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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