

# Effects of injection of acetic acid and propionic acid for total phosphorus removal at high temperature in enhanced biological phosphorus removal process

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## ABSTRACT

In summer, wastewater treatment plant total phosphorus (TP) removal efficiency is low in South Korea. The reason is because of high temperatures or significant fluctuation of inflow characteristics caused by frequent rainfall. Hence, this study tried to raise TP removal efficiency by injecting fixed external carbon sources in real sewage. Polyphosphate accumulating organisms (PAOs) and glycogen accumulating organisms (GAOs) compete to occupy microorganisms at high temperature. Propionate is known to restrain GAOs. Thus, acetate and propionate were chosen as the external carbon source in this study to find out the suitable volume and ratio of carbon source which ensured the dominance of PAOs. An external carbon source was supplied in the anaerobic reactor of the biological phosphorus removal process at high temperature (above 25 °C). TP removal efficiency was improved by injecting an external carbon source compared to that without an external carbon source. Also, it remained relatively stable when injecting an external carbon source, despite the variation in temperature. TP removal efficiency was the highest when injecting acetate and propionate in the proportion of 2:1 (total concentration as chemical oxygen demand (COD) is 12 mg/L in influent).

**Key words** | external carbon source, PAOs, phosphorus removal, temperature

## INTRODUCTION

Allowable phosphorus concentrations within industrial and sewage discharges are in place primarily to prevent eutrophication, and differ from country to country. The average total phosphorus (TP) concentration of Korea's rivers is 0.16 mg/L. This is higher than the eutrophication standard of 0.02 mg/L of the US Environmental Protection Agency (USEPA), which can cause eutrophication any time. Thus, from the beginning of 2012, the South Korean government has enforced stricter regulations than before, which limit TP effluent concentrations to <0.2 mg/L. In order to satisfy the reinforced water quality standard (which varies as 0.2, 0.3, 0.5 mg/L by regions), chemical coagulation facilities were installed after biological treatment. However, TP removal is reduced during summer, due to the higher temperatures.

Enhanced biological phosphorus removal (EBPR) from wastewater is based on the enrichment of activated sludge with polyphosphate accumulating organisms (PAOs) (Brdjanovic *et al.* 1998; Wagner & Loy 2002). PAOs under

anaerobic conditions release phosphate into solution, but upon conversion to aerobic conditions, an excess of P-uptake occurs (about two to five times the P-release amount). However, glycogen accumulating organisms (GAOs) interrupt the phosphorus removal efficiency during the summer because GAOs are more active than PAOs above 20 °C.

Temperature not only influences the metabolic activities of the microbial population, but also has an effect on the settling characteristics of the biological solids (Metcalf & Eddy 1991; Crites & Tchobanoglous 1998). Also, BPR efficiency has been reported to improve at higher temperatures (20–37 °C) (McClintock *et al.* 1993; Converti *et al.* 1995), while P removal efficiency has been observed at lower temperatures (5–15 °C) (Viconneau *et al.* 1985; Florentz *et al.* 1987). In studies performed by Panswad *et al.* (2003), the PAOs were found to be lower-range mesophiles or, perhaps, psychrophiles, and predominated only at 20 °C or lower. The GAOs were somewhat mid-range

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mesophilic organisms with an optimum temperature between 25.0 and 32.5 °C.

In order to establish more stable performance of EBPR processes, the effect of microorganisms should be evaluated adequately at high temperature. This study aimed to evaluate the effects of injected external carbon sources, acetate and propionate, and improve TP removal efficiency by the activation of the PAOs at high temperature. The target of effluent TP concentration for biological treatment was less than 0.2 mg/L.

## MATERIALS AND METHODS

### Pilot-scale plant experiment

The pilot plant is located at the J-wastewater treatment plant in Dae-gu city, Republic of Korea. This study was conducted over 6 months and focused on the relationship between external carbon source and TP removal efficiency according to temperature. This study was conducted according to a pre-anoxic  $A_2/O$  process, with acetate and propionate as the organic carbon source. The pre-anoxic  $A_2/O$  condition was based on the pre-anoxic reactor being set up in front of the  $A_2/O$  process to minimize nitrate and DO introduced to the anaerobic tank (Figure 1).

Wang et al. (2009) suggested that acetate might be a better substrate than glucose for the PAO community because its conversion to PHA (poly- $\beta$ -hydroxyalkanoates) requires energy and reducing powers that are conveniently provided by polyphosphate transformations. Acetate induces the generation of more PHA and the release of more phosphorus in an anaerobic state, and also stores more poly-P in the aerobic state, within the cells of PAOs. However, at the same time, GAOs are also more activated, whereas propionate can enrich PAOs more selectively than other organic matter, like GAOs. Therefore, acetic

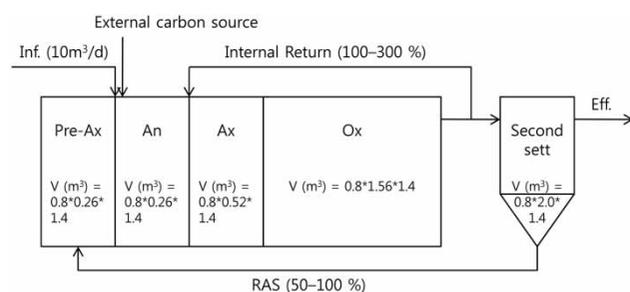


Figure 1 | Schematic diagram of pre-anoxic  $A_2/O$  process in pilot scale.

acid and propionic acid were chosen as an external carbon source to improve the activity of PAOs.

The influent was real domestic wastewater sampled from the overflow of the primary settlement tank. The inflow was 10 m<sup>3</sup>/day into an anaerobic reactor. The experiment was conducted as shown in Table 1 with various external carbon source concentrations under five conditions. Temperature was controlled using a heating device (above 25 °C). The process was operated under a solid retention time of 28 d, and the hydraulic retention times were 1.1–2.2 h (pre-anoxic, anaerobic), 2.2–3.3 h (anoxic), 5.5–8.5 h (aerobic), and 3.5–5.5 h (second settlement) in each tank. The return ratio and internal return ratio were 50–100% (Q<sub>r</sub>/Q) and 100–300% (Q<sub>r</sub>/Q), respectively.

### Sampling and analytical procedures

The EBPR process was monitored two or three times a week by sampling between 2 and 3 p biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, nitrogen, phosphate, mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), which were analyzed by *Standard Methods* (2005). PHA was analyzed by gas chromatography (GC).

Sludge samples from the pilot scale were mixed with approximately 1% formaldehyde per sample volume in order to inhibit biomass activity in the sludge. All samples were lyophilized. About 20 mg of lyophilized sludge was added to 2 mL of chloroform and 2 mL of an acidified methanol (3% H<sub>2</sub>SO<sub>4</sub>), containing benzoic acid as the internal standard. The samples were heated for 3 h at 100 °C and then 1 mL of distilled water was added and mixed. One hour of settling time was allowed to achieve phase separation. The chloroform phase containing

Table 1 | Operating modes in pilot scale

Mode	Process	Description (at 25 °C)
Mode 1	Pre-anoxic $A_2/O$	Pre anoxic – $A_2/O$
Mode 2		Adding only acetate 8 mg/L as COD (in influent to anaerobic phase)
Mode 3		Adding only propionate 8 mg/L as COD (in influent to anaerobic phase)
Mode 4		Adding acetate: propionate 8:4 mg/L as COD (in influent to anaerobic phase)
Mode 5		Adding acetate: propionate 16:8 mg/L as COD (in influent to anaerobic phase)

the PHA was transferred to another vial, dried with approximately 0.5–1 g of granular sodium sulfate pellets, and a 1  $\mu$ L sample was injected into the GC. The chromatograph was operated with a SE-54 column (30 m length  $\times$  0.25 mm I.D.  $\times$  0.25  $\mu$ m film) and helium was used as the carrier gas (1.5 mL/min). A flame ionization detection unit was operated at 300  $^{\circ}$ C (Comeau *et al.* 1998; Oehmen *et al.* 2005).

## RESULTS AND DISCUSSION

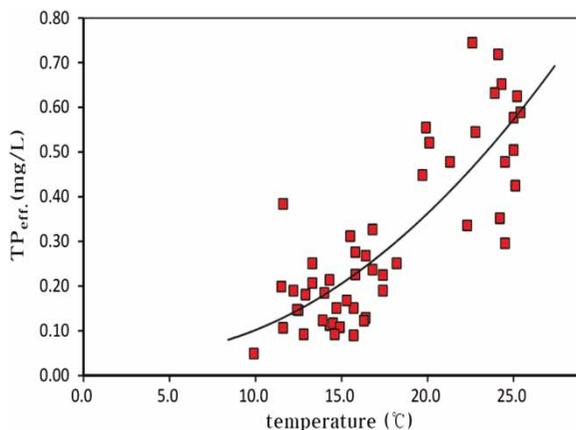
### Influences of temperature on removal of phosphorus

The relationship between annual temperature and effluent TP from J-wastewater treatment plant showed that the phosphorus removal efficiencies were generally good at temperatures less than 18  $^{\circ}$ C (Figure 2). It seems that the PAO microorganisms were depressed when the water temperature was above 20  $^{\circ}$ C. Lopez (2009) reported that GAOs have clear advantages over PAOs for substrate uptake at temperatures higher than 20  $^{\circ}$ C. Thus, the treatment efficiency was somewhat low even with the addition of an external carbon source.

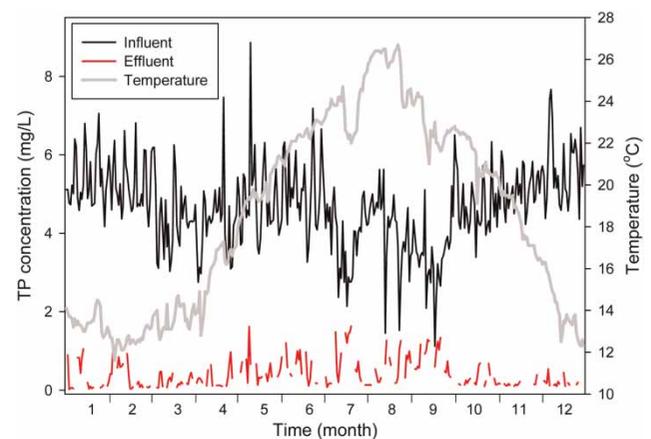
Figure 3 shows the effluent concentration of TP and the temperature of J-wastewater treatment plant in 2012. It was found that TP effluent was high and TP removal efficiency was not constant in July, August, and September.

### Effect of phosphorus removal on external carbon sources at high temperature

Two types of tests for TP removal were conducted, with and without the addition of an external carbon source. In the



**Figure 2** | Relationship between effluent phosphorus and annual temperature at J-wastewater treatment plant.



**Figure 3** | Comparison of TP effluent and temperature for a single year (2012).

first test, without the external carbon source, the average TP concentration from influent was determined to be 3.06 mg/L, while that of the effluent was 1.85 mg/L. The average TP removal efficiency was 40.6%. In the second test, with an external carbon source, the influent of TP concentration was similar to the first test, while that of the effluent varied and was a little low. As shown in Table 2, the removal efficiencies from Mode 2 to Mode 5 were 79.6, 82.8, 90.8 and 75.9%. The results indicate that the injection of external carbon sources was effective for the removal of phosphorus. The reason is assumed to be that P-release and P-uptake occurred more actively, provided that there was a sufficient amount of organic matter available to microorganisms.

The alteration of effluent phosphorus concentration at high temperature is shown in Figure 4. In Mode 2 to Mode 5, injecting an external carbon source resulted in the phosphorus removal efficiency being increased by more than 75%. When the influent TP concentration was 2.5–4.0 mg/L, effluent TP was as follows: Mode 1, 1.85 mg/L; Mode 2, 0.63 mg/L; Mode 3, 0.39 mg/L; Mode 4, 0.22 mg/L; Mode 5, 0.64 mg/L. Mode 4, injecting acetate:propionate (8:4 mg/L)

**Table 2** | Average TP removal efficiency in each mode

Mode	TP <sub>inf.</sub> (mg/L)	TP <sub>eff.</sub> (mg/L)	Rem (%)	Remark
Mode 1	3.06	1.85	40.6	Without external carbon sources
Mode 2	3.12	0.63	79.6	With external carbon sources
Mode 3	2.27	0.39	82.8	
Mode 4	2.27	0.22	90.8	
Mode 5	2.57	0.64	75.9	

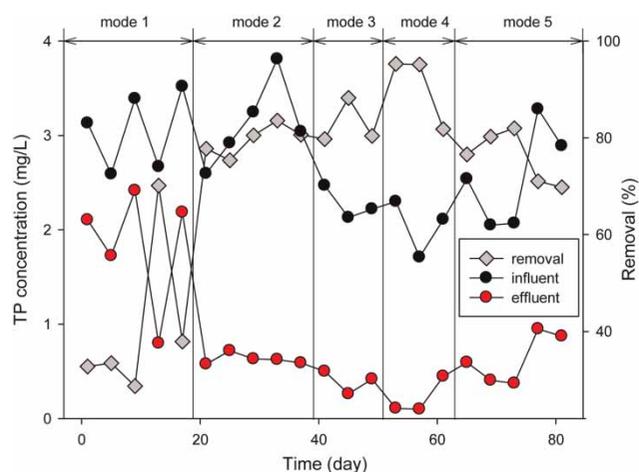


Figure 4 | Concentration of influent and effluent and removal efficiency of TP (at 25 °C).

as an external carbon source, showed the highest efficiency of TP removal, with a maximum of 95.3% removal determined. It can be expected that GAOs are suppressed by propionate. Oehmen *et al.* (2005) reported that propionate uptake of GAOs was practically negligible, which can minimize GAO growth. A study conducted by Lopez (2009) indicated a similar result and showed that an adequate acetate to propionate ratio could be used as a potential tool to suppress the proliferation of GAOs at high temperature. Mode 4 was the most effective of any mode, although a higher volume of carbon sources was injected in Mode 5. This shows that an excess of organic matter may also be a disadvantage for phosphorus removal. Too high a carbon concentration can also restrain PHA synthesis (Jiang *et al.* 2010). Wang *et al.* (2010) studied the P removal efficiencies of the A/O process. The highest P removal of  $95 \pm 1.24\%$  was achieved when using propionate as the sole carbon source (P-release: acetate > mixture > propionate; P-removal: propionate > mixture > acetate). Therefore, to obtain a high removal efficiency of phosphorus, the injecting concentration and an external carbon source must be optimized.

The amount of organic matter, such as volatile fatty acids, is low in domestic wastewater in Korea and depends on rainfall frequency. According to Reddy (1998), the readily available organic matter concentration within the initial anaerobic phase must be more than 25 mg COD/L to accomplish significant EBPR. The study by Randall *et al.* (1992) stated that the influent to the anaerobic zone of the BPR system should have a BOD5:total P ratio of >20:1 or COD:P ratio >40:1 in order to meet an effluent P level of <1.0 mg/L. Thus, the concentration of TP effluent was investigated by controlling the C:P ratio with an external carbon source.

The influent organic matter's concentration (BDCOD) had an average value of 136 mg/L. The values of C:P ratio were increased by approximately 2.7, 4, 8, when external carbon sources of 8, 12, 24 mg/L were added, respectively. It was an immaterial increase, but the effluent concentration of TP decreased dramatically. It was considered that the concentration of injected external carbon source was sufficient to increase the amount of P-release during the anaerobic phase, and as a result, P-uptake in the aerobic phase was increased substantially, even though the volume of injected external carbon source was very little. It shows that an increase of C:P ratio improved the removal of TP, whereas an excessive injection reduced the removal efficiency of TP. There was a better phosphate removal efficiency when the C:P ratio was more than 50.

According to a previous study, polyphosphate and organic phosphorus are difficult to remove by chemical treatment (Park *et al.* 2002). Figure 5 presents the relationship between org-P concentration and removal efficiency. An average org-P concentration of the influent was 0.85 mg/L and the removal efficiencies of org-P in each mode were 75.3, 82.2, 82.3 and 81.7%, respectively.

It seems that the injection of an external carbon source in biological treatment was efficient for the removal of org-P. On average, 0.12 mg/L of org-P exists in J-wastewater treatment plant. Removal of org-P is important to lower the effluent to less than 0.2 mg/L. This system could improve the TP removal efficiency by its application prior to chemical treatment.

### Metabolism of microorganism in anaerobic and aerobic phase

Shehab *et al.* (1996) reported that the recommended MLSS for P-removal should be maintained at between 1,500 and

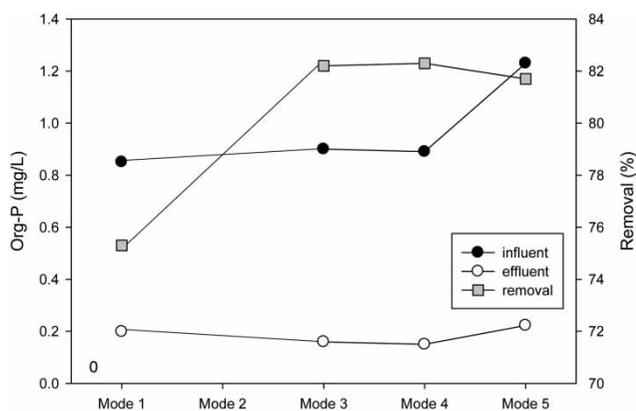


Figure 5 | Relationship between concentration of org-P and the removal efficiency in each mode.

1,700 mg/L. At the pilot plant, most MLSS in each mode was more than 1,700 mg/L. This system is suitable for use with microorganisms such as GAOs and PAOs.

Table 3 presents an average SPRR and SPUR for each mode. At Mode 1 without an external carbon source, both SPRR (specific phosphorus removal rate) and SPUR (specific phosphorus uptake rate) were low. As a result of injecting the external carbon sources, both SPRR and SPUR were increased, regardless of the kind of external carbon source which was used. The level of SPUR was higher than that of SPRR and the removal efficiency of TP was also increased. It seems that microorganisms became active under a rich carbon source.

The patterns of PHA are shown in Figure 6. In the high temperature condition, the quantity of PHB and PHV

increased five times, compared to those of the low temperature condition. Also, the concentration of TP effluent increased about four times. As the P-uptake did not work well in high temperatures, it was interpreted that the amount of PHA was increased. It can be inferred that GAO achieved a dominant position compared to PAO in the EBPR process. A higher PHA degradation led to higher SOP (soluble ortho-phosphate) uptake but did not correspond to greater SOP removal. The reason was that SOP removal was determined by SOP transformation. Moreover, SOP release was related to wastewater acetate and propionate content and SOP uptake was related to both PHA degradation and SOP release. A study of PHA according to the injection of an external carbon source is currently under way.

Table 3 | An average SPRR and SPUR in each mode

Mode	SPRR (mg $\Delta$ PO <sub>4</sub> -P/g MLVSS · d)	SPUR (mg $\Delta$ PO <sub>4</sub> -P/g MLVSS · d)	TP removal (%)
Mode 1	12.6	15.8	40.6
Mode 2	56.1	66.4	79.6
Mode 3	49.3	52.3	82.8
Mode 4	35.4	51.5	90.8
Mode 5	55.3	65.8	75.9

## CONCLUSIONS

The removal efficiency of TP was good at low temperature, but lower at high temperature in the EBPR process. When the EBPR process was operated with an external carbon source, the removal efficiency of TP increased, compared to that without an external carbon source, and the concentration of TP in the effluent was relatively stable despite temperature variation. The maximum removal efficiency in

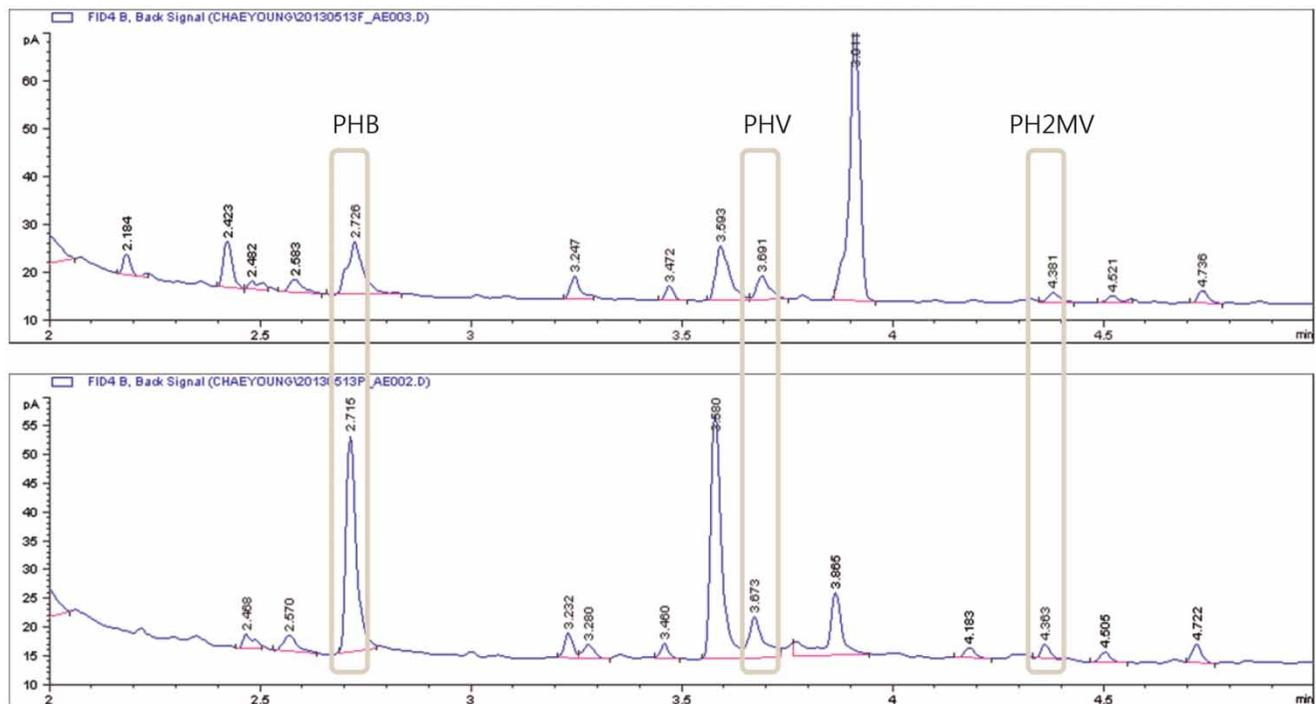


Figure 6 | Contents of PHA vs temperature in the aerobic phase (top: low temperature, bottom: high temperature).

effluent was 95.3%. In addition, org-P, which is difficult to remove by chemical treatment, was removed to a considerably higher degree than that. The resultant effluent concentration was below 0.2 mg/L in spite of the high temperature. With the addition of a rich carbon source, SPRR and SPUR were increased and the volume of PHA showed a marked difference. The BPR process can use waste sludge as the resource through excess P-uptake. Therefore, this process is advantageous in terms of resources recycling of P.

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