Research Journal of Recent Sciences _____E-ISSN 2277-2502
Vol. **5(6),** 45-49, June **(2016)** *Res. J. Recent Sci.*

Bioremediation of Hexavalent Chromium by *Pseudomonas spp.*

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Available online at: www.isca.in, www.isca.me
Received 29th April 2016, revised 8th May 2016, accepted 27th May 2016

Abstract

34 different isolates were obtained from collected sample. Out of which three strain of Pseudomonas spp. were isolated and used for evaluating their efficiency for reduction of hexavalent chromium. The effect of initial pH, temperature and incubation time on the bioreduction rate of hexavalent chromium was studied and process was optimized for bioreduction of hexavalent chromium. Maximum Hexavalent chromium removal of 42.30% by Pseudomonas isolates (UK3), 30.00% by Pseudomonas spp. (UK4) and 19.34% by Pseudomonas spp. (UK5) at 100 ppm of synthetic solution, during 5 days. On the basis of highest removal rate, Pseudomonas spp. (UK3) was selected and used for further study. The present study depicts that Pseudomonas spp. removes chromium efficiently and this could be used for industrial waste management and bioremediation of environmental contaminants.

Keywords: Hexavalent chromium, Bioremediation, *Pseudomonas*, Bioconversion.

Introduction

Chromium exhibiting in hexavalent form is the most critical, toxic and highly soluble metal pollutant generated by chemical, metal, textile and tannery industries[1-3]. Hexavalent chromium is highly toxic, teratogenic, mutagenic and carcinogenic for human being[4]. Biological reduction of hexavalent chromium offers cost-effective and environment friendly means for treatment of industrial waste contaminated with hexavalent chromium[5]. Numbers of microorganism are known having ability to undergo bioreduction of heavy metals like hexavalent chromium [6-8]. In the present study, microorganisms isolated from contaminated sites were evaluated for their efficiency for bioreduction of toxic form of hexavalent Chromium. Thus proposes the use of microorganisms for bioremediation of Hexavalent chromium contaminated site.

Materials and Methods

Sample Collection: Soil samples were collected from contaminated site from nearby vicinity of dyes and chemical industries in Palsana, Surat, Gujarat. The soil sample was mixed with distilled water and aliquots upto 10^{-6} were prepared. Each dilution was inoculated into the screening medium containing $K_2Cr_2O_7$.

Screening of microorganisms for chromium reduction: Basal medium (Bushnell-Hass medium) supplemented with 1% glucose and $K_2Cr_2O_7$ was prepared having chromium concentration of 100 ppm. 3.5 ml of sample were inoculated into 350 ml of Basal medium. The inoculated flasks were incubated on rotary shaker kept at room temperature with speed of 150 RPM for 5 days. The screening of microorganism for their ability to reduce hexavalent chromium was evaluated in terms of their tolerance and % reduction of hexavalent chromium[9].

Determination of tolerance of microorganisms against Hexavalent Chromium: The samples from inoculated basal medium were collected at an interval of 1 day, 2 day, 3 day, 4 day and 5 day. The biomass was determined by measuring absorbance at 540 nm using a SHIMADZU UV-Spectrophotometer against uninoculated basal medium with chromium as blank.

Determination of Hexavalent Chromium: Hexavalent Chromium was estimated using diphenylcarbazide method[9-10]. Chromium Standard with chromium concentration of 100 ppm to1000 ppm was prepared. 95 mL of the medium was transferred aseptically and was centrifuged and 2.0 mL diphenylcarbazide solution was added. The pH was adjusted to 2 ± 0.5 with 10% H_2SO_4 solution. The final volume was makeup to 100 mL. The absorbance was recorded spectrophotometrically at 540 nm against Basal medium as Blank.

Identification of Isolates: The inoculated basal medium was subjected to dilution upto 10^{-6}. Each dilution was plated onto Basal medium supplemented with $K_2Cr_2O_7$. The isolates were subjected to Morphological, Colonial and Biochemical characterization aids in partial identification of isolates.

Optimization for various parameters for Hexavalent Chromium reduction: Optimizations of hexavalent chromium reduction were carried out with respect to initial pH, Incubation Temperature and Incubation time.

Results and Discussion

Determination of tolerance and screening of microorganisms for chromium VI reduction: The growth of microorganism in basal medium inoculated with $K_2Cr_2O_7$ suggests tolerance of microorganism towards chromium. The increase in biomass determined by increase in optical density at 540 nm suggests tolerance against chromium[9]. On plating of aliquots on basal medium containing $K_2Cr_2O_7$, 34 different isolate were obtained.

Determination of Hexavalent Chromium: Three different bacterial species were screened depending upon their abundance growth pattern. After screening, UK1, UK3 and UK4 were found capable to reduce hexavalent chromium and used for further study. Three isolate namely UK1, UK3 and UK4 were selected and its activity were analysed showing hexavalent chromium reduction. UK1 show 42.3%, UK3 show 34.5%, and UK4 shows 29.8% reduction of hexavalent chromium after 48 hrs of incubation with chromium concentration of 100 ppm (Figure-1)[7].

Characterization of Isolates: All the three isolate were subjected to morphological, colonial and biochemical characterization aid in partial identification of isolate. The characterization of isolate mentioned in Table-1.

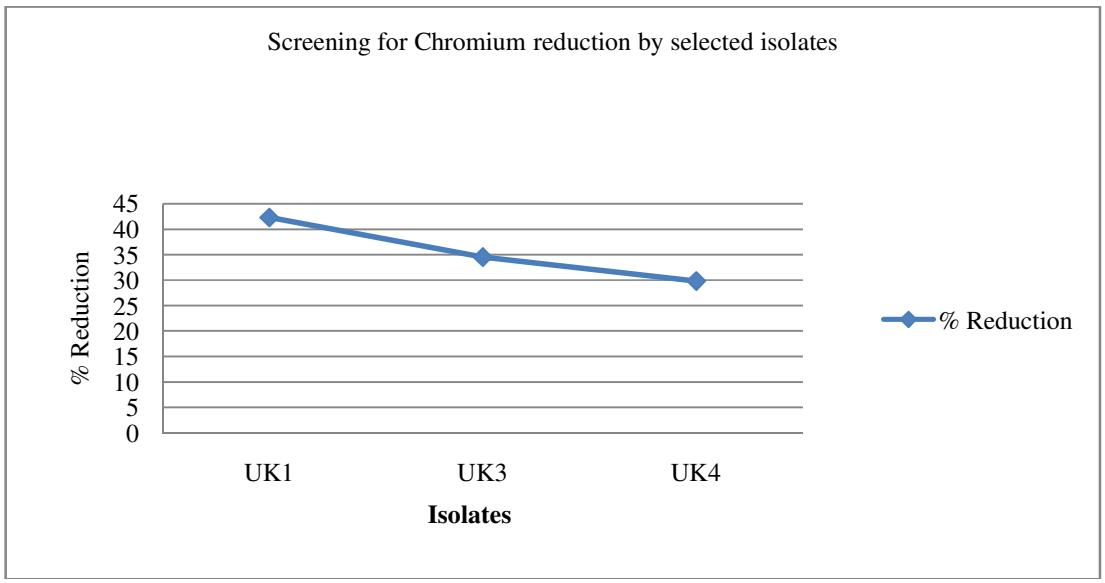

Figure-1
Screening for chromium by selected isolates

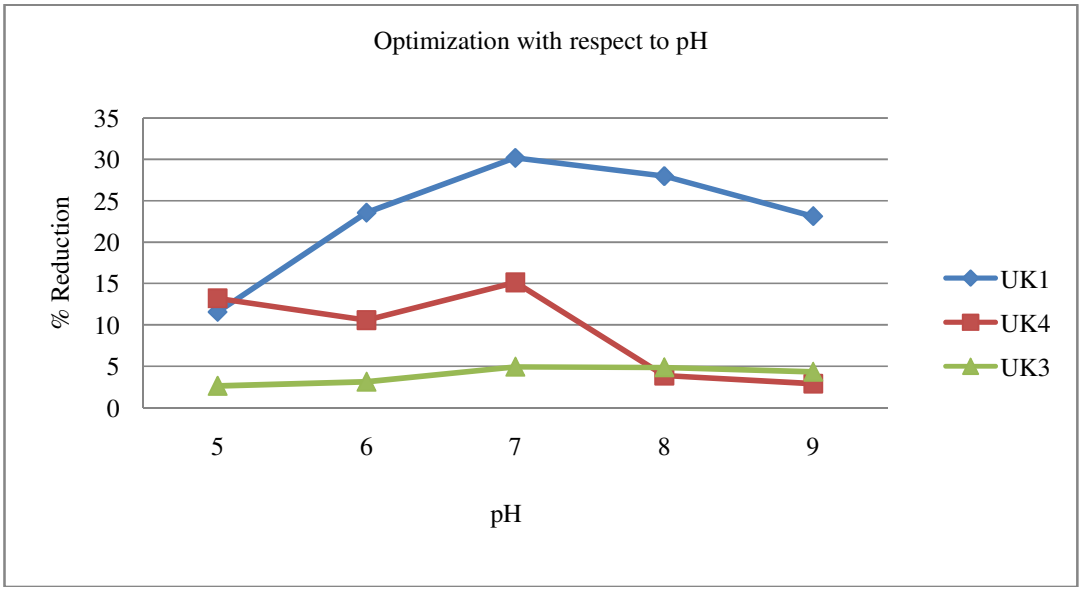

Figure-2
Optimization with respect to pH

Table-1
Characterization of Isolates

Code of Isolate	**UK1**	**UK3**	**UK4**
Morphological Characterization			
Gram Reaction and Morphological Characterization	Gram negative, rods occurring singly	Gram negative, Rod shape occurring singly	Gram negative, Rod shape occurring singly
Colony Characterization	Round, flat, transparent color colony, green pigmentation.	Round, flat, large white colour colony with Bluish green Pigmentation	Round, flat, transparent colony with entire edge having fluorescent green pigmentation
Biochemical Characterization			
Indole Utilization	-	-	-
Methyl Red Reduction	-	-	-
Vogas Proaskauer	-	-	-
Citrate Utilization	+	+	+
Gelatin Liquefaction	+	+	-
H_2S production	-	-	-
Catalase Test	+	+	+
Nitrate reduction	+	+	-
Urea utilization	+	+	+
Glucose	+	+	+
Sucrose	+	+	+
Lactose	-	-	-
Maltose	+	+	+
Mannitol	-	-	-
Fructose	+	+	+
TSI	No H2S or gas production, Acid production in butt	No H2S or gas production, Acid production in butt	No H2S or gas production, Acid production in butt

On the basis of morphological, colonial and biochemical characterization of isolates were identified as *Pseudomonas* species[10].

Optimization for various parameters for Hexavalent Chromium reduction: pH has an important role in biological system as bioreduction are pH sensitive[11]. Some of the biochemical reaction occurs at acidic, alkaline or neutral pH. The effect of pH on hexavalent chromium reduction were studied using the pH range from 5-9. The optimization result suggests all the isolate shows optimal activity at pH 7 (Figure-2). The result was in accordance with the results obtained by different authors[12-14].

The hexavalent chromium reducing ability by isolate UK1, UK3 and UK5 were greatly influenced by incubation temperature. All

the isolate exhibited reduction over the temperature range 15–30°C with chromium concentration at 100 ppm (Figure-3). The optimization result suggests all the strain activity was obtained best at temperature 30°C. The result was in accordance with the results obtained by different authors[15-17].

The Optimization for hexavalent chromium was conducted using different time at an interval of 1, 2, 3, 4 and 5 days. The optimization result suggests that UK1 show best activity at 5th day and UK3 and UK4 isolates were showing best activity at 4th day (Figure-4). The result was in accordance with the results obtained by different authors[11].

Conclusion

Microorganisms isolated namely UK1, UK3 and UK4 identified as *Pseudomonas* species by characterization of microorganisms were evaluated for their ability to reduce hexavalent chromium. It was observed that Chromium degradation can be achieved by maintaining the condition of pH 7, temperature 30°C and incubation time of 5-6 days. Thus, tolerant strain of *Pseudomonas* species can be use for bioremediation of heavy metals like Chromium that may contribute for reducing pollution load and detoxify the hazardous effect of heavy metals.

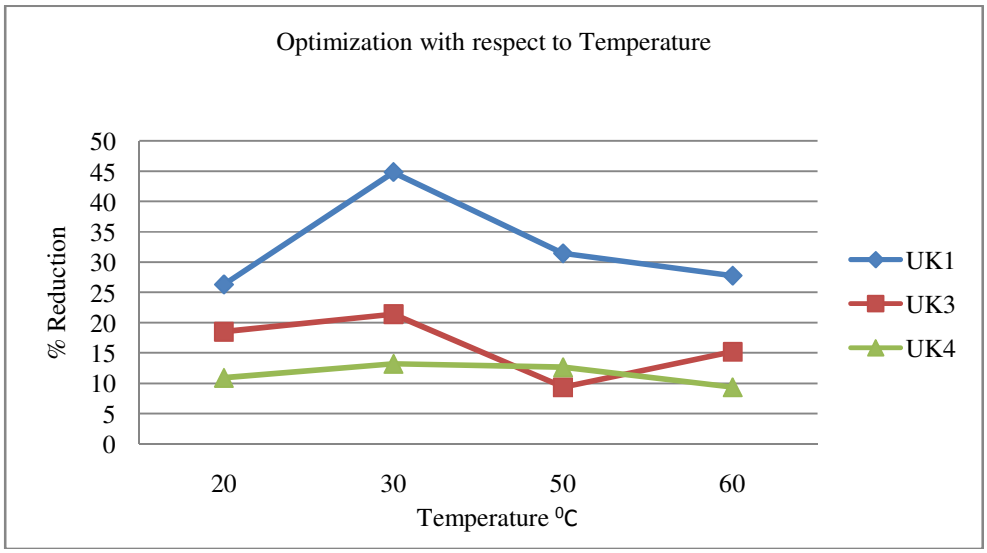

Figure-3
Optimization with respect to Temperature

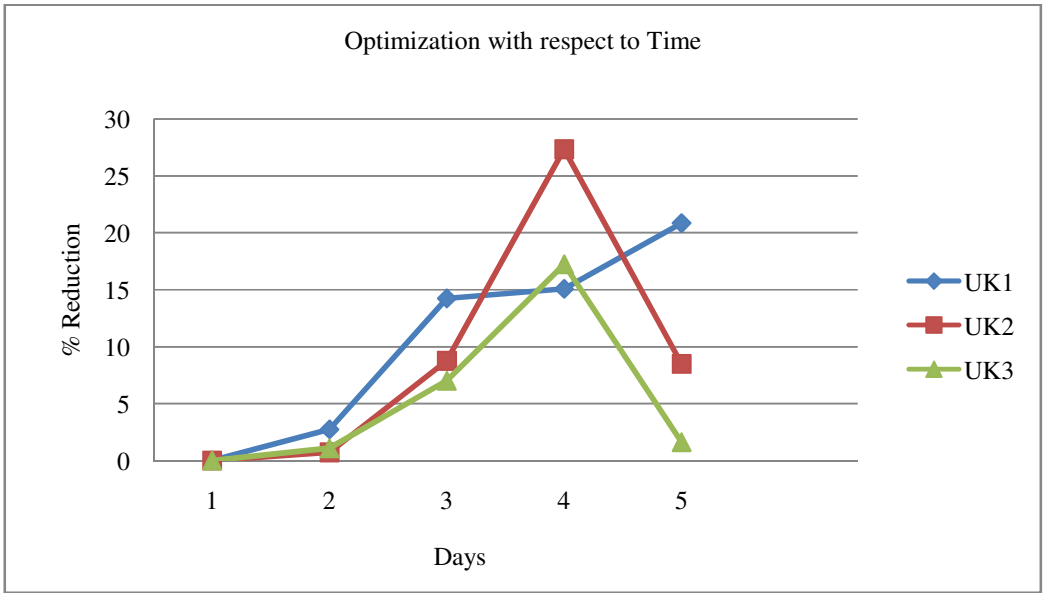

Figure-4
Optimization with respect to Time

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