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Heavy metal inhibition on an alternating activated sludge system and its comparison to conventional methods: case study of Cu^{2+}

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ABSTRACT

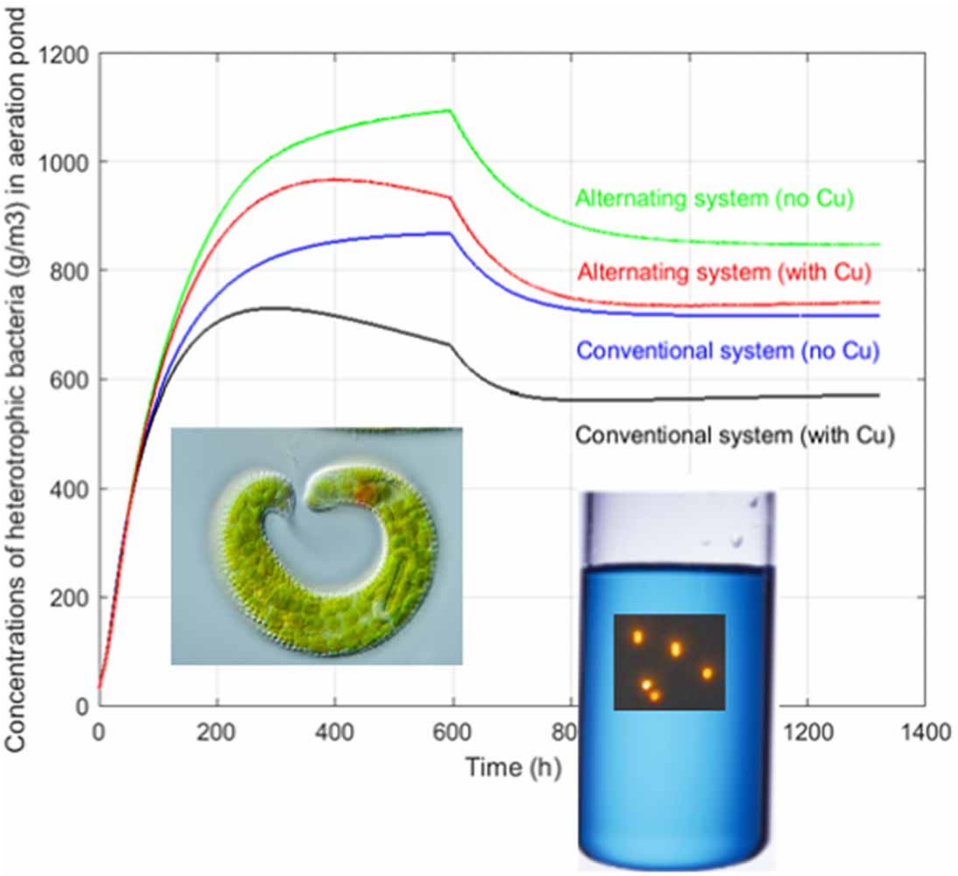
In order to understand the behaviour of wastewater treatment plants with heavy metal presence, this study evaluates the treatment process in the presence of heavy metals (Cu^{2+} as a case study) and compares it with the absence of heavy metals. An activated sludge model is improved by means of incorporating other novel inhibitory kinetic and settler models for this evaluation. To achieve this goal, a simulation algorithm is developed using the MATLAB code to detect any heavy metal influence on the aerobic and anoxic growth of heterotrophic and autotrophic biomass. The code also allows for a comparison of treatment plant performance with and without Cu^{2+} in both conventional and alternating systems. The results reveal that the presence of heavy metals, in this study for Cu^{2+} at 0.5 mg/L, in a biological treatment system, has an inhibitory effect on the heterotrophic bacteria but more so on the autotrophic bacteria growth and it prevents nitrification and denitrification, thus having a negative effect on the nitrogen removal in the alternating systems.

Key words: alternating systems, ASM3, autotrophs, copper inhibition, heterotrophs

HIGHLIGHTS

- A simulation algorithm is developed using the MATLAB code.
- The wasterwater treatment plants process is evaluated in the presence of Cu^{2+}.
- An ASM is improved by incorporation of inhibitory kinetic and settler models.
- Heavy metal influence on heterotrophic and autotrophic biomass is assessed.
- WWTP performance in conventional and alternating systems is determined.

GRAPHICAL ABSTRACT



SYMBOLS AND ABBREVIATIONS

S_{NH}	ammonium plus ammonia nitrogen
S_{N_2}	dinitrogen
S_{NO}	nitrate plus nitrite nitrogen
X_H	heterotrophic bacteria
X_A	autotrophic bacteria
X_{SS}	total suspended solids
μ	the maximum specific growth rate
b	decay rate
ASM	sludge model
COD	chemical oxygen demand
SS	suspended solids

INTRODUCTION

Activated sludge is a well-known process that has been widely used for domestic wastewater treatment in recent decades. To remove organic carbon, a biological system consists of an aeration pond and a settler. When the treated water is discharged into the special receiving medium where nitrogen removal is essential, the alternating system can be utilized instead of a simple activated sludge system. To materialize the alternating systems, the aeration pond is operated with a sequence of aerated and non-aerated periods to succeed nitrification and denitrification so that nitrogen is removed up to the required extent. However, the problem begins when industrial wastewater is mixed with domestic wastewater before treatment. Industrial wastewater discharged from industries such as textile dyeing, petroleum, metal finishing, automobile, electro-plating, and leather tanning cause heavy metals to enter into this cycle, requiring major treatment worldwide at the urban level. This is one of the most important environmental problems in the world. The precise detrimental effects generally depend on the type and concentration of the heavy metals. The most frequently encountered heavy metals present in industrial effluents are copper(Cu), mercury(Hg), zinc(Zn), lead(Pb), cadmium(Cd), iron(Fe), chromium(Cr), cobalt(Co) and nickel(Ni). There are many treatment technologies for the removal of heavy metal ions from wastewaters as ion exchange, adsorption and membrane filtration (Fu & Wang 2011). There are also other methods, such as chemical precipitation, coagulation–flocculation, and flotation.

The biological wastewater treatment may not be an efficient process due to the toxic cation effect on the biomass for wastewater with high metal concentrations. The toxicity of metals influences the microbial biomass growth and treatment efficiency inversely. If they are present in low concentrations, some metals may act as micronutrients; however, in high concentrations they may cause cell walls to break. There have been various studies carried out to understand the inhibitory effects of the heavy metals in treatment systems. Çeçen *et al.* (2010) investigated the effect of Cr, Pb, Hg, Cd and silver (Ag) on nitrifying sludge respiration, concluding that the highest inhibitory effect belonged to Ag and the lowest one to Cr (either trivalent or hexavalent, their effects were similar).

On the other hand, combined treatment for industrial and domestic wastewater in municipal WWTPs may be economically profitable; the consequences can be directed according to the difficulty of treatment and unexpected impact on biomass and end-product. Hence, there is a need to evaluate the behaviour of WWTPs with and without heavy metals in activated sludge systems. Different activated sludge models have been proposed for domestic wastewater treatment systems and they have found a place in industrial wastewater. Wu *et al.* (2016) simulated and optimized a coking wastewater biological treatment process using activated sludge model no. (ASM3; Gujer *et al.* 1999) and compared the reported model parameters for different industrial wastewaters such as pulp mill, tannery and palm oil mill. The heterotrophic growth rate constant and lysis rate constant changed between 0.78–28 and 0.03–0.76 per day, respectively. They concluded that the ASM3 model could predict the performances of coking WWTP successfully by removing chemical oxygen demand (COD) and ammonium nitrogen. Man *et al.* (2017) investigated wastewater treatment for pulp and paper mills. In their modelling and simulation studies they used ASM1 (activated sludge model no. 1; Henze *et al.* 2000), and correlated heterotrophic growth rate and lysis rate constants with temperature as maximum 9.69 per day and 1.96 per day, respectively. They concluded that ASM1 could be applied in the simulation of papermaking wastewater treatment systems.

Principi *et al.* (2006) investigated the toxicity of heavy metals in an activated sludge system for Cu^{2+}, Zn^{2+} and Ni^{2+} ions, and they determined that the nitrifiers had higher sensitivity than the heterotrophic bacteria. Additionally, the metal accumulation capability of the biomass is the highest in the presence of copper and they concluded that the presence of heavy metals reduces microbial diversity abundance in activated sludge systems. Cabrero *et al.* (1998) studied the effects of Cu^{2+} and Zn^{2+} on biomass, both separately and combined, and concluded that Cu^{2+} was more toxic than Zn^{2+}, and that when Cu^{2+} was present in concentrations up to 5 mg/L, the bio-kinetic parameters were not affected adversely. However, serious problems occurred in the system when concentration increased to 10 mg/L and higher. Dilek *et al.* (1998), indicated the toxicity effect changes with heavy metal ions, type and concentrations of organisms, and also environmental conditions such as temperature, pH, dissolved oxygen (DO), ionic strength and in the presence of other metal ions. Pamukoglu & Kargi (2007a) investigated the influence of Cu^{2+} on COD removal with hydraulic residence time and solid retention time (SRT), and concluded that COD removal percentage increased with increasing SRT both with and without copper, and the percentage of COD removal was lower at all SRTs in the presence of copper. Their experiments were performed on synthetic wastewater containing 14 mg/L Cu^{2+}. In another study by the same authors (Pamukoglu & Kargi 2007b), they revealed that the growth yield coefficient decreased and the death rate constant increased in the presence of 15 mg/L Cu^{2+}. Sun *et al.* (2016) summarized the previous studies on this subject: all heavy metals are toxic to bacterial life and inhibit the microbiological processes at moderate and high concentrations; however, these metals can stimulate microorganisms at low concentrations. They further add that Cu^{2+} inhibits the heterotrophic biomass at low concentrations and is more toxic than Pb^{2+}, Zn^{2+} and Ni^{2+}. The authors studied in detail the effect of copper on the bacterial communities in activated sludge systems and their performance. The anaerobic–anoxic–aerobic process was treated with 10, 20, and 40 mg/L Cu^{2+}. The conclusion was that Cu^{2+} had a very important negative effect on the performance of such systems. To overcome the toxicity of Cu^{2+} ion, the bacterial species showed different adaptations and tolerance levels, some species were even stimulated in high concentrations of copper. They used Illumina MiSeq sequencing analysis to classify the microbial community. The results showed that the system experienced sharp decreases in the COD and ammonia nitrogen removal rates at high copper concentrations.

In the modelling of activated sludge systems, several mathematical models have been suggested and the effects of heavy metals on the performance of these sludge systems and the growth rates have been studied. However, there have been limited attempt to examine the lysis rate constants. Pai *et al.* (2009) established a novel modelling concept to evaluate the effects of these metals on the heterotrophic growth and lysis rate. They showed that the growth rate decreased while the decay rate increased in the presence of heavy metal, with the inhibition coefficients at 1.21 and 1.82 mg/L for copper and cadmium, respectively.

Another important point is that the nitrifying microorganisms are more sensitive than those responsible for the oxidizing carbonaceous material (Stoveland *et al.* 1979). Several heavy metals and organic compounds have inhibitory effects on the net maximum specific growth rate of autotrophic biomass. To determine these effects, a respirometric technique was used by Juliastuti *et al.* (2003). This technique was applied under normal conditions when inhibition occurred by some metals (Cu^{2+} and Zn^{2+}) and by some selected organic compounds, such as chlorobenzene, trichloroethylene, phenol and ethylbenzene. In their study, the inhibition degree and the concentration of the inhibitors were determined when total inhibition occurred. They concluded that Cu^{2+} has stronger inhibitory effects than Zn^{2+}, and that the nitrification process was completely inhibited at 1.2 mg/L for both Cu^{2+} and Zn^{2+}. The IC50 (metal ion concentration required for 50% inhibition of microorganism growth) values were 0.08 mg/L and 0.35 mg/L for Cu^{2+} and Zn^{2+}, respectively. Inhibition with copper starts at concentrations above 0.05 mg/L and inhibits the nitrification process by 50% at 0.08 mg/L. Juliastuti *et al.* (2003) cited other studies on autotrophic bacteria decay and growth rate:

- The autotrophic decay rate, bA, at 20 °C is determined as 0.2 per day under aerobic conditions, and 0.1 per day under anoxic conditions (Nowak & Svardal 1993);
- In the activated sludge modelling, the reported values of bA are between 0.05 and 0.15 per day (Gujer *et al.* 1999);
- The value of the maximum specific growth rate of the autotrophic biomass (20 °C) is measured to be 0.57 per day and the specific decay rate at 0.13 per day (Lesouef *et al.* 1992);
- Martinage & Paul (2000) indicate a value of 0.15 per day for decay;
- The concentration range of heavy metals and organic compounds that inhibit nitrification are Zn^{2+} (0.08–0.5 mg/L); Cu^{2+} (0.005–0.5 mg/L) and phenol (4–10 mg/L) (Vismara 1982);
- 0.08 mg/L Zn^{2+}as the minimum inhibition threshold value, and 0.08–0.5 Zn^{+2} is the range of inhibition levels (Eysenbach 1994).

According to Juliastuti *et al.* (2003) the net maximum specific growth rate of the autotrophic biomass decreases as the concentration of heavy metals increases. The growth rate of the autotrophic biomass is 92% inhibited at 1.2 mg/L Zn^{2+}. The net maximum specific growth rate is severely reduced by Zn^{2+} at concentrations above 0.3 mg/L. The presence of Cu^{2+} in wastewater inhibits the net maximum specific growth rate of autotrophic biomass to a larger extent than Zn^{2+}. The value of the growth rate constant of autotrophic bacteria per day (μA)$-$ bA reaches 0.4 per day at 0.1 mg/L Cu^{2+}. The inhibition at this concentration is 54%, and reaches 82% at 0.5 mg/L Cu^{2+}, whereas 65% inhibition is found only at 0.5 mg/L Zn^{2+}. The autotrophic biomass is hardly inhibited by Cu^{2+} at concentrations below 0.05 mg/L, where the value of $\mu A - bA$ is close to 0.90 per day.

Buaisha *et al.* (2020) investigated the effect of Cu^{2+} and cadmium(II) on the heterotrophic bacteria growth in a conventional system, at 0.7 mg/L concentration, and found the effluent quality does not change considerably. It has to be mentioned that the change in the Cu^{2+} concentration with respect to time and the particulate and soluble difference was not considered in that study.

In summary, the treatment of industrial wastewater with domestic wastewater in biological treatment is not suitable due to heavy metals contained in industrial wastewater. Studies in this area have shown that Cu^{2+} in industrial wastewater is more toxic than Pb^{2+}, Zn^{2+} and Ni^{2+} and severely inhibits heterotrophic biomass even at relatively low concentrations (i.e. 10 mg/L) (Sun *et al.* 2016). The metal deposition capacity of the biomass was higher in the presence of Cu^{2+} than Zn^2+ and Ni^{2+} (Principi *et al.* 2006). Therefore, it is very important to determine the effects of heavy metals on biological treatment, and simulation algorithms with experiments or models can be done to understand these effects. Application of models to industrial wastewater requires modification of model parameters and the most important parameters are growth and lysis rate constants. Although there are many studies on heterotrophic growth and lysis rate constant, there are limited simulations examining growth and lysis rate constant on autotrophic bacteria. Experimental studies on Cu^{2+} inhibition are generally observed for concentrations above 10 mg/L (Cabrero *et al.* 1998; Pamukoglu & Kargi 2007a, 2007b; Sun *et al.* 2016).

However, Juliastuti *et al.* (2003) showed that for autotrophic biomass the inhibition was 54% at 0.1 mg/L Cu^{2+} concentration and the inhibition increased to 82% when the Cu^{2+} concentration reached 0.5 mg/L. No autotrophic biomass inhibition was observed at Cu^{2+} concentrations below 0.05 mg/L.

In light of the above-stated backgrounds and limitations in the available literature, in this study a dynamic model based on ASM3 (Gujer *et al.* 1999) was used to assess the behaviour of the activated sludge process at a full-scale domestic WWTP. To include nitrogen removal, an alternating system based on ASM3 (Balku 2007) is utilized. The main objective of the WWTP is to keep the concentrations of organic, nitrogen and other contaminants below the required limits. The proposed model is further extended to cover the growth and lysis rates of heterotrophic and autotrophic bacteria with metal presence, so that the model can be applied to industrial wastewater containing heavy metals. The ultimate goal is to improve ASM3 by a combination of laboratory tests and process modelling to assess the effects of heavy metals on the process of an alternating activated sludge wastewater treatment that receives the effluent from an industrial plant. This evaluation will take copper into account in the process while some of the ASM3 original default parameter values are changed according to the results of the batch experiments to predict the effects of heavy metals on activated sludge treatment plants. Finally, a new model coupled with ASM3 is proposed by considering the growth and the lysis processes in activated sludge under aerobic and anoxic conditions. The outcomes of this study are expected to enlighten the perspectives on the growth and lysis processes in activated sludge containing heavy metals, more specifically in relation to the effect of Cu^{2+} because its effects are more important than the other metals investigated in the literature.

METHODS

A simple activated sludge system consisting of an aerated basin and a settler is used. In the modelling of an activated sludge system, the wastewater flowing into the aeration basin is subjected to biological processes. Following biological treatment, the flow is delivered to the clarifiers, which is where the sludge settles down and water is discharged to the receiving medium. A portion of the settled sludge is delivered back to the biological pond to maintain high mixed-liquor suspended solids concentration. The ASM3 model is applied for the biological processes taking place in the aeration tank, and a ten-layer model proposed by Takács *et al.* (1991) is used in the settler modeling. In this study the wastewater treatment model, improved for an alternating aerobic–anoxic system (Balku 2007), is simulated to include the heavy metal effects – in this case copper – and the results are compared to the conventional system. Growth of heterotrophic and autotrophic bacteria is possible under both aerobic and anoxic conditions. There is continuous aeration in the conventional system, whereas the aeration pond is simulated for a time sequence of 0.9 h as non-aerated and 1.8 h as aerated in the alternating system. The model parameters, both kinetic and stoichiometric, are used as they are in ASM3, except the growth and decay rate constants for heterotrophic and autotrophic bacteria. The settling parameters are applied as proposed by Takács *et al.* (1991). The difference to Takács' model is that the settling model is applied to each particulate component of ASM3 and also the particulate copper at each layer of the settler. This study refers to data previously obtained in three other works (Pai *et al.* 2009; Juliastuti *et al.* 2003; and Less *et al.* 2012). The values of growth and lysis rate constants obtained from the experimental results were used to derive regression models (Equations (1) and (2)) for the heterotrophic bacteria by Pai *et al.* (2009). The R^2 values of 0.97 and 0.74 were obtained for growth and lysis constants at different copper concentrations. The experimental data covers 0–0.7 mg/L Cu^{2+} concentrations.

$$\mu_H = 4.5197e^{-0.6336\times C_{Cu}} \tag{1}$$

$$b_H = 0.1689 \times C_{Cu} + 0.3122 \tag{2}$$

where μ_H: heterotrophic growth rate constant (per day); b_H: heterotrophic lysis rate constant (per day); and C_{Cu}: concentration of Cu^{2+} (mg/L).

In the case of autotrophic bacteria, the same procedure was applied to the experimental results to derive a linear regression model (Equation (3)) for the growth constant at different copper concentration values. The lysis rate constant was used as an average value of 0.15 per day (Juliastuti *et al.* 2003). The experimental data covers 0–0.5 mg/L copper (II) concentrations.

$$\mu_A = -1.2254C_{Cu} + 0.8532 \tag{3}$$

where
μ_A: growth rate constant of autotrophic bacteria (per day);
C_{Cu}: concentration of Cu^{2+} (mg/L).

The models are not used for concentrations higher than the experimental data in this study.

In addition, the copper concentration entering the system is classified as soluble and particulate and applied to all algorithms. To ensure this, another work is referenced (Less *et al.* 2012) for the portions, and 4% and 96% are accepted as soluble and particulate parts of the entering Cu^{2+} concentration, respectively. The particulate Cu^{2+} is accepted to be settled down in the settler with the sludge, and some is recycled to the aeration tank. The Cu^{2+} concentration in the aeration pond is much higher than the inlet concentration. The Cu^{2+} concentration change with respect to time in the aeration pond is included in the calculation of the growth and lysis constants during the course of simulation at each step.

There are 12 processes and 13 variables in ASM3. All kinetic and stoichiometric parameters regarding these twelve processes are used as the defaults, except the aerobic and anoxic growth rate constants of heterotrophic and autotrophic biomass and lysis rate constant of heterotrophic biomass because they are already included from the experimental results with the presence of Cu^{2+}. The values of growth and lysis rate constants are determined by using regression models for Cu^{2+} inhibition according to the experimental results in the aeration pond. Dependently, these constant change with respect to time during the course of simulation as Cu^{2+} concentration changes. When particulate and soluble Cu^{2+} and all seven particulates in the settler's ten-layer are considered, the total state variables in the algorithm become 85.

Using the MATLAB (the language of technical computing) code (R2017a), the activated sludge process model is simulated for conventional and alternating systems, including both in the presence and the absence of Cu^{2+}. To represent a WWTP correctly, the simulation is performed over three stages. In the first stage, there is no sludge removal from the system and all the sludge is returned to the aeration pond so that the bacteria growth reaches required concentrations. The second stage is the conditioning period in which the sludge removal begins and the system fluctuates, then turns to a steady state eventually. The last stage is the normal operation period, which is thought to be steady. The durations of the start-up, conditioning and normal operation periods are 594 h, 594 h and 135 h, respectively, for the conventional system. These durations are determined by trial-and-error method to have a satisfactory growth quantity for heterotrophic and autotrophic bacteria. The corresponding time intervals for the alternating system are 440, 440 and 100 divisions, respectively, for start-up, conditioning and normal operations. These intervals correspond to 594, 594 and 135 h, respectively, for 0.9 h non-aerated and 1.8 h aerated periods sequentially. Therefore, both systems are simulated approximately for the same time period. The change in the 85 variables can be followed with respect to time during each instant of integration, and all the other dependent variables are determined accordingly.

RESULTS AND DISCUSSION

In the present study, a simulation algorithm is developed to determine the effect of metal inhibition on the activated sludge system. As a case study, copper inhibition is investigated in a conventional as well as an alternating activated sludge system. In detail, the simulation algorithm is performed for the following four cases, and the results are compared:

1. Conventional system with no Cu^{2+} addition;
2. Alternating system with no Cu^{2+} addition;
3. Conventional system with Cu^{2+} addition; and
4. Alternating system with Cu^{2+} addition.

To represent the activated sludge process, the ASM3 model is applied to microbiological processes occurring in the aeration pond, and a ten-layer settling model is applied to the settler to examine the elimination of organic pollutants. Nitrogen removal is ensured by an alternating system, and the regression models based on the experimental data are used to determine the growth and lysis rate constants of heterotrophic and autotrophic bacteria in the presence of Cu^{2+}.

The changes in the concentrations of heterotrophs (Figure 1) can be followed with respect to time during the start-up, conditioning and normal operation periods without and with copper at an inlet concentration of 0.015 mg/L for both the conventional and alternating systems. The bacteria concentrations increase during the start-up period, decrease during the conditioning period due to the start of sludge removal, and continue steadily during the normal operation period, as expected for all cases. The best heterotrophic bacteria growth is ensured by an alternating system with no copper. Once copper enters the system in a continuous manner, the concentration of heterotrophic bacteria is decreased in both systems due to Cu^{2+} inhibition.

Growth and endogenous respiration of both heterotrophic and autotrophic bacteria are possible under aerobic and anoxic conditions. The alternating system provides better conditions for both bacterial groups to achieve higher bacterial concentrations under the same conditions and for the same duration, when the aerated and non-aerated periods are appropriately selected, as shown in Figures 1 and 2. The observed changes in the autotrophic bacteria concentration (X_A) over time in alternative and conventional systems in the presence and absence of Cu^{2+} are shown in Figure 2. Figure 2 shows that the decrease in concentration of autotrophic bacteria in the presence of Cu^{2+} is greater than that of heterotrophic bacteria. It is evident from this simulation that the presence of Cu^{2+} in the biological system adversely affects the growth of both bacteria, even at low input concentrations. However, autotrophic bacterial growth is not insignificant, but more affected.

In the ASM3 model, there are 13 variables, in which all the changes are determined during the simulation with respect to time, and the ones which are important for the comparison in this study are listed in Table 1. In the aeration pond if there is no inhibition (no Cu^{2+}), most of S_{NH} (ammonium and ammonia nitrogen) oxides are inverted to S_{NO} (nitrate and nitrite nitrogen) in both systems. In the alternating system, S_{NO} is converted into S_{N2} (dinitrogen) during the non-aerated period, and therefore nitrogen removal is complete. Continuous copper addition (0.015 mg/L) to the treatment system causes a decrease in the growth of the heterotrophic bacteria, but particularly inhibits the growth of the autotrophic bacteria. The heterotrophic (X_H) concentration in the aeration pond decreases by 20.07%, and 12.57% in the conventional and the alternating systems, respectively, with Cu^{2+} addition. In the case of autotrophic bacteria (X_A), these decreases are 84.46%, and 91.76%, respectively, at the end of a 135-h normal operation period. It means that the Cu^{2+} addition or the presence of Cu^{2+} in the biological system prevents most of the growth of the autotrophic bacteria, which are responsible for the oxidation of S_{NH}. However, if the growth of these bacteria is inhibited, S_{NO} conversion is prevented in both systems and nitrogen removal could not be possible in the alternating system. To understand this effect, one can follow the inhibition of the autotrophic bacteria growth in all systems in

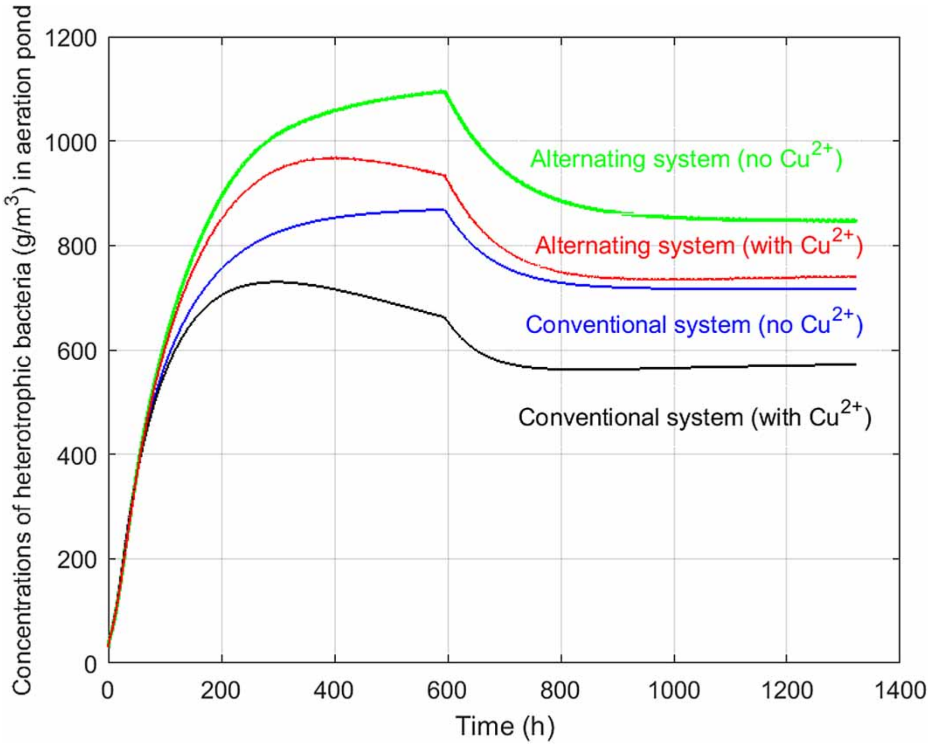


Figure 1 | Heterotrophic bacteria concentration change in aeration pond (with and without Cu^{2+}).

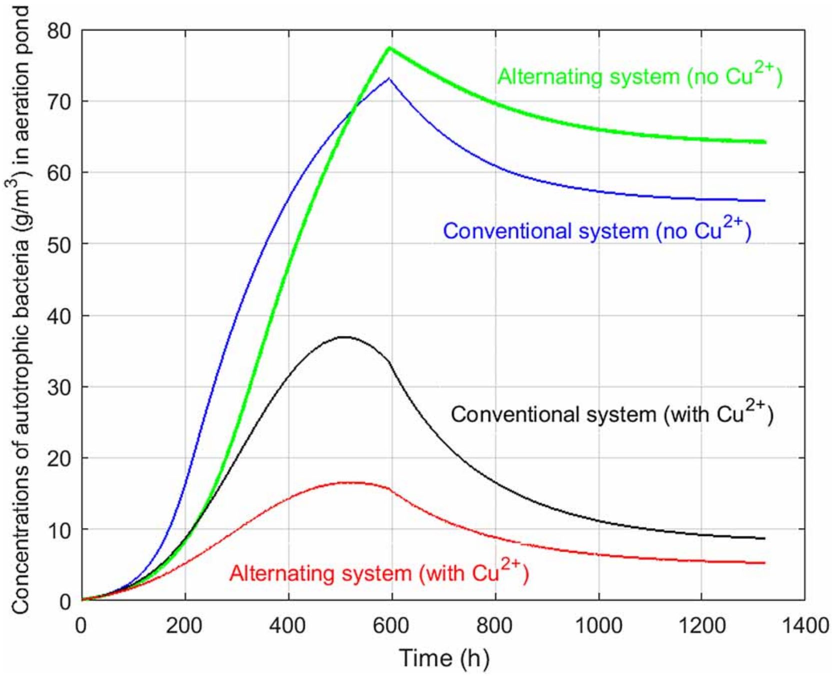


Figure 2 | Autotrophic bacteria concentration change in aeration pond (with and without Cu^{2+}).

Table 1 | Simulation results of ASM3's variables in the aeration tank and in the final effluent

ASM3 variables	**Inlet concentrations**[a] (Henze *et al.* 2000) (g/m^3)	**Aeration tank**				**Final effluent of wastewater plant**			
		Without Cu^{2+}		**With Cu^{2+} (0.015 mg/L)**		**Without Cu^{2+}**		**With Cu^{2+} (0.015 mg/L)**	
		I	**II**	**I**	**II**	**I**	**II**	**I**	**II**
S_{NH}	16.00	0.5	0.4	19.5	20.9	0.5	0.4	19.5	20.9
S_{N_2}	0.00	1.0	7.0	0.5	0.6	1.0	7.0	0.5	0.6
S_{NO}	0.00	19.7	8.2	1.9	0.2	19.7	8.2	1.9	0.2
X_H	30.00	715.6	846.7	572.0	740.3	2.0	2.3	1.7	2.1
X_A	0.10	56.0	64.3	8.7	5.3	0.2	0.2	0.0	0.0
X_{SS}	125	2,807.7	2,897.1	2,637.6	2,774.1	8.0	8.0	7.9	8.0

I, conventional system; II, alternating system; S_{NH}, ammonium and ammonia nitrogen; S_{N2}, dinitrogen; S_{NO}, nitrate and nitrite nitrogen; X_H, heterotrophic bacteria; X_A, autotrophic bacteria; X_{SS}, total suspended solids.

[a] Initial concentrations throughout the system at the beginning of the simulations at $t = 0$.

Figure 2, as well as the simulation results for concentrations of ammonia (S_{NH}), nitrite plus nitrate nitrogen (S_{NO}) and dinitrogen (S_{N2}) in Table 1. The nitrification of ammonium and ammonia nitrogen is negatively affected due to inhibition of autotrophic bacteria by Cu^{2+} and the conversion into N_2 by denitrification process is prevented in the alternating system. There are no expectations for denitrification in conventional system as a matter of course.

To specify this effect, data shown in Table 2 may be examined. In the conventional system, whereas the conversion into NO_x is 97.6% without Cu^{2+}, it decreases to 11.0% with Cu^{2+} addition. In the alternating system, this conversion is 97.5% and conversion to N_2 is 44.9%. However, NO_x conversion decreases to 3.7% with Cu^{2+} presence, and consequently, N_2 conversion decreases to 2.8%.

Table 2 | Percentage conversion of nitrogen compounds

	Conventional system		Alternating system	
	Without Cu^{2+}	With Cu^{2+}	Without Cu^{2+}	With Cu^{2+}
Conversion into NO_x	97.6	11.0	97.5	3.7
Conversion into N_2	4.7	2.3	44.9	2.8

During our simulation studies, the inlet concentration of Cu^{2+} was determined as 0.015 mg/L. On the other hand, the copper concentration in the aeration tank increased up to 0.5 mg/L when the system switched to the normal operating period. These theoretical values obtained from our simulation model are in agreement with the literature values (Juliastuti *et al.* 2003) where the copper concentration has been reported as approximately 0.5 mg/L for the growth constant of autotrophic bacteria. Again, according to the studies of the same group, the IC50 (necessary concentration for 50% growth inhibition of autotrophic bacteria) value was determined as 0.08 mg/L for Cu^{2+}. Accordingly, inhibition by Cu^{2+} starts at concentrations above 0.05 mg/L, and at 0.08 mg/L the nitrification process is inhibited by 50%. In the presence of 1.2 mg/L Cu^{2+}, the nitrification process is terminated completely.

The consequences of the decrease in the growth of autotrophic bacteria is shown in Table 3. There are no significant changes in COD_{eff} and SS_{eff} (total suspended solids) with the Cu^{2+} addition for the considered amounts in the effluent treated water. The total nitrogen concentration (TN_{eff}) is not affected in the conventional system when Cu^{2+} is in considerable amount, but most of the nitrogen remains in NH form instead of NO. The alternating system is influenced and no significant nitrogen removal is detected in this system. In other words, there should be nitrogen removal in the alternating system because the aim here is the nitrogen removal; however, if there is added Cu^{2+}, then the alternating system cannot succeed in reaching nitrogen removal. The TN_{eff} amount, however, remains the same when compared to the conventional system.

Table 4 reveals the Cu^{2+} concentration in both systems as particulate and total (particulate plus soluble). The present simulation studies take the basis of a continuous constant Cu^{2+} concentration inlet, which is 0.015 mg/L, and assumes 4% soluble and 96% particulate of total Cu^{2+} inlet. This amount of Cu^{2+} may not seem to be too high and harmful; however, during recycling, most of the sludge returns to the aeration pond along with the copper. In this way, the Cu^{2+} concentration in the aeration pond increases at first and reaches maximum value within 600 h, then it decreases slightly due to sludge removal

Table 3 | Effluent quality of conventional and alternating systems in the presence and absence Cu^{2+}

		Normal operation period			
		Without Cu^{2+}		With Cu^{2+} (0.015 mg/L)	
Parameters	Inlet concentration[a]	Conventional	Alternating	Conventional	Alternating
COD_{eff} (g/m^3)	260.1000	37.3396	37.3419	37.3120	37.3317
TN_{eff} (g/m^3)	24.9070	20.7380	9.2383	21.9621	21.6173
SS_{eff} (g/m^3)	125.0000	7.9576	7.9746	7.9387	7.9515

[a] Initial concentrations throughout the system at the beginning of the simulations at $t = 0$.

Table 4 | Cu^{2+} concentrations in the treatment systems with 0.015 mg/L Cu^{2+} inlet

	Cu^{2+} concentration (mg/L)			
	Conventional system		Alternating system	
Stream	Particulate	Total	Particulate	Total
Aeration tank exit	0.5126	0.5132	0.5151	0.5157
Effluent	0.0016	0.0022	0.0015	0.0021
Recycle and sludge	1.1345	1.1351	1.1402	1.1408

and afterwards becomes steady during the normal operation period as shown in Figure 3. Even though drawn for the conventional systems, Figure 3 resembles the alternating systems. In the treated water, the concentration of Cu^{2+} is 0.0022 mg/L (Table 4) which is not high. It can be stated that copper is also removed from the water during treatment; on the other hand, it should be mentioned that the copper concentration in the sludge is approximately 1.14 mg/L only with 0.015 mg/L inlet, and it is continuously discharged to land by sludge.

In our study, additional simulations were carried out to determine the degree of inhibition with respect to Cu^{2+} with six different Cu^{2+} inlet concentrations between 0.000 and 0.030 mg/L. Figure 4 shows the changes in the Cu^{2+} concentration in the aeration pond over time at these six different Cu^{2+} inlet concentrations. When Figure 4 is examined carefully, it is observed that the Cu^{2+} concentration in the aeration pool increases as the inlet Cu^{2+} concentration increases, as expected, and reaches its maximum value after 594 h due to the accumulation caused by recycling. At the start of the sludge removal process, there was a decrease in the Cu^{2+} concentration and then it continued steadily.

Time-dependent changes of the heterotrophic bacteria concentration in the aeration pond in Figure 5 showed a decrease in the heterotrophic bacteria concentration as the inlet Cu^{2+} concentration increased. When no Cu^{2+} enters the system (initial concentration: 0.000 mg/L Cu^{2+}), the concentration of heterotrophic bacteria rises up to 594 h. With the start of the sludge removal process, a decline is observed in that value and then the change continued in a constant manner. Similar changes were obtained when the inlet Cu^{2+} concentration was increased to 0.005 mg/L. However, when 0.010 mg/L Cu^{2+} entered the system, it was observed that the heterotrophic bacteria concentration increased up to 400 h with a lower peak value (1,000 g/m^3) compared to that of the initial value (1,100 mg/m^3). Likewise, when the inlet Cu^{2+} concentration was chosen as 0.015, 0.020, 0.025 and 0.030 mg/L, respectively, it was observed that the maximum and normal working time concentrations of heterotrophic bacteria decreased. Due to the high concentrations of Cu^{2+} in the aeration tank in the initial period (Figure 4), maximum bacterial concentrations are reached quicker and are therefore inhibited, and a decrease in heterotrophic bacteria concentrations starts before the sludge removal process.

Time-dependent changes in autotrophic bacteria concentration in the aeration pond with the selected inlet concentrations of Cu^{2+} are shown in Figure 6. As in the case of heterotrophs, the autotrophic bacteria concentrations decreases while inlet Cu^{2+} concentration rises. For the Cu^{2+} concentrations greater than 0.015 mg/L, the autotrophic bacteria concentration decreases so much that nitrogen removal is impossible under those conditions (Juliastuti *et al.* 2003).

The inlet Cu^{2+} concentration of 0.010 mg/L is a critical value as seen in Figure 6. When this value is exceeded, for example at 0.015 mg/L, the Cu^{2+} concentration in the aeration tank rises to 0.5 mg/L at 500 h before sludge removal begins, and the

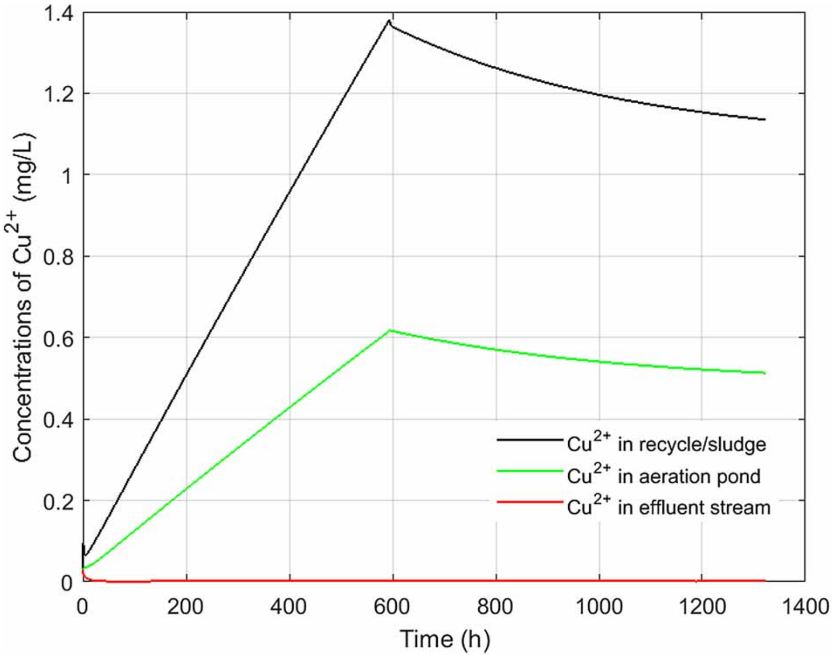


Figure 3 | The change in Cu^{2+} concentration in the conventional system.

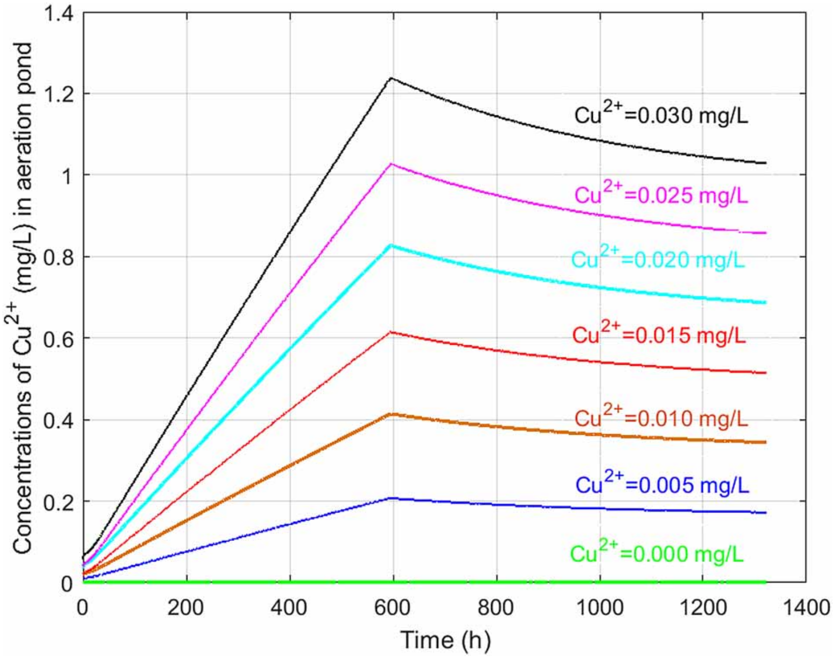


Figure 4 | The plot of Cu^{2+} concentration changes *versus* time in the aeration pond with various inlet concentrations of Cu^{2+}.

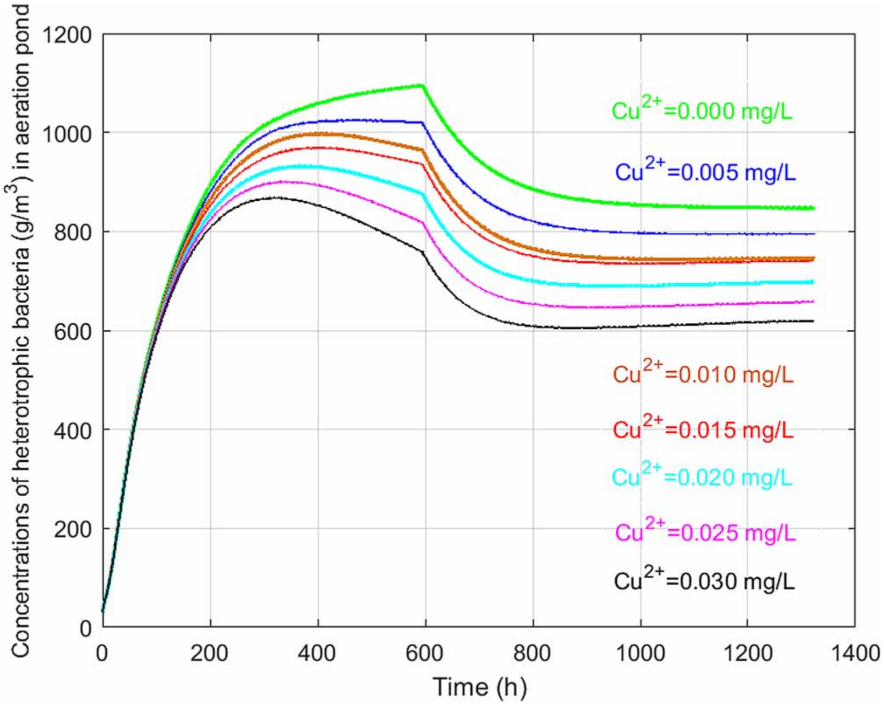


Figure 5 | Time-dependent changes in heterotrophic bacteria concentration in the aeration pond with various inlet concentrations of Cu^{2+}.

concentration of autotrophic bacteria begins to decrease due to inhibition. In the normal operating period, the concentration of autotrophic bacteria drops to 5 g/m^3, and this concentration is not sufficient for nitrogen removal. Figure 6 also shows that inlet Cu^{2+} concentrations higher than 0.015 mg/L cause complete inhibition in autotrophic bacteria.

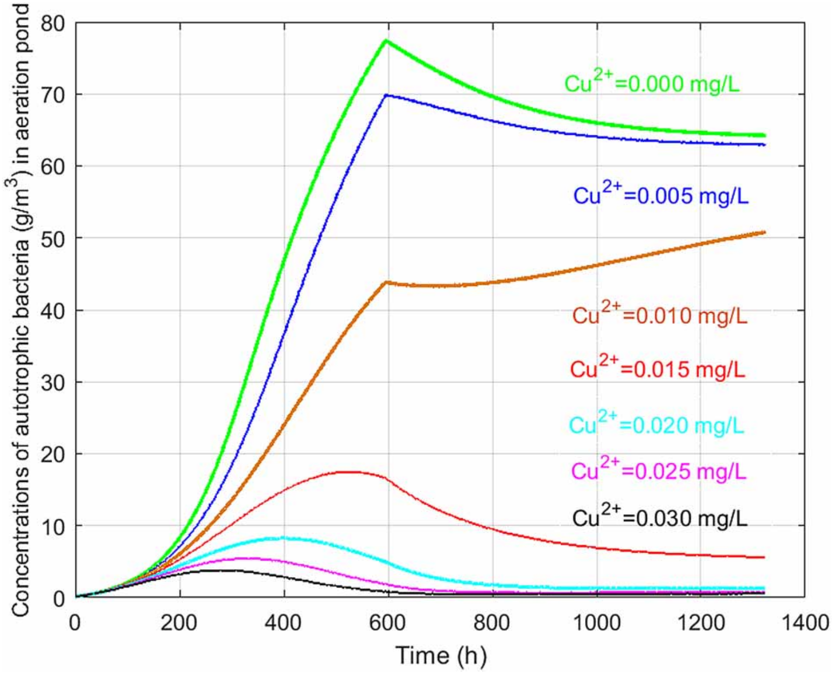


Figure 6 | Time-dependent changes in autotrophic bacteria concentration in the aeration pond with various inlet concentrations of Cu^{2+}.

The toxicity of heavy metals in an activated sludge system was investigated by Principi *et al.* (2006) for copper, zinc and nickel, and they determined that the nitrifiers had higher sensitivity than heterotrophic bacteria to these metals, and that metal accumulation capability of biomass was highest for copper case. Sun *et al.* (2016) summarized the previous studies as all heavy metals are toxic to bacterial life and inhibit the microbiological processes at moderate and high concentrations, although they stimulate microorganisms at low concentrations. Copper inhibits the heterotrophic biomass at low concentrations and is more toxic than lead, zinc and nickel. They studied the effect of copper upon the bacterial communities in activated sludge systems and their performance. The anaerobic–anoxic–aerobic process was treated with 10, 20, and 40 mg/L copper. The conclusion reached was that copper had a very important negative effect on the performance of such systems. The present study confirms that nitrifying microorganisms are more sensitive to heavy metal inhibition (in this case copper) than the microorganisms responsible for the oxidation of carbonaceous material. For this reason, alternating systems and similar ones intended for nitrogen removal are particularly affected by this inhibition. The inhibition of the autotrophic bacteria at 0.5 mg/L Cu^{+2} concentration is observed as 82% by Juliastuti *et al.* (2003), experimentally, and the simulation performed with developed algorithm in this work is 84.46% at 0.5132 mg/L Cu^{+2} concentration in the aeration pond. Therefore, it can be concluded that the proposed simulation model fits the experimental results.

CONCLUSIONS

To the influence of heavy metal presence in conventional and alternating activated sludge systems, a wastewater treatment model is developed with the help of inhibitory kinetic models – in this case, Cu^{2+}. These inhibitory kinetic models obtained from the experimental results are used in the evaluation of growth and lysis rate constants of the heterotrophic bacteria and the growth rate constant of the autotrophic bacteria in the presence of copper.

The main contribution of this study is to improve ASM3 by combining it with the other models to assess the impact of an inhibitory compound on the operation of a WWTP that receives the effluent from an industrial plant. Using a MATLAB code, the activated sludge process is simulated by coupling ASM3 with heavy metal inhibitory models as well as a settler model. The results reveal that the proposed simulation model fits the experimental results.

It can be concluded from this simulation that the presence of copper, even at a concentration of 0.5 mg/L in the biological system, 0.015 mg/L Cu^{+2} inlet, has a negative impact on the growth of heterotrophic and autotrophic bacteria. For a continuous 0.015 mg/L inlet concentration of Cu^{2+}, the heterotrophic bacteria concentration (X_H) in the aeration pond decreases by 20.07% and 12.57% in the conventional system and the alternating system, respectively, compared to the initial process containing no Cu^{2+} inlet. In the case of autotrophic bacteria (X_A), these decreases are 84.46% and 91.76%, respectively, at the end of the normal operation period in the aeration pond. It means that the addition or presence of Cu^{2+} in the biological system prevents mostly the growth of autotrophic bacteria. These bacteria are responsible for the oxidation of S_{NH} to S_{NO}. When there is no conversion to S_{NO}, it means that the conversion to S_{N2} is also inhibited and nitrogen removal could not be possible in the alternating system. In other words, the nitrification of ammonium and ammonia nitrogen is negatively affected due to the inhibition of the autotrophic bacteria growth in the presence of copper, and the conversion into N_2 upon the denitrification is prevented in the alternating system.

No important changes were observed in COD and SS (total suspended solids) in the final effluent of the conventional and alternating systems. In spite of a small change in total nitrogen value in the conventional system, it increases by 133.4% in the alternating system at concentrations of 0.5 mg/L Cu^{2+} in the aeration pond.

It can also be concluded that ASM3 can predict and evaluate the operation of an activated sludge system that receives the effluent from an industrial plant. However, this is only under the condition that the model is improved in order to include the effects of important parameters. Naturally, these parameters are subjected to characteristics of different industries and depend on the effluents they generate.

The fact that the Cu^{2+} concentration (0.0021 mg/L) in the effluent treated water is not high, causes no serious problems and implies that copper is removed from the water upon treatment in a considerable amount in the alternating system. However, the Cu^{2+} concentration in the sludge is approximately only 1.14 mg/L with 0.015 mg/L Cu^{2+} inlet concentration, and is continuously discharged to land in the wet sludge, which is an important environmental problem. The case is the similar with the conventional system.

Additional runs, in which the inlet Cu^{2+} concentration varied, reveal that Cu^{2+} concentrations greater than 0.50 mg/L (with the 0.015 mg/L inlet Cu^{2+} concentration) cause complete inhibition in alternating aerobic–anoxic systems, and that nitrogen removal is not successful, contrary to what was intended.

The results obtained in this study are consistent with the results reported in the literature. In fact, the proposed simulation model fits very well with the experimental results. For autotrophic bacteria, 82% inhibition was obtained experimentally in the presence of 0.5 mg/L Cu^{2+} (Juliastuti *et al.* 2003). This inhibition value was calculated as 84.46% with 0.5132 mg/L Cu^{2+} concentration in our simulation model.

The algorithm developed could be applied for copper and the other heavy metals and also some other inhibitory materials to understand their effects on the treatment system before the industrial wastewater containing them is connected to the urban wastewater treatment systems. Therefore, a simulation study is a good way to decide whether to include the industrial wastewater into the sewerage system or not.

An improved optimization algorithm could be used to inform decisions about which concentration of inhibitory materials can be accepted to the sewerage system and thus the collective treatment plant.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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