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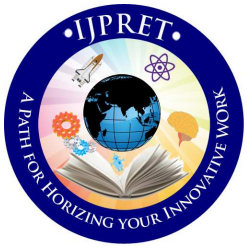

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STUDY OF CHROMIUM TOLERANCE, REMEDIATION AND ACCUMULATION BY AN INDIGENOUS BACTERIUM ISOLATED FROM ELECTROPLATING INDUSTRIAL EFFLUENT

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Abstract

Heavy metal chromium is present in many industrial effluents of which tannery & electroplating industries being responsible for environmental pollution. Conventionally chromium removal was carried out by chemical precipitation, membrane filtration and reverse osmosis. However, these methods are not completely feasible. Biosorption is a process in which solids of natural origin are adopted for removal of heavy metals. This method is a promising alternative method to treat industrial effluent because of low cost work, Chromium biosorption process is studied by isolating and characterizing chromium tolerant and reducing bacteria isolated from source of an electroplating industrial effluent. The bacterium isolated is characterized morphologically and biochemically as Gram positive rod (Bacilli). Operational parameter of Chromium remediation such as effect of pH and temperature were standardized for the isolate. Accumulation and localization of chromium in the isolate was studied using Transmission Electron Microscope (TEM). In situ reduction of toxic Cr (VI) to non toxic Cr (III) has become an alternative Bioremediating strategy.

INTRODUCTION:

Industrial pollution is one of the major matters of concern as effluents generated by the industries are the major sources of pollution (Gill et al., 1996; Volesky B, 2001; Gulfraz et la., 2002). Electroplating and tannery industries release heavy metal chromium contaminated and have been found to be carcinogenic to human as well as toxic to aquatic life (Verma et al.,2000; Bal et al., 2002; Singh et al., 2005; Parvathi et al., 2007).

Chromium exists mainly in two dominant oxidation states that are trivalent Cr (III) and hexavalent Cr (VI) forms. Cr (VI) is more mobile and water soluble as well toxic than Cr (III) (James, 1996). The toxicity of Cr is dependent on its oxidation state (Megharaj et al, 2003). Cr (VI) is a carcinogenic and toxic for all form of life. Whereas Cr (III) is an essential micronutrient for many higher organisms, it is insoluble in water and 100 times less toxic than Cr (VI).

Micro organism in nature has the ability to protect themselves from heavy metal adsorption, accumulation, uptake, methylation, oxidation and reduction. Thus micro organisms have been utilized in environmental management for a process of bioremediation with reference to chromium remediation, there is evidence of reduction of toxic Cr (VI) to less toxic Cr (III) and thus this method is safe and cost effective technology have replaced traditional treatment methods like excavation and pumping, addition of chemical reductants for precipitation and sedimentation of reduced Cr which were expensive and energy intensive (Shilpi, 2012).

The present study was aimed at the isolation of Cr (VI) tolerant and reducing bacteria from electroplating industrial effluent & standardizing the parameter for efficient remediation. An additional objective of the work is to study the tolerance level, optimize the pH and temperature and visualize the accumulation of metal inside microbial cell through electron micrograph for efficient bioremediation.

MATERIAL AND METHODS:

Sample collection and Chromium analysis:

Electroplating effluent was collected form chromium Electroplating industries located in Peenya Industrial area, Bangalore, India. The sample was stored at 4^0C to arrest any biological activity. The collected electroplating effluent was analyzed for hexavalent chromium using Atomic Absorption Spectrophotometry after nitric acid digestion.

Enrichment of Microbial Culture and Maintenance:

Microbes tolerant to Cr was enriched from electroplating effluent using Luria Bertani medium supplemented with Cr concentration of 100µg/mL in the form of $K_2Cr_2O_7$ and incubated for 24-48 h in shaker incubator at 150rpm. The enriched organism was isolated, subcultured and preserved in slants of LB Agar and glycerol stock and stored at 4^0C and 20^0C respectively.

Chromium tolerance and reduction:

The bacterial strain designated as PES B was precultured overnight in LB broth. 1% culture of 1.0 OD read at 600nm was inoculated into 100ml of LB broth in culture flask of 250ml capacity containing Cr (VI) in the concentration range of 200-1000 mg/L and 0.5% glucose and incubated at 28^0C at 150 rpm on shaker incubator. Media without Cr (VI) were also inoculated with bacteria and maintained as control. Growth of the bacteria was monitored at specific time intervals 24, 48, 72 and 96 h by measuring the optical density of the cultures at 600nm.

To check the Cr (VI) reduction ability, 1mL of culture was inoculated in LB broth containing 500mg/L concentration of Cr. The culture sample was incubated at 28^0C at 150 rpm on shaker incubator. 10mL of the culture was collected on day 1, 4, 7, 10 and 13 by giving a gap of 2 days and centrifuged at 6000 rpm for 10min at 4^0C and the supernatant was analyzed for Cr (VI) concentration and the percentage of reduction was recorded.

Optimization of process:

The optimization of condition like temperature and pH was done. For temperature optimization, 5 standards temperature like 28, 32, 35^0C was considered. Thus 1% of precultured bacteria

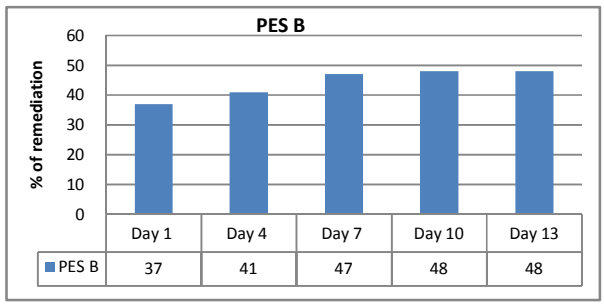
in log phase with 1.0 OD read at 600nm was inoculated into 100mL LB broth supplemented with 500mg/L concentration of Cr in the form of $K_2Cr_2O_7$ was incubated at different temperature and growth of the bacteria as well the Cr (VI) reduction ability was recorded. pH optimization was also carried out in a similar manner but using 5 pH standards as 3, 5, 7, 9, 11 which were adjusted using 1N NaOH or 1N HCl.

Study of Cr accumulation using Transmission Electron Microscope:

Localisation of adsorbed heavy metal chromium in the bacterial isolates PES B was observed with reference to a control using transmission electron microscope (TEM) (JEOL-100CX, FEI TECNAI BIO SPIRIT) at an accelerating voltage of 120kV. Cells incubated with chromium concentration of 500 mg/l at 30^{o}C in shaker incubator at 150 rpm for 24-48 h were analysed for biosorption or localization studies after Specimen preperation which includes primary fixing, post fixing, staining, dehydrating, embedding and sectioning with ultramicrotome.

RESULTS AND DISCUSSION:

Growth of isolate at different time interval:



The isolates PES B showed growth density of 0.8 OD and above at all the concentrations of Chromium exposed at different intervals of time like 24 h, 48 h, 72 h and 96h in spectrophotometric study and the results are graphically represented in Fig.2

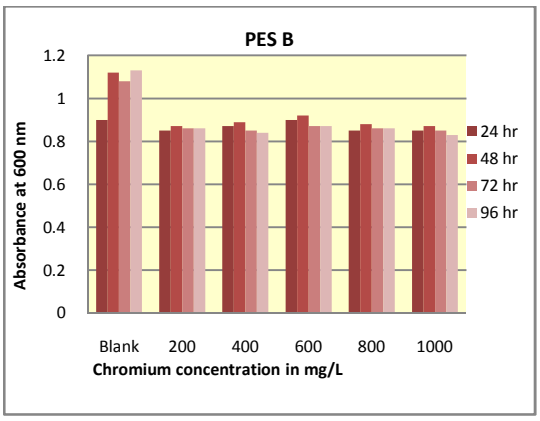


Fig.2 Graph of absorbance Vs Cr concentration for PES B

Percentage of Cr remediation at different interval of time:

The chromium remediation studies were carried out on bacterial isolate PES B at a standard tolerant concentration of 500 mg/L of chromium and grown in controlled laboratory condition for 13 days and chromium reduction was assayed using Atomic Absorption Spectrophotometer on day 1, 4, 7, 10 and 13 with a gap of 2 days. The percentage of chromium reduction is depicted in Fig. 3. A maximum of 47 % remediation was observed on Day 10 and it remains the same on day 13.

Fig.3 Graph of percentage of Cr remediation Vs time.

Percentage of Cr reduction at different pH:

The bacterial strain was inoculated in media set at different pH ranging from pH 3 to pH 11 with standard Cr concentration of 500mg/L. The blank considered was media without chromium. The remediation ability recorded at day 10 was significantly more in pH 3 and pH 7. It is reported from the literature that Chromium reductase enzyme is active in acidic to neutral conditions.

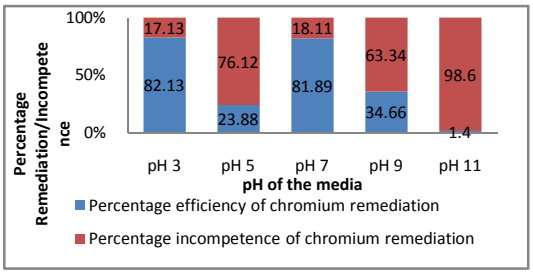


Fig. 4 Graph of percentage remediation of Cr at different pH.

Percentage of Cr reduction at different temperature:

A comparative analysis of the growth for the bacterial strain at various temperatures 28, 32 and 35^{o} C was done. A moderate growth and highest biosorption of Chromium was observed at temperature 32^{0}C and the remediation being 65 %.

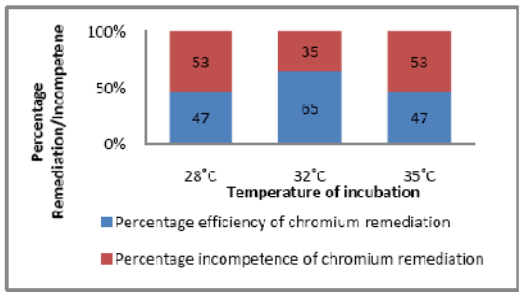


Fig. 5 Graph of percentage of Cr remediation at different temperature.

Identification of bacterial isolate:

The isolate was subjected to identification study by standard protocols like colony

characterization, morphological study using staining techniques and biochemical tests. It was evident from the results depicted in Table 1, Table 2 and Table 3 that the isolate is a Gram positive rod that is *Bacillus sp*.

The bacterial isolate was primarily identified by standard colony characterization, Morphological study, and staining and biochemical tests protocols as described in (Aneja K.R, 1996).

Table. 1 Morphology of Bacterial isolate.

Isolate	Form	Elevation	Margin	Color
PES B	Irregular	Flat	Lobate	White

Staining technique:

Gram's Staining, Endospore Staining, Negative Staining were performed.

Table. 2 Staining characteristics of isolate PES B.

Staining	Results
Gram's Staining	Positive rods
Endospore Staining	+, oval endospore
Negative Staining	Positive

Biochemical tests:

Biochemical tests listed below.

Table. 3 Results of biochemical tests of isolate PES B.

Biochemical Test	Results
Oxidase test	-
Catalase test	+
Indole test	-
Methyl red test	-
Voges-Proskauer test	+
Citrate test	+
Starch hydrolysis	+
Gelatinase test	+

Study of Cr accumulation using TEM:

It is evident from the Fig 6 that high level of heavy metal chromium is deposited in cell wall, cell membrane and cytoplasm. Thus the Gram positive rod has Cr accumulation ability thereby reducing the chromium from the environment.

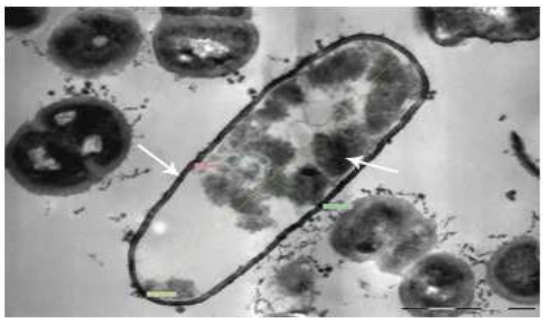


Fig. 6 Electron micrograph of PES B

CONCLUSION:

The strain isolated from high Cr (VI) Concentration has exhibited on excellent Cr (VI) reduction ability. The potential of the organism may be due to the environment stress in which the organism have grown might have forced the strain to develop an effective detoxification mechanism to survive in adverse condition of Cr (VI) contamination. It has also been reported that microorganism isolated have high tolerance to the toxicant and hence better remediation potential for both growth & Cr removal. The organism being Gram positive having thick peptidoglycan layer can accumulate Cr in it, which was evident in Electron micrograph.

The isolate can further be molecularly characterized and could be used as an efficient tool of bioremediation for heavy metal pollutants.

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