Ecological Engineering 91 (2016) 31–40

Contents lists available at ScienceDirect

Ecological Engineering

journal homepage: www.elsevier.com/locate/ecoleng

Development of biomaterial for chromium(VI) detoxification using *Aspergillus flavus* system supported with iron

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ARTICLE INFO

Article history:
Received 26 November 2014
Received in revised form
10 December 2015
Accepted 26 January 2016

Keywords:
Aspergillus flavus
Biomaterial
Biotransformation
Hexavalent chromium
Biosorption

ABSTRACT

This study was aimed to develop the growing *Aspergillus flavus* biomaterial for Cr(VI) transformation and removal from simulated wastewater. Fe^{2+} ions have significant implication as it enhanced both chromium removal as well as sticky nature of the biomaterial. The values of Δ, i.e. the differences between the frequencies ν_{as} and ν_s of carboxyl group 146.59, 139.27, 152.38 by Fe(II), Cr(VI) and mix metals ions (Fe(II) + Cr(VI)) inferred the carboxylate ions bidentate coordination. The highest value in case of mix metals ions (Fe(II) + Cr(VI)) inferred that carboxylate groups have coordinated to surface Fe^{3+} ions increasingly with greatest bridging character. Heat flow value 31.36 J g^{-1} and 51.02 J g^{-1} in Fe^{2+} and Cr(VI) amended material respectively indicated that extremely little amount of the free water content was sorbed.

1. Introduction

Chromium is considerable environmental concern, as it is widely used for industrial applications. The use of microbial biomass technology for removal of toxic heavy metal ions from wastewater has emerged as an alternative low cost, innovative to existing methods (Kapoor and Viraraghavan, 1998). A wide variety of living and nonliving biological materials are capable of removing toxic and precious metals from the waste streams and offer an economical and efficient alternative for sorption technologies (Vijayaraghavan and Yun, 2008). Bacteria, fungi and algae are also promising biosorbents, owing to their abundance and low cost (Ahluwalia and Goyal, 2007). Fungal biomass seems to be a good sorption material, because, it can be produced easily and economically using simple fermentation techniques with a high yield of biomass and growth media (Aksu and Balibek, 2007).

Common chemical reductants of Cr(VI) include sulfides, dissolved organic compounds, aqueous Fe^{2+}, and Fe^{2+} bearing minerals (Sedlak and Chan, 1997). Cr(VI) reduction through rapid abiotic reaction with ferrous iron [Fe(II)], is an alternate route to remediate Cr(VI) (Wielinga et al., 2001). In contrast, generation of Fe^{2+} by stimulating indigenous Fe(III)-reducing organisms is likely to encounter fewer regulatory hurdles, and can potentially be less expensive, especially if economical nutrient sources such as molasses or cheese whey are used (Viamajala et al., 2008).

Therefore development of innovative bioremediation technologies is urgently needed to achieve cleanup goals at contaminated sites and ensure safe water for future generations. In the present study live biomaterial (growing biomass) was applied to overcome the problems of suspended dead biomass, which is less effective in long-term application for industrial effluent treatment and will lead to an efficient economical bioremediation technology. The experiments were conducted to test the feasibility of bio-barriers for Cr(VI) remediation using *Aspergillus flavus*. The amendment of various forms of Fe enhanced the Cr(VI) transformation as well as removal efficiency. Moreover, after cell death or exposure to higher metal ions concentration, metal reduction/removal may be restored to previous levels by display of more functional groups.

2. Materials and methods

2.1. Preparation of reagents and medium

The Cr(VI) working concentration throughout the experiments was obtained by adding appropriate quantity of the autoclaved

Abbreviations: Cr(VI), hexavalent chromium; Cr(T), total chromium; ORP, oxidation reduction potential; COD, chemical oxygen demand; $NADPH_2$, nicotinamide adenine dinucleotide phosphate; PCA, principal component analysis; DSC, differential scanning calorimetry; FTIR, Fourier transformed infrared spectroscopy.

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http://dx.doi.org/10.1016/j.ecoleng.2016.01.060
0925-8574/

stock solution ($K_2Cr_2O_7$ 5000 mg L^{-1}). The microbial culture was maintained in nutrient growth medium having composition (g L^{-1}): glucose 10.0; peptone 3.0; $MgSO_4 \cdot 7H_2O$ 0.5; KH_2PO_4 1.0. The pH of growth medium was adjusted to 5–6 using 0.1 N H_2SO_4/NaOH before autoclave. The medium was sterilized by autoclaving at 1.5 psi and 121 °C for 20 min. Solid medium was prepared by adding 2% agar in nutrient broth medium where it was required. All reagents used were of analytical grade.

2.2. Microorganism and inoculums preparation

The strain used in present study was isolated from contaminated soil sample collected and was purified using standard spread plate techniques (Singh and Bishnoi, 2015). Adapted isolate was used further by preparing the fresh culture in 250 ml screw capped Erlenmeyer flasks containing 50 ml of nutrient broth medium at 120 rpm at 30 °C. The strain was routinely grown for 5 days (end of exponential phase) maintained in nutrient broth stored at 4 °C. 1 ml fully grown culture was used as inoculum during all the experiments.

2.3. Analytical methods

The culture was harvested as whole flask at predetermined intervals for various parameters estimations. Samples were filtered and separated into filtrate and cells residual. The dried biomass was measured by gravimetric methods using Whatman filter paper by drying at 105 °C for 24 h in hot air oven. Total heavy metal concentration was analyzed using Atomic Absorption Spectrophotometer (Simazdhu AAS-6300). Cr(VI) was analyzed by 1,5-diphenylcarbazide method at 540 nm with a UV/VIS spectrometer (APHA, 2005). COD was determined using the dichromate COD Titrator Model CT-15 based on the use of measurement for high-range COD (0–500 mg L^{-1}) using potassium hydrogen phthalate solution as a standard. The COD results were corrected by adding the oxygen corresponding to the chromium (Trunfio and Crini, 2010). The pH, ORP, Cr(VI) and protein contents were measured immediately whereas the filtrate was preserved as per standard methods for analysis of COD and heavy metal. The protein content in the supernatants was measured using Lowry et al. (1951). Enzymatic chromate reduction was estimated as described previously using a standard calibration curve of 0–20 mM Cr(VI) (Singh et al., 2013).

2.4. Effect of initial metal ions concentration and incubation period

Effect of metal ions strength was studied by growing *A. flavus* in 50 ml growth medium amended with 10–60 mg L^{-1} of Cr(VI). The experiments were conducted at initial pH 5 and 120 rpm incubated at 33 °C for 5 days inoculated with 1 ml of 5 days old inoculums. Parallel experiments were carried out in nutrient medium in the absence of metal ions under similar conditions. To find abiotic transformation, experiments were also carried out in medium containing metal ions to check the natural loss/transformation of Cr(VI). The effect of incubation period on Cr(VI) transformation and Cr removal was studied in glucose (at 6.0 g L^{-1}) as well as in fructose (6.0 g L^{-1}) amended medium separately at 33 °C keeping pH 5 and 120 rpm.

2.5. Biosorption capacity of growing biomass

Biosorption capacity of culture was investigated as a function of pH and initial Cr(VI) concentrations. The percentage removal of Cr(VI) was calculated from following eq.

$$\text{Removal(Cr)}\% = \frac{(C_i - C_f)}{C_i} \times 100 \quad (1)$$

The amount of Cr(VI) biosorbed, q_e (mg g^{-1}) was computed by using the following expression:

$$q_e = \frac{(C_o - C_e)}{m} \times V \quad (2)$$

where C_o (mg L^{-1}) and C_e (mg L^{-1}) are Cr(VI) concentrations before and after removal, respectively; V is the volume of medium, and m (g) is the weight of the adsorbent per liter.

The Cr(VI) reduction rate (TCR) was calculated using the Eq. (3) (Xu et al., 2011).

$$\text{TCR} = \frac{(C_o - C_f)}{\text{DCW} \times t} \quad (3)$$

TCR is Cr(VI) reduction rate mg g^{-1} (dry weight) h^{-1}, C_o the initial Cr(VI) concentration (mg L^{-1}), C_f the finial Cr(VI) concentration (mg L^{-1}), DCW the dry weight of cells (g L^{-1}), t the Cr(VI) reduction time (h).

2.6. Determination of optimum pH

In order to determine optimum pH for biosorption and biotransformation of Cr(VI), the culture was grown in 50 ml nutrient broth medium at pH ranging from 2 to 8 for 3 days at 30 °C and 120 rpm. The experiments were carried out at 30 mg L^{-1} of Cr(VI) ions concentration. The controls with culture inoculation and blank without culture having metal ions were also run parallel to check the variations in various parameters of medium owing to abiotic changes.

2.7. Effect of $FeSO_4$/$FeCl_3$ on Cr(VI) transformation and removal

To investigate effect of $FeSO_4$ on Cr(VI) transformation and removal by *A. flavus*, experiments were conducted in 50 ml of nutrient broth medium amended with 30 mg L^{-1} Cr(VI) at pH 5 and 120 rpm at 33 °C. The $FeSO_4$ concentration was varied from 0.01 to 0.2 mM using appropriate amount of sterilized stock solution of 10 mM $FeSO_4$. The control of individual metal ions i.e. Fe^{2+} (0.15 mM), without Fe^{2+} and Cr(VI), and Cr(VI) (30 mg L^{-1}) were also conducted parallel under similar conditions. The screw capped flasks were withdrawn on 3rd and 6th days for analysis of various parameters. The samples withdrawn were scanned using UV-VIS spectrophotometer at 200–800 nm at ambient temperature on 3rd day. Before scanning the samples were centrifuge in 1.5 ml at 7500 rpm for 10 min at 4 °C. The supernatant was diluted with equal volume of the double distilled water. The baseline was corrected with sterilized nutrient broth medium deficit of both Fe^{2+} and Cr(VI) ions. All experiments were duplicated with appropriate controls in each instance. Effect of various doses of $FeCl_3$ (1–45 mg L^{-1}) was studied at pH 5 and 120 rpm at 33 °C. The data generated was also analyzed by plotting the biplot using principal component analysis (PAST, 2001).

2.8. Fourier transforms infrared spectroscopy (FTIR)

FTIR spectrum of the *A. flavus* biomass was carried out in presence of 30 mg L^{-1} Cr(VI), 0.2 mM Fe(II) as $FeSO_4$ and under mix metal ions (0.2 mM Fe(II) + 30 mg L^{-1} Cr(VI)). To get the information specific to the functional group, and also on the interaction of the groups with other parts of the molecule and on the spatial properties of the groups by FTIR, the *A. flavus* was incubated at 33 °C under shaking speed of 120 rpm in 250 ml screw capped flasks for 6 days in 50 ml medium. The biomass was harvested by filtering the culture and kept in deep freezer at −80 °C for 48 h before lyophilization in 50 ml centrifuge tubes and lyophilized samples were preserved in freezer till FTIR spectrum obtained using KBr disk.