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Chromate detoxification using combination of ChromeBac™ system and immobilized chromate reductase beads

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ABSTRACT

Incomplete removal of chromium from discharge effluent of chromium-based industries is a serious problem due to its deleterious effect. ChromeBac™ is a locally developed Cr(VI) reduction system carried out by aerobic Cr(VI) resistant bacteria followed by chemical precipitation. The locally isolated Cr(VI) resistant-reducing *Acinetobacter haemolyticus* EF369508 was immobilized onto carrier materials inside a 50 L bioreactor. Mixture of 10% (v/v) liquid pineapple waste and neutralized Cr(VI) solutions (30 −60 mgL^{-1}) was fed into the bioreactor at 0.11 $m^3\ h^{-1}$. Around 90% of the initial Cr(VI) was reduced after 24 h of contact inside the bioreactor. Residual Cr(VI) was then further reduced to between 1.0 and 1.5 mgL^{-1} by immobilized chromate reductase alginate-beads packed in a 10 L flow-through column, after 15 h of contact. Some important characteristics for the chromate reductase activities for *A. haemolyticus* are as follows; not NADH-dependent, associated with CFE with notable contribution from the membrane fraction, enhanced in the presence of glucose, optimal at pH 7.0, 30 °C, in the presence of 1 mM Co^{2+} (highest) with Michaelis–Menten constant, K_m and maximum reaction rate, V_{max} of 184.47 μM and 33.3 nmol/min/mg protein respectively. Ag^{+} and Hg^{2+} inhibited the enzyme activity. This study demonstrated the potential of using immobilized chromate reductase beads to further reduce residual Cr(VI) present in the effluent of a ChromeBac™ process, hence reducing the time for overall Cr(VI) treatment process.

1. Introduction

The contamination of the water system by the toxic heavy metals such as chromium, lead, copper, zinc, cadmium, nickel, arsenic and mercury is gaining serious attention nowadays. Chromium, Cr originates from various anthropogenic sources such as alloy manufacturing, dyes and pigments, electroplating, metal finishing, petroleum refining, leather tanning, wood preservation and as corrosion inhibitor in conventional and nuclear power plants (Elangovan et al., 2010). Although Cr is able to exist in several oxidation states (−2–+6), the most stable and common forms are Cr(III) and Cr(VI) species. Cr(VI), is the most toxic form of Cr, usually associated with oxygen as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$) ions. In contrast, Cr(III) in the form of oxides, hydroxides or sulfates, is much less toxic, less mobile, less soluble under neutral pH and unable to cross cell membranes. The high solubility of Cr(VI) makes it is a very toxic and carcinogenic element, which represents a serious threat to human health, living resources and ecological system as they considered as persistent and bioaccumulative. Therefore, it is compulsory to remove Cr(VI) from water/wastewater prior to discharging into the environment. Conventional methods for Cr(VI) removal involves physico–chemical techniques which are highly expensive, inappropriate at low Cr(VI) concentration, high reagent consumption, energy requirements and generation of toxic sludge (Ahmad et al., 2015). Therefore, development of an effective system for Cr(VI) bioremediation is highly desirable.

Microbial reduction of Cr(VI) is considered as an alternative

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remediation technique for Cr(VI) contamination due to its lower cost and less sludge production. Various studies were reported on the ability of microbial species to carry out both the Cr(VI) resistant-reducing reaction including *Pannonibacter phragmitetus* LSSE-09 (Xu et al., 2011), *Staphylococcus* sp. (Ilias et al., 2011), *Lysinibacillus fusiformis* ZC1 (He et al., 2011), *Serratia* sp. (Zhang and Li, 2011), *Ochrobactrum* sp. (Francisco et al., 2010), *Bacillus* sp. (Campos et al., 1995; Camargo et al., 2003), *Leucobacter* sp. (Zhu et al., 2008), *Pseudomonas* sp. (McLean et al., 2000), *Exiguobacterium* sp. (Okeke, 2008), *Acinetobacter haemolyticus* (Zakaria et al., 2007) and others. Most of the biological systems for the treatment of Cr(VI)-containing wastewater using microbial Cr(VI) reduction are operated in batch mode (Elangovan et al., 2010). However, this system is not fully effective (compared to continuous or fixed film bioreactor system) with an eventual loss of active biomass. This was mainly due to metal toxicity and the stage of biofilm development which has not fully matured. Several improvements are needed both at the enzymatic as well as cellular levels for bacteria to work efficiently as agents for chromate bioremediation. The use of cell-free enzymes has advantages over whole cells. Cell-free enzymes are not affected by growth inhibitors, toxins, predators of microbial growth or microbial competition in the environment. Moreover, cell-free enzymes do not require transport mechanisms that may impair microbial uptake of chromate. Furthermore, cell-free enzymes can be immobilized for pollutant removal in reactors. The enzyme can be re-used and no need for post-reaction treatment of biomass (Camargo et al., 2003).

Chromate reductase facilitates the reduction of Cr(VI) to Cr(III) either in aerobic or anaerobic conditions. Aerobic Cr(VI) reduction is generally associated with a soluble fraction that utilizes NADH as an electron donor. Conversely, anaerobic Cr(VI) reduction is mediated by membrane bound cytochrome b, c and d, or cytoplasmic membrane proteins (Bae et al., 2005). To date, the use of purified, partially purified or crude enzymes in bioremediation of Cr(VI) from contaminated wastewater treatment has not been reported. The present study highlights the incorporation of an immobilized chromate reductase beads in the existing ChromeBacTM system in order to reduce the overall Cr(VI) treatment time.

2. Materials and methods

2.1. Design and operation of the pilot plant

The operation of the 50 L pilot plant of the ChromeBac™ system can be summarized as follows (Ahmad et al., 2009, 2015); in the 100 L mixing tank, Cr(VI) solution (30–60 mgL^{-1}) was mixed with liquid pineapple waste (LPW) to a final LPW concentration between 1 and 20% (v/v). The pH of the mixture was adjusted to 7.0 ± 0.5 using controller–regulated dosing of 12.5% (v/v) NaOH prior to transferring into the 150 L holding tank. Upon reaching the capacity of the holding tank, the neutralized and LPW amended Cr(VI) solution entered the 50 L bioreactor gravitationally in a down flow mode at 0.11 $m^3\ h^{-1}$. After 24 h of continuous treatment inside the 50 L bioreactor, residual Cr(VI) was passed through a 10 L flow-through column containing immobilized chromate reductase beads to further reduce the remaining Cr(VI). The effluent was then ran through a flocculation (alum) and coagulation (anionic polymer) setup where the Cr(III), total Cr, other heavy metals, color, odor and organic contents were removed from the effluent mixture. Potassium dichromate ($K_2Cr_2O_7$) stock solutions were used as source of Cr(VI) where Cr(VI) stock solution (9615.4 μM = 1000 mg/L) was prepared by dissolving 2.829 g of $K_2Cr_2O_7$ (294.18 g/mol, BDH-AR) in 1 L of DI water and filter sterilized using 0.45 μm cellulose–acetate filter (Millipore, Bedford, MA). Cr(VI) concentration was determined using the DPC method (APHA 4500, Greenberg et al., 2005) while total Cr was determined using the AAS spectrophotometer (Perkin Elmer A Analyst 400). The organic contents were determined using the COD and BOD kits (HACH, USA) and measured at 620 nm using UV–vis spectrophotometer (HACH DR4000, DR5000).

2.2. Preparation of bacterial inoculum and immobilized chromate reductase beads

The Cr(VI) resistant-reducing *A. haemolyticus* (GenBank Accession No. EF369508), an aerobic Gram–negative bacterium, was isolated from Cr(VI)–containing textile dye effluent in Kota Bharu, Kelantan, Malaysia (Zakaria et al., 2007). It was used as the primary bacteria during the inoculation process. Single colony was selected from agar plate and grown into fresh 50 mL nutrient broth for 24 h at 30 °C before transferred into 5 L bioreactor followed by growth at 50 L bioreactor at similar conditions for high biomass production. The bioreactor was packed with rubber wood sawdust (RWS) as carrier material, which was first, sieved through a 25 mm^2 pores. The RWS has specific surface areas of 3.0025, 5.8345 and 1.9806 $m^2\ g^{-1}$ determined using the BET, Langmuir and Single Point (at P/Po = 0.2002) methods plus an average pore diameter of 694.03 nm. Upon packing of the RWS into the bioreactor, it was rinsed using tap water until a clear solution was obtained. Then, the bioreactor was inoculated with 50 L bacterial cultures (10^8–10^9 CFU m L^{-1}) with continuous circulation at 0.11 $m^3\ h^{-1}$for 3 days to allow initial bacterial attachment. Following this, 50 L of 20% (v/v) of LPW at pH 7 was pumped into the bioreactor for 3 days (using similar flowrate) to allow initial development of the biofilm on the RWS. Then, 50 L of solution mixture consisting of 30 mgL-1 of Cr(VI) and 10% (v/v) LPW was introduced into the column. Sampling of the effluent fraction was carried out every hour for the first 4 h followed by 3 h gap for subsequent sampling. Each of the samples was determined for the Cr(VI) and COD values. The immobilized chromate reductase beads were prepared as follows; 10 L solution of a 24 h-grown culture broth for *A. haemolyticus* (1 g cell wet weight: 5 mL buffer) were placed in an ice bath and disrupted using a Sonics Vibra Cell 500 Ultrasonic Probe (amplitude - 9%, 50 W, pulse - 9 s, 1 s off-mode, 35 min). The sonicated fraction (containing intracellular chromate reductase) was mixed with 3% (v/v) sodium alginate solution at a ratio of 4:1 (v/v). The mixture was then homogenized and extruded drop-wise into a beaker (with stirring) containing 100 mL of 0.2 M $CaCl_2$ (110.99 g/mol) solution. Beads produced have an average diameter of 0.3–0.4 cm. The beads were left in the 0.2 M $CaCl_2$ solution to cure for 0.5–3 h priors followed by distilled water washing until the spent-wash solution reached pH 7.

2.3. Chromate reductase assay and protein estimation

Chromate reductase activity assay was carried out using modified procedures from Elangovan et al., (2010) as follows; Cr(VI) solution (48 μM) was added into a series of 1.5 mL Eppendorf tubes containing a mixture of 0.8 mL of 100 mM potassium phosphate buffer and 0.2 mL aliquots of sub-cellular fractions. The mixtures were then incubated for 6 h at 30 °C. One unit enzyme activity for chromate reductase was defined as the amount of enzyme that reduces 1 pmol of Cr(VI) per min at 30 °C while specific activity as unit of chromate reductase activity per mg protein in the CFE. Total protein was estimated via the Bradford's method using bovine serum albumin as standard (Bradford, 1976).