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Effects of nickel(II) addition on the activity of activated sludge microorganisms and activated sludge process

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Abstract

The effects of Ni(II) in a synthetic wastewater on the activity of activated sludge microorganisms and sequencing batch reactor (SBR) treatment process were investigated. Two parallel lab-scale SBR systems were operated. One was used as a control unit, while the other received Ni(II) concentrations equal to 5 and 10 mg/l. The SBR systems were operated with FILL, REACT, SETTLE, DRAW and IDLE modes in the time ratio of 0.5:3.5:1.0:0.75:0.25 for a cycle time of 6 h. The addition of Ni(II) into SBR system caused drastically dropped in TOC removal rate (k) and specific oxygen uptake rate (SOUR) by activated sludge microorganisms due to the inhibitory effects of Ni(II) on the bioactivity of microorganisms. The addition of 5 mg/l Ni(II) caused a slight reduction in TOC removal efficiency, whereas 10 mg/l Ni(II) addition significantly affected the SBR performance in terms of suspended solids and TOC removal efficiency. Termination of Ni(II) addition led to almost full recovery of the bioactivity in microorganisms as shown in the increase of specific oxygen uptake rate (SOUR) and SBR treatment performance.

Keywords: Activated sludge; Nickel; Sequencing batch reactor; Inhibitory effect; SOUR

1. Introduction

Considerable work has been done on the capability of activated sludge cultures to remove variety of heavy metals. Pb(II), Cu(II) and Zn(II) are removed relatively efficient by activated sludge microorganisms, whereas Cd(II), Ni(II) and Mn(II) are removed poorly [1,2]. Removal of heavy metals at low concentrations by the activated sludge process is particularly effective, producing effluents with minimal metal contamination. However, a loss in activated sludge viability, changes in sludge community structure [3], loss of floc structure [3,4], and/or decreases in treatment efficiency [3–6] may occur at high concentrations of heavy metals.

The deleterious effects of toxicants on biological processes are complex and generally related to the species, the solubility of the metal and the concentration of the toxicant. Toxicity may also depend upon the influent matrix such as pH, concentration of other cations and/or molecules present, suspended solids, and upon operational parameters such as sludge age [7–10].

Some investigations have indicated that the tolerance of biological treatment systems to heavy metals can be enhanced greatly by proper acclimation [9,11]. Yetis and Gokcay [12] have reported that the substrate removal efficiency of acclimatized activated sludge process was not adversely affected by the presence of Ni(II) up to a concentration of 10.0 mg/l, while 5.0 mg/l Ni(II) had some stimulatory effects.

The first stage of metal adsorption by activated sludge is rapid uptake between 3 and 10 min, in which a large quantity of metal ions is adsorbed by the cell flocs. The second stage is a slow phase, which may extend over many hours [13,14]. Mechanisms proposed for metal removal in activated sludge include physical trapping of precipitated metals in the sludge floc matrix, binding of soluble metal to extracellular polymers [15], accumulation of soluble metal by the cell, and volatilization of metal to the atmosphere.

The biosorption of heavy metal ions by activated sludge in biological wastewater treatment system could be used as an

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alternative method for heavy metal removal. As a result, it would reduce the toxic sludge generation, chemical and energy consumption, and operation cost. However, before the using of activated sludge as a biosorption for heavy metal removal, the toxic effects of metal on the bioactivity of activated sludge microorganisms and the biological wastewater treatment performance should be carried out carefully. The objective of this research is to evaluate the toxic effects of Ni(II) on the activity of activated sludge microorganisms and sequencing batch reactor (SBR) treatment performance. The inhibitory effects of Ni(II) on activated sludge microorganisms was measured by monitoring the changes of specific oxygen uptake rate (SOUR). Besides, the bioactivity and SBR system recovery were also investigated by the termination of Ni(II) addition.

2. Materials and methods

2.1. Activated sludge system operation

Two identical 5-l sequencing batch reactors (SBR 1 and SBR 2) were used to simulate activated sludge process and to provide biomass for the metal uptake experiments. In each reactor, aeration and efficient mixing were provided during FILL and REACT modes by using porous air diffusers. The temperature within the activated sludge units was kept at 25 ± 1 °C using a water bath. The SBRs were operated in 6 h per cycle and four cycles per day. Each cycle consisted of five stages: FILL (30 min), REACT (3.5 h), SETTLE (1 h), DRAW (45 min) and IDLE (15 min). In each cycle, 3 l of synthetic wastewater was added in the SBR systems during FILL mode and the same amount of treated effluent was removed during DRAW mode after settling for 1 h. The operating cycle was automated with timer-controlled feed, draw-off and aeration pumps. The SBR schematic diagram is shown in Fig. 1.

The activated sludge seed was obtained from a municipal wastewater treatment plant that received no industrial wastewater. It was acclimatized in the laboratory for 1 month by feeding it with synthetic wastewater. The synthetic wastewater consisting of base mix of bacto-peptone, sucrose, nutrients and buffer solution of the following composition (concentrations in mg/l): bacto-peptone (188), sucrose (563), NH_4Cl (344), $MgSO_4$ (49), $FeCl_3$ (11.3) and KH_2PO_4 (318) giving a TOC of 270 mg/l. The pH value of the base solution was found to be in the range of 6.3–7.1. During the treatment process, the pH was relatively unchanged.

When the systems were acclimatized to the feed, as indicated by a stable TOC removal efficiency and MLSS concentration (<10% variation), nickel(II) sulphate hexahydrate ($NiSO_4 \cdot 6H_2O$, Wako) solution was added to the synthetic wastewater to provide a constant concentration of 5 mg/l to the influents of the SBR 1. After 22 days, the Ni(II) concentration in the feed solution was increased to 10 mg/l and the reactor was operated for a further 22 days. At the end of this period, no Ni(II) was added to SBR 1 for a period of 25 days in order to investigate the recovery capability of the system. The SBR 2 was used as a control system in this studied.

The treated effluents collected from the DRAW mode in each cycle were analyzed for total organic carbon (TOC) and Ni(II) concentrations. The TOC concentration was determined by using TOC analyzer whilst Ni(II) concentration was determined by using ICPS-7000 (Shimadzu). The determination of mixed liquor suspended solid (MLSS) and mixed liquor volatile suspended solid (MLVSS) concentrations followed the standard methods.

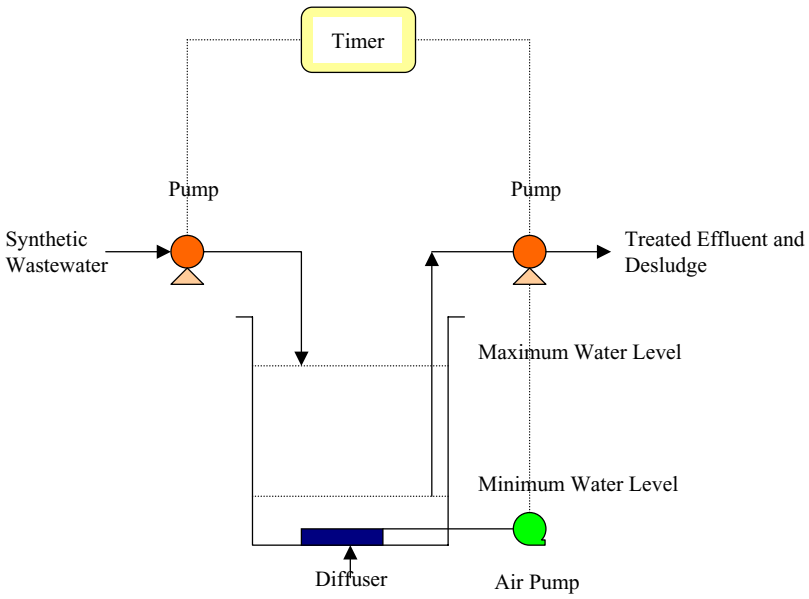


Fig. 1. Sequencing batch reactor diagram.

2.2. Sorption test

The adsorbents used in this research were powdered activated carbon (Charcoal, Powder, Wako) and activated sludge. The activated sludge was obtained from SBR system. Ni(II) uptake kinetics was studied at 25 ± 1 °C, in 120 ml plastic bottles by monitoring the soluble Ni(II) content, at appropriate time intervals for approximately 9 h, until further depletion of the Ni(II) concentration became insignificant.

The Ni(II) binding capacity of the adsorbents was determined by shaking 0.05 g of PAC or activated sludge in 100 ml of Ni(II) solution with concentration varying from 10 to 100 mg/l for a contact time of 5 h. The sorption mixture was filtered and the filtrate was analyzed for its Ni(II) concentration using ICPS-7000 (Shimadzu).

2.3. Determination of specific oxygen uptake rate (SOUR)

Specific oxygen uptake rates (SOUR) were determined to estimate the influence of Ni(II) on activities of microbes. Aerated sludge withdrawn from the SBR system was instantly put into 300 ml BOD bottle, which was subsequently filled with a fully aerated Ni(II)-containing base solution with the Ni(II) concentrations varying from 0 to 150 mg/l. DO was measured at 30 s intervals until completely exhausted. MLSS was also measured to represent the mass of microbes. The SOUR can then be computed according to the following equation:

$$\text{SOUR} = -\frac{60G}{X}\left(\frac{\text{mg O}_2}{\text{g MLSS h}}\right) \quad (1)$$

where G is the slope of the linear portion of the DO decline curve in mg/l min, X the MLSS concentration in g/l.

2.4. Kinetic study

This study was conducted to investigate the TOC removal rate in SBR system. The pseudo first-order kinetic constants of biological oxidation during the REACT mode were determined by monitoring the residual soluble TOC in the mixed liquor. Mixed liquor sample was collected in a flask at 2 min intervals from SBR system once the REACT mode began. The flasks were immediately immersed in ice bath for 1 h to quench the reaction eventually and to allow the solids to settle. The supernatant was analyzed for TOC concentration using TOC analyzer.

3. Results and discussion

3.1. Ni(II) removal from aqueous solutions by activated sludge and PAC

The removal of Ni(II) from aqueous solution by activated sludge and powdered activated carbon (PAC) was studied as a function of contact time in order to determine the equilibrium time for maximum adsorption (Fig. 2). The metal concentration decreased rapidly during the first 30 min and remained nearly constant after 3 h of adsorption, suggesting that the biosorption was fast and reached saturation within 3 h. Therefore, the equilibration time was set conservatively at 5 h for further experiments.

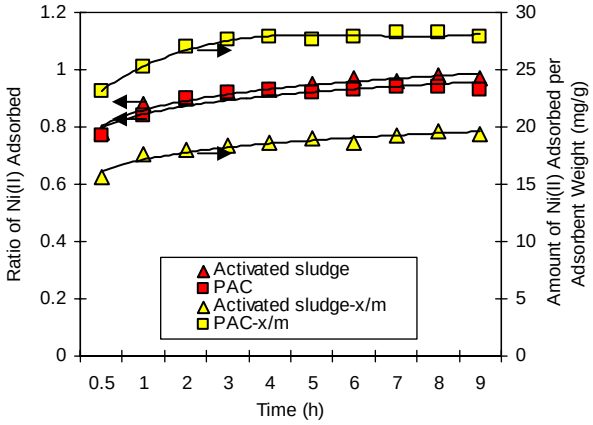


Fig. 2. Contact time study for PAC and activated sludge in Ni(II) adsorption.

Adsorption isotherms show the distribution of solute between the liquid and solid phases and can be described by several mathematical relationships such as the standard Langmuir and Freundlich adsorption isotherms. The linear form of Langmuir and Freundlich isotherms are given by the following equation:

$$\frac{C_e}{q_e} = \left(\frac{1}{bQ^0}\right) + \left(\frac{C_e}{Q^0}\right) \quad \text{(Langmuir model)} \quad (2)$$

$$\log q_e = \log K_F + (1/n)\log C_e \quad \text{(Freundlich model)} \quad (3)$$

where q_e is the amount metal adsorbed (mg/g), C_e the equilibrium concentration of the adsorbate (mg/l) and Q^0 and b are the Langmuir constants related to maximum adsorption capacity and energy of adsorption, respectively. On the other hand, K_F and $1/n$ are Freundlich constants related to adsorption capacity and adsorption intensity, respectively.

The linearized Freundlich and Langmuir adsorption isotherms of Ni(II) obtained at 25 °C were shown in Fig. 3. In view of the values of linear regression coefficients, both of the models fitted very well to the sorption data in the studied concentration range. The Freundlich and Langmuir adsorption constants evaluated from the isotherms with the correlation coefficients are given in Table 1. The magnitude of Freundlich and Langmuir constants for Ni(II)-activated sludge adsorption showed easy uptake of Ni(II) from wastewater with high adsorptive capacity of activated sludge. The value of $1/n$, which lowers than 1, indicated that the adsorption of Ni(II) by activated sludge was favorable. It was reported that if $1/n$ falls between 0.1 and 0.5, the adsorption mechanism is considered good [16]. The maximum adsorption capacity, Q^0 indicated that the

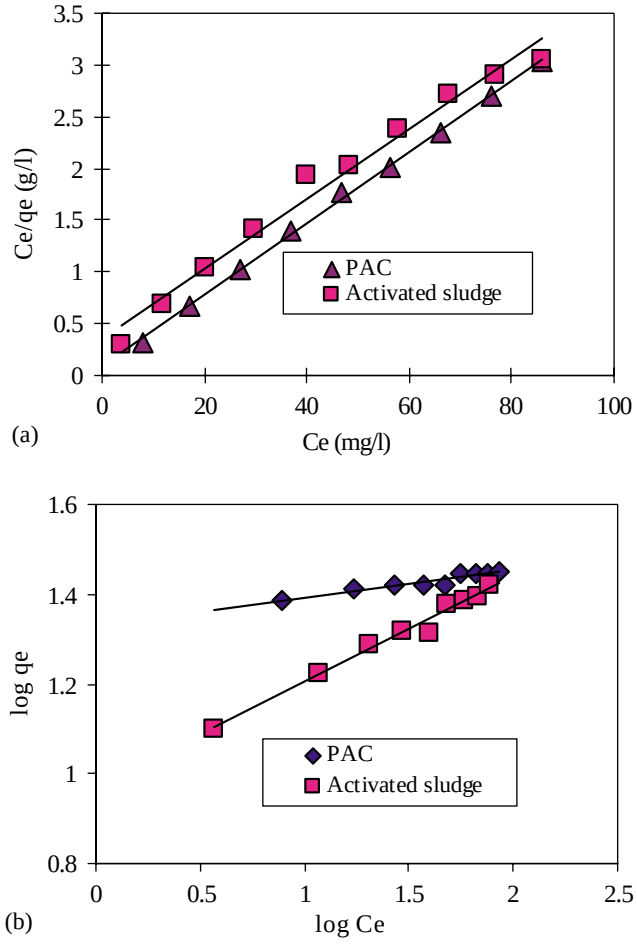


Fig. 3. (a) Langmuir, (b) Freundlich plots for PAC and activated sludge in Ni(II) adsorption.

adsorption of Ni(II) by activated sludge was comparable to PAC adsorption.

The mechanisms associated with metal sorption by activated sludge microorganisms include extracellular and intracellular metal binding and, are complex and dependent on the metal ions and the biological system [17]. The phenomenon of metal uptake by microorganisms is assumed to arise from two basic mechanisms: an initial rapid metal ion uptake due to surface binding on the cell walls (passive uptake) and a subsequent slow active uptake due to membrane transport of the metal ions into cell (active uptake) [18,19]. In most studies, it was shown that passive uptake, especially the complexation by extracellular polymeric substance is the dominant mechanism in metal removal [20,21]. On the other hand, for some metals, such as nickel, active uptake was

Table 1
Langmuir and Freundlich parameters for PAC and activated sludge in Ni(II) adsorption

Adsorbent	Langmuir parameter			Freundlich parameter		
	Q^0	$1/b$	R^2	K_F	$1/n$	R^2
PAC	33	0.384	0.998	21.3	0.064	0.935
Biomass	30	0.093	0.981	9.12	0.243	0.980

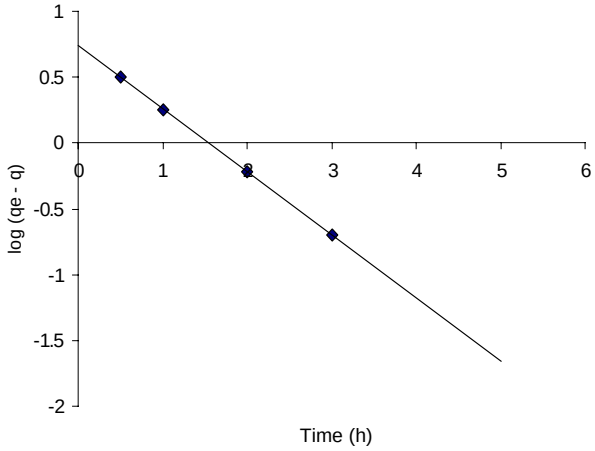


Fig. 4. Lagergren plot for adsorption of Ni(II) by activated sludge at Ni(II) concentration of 10 mg/l.

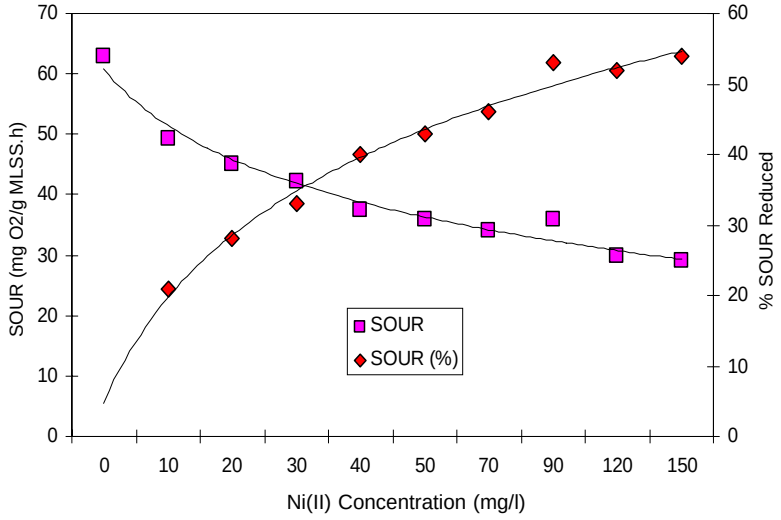


Fig. 5. Acute toxic effects of Ni(II) on activated sludge microorganisms SOUR.

indicated to be important at low concentrations [22]. The actual attachment of the metal ions on the cellular surface may include physical adsorption, complexation or chemical adsorption [23]. Cellular surfaces consist of anionic and cationic exchange sites such as amino, phosphoryl, sulfydryl and carboxylic groups that enable the metal ion adsorbed by activated sludge [24]. The net charge on the cell wall depends on the isoelectric point and the extent to which these sites are occupied by the anions and cations. However, the cells are assumed to carry out a net negative charge [18]. The polysaccharides which form a matrix around the cells maintain an extensive complexing capacity for heavy metals and have ion exchange properties.

Use of the Lagergren Eq. (4) showed that the kinetics of Ni(II) removal follows the first order expression [25].

$$\log(q_e - q) = \log q_e - \frac{k_{ad}t}{2.303} \quad (4)$$

where q is the amount of Ni(II) adsorbed (mg/g) at time t, q_e the amount of Ni(II) adsorbed (mg/g) at equilibrium time and k_{ad} the rate constant of adsorption (min^{-1}). The linearity of the plot (Fig. 4) with a correlation coefficient 0.999 showed

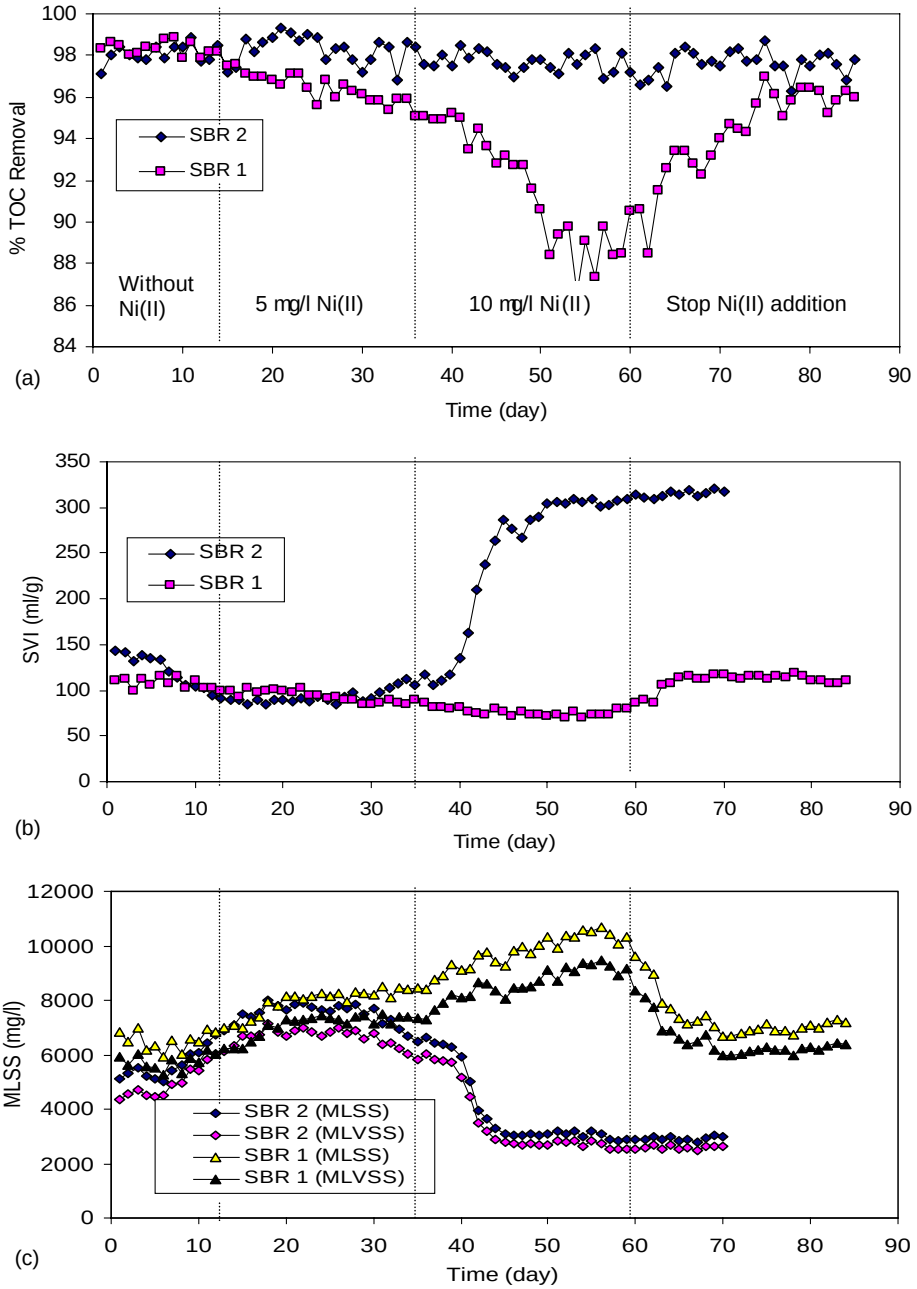
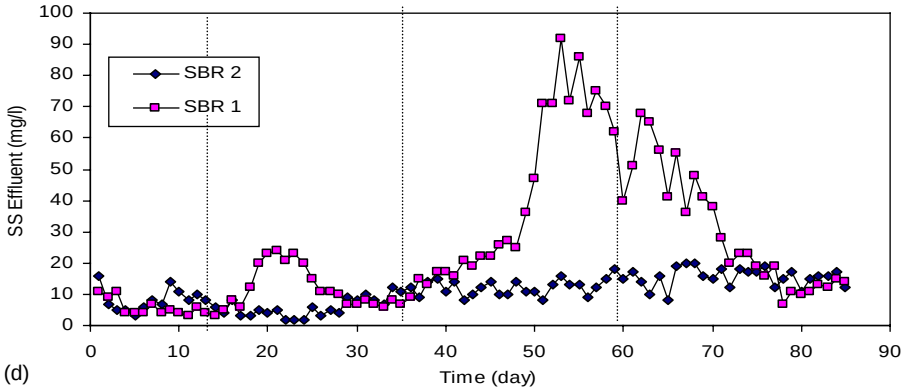
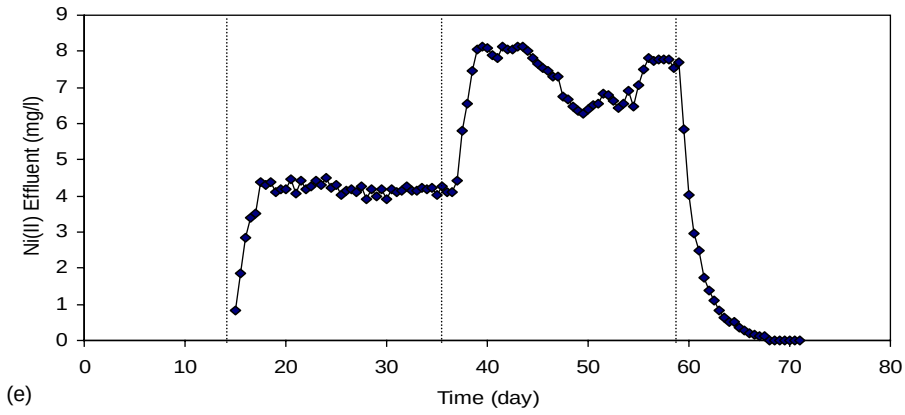


Fig. 6. (a) %TOC removal, (b) SVI, (c) MLSS, (d) SS and (e) Ni(II) effluent (f) SOUR in SBR reactors with and without the addition of Ni(II) in synthetic wastewater (SBR 1: experimental unit; SBR 2: control unit).





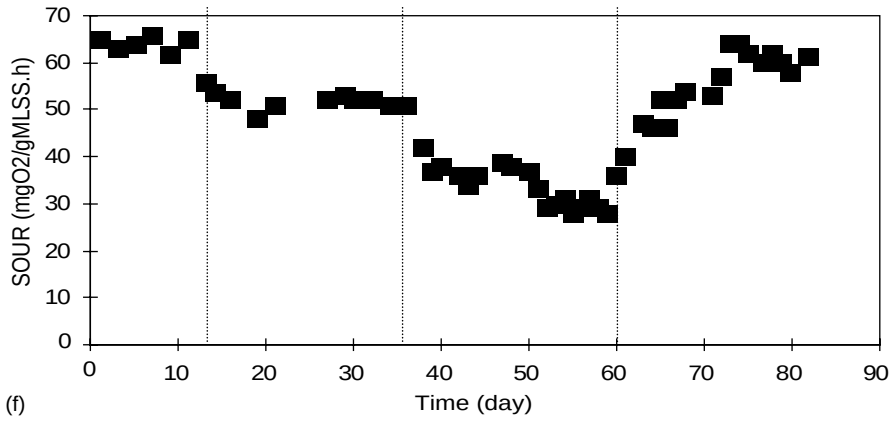


Fig. 6. (*Continued*).

that the Ni(II) adsorption by activated sludge followed first order process with rate constant of 0.0184 min^{-1}.

3.2. Acute toxic effects of heavy metals on activated sludge microorganisms SOUR

The acute toxic effects of Ni(II) on the bioactivity of activated sludge microorganisms were investigated. As shown in Fig. 5, the addition of Ni(II) into base solution caused the decrease of oxygen uptake rate by activated sludge microorganisms. This implied that there was a retardation of biodegradation process in activated sludge microorganisms due to the inhibitory effects of Ni(II). The SOUR decreased more than 30% when the added Ni(II) concentrations were higher than 30 mg/l. Therefore, to avoid extreme inhibitory effects of the Ni(II) on the activated sludge microorganisms, 5 and 10 mg/l of Ni(II) were selected to study the toxic effects of Ni(II) on the performance of sequencing batch reactor (SBR) system.

3.3. Effects of Ni(II) on the SBR performance

The effects of Ni(II) on the performance of SBR system were investigated by monitoring the total organic carbon (TOC), suspended solids (SS) and Ni(II) concentration in treated effluents. Fig. 6a shows the TOC removal efficiency

in SBR systems. A slight reduction of TOC was observed in the 5 mg/l Ni(II)-fed system as compared to the control system. The difference in TOC removal between the two SBR systems became significant after the added Ni(II) concentration was increased up to 10 mg/l. On the other word, the addition of 5 and 10 mg/l Ni(II) into SBR system deteriorated the TOC removal efficiency from 98 to 96 and 87%, respectively, due to the inhibitory effects of Ni(II) on the bioactivity of microorganisms in substrate degradation. The termination of Ni(II) addition improved the TOC removal efficiency up to 96%, almost close to the treatment performance in control system. This indicated the recovery of bioactivity in substrate degradation by activated sludge microorganisms.

As shown in Fig. 6b, the settle ability of activated sludge was almost similar in both SBR systems during the first 30 days of experiment. The good settle ability of the activated sludge with the sludge volume index (SVI) consistently remaining below 120 ml/g was ascribed to the filamentous and floc-forming bacteria were present in a balanced ratio. However, the continual use of readily biodegradable substrates (bacto-peptone and sucrose) as the source of carbon led to a gradual increase of filament abundance and caused activated sludge bulking in the control system [26]. Consequently, the settle ability of activated sludge was deteriorated as shown in the gradual increase of SVI until it reached a steady state at 300 ml/g. During this stage, the filamentous bacteria were predominated and the protruding filaments prevent

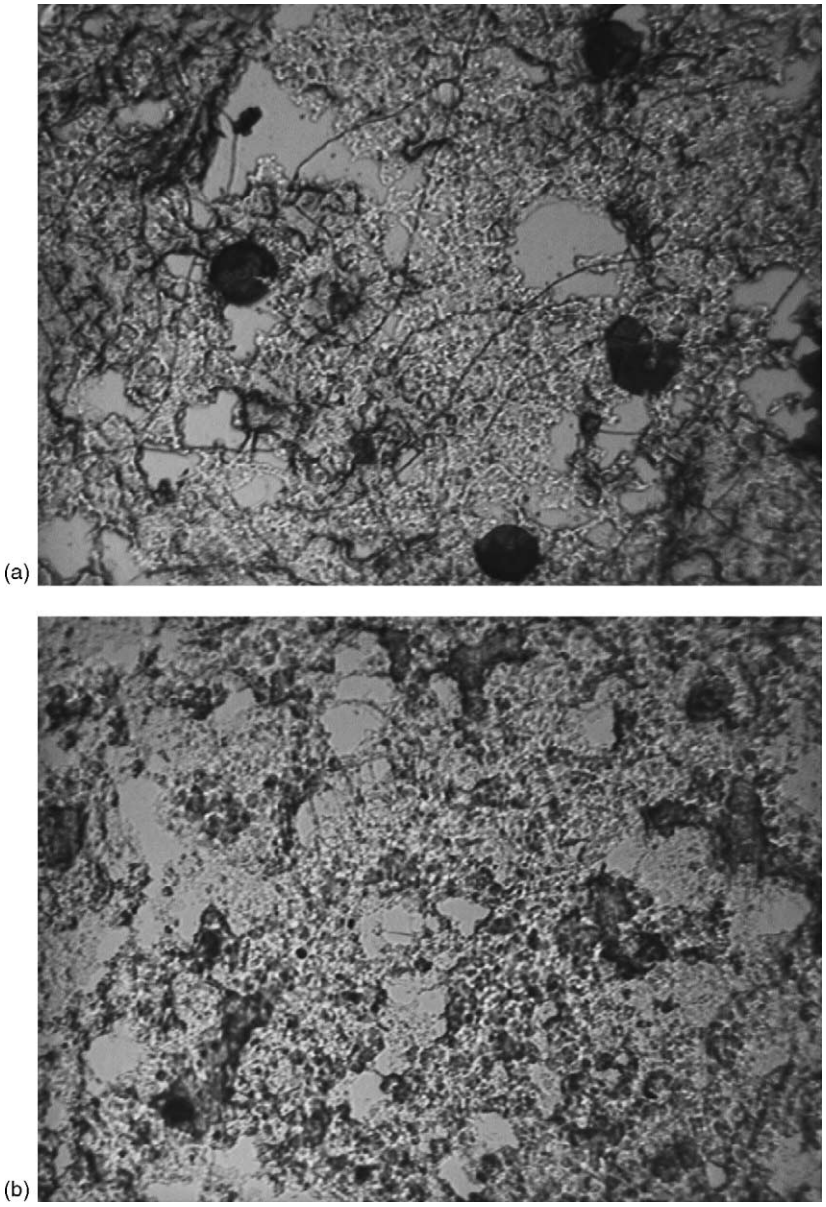


Fig. 7. Microscopy images of activated sludge. (a) SBR without Ni(II) addition, (b) SBR 1 with 5 mg/l Ni(II) addition, (c) SBR 1 with 10 mg/l Ni(II) addition, and (d) SBR 2 with a lot of filamentous bacteria.

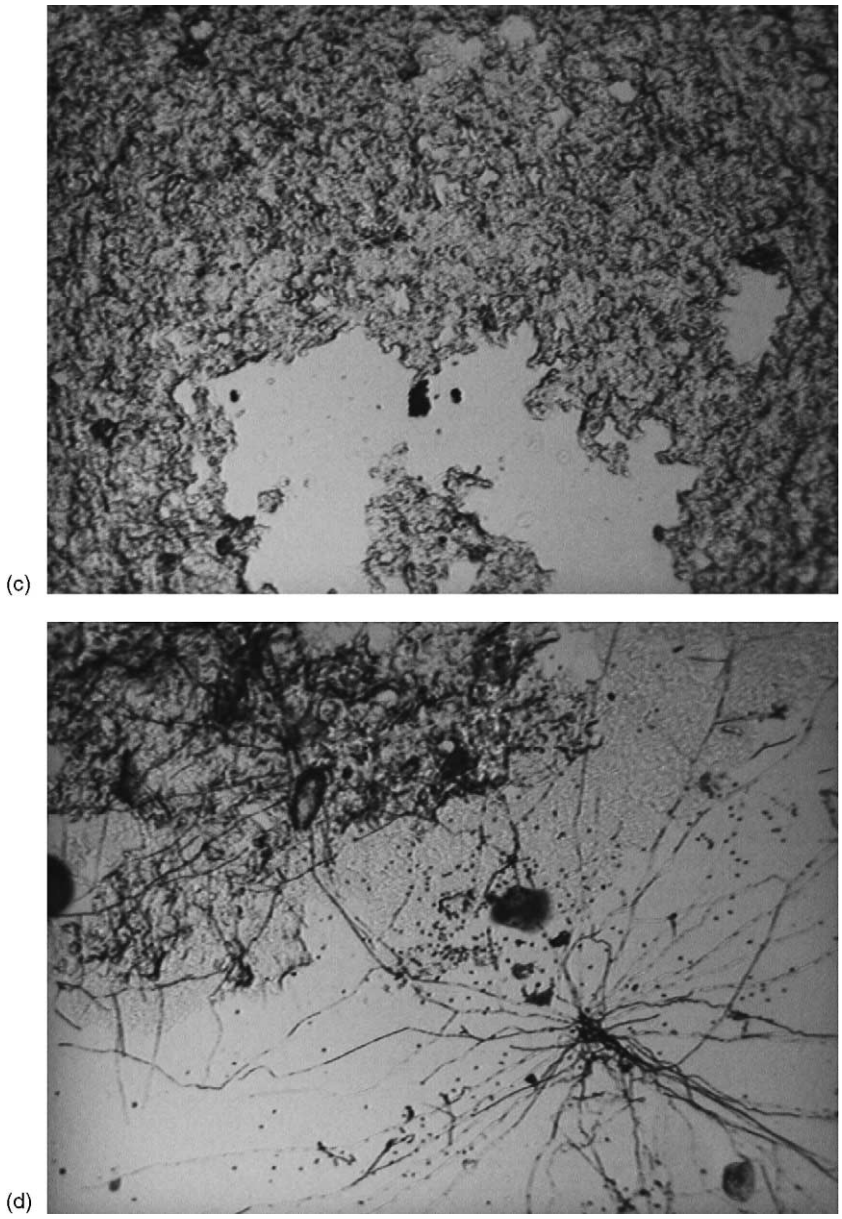


Fig. 7. (*Continued*).

the close approach of other flocs during settlement, led to sludge-settling rate decreased and thickening was poor. As a result, the activated sludge in SBR 2 was being washout during DRAW mode and it caused severe decrease of MLSS (Fig. 6c).

On the contrary, the sludge bulking was not observed in the SBR 1. The addition of 5 and 10 mg/l Ni(II) decreased the filamentous bacteria population in activated sludge as shown in the reduction of SVI from about 100 to 85 and 70 ml/g, respectively (Fig. 6b). It was observed that the present of Ni(II), initially affected the abundance of filamentous bacteria. The improved in sludge settle ability able to retain more activated sludge in SBR system and led to increase in MLSS (Fig. 6c). However, in the system with 10 mg/l Ni(II) addition, the treated effluent became cloudy and the concentration of suspended solid was increased (Fig. 6d). During this stage, the floc-formers were predominated and there was insufficient floc rigidity and small, weak flocs were produced (pin-point floc) which settle poorly. Fig. 7 shows the microscopy images of activated sludge. It was observed that the filamentous bacteria were decreased after the addition of Ni(II) in SBR 1. The outgrowth of excessive quantities of filaments from the flocs (Fig. 7d) correlated with sludge bulking in control system.

The termination of Ni(II) addition was followed by a gradual system recovery as shown in the decrease of SS and increase in TOC removal efficiency. As the system recovered from the toxic effects of Ni(II), the population of filamentous bacteria, free swimming ciliates and stalked ciliates were increased gradually. As the filamentous microorgan-

isms increased, the sludge-settling rate became lower again and led to a small amount of biomass being washout from SBR system.

Fig. 6e shows the Ni(II) removal efficiency in SBR 1. It was observed that the average of Ni(II) removed were 20 and 25% in the system with the addition of 5 and 10 mg/l Ni(II), respectively. The Ni(II) removal efficiency in the SBR system was relatively low compare to the adsorption capacity obtained in adsorption study. As a result, the Ni(II) concentration profile was collected from SBR system during FILL and REACT modes. It was found that the Ni(II) concentration decreased in the FILL mode as the Ni(II) adsorbed by biomass, and increased gradually in the REACT mode (Fig. 8). This phenomenon might due to the desorption process of Ni(II) from biomass. To re-ensure it, 200 ml of MLSS was collected from SBR 1 during IDLE mode and mixed with 200 ml of RO water. The soluble Ni(II) content was monitored at appropriate time intervals for approximately 2 days and the result is shown in Fig. 9. It was observed that the soluble Ni(II) concentration increased gradually until it reached a constant level at about 3 mg/l, due to the desorption process in biomass.

The SOUR in SBR system had been studied for about 85 days for the investigation of activated sludge microorganism activity before and after Ni(II) addition. Fig. 6f depicts the changes of SOUR in SBR system throughout the study. It was found that the SOUR decreased 23% after the addition of 5 mg/l Ni(II) and further decreased to about 44% when the Ni(II) concentration was increased to 10 mg/l. This result implied that the addition of Ni(II) inhibited the activity of microorganisms in the degradation of substrates. The increase of Ni(II) concentration enhanced the inhibitory effects and subsequently the bio-oxidation process carried out by biomass reduced. The termination of Ni(II) addition was followed by the system recovery from the toxic effects of Ni(II) as shown in the increase of oxygen uptake rate by activated sludge microorganisms. The activity of microorganisms was almost fully recovered after 12 days of the Ni(II) input termination.

3.4. Kinetic study

The Monod equation has been widely used to describe substrate removal in biological treatment processes.

$$\frac{dC}{dt} = \frac{\mu_m XC}{K_m + C} \tag{5}$$

where X is the MLVSS, μ_m and K_m the maximum and half-velocity constants and C the substrate concentration. If substrate concentration is low compared to K_m, then the C term in the denominator becomes unimportant and Eq. (5) may be approximated as a first-order reaction with respect to substrate removal:

$$\frac{dC}{dt} = \frac{\mu_m XC}{K_m} \tag{6}$$

For SBR process, a mass balance on substrate in the reactor during the REACT mode can be shown to be:

$$-\frac{dC}{dt} = \frac{\mu_m XC}{K_m} = kC \tag{7}$$

where k is the pseudo first-order constant and t the reaction time. By plotting ln TOC versus time, a relatively good fit (>0.9) for all cases indicates that the first-order formulation (Eq. (7)) provides good correlation of results (Fig. 10) [27]. This is not surprising because the degradable substrate con-

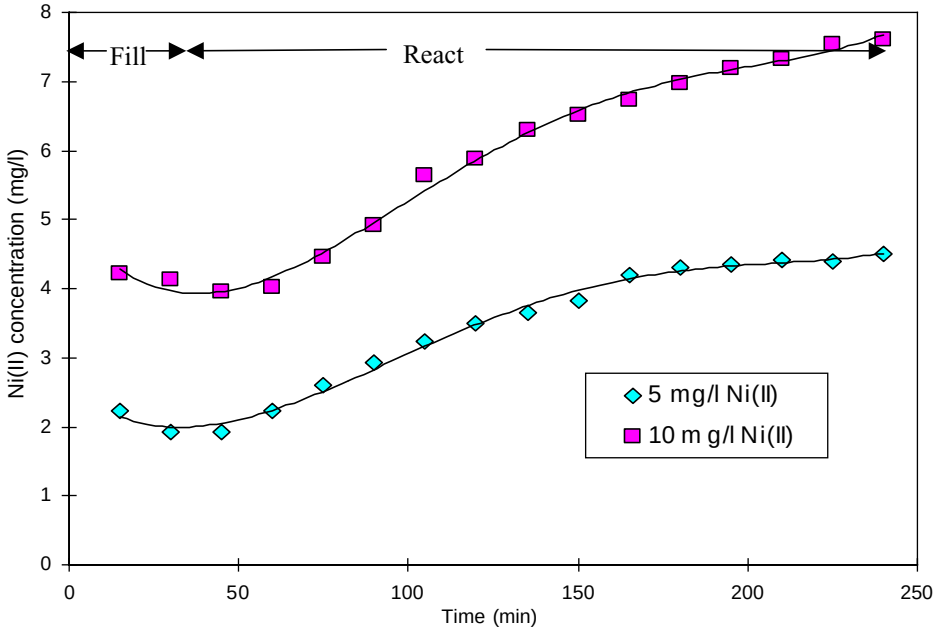


Fig. 8. Ni(II) profile during FILL and REACT modes in SBR reactor with the addition of 5 and 10 mg/l of Ni(II).

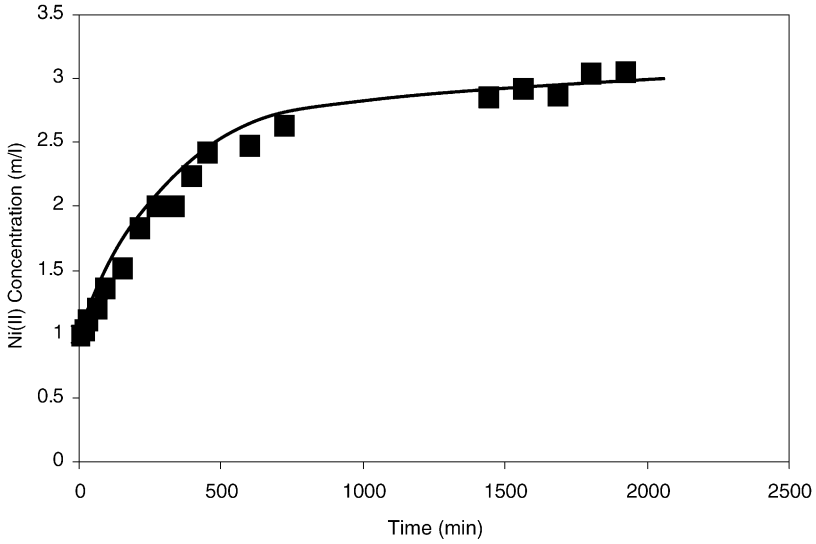


Fig. 9. Desorbed Ni(II) profile monitoring from the activated sludge treating Ni(II)-containing wastewater.

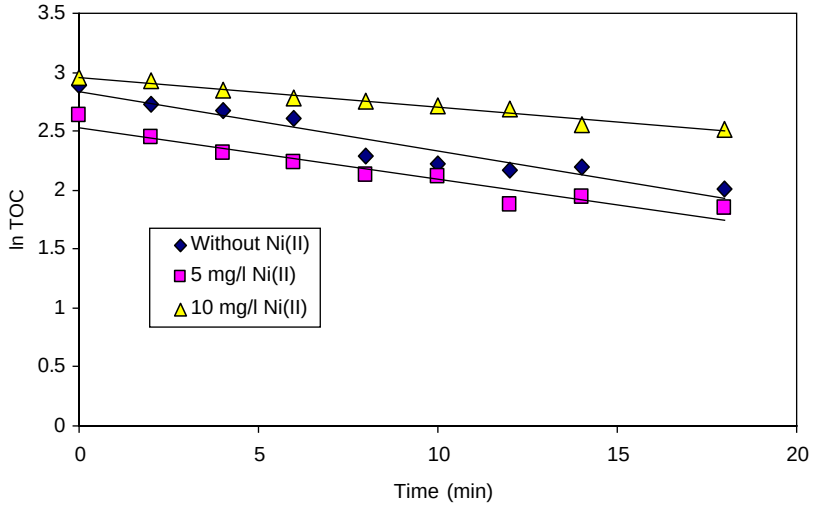


Fig. 10. Plots of ln TOC vs. time for first-order kinetic constant determination for SBR system with and without the addition of Ni(II).

centration at the beginning of the REACT mode of a cycle was very much lower than the influent TOC of the base solution (270 mg/l).

As shown in Table 2, the TOC removal rate was 76 day^{-1} in the system without Ni(II) addition. However, the addition of 5 and 10 mg/l Ni(II) reduced the k values by 22 and 51%, respectively. This implied that the added Ni(II) inhibited the activity of microorganisms in bio-oxidation process. The results in the kinetic study were agreed with the earlier results of the effect of Ni(II) on SOUR activated sludge microorganisms.

Table 2
Comparison of pseudo first-order kinetic constants

Wastewater	k (per day)	R^2
Without Ni(II)	76	0.9236
5 mg/l Ni(II)	59	0.9244
10 mg/l Ni(II)	37	0.9747

4. Conclusions

The results presented in this paper show that:

- The addition of 5 mg/l Ni(II) caused a slight reduction in TOC removal efficiency, whereas 10 mg/l Ni(II) addition significantly affected the SBR performance in terms of suspended solids and TOC removal efficiency.
- The SOUR decreased 23 and 57% after the addition of 5 and 10 mg/l Ni(II), respectively, into SBR system. In the case of kinetic study, the addition of 5 and 10 mg/l Ni(II) decreased the k value by 22 and 51%, respectively. These results indicated that the addition of 10 mg/l Ni(II) seri-

ously inhibited the activity of microorganisms in substrate degradation.

- Termination of Ni(II) addition allowed almost full recovery of activated sludge microorganisms activities, which shown in the increase of SOUR values and SBR treatment performance.

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