

Temperature Effects on Zn(II) Toxicity to Metabolisms of Polyphosphate Accumulating Organisms in Aerobic and Anaerobic Conditions

Tsai YP*, Lu MS, Lin JW, Chou CC and Hu LY

Department of Civil Engineering, National Chi-Nan University, Taiwan, Republic of China

Abstract

This study explores the influence of temperature on the tolerance of polyphosphate accumulating organisms (PAO_s) to Zn(II) in enhanced biological phosphorus removal. The results show anaerobic and aerobic metabolisms of PAO_s decreased with increasing Zn(II) concentration varying between 0-2 mg L⁻¹ and temperature indeed affected inhibitive degrees. Furthermore, Zn(II) is more toxic to anaerobic poly-P hydrolysis and glycogen degradation at 10°C than at 20 and 30°C. For anaerobic polyhydroxyalkanoate (PHA) synthesis, Zn(II) had a highly inhibitive effect at 10°C too. The inhibitions of PAO_s aerobically taking up phosphorus, degrading PHA and replenishing glycogen in the presence of Zn(II) were amplified at 10°C. The metabolism of aerobic PHA degradation at 10°C was completely terminated and the aerobic replenishment of glycogen at 10°C was also completely terminated at 0.1 mg L⁻¹ of Zn(II) due to the complete inhibition of aerobic PHA metabolism, which provides the required reducing power for synthesizing glycogen.

Keywords: Polyhydroxyalkanoates (PHAs); Zinc; Phosphorus accumulating organisms (PAO_s); Temperature; Phosphorus removal

Introduction

Though enhanced biological phosphorus removal (EBPR) is a standard technology for removing phosphorus from wastewater, the responsible organisms and relevant mechanisms are complex and not well-understood [1]. Substrate type has been identified as an important factor affecting the competition between polyphosphate accumulating organisms (PAO_s) and glycogen accumulating organisms (GAO_s), which are usually dominant in a failed EBPR [2]. In addition, temperature is also considered an important factor in practice governing the efficiency of phosphate removal in EBPR because the population competition between PAO_s and GAO_s is significantly Ref. [3] affected by water temperature. Ref. [4] found that the predominant microbial population changed from PAO_s to GAO_s when temperature gradually rose from 20°C to 35°C. Similarly [5] found PAO_s were the dominant microorganisms at low temperature (10°C), regardless of the influent carbon source or pH. However [6] found conflicting results. Heavy metal ions have also been reported to be able to inhibit biological reactions responsible for carbon, nitrogen and phosphate removals. Ref. [7] found that the presence of Cd (II) significantly changed the structure of microbial population and was toxic to the bacterial community in nutrient removal sludge, especially to the bacteria in the *Beta proteobacteria*. Monitoring of a full-scale advanced municipal wastewater treatment plant showed the P removal efficiency decreased dramatically after tin (Sn) levels in the solids fraction of the mixed liquid suspended solids (MLSS) exceeded 4 µgSn L⁻¹ [8]. A similar study by [9] showed 2 mg L⁻¹ of Cd(II) took the lead in affecting biological phosphate removal. At the level of 5 mg L⁻¹ of Cd (II), the total nitrogen removal and nitrification efficiency substantially declined and the denitrification rate was inhibited by about 61%. At the same time, the inhibition percentages of anaerobic release, anoxic and aerobic uptake rates of phosphate were about 76, 64, and 90%, respectively Ref. [10] further conducted a series of batch experiments to identify the toxic effects of Pb(II), Ni(II) and Cd(II) on nitrification and denitrification for nutrient removal sludges. Although both the inhibition kinetics of Ni(II) and Cd(II) followed first-order models, no significant inhibition to nitrification was observed for Pb(II) case, even when the Pb (II) concentration was as high as 40 mg L⁻¹. In contrast, 80% inhibition of nitrification was observed when Ni (II) or Cd (II) concentration was higher than 5 or 25 mg L⁻¹, respectively. No synergistic effect upon adding different heavy metals at one time was observed for nitrification. However, a significant synergistic effect was found for denitrification.

A similar study was conducted by [11] who investigated the toxic effects of Cu (II), Zn(II) and Cr(VI) on nitrification and denitrification in sequencing batch reactor (SBR) sludge. Ref. [12] also found the nitrifiers in SBR sludge could withstand more metal ion toxicity than those in anaerobic-anoxic-oxic (A₂O) sludge.

The mechanisms of biological phosphorus removal are very complex, especially for the competition of PAO_s and GAO_s. Many factors can potentially affect this competition, including pH, temperature, carbon sources, metal ions, sludge retention time (SRT) etc. The influences of those factors on phosphorus removal are probably not independent. Interaction might exist between one factor and the others, for example, the toxic metal effect is possibly subject to pH, temperature, SRT, and carbon source etc. Ref. [13] investigated the shock load effect of Cu (II) on PAO_s behaviors with respect to the transformations of poly-P, intracellular polyhydroxyalkanoates (PHA_s), and glycogen. They found Cu (II) inhibitory effect was reduced by increasing the pH to 7.6, revealing that pH was able to influence the endurance of PAO_s biomass to Cu (II) and there was an interaction between pH and toxic effect. Ref. [14] further experimentally demonstrated that PAO_s loss of the ability to anaerobically synthesize PHA_s and to aerobically take up phosphate under the presence of Cu (II) was due to inhibition of the enzyme activities of acetyl-CoA synthases (ACS) and of polyphosphate kinase (PPK), respectively. The apparent maximum specific activity (V_{max}) of ACS decreased with increasing Cu (II) concentration, revealing that Cu (II) has a higher affinity for free ACS than for ACS-coenzyme A complex. A similar result Ref. [15] was found in PPK activity tests, showing that Cu (II) more easily bound to free PPK than to PPK-Adenosine triphosphate complex.

Investigated the effect of Pb (II) on metabolisms of PAO_s and GAO_s fed with acetic acid or with glucose, respectively, as their sole carbon

*Corresponding author: Yung-Pin Tsai, Department of Civil Engineering, National Chi-Nan University, Puli 545, Taiwan, Republic of China, Tel: 886492918230; Fax: 886492918386; E-mail: yptsai@ncnu.edu.tw

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source. Results from trials with a high concentration of acetic acid showed there was an abnormal aerobic phosphate release. Ref. [16] found that the effect of Cu (II) on phosphate removal was significant and the SRT was an important factor influencing the endurance of PAOs to Cu (II). The PHA storage in anaerobic stage for the sludge at 10d SRT was less influenced by Cu (II) than storage at 5d or 15d SRT_s. The reaction of PAO_s to aerobically take up phosphate for the sludge at 5d or 15d SRT almost ceased at 2 mg Cu L⁻¹, whereas PAO_s in the sludge at 10d SRT retained half of their ability to take up phosphate.

To date, however, little is known about how water temperature affects the endurance of PAOs biomass to metal ion toxicity. Therefore, this study examines the shock load effect of heavy metal (Zn²⁺) on the behavior of PAOs biomass in activated sludges at different temperature levels (10, 20 and 30°C) with respect to the anaerobic phosphate release, the aerobic/anoxic phosphate uptake, the transformations of poly-P and intracellular PHA_s, as well as the utilization and replenishment of glycogen.

Materials and Methods

SBR pilot-plant

An SBR pilot-plant was used to chronically acclimatize the activated sludge without the addition of metal ions. The system had a total reactor volume of 160 L, base volume of 80 L, and effective volume of 148.5 L. It was operated at 2 cycles per day with 12 hours per cycle. The phases and durations in a cycle followed the sequence: anaerobic 150 min, aerobic 330 min, anoxic 180 min, re-aeration 10 min, sedimentation 30 min, and decantation 20 min. The SBR system was fed with a synthetic wastewater containing milk, KH₂PO₄, Urea, FeCl₃, CH₃COOH, Glucose, and NH₄Cl. The pH of the synthetic wastewater was adjusted to 6.8-7.2 with 6N NaOH. The pH in the aerobic phase of SBR system was automatically maintained at 7.2 ± 0.2 by adding a NaOH/NaHCO₃ solution, and the dissolved oxygen (DO) concentration in the aerobic phase was also controlled at 2.0 ± 0.3mgL⁻¹. The SBR system was acclimatized at the condition of 10d SRT. The batch experiments described below were carried out when the SBR system had reached a steady state condition. Other conditions are described in Ref. [15].

Batch experiments

The batch test was carried out in a 2 L acrylic, magnetically stirred reaction vessel. The sludge for the batch test was taken from the SBR system at the end of the aerobic phase. Before starting a batch test, the sludge was pre-treated according to Ref. [15]. A required amount of ZnCl₂ solution was then added into the batch reactor and the pH was adjusted to 7.5. The concentration of acetate in the batch reactor equaled 100 mg L⁻¹ as chemical oxygen demand (COD). The mixed liquid in

the batch reactor bubbling with nitrogen gas was then anaerobically incubated at a constant temperature in a water-bath tank for 120 min. Later, the mixed liquid was aerated for 240 min to examine the aerobic phosphate uptake of PAO_s.

Analytical methods

The liquid samples taken from the batch reactor were immediately filtered through Millipore filter (0.45 μm pore size) and then COD, total phosphorus (TP), PO₄³⁻-P, MLSS and mixed liquor volatile suspended solid (MLVSS) were analyzed in accordance with Standard Methods [17]. The PHA concentration was measured according to [18]. The intracellular glycogen was analyzed using the method described in [19]. The concentration of Zn²⁺ was analyzed by an Atomic Absorption Spectrometry device (Shimadzu AA-6200), and the averaged value of duplicate analysis was used in this study.

Data analysis

To compare variation in treatments, analysis of variance (ANOVA) F-test was applied to test whether the temperature, zinc concentration and their interaction significantly influenced the metabolisms of PAO_s in the anaerobic and aerobic stages. If significant factors (e.g., temperature and/or zinc concentration) were found, then the least significant difference test (LSD) was further applied to establish which particular levels of a factor were different. A paired sample *t*-test was also used to determine if means of interested variables were not statistically equal. All tests were performed at a significance level (*p*-value) of 0.05, using standard statistical software (SPSS release 10.1.0, SPSS Inc., Chicago, IL).

Results and Discussion

The average water qualities and operational conditions of the SBR system under steady state conditions are shown in Table 1. The removal rates of phosphate and total phosphorus were 99% and 98%, respectively. The data show the sludge used in batch tests was good with high carbon, nitrogen, and phosphate removal efficiencies. Before batch tests, a full cycle examination of the SBR system was conducted and the result showed the sludge in the SBR system could release 30 mgL⁻¹ of total phosphorus after 150 minutes in the anaerobic stage, indicating the predominant population of sludge in SBR system was PAO_s, rather than GAO_s. The 4, 6-diamidino-2-phenylindole (DAPI) method was also used to reconfirm this issue in the study (data not shown).

Background study - temperature effects on PAO metabolisms in the absence of Zn(II)

To understand the effect of temperature on PAO metabolisms in the absence of Zn(II), two to four times experiments were conducted as

| Water Quality Parameters | Influent (mgL ⁻¹) | Effluent (mgL ⁻¹) | Removal (%) | Operational Parameters |
|--|-------------------------------|-------------------------------|-------------|------------------------|
| Total COD | 357 ± 30.5 | 17.5 ± 6.62 | 95 | |
| Soluble COD | 309 ± 25.3 | 9.68 ± 5.23 | 96 | |
| Total P | 6.46 ± 0.44 | 0.13 ± 0.1 | 98 | |
| PO ₄ ³⁻ -P | 6.20 ± 0.36 | 0.09 ± 0.25 | 99 | |
| Total nitrogen | 51.8 ± 14. | 11.9 ± 1.2 | 74 | |
| NH ⁴⁺ -N | 30.0 ± 6.03 | ND | 100 | |
| NO _x -N | 2.39 ± 0.86 | 11.5 ± 0.83 | | |
| Suspended solids | | 8.39 ± 3.28 | | |
| MLSS (mgL) | | | | 1366 ± 59 |
| SRT (d) | | | | 10 |
| MLVSS (mgL ⁻¹) | | | | 1151 ± 53 |
| Organic loading (g COD g ⁻¹ VSS d ⁻¹) | | | | 0.29 ± 0.03 |

Table 1: Influent and effluent water qualities of the SBR pilot-scale system at steady state (n>8).

background study. The averages and standard deviations of these data were further used to statistically compare their differences.

PAO anaerobic metabolisms in the absence of Zn(II): The experimental results in Figure 1(a) show the anaerobic specific substrate utilization rates (SSUR) of PAOs at the temperatures of 10, 20, and 30°C were 17.7 ± 1.2 , 19.7 ± 6.2 , and 23.8 ± 3.6 mgCOD g⁻¹VSS h⁻¹, respectively. Statistical analysis showed the temperature effect on anaerobic substrate utilization of microorganisms was insignificant ($p=0.494$).

Figure 1(a) also shows the total phosphorus releases (TPR_s) and the specific phosphorus release rates (SPRRs) for PAOs at different temperature conditions. The PAOs released 7.8 ± 0.2 , 10.6 ± 0.2 , and 11.2 ± 1.6 mg L⁻¹ of phosphorus at 10, 20, and 30°C, respectively. The corresponding SPRRs were 3.5 ± 0.4 , 4.5 ± 0.5 , and 5.2 ± 0.1 mgP g⁻¹VSS h⁻¹. Statistical analysis showed temperature variation significantly affected anaerobic TPRs and SPRRs ($P=0.01$ and 0.002 , respectively) and the metabolic activity of PAO_s releasing phosphorus in anaerobic condition increased with increasing temperature.

From an energy viewpoint, PAO_s are primarily characterized by their ability to anaerobically take up organic substrates and store them by utilizing energy from the hydrolysis of stored poly-P without consuming any electron acceptors. When organic substrates are taken up anaerobically, they are usually converted to PHA and stored. The anaerobic conversion of acetate to PHA requires a reducing power because PHA is a more reduced compound than acetate. Only 30% of the reducing power is supplied by the citric acid (TCA) cycle, which functions under anaerobic conditions to oxidize a portion of the acetate to CO₂ and generate the reducing power in the form of nicotinamide adenine dinucleotide hydrogen (NADH). The remaining 70% of the reducing power is supplied by anaerobic degradation of intracellularly stored glycogen to acetyl-coA, together with its partial oxidation to CO₂ [20]. The metabolic function of glycogen degradation, therefore, appears to be essential for anaerobic substrate uptake, PHA synthesis, and PAO proliferation in the EBPR process [21].

In Figure 1(a) it shows the anaerobic PHAs synthesis rates at 10, 20, and 30°C were 7.9 ± 1.9 , 8.8 ± 1.6 , and 10.0 ± 1.9 mg g⁻¹VSS h⁻¹, respectively. Figure 1(a) also shows the anaerobic glycogen degradation rates at 10, 20, and 30°C were 3.3 ± 0.7 , 4.5 ± 0.3 , and 4.9 ± 1.4 mg g⁻¹VSS h⁻¹, respectively. Statistical analysis shows the temperature effects on both rates were insignificant ($P=0.40$ and 0.21 , respectively). The results are similar to that of SSUR, indicating that the anaerobic

metabolisms of PAO_s taking up substrate and consuming glycogen to supply reducing power to convert acetate to PHA are not statistically influenced by temperature variation.

PAO aerobic metabolisms in the absence of Zn(II): In Figure 1(b) it shows that the total phosphorus uptakes (TPU_s) and the specific phosphorus uptake rates (SPUR_s) for PAOs at different temperature conditions. PAOs aerobically took up 3.2 ± 0.9 , 9.2 ± 0.8 and 10.6 ± 2.3 mg L⁻¹ of phosphorus at 10, 20, and 30°C, respectively. The corresponding SPURs were 0.7 ± 0.1 , 2.0 ± 0.2 , and 2.4 ± 0.2 mgP g⁻¹VSS h⁻¹. Statistical analysis indicates temperature variation significantly affected aerobic TPU_s and SPUR_s ($P<0.001$ and <0.001 , respectively) and the aerobic TPU_s and SPUR_s increased with increasing temperature.

In Figure 1(b) it also shows the PHA_s energy consumption and glycogen replenishment at different temperature conditions. The PHA degradation rates were -1.3 ± 0.4 , 1.2 ± 0.3 , and 3.3 ± 0.6 mg g⁻¹VSS h⁻¹ for 10, 20, and 30°C, respectively. At the same time, the glycogen replenishment rates were 0.2 ± 0.6 , 2.6 ± 0.7 , and 4.0 ± 0.6 mg g⁻¹VSS h⁻¹ for 10, 20, and 30°C, respectively. It is noticeable that PAO_s might have been unable to use PHA_s as a carbon source when the temperature was as low as 10°C. At the same time, the mechanism for PAO_s aerobically replenishing glycogen was also inhibited due to the low temperature. Both PHA degradation rates and glycogen synthesis rates in the aerobic stage were significantly influenced by temperature variation in that both rates increased with increasing temperature ($p<0.001$).

Temperature effects on the endurance of PAO_s to Zn(II) toxicity: To understand how the temperature variation influences Zn(II) toxicity to PAO metabolisms, ZnCl₂ solution was added to the batch reactor at different temperature conditions. The inhibition kinetic concept proposed in [8] was used to compare Zn(II) toxic intensity according to inhibition constants (r_i) which are shown in the regression equation in each graph. The larger the constant value is, the larger the metabolic inhibition.

Anaerobic metabolisms of PAO_s in the presence of Zn(II)

In Figure 2(a) it shows the results of microorganisms anaerobically taking up substrate at different temperature conditions in the presence of Zn (II). At 10°C, the SSUR_s of microorganisms at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are 17.7, 16.9, 11.9, 11.6, 9.9, and 6.4 mgCOD g⁻¹VSS h⁻¹, respectively. At 20°C, the corresponding SSURs are 23.7, 21.3, 19.4, 18.4, 17.8, and 15.2 mgCOD g⁻¹VSS h⁻¹. At 30°C, the corresponding SSUR are 28.0, 21.3, 18.4, 11.8, 11.8, and 10.0 mgCOD g⁻¹VSS h⁻¹. The SSUR for microorganisms at anaerobic

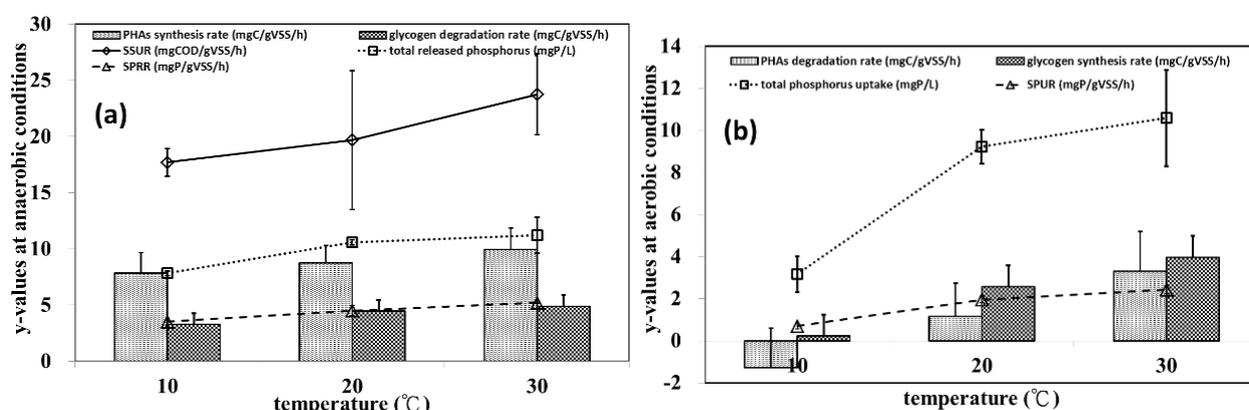
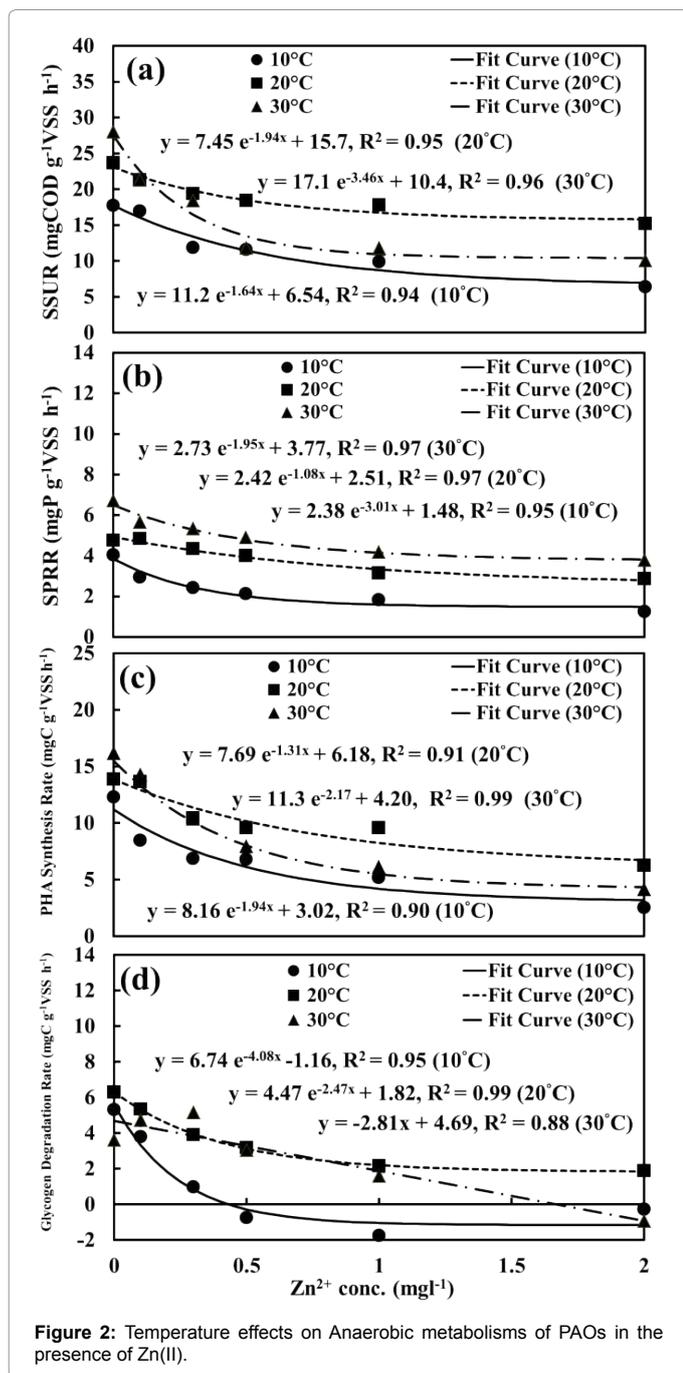


Figure 1: Temperature effects on (a) Anaerobic, and (b) Aerobic metabolisms of PAOs in the absence of Zn(II).



conditions decreased with increasing Zn(II) concentration, regardless of temperature. The inhibition constants (r_i) at temperatures 10, 20, and 30°C are 1.64, 1.94, and 3.46 L mg⁻¹, respectively, indicating Zn(II) is more toxic to substrate uptake of microorganisms at higher temperature condition (30°C) than at lower conditions (10 and 20°C).

In Figure 2(b) it shows the results of PAOs anaerobically releasing phosphorus at different temperature conditions in the presence of Zn(II). At 10°C, the SPRRs of PAOs at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are 4.0, 3.0, 2.4, 2.1, 1.8, and 1.3 mgP g⁻¹VSS h⁻¹, respectively. At 20°C, the corresponding SPRRs are 4.8, 4.8, 4.3, 4.0, 3.1, and 2.9 mgP g⁻¹VSS h⁻¹. At 30°C, the corresponding SPRRs are 6.7, 5.7, 5.3, 4.9, 4.2, and 3.8 mgP g⁻¹VSS h⁻¹. It can be seen that the hydrolysis of stored poly-P in sludge at anaerobic conditions decreased with increasing

Zn(II) concentration, regardless of temperature. The inhibition constants at temperatures 10, 20, and 30°C were 3.01, 1.08, and 1.95 L mg⁻¹, respectively, indicating Zn(II) is more toxic to poly-P hydrolysis at 10°C than at 20 or 30°C.

In Figure 2(c) it shows the results of PAOs anaerobically synthesizing PHAs at different temperature conditions. At 10°C, the PHA synthesis rates of PAOs at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 12.3, 8.5, 6.8, 6.8, 5.2, and 2.5 mg g⁻¹VSS h⁻¹, respectively. At 20°C, the PHA synthesis rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 13.9, 13.7, 10.4, 9.6, 9.6, and 6.2 mg g⁻¹VSS h⁻¹, respectively. At 30°C, the PHA synthesis rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 16.2, 14.2, 10.4, 7.9, 6.1, and 4.1 mg g⁻¹VSS h⁻¹, respectively. It can be seen that the PHA synthesis rates for PAOs at anaerobic conditions decreased with increasing Zn(II) concentration, regardless of temperature. The inhibition constants at temperature 10, 20, and 30°C are 1.94, 1.31, and 2.17 L mg⁻¹, respectively, indicating Zn(II) is more toxic to PHA synthesis at 10 and 30°C than at 20°C.

Figure 2(d) shows the results of PAOs anaerobically degrading glycogen for energy requirement at different temperature conditions. At 10°C, the glycogen degradation rates of PAOs at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 5.3, 3.8, 1.0, -0.8, -1.8, and -0.3 mg g⁻¹VSS h⁻¹, respectively. At 20°C, the glycogen degradation rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 6.3, 5.3, 3.9, 3.2, 2.1, and 1.9 mg g⁻¹VSS h⁻¹, respectively. At 30°C, the glycogen degradation rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 3.6, 4.7, 5.1, 3.0, 1.6, and -0.9 mg g⁻¹VSS h⁻¹, respectively. It can be seen that the glycogen consumption rates for PAOs at anaerobic conditions also decreased with increasing Zn(II) concentration, regardless of temperature. The inhibition constants at temperature 10 and 20°C are 4.08 and 2.47 L mg⁻¹, respectively, but the data at 30°C have a better fit with a linear regression instead of the first order model. It is noteworthy that the metabolism of glycogen degradation was completely terminated at 10°C when more than 0.5 mg L⁻¹ of zinc ions was added, indicating Zn(II) is more toxic to the glycogen metabolism at 10°C than at 20 or 30°C.

In this study, both the metabolic rates of anaerobic glycogen degradation and PHAs synthesis were reduced with increasing Zn(II) concentration, revealing that the presence of Zn(II) caused a reduction in the degradation of glycogen for production of NADH, which is essential for PHA synthesis.

Aerobic metabolisms of PAOs in the presence of Zn(II): In Figure 3(a) it shows the results of PAOs aerobically taking up phosphorus at different temperature conditions. At 10°C the SPUR levels of PAOs at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 0.9, 0.4, 0.2, -0.04, -0.2, and -0.2 mgP g⁻¹VSS h⁻¹, respectively. At 20°C, the SPUR levels at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 2.0, 1.8, 1.0, 0.6, 0.3, and -0.2 mgP g⁻¹VSS h⁻¹, respectively. At 30°C, the SPUR levels at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 3.2, 2.5, 1.3, 0.6, 0.1, and -0.4 mgP g⁻¹VSS h⁻¹, respectively. The phosphorus uptake activity for PAOs at aerobic conditions decreased with increasing Zn(II) concentration and was completely inhibited at 2.0 mg L⁻¹ of Zn (II), regardless of temperature. Furthermore, the inhibition by Zn(II) was accelerated at 10°C, since PAOs had completely stopped taking up phosphorus at 0.5 mg L⁻¹ of Zn (II). The inhibition constants at temperatures 10, 20, and 30°C were 3.94, 1.84, and 2.43 L mg⁻¹, respectively, indicating Zn(II) is more toxic to phosphorus uptake by PAOs at 10°C than at 20 or 30°C.

In Figure 3(b) it shows the results of PAOs aerobically consuming PHAs at different temperature conditions. At 10°C, the PHA degradation rates of PAOs at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II)

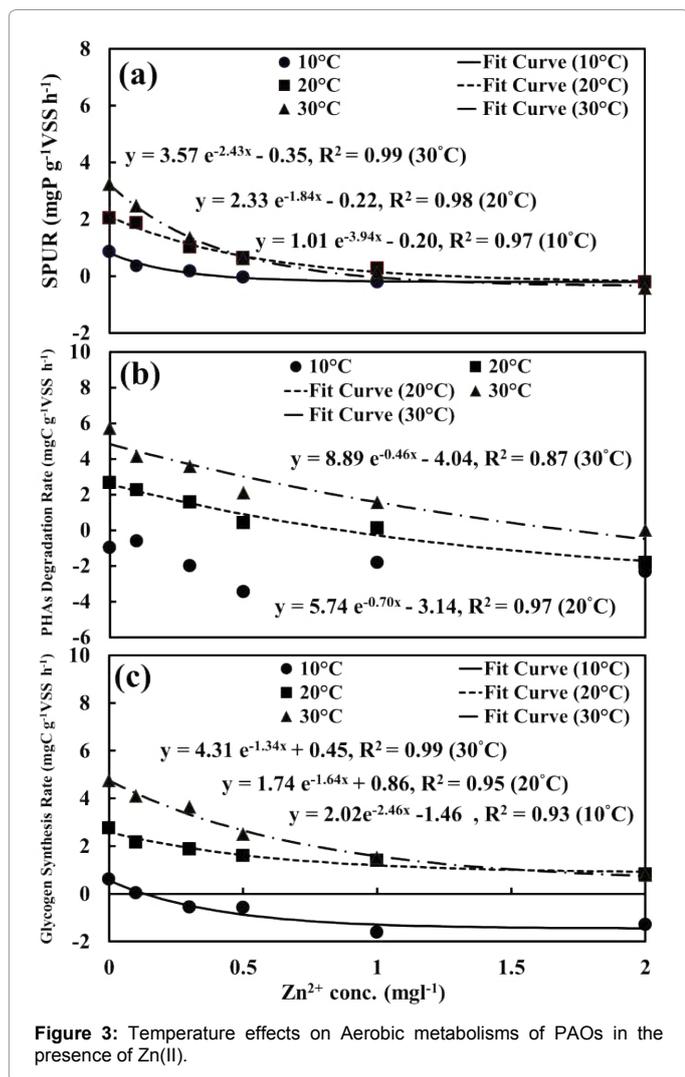


Figure 3: Temperature effects on Aerobic metabolisms of PAOs in the presence of Zn(II).

were -1.0, -0.6, -2.0, -3.4, -1.8, and -2.3 mg g⁻¹VSS h⁻¹, respectively. At 20°C, the PHA degradation rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 2.7, 2.3, 1.6, 0.4, 0.1, and -1.8 mg g⁻¹VSS h⁻¹, respectively. At 30°C, the PHA degradation rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 5.7, 4.1, 3.6, 2.1, 1.6, and 0.02 mg g⁻¹VSS h⁻¹, respectively. The PHA degradation rates for PAO_s at 20 and 30°C decreased with increasing Zn(II) concentration, regardless of temperature. The corresponding inhibition constants were 0.70, and 0.46 L mg⁻¹, respectively, whereas the aerobic PHA metabolism at 10°C completely stopped, regardless of Zn(II) concentration. Moreover, the PHA metabolisms at 20 and 30°C also stopped at 2.0 mg L⁻¹ of Zn (II).

In Figure 3(c) it shows the results of PAO_s aerobically replenishing intracellular glycogen at different temperature conditions. At 10°C, the glycogen synthesis rates of PAO_s at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 0.6, 0.03, -0.6, -0.6, -1.6, and -1.3 mg g⁻¹VSS h⁻¹, respectively. It is noteworthy that the aerobic glycogen metabolism was completely inhibited at 0.1 mg L⁻¹ of Zn(II) due to the entire inhibition of aerobic PHAs metabolism, which provides the required reducing power for synthesizing glycogen. At 20°C, the glycogen synthesis rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 2.8, 2.2, 1.9, 1.6, 1.4, and 0.8 mg g⁻¹VSS h⁻¹, respectively. At 30°C, the glycogen synthesis rates at 0, 0.1, 0.3, 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) were 4.7, 4.1, 3.6, 2.5, 1.5, and 0.8 mg g⁻¹VSS h⁻¹, respectively. The glycogen synthesis rates for PAOs

at aerobic conditions decreased with increasing Zn(II) concentration, regardless of temperature. The inhibition constants at temperatures 10, 20, and 30°C were 2.46, 1.64, and 1.34 L mg⁻¹, respectively, indicating Zn(II) is more toxic to aerobic glycogen metabolism for PAOs at 10°C than at 20 or 30°C.

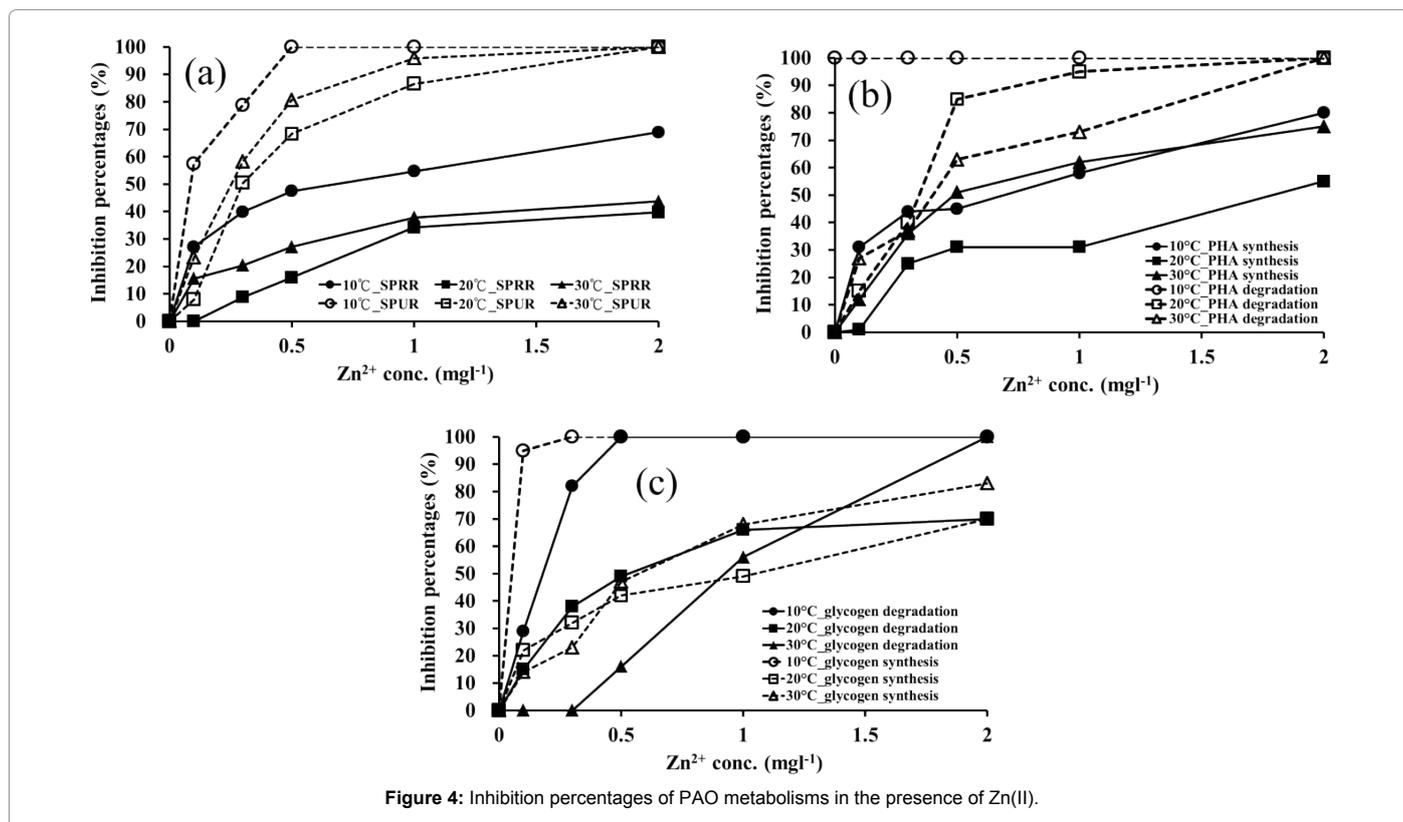
Inhibition percentages for metabolisms of PAOs in the presence of Zn(II): To understand how the Zn(II) toxicity influences PAO_s metabolisms, inhibition percentages at different Zn(II) concentration and temperature conditions were calculated and are shown in Figure 4.

Inhibition percentages of phosphorus metabolisms in the presence of Zn(II): In Figure 4(a) it shows the inhibition percentages of PAO_s anaerobically releasing phosphorus and aerobically taking up phosphorus at different Zn(II) concentrations and temperature conditions. The order of the magnitude of inhibition percentages at different temperature is 10°C >30°C >20°C for both SPRR and SPUR cases. Clearly, Zn(II) toxicity to the PAO activities of releasing and taking up phosphorus was magnified at the low temperature condition of 10°C. Furthermore, the inhibition percentages of SPUR by Zn(II) were significantly larger than those of SPRR, regardless of temperature. For example, regardless of temperature, the inhibition percentage of SPUR_s was nearly 100% at 2 mg L⁻¹ of Zn(II), whereas only 69, 40 and 44% of SPRR inhibition were found at 10, 20 and 30°C, respectively.

Inhibition percentages of PHA metabolisms in the presence of Zn(II): In Figure 4(b) it shows the inhibition percentages of PHA metabolisms at different Zn(II) concentration and temperature conditions. The order of magnitude for inhibition percentages at different temperatures was 10°C >30°C >20°C for the case of aerobic PHA degradation and 10°C ≈ 30°C >20°C for the case of anaerobic PHAs synthesis. This indicates that, similar to phosphorus metabolisms, the 10°C low temperature condition also magnified the toxicity of Zn(II) to PHA metabolisms. Moreover, similar to phosphorus metabolisms, the inhibition percentages of aerobic PHA degradation by Zn(II) were significantly larger than those of anaerobic PHA synthesis, regardless of temperature. It is noteworthy that the metabolism of aerobic PHAs degradation completely stopped at the low temperature condition (10°C), regardless of Zn(II) concentration, whereas completely stopped at 2 mg L⁻¹ of Zn(II) for 20 and 30°C. At the same time, only 80, 55, and 75% of the inhibition of anaerobic PHA synthesis were found at 10, 20 and 30°C, respectively.

Inhibition percentages of glycogen metabolisms in the presence of Zn(II): In Figure 4(c) it shows the inhibition percentages of glycogen metabolisms at different Zn(II) concentration and temperature conditions. The order of the magnitude of inhibition percentages at different temperature is 10°C >30°C >20°C for aerobic glycogen synthesis case and 10°C >20°C >30°C for anaerobic glycogen degradation case. Similar to phosphorus and PHA metabolisms, the 10°C low temperature condition also magnified the toxicity of Zn(II) to glycogen metabolisms. However, unlike phosphorus and PHAs metabolisms, the inhibition percentages of aerobic glycogens synthesis by Zn(II) are not clearly larger than those of anaerobic glycogens degradation. It is noteworthy that only 0.3-0.5 mg L⁻¹ of Zn(II) completely terminated the glycogen metabolisms at low temperature condition (10 °C).

Distinguishing the individual degrees of Zn(II) toxicity to anaerobic and aerobic metabolisms from Figures 4(a) and 4(b), it can be seen that the inhibition percentages by Zn(II) with respect to phosphorus and PHA metabolisms at the aerobic stage were larger than those at the anaerobic stage. This seemingly indicates Zn(II) is more toxic to the aerobic metabolism than anaerobic metabolism. However, it is



difficult to draw an exact conclusion with respect to this issue because Zn(II) was added at the beginning of the anaerobic stage, which was subsequently followed by the aerobic stage in a batch test of this study. Thus an interesting problem arises as to whether or not the toxic effect of Zn(II) that began at the aerobic stage included the toxic effect at the previous anaerobic stage. In other words, does the high metabolic inhibition at aerobic stage come from the direct interference of Zn(II) in aerobic metabolisms? Or, does a partial inhibition come from the indirect interference in previous anaerobic metabolisms linked to subsequent aerobic metabolisms? To verify this issue, we conducted two simultaneous series of batch tests at 20°C, one added Zn(II) at the beginning of the anaerobic stage and the other not until the end of the anaerobic stage (i.e., the beginning of the aerobic stage). We focused on the differences of phosphorus and PHA metabolisms.

Inhibition sources for phosphorus metabolisms: In Figure 5(a) it shows the inhibition percentages of PAO_s anaerobically releasing phosphorus (SPRR) and aerobically taking up phosphorus (SPUR) at different Zn(II) occurring times and concentrations. The difference of inhibition percentages of SPUR_s between Zn(II) initially presented at the anaerobic stage and at the aerobic stage is defined as the inhibition percentage only contributed by Zn(II) toxicity at the anaerobic stage. At the same time, the inhibition percentages of SPUR while Zn(II) initially presented at the aerobic stage is defined as the inhibition percentage only contributed by Zn(II) toxicity at the aerobic stage. The same concept is used for the SPRR, and then the inhibition percentages of SPRR contributed only from Zn(II) toxicity at the anaerobic stage could be obtained. All results are shown in Figure 5(b). The inhibitions of SPRR originated from Zn(II) toxicity at the anaerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are low (10, 5, and 14%, respectively). The inhibitions of SPUR that originated from Zn(II) toxicity at the anaerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) only are 2, 2, and

11%, respectively. However, the inhibitions of SPUR that originated from Zn(II) toxicity at the aerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are 42, 80, and 89%, respectively. Consequently, it is clear the majority of metabolic inhibition (>90% of total inhibitions) for PAOs aerobically taking up phosphorus came from the direct interference of Zn(II) in aerobic metabolisms, regardless of Zn(II) concentration. Only a small amount of the inhibitions (<10% of total inhibitions) came from the indirect interference in previous anaerobic metabolisms linked to subsequent aerobic metabolisms.

Inhibition sources for PHA metabolisms: In Figure 6(a) it shows the inhibition percentages of PAOs anaerobically synthesizing PHAs and aerobically degrading PHAs at different Zn(II) times of occurrence and concentrations. The same calculations for phosphorus metabolisms are applied to PHA metabolisms, and then the inhibition percentages of PHA degradation contributed only by Zn(II) toxicity at the anaerobic and aerobic stages, as well as the inhibition percentages of PHA synthesis contributed only by Zn(II) toxicity at the anaerobic stage could be obtained, as shown in Figure 6(b). Unlike SPRR, the inhibitions of PHA synthesis originated from Zn(II) toxicity at the anaerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are significant (23, 30, and 67%, respectively). The inhibitions of PHA degradation originated from Zn(II) toxicity at the anaerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are 14, 16, and 60%, respectively. And, the inhibitions of PHA degradation originated from Zn(II) toxicity at the aerobic stage for 0.5, 1.0, and 2.0 mg L⁻¹ of Zn(II) are 25, 22, and 38%, respectively. Like phosphorus metabolisms, the majority of metabolic inhibition for PAOs aerobically degrading PHAs came from the direct interference of Zn(II) in aerobic metabolisms when 0.5 or 1.0 mg L⁻¹ of Zn(II) was added (64 and 58% of total inhibitions, respectively). Only less than half inhibitions came from the indirect interference in previous anaerobic metabolisms linked to subsequent aerobic

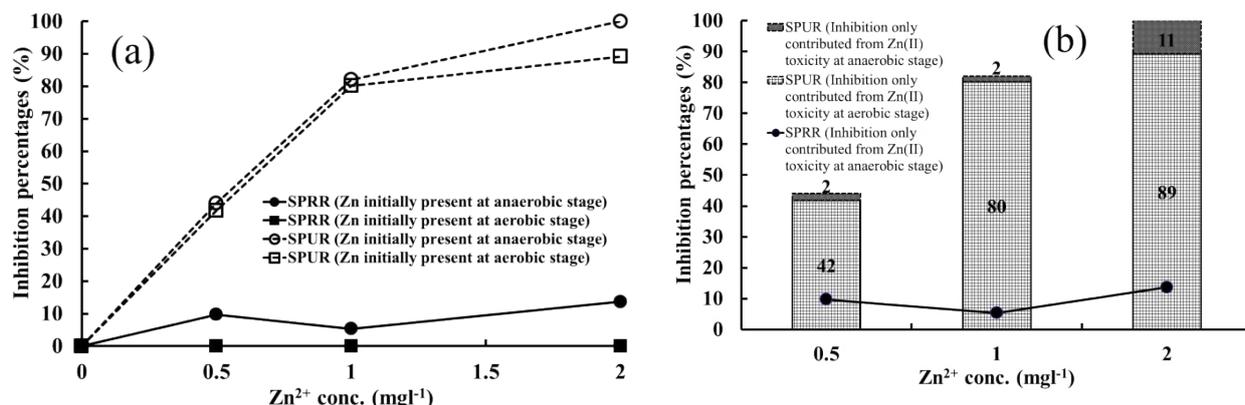


Figure 5: Contribution of inhibition percentages for Phosphorus metabolisms of PAOs in the presence of Zn(II).

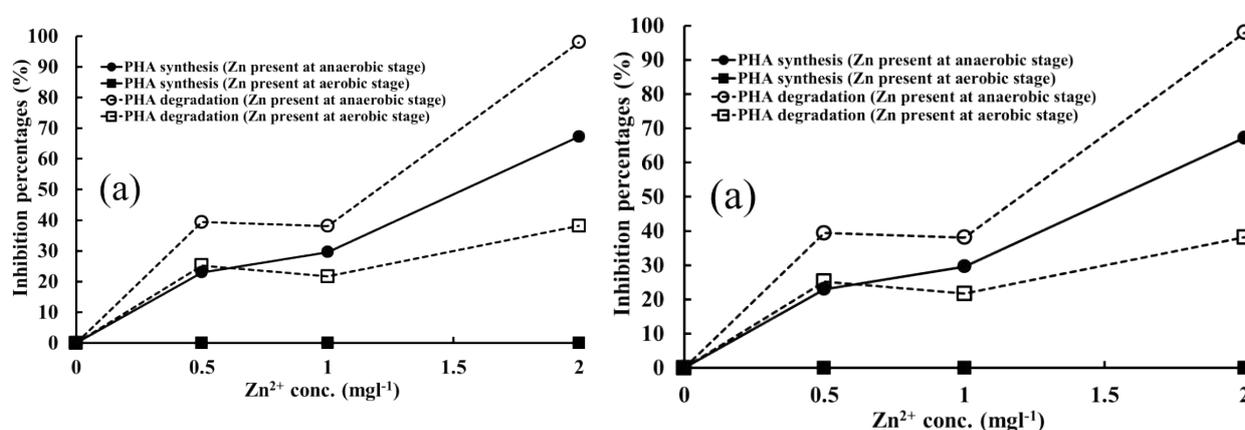


Figure 6: Contribution of inhibition percentages for PHA metabolisms of PAOs in the presence of Zn(II).

metabolisms. On the contrary, the major inhibition of aerobic PHA metabolisms came from the indirect interference in previous anaerobic metabolisms (61% of total inhibitions) while 2.0 mg L⁻¹ of Zn(II) was added. Only 39% of total inhibitions came from the direct toxic effects to aerobic PHA metabolisms. This amplification trend of indirect interference followed by increasing Zn(II) concentration for aerobic PHA degradation was consistent with the trend of direct interference for anaerobic PHA synthesis. This indicates that the degree to which PAO_s lose their ability to utilize PHAs is dependent on how much PHA was synthesized in the previous anaerobic stage under the presence of Zn (II). This is reasonable because the source of PHA_s providing for PAO use at the aerobic stage were those PHAs synthesized at the previous anaerobic stage.

Conclusions

Anaerobic and aerobic metabolisms of PAO_s decrease with increasing Zn(II) concentration varying between 0-2 mg L⁻¹ and temperature variation indeed affects inhibitive degrees. The majority of metabolic inhibition (>90% of total inhibitions) for PAO_s aerobically taking up phosphorus came from the direct interference of Zn(II) in aerobic metabolisms. Only a small amount of the inhibitions (<10% of total inhibitions) came from the indirect interference in previous anaerobic metabolisms linked to subsequent aerobic metabolisms. The results are helpful to understanding the reasons of the failure of EBPR systems under the presence of toxic substances and temperature variation.

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