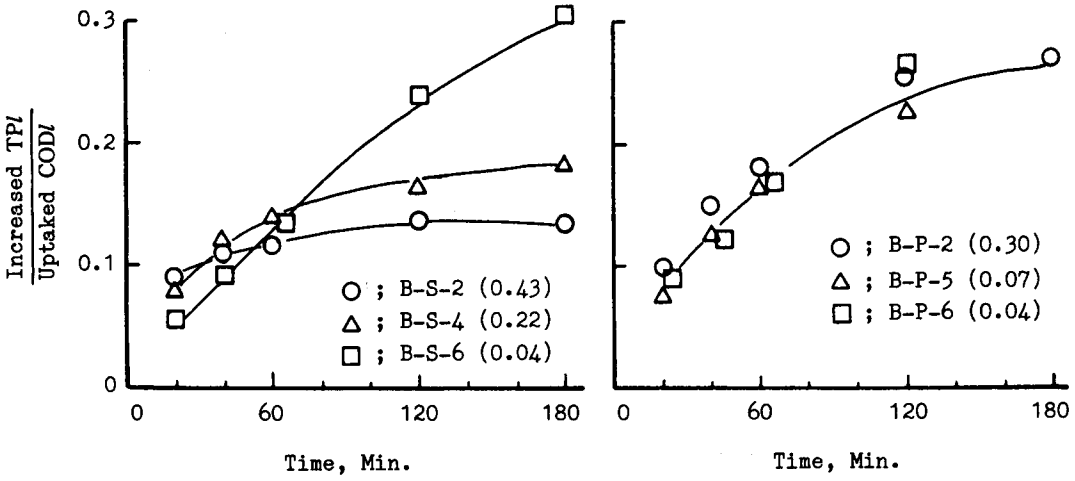

Fig. 8. Effect of substrate concentration on ΔTP*l*/ΔCOD*l*.

M ratio are low. In the case of polypeptone, ΔTP*l*/ΔCOD*l* ratios increased with time independently from the initial F/M ratios and showed the same value at any time.

3. 3 **Effect of the sort of sludge acclimated**

The condition of sludge acclimation determines the sludge characteristcs such as composition and function, namely the sort of sludge. The phosphorus and carbohydrate contents of sludge (TPs, CHs) A and B are 63, 61 mgP/gSS and 80, 89 mgCH/gSS in average, respectively. The TPs and CHs of sludge C and D are 31, 36 mgP/gSS and 70, 72 mgCH/gSS, respectively, being less than those of sludge A and B.

Fig. 9 shows the effects of the sort of sludge on the phosphorus release and subtrate uptake under anaerobic conditions. Both of the phosphorus release and substrate uptake rates of sludge A and B were larger than those of sludge C and D. It is estimated to be because the initial substrate unsaturation degrees of sludge A and B were higher than those of sludge C and D. Also, it is because the substrate uptake rates of sludge A and B were larger, despite a lesser initial carbohydrate content, than those of sludge C and D.

The transitions of the ratio of ΔTP*l*/ΔCOD*l* with time are shown in Fig. 10. The ΔTP*l*/ΔCOD*l* ratios of sludge A and B were larger than those of sludge C and D, respectively. This means that the TPs and CHs of the sludge to be used are important factors affecting the rates of the phosphorus release and substrate

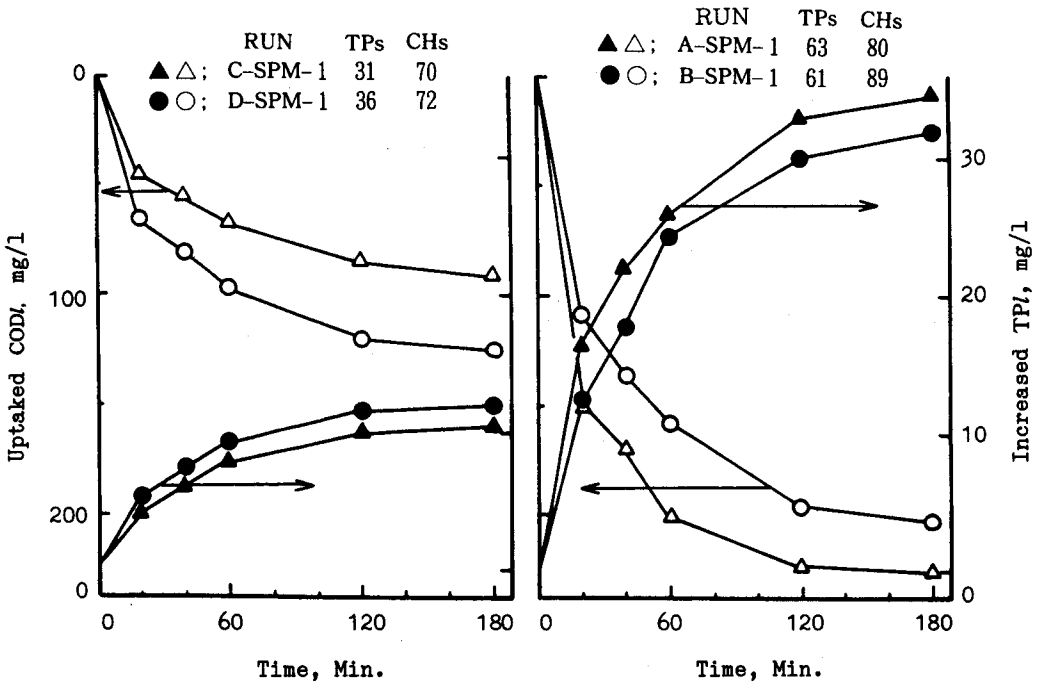

Fig. 9. Effect of sludge sort on phosphorus release and substrate uptake.

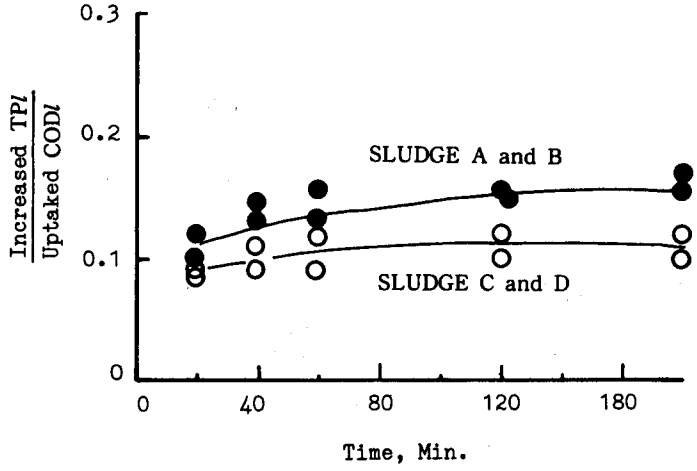

Fig. 10. Effect of sludge sort on $\Delta TPl/\Delta CODl$.

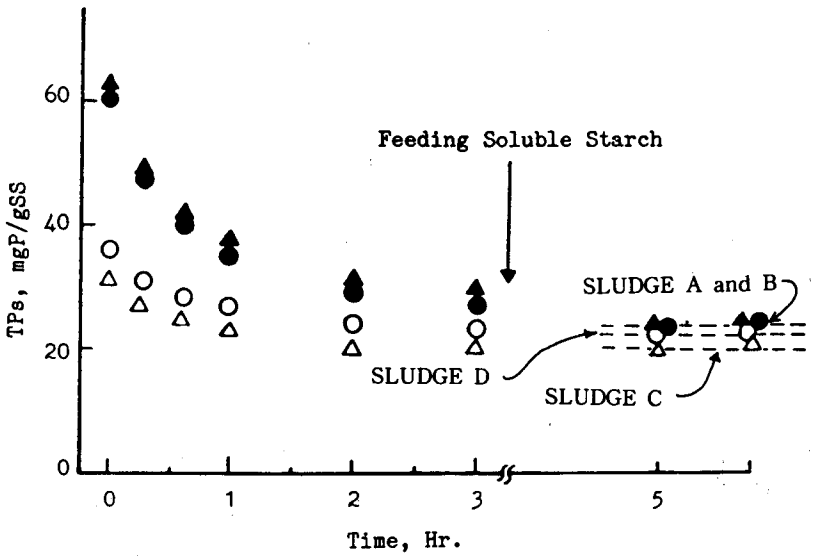

Fig. 11. Minimum phosphorus contents of sludges.

uptake under anaerobic conditions.

3. 4 **Releasable phosphorus content of sludge**

Experiments to investigate the releasable phosphorus content of sludge were performed, following the anaerobic batch experiments (RUN A–, B–, C–, D–SPM–1). by adding about 20 ml of a soluble starch solution (20 g/l) at the end of the above RUN's to prevent depletion of the substrate in the bulk liquid phase. The results are shown in Fig. 11. The phosphorus contents of each of the sludge A, B, C, and D decreased to be minimm with the elapse of time. The minimum phosphorus content of sludge (TPsm) depended upon the sort of sludge: TPsm of the sludge A and B, C, and D were 24, 19 and 22 mgP/gSS, respectively. Mino *et al.*[12] Suggested that the low molecular weight polyphosphorus functined as an energy pool under anaerobic conditions, and that the high molecular weight polyphosphorus as a phosphate reservoir for microbial growth. The values of the above TPsm (19–24 mgP/gSS) agree closely with the sum of the reported contents[12] of high molecular weight polyphosphorus, metallic bonding phosphorus, and phosphorus as basic elements of microorganism. These types of phosphorus are unchangeable under anaerobic conditions. The releasable total content of phosphorus (PR) can be, therefore, calculated as TPs minus TPsm.

4. Kinetic Model

4. 1 Basic concept of model

A kinetic model system for the phosphorus release and substrate uptake under anaerobic condition was developed, based on experimental results and literature reviews. Fig. 12 shows a schematic of reaction pathways under anaerobic conditions. The basic concepts of this scheme are as follows: (a) The uptakeable substrates in liquid phase (CLe) are adsorbed on microorganisms in sludge flocs. (b) The adsorbed substrates (CAex) are hydrolyzed and transported into the cell of the microorganisms through the cell membrain. (c) The transported substrates (CAin) are synthesized to become the end products (CP) such as PHB, PHV, and so on. (d) The releasable phosphorus in the sludge (PR) is released by the energy generating degradation of polyphosphorus during the hydrolysis and transportation of the adsorbed substrates CAex, and during the synthesis of the end products.

The following several assumptions were adopted to obtain a simplified model system, not only for an explanation of the phenomena, but also for the design and control of a treatment plant. (a) Both of the stoichiometric constants of $\Delta TPl/\Delta CODl$ related to the transportation of CAex and related to the production of CP have the same value. (b) Chemical forms of the substrates CLe, CAex and CAin are not different from each other. Consequently, the value of (COD of CLe /unit weight of the substrate) is equal to that of CA. (c) One sort of accumulated CA, namely CAex plus CAin, produces various sorts of CP, of which

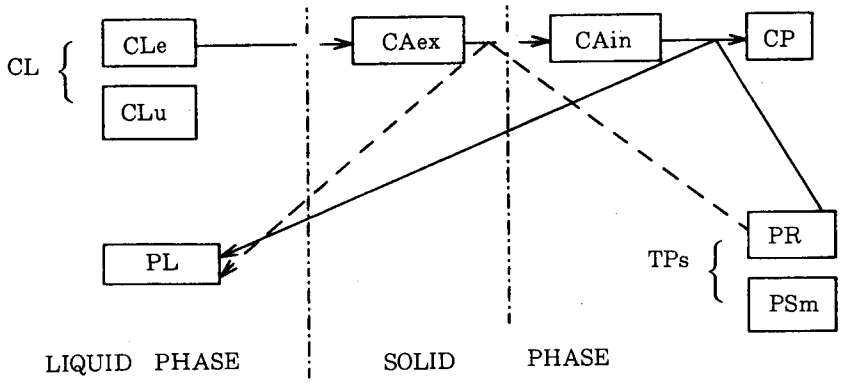

Fig. 12. The schematic pathways of phosphorus and substrate under anaerobic condition.

fractions are equal. Consequently, the amount of the produced CP can be stoichiometrically represented by the amount of released phosphorus. (d) The initial CA (CAo) is carbohydrate (CHso) only, which is completely usable for phosphorus release. (e) The concentration of active microorganism (M) is constant under anaerobic conditions. Consequently, M is equivalent to the initial MLSS concentration.

4. 2 **Stoichiometric relationship**

The total phosphorus (TP) in a batch system is constant and consists of TP*l* and TPs:

$$TPl + TPs \cdot M/1000 = \text{Constant} \quad (1)$$

where, TP*l*: Concentration of TP in liquid phase, mgP/l

TPs: Content of TP in solid phase, mgP/gM

M : Concentration of initial MLSS, mgM/l

The total COD in the system is also constant and consists of CL, CA, and CP under anaerobic conditions:

$$CL + CA + CP = \text{Constant} \quad (2)$$

where, CL: Concentration of substrate in liquid phase, mgCOD/l

CA: concentration of accumulated substrate (CAex+CAin), mgCOD/l

CP: Concentration of end products, mgCOD/l

The differential forms of Equation (1) and (2) are experssed as follows:

$$dTPl/dt = -M\ (dTPs/dt)\ /1000 \quad (3)$$

$$dCA/dt = -dCL/dt - dCP/dt \quad (4)$$

The differential form of CP is expressed as Equation (5) based on assumption (c):

$$dCP/dt = \alpha \cdot dTPl/dt, \quad (5)$$

where α: Stoichiometric constant which means produced CP per increased TP*l*, mgCOD/mgP

Substituting Equation (5) into Equation (4) yields,

$$dCA/dt = -dCL/dt - \alpha \cdot dTPl/dt \quad (6)$$

Integrating Equation (6) yields,

$$CAt = CAo + CLo - \alpha\ (PLt - PLo), \quad (7)$$

where CAo, CLo and PLo are the initial concentration. CAt and CLt represent concentrations at time t.

The value of CAo can be taken to be the same as that of the initial CHs based on assumption (e). The uptakeable CL, CLe, and the releasable phosphorus, PR, are expressed as follows.

$$CLet = CLt - CLo\ (1 - FR), \quad (8)$$

where FR: Fraction of uptakeable substrate

$$PRt = TPst - TPsm, \quad (9)$$

where TPst: phosphorus content of sludge at time t, mgP/gM

TPsm: Minimum phosphorus content of sludge, mgP/gM

Substituting the equation obtained by integration of Equation (3) into Equation (9) yields

$$PRt = TPso - TPsm + 1000\ (PLo - PLt)\ /M \quad (10)$$

4. 3 **Rates of substrate uptake and phosphorus release.**

The factors affecting the rates of substrate uptake and phosphorus release are substrate species, uptakeable substrate concentration, and sludge sort. The sludge sort can be characterized by the content of TPs, TPsm, and accumulated materials such as CHs. Based on these experimental results and stoichiometric relationships, the rates of substrate uptake and phosphorus release are expressed as the following equations in this study:

$$dCL/dt = -k_1\ (GS - G)\ M^2 \cdot CLe \cdot PR/\ (KCL + CLe)\ /\ (KPC + PR) \quad (11)$$

where, k_1: Substrate uptake rate constant, l /mgM/hr

GS: Saturation content of CA, mgCOD/mgM

G: Content of CA, mgCOD/mgM

KCL: Saturation constant of CLe, mgCOD/ l

KPC: Saturation constant of PR for substrate uptake, mgP/gM

$$dTP_l/dt = k_2 \cdot M \cdot CA \cdot PR/(KCA+CA)/(KPP+PR), \quad (12)$$

where k_2: Phosphorus release rate constant, mgP/mgM/hr

KCA: Saturation constant of CA, mgCOD/l

KPP: Saturation constant of PR for phosphorus release, mgP/gM

5. Conclusion

In order to obtain information about the effects of substrate species, substrate concentration, and sludge sort on the release of phosphorus and uptake of high polymeric substrates such as soluble starch and polypeptone under anaerobic conditions, and to obtain also information for kinetic model development, batch experiments were undertaken using the sludge acclimated in sequencing batch reactors. The main results are as follows.

(1) The specific rates of phosphorus release (SRP) and substrate uptake (SRS) in the case using soluble starch were larger than those of polypeptone.

(2) The high polymeric substrate such as soluble starch and polypeptone contained unuptakeable substrate under an anaerobic condition. The unuptakeable fraction of soluble starch was smaller than that of polypeptone.

(3) In the case using high polymeric substrate, the ratio of $\Delta TP_l/\Delta COD_l$ increased with the time of anaerobic reaction. The increase rate of $\Delta TP_l/\Delta COD_l$ ratio of polypeptone was larger than that of soluble starch.

(4) The higher the added substrate concentration was, the larger SRP and SRS were. When each of the sludge components such as TPs and CHs was constant, SRS in the case using soluble starch showed a change nearly proportional to the initial liquid COD concentration. On the other hand, SRS in the case using polypeptone showed a Monod-type change.

(5) The lesser an initial F/M ratio was, the larger the increase rate of $\Delta TP_l/\Delta COD_l$ was.

(6) When the contents of TPs and CHs of sludge used were high, SRP and SRS were larger than when the contents of those were low.

(7) The accumulated sludge contained unreleasable phosphorus under anaerobic conditions. The content of unreleasable phosphorus depended on the operating condition for sludge acclimation. The content of unreleasable phosphorus ranged from 19 to 24 mgP/gSS in this study, and agreed closely with the sum of the contents of high molecular weight polyphosphorus, metallic bonding phosphorus, and phosphorus as basic elements of the microorganism.

(8) A kinetic model system to express the phosphorus release and substrate uptake under anaerobic conditions was developed, based on experimental results and literature reviews. The kinetic equation for the rate of substrate uptake could be expressed by the saturation degree of the accumulated substrate, the concentration of uptakeable substrate in bulk liquid phase, and the amount of releasable phosphorus in sludge floc. The kinetic equation for the rate of phosphorus release could be expressed by the amount of accumulated substrate and the content of releasable phosphorus.

References

1) Barnard. J. L.; "Biological Nutrient Removal Without the Addition of Chemicals." Water Res., **9**, 485-490 (1975)
2) Fuhs. G. W. and Chen. M.; "Microbiological Basic of Phosphate Removal in the Activated Sludge Process for the Treatment of Wastewater." Microbial Ecol., **2**, 119-138 (1975)
3) Nicholls. H. A. and Osborn. D. W.; "Bacterial stress: prerequisite for biological removal of phosphorus." J. Water Poll. Control Fed., **51**, 557-569 (1979)
4) Comeau. Y. *et al.*; "Biochemical Model for Enhanced Biological Phosphorus Removal." Water Res., **20**, 1511-1521 (1986)
5) Fukase. T. *et al.*; "Studies on the Mechanism of Biological Removal of Phosphorus (in Japanese). "Jap. J. Wat. Pollut. Res., **5**, 309-317 (1982)
6) Matsuo. Y. and Miya. A.; "Anaerobic Uptake of Organics by the Activated Sludge Growth with Anaerobic Aerobic Sequence (in Japanese). " Proceedings of Environmental and Sanitary Engineering Research, **23**, 287-300 (1987)
7) Comeau. Y. *et al.*; "BIOLOGICAL PHOSPHATE REMOVAL FROM WASTEWATER-Dynamics of carbon reserves in biological dephosphatation of wastewater." Proceedings of an IAWAPRC Specialized Conference held in Rome, Italy, 39-55 (1987)
8) Tsuno. H. *et al.*; "BIOLOGICAL PHOSPHATE REMOVAL FROM WASTEWATER-A kinetic model for biological phosphorus removal incorporating intracellular organics and phosphorus pools." Proceedings of an IAWPRC Specialized Conference held in Rome, Italy. 99-110 (1987)
9) STANDARD METHODS FOR THE EXAMINATION OF WATER AND WASTEWTER, 14 th Edition, American Public Health Association, Washington, D. C., (1975)
10) Brown. M. J. and Lester. J. N.; "Comparison of Bacterial Extracellular Polymer Extraction Methods." Applied and Environmental Microbiology, **40**, 179-185 (1980)
11) Tai. S.; "Determination of Carbohydrate in Wastewater (in Japanese). " Journal of Water and Waste, **30**, 370-374 (1964)
12) Mino. T. *et al.*: "Studies on Phosphorus Composition and Phosphorus Metabolism in Activated Sludges." Journal of Japan Sewage Works Association, **20**, 229, 22-29 (1983)