RESEARCH ARTICLE

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Effect of Temperature Shock on Activities of Phosphorus-accumulating Organisms

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ABSTRACT: Four anaerobic-aerobic sequencing batch reactors with 9-day of sludge age were operated under a 12-hour cycle of 5, 290, 360, 60, and 5 minutes for the filling, anaerobic, aerobic, settling, and withdrawal periods, respectively. The results indicated that increasing temperature from the phosphate-accumulating organisms (PAOs) preferred temperature of 20°C by either pulse (5°C after every 5 days) or step (1°C per day) manner had negative impacts on the PAOs' activities, particularly on the aerobic phosphorus uptake. However, the pulse-increase scenario, especially from 30 to 35°C, caused more severe impacts than the step-increase scenario. On the contrary, the shock in a decreasing manner regardless of experimental scenarios gave positive impacts on the PAO's activities in both anaerobic phosphorus release and aerobic phosphorus uptake. Hence, operators of the enhanced biological phosphorus removal (EBPR) plants should be cautious when the weather becomes abruptly warmer due to seasonal variation or heat wave. Certain actions including external carbon supplement, sludge age alteration, or other phosphorus removal alternatives may be needed in order to maintain the performance of the EBPR system.

KEYWORDS: phosphate-accumulating organisms, PAOs, biological phosphorus removal, temperature effect, sequencing batch reactor.

INTRODUCTION

An enhanced biological phosphorus removal (EBPR) process is currently one of the most economical and practical ways to reduce high phosphorus content in wastewaters. Under sequential conditions of anaerobic and aerobic environments, the phosphate-accumulating organisms (PAOs), which are able to accumulate intracellular phosphorus in a much higher quantity than other microorganisms, become predominant. In anaerobic stage, the PAOs absorb readily biodegradable substrate by destroying their storage polyphosphate to form adenosine triphosphate (ATP) and adenosine diphosphate (ADP), sequentially, and releasing free phosphate to the bulk solution. The substrate taken up is metabolized in the following aerobic stage to produce energy some of which replenishes the polyphosphate storage pool by absorbing phosphate from the bulk solution. The amount taken up aerobically is more than that released anaerobically; hence the phosphorus in the liquid phase is reduced. Although many researchers have recently attempted to investigate the ecology and microbiology of the EBPR processes using either culture-dependent or –independent approaches, the results are still inconclusive.[1] In parallel to microbiologial approach, several investigators have tried to solve this problem through biochemical models which are developed based on mechanistic considerations of the mixed cultures in activated sludge under control conditions. These models, e.g., Comeau model,[2] Mino model,[3] metabolic model,[4-6] and enhanced culture kinetic model,[7-9] involve stoichiometry and process kinetic rate equations. Many EBPR systems have been designed and constructed by using the simplified versions of these models. Although most of them perform reliably as expected, some behave unpredictably. One possible explanation of most interest to the researchers is that, under certain operating conditions, other microorganisms are more selectively favored than the PAOs, hence diminishing the EBPR performance. The microbial group believed to be a major competitor of the PAOs are the glycogen-accumulating organisms (GAOs), which also assimilate substrates under anaerobic conditions.[10] As named, this microbial group aerobically synthesizes and stores glycogen as its primary energy source. Since polyphosphate is not involved in substrate assimilation,

there is no anaerobic phosphorus release and aerobic phosphorus uptake; hence, no EBPR. Coexistence of PAOs and GAOs in the EBPR system has been confirmed consistently by several studies.[1,11,12] The operating condition is the main factor which controls and determines the predominant species in the EBPR system.

As mentioned previously, the EBPR plant will perform exceptionally if the conditions are favorable the growth of the PAOs. However, some of these conditions get out of control. Thus, plant operators need to modify their operating conditions to relieve the adverse impacts from those uncontrollable factors in order to maintain the EBPR performance. In field practice, typical EBPR plants may experience a variation in sewage temperature according to season. Therefore, there is a strong need to evaluate the impact of temperature variation on the EBPR process. Many studies on the effects of temperature on the efficiency of the EBPR process have been conducted;[11-17] however, the results are inconsistent, possibly due to differences in thermal-stress scenario. This study concentrates on the impact of temperature shock, which is likely to happen in field practice, on EBPR performance.

Materials and Methods

Four bench-scale anaerobic-aerobic SBRs, as shown in Figure 1, were operated at 20, 25, 30, 35°C, respectively, using a 700-watt heater, a 0.2-watt chiller, a condenser, a ventilation fan, and a thermostat including a two-digit digital thermometer. Each SBR had a working volume of 16.8 liters with the filling to idle volumes of 2:1 and was maintained at 9 days of sludge age. The reactors were operated with two 12-hour cycles per day consisting of 5, 290, 360, 60, and 5 minutes for the filling, anaerobic, aerobic, settling, and withdrawal periods, respectively. The composition of the synthetic wastewater is shown in Table 1. Table 2 describes the microbial populations in each SBR as determined algebraically by Panswad *et al.*[12] using stoichiometric and kinetic data as well as the mass balance equation, with the assumption that substrate uptake in anaerobic stage was due to the PAOs (coupled with phosphorus release) and the GAOs (no phosphorus release), whereas in aerobic stage was solely by ordinary heterotrophs (OHOs). After the steady state had been reached, each vessel was subjected to thermal stress by either pulse- or step-alteration schemes as shown in Figure 2. In the pulse-alteration experiments, the system temperature was abruptly changed by 5°C after every 5 days either increasing from 20 to 35°C (Case A) or decreasing from 35 to 20°C (Case B). On the other hand, in the step-alteration experiments, the temperature was gradually altered at the rate of 1°C per day either increasing from 25 to 35°C (Case C) or decreasing from 30 to 20°C (Case D). The reactor contents at the end of anaerobic

Table 1. Composition of synthetic wastewater.

Substances	Dose (per I liter)
Nutrient broth	80 mg (COD = 80 mg)
CH_3COOH	0.20 ml (COD = 220 mg)
$(NH_2)_2CO$	only enough for cellgrowth*
KH_2PO_4	70 mg (16 mg as P)
$NaHCO_3$	420 mg
$MgSO_4.7H_2O$	2.88 mg
$FeCl_3.6H_2O$	1.5 mg
$CaCl_2$	9.6 mg

*Based on nitrogen in the excess sludge (for nitrification control)

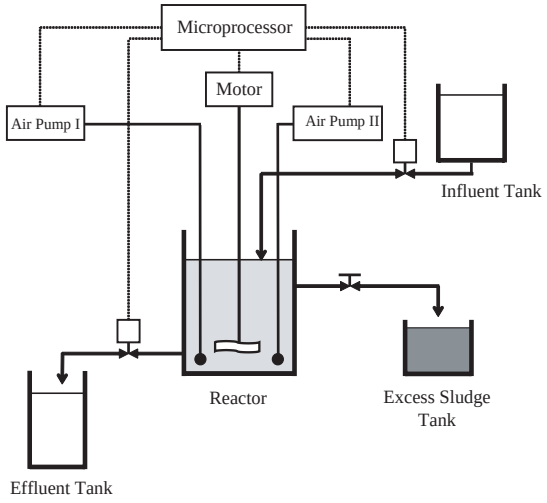

Fig 1. Experimental setup.

Table 2. Mass fraction of microbial population in controlled SBRs at steady state.*

Temperature (°C)	Application	PAOs	GAOs	OHOs
20.0	Case A	0.47-0.70	0.28-0.51	0.02
25.0	Case C	0.33-0.48	0.46-0.61	0.06
30.0	Case D	0.23-0.34	0.64-0.75	0.02
35.0	Case B	0.01-0.02	0.08-0.09	0.90

*Adapted from Panswad *et al.*[12]

Table 3. Results for pulse-increase in temperature (Case A).

Conditions	Temperature(°C)	Anaerobic COD Uptake(mg/l)	Phosphorus (mg/l)		
			Anaerobic Release	Aerobic Uptake	Effluent
1st shock	20	195	60.4	70.1	0.9
	25 (1st cycle)	158	61.7	67.0	8.0
2nd shock	25 (10th cycle)	180	64.9	70.4	6.8
	30 (1st cycle)	135	65.8	69.4	10.3
3rd shock	30 (10th cycle)	104	28.8	19.8	28.7
	35	60	19.6	4.0	38.2

and aerobic stages were monitored daily for pH, DO, temperature, COD, phosphorus, mixed liquor suspended solids, and mixed liquor volatile suspended solids. All analytical procedures were performed according to APHA.[18]

Results and Discussion

Pulse-Alteration in Temperature

The anaerobic phosphorus release changed insignificantly with the pulse-increase in system temperature (Case A) from 20 to 25°C and 25 to 30°C; however, it radically decreased as the temperature changed from 30 to 35°C as shown in Table 3. Since this anaerobic phosphorus release is a distinct characteristic of the PAOs for energy generation from the degradation of intracellular ATP, it implies that, up to the threshold limit of 30°C, an abrupt increase in temperature did not cause any potential impacts on the anaerobic activity of the PAOs. This agrees with the study of Brdjanovic *et al.*[17] which indicated that the stoichiometry of the PAOs' activities under anaerobic condition were insensitive to a short-term temperature change. Associated with the phosphorus release, the COD was taken up; nevertheless, in a decreasing manner as the temperature increased strikingly. Significant retardation of anaerobic COD uptake observed in the experiments should derive

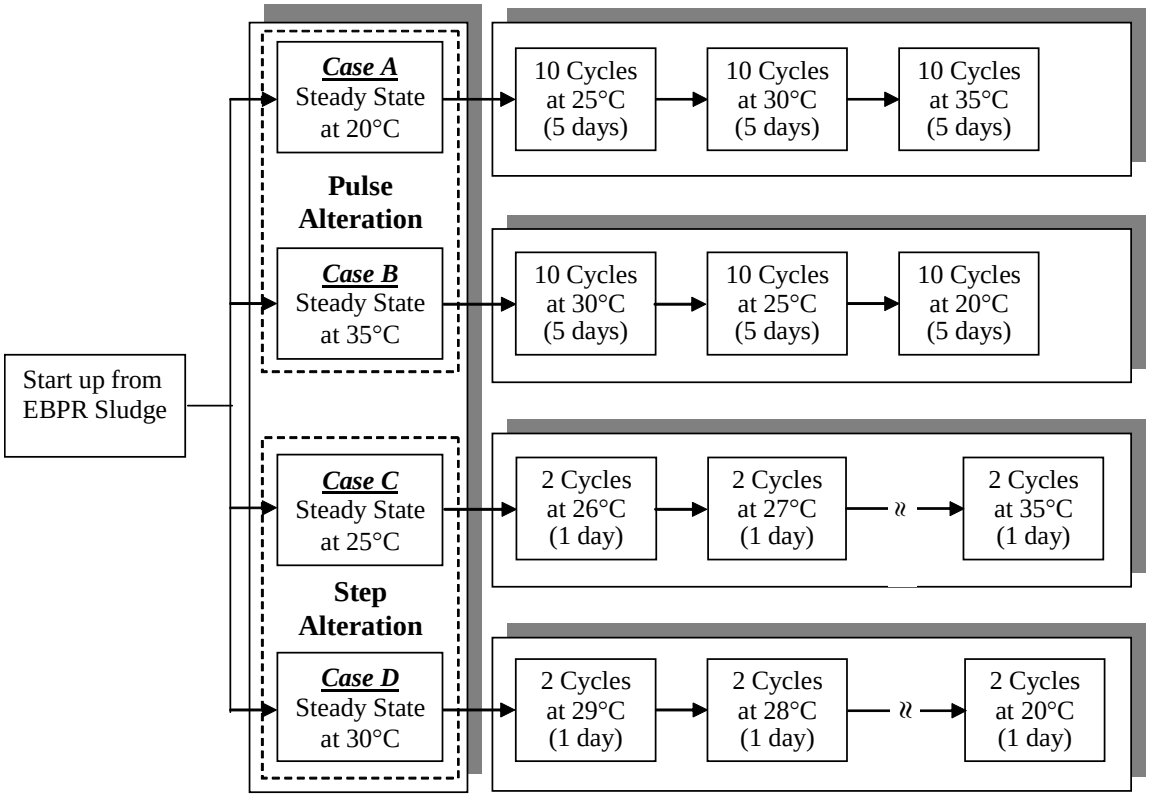

Fig 2. Experimental scheme.

Table 4. Results for pulse-decrease in temperature (Case B).

Conditions	Temperature(°C)	Anaerobic COD uptake (mg/l)	Phosphorus (mg/l)		
			Anaerobic Release	Aerobic Uptake	Effluent
1st shock	35	49	2.0	4.2	12.4
	30 (1st cycle)	61	3.0	7.1	11.4
2nd shock	30 (10th cycle)	48	4.5	8.6	9.3
	25 (1st cycle)	90	5.9	11.3	7.1
3rd shock	25 (10th cycle)	62	6.7	12.4	6.5
	20	144	12.2	19.1	4.8

from an inactivation of the GAOs which are also assimilating substrates anaerobically. This implies that the GAOs are more sensitive to a pulse increase in temperature than the PAOs. The reductions in both aerobic phosphorus uptake and intracellular phosphorus content as the temperature increased indicate that lesser portion of the metabolized energy is available for replenishing the PAOs' polyphosphate storage pool. In other words, the PAOs required more energy for cell maintenance under the abruptly temperature-increasing situation. As the temperature abruptly increased from 30 to 35°C, anaerobic phosphorus release, anaerobic COD uptake, and aerobic phosphorus uptake declined dramatically, i.e., 32, 80, and 42% reduction, respectively, indicating an inactivation of the PAOs under this high temperature-shock condition.

As the system was subjected to sudden decrease in temperature, i.e., from 35 to 30°C, 30 to 25°C, and 25 to 20°C (Case B), the anaerobic phosphorus release increased, coupled with an increase in aerobic phosphorus uptake as shown in Table 4. It is essential to point out that the values of anaerobic phosphorus release under this circumstance were much lower than typical figures for the EBPR system. This was because the initial mixed liquor used in this part and obtained from the steady-state study at 35°C of Panswad *et al.*[12] was very poor EBPR sludge containing only 1 to 2% of the PAOs (Table 2). The results indicate that the temperature shock in a decreasing manner toward 20°C does not have any harmful impacts on the PAOs but instead enhances their activities. The increasing intracellular phosphorus content from 0.03 to 0.10 mg P/mg MLVSS with the decreasing temperature from 35 to 20°C, showed that 20°C was nearer the optimum temperature for the PAOs, in agreement with the studies of Whang and Park[11] and Panswad *et al.*[12] The anaerobic COD uptake, resulting from the activities of both PAOs and GAOs, showed an increasing trend as the temperature dropped sharply. Since the increasing portions in anaerobic COD uptake was higher than those of anaerobic phosphorus release, it implies that the GAOs are more sensitive to temperature drop than the PAOs.

In conclusion, the PAOs were capable, to some extents, to tolerate an acute temperature increase within 20 to 30°C; however, they were unable to maintain their activities under shock from 30 to 35°C. On the other hand, as the temperature shock decreased toward their preference of 20°C, the consequences on the PAOs were positive, i.e., enhancing their activities in both anaerobic phosphorus release and aerobic phosphorus uptake.

Step-Alteration in Temperature

As the system temperature was gradually increased by 1°C per day from 25 to 35°C (Case C), the anaerobic phosphorus release increased slightly, despite of steady aerobic phosphorus uptake as illustrated in Table 5. This indicated that the PAOs consumed more substrate and, thus, generated more energy from metabolism. Nevertheless, they utilized more energy for cell maintenance under moderate temperature and left lesser available energy for replenishing their polyphosphate storage pool as indicated by a decreasing tendency in the P_{uptake}:$P_{release}$ ratio from 1.14 to 1.01

Table 5. Results for step-increase in temperature (Case C).

Temperature(°C)	Anaerobic COD uptake (mg/l)	Phosphorus (mg/l)		
		Anaerobic Release	Aerobic Uptake	Effluent
25	210	42.7	48.8	6.0
30	181	46.3	49.8	10.4
35	160	51.5	52.2	13.7

Table 6. Results for step-decrease in temperature (Case D).

Temperature(°C)	Anaerobic COD uptake (mg/l)	Phosphorus (mg/l)		
		Anaerobic Release	Aerobic Uptake	Effluent
30	199	33.1	36.6	9.9
25	197	39.4	44.7	7.6
20	192	47.8	55.5	4.2

as well as in the intracellular phosphorus content from 0.10 to 0.06 mg P/mg MLVSS as the temperature rose from 25 to 35°C. As a consequence, within a certain period, the PAOs would eventually exhaust all their energy resource and be unable to compete with other existing microorganisms and finally would diminish from the system. This is consistent with the steady-state study at the 35°C that showed that the PAOs accounted for only 1 to 2% of the total microbial mass in the system.[12]

Under the reverse scenario (Case D), the anaerobic phosphorus release still increased as the temperature gradually decreased from 30 to 20°C (Table 6); however as opposed to previous scenario, the P_{uptake}:$P_{release}$ ratio increased from 1.10 at 30°C to 1.16 at 20°C. The anaerobic COD uptake was still comparable during the transition period. It is important to mention that the initial sludge was taken from the 30°C steady-state study of Panswad *et al.*[12] which was dominated by the GAOs (Table 2). Hence, it can be interpreted that, as the temperature decreased towards the PAOs preference, the PAOs became more active and more competitive than the GAOs in substrate rivalry. The energy obtained from aerobic metabolism was a surplus; so the PAOs absorbed more phosphorus from the bulk solution to fulfill their polyphosphate storage pool. An increased accumulation of intracellular phosphorus content from 0.08 at 30°C to 0.11 mg P/mg MLVSS at 20°C within 10 days strongly supported this hypothesis.

Conclusion

Temperature shock in an increasing manner from 20°C, either in pulse or step scenarios, caused negative impact on the activities of the PAOs particularly on the aerobic phosphorus uptake. The pulse-increase from 30 to 35°C had more severe effects than the step-increase scenario. Temperature shock in a decreasing manner toward 20°C in both pulse and step scenarios had positive impacts on the PAOs' activities. The anaerobic phosphorus release and aerobic phosphorus uptake dramatically increased leading to lower effluent phosphorus. For field practice, EBPR-plant operators should pay more attention to when the weather becomes warmer or when there is a heat wave moving toward the area. Certain actions such as supplement of external carbon source, alteration of sludge age, or other phosphorus removal alternatives, may also be needed in order to maintain effluent phosphorus below the standard value.

Acknowledgements

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