Pergamon

*Wat. Sci. Tech.* Vol. 34, No. 1–2, pp. 25–32, 1996.

0273–1223/96 $15·00 + 0·00

PII: S0273-1223(96)00491-X

# BIOLOGICAL PHOSPHORUS REMOVAL PROCESSES – EFFECT OF pH ON ANAEROBIC SUBSTRATE METABOLISM

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## ABSTRACT

Effect of pH on the anaerobic acetate metabolism of biological phosphorus (P) removal processes was determined using anaerobic-aerobic activated sludge cultured with mainly acetate and containing a 12% or an 8% of total biomass as P (Px). The acetate uptake coupling to phosphate (Pi) release was found to be kinetically and thermodynamically influenced by pH. In the pH range 5.0-6.5, the acetate uptake rate (AUR) increased linearly with pH from 0 to about 50 (mg C/g VSS/h), and the Pi release rate (PiRR) from 20 to about 50 (mg P/g VSS/h). In the pH range 6.5-8.0, AUR remained at a constant range but PiRR continuously increased. Above pH 8.0, both AUR and PiRR started to decrease. With regard to the molar ratio of Pi released per acetate taken up (Pi/Ac), it was about 1.0 or 0.70-0.75 in the pH range 5.5-6.5, and proportionally increased to 1.75 or 1.50 in the pH range 6.6-8.5 for sludge containing a 12% Px or an 8 % Px, respectively. Apparently, acidic pH inactivated the acetate metabolism, and basic pH stimulated too much Pi release, resulting an increase in energy consumption for acetate uptake (i.e. the Pi/Ac ratio). As a compromise, an optimum pH 6.8±0.7 was proposed for anaerobic acetate metabolism, because that a relatively high AUR with less energy consumption can be maintained by the bacteria that respond for biological P removal. 

## KEYWORDS

Acetate metabolism; activated sludge; pH gradient; pH; biological phosphorus removal; polyphosphate; polyphosphate-accumulating bacteria; proton motive force; thermodynamics.

## INTRODUCTION

The enhanced biological phosphorus removal (EBPR) method has been applied in wastewater treatment plants for removing dissolved phosphate (Pi) from waste streams (Hong *et al.*, 1984; Pitman *et al.*, 1988). This method is to enrich or select certain bacteria capable of absorbing and storing Pi as cellular polyphosphate (polyP) with a P content (Px) at least two to three times higher than usual microorganisms. Later on, the polyP-accumulating bacteria (PAB) are separated from the supernatant in a settling tank, where the P-free supernatant is discharged into receiving water bodies, and the bacteria are either circulated back to the process or wasted.

For proper functioning of EBPR, repetitive anaerobic and aerobic zones and suitable substrates addition (i.e. simple acids) in the anaerobic zone are pre-requisite ( Barnard, 1974; Fukase *et al.*, 1985; Jenkins and Tandoi, 1991). It is because that the bacteria promoting EBPR can hydrolyze polyP to Pi to provide energy for substrate uptake and conversion to internal carbon storage products under the initial anaerobic conditions (Comeau *et al.*, 1986; Mino *et al.*, 1987). Under subsequent aerobic conditions where external substrates are absent, the bacteria can utilize the stored substrate to produce energy for growth and for polyP accumulation, successfully out-competing other strictly aerobic bacteria. Thus, one of the means to optimize EBPR processes will be to promote the growth of these bacteria, or to optimize their substrate uptake under anaerobic conditions.

According to recent reviews (Toerien *et al.*, 1990; Yeoman *et al.*, 1988b), factors affecting the anaerobic substrate metabolism of PAB in EBPR processes are poorly known yet. For example, no discussion was given on the organic loading in the anaerobic zone or on the sludge recycling rate. With regard to some factors such as temperature or anaerobic retention time studied, only effects on Pi release but not on substrate metabolism was reported, and, in fact, no optimum conditions were suggested for these two factors. Further, the manual proposed (U. S. EPA, 1987) and the mathematical model designed (Henze *et al.*, 1994) for EBPR processes provided no information on the key operational parameters. As a consequence, deterioration of EBPR processes was often reported (Cech and Hartman, 1993; Fukase *et al.*, 1985; Matsuo *et al.*, 1982; Matsuo, 1994; Yeoman *et al.*, 1988a).

The effect of pH on the substrate metabolism under anaerobic conditions was seldom addressed (Toerien *et al.*, 1990; Yeoman *et al.*, 1988b). Only one study (Smolders *et al.*, 1994) indicated that anaerobic release of Pi was pH-dependent, but did not suggested optimum pH for substrate uptake. Thus, this study further determines the pH effect on the kinetics and thermodynamics of the anaerobic substrate metabolism of PAB using lab-scale anaerobic-aerobic batch-type reactors simulating EBPR processes. Based on the consideration of substrate uptake kinetics and energy efficiency, an optimum pH range is recommended.

## MATERIALS AND METHODS

Anaerobic-aerobic activated sludge was cultivated in two 2 l sequential batch reactors with a medium containing minerals, acetate, and peptone as previously described (Liu *et al.*, 1994). A P/total organic carbon (TOC) ratio (wt/wt), 10/100~20/100, in the medium was used for selecting the growth of polyP-accumulating bacteria in sludge. The reactors were operated on a fill-and-draw basis with a 3-h batch cycle consisting of a 50-min anaerobic phase, an 80-min aerobic phase, and a 50-min sedimentation phase. The anaerobiosis was achieved by nitrogen gassing, and the aerobic condition by an air pump with a flow rate of 1.5 l/min. The TOC loading was 0.6 kg $C/m^3 \cdot d$; the sludge retention time, 7.5-8.0 d; and the hydraulic retention time, 6 h. After a steady state operation for 5 wk, the sludge was used for determining the effect of pH on the acetate uptake and the Pi release under anaerobic conditions.

The batch experiments were carried out in a custom-made anaerobic apparatus (Fig. 1). Eighty ml diluted fresh sludge from the reactor at the end of the aeration period was dispensed into a 100 ml Pyrex bottle along with resazurin (final concentration, 1 mM) as a redox indicator. The bottle was then capped with a rubber stopper, and gassed with $N_2$ at 0.5 l/min for 20-30 min. Ten minutes before and during a batch test, the pH in the sludge mixture was regulated to a desired level ($\Delta$pH, $\pm$ 0.1) using a pH controller. To start the test, $N_2$ was introduced only to the head space of the bottle, then 1 ml 360 mM acetic acid stock solution (pH pre-adjusted) was injected into the bottle. The designed carbon loading, about two times higher than at the start of the anaerobic stage in the reactor, was 50 mg C/g VSS. Bulk concentrations of Pi and acetate were measured every 5, 10, 15, or 20 min. Two to three replicates were conducted at several specific pH values in the range 5.0-8.5.

Activated sludge samples taken from the anaerobic batch experiments were immediately filtered through a 0.45 $\mu$m filter (Millipore) before further determination. Analytic methods for the determination of substrate and Pi concentrations were the same as previously described (Liu *et al.*, 1996). Unless otherwise stated, other analysis used in this study was performed according to *Standard Methods* (APHA, 1989).

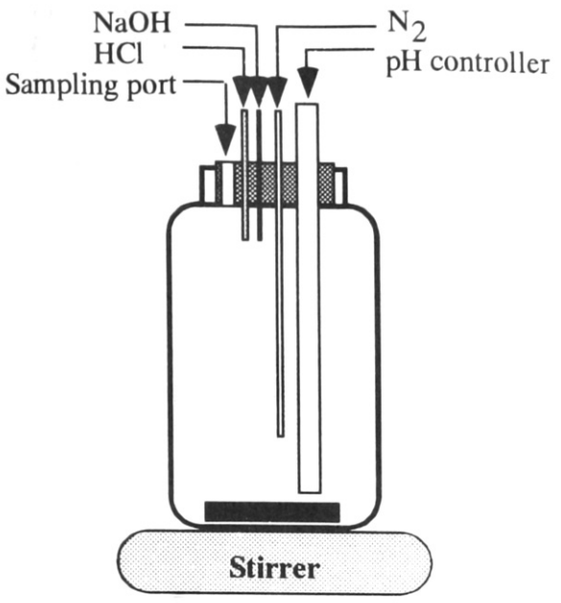


**Fig. 1.** Setup of an anaerobic testing apparatus.

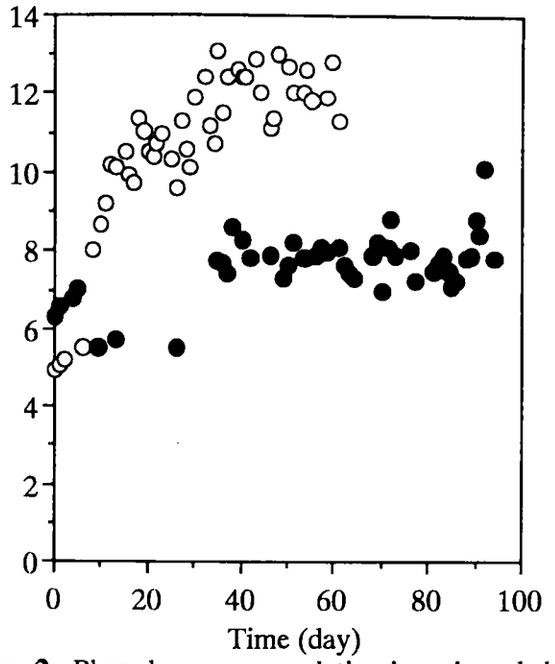


**Fig. 2.** Phosphorus accumulation in activated sludge. P/TOC ratio (w/w): ○, 20/100; ●,10/100.

## RESULTS

After 30 d acclimation in the anaerobic-aerobic batch-type reactors, the Px of sludge in those two reactors reached 12-13% or 8-9%, respectively (Fig. 2). At the same time, good TOC reduction and complete Pi removal were obtained. The difference in the Px between two reactors was due to the difference of the P/TOC feeding ratio applied.

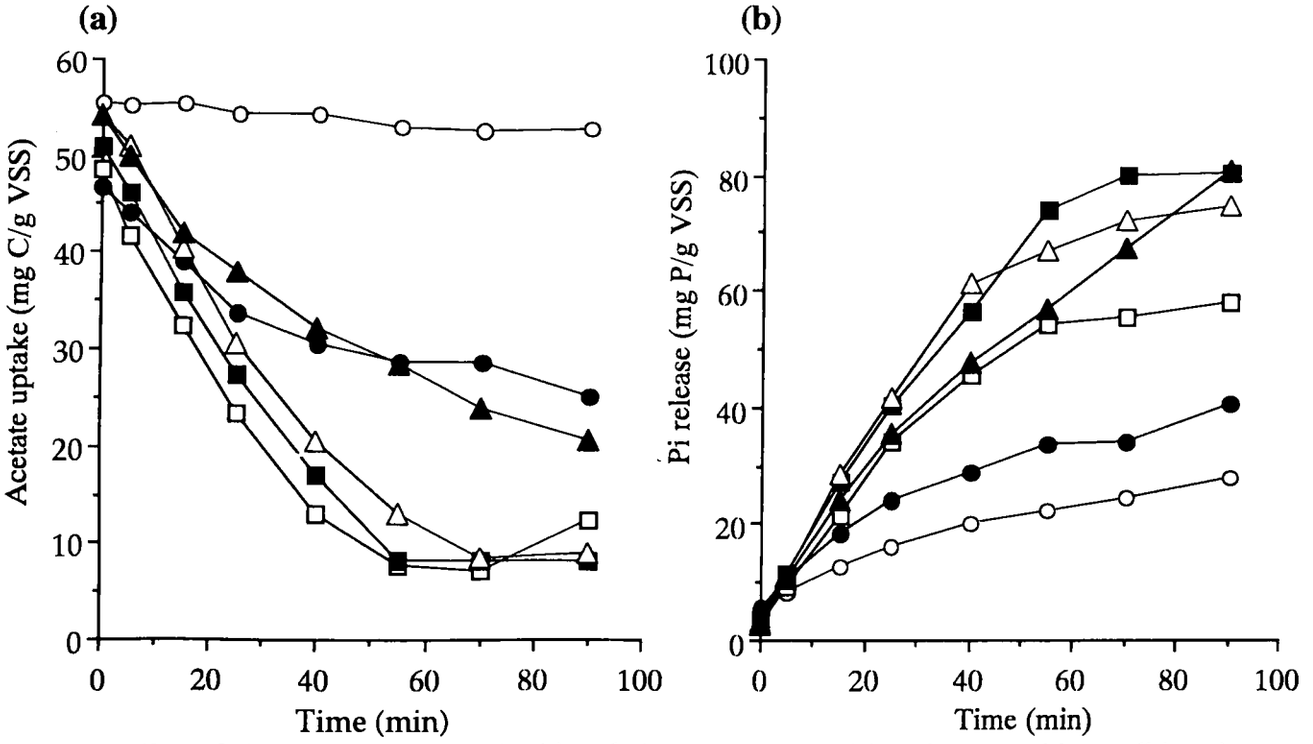


**Fig. 3.** Effect of pH on the acetate uptake (a) and phosphate release (b) under anaerobic conditions in sludge with a 12% Px. pH 5.0, ○; pH 5.7, ●; pH 6.5, □; pH 7.1, ■; pH 7.8, Δ; pH 8.6, ▲.

The amount and rate of the acetate uptake and the Pi release in sludge with an 8 or a 12% Px were found to be highly affected by pH. Figure 3 shows acetate uptake and Pi release profiles of sludge accumulating a 12% Px in the pH range 5.0-8.6 under anaerobic conditions. The amounts of acetate taken up, about 40-45 mg C/ g VSS, at pH 6.5, 7.1 and 7.8 were the highest. In contrast, the maximum amount of Pi released, about 80 mg P/g VSS, was observed in a different pH range 7.1-8.6, and represented about 40% of total P in the sludge (wt/wt). Obviously, acidic pH had a negative effect on both acetate uptake and Pi release. Basic pH inhibited the uptake of acetate, and stimulated more Pi release than at acidic pH. Similar results were observed using activated sludge containing an 8% Px (results not shown).

The acetate uptake rate (AUR) and the Pi release rate (PiRR) of sludge containing an 8 or a 12% Px in the pH range 5.0-8.5 were calculated and indicated in Fig. 4. Clearly, the sludge with a 12% Px has higher AUR and PiRR than that with an 8% Px. In the sludge with a 12% Px, both AUR (mg C/g TSS·h) and PiRR (mg P/g TSS·h) increased linearly to 50 and 65, respectively, between pH 5.0 to 6.3. In the pH range 6.3-8.2, AUR remained at a constant level but PiRR still increased to 115. Above pH 8.0, both AUR and PiRR started to decrease. Similar pH-dependence of AUR and PiRR were observed with the sludge containing an 8% Px.

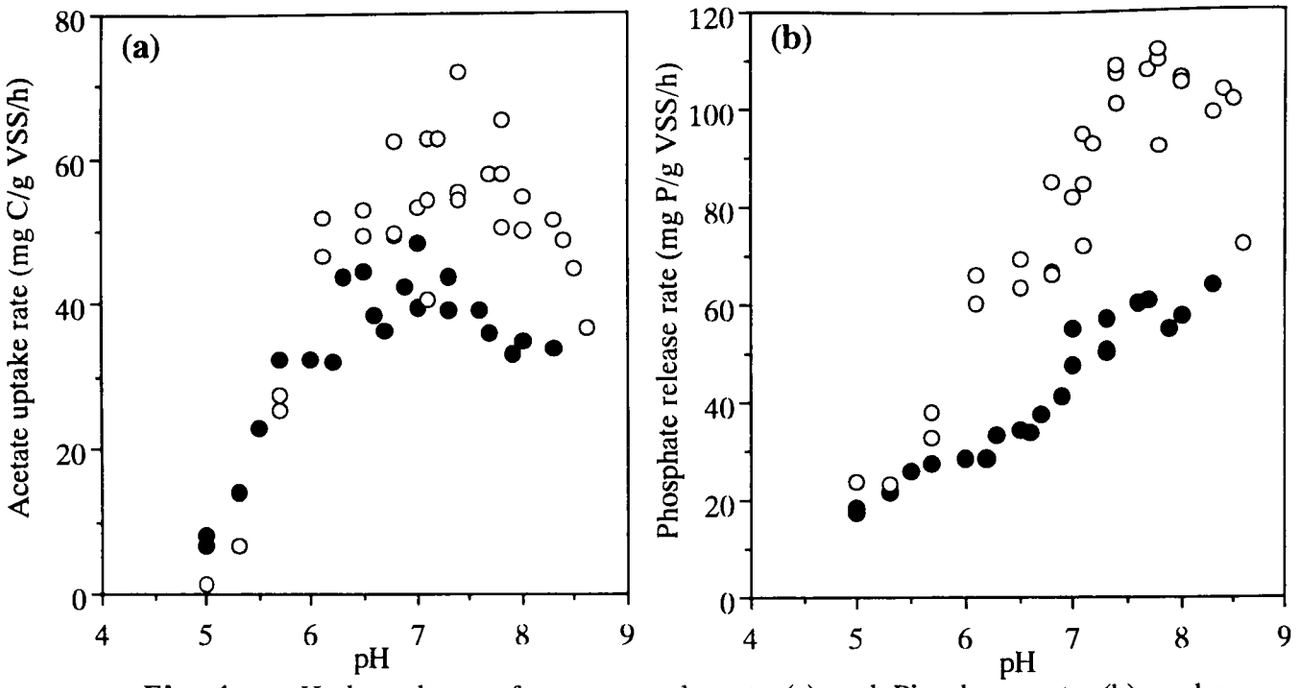


**Fig. 4.** pH dependence of acetate uptake rate (a) and Pi release rate (b) under anaerobic conditons with different Px (sludge Px: ○, 12%; ●, 8.0%).

We further examined the Pi release/acetate uptake (Pi/Ac) molar ratio that was calculated from the AUR and the PiRR. Figure 5 indicates that the Pi/Ac ratio increasing with the Px of sludge in the pH range 5.5-6.5 was about 1.0 for sludge containing a 12% Px, or 0.70-0.75 for sludge containing an 8 % Px. At pH above 6.7, a proportional increase of Pi/Ac ratio with pH was observed in both sludges, suggesting that more Pi release or more energy consumption was necessary for the acetate uptake anaerobically. Still optimum pH for acetate metabolism could not be reached.

## DISCUSSION

Anaerobic acetate metabolism of bacteria responding for EBPR processes have been consistently studied (Comeau *et al.*, 1986; Fuh and Chen, 1975; Mino *et al.*, 1987). Generally, the acetate uptake stoichiometrically couples to the Pi release under anaerobic conditions. The acetate taken up is further converted to internally-stored carbon reserve, polyhydroxybutyrate (PHB). The primary ATP source for the uptake is derived from the hydrolysis of polyP, resulting in Pi release.

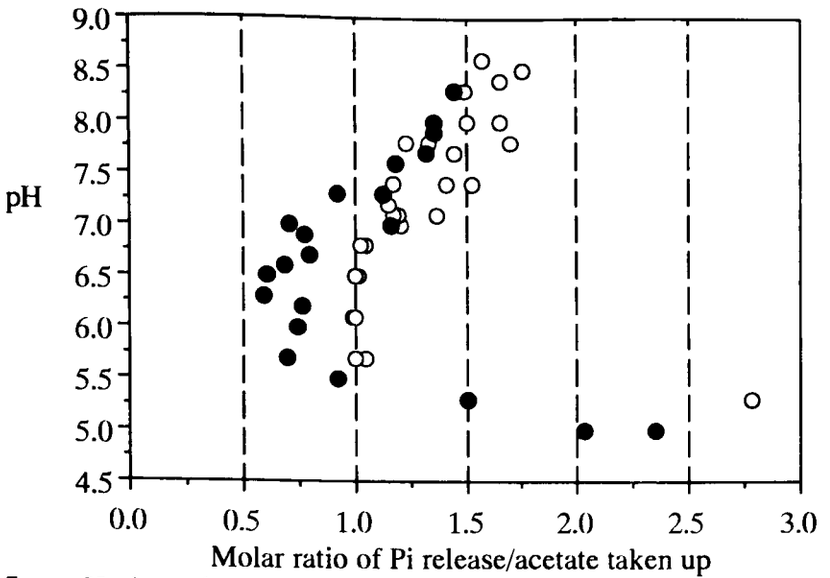


**Fig. 5.** pH dependence of the Pi release/acetate uptake molar ratio in sludge with different Px (sludge Px: ○, 12%; ●, 8.0%).

In addition, Smolders *et al.* (1994) found that the Pi release was kinetically and thermodynamically influenced by pH in the range 5.5-8.2, while acetate uptake remained unchanged. Our results differed from that of Smolder *et al.* (1994) in that the optimum pH for AUR or the conversion of acetate to PHB occurred in a rather narrow region 6.0-8.2 for sludge containing a 12% Px (Fig. 4). Below or above this region, the amount and rate of acetate uptake significantly decreased. We think that this decrease on the kinetics of PHB synthesis from acetate was due to a direct effect from pH, but not due to the effect of PiRR though Pi release is linked to acetate uptake.

With further attention to the thermodynamics of acetate uptake, Harold (1986) indicated that most substrate transport through cell membrane occurs against an electrochemical potential gradient, thus requires energy coupling. Accordingly, Smolders *et al.* (1994) indicated that the transport energy ($\Delta G^{o"}$) required to move one mole of acetate against the electric potential difference ($\Delta\Psi$) across the cell membrane and against its concentration gradient (Cin/Cout) can be represented by:

$$\Delta G^{o"} = n\Delta\Psi + 2.3nRT\log(C_{in}/C_{out}) \quad \text{(kJ/mol)} \quad \text{..... (1)}$$

where

- $\Delta G^{o"}$: acetate transport energy (kJ/mol)
- $\Delta\Psi$: electric potential difference (kJ/mol)
- n: the charge of the transported acetate per mol
- $C_{in}$: the internal concentration of acetate (mol)
- $C_{out}$: the bulk concentration of acetate (mol)
- R: gas constant (kJ/K·mol)
- T: temperature (K)

Further, electric potential difference is a function of proton motive force ($\Delta p$) and pH gradient (interior alkaline) and can be described by:

$$\Delta\Psi = \Delta p + 2.3RT(pH_{in} - pH_{out}) = \Delta p + 2.3RT\Delta pH \quad \text{(kJ/mol)} \quad \text{..... (2)}$$

where

- $\Delta p$: proton motive force (kJ/mol)
- $pH_{in, out}$: pH inside cell, pH outside cell (-)

Thus, Equ (1) representing the energy for the uptake of acetate becomes:

$$\Delta G^{o"} = n(\Delta p + 2.3RT\Delta pH) + 2.3nRT\log(C_{in}/C_{out}) \quad \text{(kJ/mol)} \quad \text{..... (3)}$$

The necessary work can be provided by the hydrolysis of ATP originated from polyP degradation under anaerobic conditions (Comeau *et al.*, 1986), since electron transport chain, membrane-bound ATP-ase enzyme, or NADH oxidation for the maintenance of pH gradient (Harold, 1977) does not function under anaerobic conditions.

With the assumption that $pH_{in}$ and $\Delta p$ of a cell is kept constant (Harold, 1986), Equ (3) well supports the experimental results of Smolder *et al.* that an increase in the Pi/Ac mol ratio with pH in the range 5.5-8.5. However, our results showed that the pH-dependence of the Pi/Ac ratio occurred in the range 6.6-8.6, or 6.3-8.4 for sludge with a 12% Px or an 8% Px, respectively (Fig. 5). In the pH range 5.5-6.4, the Pi/Ac ratio stayed at a constant level, and below pH 5.5, the ratio greatly increased. In fact, Padan *et al.* (1981) indicated that in most bacteria, $pH_{in}$ in a cell is kept relatively constant in a rather narrow pH range (2 units difference), and decreases or increases when below or above the pH range, respectively. Baronofsky *et al.* (1984) also reported in *Clostridium thermoaceticum* that $\Delta p$ and $pH_{in}$ is not constant in the pH range 5.0-7.0, and a significant decrease of $pH_{out}$ to 5 ultimately caused the collapse of $\Delta pH$ and $\Delta\Psi$, resulting a cessation of substrate metabolism. These two studies may support our observations that no uptake of acetate at pH 5.0, a decrease of both AUR and PiRR at pH above 8.2 (Fig. 4), and a greatly increase of the Pi/Ac ratio below pH 5.5 (Fig. 5).

In addition to the pH effect, the Px of sludge also influenced the acetate metabolism (Fig. 4 and 5). Possibly, the microbial population of sludge with a lower Px may consist of members other than PAB, such as glycogen-accumulating bacteria (Cech and Hartman, 1992; Liu *et al.*, 1996; Matsuo, 1994) that do not release Pi during acetate uptake. Participation of this population in the acetate metabolism may greatly influence AUR, PiRR, and Pi/Ac ratio. This possiblity will be further elucidated in a forthcoming paper.

Based on the above discussion of acetate metabolism, the optimum pH range was suggested. Figure 6 combines the AUR and its related energy efficiency observed in the sludge with a 12% Px. A pH value of $7.3\pm0.5$ is suggestively the best for maximizing the AUR or the prefominance of PAB, though more energy is necessary at a pH above 7.5. A pH range 5.7-6.8 is the best for an effective use of polyP (i.e. less Pi

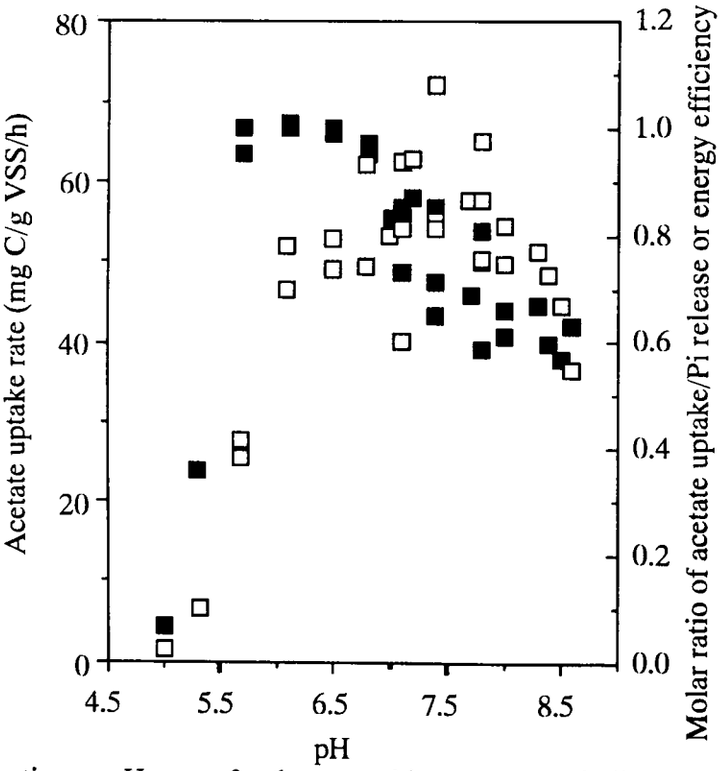


**Fig. 6.** An optimum pH range for the anaerobic acetate metabolism of biological phosphorus removal processes using sludge with a 12% Px (□, acetate uptake rate; ■, molar ratio of acetate uptake/Pi release).

release), resulting a relatively stable P removal because of less energy required for Pi uptake under subsequent aerobic conditions. As a compromise, a pH value of 6.8±0.7 was recommended for the acetate metabolism of PAB.

## ACKNOWLEDGMENT

The authors thank members of the Microbial Population Dynamics lab, National Institute of Bioscience and Human-Technology, Japan for providing the lab space and facilities during the course of this study.

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