# KINETICS OF BIOLOGICAL PHOSPHORUS BEHAVIOR IN SEQUENTIAL BATCH REACTOR UNDER ANAEROBIC/AEROBIC CONDITION

CHO-HEE YOON AND MOTOYUKI SUZUKI
*Institute of Industrial Science, University of Tokyo, Tokyo 106*

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**A kinetics study of biological phosphorus release/uptake in biomass under anaerobic/aerobic (A/O) condition was made in a laboratory sequential batch reactor (SBR) system. The ratios of P/BOD and TN/BOD of influent feed solution for increasing the intracellular phosphorus must be greater than about 0.01 and 0.13 in this system. Phosphorus uptake was correlated to the uptake of $K^+$ and $Mg^{2+}$ as $\triangle K/\triangle P = 0.28$, $\triangle Mg/\triangle P = 0.36$ by molar ratios.**

**The rate of phosphorus release for endogeneous respiration of the biomass under anaerobic condition can be described by using a Monod-type equation with respect to phosphorus content in the biomass, and the values of the rate constant, $K_{rp}$, and the saturation constant, $K_{kp}$, are $1.1 \times 10^{-3}\,h^{-1}$ and $1.4 \times 10^{-3}$ kg/kg respectively. The rate of luxurious phosphorus uptake at the aerobic stage can be expressed by a first-order kinetic equation based on $Px_m$-Px with the rate constant, $K_u$, is $0.017\,h^{-1}$.**

## Introduction

In the past several years, a considerable amount of research has been devoted to studying the mechanism of enhanced biological phosphorus removal, and it has been reported that phosphates as polyphosphates in biomass could be increased by using anaerobic/aerobic (A/O) stages.[10,12,13]

Clarification of the behavior of phosphorus in the biomass, therefore, is important for understanding enhanced biological phosphorus removal. In spite of its significance, however, there have been fewer quantitative studies than qualitative studies of phosphorus, organic carbon, nitrogen, metallic cations and other factors. Therefore, a confirmed quantitative knowledge of the effects of these parameters on phosphorus behavior in the biomass must be gained in order to investigate kinetic mechanisms and to design stable operation conditions of a biological phosphorus removal system. We have found that phosphorus release from the biomass could basically be attained by controlling biodegradable organic carbon (BOC) concentration in the bulk solution under anaerobic condition.[14,15]

However, even in the absence of BOC, release of phosphorus from the biomass occurs gradually. Simple correlation of phosphorus release and BOC uptake was not possible. Then an explanation was proposed on the basis of a hypothesis that phosphrous accumulated under aerobic condition can be used to supply energy for endogenous respiration of the biomass.[8]

The specific objectives of this study are two-fold; to examine the characteristics of accumulating phosphorus in the biomass by varying parameters such as phosphorus, nitrogen and metallic cations, and to develop a kinetic model of the biological phosphorus removal process under A/O conditions.

## 1. Experimental Apparatus and Procedures

A sequential batch reactor (**SBR**) of $5 \times 10^{-3}\,m^3$ was equipped with a mechanical stirring device and fitted with a stone diffuser connected to an air compressor. Temperature in the SBR was maintained at $293 \pm 1$ K during the whole operation. Nitrogen gas was introduced to the surface of the bulk solution during the anaerobic period. Compressed air was supplied to a reactor at the aerobic stage at an air flow rate of $1 \times 10^{-3}\,m^3$/min. The liquid volume in the reactor after the fill stage was $5 \times 10^{-3}\,m^3$, and $3 \times 10^{-3}\,m^3$ was removed at the draw stage. The 8 h basic cycle mode operation, shown in **Fig. 1**, is composed of a 4 h anaerobic stage, a 3 h aerobic stage, 45 min settling and a 15 min draw step. Substrate was supplied to the reactor instantaneously at the initial stage of the anaerobic step. Biomass used in this study was obtained from activated sludge, cultivated by using synthetic substrate under aerobic condition. Biomass was transferred to the SBR and acclimatized more than six months prior to starting-up measure-

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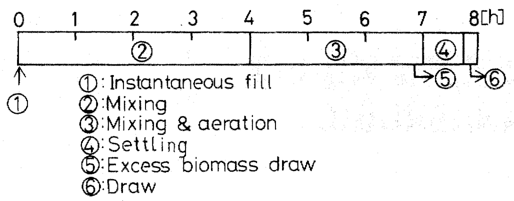


**Fig. 1.** Time schedule during a cycle

ment. Constant concentration of biomass was achieved by withdrawing an excess amount of growth prior to 5 min settling in a cycle. All the procedures mentioned above were controlled through an A/D converter by a microcomputer (NEC Co., PC-8801).

The chemical composition of the feed solution is specified in **Table 1**, whose organic substrate consisted of glucose and polypeptone. All the nutrients except phosphorus and nitrogen were maintained unchanged during the experimental period. The ranges of operation parameters employed in this study are summarized in **Table 2**.

The filtered samples of the mixed liquor, produced by using glass fiber filter with a pore size of 1 $\mu$m, were analyzed for total organic carbon (TOC), total phosphorus (TP), total nitrogen (TN), $PO_4$-P, $NH_4$-N and $NO_3$-N, potassium (K), magnesium (Mg) and calcium (Ca) concentrations. Biomass concentration (MLSS) and intracellular phosphorus content were analyzed from the filter residues.

TOC analysis was done by a TOC analyzer (TOC-10B, Shimadzu Co.) and K, Mg and Ca were measured with an atomic absorption analyzer (Model 11-551 AA/AE, Perkin Elmer Co.). All the other measurements were performed according to the procedures described in "Analytical Methods".[7]

## 2. Results and Discussion

### 2.1 Phosphorus behavior in the bulk solution through each cycle

Phosphorus concentration changes in the bulk solution during a cycle in the dynamic steady state are shown in **Fig. 2** for the cases of influent P concentrations ($P_i$) of 4, 6, 12 and $24\times10^{-3}$ kg/m$^3$ and initial biomass concentrations ($X_0$) of 2.5, 4.5 and 6.5 kg/m$^3$ as MLSS. Influent TOC concentration is kept at about 0.28 kg/m$^3$. At the influent phosphorus concentration of $4\times10^{-3}$ kg/m$^3$, i.e., $P_1$/BOD = 0.01, phosphorus release from the biomass did not occur, and it ingested into the biomass adversely even in the anaerobic stage during about 30 min after feeding substrates. All the phosphorus may have been consumed for the growth of biomass. Therefore, phosphates could not be accumulated luxuriously in the biomass when the ratio of influent P/BOD is smaller than about 0.1, even if the A/O system was used. This value is roughly in agreement with the experimental observation of Nakamura *et al.*[11] In the cases of 6, 12 and $24\times10^{-3}$ kg/m$^3$ of of $P_i$, on the other hand, these concentration changes apparently showed the release and uptake of phosphorus by biomass at A/O stages. For the case of $6\times10^{-3}$ kg/m$^3$, however, phosphorus release from the biomass at the initial anaerobic stage, which might possibly be related to the uptake of BOC, is rapid but after the initial hour such release hardly occurred. This phenomenon can easily be accounted for by considering that releasable phosphates were exhausted in the biomass. It indicates that the amount of phosphorus in the bulk solution may not be enough to satisfy the biomass,

**Table 1.** Feed solution characteristics

| Component | Unit [kg/m$^3$] |
|---|---|
| glucose | 0.5 |
| polypeptone | 0.2 |
| $KH_2PO_4$ | 0.015–0.1 |
| $(NH_4)_2SO_4$ | 0–0.2 |
| $MgSO_4\cdot7H_2O$ | 0.15 |
| NaCl | 0.05 |
| $CaCl_2\cdot2H_2O$ | 0.02 |
| $NaHCO_3$ | 0.15 |

**Table 2.** Operation parameters in this study

| Parameters | Performance range |
|---|---|
| influent $BPD_5$* | 0.4 kg/m$^3$ |
| influent P | $4$–$24\times10^{-3}$ kg/m$^3$ |
| influent TN | $25$–$70\times10^{-3}$ kg/m$^3$ |
| BOD loading | 0.1–0.3 kg/kg·d |
| BC** | 2.5–6.5 kg/m$^3$ |
| BA*** | 5–11 d |

* $BOD_5$; TOC × 1.42
** BC; biomass concentration
*** BA; biomass age

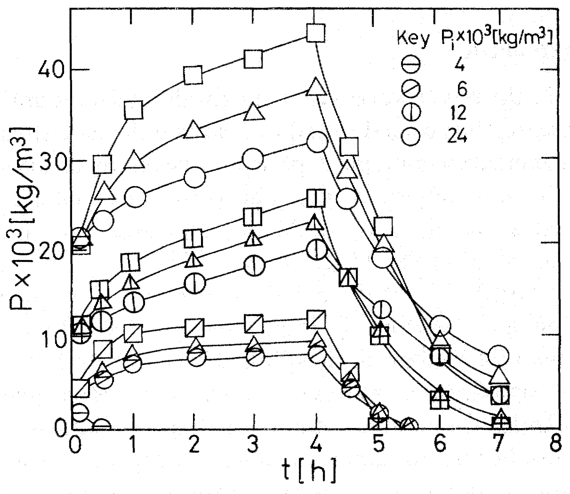


**Fig. 2.** Changes in $P$ concentration in the bulk solution during a cycle of SBR ($X_o$=6.5: □, 4.5: △, 2.5: ○)

which is able to accumulate more phosphorus during aeration periods as is shown in Fig. 2. In the case of $24 \times 10^{-3}$ kg/m$^3$ of $P_i$ and 6.5 kg/m$^3$ of MLSS, $20.5 \times 10^{-3}$ kg/m$^3$ of $P$ concentration at the initial anaerobic stage rose to a concentration as high as $46 \times 10^{-3}$ kg/m$^3$ at the end of that stage in the bulk solution. An approximately $25.5 \times 10^{-3}$ kg/m$^3$ increase in $P$ concentration in the bulk solution during the anaerobic stage can be accounted for by its release from the biomass in order to accumulate organic carbon and for aerobes to supply energy during their endogenous respiration at the anaerobic stage.

As mentioned above, it should be noted that the phenomenon of phosphorus release from the biomass during the anaerobic steps is divided into two distinct phases, especially according to the presence of BOC in the bulk solution. During the period when BOC exists, as discussed in our previous paper,[15] the rate of phosphorus release from the biomass is rapid and is controlled by a function of its concentration in a form of a modified Monod-type equation. The period after all BOC is consumed will be discussed in more detail in section 2.4.

On the other hand, under aerobic condition the uptake of phosphorus by the biomass started immediately when the aeration started. In the case of influent $P$ concentration of $6 \times 10^{-3}$ kg/m$^3$, phosphorus uptake is completed within 1 h after starting the aeration regardless of the biomass concentration. This suggests that the amount of phosphorus in the bulk solution may not be sufficient although the biomass still has the capability of $P$ uptake. For the case of influent phosphorus concentration of $24 \times 10^{-3}$ kg/m$^3$ with three concentrations of the biomass, the profiles of phosphorus concentrations in the supernatant solution with time are plotted in **Fig. 3** in semi-log scale. As can be seen from Fig.3, the reaction of phosphorus uptake by the biomass can be described by first-order reaction kinetics. It is likely that the quantity of P taken up into the biomass is stored in the form of polyphosphates as high energy sources and used for its growth, as reported by many investigators.[4,9)]

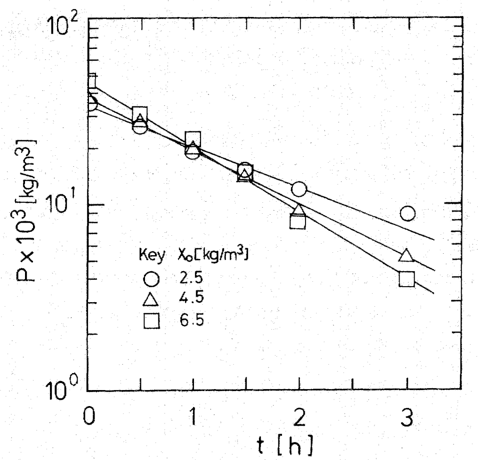


**Fig. 3.** Profiles of $P$ concentration in the bulk solution during aerobic period ($P_i$: 0.024 kg/m$^3$)

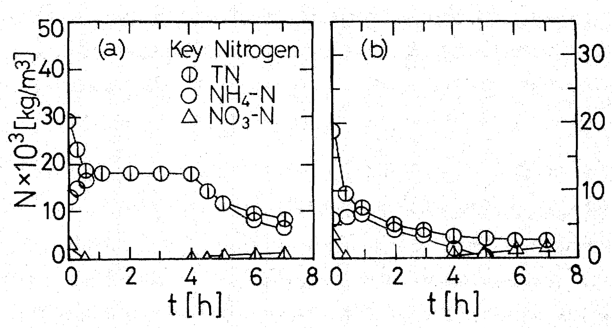


**Fig. 4.** Changes in nitrogen concentration in the bulk solution during a cycle period ($X_o$: 6.5 kg/m$^3$)

**2.2 Nitrogen behavior in the bulk solution during one cycle**

Nitrogen (TN, $NH_4$-N and $NO_3$-N) concentration changes in the bulk solution during one cycle period are illustrated in **Fig. 4** for the influent concentrations of $55 \times 10^{-3}$ (a) and $40 \times 10^{-3}$ kg N/m$^3$ (b) as TN with 6.5 kg/m$^3$ of biomass as MLSS. TOC and P concentrations of the feed solution are kept at about 0.28 kg/m$^3$ and $24 \times 10^{-3}$ kg/m$^3$ respectively.

TN concentration changes in the bulk solution decreased immediately in the initial hour of the anaerobic stage. It is likely that changes from organic-nitrogen to $NH_4$-N and uptake into the biomass occur in this period. After 2 h of the initial anaerobic stage, in Case (a), TN concentration changes in the bulk solution no longer occurred and the nitrogen was mostly in the form of $NH_4$-N.

But in Case (b), $NH_4$-N concentration in the bulk solution decreased gradually, which means that consumption occurred with the growth of facultative biomass. Under aerobic condition, on the other hand, $NH_4$-N concentration in Case (a) decreased with time but in Case (b) most of the nitrogen was consumed out in the mixed solution during anaerobic period. Furthermore, the degree of nitrification in this study was very low in both cases, as shown in Fig. 4. In Case (a), this implies that $NH_4$-N is used only for the growth of heterotrophic biomass. In Case (b), nitrogen under aerobic condition probably limited growth of the aerobes that could accumulated phosphorus. From these results it may be deduced that the influent nitrogen concentration has a significant effect on increasing phosphorus content in the biomass since there was no difference in feed composition except for nitrogen components between Case (a) and Case (b).

Phosphorus content in the biomass in the dynamic steady-state condition in Cases (a) and (b) were 6.1% and 3.6%. In addition, the relationship between TN/BOD of influent feed solution and the ratio of phosphorus content per unit biomass is shown in **Fig. 5**. The increase of the ratio in influent feed solution above 0.13 certainly improved phosphorus content in the biomass. This result indicates that in the SBR system the TN/BOD ratio of influent feed must be kept greater than about 0.13.

### 2.3. Relationship between phosphorus and cations uptakes

**Figure 6** shows the relationship between the amount of phosphorus uptake and $Mg^{2+}$ and $K^{+}$ uptake under aerobic condition. The molar ratios of $Mg^{2+}$ and $K^{+}$ to phosphorus taken up by the biomass were found to be 0.28 and 0.36 respectively. These values are similar to that reported by Comeau *et al.*[2]

$Mg^{2+}$ and $K^{+}$ concentrations in the bulk solution under anaerobic conditions also followed the change in phosphorus concentration.

But $Ca^{2+}$ concentration was almost constant under anaerobic condition. Calcium phosphate precipitation under the conditions of this study, therefore, did not show a significant contribution to phosphorus behavior. These results indicate that the ratios of Mg/P and K/P of the influent feed must be greater than about 0.28 and 0.36 for the biological process of enhanced phosphorus removal under A/O conditions. However, it could not be confirmed in this study that a chemical complex composed of polyphosphates with such metallic cations as $Mg^{2+}$ and $K^{+}$ are formed into the biomass.

### 2.4 Rate of P release during endogenous respiration from biomass at the anaerobic stage

The postulated model for phosphorus release from biomass under anaerobic condition is composed of two phases according to the difference in its release rate as mentioned above. The difference arises from whether BOC was present or not.

When there was no BOC in the bulk solution, polyphosphates stored in the biomass were used as an energy source for endogenous respiration of the biomass and as a result the biomass released phosphorus from the polyphosphates pool.

If biomass growth is negligible under anaerobic condition, the mass balance for phosphorus between the solution and the biomass is given by

$$V \cdot dP/dt = -V \cdot X \cdot (dPx/dt) \qquad (1)$$

In Eq. (1), the term on the right-hand side represents phosphorus release as an energy source for endogenous respiration of the biomass. During this period, the rate of phosphorus release is assumed to be described as a Monod-type equation with respect to releasable phosphorus content, $(Px-Pe)$, where $Px$ is the total phosphorus content per unit biomass and $Pe$ is the essential phosphorus content per unit biomass. The rate of phosphorus release is given by:

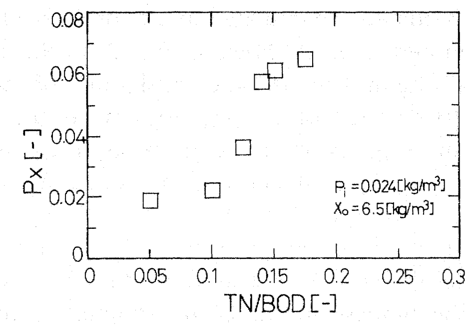


**Fig. 5.** Effect of TN/BOD ratio in the influent feed solution on $P$ content in the biomass

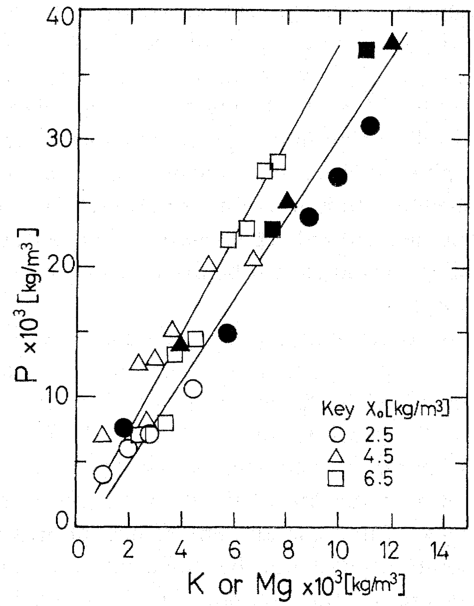


**Fig. 6.** Relation between $P$ and $K^{+1}$, $Mg^{+2}$ (open key: $Mg^{+2}$; closed key: $K^{+1}$)

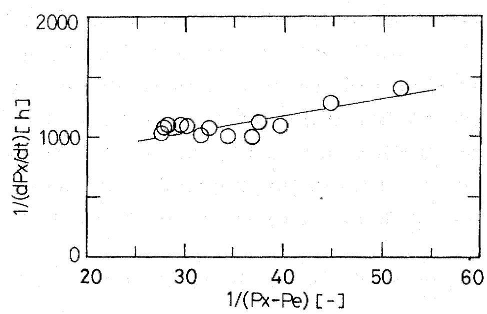


**Fig. 7.** Lineweaver-Burk plot

$$dPx/dt = -K_{rp} \cdot (Px-Pe)/\{K_{kp}+(Px-Pe)\} \qquad (2)$$

The Lineweaver-Burk plot of the measurements according to Eq. (2), and the regression line, are shown in **Fig. 7**. The values of rate constant, $K_{rp}$, and the saturation constant, $K_{kp}$, are $1.1 \times 10^{-3}\,h^{-1}$ and $1.4 \times 10^{-3}$ kg/kg, respectively. The value of $Pe$ adopted in this study is 0.015 kg/kg, which was reported by Ide *et al.*[6] Apparently, the simple Monod-type expression can be used to described the

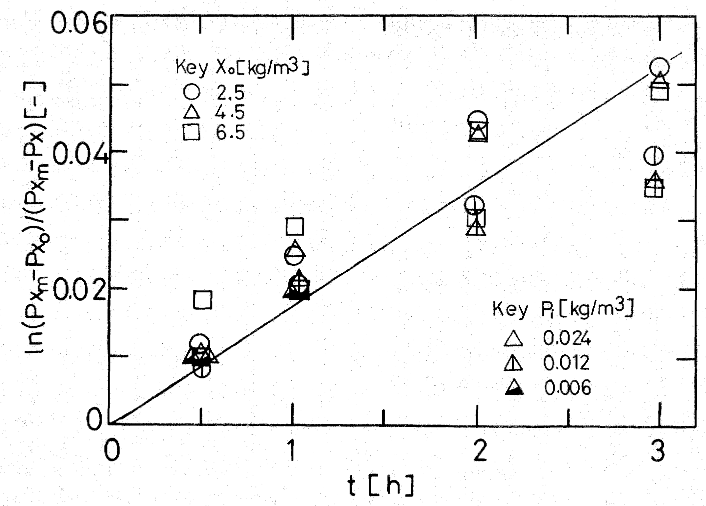

**Fig. 8.** Rates of $P$ uptake during aerobic period

kinetics of phosphorus release for endogenous respiration of biomass. This also suggests that the saturation constant, $K_{kp}$, plays a significant role in phosphorus release at low concentration.

### 2.5 Rate of phosphorus uptake by biomass under aerobic condition

The mass balance equation of phosphorus per unit volume during aerobic periods is written by considering both luxurious phosphorus uptake and growth of the biomass:

$$-dP/dt = d(X \cdot Px)/dt = X \cdot dPx/dt + Px \cdot dX/dt \quad (3)$$

The rate of luxurious phosphorus uptake is assumed to be expressed by first-order kinetics with regard to the remaining phosphorus capacity, $(Px_m - Px)$, where $Px_m$ is the maximum accumulative phosphorus content in the biomass as follows:

$$dPx/dt = K_u \cdot (Px_m - Px) \quad (4)$$

The growth rate of biomass is written as:

$$dX/dt = \mu \cdot X \quad (5)$$

where $K_u$ and $\mu$ are the rate constant of luxurious phosphorus uptake and the specific growth rate.

Rearranging and integrating Eq. (4) from $Px_o$ at time $t=0$ to $Px$ at time $t=t$ rsults in the following equation:

$$\ln(Px_m - Px_o)/(Px_m - Px) = K_u \cdot t \quad (6)$$

A plot of $\ln(Px_m - Px_o)/(Px_m - Px)$ vs $t$ for different values of $X_0$ and $P_i$ is shown in **Fig. 8**. The rate constant, $K_u$, can be obtained from the slope of a straight line through the origin drawn as a regression line. The maximum allowable phosphorus content, $Px_m$, is assumed here as 0.14 kgP/kg MLSS and $\mu$ is assumed as $0.04\,h^{-1}$, as reported by Wentzel *et al.*[16] The resulting value of $K_u$ is $0.017\,h^{-1}$. It should be kept in mind that the present estimation method has two potential problems: 1) difficulty in

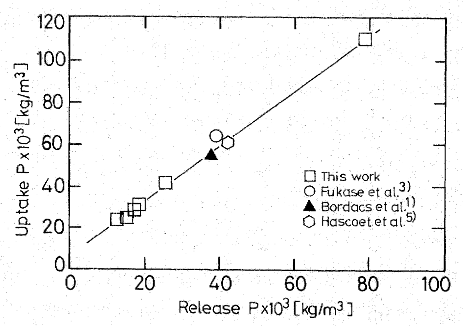

**Fig. 9.** Correlation between $P$ release and uptake in the biomass

accurately distinguishing the ratio of the amount of phosphates accumulating-biomass to that of phosphates non-accumulating-biomass, and 2) neglected factors such as the behavior of stored organic carbon in the biomass and the pH.

### 2.6 Relationship between *P* release and uptake

As mentioned above, when the biomass concentration is kept at about $6.5\,kg/m^3$ and the influent phosphorus concentration is varied in the range from $6 \times 10^{-3}\,kg/m^3$ to $24 \times 10^{-3}\,kg/m^3$, the relationship between the amount of phosphorus release and uptake in the biomass, together with those reported by Fukase *et al.*,[3] Bordacs *et al.*[1] and Hascoet *et al.*,[5] is shown in **Fig. 9**, where the amounts of release and uptake in the biomass were measured at the dynamic steady-state condition. As shown in Fig. 9, the higher the phosphorus release in the aerobic condition, the higher becomes the phosphorus uptake in the aerobic condition. These results are almost correlated by a linear relation.

$$(P\ \text{uptake}) = 1.34 \cdot (P\ \text{release}) + 7.5 \quad (7)$$

Here, higher phosphorus uptake mean reingestion of the phosphorus released as well as intake of the influent phosphorus. From this relation, it might be concluded that its release under anaerobic condition is the most important factor in biological phosphorus removal process by using the A/O method. It is, however, not yet clear biochemically why phosphorus uptake is controlled by its release. Moreover, this point is necessary to elucidate the reasons.

## Conclusion

The behavior of phosphorus in biomass under A/O conditions was examined by using an SBR and the results are summarized as follows:

1) Phosphorus release and uptake are affected by the phosphorus content in the biomass. In the A/O process system, P/BOD and TN/BOD ratios of the influent feed solution should be greater than 0.01 and 0.13 respectively for increasing the phosphorus content in the biomass.

2) The estimated $\triangle$Mg/$\triangle$P and $\triangle$K/$\triangle$P molar ratios during the uptake process at the aerobic stage are 0.36 and 0.28, respectively.

3) Kinetic constants of phosphorus release, $K_{rp}$, $K_{kp}$ and that of phosphorus uptake, $K_u$, where obtained by using the proposed kinetic model. These values are $1.1 \times 10^{-3}\,\mathrm{h}^{-1}$, $1.4 \times 10^{-3}$ kg/kg and $0.017\,\mathrm{h}^{-1}$, respectively.

4) The extent to which phosphorus uptake at the aerobic stage occurs can be correlated to the amount of phosphorus release at the anaerobic stage.

### Acknowledgement

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### Nomenclature

| | | | |
|---|---|---|---|
| $K_{rp}$ | = | rate constant defined by Eq. (2) | [$h^{-1}$] |
| $K_u$ | = | rate constant defined by Eq. (4) | [$h^{-1}$] |
| $K_{kp}$ | = | saturation constant | [kg/kg] |
| $P$ | = | phosphorus concentration | [kg/$m^3$] |
| $Pe$ | = | essential $P$ content in biomass | [kgP/kg MLSS] |
| $Px$ | = | total $P$ content in biomass | [kgP/kg MLSS] |
| $t$ | = | time | [h] |
| $X$ | = | biomass concentration | [kg/$m^3$] |
| $V$ | = | volume of reactor | [$m^3$] |
| $\mu$ | = | specific growth rate | [$h^{-1}$] |

⟨Subscripts⟩

| | | |
|---|---|---|
| $i$ | = | influent |
| $m$ | = | maximum |
| $o$ | = | initial |

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