Inhibitory effect of copper on enhanced biological phosphorus removal

Guangxue Wu and Michael Rodgers

ABSTRACT

Copper inhibition of enhanced biological phosphorus removal (EBPR) was examined in batch experiments under anaerobic and aerobic conditions. Inhibition was represented by both acetate uptake and phosphorus release coefficients under anaerobic conditions, and by a phosphorus uptake coefficient under aerobic conditions. The results showed that copper inhibition of EBPR occurred mainly during aerobic phosphorus uptake and a first-order phosphorus uptake coefficient can be better used to describe the inhibition effect. For the aerobic phosphorus uptake using the EBPR activated sludge, (i) copper inhibition started at 0.07 mg/l, (ii) 50% and 100% inhibition occurred at 0.30 mg/l and 0.53 mg/l, respectively, and (iii) the inhibition constant was 0.48 mg/l.

Key words | copper, enhanced biological phosphorus removal, heavy metals, inhibition

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INTRODUCTION

Compared with nitrogen, phosphorus is usually the limiting nutrient causing eutrophication in fresh water bodies, and its removal has been recently receiving much attention. In comparison with physical and chemical methods, biological phosphorus removal methods are much more environmentally friendly and sustainable due to less chemical addition, less waste sludge production, and less capital and operating costs. Therefore, biological phosphorus removal processes have been applied world-wide to remove phosphorus from wastewater. Biological phosphorus removal is achieved by enhanced biological phosphorus removal (EBPR), in which alternating anaerobic and aerobic conditions are applied and polyphosphate accumulating organisms (PAOs) are enriched. Under the anaerobic condition, PAOs take up organic carbon in the wastewater and store it as polyhydroxyalkanoates using polyphosphate as the energy source and glycogen as the reducing equivalent; while under the following aerobic condition, PAOs use polyhydroxyalkanoates as the carbon and energy sources for taking up phosphorus, reproducing, and replenishing glycogen (Mino *et al.* 1998). More phosphorus is taken up by PAOs under the aerobic condition than is released under the anaerobic condition, and as a consequence, phosphorus is removed from wastewater by removing waste sludge from the system. Many environmental factors affect the EBPR process, and the following factors have been investigated in detail: temperature, carbon concentration and type, and pH (Oehmen *et al.* 2007). However, only limited studies have been carried out on the effect of heavy metals on EBPR, and these include copper, zinc, cadmium, and tin (Chiesa *et al.* 1987; Ting *et al.* 1994; Rayne *et al.* 2005; Tsai *et al.* 2006).

With increasing industrialization, wastewater containing various concentrations of heavy metals may arise in cities. In wastewater treatment processes, trace amounts of heavy metals are required for microbial metabolisms, while a high concentration of heavy metals can inhibit these biological processes. Copper is one of the common heavy metals in industrial wastewater, with concentrations varying from several hundred mg/l from plating waste to less than 1 mg/l from acid mine drainage (Patterson & Jancuk 1977). A high concentration of copper can cause toxicity in microorganisms by: (i) inhibiting metabolic enzymes;

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(ii) denaturing proteins; and (iii) combining with the thiol compounds within cells (Dilek *et al.* 1991). The inhibition of copper on carbon removal, nitrification and phosphorus removal in activated sludge systems has been examined (Chiesa *et al.* 1987; Ting *et al.* 1994; Gernaey *et al.* 1997; Wong *et al.* 1997; Gutierrez *et al.* 2002; Juliastuti *et al.* 2003). Limited studies at copper concentrations above 1 mg/l showed that complete aerobic phosphorus uptake inhibition was observed but no kinetic parameters were reported (Chiesa *et al.* 1987; Ting *et al.* 1994).

The objectives of this study were to: (i) examine copper inhibition of EBPR activities during anaerobic acetate uptake or phosphorus release, and aerobic phosphorus uptake; and (ii) obtain kinetic parameters for copper inhibition of EBPR.

MATERIALS AND METHODS

EBPR acclimation

A 3-litre sequencing batch reactor (SBR) was operated at 10°C in a temperature-controlled room for EBPR acclimation. The SBR had four cycles per day and each cycle comprised the following phases: fill (15 min), anaerobic (105 min), aerobic (180 min), settle (40 min) and draw/idle (20 min). The reactor was constantly stirred with a magnetic stirrer (Agitatore Magnetico Tipo AGE, VELP Scientifica, Italy) that rotated at 500 rpm during the fill, anaerobic and aerobic phases; during the aerobic phase, air was supplied with an air diffuser located at the bottom of the reactor. In each cycle, 1.5 L of fresh synthetic wastewater were fed into the reactor. In three of the cycles, 1.5 L of treated settled wastewater were decanted from the reactor, while in the fourth cycle, 1.2 L of treated settled wastewater were decanted from the reactor and a mixed liquor volume of 300 ml was taken just before the end of the aerobic phase. As a result, an average hydraulic retention time of 12 h was maintained, and a solids retention time of 10 days was achieved if no solids loss occurred during the settle phase.

The components in the synthetic wastewater were: 300 mg/l sodium acetate, 12 mg/l yeast extract, 80 mg/l NH_4Cl, 95 mg/l K_2HPO_4, 67 mg/l $MgSO_4{\cdot}7H_2O$ and 11 mg/l $CaCl_2{\cdot}6H_2O$. Trace elements (1 ml) were added following Barat *et al.* (2008). Concentrated sulfuric acid (7 drops in 10 L) was added to the synthetic wastewater to adjust pH to approximately 6.8. The reactor was seeded with activated sludge taken from another laboratory-scale EBPR reactor.

Batch experiments

Batch experiments were carried out to examine the effect of various copper concentrations on: (i) anaerobic acetate uptake and phosphorus release, and (ii) aerobic phosphorus uptake. Copper concentrations of 1, 2, 5 and 10 mg/l were examined under both anaerobic and aerobic conditions. The results showed that aerobic phosphorus uptake was completely inhibited when the copper concentration was above 1 mg/l. On finding that the 1 mg/l concentration of copper inhibited the aerobic phosphorus uptake, the effect of copper concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 and 1 mg/l on aerobic phosphorus uptake was examined to obtain the copper inhibition kinetic parameters. The 300 ml volumes of activated sludge mixed liquor that were withdrawn from the SBR at the end of the aerobic phase were used in the batch experiments. Three replications were carried out for both the anaerobic and aerobic conditions and the average results are presented.

The protocol for the anaerobic experiments was as follows: (i) the 300 ml mixed liquor from the parent SBR were purged with nitrogen gas for 3 min, and then mixed with 300 ml of synthetic wastewater (also pre-purged with nitrogen gas) with the same composition as that fed into the parent SBR, except that the sodium acetate concentration was doubled; (ii) 80 ml of the new mixture were taken and placed into five 125 ml serum bottles (Sigma-Aldrich, Ireland). (iii) stock copper solutions (0.8 ml of 100 ×) were added to four of the serum bottles to achieve initial copper concentrations of 1, 2, 5 and 10 mg/l; (iv) the serum bottles were then purged with nitrogen gas, sealed with rubber stoppers and mixed at 250 rpm (Orbital Shaker SSL1, Barloworld Scientific Ltd., UK) at 10°C; and (v) samples were then taken from the serum bottles at intervals to measure orthophosphate (PO_4-P) and sodium acetate concentrations.

For the aerobic batch experiments, the procedure was: (i) the 300 ml of mixed liquor from the SBR were mixed

with 300 ml of synthetic wastewater with the same composition as that fed into the parent SBR, and then incubated for 2 h under the anaerobic condition at 10°C; (ii) after the 2 h anaerobic incubation period, 80 ml of the mixture were taken and placed into seven 250 ml glass flasks; (iii) stock copper solutions (0.8 ml of 100 ×) were added to six glass flasks to achieve initial copper concentrations of 0.1, 0.2, 0.3, 0.4, 0.5 and 1 mg/l; (iv) the open-mouth glass flasks were shaken at 250 rpm (Orbital Shaker SSL1, Barloworld Scientific Ltd., UK) at 10°C with samples being taken at intervals to measure the PO_4-P concentrations.

Analytical methods

PO_4-P was analyzed using a Konelab 20 analyzer (Thermo Clinical Labsystems, Vantaa, Finland). Sodium acetate concentrations were measured using a high performance liquid chromatography (HPLC, Agilent 1200, Agilent Technology, USA) with a UV index detector and an Aminex HPX-87H column (Bio-Rad, USA). Separation during HPLC tests was achieved using a mobile phase of 1‰ (vol/vol) H_2SO_4 at a flow rate of 0.6 ml/min, a column temperature of 65°C, and a detector temperature of 40°C. Suspended solids (SS) and volatile suspended solids (VSS) were determined according to standard methods (APHA 1995).

The dynamics of acetate uptake and PO_4-P release are described by zero-order equations against time (Equation (1)), and aerobic phosphorus uptake is described by both zero-order and first-order equations against time (Equations (1) and (2)).

$$C_t = Vt + A \tag{1}$$

$$C_t = C_0 e^{-kt} \tag{2}$$

where C_t is the concentration of sodium acetate or PO_4-P at time t, V is the zero-order reaction coefficient, k is the first-order reaction coefficient, and A is a constant.

Inhibition is described by Equation (3) based on zero-order or first-order reaction coefficients as follows:

$$\%\,\text{inhibition} = \frac{V_I}{V_0} \times 100 = \frac{k_I}{k_0} \times 100 \tag{3}$$

where V_I and V_0 are acetate or phosphorus zero-order reaction coefficients at conditions with and without the copper addition, respectively; and k_I and k_0 are phosphorus first-order reaction coefficients at conditions with and without the copper addition, respectively.

The inhibition constant (K_i) and the start copper inhibition concentration (I_a) can be obtained from Equation (4) (Ren & Frymier 2003):

$$\frac{100}{100 - \%\,\text{inhibition}} = \left(1 - \frac{I_a}{K_i}\right) + \frac{I}{K_i} \tag{4}$$

where I is the applied initial copper concentration.

RESULTS AND DISCUSSION

In this EBPR study, the SBR influent and effluent PO_4-P concentrations were 17.2 mg/l and less than 0.34 mg/l, respectively, resulting in over 98% PO_4-P removal. For the removed activated sludge at the end of the aerobic phase, the SS concentration was 1.98 g/l and the VSS concentration was 1.18 g/l. The following batch experiments were carried out after more than 90 days acclimation.

Anaerobic acetate uptake and phosphorus release occurred under all the applied copper concentrations ranging from 0 mg/l to 10 mg/l (Figure 1). However, around 55% inhibition was obtained at the copper concentration of 1 mg/l; more than 80% inhibition was observed when the copper concentration was above 2 mg/l; while complete inhibition did not occur even under the copper concentration of 10 mg/l (Figure 2). Similar inhibition trends and percentages were obtained by using zero-order acetate uptake coefficients or zero-order phosphorus release coefficients because phosphorus release was mainly used as the energy source for anaerobic acetate uptake by PAOs in EBPR processes. The finding of increasing anaerobic inhibition of EBPR as copper concentrations increased is different from the result of Chiesa *et al.* (1987) where no significant inhibition on anaerobic phosphorus release was observed with copper concentrations ranging from 0 mg/l to 10 mg/l. The difference could be due to different operating conditions applied, such as acclimating temperature (10°C in this study vs 22°C in Chiesa *et al.* (1987)), initial SS concentration (0.99 g/l vs 1.7 g/l), and carbon type

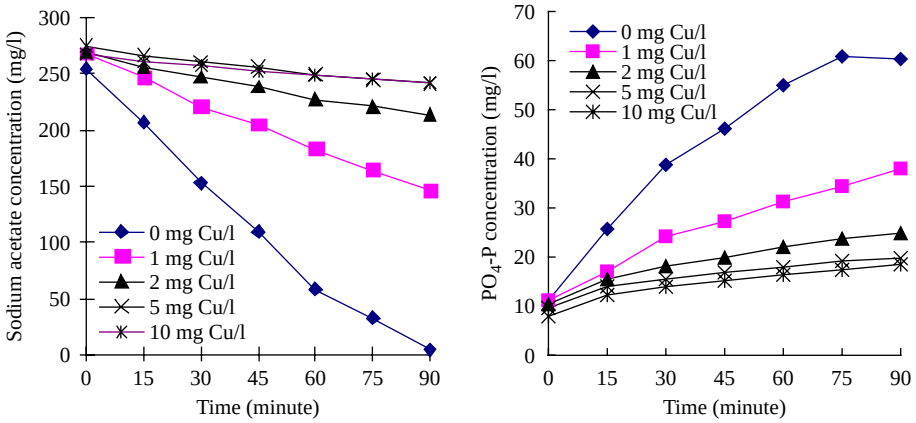

Figure 1 | Anaerobic dynamics of soluble sodium acetate and PO_4-P under copper concentrations from 0 mg/l to 10 mg/l.

(acetate vs acetate/glucose), and these may affect the physical characteristics and microbial composition of the acclimated activated sludge. There were no statistically significant differences for the P/C ratio (phosphorus released/acetate uptake) among all test conditions, with the value of 0.4 mol-P/mol-C. This result indicated that phosphorus release was mainly contributed by activities of PAOs rather than cell lysis due to the addition of copper. In the study of Ting *et al.* (1994), cell lysis caused by high copper concentrations was observed, reflected by an increase in the soluble total organic carbon concentrations. In the study of Chiesa *et al.* (1987), the amount of phosphorus released was slightly increased with increasing copper concentrations, which was proposed to be due to higher maintenance requirements under high copper concentrations. This phenomenon was not clearly shown in the anaerobic experiment in the present study.

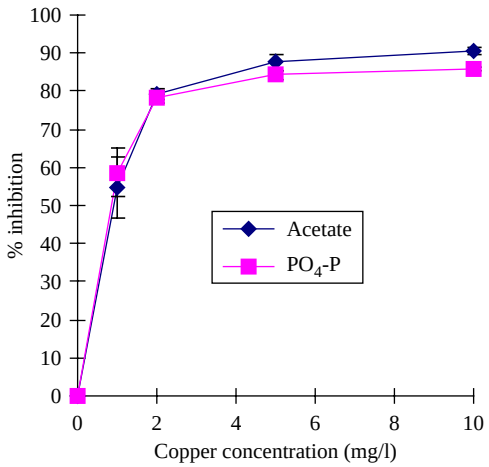

Figure 2 | Inhibition of microbial activity on anaerobic acetate uptake and phosphorus release under copper concentrations from 0 mg/l to 10 mg/l based on the zero-order regression.

In the aerobic batch experiment, with the copper concentration above 1 mg/l, phosphorus release rather than phosphorus uptake was observed (Figure 3). The release of phosphorus could be due to the secondary phosphorus release for the purpose of maintenance rather than cell lysis as discussed under anaerobic conditions. This result indicates that EBPR was completely inhibited with the copper concentration above 1 mg/l under aerobic conditions. Therefore, only results from copper concentrations below 1 mg/l are presented and discussed. Phosphorus uptake occurred with copper concentrations ranging from 0 mg/l to 0.5 mg/l. However, phosphorus uptake decreased with increasing copper concentrations (Figures 3 and 4).

Compared with inhibition under anaerobic conditions, copper had a higher inhibition percentage on EBPR activities under aerobic conditions with the same copper concentration applied (Figures 2 and 4). For example, more than 80% inhibition was obtained when the copper concentration was above 0.5 mg/l under aerobic conditions, while the same percentage inhibition required the copper concentration to be above 2 mg/l under anaerobic conditions. This indicates that copper inhibition on EBPR occurs mainly through inhibiting aerobic metabolisms. Under anaerobic conditions, there is little biomass growth with only storage of organic substances occurring at low enzyme activity; while under aerobic conditions, biomass growth occurs with greater enzyme activity, leading to

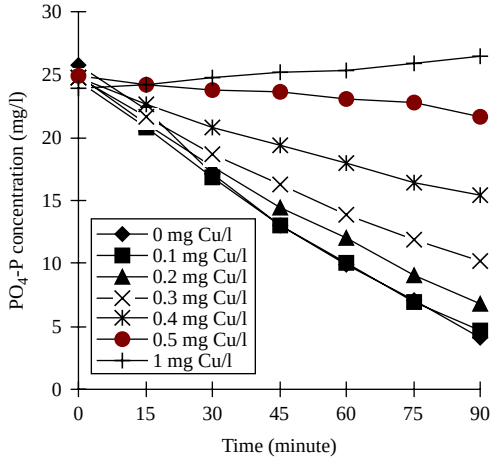

Figure 3 | Aerobic dynamics of soluble PO_4-P under copper concentrations ranging from 0 mg/l to 1 mg/l.

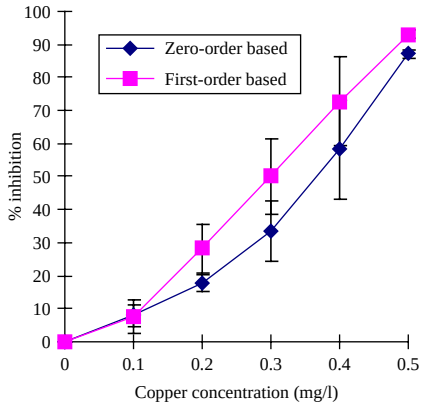

Figure 4 | Inhibition of microbial activity on aerobic phosphorus uptake under copper concentrations from 0 mg/l to 0.5 mg/l based on both the zero-order and the first-order regressions.

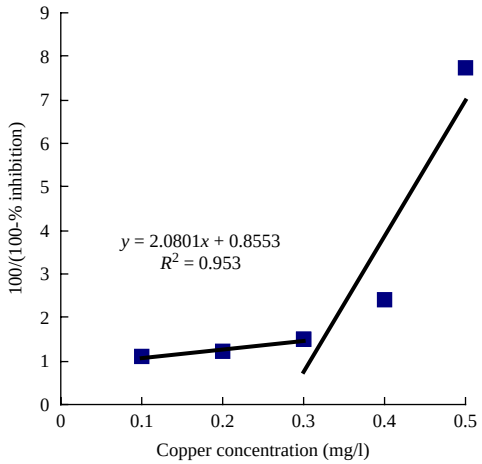

Figure 5 | Regression of 100/(100 − % inhibition) with copper concentrations. The inhibition constant was determined as the inverse of the slope of the regressed line (the first three data points).

increased inhibition. Heavy metal toxicity should be similar to disinfectant toxicity, because the gene responsible for these two processes can be co-selected (Baker-Austin *et al.* 2006). Microorganisms with low growth rates can tolerate high concentrations of disinfectants (Walker & Marsh 2007), and the same mechanism may also be applied to PAOs, which can tolerate a high copper concentration under anaerobic conditions without biomass growth.

The %inhibitions under aerobic conditions based on the zero-order coefficient, Equation (5), (linear-based regression, Equation (1)), and based on the first-order coefficient, Equation (6), (exponential-based regression, Equation (2)) are compared in Figure 4, showing that inhibition based on the first-order coefficient fits the data marginally better than that based on the zero-order coefficient:

$$\%\ \text{inhibition} = 198.45I - 18.574\ (R^2 = 0.9613) \quad (5)$$

$$\%\ \text{inhibition} = 215.21I - 14.164\ (R^2 = 0.9997) \quad (6)$$

The reason could be that phosphorus uptake is mainly controlled by the degradation of intracellular stored carbon (polyhydroxyalkanoates), and aerobic degradation of intracellular stored carbon could be better described by the first-order equation (Beun *et al.* 2002). The results suggest that: (i) utilization of different carbon types - intracellular or extracellular - should be emphasized in studies on microbial inhibition by heavy metals; and (ii) results should be presented based on microbial metabolic characteristics. From Equation (6), under aerobic conditions, the start inhibition copper concentration (I_a) on EBPR was 0.066 mg/l, the EC50 (the concentration at which 50% inhibition occurs) was 0.30 mg/l, and the complete inhibition concentration was 0.53 mg/l. As to copper inhibition on nitrification, Juliastuti *et al.* (2003) obtained that the start inhibition concentration was 0.05 mg/l and the complete inhibition concentration was 1.2 mg/l. The substrate, reaction time, initial food to microorganism ratio, and test method, can affect the heavy metal toxic test results greatly (Cokgor *et al.* 2007). Therefore, a high variation in the obtained EC50 values occurs, such as from 32 mg/l to 123 mg/l for heterotrophs (Wong *et al.* 1997; Gutierrez *et al.* 2002), and from 0.08 mg/l to 173 mg/l for nitrification (Gernaey *et al.* 1997; Juliastuti *et al.* 2003). However, to

date, there are no reported literature EC50 values for heavy metal inhibition on EBPR processes.

By plotting 100/(100 − % inhibition) against copper concentrations (Figure 5), it showed that there was an abrupt increase in the 100/(100 − % inhibition) value when the copper concentration was above 0.3 mg/l. The copper inhibition is non-competition inhibition, and the data should have a good linear relationship. However, this is not the case as shown in Figure 5. A similar phenomenon was also observed by Ren & Frymier (2003) when studying inhibition of activated sludge by chromium (Cr), which was due to the Cr transformation between Cr^{3+} and Cr^{6+}. The underlying mechanism responsible for the abrupt increase in inhibition of EBPR by copper is unclear. By regression using Equation (4) (the first three data points in Figure 5), K_i of 0.48 mg/l and I_a of 0.07 mg/l were obtained. These values are much lower than K_i of 4.51 mg/l and I_a of 9.53 mg/l for copper inhibition on luminescent bacteria obtained using the same method (Ren & Frymier 2003). The start copper inhibition concentration of 0.07 mg/l was similar to 0.066 mg/l obtained from Equation (6), and both are reasonable compared with the experimental data. However, the calculated start copper inhibition concentration from Equation (5) was 0.094 mg/l, which was higher than those values of 0.066 mg/l and 0.07 mg/l. This further indicated that the first-order equation might be more suitable to describe the inhibitory effect of heavy metals on EBPR.

CONCLUSIONS

From the batch experiments on inhibition of EBPR by copper, the following conclusions can be obtained.

- Inhibition of microbial activity by copper on EBPR was mainly through the inhibition of aerobic metabolisms. For example, at the copper concentration of 1 mg/l, complete inhibition of aerobic BEPR activities was observed, while only 55% inhibition was obtained under anaerobic conditions.
- From analysis of the aerobic phase results on the inhibition of EBPR by copper, the following kinetic parameters were obtained: a start copper inhibition concentration of 0.07 mg/l, a 50% inhibition concentration of 0.30 mg/l, a complete inhibition concentration of 0.53 mg/l, and an inhibition constant of 0.48 mg/l.
- Evaluation of the copper inhibition of aerobic phosphorus uptake based on the first-order phosphorus uptake coefficient was marginally better than that based on the zero-order phosphorus uptake coefficient, with a better linear relationship obtained by the former method.

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