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Chromium VI Detoxification by *P. fluorescens* Adopted from Tannery Effluent under Optimal Condition

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Abstract The present study is aimed to isolate and evaluate chromium reduction by the indigenous bacterium *Pseudomonas fluorescens* in tannery effluents collected from airport, Tiruchirappalli. Chromium resistant pattern was studied by plate method and reduction was studied by shake flask method. Isolate *Pseudomonas fluorescens* biochemically characterized as Gram negative siderophore producing bacteria and found to tolerate 1000 ppm Cr VI. Among the pH best growth was found at pH 6 showed Lag Growth phase was observed on 4 h at nonchromium condition and 6 h for chromium supplemented medium at 100 ppm and 7 h at 1000ppm. The growth was retreated by chromium but not arrested. The yield of the bio-surfactant was relatively higher in pH 8. Medium with yeast extract, pH 8 comparatively better than other nitrogen in chromium reduction. Maximum Chromium removal efficiency was 82% under chitosan and 78% by EDTA supplemented medium. Other nitrogen sources were less significant. In pH 8 under yeast extract and glucose with EDTA and 68% chromium reduction was noted without EDTA. Medium with chitosan showed enhanced reduction from 55 % to 88%. Other nitrogen sources like peptone, casein, ammonium nitrate showed 60-70% reduction in the presence of EDTA and reduction was reduced to 50% without EDTA. The increase of dextrose strength does not influence the reduction of chromium and maximum activity was found at 2% same as 10%.

Keywords: *Pseudomonas fluorescens, Chromium, EDTA, Chitosan, Reduction*

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1. Introduction

Chromium occurs in the environment in two forms i.e. as trivalent and hexavalent. The reduction of Cr(VI) to Cr(III) is much needed potential detoxification process for cleaning of ecosystem from heavy metal pollution. Environment is globally affected by discharging chromium from different industrial sectors [1]. Currently available conventional methods are not only high cost but also less effective to remove chromium from soil and water. Therefore researchers focus on efficient strategy in removing the bulk of metal from solution at moderate to higher concentrations [2]. Many microorganisms have been reported to reduce high soluble and toxic Cr^{6+} to less soluble or less toxic Cr^{3+}. Heavy metal removal efficiency by microorganisms is proven and number of experiments are required to optimize it for better *in situ* application [3]. The non-biodegradable aggregation in environment ensures the presence of heavy metals [4]. Biosurfactant are surface active agents plays vital role in metal desorption by forming complexes with free, non-ionic metals in solution. The mechanisms involved in biosurfactant is metal binding it includes ion exchange, precipitation dissolution, counter-ion association and electrostatic interaction. The hydrophobic (non- polar) part of the biosurfactant is insoluble in water and it could have a long-chain fatty acids, hydroxyl fatty acids or α-alkyl-β-hydroxy fatty acids. The hydrophilic (polar) end can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol [5,6]. The bacteria interact with phosphate, carboxyl, hydroxyl and amino groups, these groups would create metal interaction with bacteria [7]. The microbes resistant to chromium has the property of chromium reduction due to the presences of chromium reductase enzyme [8]. Chitosan is a natural copolymer of β-(1 → 4)-d- glucosamine and β-(1 → 4)-N-acetyl-d-glucosamine derived from chitin and due to their low-cost and high contents of amino and hydroxyl functional group used as an absorbent to remove pollutants like chromium [9]. Chitin is the most abundant renewable, natural resource after cellulose. Chitin and its end product are biomolecules which are excessively potential, along with flexible biological activities that demonstrate biocompatibility and biodegradability. Chitin has a unique properties like biodegradability, biocompatibility and non-toxicity [10]. Ethylene diamine tetra acetic acid (EDTA), a kind of synthetic APCA, has been widely used in environment because of its persistence's. EDTA can

dissolve more in alkaline solution, attach to a metal surface particle and increase the rate of migration of a material. The effect of pH may affect the stability and effectiveness of a metal.

2. Materials and Method

2.1. Effect of Chromium on Growth Rate

P. fluorescens isolated from leather tannery site permitted to grow on yeast extract glucose broth contain 25, 50, 100, 250, 500 and 1000 ppm of potassium dichromate. 100 ml of broth with respective concentration of chromium (VI) is inoculate with 10% v/v 24 h *P.fluorescens* and kept under shaking for 6 h and then incubated at 37°C for 18 h. growth rate was recorded after 24 h at 600 nm.

2.2. Effect of pH on Biosurfactant Screening

Chromium removal medium (CRM) contain $MgSo_4$ (0.02 g/l), Na_2HPO_4 (2 g/l) and glucose (2 g/l) were prepared with yeast extract and pH was altered to obtain 2, 4, 6 and 8. Culture was inoculated, cell free culture filtrate was taken by centrifugation and used for bio-surfactant screening by oil displacement test.

2.3. Oil Displacement Test

In this test, 14 ml of distilled water was added to a petri dish which is 90 mm in diameter. 1ml of diesel was added to the water surface. To this setup 50 µl of cell free culture filtrate was loaded on to the oil surface and the diameter of clear halo zone was measured after 30sec.

2.4. Emulsification Capacity

A mixture of 5 ml hydrocarbon and 2.5 ml cell free extract obtained after the centrifugation of sample culture were taken in a test tube and homogenized by vortexing for 2 min. The emulsion activity was investigated after 24 hours and the emulsification index (E24) was calculated by the total height of the emulsion by the total height of the aqueous layer, then multiplied by 100. The results were compared with SDS as positive control.

$$E24 = \frac{Height\ of\ the\ emulsification\ layer}{total\ height\ of\ mixture} \times 100\%$$

2.5. Effect of Nitrogen

Chromium removal medium (CRM) contain $Mgso_4$ 0.02 g/l, Na_2HPO_4 2 g/l and glucose 2 g/L enriched with 100 ppm of chromium (VI) was prepared. 1 % of nitrogen source like yeast extract, peptone, casein, ammonium nitrate were selected and supplemented. Culture were inoculated and reduction of chromium was estimated after 24h.

2.6. Effect of EDTA

CRM enriched with yeast extract with PH 8 was prepared and added with 10 ppm of EDTA Culture were inoculated and reduction of chromium was estimated after 24 h.

2.7. Effect of Chitosan on Cr Reduction

CRM enriched with yeast extract with pH-8 supplemented with 10 mg/L of chitosan solubilized in acetic acid were added. Culture were inoculated chromium was estimated after 24 h.

2.8. Estimation of Chromium VI

The total concentration of Cr (VI) in the broth was determined spectrophotometrically using 1,5-diphenylcarbazide as complexing agent. A standard stock solution of 500 ppm was prepared by dissolving 0.05 g dried K_2CrO_4 (99% purity). A 1,5-diphenylcarbazide (DPC) solution was prepared by dissolving 250 mg of DPC in 50 mL methanol. Working standard Cr(VI) concentrations of 10 to 100 ppm were prepared from the stock solution. The pH of solutions was adjusted to 2 with phosphoric acid and diluted with sulfuric acid before complexation. The absorption concentration calibration curves were plotted with a correlation coefficient. The linear regression of the standard graph was used for the estimation of chromium present in the solution. The chromium removal percentage was calculated using the following formula

$$E = \left(A - B / A\right) \times 100$$

Where A = Initial metal ion concentration, B= final metal ion concentration.

3. Result and Discussion

Gram negative, KOH positive cells with greenish transparent colonies were selected and identified as *Pseudomonas fluorescens.* The biochemical characters are given in Table 1. Different concentration of Cr (VI) was tested against *Pseudomonas fluorescens* to find out the minimum concentration of Cr (VI) that could inhibit the growth of *P. fluorescens.* Figure 1. reveals that medium without chromium shows luxurious growth with maximum OD- 0.094 within 24 hours, good growth rate was noted at 100-250 ppm. Moderate growth was obtained at 500 ppm and less moderate at 750 ppm. *P. fluorescens* found to tolerant at the highest concentration of Cr. These result indicates that isolate can survive even at a high concentration up to 1000 ppm. Calibration curve was plotted from standard and the R^2 was derived as 0.96. From the exponential growth phase the generation time was calculated and given in Figure 2. The doubling time was 38.5 min for cells grown without chromium. The same doubling time was recorded in 100 ppm. It was noted that, the increasing concentration may slow down the growth and increase the doubling time. The extracellular biosurfactant from cell free culture filtrate under different pH were tested and given in Table 2. The oil collapse test shows biosurfactant activity was higher in pH-8 and moderate at pH-6. Further the biosurfactant extracted from *P.fluorescens* maximum emulsification index 58% at pH 8. The biosurfactant production was moderate at pH 6 and less significant at

other pH. The biosurfactant producing bacteria tolerant to chromium capable to produce Cr reductase was tested under different nitrogen. The reduction of hexavalent chromium to trivalent Cr on chromium reduction medium (CRM) was given in Figure 3. The percentage of chromium reduction was 64≥48≥38≥48 for yeast extract≥peptone≥ casein≥ammonium nitrate. Altering the nitrogen base on CRM shows variation in reduction. The reduction was significantly enhanced under chromium reduction medium contain EDTA and chitosan. Maximum reduction was found in chitosan polymer (88%) followed by EDTA (72%). The previous study find out that *Pseudomonas* sp are temperament to hexavalent chromium up to 2000 ppm. The isometry was declined when the concentration of chromium is elevated. The indefinite amount of growth was noted on log phase [11]. The endurance nature of *Pseudomonas* sp may drop off when the chromium content in the medium is enhanced because of the reduction in biosorption site of bacterial surface [12]. The species of *Pseudomonas* contain exopolysaccharides with anionic functional groups, which incorporated in biosorption of heavy metals [13]. According to early studies, maximum bio-surfactant produced by *Pseudomonas* sp181 was attained by maintaining the pH-7.0 at 37°C 120 hours of incubation [14]. According to many researches, maximum amount of Cr(VI) reduction was achieved by *Pseudomonas* sp. under aerobic conditions and it was unconnected to purge [15].

Table 1. Biochemical character of isolate

CODE	COLONY MORPHOLOGY	KOH	Gram stain	IMViC	Nitrate	Catalase	Oxidase
S3	Green small circular, translucent, raised,	+	- rod	-/+/-/+	-	+	+

Table 2. Oil collapse test and Emulsification index

pH	Oil collapse(mm)	Emulsification index (%)
2	3	12
4	5	18
6	9	36
8	14	58
10	2	23

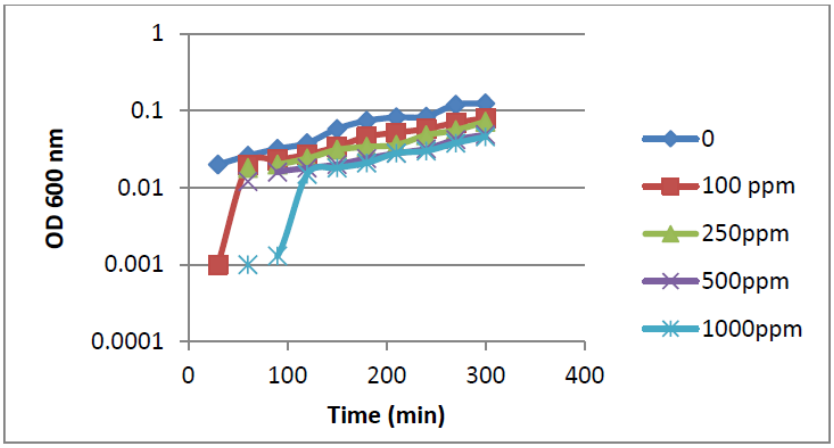

Figure 1. Growth curve of *P.fluoresence* under chromium

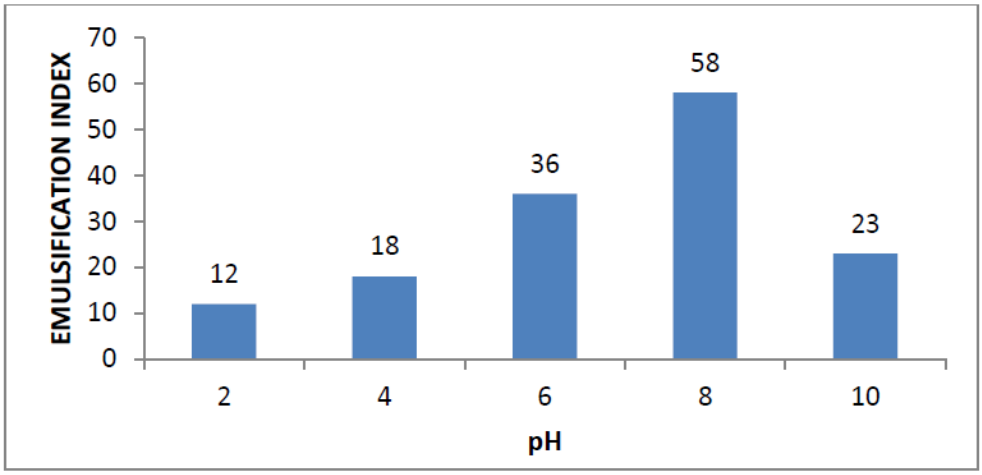

Figure 2. Effect of pH on biosurfactant production

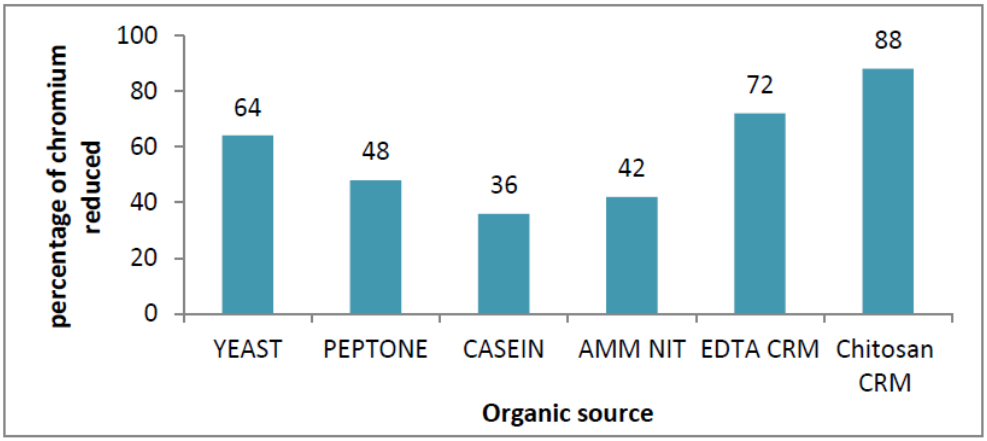

Figure 3. Effect of Nitrogen on chromium reduction

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