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Biological chromium(VI) reduction using a trickling filter

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Abstract

A pilot-scale trickling filter was constructed and tested for biological chromium(VI) removal from industrial wastewater. Indigenous bacteria from industrial sludge were enriched and used as inoculum for the filter. Sodium acetate was used as carbon source and it was found to inhibit chromate reduction at high concentrations. Three different operating modes were used to investigate the optimal performance and efficiency of the filter, i.e. batch, continuous and SBR with recirculation. The latter one was found to achieve removal rates up to 530 g Cr(VI)/m^2 d, while aeration was taking place naturally without the use of any external mechanical means. The low operating cost combined with the high hexavalent chromium reduction rates indicates that this technology may offer a feasible solution to a very serious environmental problem.

Keywords: Chromate; Biological removal; Trickling filter; Sequencing batch reactor; Recirculation

1. Introduction

Chromium is one of the most toxic heavy metals discharged into the environment through various industrial wastewaters, and has become a serious health problem. Metal plating, tanneries and industrial processes using catalysts discharge worldwide huge amounts of chromium every year. The effluents of these industries contain Cr(VI) and Cr(III) at concentrations ranging from tenths to hundreds of milligrams/liter. While Cr(VI) is highly toxic and is known to be carcinogenic and mutagenic to living organisms [1], Cr(III) is generally only toxic to plants at very high concentrations and is less toxic or non-toxic to animals [2]. The discharge of Cr(VI) to surface water is regulated to below 0.05 mg/l by the US EPA [3] and the European Union [4], while total Cr, including Cr(III), Cr(VI) and its other forms, is regulated to below 2 mg/l [3].

At present, the most commonly used technology for treatment of heavy metals in wastewaters is chemical precipitation. Conventional chemical treatment involves reduction of Cr(VI) to Cr(III) by a reducing agent under low-pH conditions and subsequent adjustment of solution pH to near neutral ranges to precipitate Cr(III) as hydroxides [5]. However, this method is not completely satisfactory because of the large amount of secondary waste products due to various reagents used in the above-mentioned processes.

Biological treatments arouse great interest because of their lower impact on the environment as opposed to chemical treatments. Recent studies have shown that certain species of bacteria are capable of transforming hexavalent chromium, Cr(VI), into the much less toxic and less mobile trivalent form, Cr(III) [6,7]. Bacteria may protect themselves from toxic substances in the environment by transforming toxic compounds through oxidation, reduction or methylation into more volatile, less toxic or readily precipitating forms.

The processes by which microorganisms interact with toxic metals enabling their removal/and recovery are bioaccumulation, biosorption and enzymatic reduction [8]. Microbial heavy metal accumulation often comprises of two phases. An initial rapid phase involving physical adsorption or ion exchange at cell surface and by a subsequent slower phase involving active metabolism-dependent transport of metal into bacterial cells [9]. Biosorption is a metabolism-independent process and thus can be performed by both living and dead microorganisms. This adsorption is

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based on mechanisms such as complexation, ion exchange, coordination, adsorption, chelation and microprecipitation, which may be synergistically or independently involved [10]. Enzymatic reduction of Cr(VI) into Cr(III) is believed to be one of the defense mechanisms employed by microorganisms living in Cr(VI)-contaminated environments. The reduced Cr(III) may precipitate as chromium hydroxide in neutral pH range [11].

Most of the previous studies on biological reduction of Cr(VI) were conducted in batch reactors (flasks) using mainly pure cultures. For instance, Wang and Xiao [12] studied several factors affecting hexavalent chromium reduction in pure cultures of bacteria in flasks. Wang and Shen [5] studied the kinetics of Cr(VI) reduction by pure bacterial cultures in flasks. Shakoori et al. [13] isolated a dichromate-resistant Gram-positive bacterium from effluent of tanneries and used flasks as batch reactors. Fein et al. [14] used pure bacterial cultures in flasks to study the non-metabolic reduction of Cr(VI) by bacteria under nutrient-absent conditions. Srinath et al. [8] studied Cr(VI) biosorption and bioaccumulation by pure cultures of chromate resistant bacteria in flasks. Megharaj et al. [15] studied hexavalent chromium reduction in flasks, by pure cultures of bacteria isolated from soil contaminated with tannery waste.

Recently, continuous-flow and fixed-film bioreactors were also used for biological reduction of Cr(VI). Shen and Wang [16] demonstrated Cr(VI) reduction in a two-stage, continuous-flow suspended growth bioreactor system. *Escherichia coli* cells grown in the first-stage completely mixed reactor were pumped into the second-stage plug-flow reactor to reduce Cr(VI). Chirwa and Wang [11] demonstrated the potential of fixed-film bioreactors for Cr(VI) reduction. This was the first report on Cr(VI) reduction through biological mechanisms in a continuous-flow laboratory-scale biofilm reactor without the need to constantly resupply fresh Cr(VI)-reducing cells. *Bacillus* sp. was used in this work for the transformation of Cr(VI) into Cr(III).

Virtually all the previous studies on biological reduction of Cr(VI) were conducted in laboratory scale apparatus (reactors), using sterilized conditions and pure cultures of microorganisms. The present study is the first to report on Cr(VI) biological reduction in a pilot-scale trickling filter using mixed culture of microorganisms, originating from an industrial sludge. The operation of the trickling filter as a Sequencing Batch Reactor (SBR) with recirculation led to significantly high Cr(VI) reduction rates, thus promising a feasible technological solution to a serious environmental problem.

2. Materials and methods

2.1. Media

The influent feed to the bioreactor was prepared by dissolving 1 g NH_4Cl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.001 g $FeSO_4{\cdot}7H_2O$, 0.001 g $CaCl_2{\cdot}2H_2O$, 5 g $CH_3COONa{\cdot}3H_2O$ and 0.5 g K_2HPO_4 in 1.0 l of tap water.

2.2. Reagents

Stock Cr(VI) solution (500 mg/l) was prepared by dissolving 141.4 mg of 99.5% $K_2Cr_2O_7$, previously dried at 103 °C for 2 h, in Milli-Q water and diluting to 100 ml. Diphenyl carbazide solution was prepared by dissolving 250 mg of 1,5-diphenylcarbazide in 50 ml of HPLC-grade acetone and storing in a brown bottle. Potassium hydrogen phthalate standard (KHP) was prepared by dissolving 425 mg in distilled water and diluting to 1000 ml. Digestion solution was prepared by dissolving 10.216 g $K_2Cr_2O_7$, previously dried at 103 °C for 2 h, in 500 ml distilled water, 167 ml conc. H_2SO_4 and 33.3 g $HgSO_4$ and diluting to 1000 ml (for the determination of the COD values).

1,5-Diphenylcarbazide was purchased from Fluka Chemical, potassium dichromate was purchased from Sigma Chemical Co. All the others chemicals were purchased from Riedel-de Haen.

2.3. Analytical methods

During all experiments, hexavalent chromium concentration, pH, temperature, dissolved oxygen concentration and TOC measurements were made on a daily basis. Samples were filtered through 0.45 μm –Millipore filters (GN-6 Metricel Grid 47 mm, Pall Corporation). Hexavalent chromium concentration was determined by the 3500-Cr D Colorimetric method according to Standard Methods for the Examination of Water and Wastewater [17]. Total organic carbon measurements (TOC) were conducted in order to determine the feed sodium acetate concentration both in the liquid culture (chemostat) and the liquid volume of the bioreactor, following the methods described in Standard Methods for the Examination of Water and Wastewater [17] by using, Total organic carbon analyzer (TOC-V_{CSH}, SHIMAZDU Corporation, Japan). Total chromium concentration measurements were made according to Standard Methods for the Examination of Water and Wastewater [17] using an atomic absorption spectrophotometer (model AAS-700, Perkin-elmer) (results not shown for total chromium concentrations).

2.4. Isolation and enrichment of indigenous bacteria

Samples of industrial sludge were taken from the Hellenic Aerospace Industry S.A. In order to grow bacterial strains able to reduce hexavalent chromium, a sludge sample of 10 g was added in a 2 l Erlenmeyer flask and was diluted in an acetate-minimal medium and concentrated chromium solution (in the form of $K_2Cr_2O_7$) resulting in a final hexavalent chromium concentration of 50 mg/l. The final volume of the solution was 1 l. Acetate-minimal medium (AMM) was comprising (per litre) 1 g NH_4Cl, 0.2 g $MgSO_4{\cdot}7H_2O$, 0.001 g

$FeSO_4 \cdot 7H_2O$, 0.001 g $CaCl_2 \cdot 2H_2O$, 5 g $CH_3COONa \cdot 3H_2O$, 0.5 g yeast extract and 0.5 g K_2HPO_4 in 1.0 l of tap water (micronutrients were supplied using tap water as diluent), while the final pH of the nutrient solution was adjusted to 7. The solution was kept under oxic conditions through aeration and mixing while nutrients and hexavalent chromium were added according to the observed chromium reduction. An intense chromium reduction was observed after 30 days of cultivation. Bacterial samples taken at this point showed, using 16S rRNA sequencing [18–20], that the dominant bacterial species of the mixed culture was *Acinetobacter* sp. Francisco et al. [21] also report that they identified bacteria belonging to the genus *Acinetobacter* in a chromium-contaminated activated sludge. The cell density of the liquid culture was determined as optical density at 600 nm on a JASCO V-530, spectrophotometer. The liquid culture was used to inoculate the bioreactor at the beginning of its operation.

2.5. Reactor system

The pilot-scale trickling filter (Fig. 1) consisted of a Plexiglas tube, 160 cm high and 9 cm i.d. This pilot-filter height is typical of a full-scale industrial filter. Since it is the loadings (hydraulic and chromium) per unit cross-sectional area that matter, no scale-up is necessary. The support material was gravel with a mean diameter of 5.5 mm, and specific surface area of 1059 m^2/m^3, while the depth of the support media was 143 cm and the filter porosity 0.4. The gravel was brought from a beach of Lefkas island, Greece. The filter media was loaded on the filter and washed with water from the supply network for 48 h. The support material was not flooded for flow rates up to 3000 ml/min or hydraulic loadings up to 680 m^3/m^2 d.

At the top of the filter, there was a fixed nozzle, which distributed the incoming solution evenly to the whole filter surface. The filter was also equipped with an underdrain system to collect the treated water and any biological solids that would detach from the media in the case of continuous operation mode. Along the filter depth there were 10 sampling ports for chromium concentration measurements in the bulk liquid. Thus, it was possible to have an experimental assessment of the chromium concentration along the filter depth and control the homogeneity of the bulk liquid concentration. Filter backwashing was necessary from time to time due to pore clogging from Cr(III) precipitates and was performed using high water and air velocities upwards.

Throughout all experiments water temperature was fairly constant at about $28 \pm 1\,^{\circ}C$ using a heat exchanger, while ambient temperature was about 26 °C (room temperature). The pH ranged from7.2 to 8.87 and the concentration of the dissolved oxygen in the liquid phase was physically maintained at a near constant level of 4.5 mg of DO/l in the reactor. The above measurements were performed using the Hanna pH211 pH meter, and the Hanna HI9143 dissolved oxygen meter, respectively. The use of a trickling filter has the advantage of not requiring an external air supply, since air is naturally convected through the filter due to the temperature difference between the interior and exterior of the filter. Mechanic aeration was added at the bottom of the filter only under batch operation.

The pilot-scale trickling filter was first operated as a batch reactor, subsequently as a sequencing batch reactor with recirculation, and finally as a continuous flow reactor. The recirculation was provided in order to obtain completely mixed flow pattern in the bioreactor, since the formation of chromate precipitates was leading to spatial heterogeneity

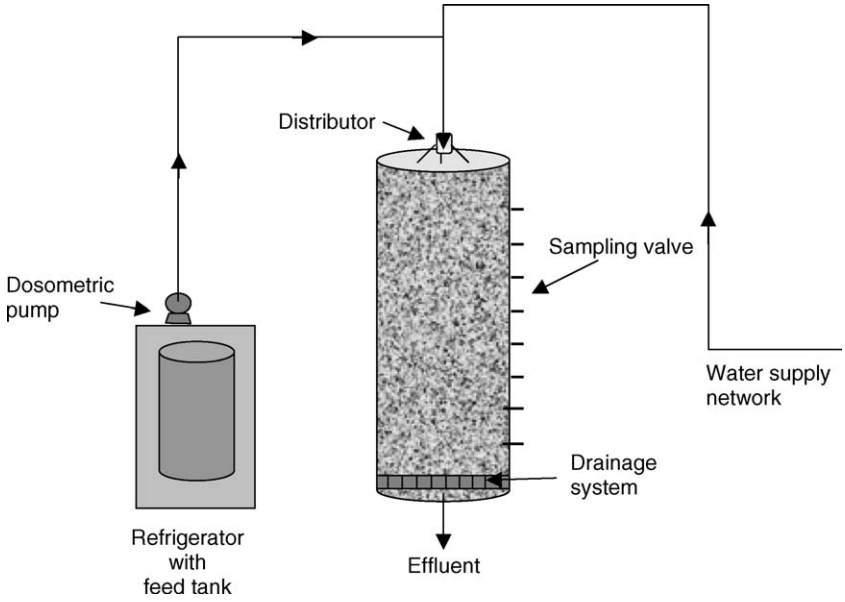

Fig. 1. Schematic drawing of the pilot-scale trickling filter arrangement.

and consequently to insufficient exploitation of the filter. The discrete values of 960, 2400 and 3200 ml/min were tested for the recirculation stream. Since no substantial difference was observed the value of 2400 ml/min was adopted as it was providing efficient filter wetting without flooding.

3. Experimental results

Before starting biological chromium reduction, an experiment was performed in order to investigate possible physicochemical chromium reduction under the specific experimental conditions. For this reason the clean filter (without any bacteria) was loaded with nutrients and chromium at a final concentration of 30 Cr(VI)mg/l, under batch operating mode. Mechanical aeration was added to the system and for a period of 8-h operation (Fig. 2) no substantial change of the concentration of hexavalent chromium was observed, indicating the absence of physicochemical reduction of hexavalent chromium. The addition of sodium acetate did not alter the above observation. Therefore, addition of bacteria was apparently necessary in order to achieve hexavalent chromium reduction.

3.1. Batch operation—reactor startup

The pilot-scale trickling filter was inoculated with enriched bacteria from the industrial sludge of the Hellenic Aerospace Industry. It was operated as a batch reactor for a period of about 50 days, to ensure attachment of the bacterial culture to the support media and development of a of biofilm layer (startup), and to investigate the maximum chromate reduction rate under this operating mode.

In the first cycle the filter was loaded with 8 mg/l hexavalent chromium (in the form of $K_2Cr_2O_7$) mixed with the influent feed to ensure bacterial growth. Sodium acetate was used in excess (5 g/l) in order to avoid growth limitation by carbon throughout the experiment. After 8 days a 47% reduction was observed under fully aerobic conditions (Fig. 3). The hexavalent chromium reduction rate was the highest observed up to this point. However, after this point there is a significant drop in reduction rate, as observed in preliminary experiments, which leads to a long time required for total reduction. Thus, in order to accelerate startup and keep a high reduction rate the system was reloaded at this point. Nutrients and hexavalent chromium were added (as well as at the beginning of each cycle) to reach the initial concentration of 8 mg/l. After another 8 days operation, maximum reduction rate and a 69% reduction of hexavalent chromium were observed, while during the third operating cycle the reduction of hexavalent chromium reached to 82%. During the fourth operating cycle the filter reached fully hexavalent chromium reduction in only 6 days and at this point we considered the startup period completed and the filter ready for full operation.

Nutrients were added again as well as hexavalent chromium this time to a concentration of 30 mg/l. This feed concentration value was used in all experiments as it was considered an extreme upper limit for the wastewater effluent of the Hellenic Aerospace Industry. A new series of operating cycles was performed, as in Fig. 3, until a minimum period of about 19 h was needed for complete reduction (100% removal) of 30 mg/l hexavalent chromium (Fig. 4). For the filter diameter of 9 cm or cross-sectional area of 63.62 cm^2, filter removal rate for the batch operating mode was about 18 g Cr(VI)/m^2 d.

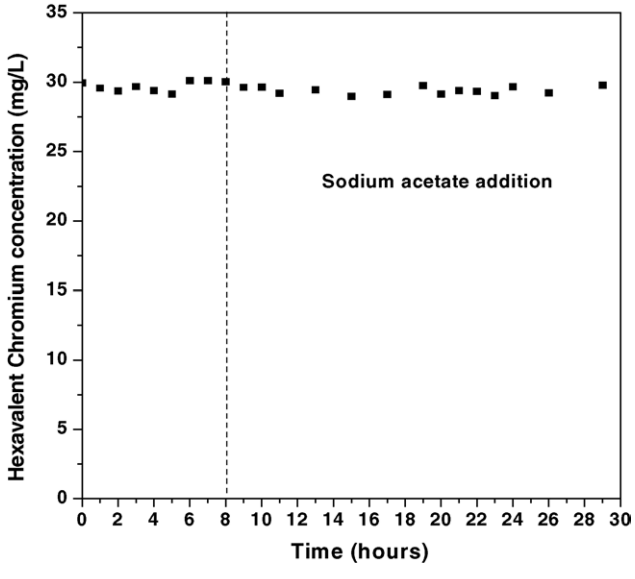

Fig. 2. Evolution of hexavalent chromium concentration in the clean (without bacteria) pilot-scale trickling filter with the addition initially of air and in sequence of air and sodium acetate.

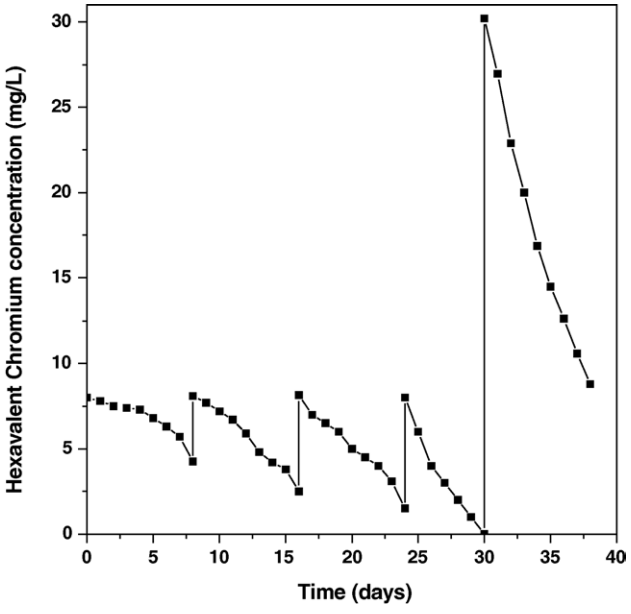

Fig. 3. Startup of the pilot-scale trickling filter for the biological reduction of hexavalent chromium.

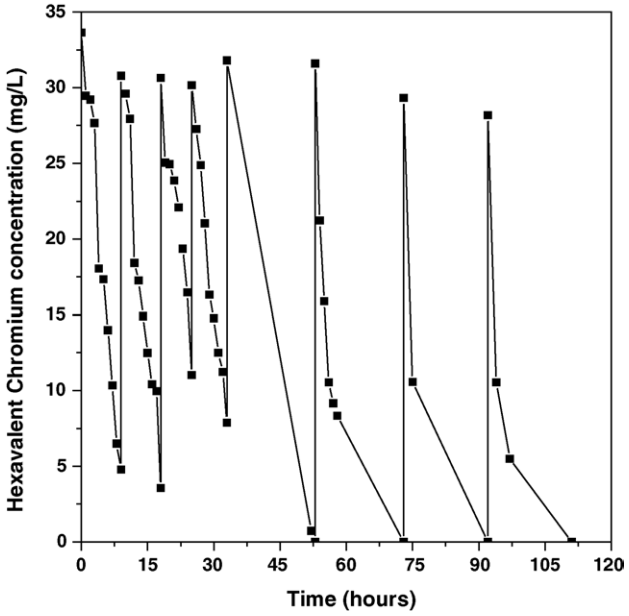

Fig. 4. Operating cycles of the filter under batch operation. Duration of minimum stable cycles: 19 h.

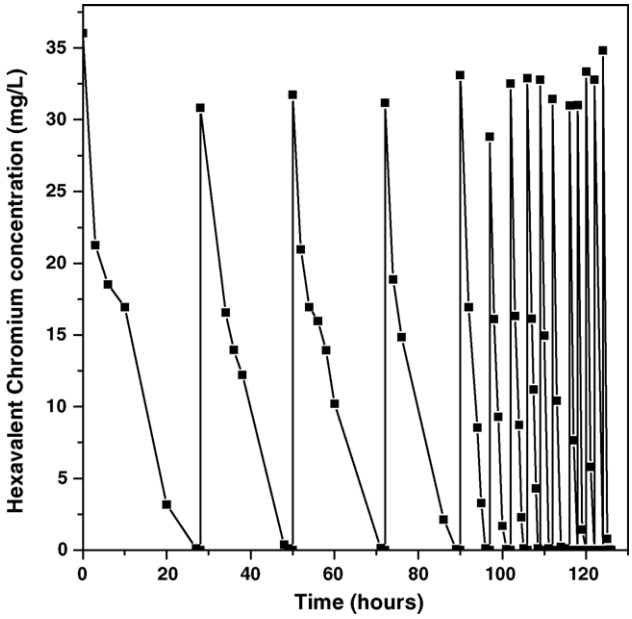

Fig. 5. Operating cycles of the filter under SBR operation with recirculation. Duration of minimum stable cycles: 40 min.

3.2. SBR operation with recirculation

The reduction of Cr(VI) to Cr(III) was followed by the formation of sediments, which caused obstruction of the flow along the filter depth (partial or complete pore clogging) and consequently insufficient exploitation of the entire filter volume. In order to overcome sediment formation and make better use of the entire filter, we switched the filter to sequencing batch reactor operating mode with recirculation. The filter was loaded with nutrients and hexavalent chromium solution to a final concentration of 30 mg/l. After several operating cycles the period was reduced to only 40 min (Fig. 5).

Fig. 6 shows the reduction of hexavalent chromium and the consumption of sodium acetate during an operating cycle of 40 min. It is obvious that sodium acetate is in excess (390 mg/l as organic carbon) while only about 120 mg/l of organic carbon is necessary for the reduction of about 33 mg/l of hexavalent chromium.

A reduction of the organic carbon to 265 mg/l did not alter the duration of the operating cycle, which remained at 40 min (Fig. 7a). Further reduction of the organic carbon to about 150 mg/l reduced even further the duration of the operating cycle to only 30 min (Fig. 7b), while for lower organic carbon concentrations down to 100 mg/l the duration of the operating cycles remained constant at 30 min. Reduction of the organic carbon concentration to about 20 mg/l, led to carbon source limitation as shown in Fig. 7c. From this figure it is apparent that the bulk sodium acetate concentration is consumed in the first 20 min while slow desorption of organic carbon from the biofilm structure leads to very slow reduction of the remaining hexavalent chromium.

For the of 30 min operating cycle a hexavalent chromium reduction rate of 535 g Cr(VI)/m^2 d was achieved. This type of operating mode accomplished the highest reduction rate, since the recirculation provided complete mixing in the bioreactor and therefore spatial homogeneity. In samples taken from the effluent of the reactor the Cr(VI) concentration at the end of each recirculation cycle was found well below the permitted limit of Cr(VI) for drinking water (0.05 mg/l).

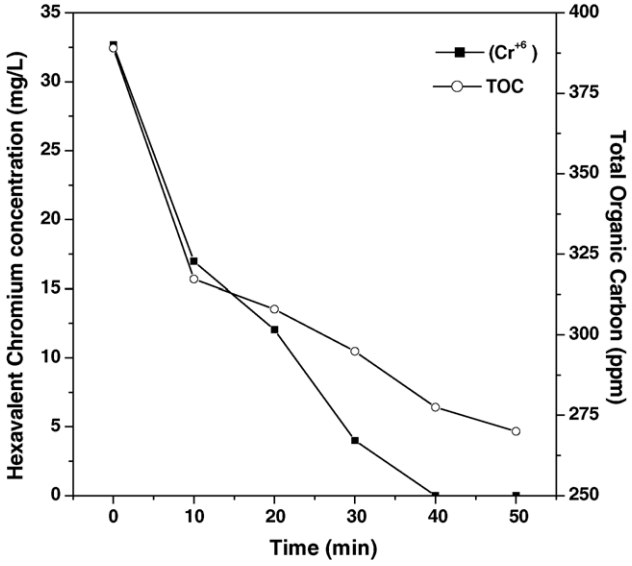

Fig. 6. Hexavalent chromium reduction and sodium acetate consumption during an operating cycle of 40 min (SBR operation with recirculation).

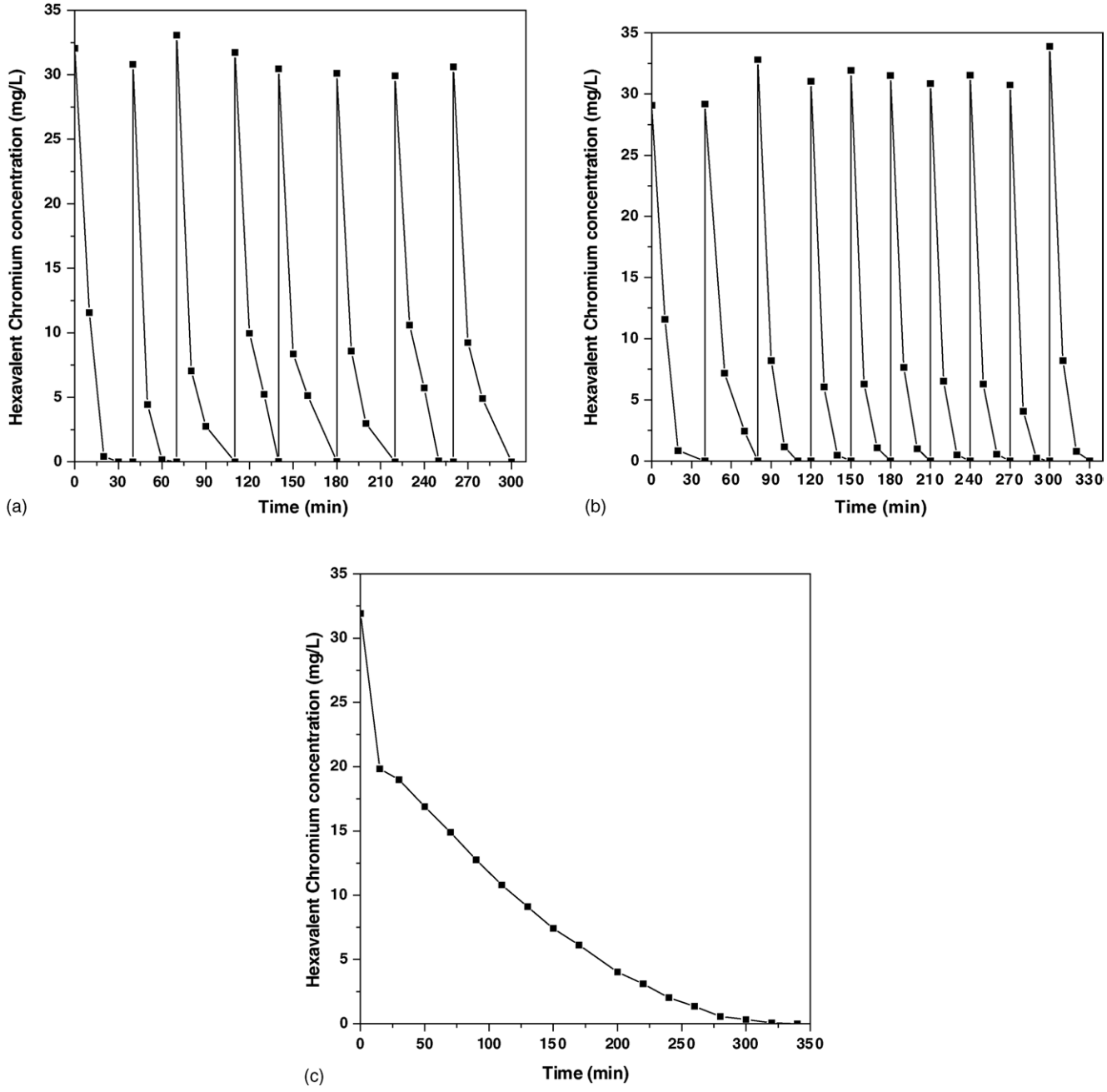

Fig. 7. Operating cycles of SBR operation with recirculation for various sodium acetate concentrations: (a) 265 mg C/l; 40 min, (b) 150 mg C/l; 30 min, (c) 20 mg C/l; 340 min.

3.3. Continuous operation

Finally, the continuous operating mode was tested. This operating mode (gravity flow) ensures fully aerobic conditions without the need for any external aeration source. However, the use of the continuous operating mode did not improve filter performance. The maximum chromate removal rate (200 g Cr(VI)/m^2 d) was achieved for a volumetric flow rate of 30 ml/min resulting in a retention time of only 1.3 min, while for higher flow rates chromate removal rate was less.

4. Discussion

At present the most commonly used technology for treatment of heavy metals in wastewater is chemical precipitation. Conventional chemical treatment involves reduction of Cr(VI) to Cr(III) by reducing agent under low pH (2–3) conditions and subsequent adjustment of solution pH to near neutral ranges to precipitate Cr(III) as hydroxides [5]. Thus, chemical Cr(VI) reduction requires energy input and large quantities of chemicals. Biological reduction could, therefore, provide a useful alternative economical process

[20]. However, until now, the biological chromium reduction was tested only in small laboratory devices, in suspension processes, and using pure cultures. Recently, attempts using attached growth processes appeared in the literature with promising results. This is the first time it was attempted to attack the serious industrial environmental problem of hexavalent chromium disposal, using mixed cultures and a pilot-scale attached growth process bioreactor.

The inability of maintaining pure cultures under industrial conditions made it necessary to look for mixed cultures able to overcome industrial scale difficulties and possible contamination. As discussed earlier, we searched for indigenous bacterial population in a contaminated industrial sludge while the dominant species in the culture we used was found to be *Acinetobacter* sp. It is worth noting that we did not use aseptic (sterilized) conditions while at the same time we used tap water and sodium acetate as carbon source. The operating concentration of 30 mg/l of hexavalent chromium that was used, is extremely high, since in the common Hellenic Aerospace Industry untreated effluents is about only 5 mg Cr(VI)/l. On the other hand concentrations up to 120 mg/l did not seem to be toxic for the bacterial culture we used, indicating that the specific culture appears to be resistant in an actual industrial environment.

The use of an attached growth system provides the necessary surface for the development of biofilm structures. Biofilms provide high biomass concentration per unit volume, while bacteria can remain in the reactor for unlimited time, thus allowing the bacteria better adjustment to the environmental conditions. The height of the pilot-scale trickling filter is the same as that of industrial filters and since it is the loadings (hydraulic and chromium) per unit cross-sectional area that matters, no scale-up is necessary. Trickling filter also does not require an external mechanical aeration source (except for the case of batch operating mode), since air is naturally convected due to the temperature difference between the interior and ambient air.

Sequencing batch reactor operating mode with recirculation proved to be a very effective way for hexavalent chromium reduction. The achieved removal rate of about 0.5 kg Cr(VI)/m^2 d indicates that a trickling filter with a diameter of only a few meters (2–3 m) and the same height as the pilot-scale filter could replace several tanks, basins, processes and, of course addition of chemicals at the particular industry. The reduction of the capital and operating cost is enormous.

The results from this work are very promising and indicate that biological hexavalent chromium reduction could prove a very simple, economical and effective method for the treatment of industrial effluents.

5. Conclusions

Hexavalent chromium is a strong toxic and carcinogenic agent and it should be removed before disposed to the environment. Its reduction to the less dangerous trivalent form seems to be a promising solution. In this work a new approach for biological hexavalent chromium reduction was introduced. The use of indigenous bacterial populations provides a certain advantage and ensures durability under various operating conditions. Trickling filters proved very promising devices providing a support material for consistent biofilm structure development while minimizing operating cost since physical aeration is adequate for bacterial needs. SBR operation with recirculation proved to be a very effective operating mode, since it ensures even wetting of the filter and distribution of the precipitates all over filter volume. The high removal rates of hexavalent chromium that were achieved indicate a feasible, economical and efficient technique for biological hexavalent chromium removal from industrial wastewater effluents.

Acknowledgments

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