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Performance evaluation of sulfate reducing bacteria in removing lead, chromium and nickel by anaerobic packed bed reactor

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ABSTRACT

Synthetic wastewater containing Pb, Cr (VI), and Ni was treated by sulfate reducing bacteria (SRB) in a bench scale, down-flow, anaerobic packed bed reactor filled by ceramic saddle as media. Synthetic wastewater was made in the laboratory, and SRB cultures and their adaptation were done before beginning of the experiments. The experiments were conducted in 12 and 24 h of operation under sulfate concentrations of 800 and 1,600 mg l^{-1}. Various concentrations of Pb (5, 150, and 300 mg l^{-1}), Cr (VI) (5, 50, and 100 mg l^{-1}), and Ni (5, 15, and 30 mg l^{-1}) were used in these experiments. Heavy metals removal efficiencies for the concentrations of 300 mg l^{-1} Pb, 100 mg l^{-1} Cr (VI), and 30 mg l^{-1} Ni in 12 h of operation under sulfate concentration of 800 mg l^{-1} were 99.6, 98.11, and 94.7%, respectively. The main mechanisms of the removal in this work were precipitation of Pb, Cr (VI), and Ni as metal sulfides as well as adsorption on the SRB mass. The results from the present study indicate that SRB mass in the down-flow anaerobic packed bed with ceramic saddle as media is efficient for Pb, Cr (VI), and Ni removal from industrial wastewater.

Keywords: Heavy metals removal; Sulfate reducing bacteria; Anaerobic packed bed reactor; Lead; Nickel; Chromium (VI)

1. Introduction

One of the main toxics and non-biodegradable compounds which could be found in the wastewater is heavy metals. These compounds could be released into the environment through industrial wastewater effluents and agricultural activities as well [1,2]. Based on the researches performed about Pb, Cr (VI), and Ni impacts on human health, theses toxic elements have been known as human poisoning [3]. Most common industries having high concentrations of these three toxic elements in their wastewater effluents are metal finishing operations, plating industrial, mining, electronic industries and chemical, cadmium-nickel battery, leather and textile manufacturing [4,5]. Nickel has high toxic properties in large doses, because it is required for human body only in small amounts [6]. Chromium (VI) tends to accumulate in aquatic life and once this toxic element enters food chain, it can cause kidney and liver damage to human body and cancer as well [7]. Short-term exposure to high levels of lead has serious health effects like vomiting, diarrhea, convulsion, coma, or even death. In addition, long-term exposure to low levels lead

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can also have harmful effects, especially on infants, young children, and fetus [8].

Several processes have been used for heavy metals removal from wastewater, including precipitation, coagulation, reduction, membrane processes, ion exchange, adsorption, electro-coagulation, and application of nanotechnology [9–21]. However, these various methods have their own restrictions mostly related to the high operating costs or generating solid wastes that are difficult to treat [22,23]. Also, removal efficiency of precipitation is lower than the limits required by regulatory agencies [24], and both precipitation and ion exchange have limitations for dilute metal wastes [25–27]. In addition, these methods produce toxic sludge, and require high energy [3,28]. Therefore, use of alternative methods for heavy metals removal is highly needed. In recent years, several methods have been suggested for heavy metals removal from wastewater effluents; biological process is one of these methods [29–32]. There is increasing interest on application of sulfate reducing bacteria (SRB) for heavy metals removal under different conditions [31,33–35]. The SRB generate sulfide (S^{-2}) and alkalinity under anaerobic conditions, and sulfide reacts with heavy metals present in wastewater. This reaction causes formation of metal sulfide precipitates having low solubility in wide range of pHs. The heavy metals can be adsorbed and/or co-precipitate with metal sulfide [36].

In a study conducted by Jong [37], SRB were applied in an up-flow, anaerobic packed bed (UAPB) reactor as an alternative method for heavy metals removal from wastewater effluents. However, a down-flow, anaerobic packed bed (DAPB) reactor was used in the present study regarding its advantages, including low costs, lower energy requirement, simplicity of construction, lower probability of clogging than in the up-flow mode, capable of handling a wide variety of waste concentrations [37,38]. In addition, ceramic saddle media used in this work has several benefits such as high surface area, high void fraction, low bulk density, low gas phase pressure drop, and having convective properties that are helpful for keeping monotonous temperature in the reactor; however, few experimental studies have been conducted using this media as bio-trickling filter. The aim of the present study was, therefore, performance evaluation of the SRB in the DAPB reactor with ceramic media for Pb, Cr (VI) and Ni removal.

2. Materials and methods

2.1. Bioreactor

These experiments were done in a bioreactor constructed from glass with 10 L volume, an overall height of 300 mm, and an internal diameter of 200 mm. The reactor was equipped with three ports upon the bioreactor for feeding, gas discharge and heat supply, and a port at the bottom for sampling having internal diameter of 10 mm. The media used as SRB culture inside the reactor was made of 6.5 mm fractions of ceramic saddle, and 50% of the reactor's volume was filled by these fractions. Porosity of the media and approximate net empty volume for the wastewater was 66.67% and 5 ± 0.1 L, respectively. The reactor designed for this work operated as continuous down-flow. Schematic diagram of the DAPB reactor is shown in Fig. 1.

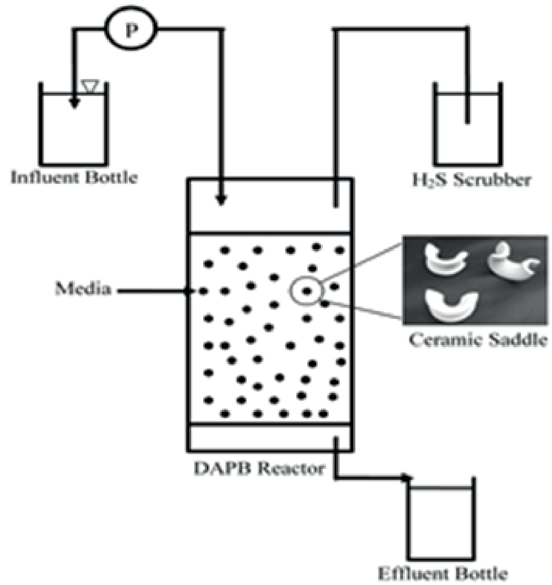

Fig. 1. Schematic diagram of the down-flow, anaerobic packed bed (DAPB) reactor.

2.2. Culture of SRB

At the beginning, the reactor was filled by influent of Shush wastewater treatment plant as microbial inoculation. The selected influent had anaerobic condition, and circumstances of the reactor were adjusted to cause the growth of SRB mass as dominant species. Desired conditions are as follows: anaerobic condition in all the times, temperature of 30°C–32°C, high sulfate concentration of 1,600 mg l^{-1}, COD of 1,500 mg l^{-1} [39,40]. Besides, the COD/SO_4 ratio was always kept below 1. When efficiency of sulfate removal had reached to >99%, the reactor was ready for the operation. It is noteworthy that this part of the work took 2 months long.

2.3. SRB adaptation

According to the heavy metals' toxicity for organisms in aquatic environments, suddenly introducing the high concentrations of the elements to the reactor might be dangerous for SRB mass. Low concentrations of the each toxic element were, therefore, added through the synthetic wastewater influent to the reactor prior to the beginning of the experiments, and was continued until sulfate removal reached to >99%. SRB bacteria were preconditioned by lactate to be able to neutralize the initial drop of pH in the reactor, as suggested by Jong and Parry [37].

2.4. Influent wastewater

In this work, according to a research conducted by Deveci and Delaloglu [41], synthetic wastewater was made in the laboratory and used as influent wastewater. Characteristics of the synthetic wastewater are given in Table 1. Sodium lactate and sodium sulfate added to the bioreactor through the influent served as organic carbon source for growth and sulfate source, respectively. In addition, mixture of lactic acid and NaOH 6 N with concentration of

Table 1
Characteristics of the synthetic wastewater

Chemical compounds	Sulfate concentrations	
	1,600 mg l^{-1}	800 mg l^{-1}
Ammonium chloride (NH_4CL)	1	1
Magnesium sulfate ($MgSo_4$, $7H_2O$)	2	1
Di-potassium hydrogen orthophosphate (K_2HPO_4)	0.5	0.5
Chloride calcium hydrate ($CaCL_2$, $2H_2O$)	0.1	0.1
Sodium chloride (NaCL)	3	3
Calcium carbonate ($CaCo_3$)	1	1
Ammonium ferrous sulfate (Fe $(NH_4)_2(SO_4)_2$, $6H_2O$)	Negligible	Negligible
Sodium sulfate (Na_2SO_4)	0.9	0.45

3.5 ml l (1/2 ratio) was used as COD. Moreover, the pH of the influent wastewater to the reactor was adjusted to 6.5–7.5. Synthetic Pb, Cr (VI), and Ni in the influent were made of lead nitrate [Pb $(NO_3)_2$], potassium dichromate [$K_2Cr_2O_7$], and hydrated nickel chloride [$NiCl_2$, $6H_2O$], respectively. The selected concentrations of the heavy metals in this work were based on the degree of heavy metals' toxicity for organisms in anaerobic systems (Ni>Cu>Cb>Cr>Pb), which nickel and lead have the highest and lowest toxicities, respectively [42]. The concentrations of Pb (5, 150, and 300 mg l^{-1}), Cr (VI) (5, 50, and 100 mg l^{-1}), and Ni (5, 15, and 30 mg l^{-1}) were used in these experiments. The continuous bioreactor was operated in two different operation times of 12 and 24 h, and each one was tested under different sulfate concentrations of 800 and 1,600 mg l^{-1}.

3. Results and discussion

3.1. The SRB behaviors in the reactor

Characteristics of industrial wastewater have important roles in SRB mass behaviors in the reactor; high concentrations of the heavy metals in the reactor decreased sulfate removal from anaerobic systems. Therefore, it was needed to adapt the SRB mass with the toxic elements. SRB adaptation with heavy metals prior to the beginning of the operation in this work had some advantages, including more stability of SRB mass in high concentrations of the heavy metals, higher sulfate reduction rate during the operation, and minimization of retention time for heavy metals removal. Concentrations of sulfate and its reduction during the whole operation time are illustrated in Fig. 2. As shown in the figure, high levels of bacterial activity in the reactor enforce the use of lactate to reducing sulfate, so high levels of lactate was required for the SRB mass after adaptation. On the other hand, it implies that the high levels of sulfate and lactate concentrations can produce high concentrations of sulfide in the bioreactor, which is important for the heavy metals removal. Sulfide concentration was rapidly increased at the initial stage of the operation times of 12 and 24 h, but its concentration at the both operation times was suddenly decreased which is due to metals precipitation. At the operation time of 24 h and influent sulfate concentration of 1,600 mg l^{-1}, sulfide concentration was gradually increased after 12 h of the operation time which is probably due to decrease in heavy metal concentrations. At the operation time of 12 h and influent sulfate concentration of 800 mg l^{-1}, more concentration of sulfide was precipitated as metals precipitation. In addition, because of pre-adaptation of SRB mass to heavy metals and range of pH, consumption rate of sulfate was higher than those observed by other studies [37]. In our study, therefore, sulfate concentration of 800 mg l^{-1} in the operation time of 12 h produced acceptable results. Fig. 2 shows the variations of pH during 24 h of operation time. As can be seen from the figure, introduction of low pH wastewater caused decrease pH in the reactor, but it gradually increased to higher than 7 in the operation time of 6 h due to adaptation potential of SRB bacteria. This is mainly because of the fact that the SRB bacteria were preconditioned by lactate, as mentioned in Section 2.3. Afterwards, the pH remained almost constant over the rest of operation time, because this pH was ideal for heavy metals removal by SRB bacteria, which is quite consistent with the results found by Jong and Parry [37].

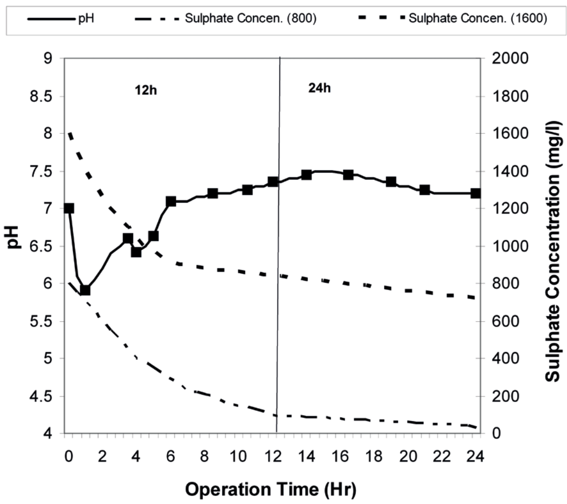

Fig. 2. Variations of pH and sulfate concentrations over the operation time.

In several studies performed by [35,43,44], sulfate removal was decreased in initial days of operation. Researchers in those studies suggested that SRB mass in the reactor was affected by heavy metals' toxicity. As mentioned above, nickel, among the other heavy metals, has the highest toxicity for the organisms in the anaerobic systems. However, the SRB mass adaptation in this work diminished the toxicity effects of nickel in the reactor and also caused 93% removal in Ni concentration of 30 mg l^{-1} after only 12 h as shown in Fig. 5(b). We suggested that the low retention time in this study is due to the compatibility of the SRB mass with the influent wastewater and also keeping relatively constant temperature (30°C–32°C) during the operation time.

3.2. Bioreactor performance

The reactor was tested for Pb, Cr (VI), and Ni after 12 and 24 h of the operation under different sulfate concentrations of 800 and 1,600 mg l^{-1}. Sampling time was at the

Table 2
Effect of sulfate concentrations on heavy metal removal efficiency

Heavy metal	Influent sulfate concentration = 800 PPM					Influent sulfate concentration = 1,600 PPM			
	Inf. Pb Con. (mg L^{-1})	Effl. Con. (12 h) (mg L^{-1})	Effl. Con. (24 h) (mg L^{-1})	Rem. % (12 h)	Rem. % (24 h)	Effl. Con. (12 h) (mg L^{-1})	Effl. Con. (24 h) (mg L^{-1})	Rem. % (12 h)	Rem. % (24 h)
Pb	6.14	1	0.7	83.7	88.6	0.4	0.35	93.4	94.3
	157.3	0.9	0.9	99.4	99.4	3	0.5	98	99.6
	297.3	1.1	0.9	99.6	99.7	0.9	0.95	99.7	99.68
Cr	3.68	1.2	1.05	67.4	71.47	0.15	0.2	95.9	94.56
	64.64	1.71	1.4	97.35	97.8	1	1.1	98.45	98.2
	111.4	2.1	2	98.11	98.2	1.1	1	99	99.1
Ni	6.18	2.5	2.6	59.5	57.96	1.85	1.8	70	70.8
	16.27	2.6	2.7	84.01	83.4	2.1	2.2	87.09	86.47
	51.34	2.7	2.8	94.74	94.54	2.58	2.5	94.97	95.13

ends of the two different operation times. After running the system, pH and sulfide concentrations were increased, while metals and sulfate concentrations were decreased in 12 and 24 h operation times. Jong and Parry [37] reported that pH and sulfate removal were increased during 14 days operation, which is in line with the results from our study. The results of the Pb, Cr (VI), and Ni removal in the reactor at the two operation times show the minor differences between different sulfate concentrations of 800 and 1,600 mg l^{-1} in the synthetic wastewater. These results are shown in Table 2.

The reactor was filled by ceramic saddle fractions as media and temperature inside the reactor was kept at 30°C–32°C in the all operation periods by an aquarium heater located at the top of the reactor. Ceramic saddle fractions inside the reactor can lead to SRB growth in high concentrations, because these fractions have high porosity and make large surface area for the bacterial growth. After a while, the bacterial growth and metal precipitates at the surface of the media cause decreased net empty volume of the wastewater in the reactor, so the wastewater down-flow could be clogged. The clogging of the bioreactor column was avoided by increasing the down-flow velocity.

3.3. The heavy metal removal

The effluent wastewater of the reactor was analyzed for the Pb, Cr, and Ni removal after the operation periods of 12 and 24 h. The Pb removal efficiencies for Pb concentrations of 5, 150 and, 300 mg l^{-1} after 12 and 24 h under sulfate concentrations of 800 and 1,600 mg l^{-1} are shown in Fig. 3, respectively. Based on the results, the effluent concentrations of the Pb at the three different influent concentrations were under 1 mg l^{-1} Pb, which is in accordance with the effluent standards for industrial wastewater. For example, the Pb removal efficiencies for concentration of 300 mg l^{-1} after 12 h of operation under sulfate concentrations of both 800 and 1,600 mg l^{-1} were >99%. Teekayuttasakul and Annachhatre [45] studied Pb removal from industrial wastewater by SRB mass and found that Pb removal efficiency was 99%, which is in line with the results of our study. Table 2 shows the effect of sulfate concentrations on Pb removal efficiency. When

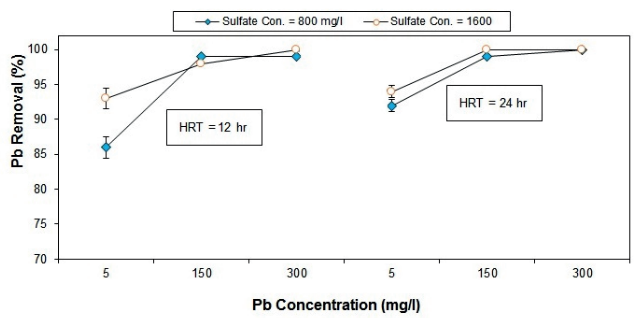

Fig. 3. Pb removal efficiencies under sulfate concentrations of 800 mg l^{-1} and 1,600 mg l^{-1}.

sulfate concentration of 1,600 mg l^{-1} was introduced along the synthetic wastewater to the system, the removal efficiency was slightly higher than that under sulfate concentration of 800 mg l^{-1}. This can be attributed to the high sulfide production under the sulfate concentration of 1,600 mg l^{-1}, which is important for heavy metal removal in the reactor.

Cr (VI) removal efficiencies for Cr concentrations of 5, 50, and 100 mg l^{-1} after 12 and 24 h under sulfate concentrations of 800 and 1,600 mg l^{-1} is shown in Fig. 4. Cr (VI) reacts with sulfide in the reactor and produces Cr (III) in the effluent. The effluent standard for Cr (III) is 2 mg l^{-1}, and the Cr (III) concentrations at the end of the 12 and 24 h of operations under sulfate concentrations of both 800 and 1,600 mg l^{-1} were quite less than the standard. For example, the Cr (VI) removal efficiencies with Cr (VI) concentration of 100 mg l^{-1} after 12 h of operation under sulfate concentrations of both 800 and 1,600 mg l^{-1} were >98%. Fude and et al. [46] studied the Cr (VI) removal from metal-refinishing wastewater by SRB mass, and reported that Cr (VI) removal efficiencies for concentration range of 50–2,000 mg l^{-1} were 80%–95%, which is consistent with the results from the present study. Fig. 4 illustrates the effect of sulfate concentrations on Cr (VI) removal. The chromium (VI) removal efficiencies in high levels of Cr (VI) are higher than those in low concentrations. When Cr (VI) concentrations are high in the wastewater, it causes more contact between sulfide

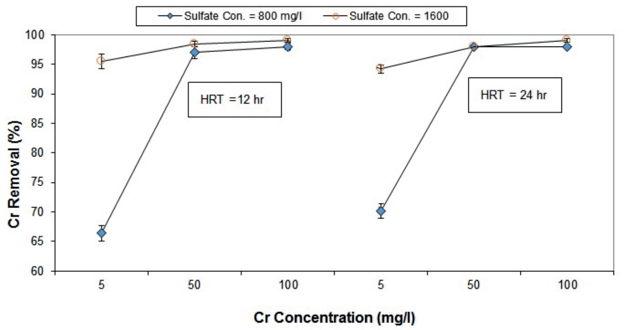

Fig. 4. Cr effect of sulfate concentrations on Cr removal efficiency.

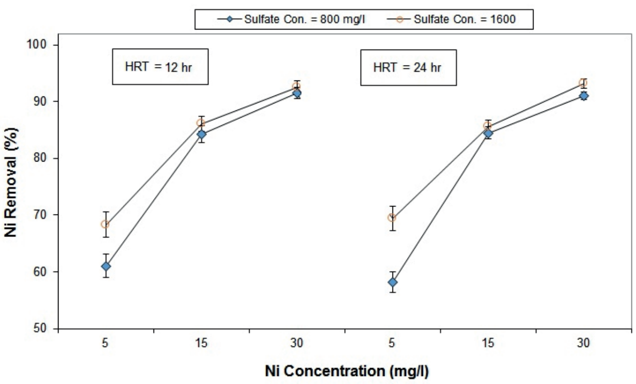

Fig. 5. Effect of sulfate concentrations on Ni removal efficiency.

and chromium (VI) occurs, so more metal-sulfide would be produced in the reactor. The heavy metals in the reactor attach to the surface of the metal-sulfide precipitates, so further removal may occur due to adsorption on the SRB mass.

Ni removal efficiencies for the concentrations of 5, 15, and 30 mg l^{-1} Ni after 12 and 24 h under sulfate concentrations of 800 and 1,600 mg l^{-1} is shown on Fig. 5. For example, Ni removal efficiencies with Ni concentration of 51.34 mg l^{-1} after 12 h of operation under sulfate concentrations of 800 and 1,600 mg l^{-1} were 94.7% and 94.9%, respectively. Jong and Parry [37] studied the removal of sulfate and heavy metals by SRB mass and reported that nickel removal efficiency among the other heavy metals was 97.5% after 7 d, which is in accordance with the results of our study. Fig. 5 illustrates the effect of sulfate concentrations on nickel removal. The lower Ni removal efficiency than those for Pb and Cr (VI) in this work is related to the high toxicity of Ni for organisms in the anaerobic systems and high solubility of the precipitates formed.

4. Conclusion

In this work, Pb, Cr (VI), and Ni removal by SRB mass in DAPB reactor was investigated. The SRB mass adaptation caused decreased operation time of the heavy metals (Pb, Cr (VI), and Ni) removal from the influent wastewater. Sulfate removal efficiency at the ends of the both culture of SRB and the adaptation of SRB mass was > 99%. After synthetic wastewater was introduced to the system, sulfide concentration was increased in the bioreactor, but its concentration was suddenly decreased which is probably due to precipitating as metals precipitation. In addition, because of the SRB mass adaptation by lactate, pH almost remained above 7 during the operation time which is ideal condition to remove heavy metals by SRB bacteria. Heavy metals removal efficiencies for the concentrations of 300 mg l^{-1} Pb, 100 mg l^{-1} Cr (VI), and 30 mg l^{-1} Ni in 12 h of operation under sulfate concentration of 800 mg l^{-1} were 99%, 98%, and 92%, respectively. The main mechanisms of removal in this work were precipitation as metal sulfide and adsorption on SRB mass. Similar mechanisms have been reported in other studies. The results indicate that SRB mass in the DAPB reactor with ceramic saddle as media is efficient for Pb, Cr (VI) and Ni removal from synthetic wastewater.

Competing interests

The authors declare that they have no competing interests.

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