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Efficiency of *Penicillium chrysogenum* PTCC 5037 in reducing low concentration of chromium hexavalent in a chromium electroplating plant wastewater

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Abstract

The effectiveness of *Penicillium chrysogenum* was evaluated for reducing Cr(VI) from the wastewater of a chromium electroplating plant. Statistically-based experimental designs were applied to optimize the condition for reducing Cr(VI) to Cr(III). By applying Plackett–Burman factorial design and central composite design as the optimization step, attempts were made to identify optimal values of the three factors that bringing about maximum microorganism activity and therefore maximum hexavalent chromium(VI) bioreduction. It was found that each gram of *P. chrysogenum* of dry biomass condition could reduce 66 mg of Cr(VI) to Cr(III) in the wastewater of the chromium electroplating plant.

Keywords: Industrial wastewater; Hexavalent chromium; Reduction; *Penicillium chrysogenum*

1. Introduction

As it is well known, the increasing of industrial and technological activities has negative impact on environment, and substantially contributes to the destruction of environment in which that development is occurring, and on occasion, in neighboring communities. Therefore, the introduction of cleaner technologies is not only socially responsible, but has also been shown to lead to increased productivity, competitiveness and profitability.

A cause of industrial activities is the environmental pollution by heavy metal, which is a red-hot issue (Parkasham et al., 1999; Lloyd and Lovely, 2001). Chromium(VI), is one of these toxic metals which is found in the environment commonly in hexavalent Cr(VI), and trivalent Cr(III) forms. Cr(VI) is extremely toxic, and is transported to the environment by means of many occupational sources, such as paints, metal finishes, steel, including stainless steel manufacturing, alloy cast iron, chromium electroplating, wood treatment and leather tannins (Sudha and Abraham, 2001; Muter et al., 2001a; Park et al., 2001; Donghee et al., 2003). The hexavalent form of chromium is mutagenic, and categorized by EPA as cancerous material. But Cr(III) is thousand times less mobile and toxic in the environment than Cr(VI), and therefore efforts should be taken to reduce the toxic Cr(VI) to the less toxic Cr(III) by mean of different available methods (Cervantes et al., 2001; Sudha and Abraham, 2001; Muter et al., 2001b; Park et al., 2001; Donghee et al., 2003).

Elimination of Cr(VI) from industrial wastewater is commonly done by means of physico-chemical methods, which are complex processes, expensive, and also result in the formation of secondary source of environmental pollution. A major draw back of these methods is the lack of ability for removal of Cr(VI) in low and minute concentrations. The most versatile, efficient, and also economical methods to overcome the problem of reduction of Cr(VI)

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in low concentrations are biological methods which offer a "natural" way of addressing environmental problems (Veglio and Beolchini, 1997; Parkasham, 2001; Sudha and Abraham, 2001; Muter et al., 2001a,b; Donghee et al., 2003; Park et al., 2001; Cossich et al., 2002). Application of microbial system, and plant biomass for this purpose is competing with great success against traditional and conventional technologies (Veglio and Beolchini, 1997; Ehrlich, 1997; Lloyd and Lovely, 2001; Sudha and Abraham, 2001; Cervantes et al., 2001).

In this study, attempts were made to reduce low concentration of Cr(VI) in a wastewater of a chromium electroplating plant by *Penicillium chrysogenum* microorganism. The microbial Cr(VI) reduction activities are either plasmid- or membrane-associated phenomena (Chen and Hao, 1998). Therefore, culture conditions, and constituents highly affect the efficiency of Cr(VI) reduction. To evaluate the relative importance of various constituents within a complex medium on *P. chrysogenum* growth, and sporulation, and therefore Cr(VI) reduction a Plackett–Burman design (Plackett and Burman, 1946) was employed. Further, optimization of important independent variables was investigated using central composite design (Jettwu and Hamada, 2000; Montgomery, 2005).

2. Methods

2.1. Microorganism and culture conditions

P. chrysogenum PTCC5037 was kindly provided by the Biotechnology Department of Iranian Research Organization for Science and Technology (IROST), Tehran, Iran, incubated over Potato- Dextrose- Agar (PDA) slant and stored at 4 °C (±1 °C) in the refrigerator.

Spores of *P. chrysogenum* from a 7 days old slant were suspended in sterile water. Number of spores in this suspension was determined using a hemocytometer. Spores were cultivated at 30 °C in 500 ml flasks containing 100 ml of sterile culture medium with stirring on a rotary shaker (160 rpm). Number of spores, pH, carbon, nitrogen and phosphorous sources, and other constituents were as indicated in Table 1. After 72 h of cultivation, 1 ml of the diluted chromium electroplating plant wastewater was added to the grown, culture so that the amount of Cr(VI) concentration was equaled to 50 mg/l. All experiments were conducted in duplicates and the results were obtained as the average of two experiments under the same conditions.

Table 1
Variables under investigation in the Plackett–Burman experimental design (culture condition)

Variable number	Variable	Low level (−1)	High level (+1)
1	Glucose (g/l)	5	15
2	Spore	5×10^7	15×10^7
3	pH	4	7
4	$NaNO_3$ (g/l)	0.5	1.5
5	$CaCl_2$ (g/l)	0.05	0.5
6	$MgSO_4$ (g/l)	0.05	0.1
7	KH_2PO_4 (g/l)	1.5	5
8	K_2HPO_4 (g/l)	0.25	0.75
9	$FeSO_4$ (g/l)	0.002	0.01
10	Na_2HPO_4 (g/l)	0.15	0.3

2.2. Measurement of chromium(VI) reduction

At appropriate time intervals (Fig. 1), aliquots of 5 ml were taken under sterile conditions and centrifuged at 2000*g* for 10 min. The remaining chromium(VI) concentration in the cell-free supernatant was determined spectrophotometrically at 540 nm, using diphenyl carbazide as the complexing agent (Eaton et al., 1995). A Pye Unicam SP6-500 UV spectrophotometer was used for absorbance measurements. The concentration of Cr(VI) was found by deducing the concentration of the test samples from those of the blank and then divide the results by the concentration of the blank (flasks without cells).

Total concentration of chromium (i.e. Cr(VI) + Cr(III)) was measured by means of atomic absorption (AA), Perkin Elmer 460.

The initial concentration of Cr(VI) in the untreated effluent from the chromium electroplating plant was high (about 8.5 g/l). This was diluted so that when 1 ml aliquots of this wastewater was added to the culture media after 72 h of cultivation, the initial concentration of Cr(VI) became 50 mg/l. Although the concentration of Cr(VI) in the media was considered as a low concentration, still it was much higher than the permissible limit of 0.05–1 mg/l (Thyagarajan, 1992; Baran et al., 2007).

2.3. Determination of glucose concentration and dry cell mass

The supernatant mentioned in Section 2.2 was used to measure glucose consumption in the growth medium, using Nelson (1944) and Somogyi (1952) method.

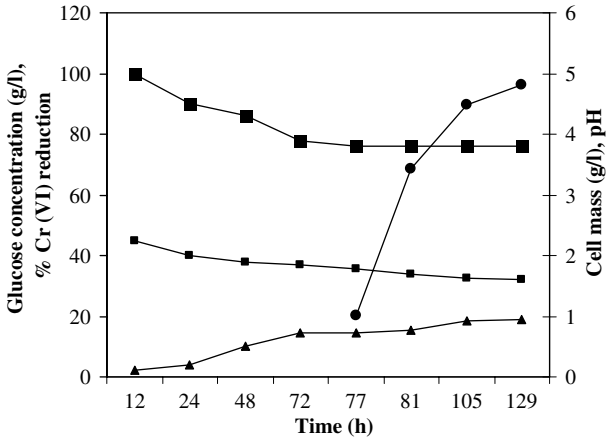

Fig. 1. Variation of Cr(VI) reduction, % (●); glucose concentration, g/l (■); cell mass concentration, g/l (▲) and pH (■) pH with time. Cr(VI) (50 mg/l) was added at 72 h of cultivation.