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Effect Of Physicochemical Parameters Of Bioremediation on Barium.

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Abstract*— Biosorption is becoming a promising alternative to replace or supplement the present removal processes of organic pollutants from wastewater (Aksu, 2005). In this study, Barium adsorption bacteria was isolated from different areas of Mangampeta, Kadapa (dist.) and the biosorption of Barium by these isolates were evaluated for the applicability of Barium removal from Barium polluted areas. Among the thirty isolates from six samples, one isolate (R1) showed the potential for high barium adsorption and was selected for further studies. Gram staining, biochemical tests and 16s RNA sequencing revealed R1 as *Pseudomonas* species Incubation period, temperature, pH, supplementation of different carbon sources and nitrogen sources was optimized for Barium adsorption. The effect of mutation, both physical and chemical was conducted to test the ability of adsorption of Barium by *Pseudomonas sps.

***Keywords:* Biosorbtion, *Pseudomonas* species, Physical mutation (U.V), Chemical mutation (EtBr).**

I. INTRODUCTION

Environmental pollution is one of the major global problems which require immediate action. It has a negative impact on health, social and economic status of a nation. Heavy metal pollution being one among the different kinds of the environmental issues, it has been one of the serious environmental problem. Environmental pollution from hazardous metals and minerals can arise from natural as well as anthropogenic sources. (Indian National Science Academy, 2011). Barium is one among the heavy metal polluters. Barium is used for a variety of industrial purposes, as a heavy additive in oil-well-drilling mud; in the paper and rubber industries; as a filler or extender in cloth, ink, and plastics products; in radiography; as getter (scavenger) alloys in vacuum tubes; and so on. The length of time that barium will last in the environment following release to air, land, and water depends on the form of barium released. (U.S. Public Health Service,1992).

The general population is exposed to barium through consumption of drinking water and foods, usually at low levels. Workers in barium mining or processing industries and individuals who reside near such industries might be exposed to relatively high levels, primarily through the inhalation of fugitive dust containing barium compounds (U.S. Public Health Service, 2007). Short term exposure can cause vomiting, abdominal cramps, diarrhea, difficulties in breathing, increased or decreased blood pressure, numbness around the face, and muscle weakness. Large amounts of barium intake can cause, high blood pressure, changes in heart rhythm or paralysis and possibly death (Martin and Griswold, 2009).

Traditional physicochemical processes for remediation of soil polluted sites are expensive and often do not permanently alleviate the pollution hazard. Biosorption is becoming a promising alternative to replace or supplement the present

removal processes of organic pollutants from wastewaters. Biosorption of these types of hazardous organics by selected live and dead microoganisms has been investigated by various workers. (Aksu, 2005).

The current study is aimed at optimizing physicochemical parameters for Barium adsorption. Parameters like incubation period, temperature, pH, carbon sources and nitrogen sources were varied. Mutational studies were conducted to further test the adsorption capacity of Barium by the bacterial strain.

II. MATERIALS AND METHOD

A. Collection and Isolation of Barium adsorption microorganisms.

B. Collection of soil sample:

The soil samples were collected in agriculture, rural, water, mining soils in Mangampeta, Kadapa (dist.). Samples were collected in a pre-sterilized zip lock covers, from the depth of 0-20cm using a sterile spatula. Precautionary measures were taken to minimize the contamination. The samples were then brought to the laboratory for the isolation of barium adsorption microbes. The soil samples were mixed well and processed on the same day.

C. *Isolation of micro organisms:*

D. Preparation of Media:

Specific medium for screening of barium adsorption bacteria called Minimal salt medium (MSM) was used. All the components of the medium were individually weighed and dissolved in 1000ml distilled water. The media was autoclaved at 121^0 C and 15 lbs pressure for 20 minutes. 10g of barium was added under aseptic condition.

E. *Serial dilution*

One gram of soil was mixed in 10ml of 1% sterile saline water to prepare a stock solution giving a 10-fold dilution. The samples were serially diluted up to five dilutions and about 1000µl of the diluted samples from 10^{-3}, 10^{-4} and 10^{-5} were inoculated onto the plates containing MSM media with barium. The plates were incubated at 37^0C for 24 hours to screen for Barium adsorption bacteria.

F. Maintenance of pure culture:

The isolated bacteria were cultured on nutrient agar to prepare a pure slant. They were sub-cultured at regular intervals and incubated at 37^0C. The pure cultures were then screened for barium adsorption bacteria.

G. Identification of the isolates:

The bacterial isolates obtained were maintained in pure culture on nutrient agar slants, all the agar slants were refrigerated at 4^0C until used. Based on colony morphology, Gram character, microscopic characters, biochemical tests the potential bacterial isolates were identified.

H. BIOCHEMICAL CHARACTERIZATION:

Different mediums were used for the biochemical characterization of the isolated and selected bacteria for identification according to Bergey's Manual of Determinative Bacteriology. The tests performed were indole, methyl-red, Voges -Proskauer, citrate utilization, oxidase, catalase, urease, starch hydrolysis, hydrolysis of casein, gelatin hydrolysis, hydrogen sulphide production and carbohydrate fermentation of lactose, sucrose, fructose and mannitol.

I. *GROWTH CURVE*

The bacteria inoculated in the MSM broth containing 5g of barium was incubated at 37^0C for 96hrs. The bacterial growth was checked at 600nm against a suitable blank.

J. *BARIUM ADSORPTION ANALYSIS BY SPECTROPHOTOMETER*:

To check for adsorption, 5ml of the un-inoculated and inoculated broth was centrifuged at 10,000rpm for 10 minutes. The supernatant was used for spectrum analysis.

Spectrum analysis:

Reagent solution preparation**:**

Reagent was prepared by mixing 25 ml of Glycerol, 15 ml of Concentrated Hcl and 50 ml of 95% of Isopropyl alcohol. 37.5grams of Sodium chloride was added to the above mixture. The volume was made up to 250 ml of distilled water.

Standard solution preparation:

Standard solution was prepared by dissolving 1.479 grams of sodium sulphate in 100 ml of distilled water and was used for quantification of barium adsorption.

Quantification of Barium was conducted as per IS: 3025 (part 24) 2003 and has been tabulated in table 1

Table 1 :

	Standard 1	Standard 2	Standard 3	Standard 4	Sample	Blank
NaSo4	1 ml	2 ml	3 ml	4 ml	2ml(of inoculated media)	2ml.Of water
Conditioning reagent	5 ml	5 ml	5 ml	5 ml	5 ml	5 ml
Bacl2	1 ml	1 ml	1 ml	1 ml		
Water	3 ml	2 ml	1 ml		3 ml	3 ml

K. DIFFERENT CONCENTRATION OF BARIUM:

Different concentration of Barium Sulphate (50ppm, 100ppm, 150ppm and 200ppm) were incorporated in the MSM medium to check for the extant adsorption by the bacterial isolates..

L. EFFECT OF TEMPERATURE:

The bacterial cultures inoculated in MSM broth and were incubated at different temperatures (20^0C, 25ºC, 37^0C and 55^0C). After 96 hours of incubation the broths were checked for adsorption at 420nm against a suitable blank.

M. EFFECT OF pH:

The bacterial cultures inoculated in MSM broth and were incubated at different pH (2, 3, 4, 5,6,7,8 and 9). After 96hrs of incubation the broths were checked for adsorption at 420nm against a suitable blank.

N. EFFECT OF CARBON SOURCE ON BARIUM ADSORPTION:

Adsorption of Barium was observed by the addition of different carbon sources like sucrose, starch, lactose, dextrose and cellulose to MSM media. After 96hours of incubation the broths were checked for adsorption at 420nm against a suitable blank.

O. EFFECT OF DIFFERENT CONCENTRATION OF CELLULOSE:

Varied concentrations of Cellulose (1%, 2%, 3%, 4%, and 5%) were supplemented to the MSM media. The *Pseudomonas Species* were inoculated and incubated for 96hrs. After 96hours of incubation the broths were checked adsorption at 420nm against a suitable blank.

P. EFFECT OF NITROGEN SOURCE ON BARIUM ADSORPTION:

During this study, organism were supplemented with additional nitrogen sources. Different nitrogen sources like $NaNO_3$, KNO_3, Tryptone, Peptone and Ammonium Nitrate were added to MSM media and organisms were inoculated. After 96hours of incubation the broths were checked for adsorption at 420nm against a suitable blank.

Q. EFFECT OF DIFFERENT CONCENTRATION OF AMMONIUM NITRATE:

Varied concentrations of Ammonium Nitrate (1%, 2%, 3%, 4%, and 5%) were supplemented to the MSM media. The *Pseudomonas Species* were inoculated and incubated for 96hrs. After 96hours of incubation the broths were checked adsorption at 420nm against a suitable blank.

R. MUTATIONS:

Two types of mutations were conducted.

S. Physical mutation:

To perform physical mutation, nutrient agar medium was prepared and was poured in five petriplates. A loop full of culture in 0.5 ml of sterile distilled water was suspended. 100µl of the bacterial suspension was inoculated and spread plate technique was carried out using a sterile swab. Four petriplates were subjected to U.V radiation for 5, 10, 15 and 20 minutes. Control (without UV exposure) along with the inoculated petriplates was incubated at 37^0C for 24hrs. After incubation, the bacterial cultures were inoculated in MSM broth containing barium and were incubated for 96hrs at 40^0C. Adsorption at 420nm was checked against the blank, after incubation.

T. CHEMICAL MUTATION:

2µl, 4 µl, 6 µl, 8 µl and 10 µl of 10% EtBr were added to nutrient broth medium. *Pseudomonas species* was inoculated to the broths and incubated at 37^oC for 24hrs. Following the incubation the bacterial culture was inoculated to the MSM media containing barium and was kept for incubation at 96hrs at 40^oC. Adsorption at 420 nm was checked against the blank, after incubation.

III. RESULTS:

A.Isolation and identification:

Five soil samples and one water sample were collected from Mangampeta minning area. The soil samples were subjected to isolate the organism by serial dilution using MSM media along with barium. After incubation at 37°C for 48 hrs found out clear zones surround the organism.

Thirty isolates were obtained from five soil samples and one water sample. All the isolates were screened for barium adsorption. Isolate R1exhibited maximum adsorption and hence chosen for further studies.

Isolate was identified as *Pseudomonas* species based on Gram staining, biochemical tests and 16s RNA sequencing compared with the phylogeny.

B. Growth curve and adsorption:

Logarithmic growth phase of the isolate, *Pseudomonas sp.* (Fig1).Was exhibited at 0.14 O.D against 600nm after 96hr incubation. From the spectrum analysis, the adsorption maxima was at 420 nm. The rate of adsorption increased with the incubation period reaching maximum 0.622 O.D at 420 nm as per IS:3025 (part24) 2003. The Barium adsorption of different concentration of Barium (50, 100, 150 and 200ppm) exhibited varied percentage of adsorption from 10% to 44%. Maximum Adsorption was recorded in 100ppm with 44%.(Fig.2).

C. Effect of temperature:

Incubation temperature plays a role in biosorption and an increase in temperature is found to reduce biosorption capacity of the biomass (Pavasant *et al..*, 2006) Isolate, *Pseudomonas Species*, was able to adsorb the barium over a wide range of incubation temperature (Fig3); adsorption percentage varied from 8 to 67%. The highest was at incubation 40 □C.

D. Effect of pH:

Both the cell surface binding sites and the availability of metal and solution are related to pH. (Tarangini and satpathy,2009).

From the data (Fig4), the isolate, *Pseudomonas Species*, Maximum adsorption of 58.18% occurred at pH 4.

E. Effect of different carbon sources:

Microorganisms acquired nutrients ,electrons and energy from their environments to support growth bio degradation of organic substrates provide microorganisms with energy and building materials that are used for growth of new cells ,cell maintenance and co-metabolism of other less degradable substances (Annadurai *et al.*,2008).In general, microorganisms grow mostly in medium supplemented with additional substrates (Harder *et a.l*,1982).Hence growth could be manipulated by addition of two or more nutrients simultaneously(Rutgers *et al.*,1990;Egli,1991;Egli;1995). In the present study, maximum adsorption was obtained by metabolizing cellulose as the carbon source among dextrose, lactose, sucrose and starch (Fig 5).Adsorption percentage ranged between35.65% to 64.34%. Medium supplemented with cellulose exhibited 64.34% respectively. The concentration of cellulose in the medium, 10g/L, was optimum for barium adsorption among 20g/L, 30g/L, 40g/L and 50g/L (Fig 6).

F. Effect of different nitrogen sources:

Nitrogen is an important nutrient factor for the growth of the microorganism (Pattanayak *et a.l*,2014). The present study data indicated there was influence of the nitrogen sources in the ability of the organism to adsorb barium,maximum adsorption was recorded in the medium supplemented with Ammonium nitrate. Adsorption percentage was 60.24% in the medium supplemented with Ammonium nitrate and 48.44% with Sodium nitrate. The adsorption was decreased to as low as 3.72% ,7.453% and6.211% with the media supplemented with tryptone, potassium nitrate and peptone respectively(Fig.7). The concentration of Ammonium nitrate in the medium, 10g/L, was optimum for barium adsorption among 20g/L, 30g/L, 40g/L and 50g/L (Fig 8).

G. Physical mutation:

Random mutagenesis induces mutations in the organisms that improve biodegradation ability of the microbial strain (Gopinath *et al*., 2009). The mutants adsorb the barium on different exposure periods to U.V wavelength (320nm to 340nm) ranging from 17% to 64%. The adsorption was maximum at ten minutes exposure to U.V mutagen (Fig9) .

H. Chemical mutation:

Decreased percentage of adsorption was recorded with increased in the concentration of Etbr. Range of adsorption 18.75% to 59.821% by the mutants have been recorded on exposure to with varied concentration of EtBr. 59.82% of adsorption was exhibited in the medium containing 10µL of EtBr (Fig 10).

IV. CONCLUSION:

In this study, Barium adsorption bacteria was isolated from heavy metal contaminated environments and the applicability of their adsorption was evaluated at laboratory scale. The optimum conditions for the growth and Barium adsorption was determined. The optimal temperature and pH for the isolate R1 was 40˚C and 4 respectively. The influence of carbon and nitrogen supplementation on the adsorption capacity of the R1 isolate was examined. Cellulose and ammonium nitrate presented a greater influence on the adsorption property of the isolate. Mutational studies had an impact on the adsorption of Barium by the isolate. The isolate didn't lose its Barium adsorption property on application of mutagens. The highest adsorption was recorded in ten minutes of exposure to U.V and 10µL of EtBr. Hence from this experimental study, the isolate *Pseudomonas Species* can be used in bioremediation in Barium polluted areas.

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Figures:

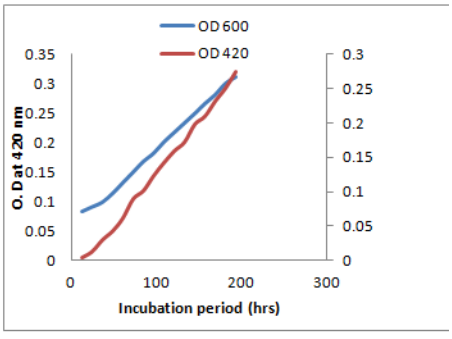

Fig. 1: Growth curve

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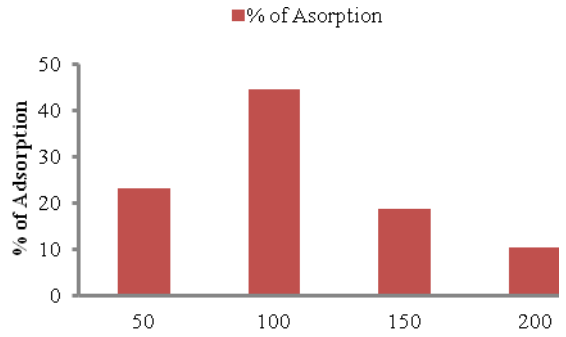

Fig 2: Different concentration of Barium (ppm)

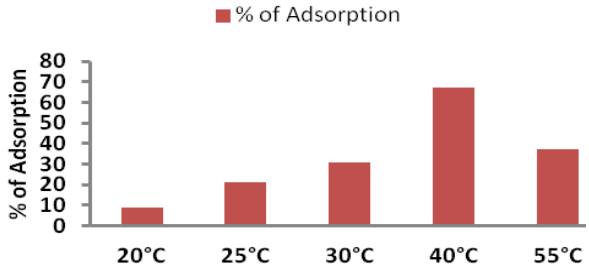

Fig 3:Temperature

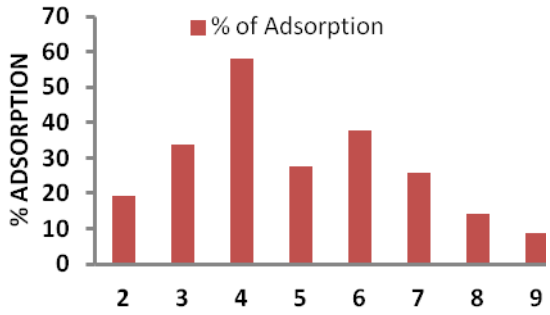

Fig 4:PH

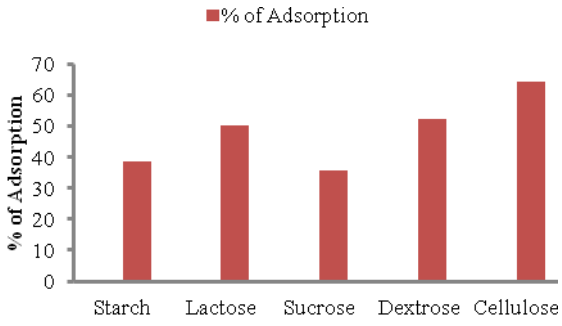

Fig5:CarbonSource(10G/l)

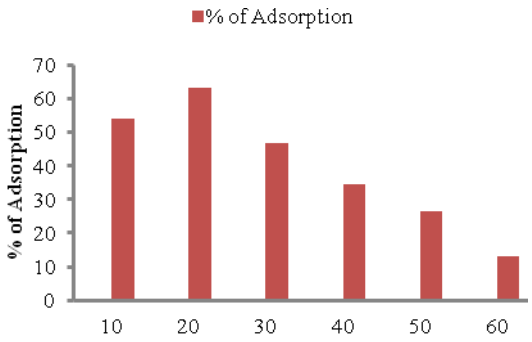

Fig 6:Different Concentration of Cellulose(g/l)

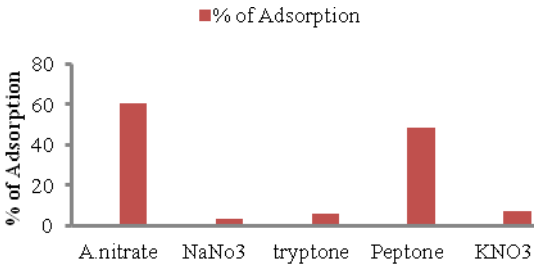

Fig 7:Nitrogen Source(10g/l)

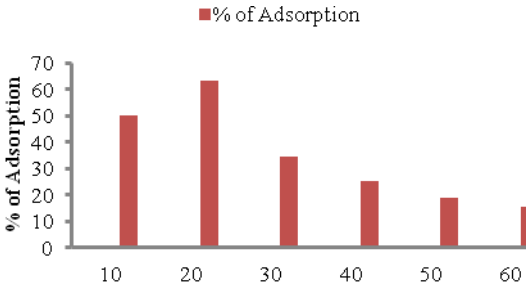

Fig 8:Different Concentration of Ammonium Nitrate(g/l)

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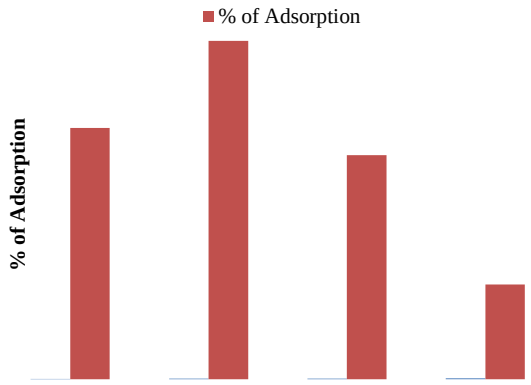

Fig 9: U.V exposure(minutes)

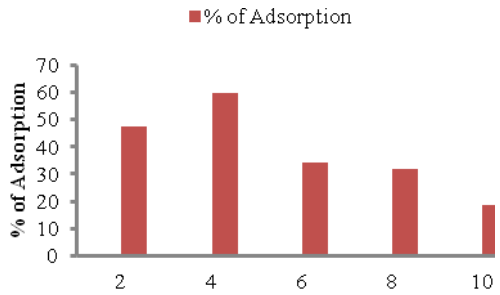

Fig 10: Volume of EtBr(mul)

Fig 11:Phylogeny