International Journal of Enhanced Research in Science, Technology & Engineering
ISSN: 2319-7463, Vol. 11, Issue 3, March-2022, Impact Factor: 7.957
Presented at "LIFECONN2022", Organized by BNN College, Bhiwandi on 7th March, 2022

Thrust Area: Bioremediation
Optimisation of Factors Affecting Biodegradation of Hexavalent Chromium by Arthrobacter, Isolated From Soil and Sewage Samples

Sandhya. R. Mulchandani, Saraswati.N.Patel

Department of Microbiology, Smt. Chandibai Himathmal Mansukhani College, Ulhasnagar - 421003, Maharashtra, India.

ABSTRACT

Arthrobacter are commonly found in soil and sewage environments because of their nutritional versatility. They have the capability to degrade a wide array of organic and inorganic pollutants. Hexavalent chromium,a toxic and carcinogenic pollutant, is widespread throughout the environment because of its use in dyes, pigments, refractory material, leather tanning and electroplating. Several species of Arthrobacter can not only grow in the presence of hexavalent chromium, they can also reduce it to trivalent chromium; it's less toxic form. The Arthrobacter isolates A11 and A40 were found to be potential biodegradors of hexavalent Chromium. The optimum conditions of biodegradation of Chromium by these isolates, identified as Arthrobacters, by 16SrRNA sequencing, were determined. The two categories of factors used were nutritional and physicochemical. Glucose, lactose, sucrose, sodium nitrite, casein hydrolysate and peptone were employed to check their effect on degradation. The effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation was also studied. Both these isolates showed maximum degradation of Chromium in presence of lactose and casein, room temperature, pH 7, 48 hours of incubation, static conditions, inoculum density of 1(optical density), inoculum volume of 3% and salt concentration of 0.5% in the medium.

Key words: chromium, pollutants, physicochemical, salinity, toxic.

INTRODUCTION

Hexavalent chromium, a toxic pollutant is widely spread in the environment. It is used in tanning of leather, dyes, pigments, refractory material, and electroplating. From the two forms of chromium, hexavalent chromium is hundred times more toxic than trivalent chromium because of its oxidation state. It is also much more soluble in water and thus seeps into ground water very easily. Very few organisms can grow in the presence of hexavalent chromium. Arthrobacter can not only grow in the presence of hexavalent chromium, it can also reduce it its less toxic form of trivalent chromium [1]. Thus, Arthrobacter can be used for bioremediation of chromium contaminated niches.

The choice and successful implementation of bioremediation technologies on contaminated areas depends upon the characteristics of the contaminated site, mechanism and a complex system of many factors that affect biodegradation processes. It is extremely important to consider and understand those limiting factors [2, 3].

If the substrate/nutrient in the surroundings of the microorganism is novel, presence of easily degradable carbon source in the medium initiates metabolism and simultaneously co-metabolize the novel substrate/pollutant. It may also affect the degradation [2, 4]. Nitrogen sources are reported to hasten the rate of degradation to a certain extent [5].

The solubility, the bioavailability of the molecules and degradation of compounds increases with temperature. The degradation activity is affected at low temperature due to change in optimum temperature for functioning of enzymes [6]. Chromium degradation/removal is better at acidic pH. Many sites contaminated with pollutants are not at the optimal pH.The degradation percent of the compound increases with time due to initial catabolite repression and the time taken by the microbe to synthesize enzymes necessary for degradation. However, with increasing time percent degradation may decrease due to accumulation of toxic products affecting the viability of microbes or due to saturation of active sites of

enzymes [7].For successful biodegradation this microbe's number should not be lower than 10^3 microbes per gram of soil and should have high catabolic activity. On one hand a smaller number of cells will slow the rate of degradation, but on other hand denser culture may not be available because of cell packing [8]. Less volume inoculum and more dense culture may not serve the purpose because the increase in number of cells will again take a lag phase to multiply [9]. Dissolved molecular oxygen is required for respiration of the microbe and for degradation pathways. At sufficient depths and in deep water sediments, degradation of pollutants can turn anaerobic when the oxygen supply is depleted. Oxygen has been shown to be the rate limiting for the activity of such organisms [10]. Microorganisms are typically well adapted to cope with the wide range of salinities common to the world's ocean. However, increasing salinity in aquatic environments has had a negative impact on the biodegradation of various pollutants of soil and water ecosystems [11, 12].

Two categories of limiting factors studied in the current work were nutritional and physicochemical factors and their effect on biodegradation by two identified Arthrobacter isolates. Glucose, lactose, sucrose, sodium nitrite, casein hydrolysate and peptone were employed to check their effect on degradation. The effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation was also studied.

MATERIALS AND METHODS

The method used for chromium estimation i.e; diphenyl carbazide method, remains same throughout the study**.** All experiments were carried out in triplicates. Positive and negative controls were kept wherever applicable. Appropriate dilutions were carried out as required.

Materials

Standard Chromium estimation

Standard solution of $K_2Cr_2O_7$ (100 mcg/ml), Orthophosphoric acid and Diphenyl carbazide reagents.
Sterile test tubes and pipettes, Centrifuge and Colorimeter.

NUTRITIONAL FACTORS

Culture suspensions of identified Arthrobacter isolates A11 and A40.

i. Effect of carbon sources

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$ and100 mgs (1 gm/lit) of 3 selected carbon sources in 3 different flasks-2 sets

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$ and no carbon source -3 flasks as controls
Carbon sources used - glucose, lactose and sucrose.

ii. Effect of Nitrogen sources

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$ and 100 mgs (1 mg /ml) of 3 selected nitrogen source in 3 different flasks -2 sets.

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$ without nitrogensource ـ3 flasks as controls
Nitrogen sources used - sodium nitrite, casein hydrolysate and peptone.

2. PHYSICOCHEMICAL FACTORS

Effect of temperature

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$- 5 flasks for 5 different temperatures (4, RT, 37, 45 and 55^0C) – 2 sets and 1 set of flasks as control for each temperature.

Effect of pH

Sterile 100ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$-5 flasks with different pH (5, 6, 7, 8 and 9) – 2 sets and 1 set of flasks as control for each pH.

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$.. 2 flasks plus 1 flask as control.

Effect of Incubation time

Sterile 100ml Mineral salt broth media containing 100 mcg/ml. of $K_2Cr_2O_7$.. 2 flasks plus 1 flask as control.

Effect of inoculum density

Culture suspensions of identified Arthrobacter isolates A11 and A40 with 5 different inoculumdensities 0·6, 0·7, 0·8, 0·9 and 1.0 (at 660 nm).
Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$.. 5 flasks for 5 different inoculum densities –2 sets and 1 flask as control .

Effect of inoculum volume

Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$.. 5 flasks for5 different inoculum volumes of cultures–2 sets and 1flask as control.
Sterile 100 ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$..2 flasks-2 sets and 2 flasks as control .

Effect of aeration

Sterile 100ml Mineral salt broth media containing 100 mcg/ml of $K_2Cr_2O_7$..2 flasks-2 sets and 2 flasks as control,Shaker.

Effect of salt concentrations

Sterile 100 ml Mineral salt broth medium containing 100 mcg/ml of $K_2Cr_2O_7$ .5 flasks with 5 different salt concentrations of 0.5, 1, 1.5, 2, 3 and 4 %– 2 sets and 1 set of flasks as controls.

Method

Degradation assay for chromium. Hundred ml of sterile Mineral salt liquid medium containing $K_2Cr_2O_7$ and different carbon/nitrogen sources were taken in different flasks and inoculated with 2 ml of Arthrobacter identified isolates A11 and A40 (0·5 @ 660 nm). A similar set up was done for both the isolates. A control flask without any carbon /nitrogen sources was also kept. They were all incubated at **RT-room temperature** for 48 **hrs-hours**. Control flask without any culture was also included. A similar set up was employed for physicochemical conditions as mentioned above.

Aliquots were removed from medium at end of 24 and 48 hrs and hexavalent chromium estimation was done by diphenyl carbazide method. They were diluted as and when required. In acid solution, diphenyl carbazide and hexavalent chromium form a soluble red-violet product that absorbs light at 540 nm which is read colorimetrically. A set of standards was run by using potassium dichromate standards ranging from 5-100 mcg/ml [13, 14]. Standard graph was plotted and the unknown values were determined from the graph. Degradation Percent was calculated by the standard formula.

RESULTS AND DISCUSSION

Standard Chromium estimation

Standard hexavalent Chromium estimation by was carried out by diphenylcarbazide method and the values were obtained from standard graph depicted in figure 1.

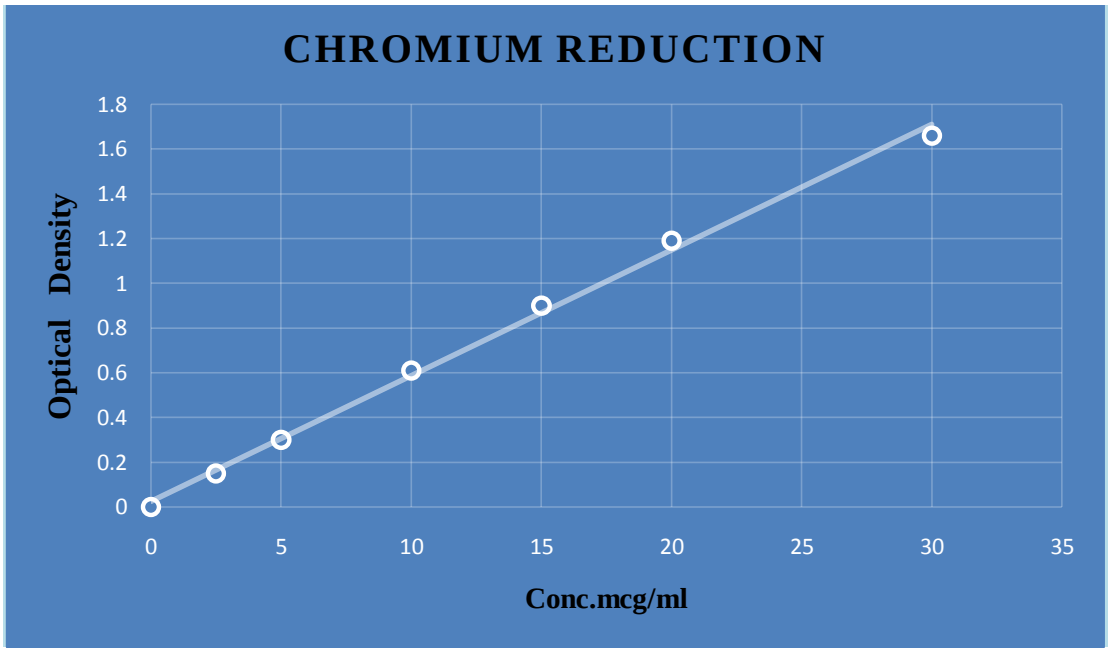

Figure:1 Standard hexavalent chromium reduction graph

1. Nutritional Factors

The results of effect of Carbon and Nitrogen sources on biodegradation by Arthrobacter isolates A11 and A40are tabulated in **tables 1and 2.**

i. Effect of carbon sources

Table:1 Degradation of Chromium in presence of different carbon sources, by Arthrobacterisolates A11 and A40

Isolate/Carbon source Hrs-hours	**Glucose**		**Lactose**		**Sucrose**	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	27.5 %	53.6%	**30.6%**	**60.1%**	5%	7%
A40	58.73%	58%	**59.7%**	**63.2%**	4%	7.3%

ii. Effect of Nitrogen sources

Table:2Degradation of Chromium in presence of different nitrogen sources at 24 and 48 hrs, by Arthrobacterisolates A11 and A40.

Isolate/Carbon source	**Peptone**		**Casein hydrolysate**		**Sodium nitrite**	
	24 hrs	48 hrs	**24 hrs**	**48 hrs**	24 hrs	48 hrs
A11	22.5 %	51.6 %	**28.6 %**	**61.2 %**	4 %	7.4 %
A40	41.73 %	43.7 %	**55.7 %**	**60.2 %**	5 %	6.3 %

2. PHYSICOCHEMICAL FACTORS

The results of effect of temperature, pH, incubation time, inoculum density and volume, aeration and salinity, on degradation by Arthrobacter isolates A11 and A40are tabulated in **tables 3 to 9.**

i. Effect of temperature

Table:3 Degradation % of Chromium at different temperatures by byArthrobacterisolates A11 and A40at24 and 48 hrs

Isolate/Temp	4^0C		RT		37^0C		45^0C		55^0C	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	1%	3%	**29.5 %**	**63.2 %**	26.1 %	55%	22.5 %	51.6 %	22%	50%
A40	1%	3%	**45.7 %**	**62.9 %**	41.7 %	54%	40%	43.7 %	36%	40%

ii. Effect of pH

Table:4 Degradation % of Chromium at different pH by Arthrobacter isolates A11 and A40 at 24 and 48 hrs

Isolate/pH	5		6		7		8		9	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	0%	0%	12.5 %	30.75 %	**30.5 %**	**49.97 %**	35%	38.1 %	32.5 %	33.8 %
A40	5%	0.5%	7.5%	23.12 %	**67.5 %**	**72.37 %**	47%	49.3 %	27%	29.8 %

iii.Effect of Incubation time

Table:5 Degradation % of Chromium by byArthrobacterisolates A11 and A40,at 24 and 48 hrs

Isolate / Incubation time	24 hrs	48 hrs
A11	27.5%	**53.6%**
A40	58.73%	**58%**

iv. Effect of inoculum density

Table:6 Degradation % of Chromium using different inoculum densities by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/ Inoculum density	0.6		0.7		0.8		0.9		1	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	12%	14.7 %	17%	20.4 %	20%	22%	29%	48%	**32.1 %**	**51.7%**
A40	19.6%	21.4 %	25.9 %	27%	50%	51%	66.4 %	70.11 %	**64.5 %**	**74.7%**

v. Effect of inoculum volume

Table:7 % degradation of Chromium using different inoculum volumes by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/Inoculum volume - %	1		2		3		4		5	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs

A11	17.6 %	19%	28.9 %	32.6 %	**35.5 %**	**52.97 %**	26.9 %	25%	10.6 %	10%
A40	20%	25%	60.1 %	64.5 %	**69.5 %**	**75.37 %**	58.1 %	50%	13%	11%

vi. Effect of aeration

Table:8 Degradation % of Chromium under Static and Shaker conditions by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate / Aeration condition	**Shaker**		**Static**	
	24 hrs	48 hrs	24 hrs	48 hrs
A11	27.5%	**53.6%**	27.5%	**53.6%**
A40	58.73%	**58%**	58.73%	**58%**

vii. Effect of salinity

Table:9 Degradation % of Chromium using different salt concentrations in medium, by Arthrobacter isolates A11 and A40, at 24 and 48 hrs

Isolate/Salt concentration%	**0.5**		**1**		**2**		**4**		**6**	
	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs	24 hrs	48 hrs
A11	**67.9%**	**77.2%**	30%	40%	14%	14.1 %	9%	9.3 %	0%	3.6%
A40	**41.5%**	**49.9%**	19%	31.2 %	8%	8.2%	7%	7.4 %	0%	5.5%

CONCLUSION

Arthrobacter a common occurant in soil and sewage habitats, can biodegrade multitude of pollutants including hexavalent Chromium. The modern times need to develop microbial inocula for bioremediation of such contaminated niches. It is necessary to know the degrading abilities of native microbial populations [15]. The use of microorganisms in bioremediation requires the optimization of parameters used in cultivating the organism.If the optimum conditions for degradation are provided to microbe, biodegradation can be achieved efficiently. A widely used approach to bioremediation involves biostimulation, thatcan be achieved through changes in pH, moisture, aeration, or additions of electron donors, electron acceptors or nutrients. Bioremediation can also be achieved through bioaugmentation [16]. Biostimulation and bioaugmentation, are not mutually exclusive. Hence optimum nutritional and physicochemical conditions were determined that can be employed for chromium degradation by Arthrobacter isolates. Maximum degradation of chromium was found to be in presence of lactose and casein hydrolysate as carbon and nitrogen source respectively, in the medium, by Arthrobacter isolates A11 and A40. Theycould carry out maximum degradation of chromium at room temperature, pH 7 in 48 hrs, at an inoculum density of 1, inoculum volume of 3%, static condition and a salt concentration of 0.5%in the medium.The isolates appear to be not fastidious and due to their all-pervasive presence in soil, can beexplored to remediate subsurface pollution and environmental clean-up by bioremediation. An important challenge that remains to be overcome for applying itpractically, is to develop strategicbioremediation that are feasible technically and economically [15].

ACKNOWLEDGEMENTS

We are thankful to our principal for providing us with facilities and encouragement for carrying out this project at Smt.C.H.M College, Ulhasnagar.

REFERENCES

[1]. Meghraj, M., Toxicity of Hexavalent Chromium & its reduction by bacteria isolated from soil contaminated with tannery wastes, Current Microbiology, **47**: 51-54, 2003.

[2]. Sihag, S., Pathak, H., & Jaroli, D. P. Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons. International Journal Pure Applied Biosciences, 2(3), 185-202, 2014.
[3]. Zhao, X., Wang, L., Du, L., Yang, J., Dong, J., & Ma, F. Optimization of culturing conditions for isolated Arthrobacter sp. ZXY-2, an effective atrazine-degrading and salt-adaptive bacterium. RSC advances, 7(53), 33177-33184, 2017.
[4]. Guo, Y., Rene, E. R., Wang, J., & Ma, W. Biodegradation of polyaromatic hydrocarbons and the influence of environmental factors during the co-composting of sewage sludge and green forest waste. Bioresource technology, 297, 122434,2020.
[5]. Aamr, A., & Cuiling, J. Bacterial influence on textile wastewater decolorization. Journal of Environmental protection, 2012.
[6]. Zekri, A.Y. and Omar, C. Effect of Temperature on Biodegradation of Crude Oil Energy Sources, (27), 233–244, 2005.
[7]. Arora, N. K., Fatima, T., Mishra, I., Verma, M., Mishra, J., & Mishra, V. Environmental sustainability: challenges and viable solutions. Environmental Sustainability, 1(4), 309-340, 2018.
[8]. Masciandaro, G., Macci, C., Peruzzi, E., Ceccanti, B., & Doni, S. Organic matter–microorganism–plant in soil bioremediation: a synergic approach. Reviews in Environmental Science and Bio/Technology, 12(4), 399-419, 2013.
[9]. Masciandaro, G., Macci, C., Peruzzi, E., Ceccanti, B., & Doni, S. Organic matter–microorganism–plant in soil bioremediation: a synergic approach. Reviews in Environmental Science and Bio/Technology, 12(4), 399-419, 2013.
[10]. Best, M. A., A. W. Wither, and S. Coates. "Dissolved oxygen as a physico-chemical supporting element in the Water Framework Directive. "Marine pollution bulletin 55, (1-6), 53-64, 2007.
[11]. Das, N., & Chandran, P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology research international, 2011.
[12]. Ghosal, D., Ghosh, S., Dutta, T. K., &Ahn, Y. Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. Frontiers in microbiology, 7, 1369,2016.
[13]. Trivedi R. K., Goel P. K; Chemical and biological methods for water pollution studies, Environmental Publications, 1994.
[14]. http://www.caslab.com/Test-Methods-Search/PDF/USGS-Method-I-1230-85.pdf.
[15]. Kumar.A 1, Bisht.B.S 1Joshi.V.D2, Dhewa. TReview on Bioremediation of PollutedEnvironment: AManagement Tool.International journal of environmental sciences Volume 1, No 6, 2011.
[16]. Sylvia, D. M., Fuhrmann, J.F., Hartel, P.G., and D.A Zuberer (2005). "Principles and Applications of Soil Microbiology." New Jersey, Pearson Education Inc.