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Hexavalent chromium reduction in a sulfur reducing packed-bed bioreactor

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ABSTRACT

The most commonly used approach for the detoxification of hazardous industrial effluents and wastewaters containing Cr(VI) is its reduction to the much less toxic and immobile form of Cr(III). This study investigates the cleanup of Cr(VI) containing wastewaters using elemental sulfur as electron acceptor, for the production of hydrogen sulfide that induces Cr(VI) reduction. An elemental sulfur reducing packed-bed bioreactor was operated at 28–30 °C for more than 250 days under varying influent Cr(VI) concentrations (5.0–50.0 mg/L) and hydraulic retention times (HRTs, 0.36–1.0 day). Ethanol or acetate (1000 mg/L COD) was used as carbon source and electron donor. The degree of COD oxidation varied between 30% and 85%, depending on the operating conditions and the type of organic carbon source. The oxidation of organic matter was coupled with the production of hydrogen sulfide, which reached a maximum concentration of 750 mg/L. The biologically produced hydrogen sulfide reduced Cr(VI) chemically to Cr(III) that precipitated in the reactor. Reduction of Cr(VI) and removal efficiency of total chromium always exceeded 97% and 85%, respectively, implying that the reduced chromium was retained in the bioreactor. This study showed that sulfur can be used as an electron acceptor to produce hydrogen sulfide that induces efficient reduction and immobilization of Cr(VI), thus enabling decontamination of Cr(VI) polluted wastewaters.

1. Introduction

Hexavalent chromium is considered as acutely toxic, teratogenic and carcinogenic [1,2]. Contamination of soil, surface- and groundwater with chromium is a worldwide problem and is the result of its extensive use in numerous industrial processes such as production of alloys and mainly stainless steel, metal plating, leather tanning and wood treatment [3,4]. Although chromium exists in oxidation states varying between −2 and +6, Cr(VI) and Cr(III) are the most dominant ions present in industrial wastewaters [4]. In contrast to Cr(VI), the hydroxide of trivalent chromium is characterized by limited solubility at neutral pHs as well as by low availability for biological uptake. Cr(III), when present in low concentrations, is essential for human nutrition, whereas at high concentrations it is toxic to plants. Hence, the most commonly used approach to detoxify chromium containing solutions is the reduction of Cr(VI) to Cr(III) and its immobilization as amorphous hydroxide ($Cr(OH)_3$), which is either adsorbed or precipitated at slightly acidic or neutral pHs [5–9]. Chung et al. [9] reported that the maximum precipitation rate of $Cr(OH)_3$ occurs at pH around 8.0, while at a pH below 7.0 Cr(III) may not be present in solid form.

Cr(VI) can be reduced by chemical or biological means. Zero-valent iron, ferrous iron [3,5,7] and dissolved sulfide [10] are the most commonly used reagents in environmental systems for chromate reduction. Although chemical reduction of Cr(VI) with the use of zero-valent or ferrous iron is quite efficient, the main disadvantages of the process are the high cost of chemicals and the production of big volumes of sludge. Microbial reduction of Cr(VI) is one of the approaches used for the detoxification of solutions containing Cr(VI) [11]. Literature data on Cr(VI) toxicity are rather controversial. Several studies mention that Cr(VI) is toxic to activated sludge at concentrations above 5 mg/L, whereas other studies reported stimulation of bacterial growth up to 25 mg/L. However, it is mentioned that a high concentration of Cr(VI) inhibits activated sludge growth and 80 mg/L have been identified as lethal dose [12]. Several other studies reported that it is difficult to continuously remove Cr(VI) from solutions without intermittently reseeding a biological system [13]. Shen and Wang [14] studied a two-stage system where *Escherichia coli* cells grown aerobically in a completely mixed reactor (first-stage) pumped into an anaerobic plug-flow reactor to reduce Cr(VI) (second-stage) and reported that almost

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complete removal of Cr(VI) was achieved in the plug-flow reactor under specific operating conditions. The efficiency of the plug-flow reactor was significantly affected by the concentration of Cr(VI) in the feed, while the rate of Cr(VI) reduction decreased with time.

The main advantages of the use of biogenically produced hydrogen sulfide for the removal of Cr(VI) from contaminated solutions are the high reduction efficiency and the low cost of chemicals. The production of hydrogen sulfide via sulfate reduction for the biotreatment of acid mine drainage has been extensively studied (for a review, see Kaksonen and Puhakka [15]). Although the reduction of Cr(VI) under sulfidogenic conditions has been well demonstrated, only few studies are available in the literature [6,11,16]. Furthermore, it should be underlined that hydrogen sulfide, as a very effective reducing agent, is responsible for the reduction of Cr(VI) under sulfate-reducing conditions such as those prevailing in marine environments [10].

Although hydrogen sulfide is able to detoxify chromium containing solutions, other media contaminated with Cr(VI), such as groundwater, may not contain sufficient sulfate to enable its generation. In this case, elemental sulfur is used as electron acceptor to enable production of hydrogen sulfide. In the presence of an electron donor, such as acetate, elemental sulfur is reduced by *Desulfuromonas* to hydrogen sulfide, according to reaction (1) [17], which can be then used for the chemical reduction of Cr(VI) (reaction (2)) [10,11].

$$CH_3COOH + 4S^0 + 4H_2O \rightarrow 2H_2CO_3 + 4H_2S \quad (1)$$

$$2CrO_4^{2-} + 3H_2S + 4H^+ \rightarrow 2Cr(OH)_3 + 3S^0 + 2H_2O \quad (2)$$

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (3)$$

A major advantage of using elemental sulfur instead of sulfate as electron acceptor is that it requires four times less electron donor for the production of the same amount of hydrogen sulfide (reactions (1) and (3)). Although few studies have investigated the simultaneous Cr(VI) and sulfate reduction using sulfate reducing bacteria [6,11,16], the use of elemental sulfur as electron acceptor to generate hydrogen sulfide which is used for the reduction of Cr(VI) has not yet been studied. Elemental-sulfur is non-toxic, insoluble in water, stable under ambient conditions, and readily available. It can be also used as support material in bioreactors for sulfur reducing bacteria. Hence, the present study investigates the efficiency of biological removal of Cr(VI) in a packed-bed bioreactor in which elemental sulfur serves as electron acceptor for the production of hydrogen sulfide and the subsequent reduction of Cr(VI).

2. Materials and methods

2.1. Abiotic-chemical reduction of Cr(VI)

In order to explore the abiotic reduction of Cr(VI) with elemental sulfur, a batch experiment was carried out in the absence of biomass. In this experiment, an anaerobic reactor containing all required micro and macro nutrients, 5 g elemental sulfur, and 2 mg/L Cr(VI) was operated at 30 °C for 24 h.

In the second batch test, the chemical reduction of Cr(VI) was evaluated to confirm the stoichiometry of its reduction by hydrogen sulfide. 150 mL serum bottles were filled with 100 mL distilled water containing 100 mg/L Cr(VI) and covered with rubber septa and aluminum caps. The bottles were purged with N_2 gas for 5 min to remove oxygen. Effluent from a sulfur-reducing bioreactor (Section 2.2) containing the stoichiometric amount of hydrogen sulfide as indicated by reaction (2) (9.5 mg HS^-) was added to the bottles using a syringe. The bottles were incubated overnight at 30 °C in a shaking incubator operating at 100 rpm. Four runs were performed: two of them were controls and did not involve addition of hydrogen sulfide whereas hydrogen sulfide was added in stoichiometric amount to the other two parallel runs. The bottles were sampled at the start and the end of the incubation period in order to determine pH, and the concentration of hydrogen sulfide and Cr(VI).

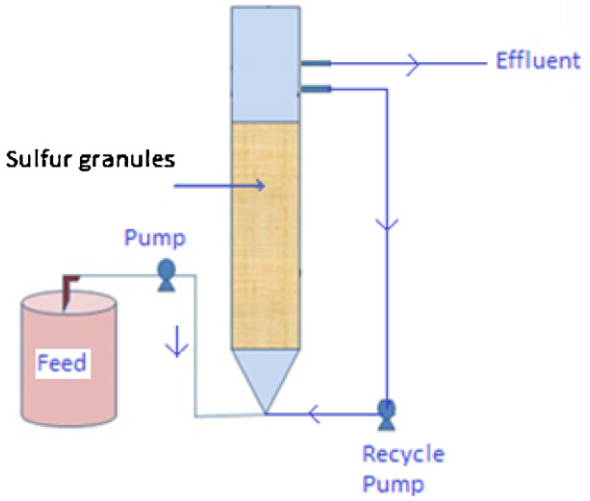

Fig. 1. Schematic representation of the column reactor used in this study.

In a third experiment, which was identical to the second one, except that biogenically produced hydrogen sulfide was added to the 1 L vessels. Sampling was done at specific time intervals and subsequent determination of the Cr(VI) reduction rate.

2.2. Bioreactor set up and operation

A laboratory glass column bioreactor with an empty bed volume of 500 mL was used (Fig. 1). The reactor was filled with commercially available elemental sulfur (3–5 mm, supplied from Microtek Ltd., Turkey) as support material and electron acceptor and covered with aluminum foil to prevent growth of phototrophic bacteria. Sulfate reducing sludge obtained from an anaerobic baffled reactor treating acid mine drainage was used as inoculum [18]. The reactor operated in batch mode for 7 days after inoculation, and then in continuous up-flow mode at 28–30 °C in a temperature controlled room. The feed contained micro and macro nutrients (56 mg/L KH_2PO_4, 110 mg/L NH_4Cl, 11 mg/L ascorbic acid and 50 mg/L yeast extract) and ethanol or acetate as electron donor and carbon source (1000 mg/L as COD). The feed was supplemented with 1000 mg/L $NaHCO_3$ to maintain the pH at neutral values, as well as with $K_2Cr_2O_7$ to obtain the desired Cr(VI) concentration (Table 1). All chemicals were purchased from Merck (Germany). The feed solution was kept refrigerated at 4 °C prior to use to prevent COD removal, sulfate reduction, and metal precipitation.

Synthetic wastewater was fed into the bioreactor (500–1400 mL/day) using a peristaltic pump to maintain the desired HRT (Table 1). The effluent was recirculated in the bioreactor at a ratio (flow rate of wastewater/flow rate of recirculated effluent) of 500 until day 142 in order to dilute feed, increase mass transfer and enable reactor operation in a completely mixed mode. HRT was calculated by considering the empty bed volume of the bioreactor and the feed flow rate without taking recirculation into account. After day 142, no recirculation took place in order to prevent loss of H_2S to the gas phase and thus increase its concentration in the liquid phase. Sampling of the reactor feed and the effluent was carried out 3 times a week to determine pH, alkalinity and COD, as well as the concentration of Cr(VI) and

Table 1
Operational conditions of the bioreactor.

Periods	1	2	3	4	5	6	7	8	9	10
Days	0–63	64–74	75–107	108–119	120–168	169–185	186–203	204–214	215–240	241–255
Electron source (1000 mg/L COD)	EtOH	EtOH	EtOH	EtOH	EtOH	EtOH	EtOH	EtOH	Ac	Ac
Temperature (°C)	22–31	32 ± 2	32 ± 2	32 ± 2	32 ± 2	30 ± 1	29 ± 1	30 ± 1	30 ± 2	30 ± 2
Cr(VI) (mg/L)	0	50	0	0	5	5	10	10	0	10
HRT (days)	0.36	0.36	1	0.7	0.7	1	1	0.5	0.5	0.5

EtOH: ethanol; AC: acetate.

dissolved hydrogen sulfide. The effluent was also sampled once a week to determine the concentration of total residual chromium.

The performance of the ethanol- or acetate-fed bioreactor was assessed for a period of 255 days, which was divided into 10 sub-periods (Table 1). In the first period (period I, days 0–63), the reactor was fed with Cr(VI) free wastewater to allow enrichment of the ethanol-oxidizing sulfur-reducing bacteria. In this period the effect of temperature, which varied between 22 and 31 °C, on the reactor performance was also evaluated. In the second period, the effect of high Cr(VI) concentration (50 mg/L) on the reactor performance at an HRT of 0.36 days was evaluated. In the third period, the reactor performance was assessed in the absence of Cr(VI) using an increased HRT of 1 day. In the following periods, up to period 8, the effect of different Cr(VI) concentrations on the bioreactor performance was investigated. Finally, in the last two periods, 9 and 10, the degree of acetate oxidation was assessed in the absence or presence of Cr(VI).

2.3. Analytical techniques

Liquid samples were centrifuged at 3000 g for 10 min using a Hettich Rotofix 32 centrifuge, prior to determination of the concentrations of dissolved hydrogen sulfide, Cr(VI) and COD in the supernatant. The total concentration of hydrogen sulfide was determined spectrophotometrically using a Shimadzu UV-1601 spectrophotometer according to the method of Cord-Ruwisch [19]. Both COD and alkalinity were determined using APHA standard methods [20]. Prior to COD determination, samples were acidified to a pH less than 2 by addition of concentrated H_2SO_4 and then purged with N_2 gas for approximately 5 minutes to remove H_2S. For the determination of alkalinity, unfiltered samples were titrated by 0.1 M HCl to a pH endpoint of 4.5. Soluble Cr(VI) was determined using the diphenyl carbazide method [20]. Total chromium concentrations were determined with an ICP-OM (Perkin Elmer Optima 5300) atomic emission spectrophotometer. All measurements were performed in duplicate and mean values of the results are presented. When the standard deviation was larger than the size of the plotting symbol, ±error bars are shown.

3. Results

3.1. Hydrogen sulfide production and COD oxidation in the bioreactor

The performance of the bioreactor over its entire operation period is presented in Figs. 2 and 3. Table 2 provides data about the average steady-state performance of the bioreactor in each period. In the first period, the effect of temperature on the production rate of hydrogen sulfide and the degree of COD oxidation was evaluated in the absence of Cr(VI). It is seen from the experimental data that temperature had a significant effect on reactor performance as decrease of temperature from 28 °C to 22 °C on day 37 resulted in decreased concentrations of hydrogen sulfide from 367 to around 130 mg/L. On the other hand the concentration of COD in the effluent increased from around 120 to 700 mg/L.

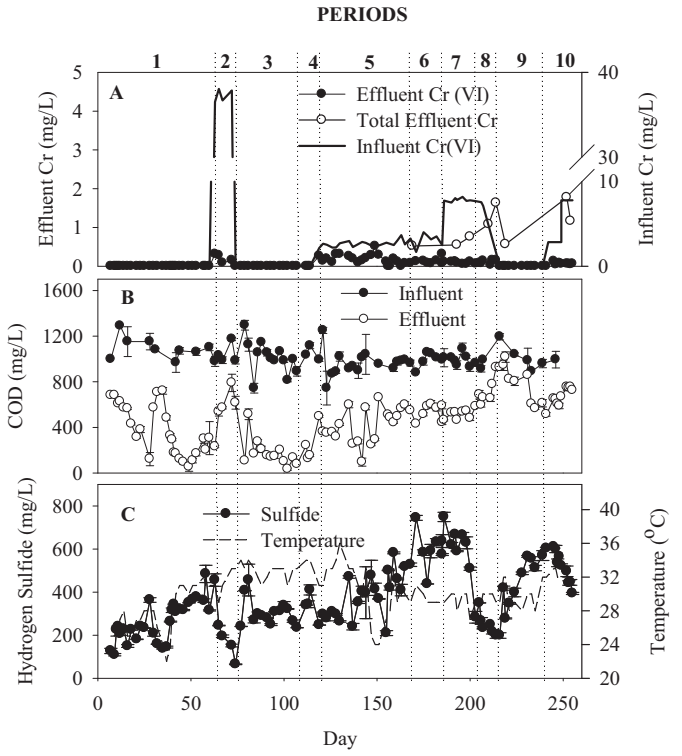

Fig. 2. Cr(VI) and total chromium (A), COD (B), hydrogen sulfide and temperature (°C) variations during bioreactor operation.

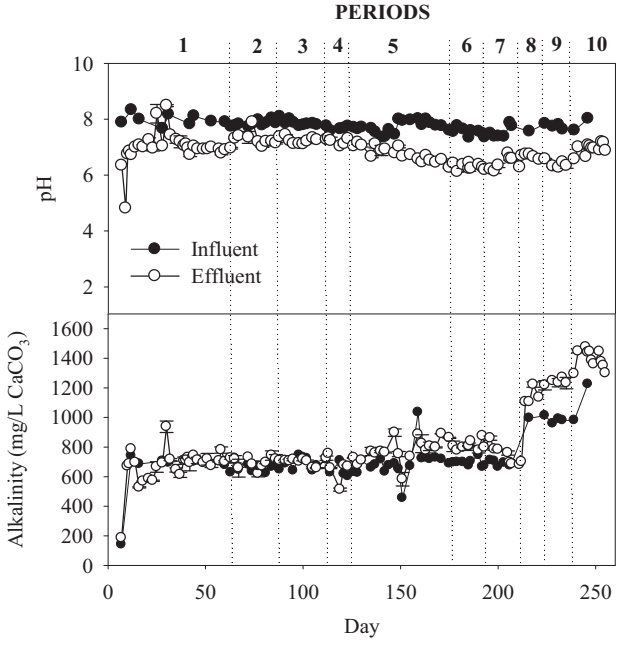

Fig. 3. Variations of feed and effluent pH (A) and alkalinity concentrations (B) during bioreactor operation.

Table 2
Performance of the bioreactor during steady-state operation.

Periods	1	2	3	4	5	6	7	8	9	10
Days	0–63	64–74	75–107	108–119	120–168	169–185	186–203	204–214	215–240	241–255
Effluent COD (mg/L)	321 ± 14 (78%)	629 ± 112 (37%)	149 ± 66 (85%)	175 ± 69 (83%)	521 ± 169 (48%)	528 ± 68 (47%)	509 ± 48 (50%)	634 ± 96 (37%)	703 ± 274 (30%)	683 ± 65 (32%)
Hydrogen sulfide (mg/L)	267 ± 98	164 ± 76	288 ± 31	364 ± 73	488 ± 62	593 ± 96	626 ± 67	300 ± 101	429 ± 143	512 ± 74
Cr(VI) (mg/L)	–	0.17 ± 0.13 (99.7%)	–	–	0.17 ± 0.12 (96.6%)	0.1 ± 0.03 (98%)	0.11 ± 0.08 (99%)	0.1 ± 0.04 (99%)	–	0.07 ± 0.027 (99.3%)
Total Cr (mg/L)	–	ND	–	–	ND	0.54 ± 0.02 (89%)	0.66 ± 0.15 (94%)	1.4 ± 0.39 (86%)	–	1.47 ± 0.43 (85%)

ND: not determined, and the values in parentheses represent degrees of removal.

Increase in temperature from 22 °C to 31–32 °C for a period of 22 days (days 42–63) resulted in increased concentrations of hydrogen sulfide (350–400 mg/L) and decreased concentrations of COD (60–150 mg/L) in the effluent (Fig. 2, period 1).

In the second period, the effect of high Cr(VI) concentrations on the reactor performance was investigated. It is underlined that when the theoretical concentration of Cr(VI) in the feed increased to 50 mg/L, the measured Cr(VI) concentration was only 37 mg/L, due to the immediate reduction of Cr(VI) to Cr(III) by the nutrients present in solution. No further reduction of the Cr(VI) concentration was noticed after storage of the feed at 4 °C. The experimental data show clearly that high Cr(VI) concentrations inhibit the activity of sulfur-reducing and ethanol-oxidizing bacteria, so the concentration of COD in the effluent increased to around 800 mg/L while the concentration of hydrogen sulfide decreased to around 65 mg/L (Fig. 2). Despite this, almost complete reduction of Cr(VI) was observed and the residual Cr(VI) concentration in the effluent was 0.17 ± 0.13 mg/L (Table 2).

In the third period, where no Cr(VI) was present in the feed, the HRT was increased to 1 days in order to recover system performance. As a result, the reactor efficiency improved: the concentration of COD in the effluent decreased to 150 mg/L and the concentration of hydrogen sulfide increased to 300 mg/L.

In the fourth period the HRT was decreased to 0.7 days while in the fifth period the feed was amended with 5 mg/L Cr(VI). At this stage a malfunction of the heating unit occurred (from days 147 to 157) and resulted in a decrease in bioreactor temperature to 25–28 °C and an increase in the concentration of COD in the effluent to 500 mg/L. As mentioned earlier no recirculation took place after day 142 and thus the concentration of hydrogen sulfide increased sharply (Fig. 2), due to the decrease in the amount of hydrogen sulfide escaping to the gas phase as a result of less intensive mixing (Table 2). Although in the fourth period the degree of COD removal was much higher compared to the fifth period (83% instead of 48%), the concentration of hydrogen sulfide in the fifth period showed the opposite trend (Table 2).

In the sixth period, despite the fact that the HRT was increased to 1 days and the concentration of Cr(VI) was maintained at 5 mg/L, no improvement in COD removal efficiency was noted. Although increased concentrations of hydrogen sulfide were recorded when no recirculation took place (day 142), the degree of COD removal decreased appreciably (periods 1–4: with recirculation, periods 5–10: without recirculation).

In the seventh period, the concentration of Cr(VI) in the feed was increased to 10 mg/L, without any noticeable effect on the degree of COD removal, concentration of hydrogen sulfide and overall reactor performance. In the eighth period, when the HRT was decreased again to 0.5 day, the reactor performance decreased significantly as the degree of COD removal and concentration of hydrogen sulfide dropped to 37% and 300 mg/L, respectively (Table 2).

In the ninth period, ethanol was replaced with acetate in the reactor feed and the degree of acetate oxidation in the absence of Cr(VI) was investigated. Although the degree of COD removal was almost 30% at HRT 0.5 day, the concentration of hydrogen sulfide remained at quite high levels and averaged 429 mg/L. In the tenth period, the performance of the acetate-fed bioreactor was only slightly affected by the presence of 10 mg/L Cr(VI) in the feed and the concentration of hydrogen sulfide increased to 512 mg/L.

The theoretical concentration of hydrogen sulfide in the bioreactor was calculated using the stoichiometry of reaction (1), which suggests that the oxidation of 1 mg COD produces 2 mg of hydrogen sulfide (H_2S–S). Fig. 2C compares the theoretical and measured hydrogen sulfide concentrations. It is shown that until day 142 when the effluent was recirculated in the bioreactor, the theoretically calculated concentrations of hydrogen sulfide were much higher than the measured ones. However, when no recirculation

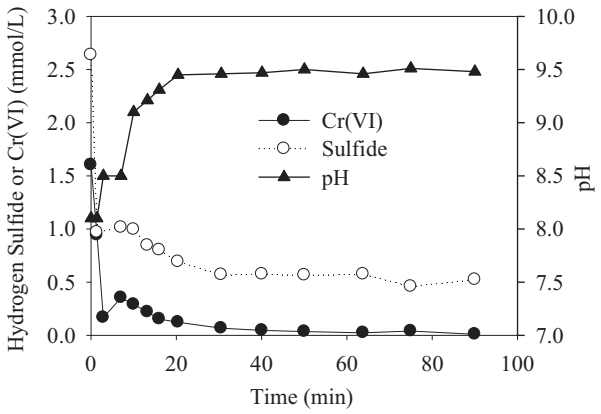

Fig. 4. Variations of Cr(VI), hydrogen sulfide and pH in batch kinetic experiments.

took place, the theoretical concentrations were very similar to the measured ones.

The variation of influent and effluent pH and alkalinity vs. time is shown in Fig. 3. Effluent pH is slightly lower than that in the feed, probably due to the production of CO_2 as a result of organic matter oxidation. Although feed and effluent alkalinity values were quite similar until period 9, increased effluent alkalinity was recorded when ethanol was replaced with acetate.

3.2. Chromium removal

3.2.1. Batch tests

Batch adsorption tests revealed that abiotic reduction of Cr(VI) by elemental sulfur or adsorption on elemental sulfur is negligible.

The second set of batch tests was conducted to determine whether Cr(VI) reduction occurs chemically with the produced hydrogen sulfide as well as to confirm the stoichiometry of reaction (2). In the control tests, when no hydrogen sulfide was used, the concentration of Cr(VI) decreased by almost 10%, from 96 mg/L to 85.5 mg/L. In the tests involving the addition of the stoichiometric amount of hydrogen sulfide, the concentration of Cr(VI) decreased from 96 mg/L to less than 0.1 mg/L, while the concentration of hydrogen sulfide decreased from 91 mg/L to less than 0.07 mg/L. Hence, batch tests confirmed that Cr(VI) reduction proceeds according to the stoichiometry of reaction (2).

In the third set of batch tests, the rate of Cr(VI) reduction by biogenically produced hydrogen sulfide was investigated (Fig. 4), where the initial concentrations of Cr(VI) and hydrogen sulfide were 1.6 mmol/L (83.35 mg/L) and 2.64 mmol/L (84.4 mg/L), respectively. Experimental results revealed that the reaction progressed quite fast and the concentration of Cr(VI) decreased from 1.6 mmol/L (83.4 mg/L) to 0.94 mmol/L (49 mg/L) within 1.4 min whereas afterwards the rate of Cr(VI) reduction, which was completed in 90 min, dropped substantially (Fig. 4). According to the stoichiometry of reaction (2), the concentration of hydrogen sulfide at the end of the test should have been 0.25 mmol/L (7.89 mg/L). However, the determined value was slightly higher and reached 0.52 mmol/L (16.77 mg/L), as part of Cr(VI) was probably reduced by the organics or inorganics, which were present in the bioreactor effluent added to the batch tests to supply the required amount of hydrogen sulfide. The pH increased from 8.1 to around 9.5 as a result of acid consumption during the reduction of Cr(VI) according to reaction (2) (Fig. 4).

3.2.2. Continuous bioreactor tests

Sulfur reduction coupled with organic oxidation produces hydrogen sulfide according to reaction (1). The variation of the hydrogen sulfide concentration versus time is illustrated in Fig. 2. The maximum hydrogen sulfide concentration of around 750 mg/L was recorded in the seventh period, whereas the lowest concentration was recorded in the second period when the highest influent Cr(VI) concentration was used.

The produced hydrogen sulfide was used to reduce and immobilize soluble Cr(VI) according to reaction (2). Influent and effluent soluble Cr(VI) and total chromium concentrations are presented in Fig. 2. Table 2 shows the average effluent Cr(VI) and total chromium concentrations as well as the respective degrees of removal in each period. The differences observed between total chromium and Cr(VI) concentrations denote the concentration of soluble Cr(III).

4. Discussion

4.1. Biological Cr(VI) reduction and precipitation of Cr(III)

Chromium causes human toxicity and thus the World Health Organization (WHO) and the European Commission (Water Directive98/83/EC) have recommended that its maximum allowable concentration in drinking water should not exceed 50 μg/L [21]. Hence, it is important to develop efficient treatment technologies in order to remove chromium from wastewaters and industrial effluents and thus prevent contamination of water resources and reduce the risk for humans. In the present study, the concentration of total chromium in the effluent of a sulfur packed bioreactor was similar to the Cr(VI) concentration, indicating that almost all Cr(III) was either precipitated or adsorbed and was retained in the system in solid form (Fig. 2 and Table 2). The relatively higher concentrations of total chromium observed in the effluent in the eighth and tenth period were the result of increased concentrations of Cr(III) due to the shorter HRT (0.5 day) applied during these periods. Although the average pH in the bioreactor (6.9 ± 0.5, Fig. 3) was slightly lower than the pH range required for minimum Cr(III) solubility (7.5–9.0 according to Chung et al. [9]), the concentration of Cr(III) in the effluent was very low (<1.5 mg/L, Table 2) and the degree of total chromium removal reached 90%. Hence, the hydrogen sulfide produced by the reduction of sulfur can be effectively used for the reduction of Cr(VI). To the best of our knowledge, this is the first study showing that elemental sulfur can be efficiently used as electron acceptor for the generation of hydrogen sulfide, that subsequently chemically reduces dissolved Cr(VI) to Cr(III) which precipitates in the bioreactor.

It is known that pure and mixed bacterial cultures have the ability to reduce Cr(VI) to Cr(III) under aerobic or anaerobic conditions [6,7,12,14,21–25]. In order to achieve efficient chromium removal, Cr(III) should be present in solid form so that it can be easily removed from the system during the subsequent solid/liquid separation [9,23]. Table 3 compares the results of the present and other studies. Elangovan and Philip [13] evaluated the performance of various bioreactors (aerobic suspended, aerobic attached and anoxic attached growth bioreactors) under different operating conditions and determined the degree of Cr(VI) reduction. All reactors were inoculated with chromium reducing *Arthrobacter rhombi-RE* isolated from a soil contaminated with chromium. Although almost complete reduction of Cr(VI) was achieved, the concentration of total chromium in both the influent and effluent was almost the same, indicating that all Cr(III) was present in the aqueous phase (Table 3). In another study, Chirwa and Wang [22] studied the reduction of Cr(VI) by *Bacillus* sp. in an aerobic packed-bed bioreactor, operating under varying influent concentrations of Cr(VI) (10–200 mg/L) and HRTs (6–24 h). Although almost complete reduction of Cr(VI) was achieved, the concentration of Cr(III) in the effluent was almost similar to the concentration of Cr(VI) in the influent, indicating that Cr(III) was not retained in the system.

Table 3
Comparison of biological treatment systems for Cr(VI) removal.

Reactor system	Microorganism	Initial Cr(VI) (mg/L)	Cr(VI) reduction efficiency (%)	Total Cr removal efficiency (%)	Reference
Aerated packed-bed	*Bacillus* sp.	10–200	~100	~ 0	[22]
Packed-bed	*Acinetobacter haemolyticus*	15	97	~15	[28]
Draft-tube airlift	*Candida* sp.	78	~100	~3	[29]
Zeolite packed column	*Arthrobacter viscosus*	100	100	73	[30]
Aerobic suspended growth		18–20	20–90	2–4	
Aerobic attached growth	*Arthrobacter rhombi-RE*	18–36	50–98	~0	[13]
Anoxic attached growth		18–36	50–98	~0	
Aerobic activated sludge (AS)		0.5–5	37–45	37–45	
Anoxic–aerobic AS	Activated sludge	1.0	80	80	[25]
Anaerobic–anoxic–aerobic AS		1.0	84	84	
Sand column	Indigenous consortium	12	39.1–63.6	NM	[7]
Denitrifying hydrogen-based membrane biofilm	Mixed-culture biofilm	0.25–1.0	45–63	~0	[8]
Sulfidogenic two-stage packed-bed reactor system	Mixed sulfate reducers	225–352.5	~100	NM	[11]
Sulfidogenic batch reactor	Mixed sulfate reducers	26 (500 μmol/L)	88	70	[6]
Sulfur reducing packed-bed bioreactor	Mixed sulfate reducers	5–50	~100	85–95	This study

NM: not mentioned.

Chung et al. [9] reported that although Cr(VI) was converted to Cr(III) in a hydrogen-based membrane biofilm reactor, the concentration of total chromium in the effluent was not affected, indicating that the produced Cr(III) was present in the system in soluble or colloidal (less than 0.2 μm) form. Minimum solubility of Cr(III) was observed at pH 8.0, while the optimum pH range required for low concentrations of Cr(III) in the effluent was 7.5–9.0.

Although several studies have investigated the reduction of Cr(VI) by pure cultures, data on Cr(VI) reduction by activated sludge are scarce. Stasinakis et al. [25] investigated the reduction of Cr(VI) by activated sludge and evaluated the use of conventional continuous flow activated sludge systems for the treatment of Cr(VI) containing wastewaters. For all Cr(VI) concentrations tested (0.5–5 mg/L), reduction was almost 40% while the use of an anoxic and anaerobic zone ahead of an aerobic reactor increased the degree of Cr(VI) reduction to almost 80%. The concentration of Cr(III) in the effluent was very low while the average degree of chromium removal was similar to the average degree of Cr(VI) reduction to Cr(III), which was adsorbed on suspended solids. Although the efficiency of the activated sludge process in terms of Cr(VI) reduction was lower than that recorded when pure aerobic cultures were used, the degree of Cr(III) removal and hence of total chromium was higher (Table 3).

The production of hydrogen sulfide in the packed-bed bioreactor was directly related to the utilization of organic matter (ethanol, acetate) (Fig. 2). The present study indicates that when no effluent recirculation took place (after day 142), the degree of COD removal decreased appreciably (Fig. 2 and Table 2), which might be due to the increased concentration of hydrogen sulfide and, hence, the toxicity on sulfur reducers. At the same time, the theoretical concentration of hydrogen sulfide decreased due to the decreased rate of COD oxidation in the system (Fig. 2). When effluent was recirculated, the theoretically calculated concentrations of hydrogen sulfide were much higher than the measured ones, probably due to the loss of hydrogen sulfide to the gas phase at relatively low pH (6.9 ± 0.5) or the formation of polysulfides as a result of the reaction between hydrogen sulfide and elemental sulfur [27]. Recirculation of the effluent establishes completely mixed conditions within the reactor, which may facilitate the escape of hydrogen sulfide to the gas phase. Chung et al. [9] mention that completely mixed conditions are established when a recirculation ratio of 150 is used. When no recirculation took place, losses to the gas phase decreased and the theoretically calculated concentrations of hydrogen sulfide were similar to the measured ones.

4.2. Reduction of Cr(VI) via sulfide produced from the reduction of elemental sulfur

In the present study, elemental sulfur was used instead of sulfate under the assumption that Cr(VI) present in sulfate deficient wastewaters can be effectively reduced by hydrogen sulfide produced from the reduction of elemental sulfur. Batch experiments showed that Cr(VI) reduction to Cr(III) occurs chemically coupled with the oxidation of hydrogen sulfide to sulfur (Fig. 4), following the stoichiometry of reaction (2). Stoichiometric addition of hydrogen sulfide to a solution containing 96 mg/L Cr(VI) resulted in almost complete removal of both Cr(VI) and hydrogen sulfide. The reduction of Cr(VI) was visually monitored through the disappearance of the yellow color of the chromate containing solution and the formation of a green/violet Cr(III) precipitate. Reduction of Cr(VI) resulted in consumption of protons and increase of pH from 8.1 to 9.5 (Fig. 4) in batch test, which can thus facilitate precipitation of Cr(III) as $Cr(OH)_3$ [9].

It has been reported in the literature that Cr(VI) can be effectively reduced chemically by hydrogen sulfide [10] while sulfate reducing bacteria can be also used to directly reduce certain metals, such as Cr(VI), U(VI) or As(V) [11]. Kim et al. [10] investigated reaction stoichiometry, kinetics and mechanisms of Cr(VI) reduction by hydrogen sulfide and reported that the reduced chromium was present as $Cr(OH)_3$ while hydrogen sulfide was oxidized to sulfur according to reaction (2). The overall reaction was second-order, i.e., first-order with respect to Cr(VI) and hydrogen sulfide, while the reaction rate increased as pH decreased, and pH dependence correlated well with the concentration of protonated hydrogen sulfide. The rate of Cr(VI) reduction increased sharply when the pH was less than 8.0 due to the increased concentration of H_2S in the system [10]. It is believed that in the present study the high efficiency in terms of Cr(VI) reduction may also be due to the fact that the bioreactor operated at a pH (6.9 ± 0.5) lower than 8.0.

It is known that the main Cr(VI) removal mechanism in the absence of sulfate is the dissimilatory reduction of Cr(VI) to Cr(III) by sulfate reducing bacteria [16], while in sulfate-rich environments Cr(VI) may be reduced biologically [6] or chemically coupled with the oxidation of hydrogen sulfide to elemental sulfur according to reaction (2) [11]. Smith and Gadd [6] investigated the ability of sulfate-reducing bacterial biofilms to reduce 500 μmol/L Cr(VI) to insoluble Cr(III) over a period of 48 h, using lactate as electron donor in the presence of sulfate and reported that the degree of total chromium removal reached 88% and that 80% of total chromium precipitated in the reactor. Although Cr(VI) did not have a significant effect on carbon utilization, it severely affected reduction

of sulfate and resulted in the generation of very low levels of hydrogen sulfide. Similarly, Tucker et al. [26] suggested that the dissimilatory reduction of Cr(VI) by *Desulfovibrio desulfuricans* is mediated by enzymatic reactions. On the contrary, Chang and Kim [11] reported that Cr(VI) reduction occurs chemically in sulfate-rich environments (reaction (2)) with the hydrogen sulfide produced by sulfate reducing bacteria. Data derived from the present study support the findings of Chang and Kim [11] as in the presence of Cr(VI), high concentrations of hydrogen sulfide and a high degree of organic oxidation were observed in the system (Fig. 2), indicating that Cr(VI) reduction occurs chemically by the hydrogen sulfide. Also, the almost instant (in less than 2 min) reduction of Cr(VI) with the biogenically generated hydrogen sulfide (Fig. 4) proves the chemical nature of the process.

5. Conclusion

The present study shows that elemental sulfur can be effectively used as support medium and electron acceptor in a packed bed bioreactor to generate hydrogen sulfide, which almost instantly reduces Cr(VI), to Cr(III). Temperature has a significant effect on hydrogen sulfide generation as a decrease in temperature from 28 °C to 22 °C resulted in noticeably decrease in the rates of hydrogen sulfide generation and COD oxidation. In general, Cr(VI) reduction and the degree of total chromium removal exceeded 97% and 85%, respectively, while the reduced chromium was retained in the bioreactor. The experimental results thus confirm that sulfur reducing packed-bed bioreactors can be used for the efficient removal of Cr(VI) from sulfate deficient industrial effluents and wastewaters. Additional studies are required to justify the role of microbial community inside the bioreactor, further elucidate reaction mechanisms and accurately design in situ (in bioreactors) and ex situ (in reactive bio-barriers) applications for the treatment of waters contaminated with Cr(VI).

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