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Detoxification and accumulation of chromium from tannery effluent and spent chrome effluent by *Paecilomyces lilacinus* fungi

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ABSTRACT

The tannery industry process involves chromium (Cr) salts as a main constituent of the process. The Cr recovery is a part of the process where other salts are used to achieve separation and recovery for using Cr back in the process. The process steps may contain both forms of Cr [Cr(VI): hexavalent and Cr (III): trivalent]. The recovery of Cr from tannery industry effluent through biological systems is much needed. The diverse physicochemical characteristics of these effluents may limit the growth of microorganisms and hence the limitation towards possible practical application of microorganisms in real industrial effluent conditions. The present study attempted the ability of the Cr-resistant fungus *Paecilomyces lilacinus* [isolated through an enrichment culture technique at 25 000 mg l^{-1} of Cr(III)] to grow and remove Cr [Cr(VI) and Cr(III)] from two physicochemically different undiluted tannery industry effluents (tannery effluent and spent chrome effluent) in the presence of cane sugar as a carbon source. Such attempts are made keeping in view the potential integration of biological processes in the overall Cr removal and recovery processes to improve its efficiency and environmental sustainability. The fungus has broad pH tolerance range and can reduce Cr(VI) both in acidic (pH 5.5) and alkaline (pH 8.0) conditions. The fungus showed the ability to remove Cr(VI) (1.24 mg l^{-1}) and total Cr (7.91 mg l^{-1}) from tannery effluent below the detection level within 18 h and 36 h of incubation, respectively, and ability to accumulate 189.13 mg Cr g^{-1} of dry biomass within 600 h of incubation from spent chrome effluent [containing 3731.4 mg l^{-1} of initial Cr(III) concentration].

At 200 mg l^{-1} of Cr(VI) in growth media, with 100% detoxification and with only 10.54% of total Cr accumulation in the biomass, *P. lilacinus* showed Cr(VI) reduction as a major mechanism of Cr(VI) detoxification. The time-course study revealed the log phase of the growth for the maximum specific reduction of Cr(VI) and stationary phase of the growth for its maximum specific accumulation of both the forms of Cr [Cr(III) and Cr(VI)] in its biomass. In growth media at 50 mg l^{-1} and 200 mg l^{-1} of Cr(VI), *P. lilacinus* showed 100% reduction within 36 h and 120 h of incubation, respectively. The high degree of positive correlation and statistically high degree of relationship ($r^2 = 0.941$) between the fungal growth and % Cr(VI) reduction by the fungus support the role of metabolically active cellular growth in Cr(VI) reduction by the fungus. Results indicate that expanded solid (sludge) retention times (SRTs) (stationary phase) can be recommended for the removal of Cr(III) through accumulation. In case of Cr(VI), reduction needs a priority; therefore, a non-expanded SRT is recommended for designing a continuous-flow completely stirred bioreactor so that a log phase of cellular growth can be maintained during the reduction process. This study reveals the strong potential of *P. lilacinus* fungi for the removal of Cr from tannery effluent and spent chrome effluent.

1. Introduction

Chromium (Cr) compounds are used in industries for chrome plating, wood preservation, textile dyeing and pigmentation, manufacturing pulp and paper, and tanning. The wastewater resulting from these industrial processes contains high concentration of Cr, which contaminates the natural environment,

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affecting human health (McLean and Beveridge, 2001; Congeevaram et al., 2007). The tannery industry, which commonly uses basic Cr(III) sulphate [$Cr(H_2O)_5(OH)SO_4$] for tanning process, is a major cause for high influx of Cr to the biosphere, accounting for 40% of the total industrial use (Barnhart, 1997). In nature, Cr exists in two stable forms Cr(III) and Cr(VI). Characteristics like higher solubility in water, rapid permeability through biological membranes, and subsequent interaction with intracellular proteins and nucleic acid makes Cr(VI) comparatively more toxic than Cr(III) (Congeevaram et al., 2007). Due to the difference in the toxic nature of both the forms of Cr, the discharge of Cr(VI) into surface water is regulated to below 0.05 mg l^{-1} by the US EPA, while the total Cr [including Cr(III), Cr(VI) and its other forms] is regulated to below 2 mg l^{-1} (Baral and Engelken, 2002).

The conventional physical and chemical methods used for removal of heavy metals from the effluent, such as precipitation with carbonates, sulphides and hydroxide, adsorption on activated carbon, use of ion-exchange resins and membrane-separation processes, are responsible for generation of pollution and are not cost-effective (Volesky and Holzen, 1995; Kratochvil et al., 1998; Camargo et al., 2003). An alternative to these methods is the removal of heavy metal contaminants by microorganisms. The metal removal ability of microorganisms, including bacteria (Cheung and Gu, 2005; Thacker et al., 2007), microalgae (Kratochvil et al., 1998; Matsunaga et al., 1999; Gupta et al., 2001a, b; Gupta and Rastogi, 2008) and fungi (Tobin and Roux, 1998; Srivastava and Thakur, 2006), has been studied extensively. Fungi, in general, are well-known for their ability to biosorb and bioaccumulate metals (Pillichshammer et al., 1995; Dursun et al., 2003; Nouri et al., 2005; Park et al., 2005) and have also been reported to be involved in reduction (biotransformation) of Cr(VI) to Cr (III) form (Pal, 1997; Gouda, 2000; Acevedo-Aguilar et al., 2006; Morales and Cristiani, 2008). The common Cr(VI) detoxification mechanisms reported in Cr-resistant microorganisms are periplasmic biosorption, intracellular bioaccumulation and biotransformation through direct enzymatic reaction (Lovley, 1993; Lee et al., 2000; Valls et al., 2000) or indirectly with metabolites (Camargo et al., 2003). In Cr(VI)-resistant filamentous fungi, such as *Aspergillus* (Gouda, 2000; Acevedo-Aguilar et al., 2006), *Penicillium* (Acevedo-Aguilar et al., 2006), *Trichoderma* (Morales and Cristiani, 2008) and *Phanerochaete* (Pal, 1997), the Cr(VI) detoxification through transformation of Cr(VI) to Cr(III) form was observed due to cellular metabolism processes based on the reducing power of carbon sources.

The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures. This gives microorganisms an ability to bind metal cation (Chen and Hao, 1998). Metal removal through biosorption is the process in which live/dead microbial biomass or adsorbents developed from biological and industrial waste materials (Gupta et al., 1997; Srivastava et al., 1997; Gupta et al., 2001a, b; Gupta et al., 2009) is simply used as an adsorbent (Volesky, 2001), whereas the bioaccumulation process involves using growing biomass in the removal or binding of metal ions (Dursun et al., 2003). Recent reports employing growing cultures of bacteria, fungi and marine microalgae indicate that intracellular metal levels are often higher than the biosorbed ones (Kapoor et al., 1999; Matsunaga et al., 1999; Perez-Rama et al., 2001; Kader et al., 2007). Moreover, on one hand, the biosorption methods are often sensitive to ambient conditions, such as pH, ionic strength and the presence of organic or inorganic ligands, while, on the other hand, it lacks specificity in metal binding (Baudet et al., 1988; Kumar et al., 2008). Application of active and growing cells might be a better option due to their ability of self-replenishment, continuous metabolic uptake of metals after physical adsorption, and the potential for optimization through development of resistant species and cell surface modification (Wilde and Benemann, 1993; Sandau et al., 1996; Malik, 2004). Apart from this, using growing cultures in bioremoval could avoid the need for a separate biomass-production process, for example, cultivation, harvesting, drying, processing and storage prior to the use.

Though it is a well-known fact that biological treatment could significantly reduce the costs for chemicals and energy compared with conventional physical and chemical treatment, there are still some problems in applying this to the real wastewater treatment processes due to the death of microbial cells in high concentrations of Cr, low reducing and accumulation rate compared with the high generation rate of wastewaters, continuous supplies of expensive nutrients or chemicals and difficulty in the separation of cells after treatment (Park et al., 2005; Katarzyna, 2007; Vijayaraghavan and Yeoung-Sang, 2008). These existing application gaps were the reason behind isolating a Cr-resistant fungus, identified as *Paecilomyces lilacinus*, from the tannery sludge and evaluating the potential of growing fungal cells for Cr(VI) detoxification and Cr(III) accumulation at higher concentrations both in growth media and in tannery industry effluent. The physicochemical characteristics, such as pH, Cr concentration, chemical oxygen demand (COD) and biological oxygen demand (BOD) of discharged tannery industry effluent, vary from industry to industry (Tobin and Roux, 1998; Nouri et al., 2005; Prigione et al., 2009; Ramteke et al., 2010), which may inhibit the growth of microorganisms and the overall Cr-removal efficiency. Therefore, the ability of microorganisms to survive and remove Cr from physicochemically diverse tannery industry effluent is warranted. Considering this, the potential of *P. lilacinus* in case of real industrial effluent was evaluated by studying the ability of the fungus to remove Cr from two physicochemically different tannery industry effluents (spent chrome effluent and tannery effluent). The spent chrome effluent is the effluent generated during the process of chrome tanning of hides and skins, whereas tannery effluent is the final composite effluent discharged from the leather industry to common effluent-treatment plants (CETPs) for final treatment. Further, the effect of growth phase on Cr(VI) detoxification and Cr(III) accumulation was studied to generate some basic data, which will be important in designing solid (sludge) retention time for a continuous-flow completely stirred (CFCS) bioreactor in future, which is a general reactor type for wastewater treatment plants for efficient Cr(VI) detoxification and Cr(III) accumulation by fungi.

2. Materials and methods

2.1. Isolation and characterization of fungi

The Cr-resistant fungi were isolated from tannery sludge [containing 25 000 mg l^{-1} of Cr(III)] through an enrichment culture technique. The sludge sample was collected from a tannery waste disposal site in Kanpur, India. For isolation of fungi, 1 g of contaminated sludge sample was inoculated in a 500-ml Erlenmeyer flask containing 100 ml of potato dextrose broth (PDB) media with initial Cr(III) concentration of 5000 mg l^{-1} [source $Cr_2(SO_4)_3$, CDH Lab Chemicals, India]. The flask was incubated in a shaking incubator (Innova 4080, New Brunswick Scientific, USA) at 30 °C, 100 rpm in dark conditions. Starting with 5000 mg l^{-1} of Cr (III), the successive Cr(III) concentrations used for culture enrichment were 10 000 mg l^{-1}, 15 000 mg l^{-1}, 20 000 mg l^{-1} and 25 000 mg l^{-1} in PDB media. The cultures were enriched by successively transferring the 2% v/v of culture aliquot from lower to the higher concentrations after every 360 h of incubation. After completion of incubation at final concentration of Cr(III) (25 000 mg l^{-1}), a Cr-resistant fungus was isolated from broth using a serial dilution and plating technique on potato dextrose agar