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Effect of acclimatization of microorganisms to heavy metals on the performance of activated sludge process

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Abstract

Although selected heavy metals (HMs) stimulate biological reactions at low concentrations, all HMs are toxic to microorganisms (MOs) at moderate concentrations and can cause inhibitory effects on the biological processes. Therefore, MOs must be acclimated to HMs or other toxic substances present in wastewaters (WWs) before they are used in an activated sludge process (ASP). In this study, combined effect of Cu^{2+} and Zn^{2+} ions in a synthetic WW on the efficiency of a laboratory-scale ASP without recycle was investigated using acclimated MOs at different extents.A synthetic feed solution of 1222 mg L^{-1} proteose-peptone (corresponding to 1300 mg COD L^{-1}) served as a source of carbon. Cu^{2+} and Zn^{2+} ions at different concentrations (1.5, 4.5 and 9, 27 mg L^{-1}, respectively) were introduced in the feed to a continuously stirred activated sludge reactor at different hydraulic residence times (2–40 h) keeping pH, temperature and stock feed composition constant. The combined effects of copper and zinc ions were determined by mixing these metallic ions at the specified combinations of concentrations such as "1.5 mg L^{-1} of Cu^{2+} + 9 mg L^{-1} of Zn^{2+}" and "4.5 mg L^{-1} of Cu^{2+} + 27 mg L^{-1} of Zn^{2+}". It was observed that using seed MOs acclimatized to two times of the combined threshold concentration of these HMs for an unduly long period of time (1–4 months) caused adverse effects on the ASP performance. Besides, it was found that usual inhibition effects of these HMs were enhanced with increasing period of acclimation. Substantially lower substrate removal efficiencies were obtained with acclimatized MOs than those obtained with non-acclimatized MOs. At the higher initial substrate concentration of 2500 mg COD L^{-1}, substrate-inhibition occurred causing a decrease in the specific growth rate constant (k); however, HM inhibition was suppressed, resulting to about 20% increase in treatment efficiency of the ASP. It can be concluded that the time period necessary for acclimatization of seed MOs must be adjusted carefully with concentrations of HMs lower than their threshold concentrations to achieve an optimal operation of an aerobic biological process.

Keywords: Activated sludge process; Bio-kinetic models; Inhibition; Threshold concentration; Acclimatization

1. Introduction

The presence of heavy metals (HMs) in wastewaters (WWs) poses an important problem for the environment as well as for the treatment process, because the disposal of both the treated water and activated sludge contaminated with HMs give rise to detrimental impacts on the environment. The aquatic life in rivers and lakes receiving these disposal streams is harmed to a great extent. Also, activated sludge fertilizers containing HMs lead to accumulation of these metals in soil and produce harmful effects on vegetation, animals and humans along the food chain [1,2].

The response of a well-known aerobic biological process, namely the activated sludge process (ASP), containing a heterogeneous mixture of microorganisms (MOs) is difficult to predict as various MOs have varying sensitivities to the factors imposed upon them. Therefore, studying the effects of HMs on the ASP becomes important in predicting the possible response of the system and in performing the necessary modifications accordingly. Although the extent and period of acclimatization of MOs to HMs are of utmost importance for a successful ASP, very few studies give detailed information on this topic. It is reported that low concentrations of HMs can stimulate biological systems by increasing the rates of reactions [3]. Further increase in HM concentration causes the inhibition of the system. Finally, the biological treatment system fails as the rate of reaction approaches to zero at relatively high metal concentrations.

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Nomenclature

a, b	constants in Eq. (9)
ASP	activated sludge process
ASR	activated sludge reactor
COD	chemical oxygen demand (mg O_2 L^{-1})
E	enzyme species
HM	heavy metal (HMs)
HRT	hydraulic residence time (h)
k	specific growth rate constant (h^{-1})
k_0	maximum specific growth rate constant (h^{-1})
k_d	decay rate of microorganisms (h^{-1})
K_m	substrate saturation constant (mg COD L^{-1})
M	total influent HM concentration in Fig. 3 (mg L^{-1})
MLSS	mixed liquor suspended solids (mg solids L^{-1})
MO	microorganism (MOs)
PAC	powdered activated carbon
Q	volumetric flow rate of feed into the reactor (L h^{-1})
r_X	biomass growth rate (mg L^{-1} h^{-1})
S	substrate concentration (mg COD L^{-1})
S_0	initial substrate concentration (mg COD L^{-1})
t	time (h)
TE	treatment efficiency, or COD removal efficiency (%)
V	reactor volume (L)
WW	wastewater (WWs)
X	biomass concentration (mg MLSS L^{-1})
X_0	initial biomass concentration (mg MLSS L^{-1})
Y	yield constant, bio-kinetic parameter

Greek symbols

θ	hydraulic residence time (h)
θ_s	sludge age (h or day)

The concentration value of a metal at which the system begins to be adversely affected by that metal is referred to as its 'threshold concentration' [4]. Some researchers claim that the metal concentration causing the complete failure of the biological system is higher than its threshold concentration by some orders of magnitude [5]. It was also stated that up to 1 mg L^{-1} copper had no adverse effect on the treatment efficiency [6]. Some others reported this value as 10 mg L^{-1} copper in a continuous feed [7], and 25 mg L^{-1} copper as a slug dose [8]. Gikas and Romanos [9] found that Cr^{6+} stimulated microbial growth for concentrations up to about 25 mg L^{-1}, exhibiting maximum growth stimulation at 10 mg L^{-1}, whilst the lethal dose was found to be between 80 and 160 mg L^{-1}. On the other hand, Cr^{3+} was also found to stimulate microbial growth for concentrations up to about 15 mg L^{-1} (with a maximum stimulation concentration at 10 mg L^{-1}), whilst the lethal dose was found to lie between 160 and 320 mg L^{-1}. The results indicated that Cr^{6+} was more toxic to biomass at relatively high concentrations (higher than 70 mg L^{-1}) whilst it had a more pronounced growth stimulation effect at relatively smaller concentrations (less than 25 mg L^{-1}), compared with effect at relatively smaller concentrations (less than 25 mg L^{-1}) of Cr^{3+}. Poon and Bhayani [8] reported that the toxicity of zinc was moderate and sewage bacteria were sustained up to a zinc concentration of 100 mg L^{-1}. However, the number of studies concerning the combined effects of HMs is relatively small. Barth et al. [10] studied the mixture of Cr, Cu, Ni and Zn, and concluded that no significant effects on the efficiency of the system were observed. Cabrero et al. [11] investigated the combined effects of Cu^{2+} and Zn^{2+} ions on the activated sludge growth kinetics during batch growth experiments and reported that these two HMs acted neither synergistically nor antagonistically. They could fit the experimental data to a sigmoidal model, including a lag period with almost no change in the growth rate constant due to the inhibition caused by HMs, since the Monod model could not provide an adequate description of microbial growth behavior due to the mixed culture character of activated sludge.

Inactivation of one or more critical enzymes by the HMs in the medium is the most widely held theory used to explain the effects of inhibition levels of HMs to the MOs [1]. The readjustment of the enzyme balance in the cell to provide extra enzymes in replacing the damaged enzymes or the construction of new shunt pathways around the inhibited enzymes [12] depends on the acclimation capacity of MOs, since they have limited amount of energy to utilize for enzyme readjustment. There are many studies with acclimated MOs, but the acclimation procedures are not well explained in most of these studies. On the contrary, Yurt [13] and Beyenal et al. [14] clearly described the acclimation procedure and its duration. It was reported that MOs acclimated to two times of the combined threshold concentration of copper and zinc exhibited good performance [14]. The studies performed by the research group [13–16] of the present authors showed that the period of time passing for acclimatization between the sets of experiments affected the kinetic behavior of the system. The objective of this paper is to explain qualitatively and quantitatively how the time period of acclimatization affects the performance of the ASP.

2. Experimental

2.1. Experimental set-up

Two parallel laboratory-scale once-thro' activated sludge systems (without recycle) (Cole Parmer Applikon-Z611000004, Holland, and Gallenkamp FER-195-020W, England) were used in the experiments. The working liquid volumes of the completely mixed reactors are 2 and 1 L in the Cole Parmer and Gallenkamp modular series, respectively.

Peristaltic pumps equipped with silicon tubings were used for reactor feeding and discharge. Aeration and efficient mixing were provided in both reactors. The temperature and pH within the activated sludge units were controlled at 25 ± 1 °C and 7 ± 0.1, respectively. The pH of the medium was kept constant around the specified value by a controller; two peristaltic pumps were used to feed 0.1N H_2SO_4 or 0.1N NaOH automatically to the reactor. In the experiments, a synthetic WW was used with a composition given in Table 1. Proteose-peptone, at a concentra-

Table 1
Composition of synthetic wastewater

Constituents	Concentration (mg L^{-1})
Proteose-peptone	1221.7
NaCl	407.4
Na_2SO_4	44.6
K_2HPO_4	44.6
$MgCl_2{\cdot}6H_2O$	3.7
$FeCl_2{\cdot}2H_2O$	3.7
$CaCl_2{\cdot}2H_2O$	3.7
$MnSO_4$	0.057
H_2MoO_4	0.031
NaOH	0.008
$ZnSO_4$	0.046
$CoSO_4$	0.049
$CuSO_4$	0.076

tion of 1221.7 mg L^{-1} corresponding to S_0 = 1300 mg COD L^{-1}, was the source of organic carbon. In adjusting the studied concentrations of Cu^{2+} and Zn^{2+} ions in the inhibition experiments and in the experiments devoid of heavy metals (Cu^{2+} = 0 and Zn^{2+} = 0), minute amounts of copper and zinc as nutrients in the synthetic feed solution (Table 1) were accounted for.

2.2. Experimental method

A mixed culture of MOs from a sewage treatment plant was used in the experiments. The MOs were acclimated to the metals up to two times their threshold concentrations, which were 15 and 90 mg L^{-1} for $Cu^{2+}$and Zn^{2+}, respectively from the literature [8]. Acclimatization procedure is given in detail elsewhere [13]. These experiments were started with the inoculation of the synthetic WW (S_0 = 1300 mg COD L^{-1}) devoid of HM, with 5% (v/v) acclimated seed MOs in the reactor, which was operated batchwise for 3 days until a healthy culture was obtained. Then, the continuous operation was started by feeding the synthetic WW at a desired volumetric flow rate corresponding to the desired hydraulic residence time. After operating the system for about 12–44 days, depending on the residence time, a steady-state was established, and the effluent was analyzed for the substrate (S), and biomass (X) concentrations. The concentration of substrate was determined in the centrifuged sample aliquots by standard COD analysis and the biomass concentration was determined gravimetrically [17]. At each residence time, a steady-state was reached when the effluent S and X concentrations remained constant with respect to time, within ±4% in S and ±10% in X.

The following sets of experiments were performed with the synthetic WWs containing HMs at different concentrations keeping the inlet substrate concentration at 1300 mg COD L^{-1}. The experiments were repeated also at the inlet substrate concentration of 2500 mg COD L^{-1} for the chosen metal concentrations of Cu^{2+} and Zn^{2+}.

2.3. Experiment sets

- Set-1: S_0 = 1300 mg COD L^{-1}, Cu^{2+} = 0 mg L^{-1}, Zn^{2+} = 0 mg L^{-1} non-acclimatized seed [13,14].
- Set-2: S_0 = 1300 mg COD L^{-1}, Cu^{2+} = 1.5 mg L^{-1}, Zn^{2+} = 9.0 mg L^{-1} acclimatized seed [13,14].
- Set-3: S_0 = 1300 mg COD L^{-1}, Cu^{2+} = 0 mg L^{-1}, Zn^{2+} = 0 mg L^{-1} acclimatized seed [15].
- Set-4: S_0 = 1300 mg COD L^{-1}, Cu^{2+} = 4.5 mg L^{-1}, Zn^{2+} = 27 mg L^{-1} acclimatized seed [15].
- Set-5: S_0 = 1300 mg COD L^{-1}, Cu^{2+} = 0 mg L^{-1}, Zn^{2+} = 0 mg L^{-1} acclimatized seed [16, this work].
- Set-6: S_0 = 2500 mg COD L^{-1}, Cu^{2+} = 4.5 mg L^{-1}, Zn^{2+} = 27 mg L^{-1} acclimatized seed [16, this work].

2.4. Data analysis

The treatment efficiency is calculated from Eq. (1):

$$\mathrm{TE}\,(\%) = \frac{100(S_0 - S)}{S_0} \tag{1}$$

The bio-kinetic parameters k_0 and K_m are obtained from the intercept and slope of (1/k) versus (1/S) plot (Lineweaver–Burk plot), respectively, according to Eq. (2) [18]:

$$\frac{1}{k} = \frac{K_m}{k_0 S} + \frac{1}{k_0} \tag{2}$$

The yield factor Y, and the MO decay rate constant k_d are obtained from the "$(S_0 - S)/X$ versus θ" graph according to Eq. (3) [18]:

$$\frac{S_0 - S}{X} = \frac{k_d \theta}{Y} + \frac{1}{Y} \tag{3}$$

The relationships among the hydraulic residence time, decay rate of MOs and the net specific growth rate of biomass can be obtained from the material balances written for biomass and substrate around the activated sludge reactor (ASR) as follows

$$V\left(\frac{dX}{dt}\right) = QX_0 - QX + (r_X - k_d X)V \tag{4}$$

where V is the volume of ASR; Q the inlet volumetric flow rate; X the biomass concentration in the ASR, and in the effluent from the ASR; r_X the biomass growth rate; X_0 the inlet MO concentration to the ASR; k_d the decay rate constant for MOs; $\theta = V/Q$ is the hydraulic residence time. The substrate balance around the ASR is given as

$$V\left(\frac{dS}{dt}\right) = QS_0 - QS + (r_S)V \tag{5}$$

At the steady-state, the substrate consumption rate ($-r_S$) becomes

$$-r_S = \frac{S_0 - S}{\theta} \tag{6}$$

The relationship between growth rate of MOs and substrate consumption rate is

$$r_X = Y(-r_S) \tag{7}$$

For a steady-state condition, the following equation can be obtained from Eqs. (4)–(7):

$$k = Y\frac{S_0 - S}{\theta X} - k_d \quad (8)$$

3. Results and discussion

3.1. Effect of acclimatization

For each run, the net specific growth rate constants were calculated from Eq. (8), after determining Y and k_d from the intercept and slope of the line plotted according to Eq. (3). The bio-kinetic behavior of the ASP for the Sets (1–6) are shown in Fig. 1, as "k versus S" plots for the purpose of comparison. These sets of results were obtained from the studies conducted by our research group [13–16] in the course of time.

The bio-kinetic data of Set-1 (devoid of HMs in the synthetic WW, with non-acclimated MOs) and Set-5 (devoid of HMs in the synthetic WW, with acclimated MOs) experiments fitted to the Monod model. In Set-1, the bio-kinetic constants Y and k_d were determined as 0.34 mg MLSS/mg COD and 0.0008 h^{-1}, respectively. The other bio-kinetic constants k_0 and K_m were 0.33 h^{-1} and 187 mg COD L^{-1}, respectively, from the Lineweaver–Burk plot [18]. However, in Set-5, the bio-kinetic constants were determined as $Y = 0.32$ mg MLSS/mg COD, $k_d = 0.035\ h^{-1}$, $k_0 = 0.56\ h^{-1}$ and $K_m = 789$ mg COD L^{-1}. As it is seen, the bio-kinetic constants k_0, K_m and k_d increase while Y slightly decreases in the case of acclimatized seed (Set-5). An increase in k_0 may be a deceiving result; it may be due to the release of additional substrate to the reaction medium from the decomposition of dead cells, because the increasing death rate of cells is indicated by the greater decay rate constant of the Set-5 experiments. For an unduly long period of acclimatization, it is apparent that readjustment and recovery of damaged enzyme balance in the cell could not be provided due to the limited acclimation capacity (energy) of the MOs [1]. Also, a higher value

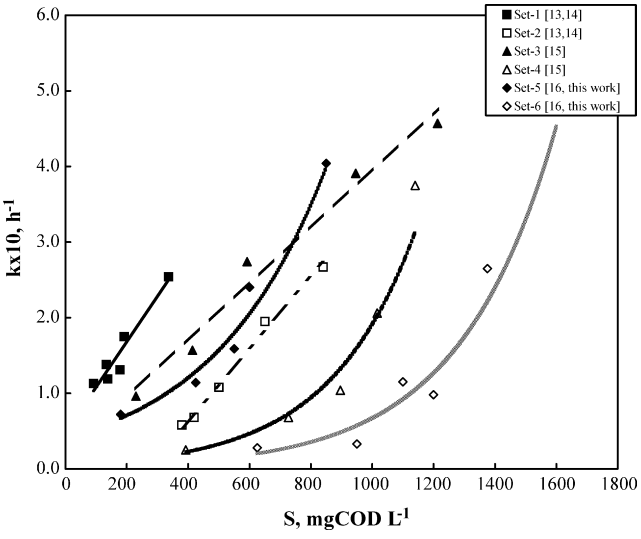

Fig. 1. Net specific growth rate constant "k" vs. steady-state effluent substrate concentration "S", for different sets of experiments.

Table 2
Comparison of treatment efficiencies of Set-1 and Set-5

Θ (h)	Set-1		Set-5		ΔTE (%)
	TE (%)	X (mg L^{-1})	TE (%)	X (mg L^{-1})	
8.13	89.76	351	67.3	230	22.46
4.06	74.15	315	53.84	200	20.31

of K_m in Set-5 shows that the specific growth rate becomes less sensitive to the changes in the substrate concentration due to the damaged enzyme balance in the MO cells, supported by the lower treatment efficiencies in Set-5 compared with those of Set-1 (Table 2). Treatment efficiencies (%TEs) in terms of %COD removals, and MO concentrations at almost the same residence times of Set-1 and Set-5 experiments are given in Table 2; the treatment efficiencies are about 20% smaller for the ASP using acclimatized MOs [15] than those using non-acclimatized ones [13,14] at the same hydraulic residence times (HRTs).

Comparing the kinetic behavior of the Set-1 with that of the Set-5 further, the negative effect of long acclimatization period was easily observed in Fig. 1 with the resulting lower net growth rate constants (k values) at all the steady-state effluent substrate concentrations (S values). Since the effluent S concentration was a dependent variable, it was different from run to run depending on the operated hydraulic residence time (HRT). Only the inlet substrate concentration and HRT were the independent variables that could be chosen as desired. At the start of this research, the range of HRTs was chosen as 3–8.5 h in the Set-1; then in the continuation of the research, this range was decided to be widened from 2.5 to 40 h; therefore, comparisons should be made at almost the same HRTs, everything else being the same, rather than at the same effluent substrate concentrations, which varied depending on the operating conditions. Exponential relationship replaced the linear relationships observed in "k versus S" plots as the extent of acclimation increased. In the Set-1, Set-3 and Set-5 experiments, the first two yielded linear relationships as the fitted equations to the data will be given in the next section, while the Set-5 results show a good fit to an exponential model. The inlet substrate concentration in the wastewaters was kept almost the same (around 1300 mg COD L^{-1}) in all the experiments except in the Set-6, where the effect of a higher inlet substrate concentration ($S_0 = 2500$ mg COD L^{-1}) on the kinetic behavior of the system was investigated.

3.2. Combined effects of heavy metals

It was observed that the color of mixed liquor in the reactor exhibited changes. In the experiments devoid of HMs (Sets 1, 3 and 5), the color of the mixed liquor was brownish yellow; however, in the Set-2 experiments (at 1.5 mg L^{-1} of Cu^{2+} and at 9.0 mg L^{-1} of Zn^{2+}), color of the mixed liquor turned to greenish yellow. Also, formation of filamentous organisms was detected [13,14]. These were the signs of adverse effects of HMs on the ASP. However, as seen in Fig. 1, "k versus S" plot of the Set-2 still showed a good fit to a linear relationship, which suggested that MOs acclimated for a relatively shorter period of

time and at the lower concentration levels of the influent HMs, were not severely damaged. At the higher concentration levels of the influent HMs (i.e., 4.5 mg L^{-1} of Cu^{2+} and 27 mg L^{-1} of Zn^{2+}, in the Set-4), the color of the mixed liquor in the reactor further changed; it became dark and approximated to dirty light green. This adverse effect was also observed by the changing "k versus S" relationship from the linear to an exponential one with the increasing concentrations of the influent HMs (Fig. 1).

The kinetic data from the experimental Sets of 2, 4 and 6 (Fig. 1) did not fit to the Monod model in the presence of HMs, using acclimatized seed MOs, under severe conditions of two times the threshold concentrations of HMs for a long period of time (1–4 months). Application of the Monod model to the data (Sets 2, 4 and 6) yielded physically unacceptable negative biokinetic parameters (k_0 and K_m) in the Lineweaver–Burk plots. As a matter of fact, it was known that the Monod equation was applicable to a single type of microorganism growing in a well-defined medium where all nutrients were in excess except for carbon source, which was the limiting substrate [11]. Therefore, it was not a successful attempt to extend the Monod model to more complex systems of wastewater treatment, where many enzymes released by various MOs were inhibited in different ways by the toxic compounds [19]. Also Cabrero et al. [11] reported that a sigmoidal model rather than a Monod model was acceptable; in the sigmoidal model, k versus S plots show an S-shaped behavior including the lag period in the microbial growth due to inhibition caused by HMs, while the lag period is not incorporated in the Monod model. Additionally it would be worth to mention here that in the work by Tyagi [20], the inhibitory effects of HMs on the microbial growth in wastewater were described in terms of the variation of net specific growth rate of MOs [19,20] as it was done in this study.

As is understood from the present results, the literature values for the threshold concentrations of copper (15 mg L^{-1}) and zinc (90 mg L^{-1}) used in the acclimation procedure were unnecessarily high; Juliastuti et al. [19] also have reported that the inhibition threshold concentrations of HMs available in the literature were significantly overestimated values compared to their experimental findings. Another supporting evidence to the above argument was the results reported by Vismara [21] where the determined concentration ranges for Cu^{2+} and Zn^{2+} inhibitions of nitrification were at very low concentration levels of 0.005–0.5 and 0.08–0.5 mg/L, respectively.

The above-given information from the recent literature [19–22] supported that the application of an acclimatization procedure at very high concentrations of the studied HMs for an unduly long period of time probably yielded highly denatured MOs, which caused significant changes in the microorganism population and in their enzymatic pathways, thus complicating the analysis of the experimental results. Also, more than one metal often gave rise to interactive effects, which were extremely complex [19]. Cabrero et al. [11] investigated the individual and combined effects of copper (Cu^{2+}) and zinc (Zn^{2+}) on the activated sludge growth phenomena. Data at four different zinc concentrations ranging from 1 to 20 mg L^{-1}, a lower MLSS concentration was observed for all the experiments in comparison

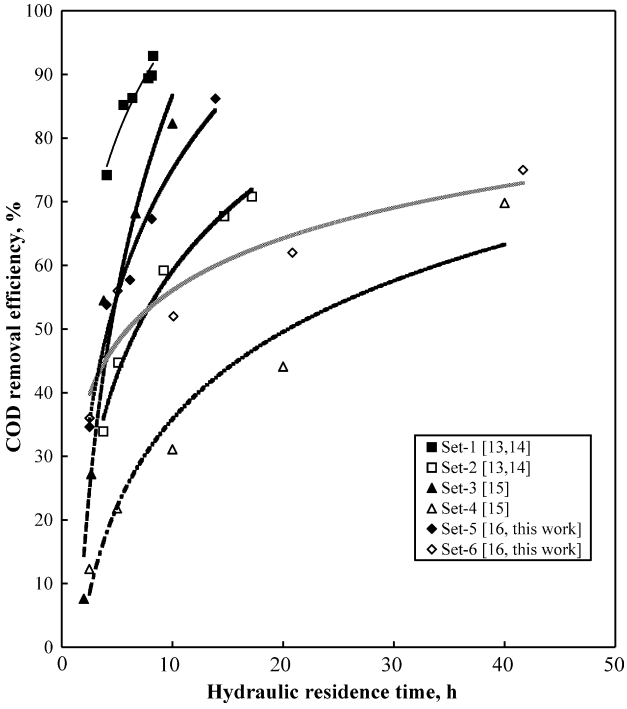

Fig. 2. COD removal efficiency (%) vs. hydraulic residence time.

with the reference (control) reactor except the one performed with 1 mg L^{-1} of zinc, where a slight stimulating effect was detected. Results indicated that 10 mg L^{-1} of zinc was more than enough to inhibit sludge growth. When the individual effect of copper was studied, no copper concentration causing any stimulation was observed, as happened with 1 mg L^{-1} of zinc; comparatively, the toxic inhibitory effect of copper was found to be considerably higher than that of zinc in all the experiments. Also sharp decreases in the maximum growth rate and biomass yield parameters were observed for the wastewater containing over to 5 mg L^{-1} of Cu^{2+} ions, showing that this concentration was the limit at which microbial growth began to be substantially inhibited by copper. Cabrero et al. [11] concluded that combinations of copper and zinc introduced to the wastewater might produce serious upsets in the biological treatment system, especially when the main metal-dosed in the system was copper. Besides, Utgikar et al. [23] reported that the effect of copper was qualitatively and quantitatively different from that of zinc. The toxic effect of copper unboundedly (exhibited no maximum) and exponentially increased with concentration. On the other hand, the toxic effect of zinc indicated an asymptotic exponential rise to a maximum with concentration. The inhibitory effects of the binary mixtures of copper and zinc were reported to be substantially higher than those of the individual metals, indicating synergistic interactions between the two metals. It was also concluded that further work was essential for predicting the toxic effect of metal mixtures [23].

In Fig. 2, %COD removal efficiency (%TE) versus HRT plots are shown for all the experimental sets. The treatment efficiencies in the experiments devoid of HMs are higher, and the treatment efficiencies decrease with the increasing time of acclimation and increasing concentrations of HMs in the influent

wastewaters. The curve for the Set-6 is above that of the Set-4, showing the beneficial effect of the increased influent substrate concentration in decreasing the HM inhibition. However, the increase in treatment efficiency with the increasing HRT is at a very slow rate; because the curve reaches a plateau with time due to the mechanism of substrate inhibition [24]. In all the sets of experiments, it is observed that the COD removal efficiency increases with the increasing sludge age [1] or HRT, which is equal to sludge age for an ASP without recycle.

3.3. Effect of influent substrate concentration

Experiment Set-6 was aimed to observe the effects of HMs on the ASP at a higher inlet substrate concentration of 2500 mg COD L^{-1}, keeping Cu^{2+} and Zn^{2+} concentrations at the same levels of 4.5 and 27 mg L^{-1}, respectively, as in the Set-4 experiments. The influent wastewater strength was reported in the previous studies [1,25,26] as one of the three important independent variables in the formation of metal–enzyme complexes; it was further explained that the effect of inhibitory levels of metal cations on MOs showed itself when the cation reacted with active cellular components such as respiratory enzymes to form stable inactive complexes. The two other variables were metal concentration, mixed liquor suspended solids (MLSS) besides the influent substrate concentration in the wastewater. The relative concentrations of these variables affected the proportion of cellular enzymes tied up in forming complexes with metals, thus changed the extent of metal toxicity [1,26]. It was claimed that these inactive enzymes might be reversed to an active form by dilution of the metals or by the addition of uncomplexed ligands or chelating agents to the medium. In other words, a decrease in influent metal concentration, an increase in MLSS, or an increase in substrate concentration of the influent wastewater would decrease the proportion of active cellular enzymes that were tied up as complexes with the metals and thereby would yield better performance of the biological process [1,26]. As understood from this widely accepted theory, the effect of HMs was not only a function of the total influent HM concentration, but also a function of the ratio of total HM concentration to the steady-state biomass concentration. Fig. 3 shows %COD removal efficiency versus ratio of total influent HM concentration to steady-state biomass concentration (*M*/*X*, as mg metal/g dry solids) for the Sets 2, 4 and 6. Also, a comparison of %TEs and steady-state biomass concentrations at almost the same residence times are shown in Table 3 for the Set-4 and Set-6 experiments, where the results indicate about 20% increase in the TE, at the higher substrate level of 2500 mg COD L^{-1}, keeping all the other parameters the same. This is due to the increase in the steady-state biomass concentrations, precipitation and complexation of heavy metals at the higher substrate level, which resulted in relatively lower HM concentration in the liquid phase. The results of the present study are found to be in agreement with the literature, as seen in Fig. 3, where the COD removal efficiency increases with the decreasing *M*/*X* ratio. The higher treatment efficiencies in the Set-6 experiments can be attributed to the suppression of HM inhibition at the higher level of influent substrate concentration (S_0 = 2500 mg COD L^{-1}). Thus, the curve for the Set-6 obtained at the higher levels of influent HMs almost coincided with that for the Set-2 at the lower levels of the HMs. A similar effect was observed by Ong et al. [22] with the addition of powdered activated carbon (PAC) into the reactor, where the combined toxic effects of Cu^{2+} and Cd^{2+} were reduced in accordance with the above-given theory. On the other hand, the increase of the influent substrate concentration from 1300 to 2500 mg COD L^{-1} keeping the inlet metal concentrations the same, most probably caused substrate inhibition resulting in the lower *k* values for the Set-6 in comparison with those for the Set-4 (Fig. 1), due to the possible formation of inactive complexes between excess substrate molecules and enzymes; because substrate inhibition being a special case of uncompetitive inhibition, occurs at high substrate concentrations in about 20% of all known enzymes. It is primarily caused by more than one substrate molecule binding to an active site meant for just one, often by different parts of the substrate molecules binding to different subsites within the substrate binding site. If the resultant complex is inactive, this type of inhibition causes a reduction in the rate of reaction at high substrate concentrations [24]. This point may need further investigation to be clarified.

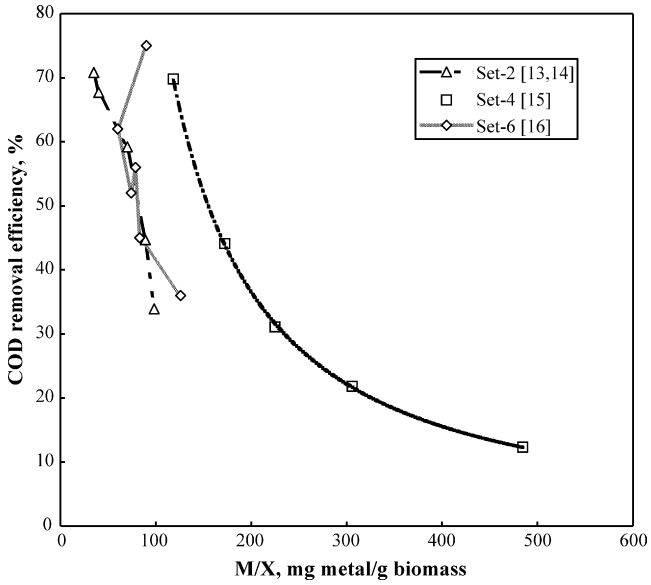

Fig. 3. COD removal efficiency (%) vs. (*M*/*X*) ratio.

Table 3
Treatment efficiencies of Set-4 [15] and Set-6 [16] showing the effect of inlet substrate strength at the same influent metal concentrations

Θ (h)	Set-4		Θ (h)	Set-6		ΔTE (%)
	TE (%)	X (mg L^{-1})		TE (%)	X (mg L^{-1})	
40	69.84	266	41.66	75	350	5.16
20	44.07	183	20.83	62	525	17.93
10	31.07	140	10.10	52	400	20.93
5	21.84	103	5.05	56	475	34.16
2.5	12.31	65	2.52	36	250	23.69

3.4. Models fitted to experimental data

The best-fitting model equations to the data of each experimental set were obtained in terms of the variation of net specific growth rate constant (k) of MOs and steady-state effluent substrate concentration (S). These results showed that the data of Sets (1–3) experiments fitted to a line equation, while those of Sets (4–6) suggested an exponential relationship as

$$k = a\exp(bS) \quad (9)$$

where a and b are constants. These obtained relationships can be given with their corresponding regression coefficients, R^2 as follows

$$\text{Set-1}: \quad k = 0.006S + 0.4795, \qquad R^2 = 0.9247 \quad (10)$$

$$\text{Set-2}: \quad k = 0.0047S - 1.2496, \qquad R^2 = 0.9925 \quad (11)$$

$$\text{Set-3}: \quad k = 0.0038S + 0.1883, \qquad R^2 = 0.9779 \quad (12)$$

$$\text{Set-4}: \quad k = 0.0558\exp(0.0035S), \qquad R^2 = 0.9749 \quad (13)$$

$$\text{Set-5}: \quad k = 0.4148\exp(0.0027S), \qquad R^2 = 0.9664 \quad (14)$$

$$\text{Set-6}: \quad k = 0.0279\exp(0.0032S), \qquad R^2 = 0.9119 \quad (15)$$

In analyzing these equations, it seems logical to argue that the behavior fits to a linear relationship when the influent HM concentrations are either zero as in the Set-1 and Set-3 or have very low values as in the Set-2, where the negative intercept indicates still the existence of HM inhibition and therefore the inadequacy of the Monod model. The exponential relationship in the case of the Set-5 data, in spite of the absence of any HM ions in the influent, may be due to the adverse effect of the longest period of acclimation for MOs used in those experiments. In comparing Eqs. (13)–(15), it can be seen that the constant "b" in the exponential model equation, Eq. (9), may be a factor reflecting the HM inhibition effect depending on the influent HM concentrations; the constant of 0.0027 in the Set-5 (Eq. (14)) is smaller than both of the values of 0.0032 and 0.0035 in Eqs. (15) and (13), respectively. The slightly smaller value of 0.0032 than 0.0035 may be attributed to the suppression of HM inhibition by the higher influent substrate concentration in the Set-6 compared to that in the Set-4, although the influent metal concentrations were the same in both of these sets of experiments. The other constant "a" in Eq. (9) may be a factor reflecting the extent of damage and denaturing of enzymes and enzymatic pathways due to the prolonged and severe acclimation of MOs (the constant being 0.4148 in the Set-5); it is reduced to 0.0558 due to the additional adverse effects of HMs at the higher level of influent metal concentrations ($Cu^{2+} = 4.5\,mg\,L^{-1}$, $Zn^{2+} = 27\,mg\,L^{-1}$, $S_0 = 1300\,mg\,COD\,L^{-1}$, in the Set-4). The constant "a" becomes even lower, such as 0.0279 in Eq. (15) due to the additional effect of substrate inhibition probably occurring in the Set-6 ($Cu^{2+} = 4.5\,mg\,L^{-1}$, $Zn^{2+} = 27\,mg\,L^{-1}$, $S_0 = 2500\,mg\,COD\,L^{-1}$), at the higher level of substrate concentration, keeping the influent HM concentrations the same as those in the Set-4.

These expressions can be used for the practical purposes such as in the scale-up, design and simulation studies of similar systems.

4. Conclusions

The effect of acclimatization of microorganisms to heavy metals (HMs) on the performance of activated sludge process was investigated. A synthetic feed solution of 1222 mg/L proteose-peptone (corresponding to 1300 mg COD L^{-1}) served as a source of carbon. The combined effects of copper and zinc ions were determined by introducing these metallic ions at the specified combinations of concentrations such as "1.5 mg L^{-1} of Cu^{2+} + 9 mg L^{-1} of Zn^{2+}" and "4.5 mg L^{-1} of Cu^{2+} + 27 mg L^{-1} of Zn^{2+}" into the wastewater fed continuously to the stirred activated sludge reactor at different hydraulic residence times (2–40 h), at a temperature of 25 ± 1 °C and pH of 7 ± 0.1. It was observed that using seed MOs acclimatized to two times of the combined threshold concentration of these HMs for an unduly long period of time (1–4 months) caused adverse effects on the ASP performance. Besides, it was found that usual inhibition effects of these HMs were enhanced with the increasing period of MO-acclimation. Substantially lower substrate removal efficiencies were obtained with acclimatized MOs than those obtained with non-acclimatized MOs. At the higher initial substrate concentration of 2500 mg COD L^{-1}, substrate-inhibition occurred causing a decrease in the specific growth rate constant (k); however, HM inhibition was suppressed, showing itself with about 20% increase in treatment efficiency of the ASP.

Experimental data obtained at different operating conditions (e.g., with and without HMs in the feed, low and high influent substrate concentrations, various values of hydraulic residence time) were fitted to the model equations with the regression coefficients being in the range of 0.92–0.99. A linear relationship between net specific growth rate constant of MOs (k) and the effluent steady-state substrate concentration (S) obtained with non-acclimated MOs and a wastewater devoid of HMs, was replaced by an exponential model of $k = a\exp(bS)$, showing the best fit to the data obtained with the MOs acclimatized for longer time durations and at the higher influent HM and substrate concentrations in the feed wastewaters. The results suggest that the model parameter "a" may get smaller values with the increasing damage and degree of denaturing in MOs, enzymes and enzymatic pathways due to severe and long acclimation procedure, with the increasing HM and substrate concentrations in the influent wastewaters, while the other parameter "b" may be used as a factor quantifying the effect of HM inhibition depending on the influent HM concentrations. Its value may increase directly with the degree of HM inhibition.

It can be concluded that the time period necessary for acclimatization of seed MOs must be adjusted carefully with concentrations of HMs lower than their threshold concentrations to achieve an optimal operation of an aerobic biological process.

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