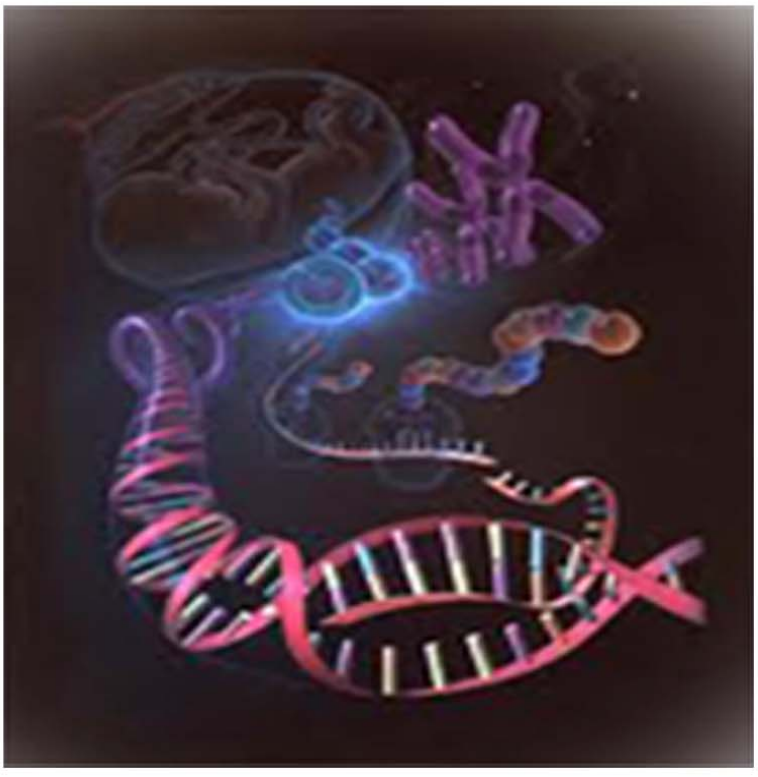
IJLBPR

ISSN 2250 - 3137
Vo1. 2, No. 3, July 2013

International Journal of

Life Sciences Biotechnology and Pharma Research



International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA

www.ijlbpr.com

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International Journal of Life Sciences Biotechnology and Pharma Research

ISSN 2250-3137 www.ijlbpr.com
Vol. 2, No. 3, July 2013

Research Paper

BIOREMEDIATION OF HEXAVALENT CHROMIUM (VI) AND OIL AND GREASE FROM PETROLEUM REFINING INDUSTRIAL EFFLUENTS BY USING *BACILLUS SUBTILIS*

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Rapid industrialization in the developing countries increases the problem of environmental pollution by improper discharge of industrial effluents. In this present study bioremediation of hexavalent chromium and oil and grease from petroleum refining industries were carried out. Using *Bacillus subtilis* species 1 and 2 for the removal of hexavalent chromium and oil and grease from the industrial waters were achieved and percentage removal of hexavalent chromium in untreated and treated was 100% and 99.8% and 100 and 99.1%. For oil and grease was 98.7 and 99.1% and 98.4 and 99.5%. These results revealed that bacillus subtilis was a suitable organism for hexavalent chromium removal.

Keywords: Petroleum refining waters, Physico-chemical parameters, *Bacillus subtilis*, Bioremediation, Hexavalent chromium, Oil and grease

INTRODUCTION

Industrialization in countries has brought several novel environmental problems of water land and air pollution with hazardous chemicals including radio nuclides ultimately leading to severe deterioration of environmental quality. The major pollution of land and water pollution are industrial and domestic wastes which are discharged into land and aquatic systems. Industrial effluents from petroleum, tanning industries, chrome plating industries, and electroplating units release large amounts of heavy metal chromium pollution into land and water systems (Salunke *et al.,* 2006). Heavy metals like mercury, cadmium, arsenic, chromium, thallium and lead, etc., are metallic compounds with high density and toxic at low concentrations. The main objective of this study is to remove hexavalent chromium from petroleum refining industries. Hexavalent chromium is more toxic and stable and hazardous as it can cause skin rashes and when ingested internally causes stomach ulcers, respiratory problems, kidney and liver damage, alteration of genetic material causes death. World

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health organization standards for hexavalent chromium in drinking waters is 0.05 mg/L and according to central pollution control broad permissible limit of hexavalent chromium in industrial effluents is 2-5 mg/L. Focusing on its toxicity and harmful effects the United States of Environmental Protection Agency (USEPA) characterized hexavalent chromium as a priority pollutant (Smith *et al.,* 2002). Several methods have been proposed in literature for industrial effluents but in recent years microbes draw attention due to their tremendous ability to degrade variety of toxic compounds and thereby improving water quality (Boominathan *et al.,* 2007). It was stated that microbes cannot degrade or remove heavy metal pollution but can transform from toxic state to non toxic state (biotransformation) (Garbisu *et al*., 2003). Microbes remove metals through biosorption in which live or dead cells may bind or concentrate metals from aqueous solutions (Naja *et al*., 2006). Microbes have high surface area to volume which makes them to use for bioremediation. Many species of soil bacteria can use petroleum hydrocarbons as a sole source of carbon and energy. Scientists (Shukla *et al.,* 2007) isolated 4 strains of bacteria resistant to high concentration of chromium form tannery effluents which showed great potential for chromium tolerance. Isolation of microbes from the source itself gives better results for bioremediation. In this study petroleum influents and effluents were collected and analyzed for physico chemical and microbiological parameters and microbes isolated from influents and effluents were used for bioremediation of hexavalent chromium and oil and grease.

MATERIAL AND METHODS

Sampling of Industrial Waters

Treated and untreated petroleum industrial waters from Visakhapatnam were collected in a clean hygienic 1 l polythene bottles with tight seal. These were brought to laboratory and immediately acidified with addition of concentrated hydrochloric acid to avoid microbial growth. Samples were stored at 4^0C for further analysis.

Sample Analysis

Estimation of DO, BOD and microbiological analysis were carried on the same day. 1 ml of the sample was serially diluted and plated on nutrient agar plates through pour plate method. Plates were incubated at 30^0C for 48 h. Preliminary identification of microbes was carried out by gram's staining, colony morphology on nutrient agar plate, biochemical tests of isolate. All the physico chemical analysis was carried out for the samples collected by following APHA standards.

Bioremediation of Hexavalent Chromium

Isolated microbial species on nutrient agar were cultured in 100 ml nutrient broth in 250 ml Erlenmeyer conical flask incubated for 24 h at 30^0C at 120 rpm in shaking. These cultures were harvested by centrifuging at 5000 rpm for 15 min. cells were washed with phosphate buffer thrice and suspended in phosphate buffer for further tests. 100 ml of sterile untreated and treated petroleum refining industrial effluents were inoculated with 2 ml of washed cell culture suspended in phosphate buffer and incubated for 24 h at 30^0C. These inoculated industrial waters were centrifuged at 5000rpm for 15 min and supernatant was used for chromium analysis.

Bioremediation of Oil and Grease

100 ml of sterile untreated and treated petroleum refining industrial effluents were inoculated with 2 ml of washed cell culture suspended in

phosphate buffer and incubated for 24 h at 30^{0}C. These inoculated industrial waters were centrifuged at 5000 rpm for 15 min and supernatant was used for oil and grease analysis. Inoculation was carried out in triplets (n=3).

ANALYTICAL METHODS

Physical Analysis

All the standard parameters for waste water analysis were carried out according to APHA methods 1995. pH was checked with pH digital meter (Elico pH meter). For conductivity and turbidity analysis Nephlorometric methods were followed. For Total Solids (TS), Total Suspended Solids (TSS), and Suspended Solids (SS) gravimetric methods were followed. For DO analysis wrinkler's method was followed. For total hardness, calcium and magnesium hardness titrimetric method was used. Inoculation of microorganisms was carried out in triplets (n=3).

Chemical Analysis

Chemical studies were estimated by spectrometric methods. Nitrates were estimated by using PDA method, hexavalent chromium was estimated by using Diphenyl carbazide method

Table 1: Physico-Chemical Parameters

Parameter Name	Untreated	Treated
pH	1.19	6.41
Conductivity	76.4mM	0.918mM
Turbidity	0.85NTU	8.8NTU
DO	1.2mg/L	9.6mg/L
BOD	1560mg/L	850mg/L
Calcium hardness	3900mg/L	2120mg/L
Magnesium hardness	500mg/L	70mg/L
Chlorides	800mg/L	280mg/L
Sulphates	Nil	Nil
Ammonia	55mg/L	10mg/L
Nitrates	45mg/L	259.6mg/L
Nickel	Nil	Nil
Hexavalent chromium	14.2mg/L	7mg/L
Iron	Nil	Nil
Oil and grease	300g/L	1000mg/L
TSS	6000mg/L	800mg/L
TDS	800mg/L	800mg/L
SS	300g/L	800mg/L
Copper	Nil	Nil

(Vogel *et al.,* 1985), nickel was analyzed by using Dimethyl gloxime method, ammonia by using Nesselers method, copper by neocuprine method, for oil and grease shake flask method was followed (APHA, 1995).

Microbiological Analysis

For microbiological analysis methods employed were MPN and total plate count.

RESULTS AND DISCUSSION

Physico Chemical Parameters

Physico Chemical Characteristics of Petroleum Refining Waters

The physico chemical analysis of petroleum refining industrial waters revealed that many parameters are not in permissible limit. Basing on pH of treated and untreated industrial waters treated industrial water is still below the permissible level. Chemical parameters such as DO, BOD, TSS, TDS, SS, chlorides, calcium and magnesium are too high indicating pollution of waters. Wastes containing high BOD levels deplete oxygen levels in particular sector (Tariq *et al.,* 2006). A high organic load is evident from the given table. High organic load stimulates algal growth in waters which indirectly depletes oxygen levels in waters. The concentration of nitrates in treated effluent indicates nitrate pollution which is more dangerous to human health. The concentration of oil and grease is also high which is above the permissible limit. The amount of hexavalent chromium found in treated sample is also high indicating hexavalent chromium pollution. The results for the total plate count and MPN tests were below the permissible level.

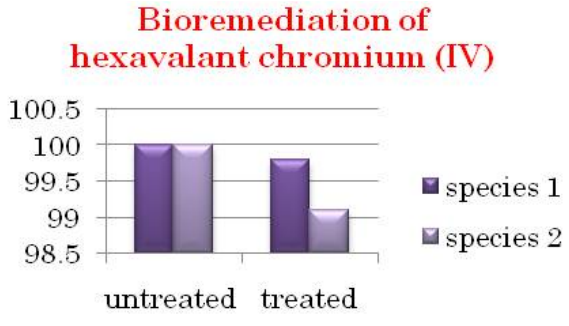
Isolation of *Bacillus subtilis*

Irregular undulate opaque colonies were observed on nutrient agar plates. After gram's staining procedure of these colonies, small gram positive bacilli were observed. Further studies for biochemical tests like VP test, mannitol fermentation gave positive results which confirmed the isolates as *Bacillus subtilis*. Clear spore was visible in gram's staining in 8 days old cultures. Inoculation in starch agar gave clear zone around the streak in culture dish which gave positive test for amylase production.

Bioremediation of Hexavalent Chromium

Bacillus subtilis was found to be suitable organism for bioremediation of variety of toxic compounds. Microbes remove toxic heavy metals by biosorption method. The study revealed that removal of hexavalent chromium from untreated and treated industrial waters was 100 and 99.8% by using live *bacillus subtilis* species 1. The percentage removal of species 2 is 100 and 99.1% (Graph 1). The results indicate that species 1 and 2 are more efficient in untreated industrial waters where as in case of treated water species 1 is more efficient in removal of hexavalent chromium. Based on the physico chemical parameters of the pH of the untreated waters were high which may be favorable for the

Graph 1

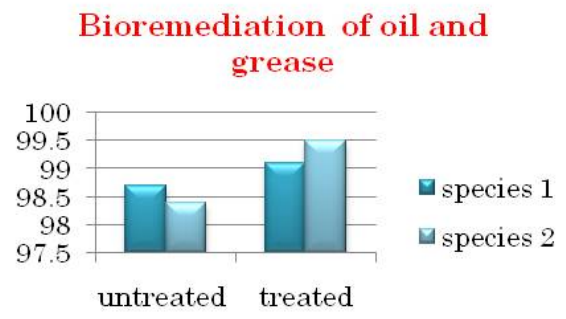


100% removal of hexavalent chromium.(Table 1) It was in literature (Seema Tarannum *et al.,* 2012) that high acidic pH may favor high percentage removal of hexavalent chromium from the untreated industrial waters. Surajana and Manas (2009) stated that pH of the effluent may play key role for effective biosorption of chromium. The incubation temperature was at 30^0C which gave better results for the experiment. Chavertudi (2011) stated that bacillus sp can remove high concentrations of chromium at 30^0C temperature. May be this condition favored high percentage results in this experiment.

Bioremediation of Oil and Grease

Bacillus subtilis is known to produce biosurfactant which reduces oil and grease naturally. Basing on this aspect *Bacillus subtilis* was selected for bioremediation of oil and grease. In the present study percentage removal of oil and grease by *Bacillus subtilis* species 1 is 98.7 and 99.1% and subtilis species 2 is 98.4 and 99.5% (Graph 2). The results in this study reveals that species 1 and 2 are more are less same in removal of oil and grease. When compared to oil and grease removal from petroleum refining waste waters *Bacillus subtilis* species 1 and 2 are more suitable for the removal of hexavalent chromium. In this study growth parameters were not studied and no acclimatization of the organism was carried out for the hexavalent chromium removal. The main purpose of this study was to examine the *in situ* effect of bioremediation of hexavalent chromium and oil and grease. Further research is needed in area of acclimatization of organism to higher concentrations of toxic compounds to improve its bioremediation efficiency.

Graph 2



CONCLUSION

Bacillus subtilis was well known for its bioremediation ability for hexavalent chromium *insitu* and *ex situ*. In this study isolation of bacillus subtilis from petroleum refining industry and study of physico chemical parameters of the untreated and treated industrial wastewaters were carried out to assesses the permissible limit. From the results it was known that many parameters were not in permissible limit. The isolated *Bacillus subtilis* were used to bioremediate hexavalent chromium and oil and grease of untreated and treated wastewaters. To know more about biosorption efficiency of this microorganism growth parameters and acclimatization to high concentrations should be carried out.

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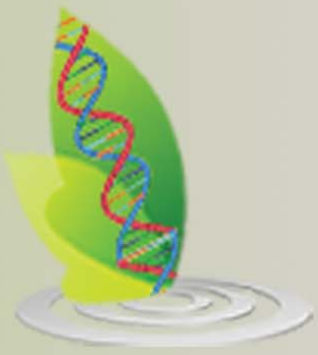

International Journal of Life Sciences Biotechnology and Pharma Research
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
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ISSN 2250-3137