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Biosorption of Heavy Metal Using Bacteria Strain and Its Optimization

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Abstract: Soil and water pollution is becoming one of major burden in modern Indian society due to industrialization. Though there are many methods to remove the heavy metal from soil and water pollution but biosorption is one of the best scientific methods to remove heavy metal from water sample by using biomolecules and bacteria. Biosorbent have the ability to bind the heavy metal and therefore can remove from polluted water. Currently, we have taken the water sample from Ballendur Lake, Bangalore. Which is highly polluted due to industries besides this lake. This sample of water was serially diluted to 10-7. 10-4 and 10-5 diluted sample was allowed to stand in Tryptone Glucose Extract agar media mixed with the different concentrations of lead acetate for 24 hours. Microflora growth was observed. Then we cultured in different temperature, pH and different age of culture media. Finally, we did the biochemical test to identify the bacteria isolate and we found till genus level, it could be either Streptococcus sp. or Enterococcus sp.

Keywords:

INTRODUCTION

The Human civilization has developed ample resources for its comfort, one out of which is "Industrialization". It has proved a boon as well as a curse to the society. The release of industrial waste in the water bodies has not only affected the human race but has also degenerated the marine life. Industrial effluents contain heavy metals like cadmium, mercury, lead etc. which are poisonous in nature and have a long half-life.

Physico-chemical methods, such as chemical oxidation, chemical precipitation, chemical reduction, electrochemical treatment, filtration, and evaporative recovery and membrane technologies have been widely used for removing heavy metal ions from industrial wastewater. However most of these methods are ineffective and expensive, it becomes very difficult to remove when the heavy metal ions are in the solution containing in the order of 1-100 mg range.(Volesky, 1990a; Volesky, 1990b)

Microorganism uptake heavy metal from polluted water and soil through actively (bioaccumulation) or passively (biosorption). (Shumate and Strandberg, 1985; Andres et al.) Since living system often requires the addition of nutrient and hence increase biological oxygen demand and chemical demand in the effluent, studies for large scale shows that biosorption process is more effective than bioaccumulation process. (Brown and Lester1982; Ajmal et al.)

The current project is mainly focused on lead Biosorption as it is the main stream contaminants found in the Indian water bodies as per ministry of water resources. Lead has a reputation of cumulative toxic which affects the multiple body functions such as kidney, lung, brain, reproductive organs and much more. It mainly affects the children as they have better absorption than adults as high blood brain barrier permeability.

Solution to this kind of pollution has been paved by "Biotechnology" and is in the form of "Biosorption". Biosorption is cheap and effective biological process, in which the cellular structure of microorganism concentrates and binds the contaminants such as heavy metal and toxic substance.

Biosorption occurs in the microorganism, mainly by two ways, either metabolically by the living organism or it's the chemical functional groups which help the dead or living organism in the metal uptake. Metal-sequestering properties of non-viable biomass provide a basis for a new approach to removing heavy metals when they occur at low concentrations (Volesky, 2001). When metals are dissolved in huge Volume at relatively low concentrations, the methods become generally ineffective (e.g., less than 100 mg/L) (Patterson, 1985). The research is for efficient and particularly cost-effective remedies (Volesky, 2001; Blöcher et al., 2003).It was only in the 1990s that a new scientific area developed that could help to remove metals: sorption by employing biomasses (Biosorbents).

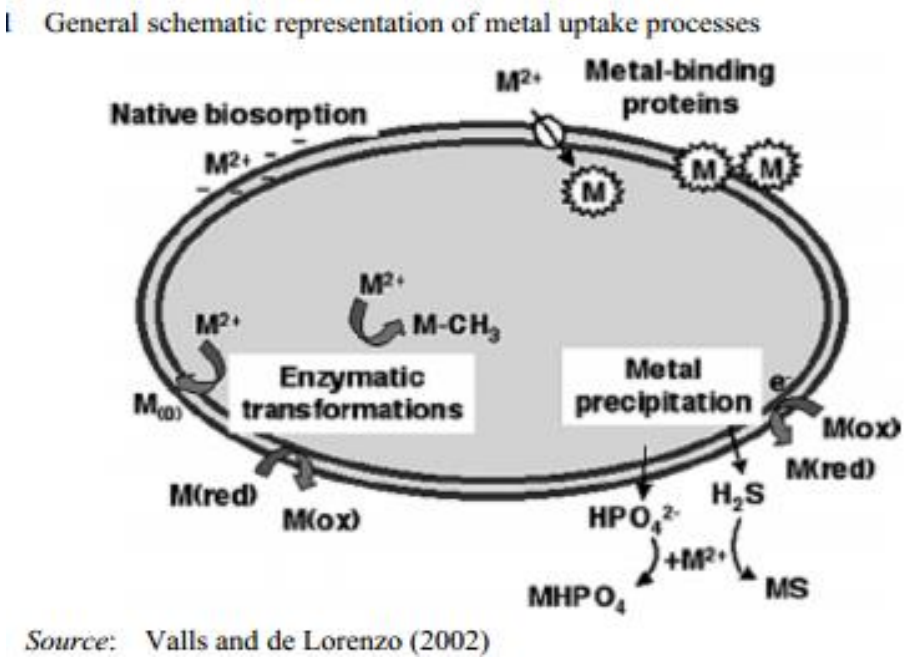

Figure 1: Schematic Representation of metal uptake process

The real challenge in the project underlie, was to isolate the organism which can bioabsorb the desired metal. To attain this, the water sample was collected from Bellandur Lake which is considered to be a highly polluted lake of Bangalore. This sample of water was serially diluted to 10-7. 10-4 and 10-5 diluted sample were allowed to stand in Tryptone Glucose Extract Agar media mixed with the different concentrations of lead acetate for 24 hours. Microflora growth was observed. Those with luxurious were selected and individually multiplied. Further, they were grown in broth to determine their efficiency.

Since, Lead has been the target since the beginning, so the amount of lead bioabsorb by the microflora has been determined by the technique called atomic absorption spectrometry. It is an analytical technique that measures the concentrations of an element. Later, microflora was tested on different parameters like temperature, pH and culture age as they can be helpful in enhancing the efficiency.

Water is the limited resource, which is depleting as the day pass on. Techniques like biosorption are cheaper and quite effective and thus should be adopted, they can lest treat water which can be consumed for general purpose.

AIMS AND OBJECTIVE

Aim: to isolate and characterisation of lead resistant bacteria and study for the extent of biosorption by the microorganism.

Objective:

- Collection of soil sample contaminated with heavy metals
- Screening for lead resistant bacteria.
- Effect of pH on biosorption of lead.
- Effect of temperature on biosorption of lead.
- Effect of culture age on biosorption of lead.
- Identification of isolated by the biochemical test.

MATERIALS AND METHODS

1. Collection of soil sample

The soil sample was collected from different location of Bellandur Lake.The Lake is situated in the southeast of the city of Bangalore and is the largest lake in the city. It is a part of Bellandur drainage system that drains the southern and the southeastern parts of the city. The lake is a receptor from three chains of lakes upstream and has a catchment area of about 148 square

kilometers (37,000 acres). Water from this lake flows further east to the Varthur Lake, from where it flows down the plateau and eventually into the Pinakani river basin. It is currently highly polluted with sewage.

For the collection of the sample, the soil was collected from different locations around the area surroundings the Bellandur lake and that soil was used for analysis and identification of lead resistant bacteria. The map of the lake is given in the following figure 2.

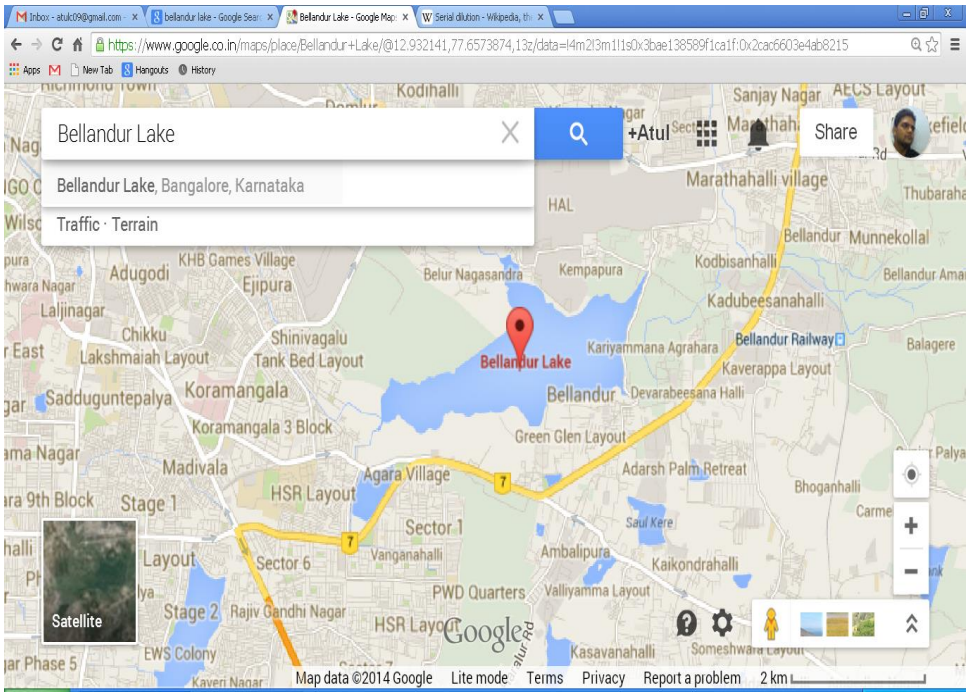

Figure 2: Map of Bellandur Lake

2. ISOLATION OF LEAD RESISTANCE BACTERIA

A. Serial dilution

Serial dilution is a technique to reduce the load or quantity of the organism.1gm of soil sample was taken and mixed in a 9 ml of double distilled water and dissolved well. Serially from the previously dissolved sample 1ml of the sample was transferred to a tube containing 9ml of double distilled water. Similarly, the dilutions are made till $10^{-7.}$

B. Media Preparation

For the growth of microorganism **Tryptone Glucose Extract Agar Media (TGEAM)** media was used. This medium is recommended for the plate count of the aerobic organism in milk, dairy products, Soil, water etc. It's composition as follows;

Table 5: Tryptone Glucose Extract Agar Media Composition

Component	**gm/l**
Glucose	1gm
Beef Extract	3gm
Tryptone	5gm
Agar	15gm
Ph	6.7

C. Metal Stock Solution

Lead (II) acetate (Pb $(CH_3COO)_2$), also known as lead acetate, lead diacetate, plumbous acetate. It is a white crystalline chemical compound with a sweetish taste. It is made by treating lead (II) oxide with acetic acid. Like other lead compounds, it is toxic.

Lead acetate is soluble in water and glycerin. With water, it forms the trihydrate, $Pb(CH_3COO)_2 \cdot 3H_2O$, a colorless or white efflorescent monoclinic crystalline substance.10 gm of lead acetate was dissolved in 50ml of distilled water to get a final concentration of 200 mg/ml.

D. Incubation

The TGEA media was prepared and was mixed with the different concentrations of lead acetate i.e. 50 mg, 100 mg, 150 mg, 200 mg, 300 mg, 400 mg, 500 mg for 50ml. The media was later autoclaved to maintain the sterile conditions. Later the media was allowed to solidify. The serially diluted sample i.e. 10^{-4} and 10^{-5} were selected and 100 µl samples were spread over the TGEAM media containing lead acetate. It was further incubated at 37 ºC for 24 hours.

3. Selection of Lead Resistant Bacteria

After 24hrs, good growth was observed at 50 mg and 100 mg concentrations. Out of many colonies, four strains which showed the very good growth were selected and were further sub-cultured to maintain the pure cultures on agar slants. The strains were labelled as Pb3, Pb4, Pb6, and Pb10.Pb3, Pb4 andPb6 showed luxurious growth at 50 mg and Pb6 showed luxurious growth even at 100 mg/ml concentration.

4. Lead Biosorption studies using Atomic Absorption Studies

Atomic absorption spectrometry (AAS) is an analytical technique that measures the concentrations of elements. Atomic absorption is so sensitive that it can measure down to parts per billion of a gram (µg dm^{-3}) in a sample. The technique makes use of the wavelengths of light specifically absorbed by an element. They correspond to the energies needed to promote electrons from one energy level to another higher energy level.

5. Preparation of Broth for Biosorption studies

For Biosorption studies, the selected strains i.e. Pb3, Pb4, Pb6, and Pb10 were inoculated into TGE broth containing 50 mg and 100 mg lead acetate and the flask were incubated on a rotary shaker for 24 hours at120 rpm.

After 24 hours, the growth was observed in all the flasks. For Atomic Absorption Analysis, the culture was centrifuged at 10,000 rpm for 10 minutes, where the supernatant was discarded and the bacterial pellet was washed with 1N NaOH thrice. After NaOH treatment, the pellet was treated with 67% HNO_3 and 30% H_2O_2 solution (67 ml HNO_3 + 30ml H_2O_2 + 3ml H_2O). This process degrades the cell wall of the bacteria and helps in dissolving the metal. Finally, the sample was sent to Environmental Health and Safety Research & Development Centre (EHSR&D), Bangalore to determine the amount of biosorption by the bacterial isolates.

6. Effect of pH on Biosorption of Lead by Pb6 isolate

The Results from Environmental Health and Safety Research & Development Centre (EHSR&D) indicated the highest Biosorption by the strain Pb6. Thus strain Pb6 was selected for the further studies.

To study the effect of pH on Biosorption, TGE Broth media was prepared with varying pH concentration i.e. 5.7, 6.2, 7.2, 7.7. The media were inoculated with isolate PB6 and were incubated on a rotary shaker at 120 rpm and the samples were taken at different time intervals i.e. 24, 48, 72 Hours to study the effect of different pH on biosorption at different time intervals.

7. Effect of Temperature on Biosorption of Lead by Strain Pb

To study the effect of Temperature on Biosorption, TGE Broth media was prepared and inoculated with isolate PB6 and was incubated at different temperatures i.e. 33ºC, 35ºC, 37ºC and 39ºC on rotary shaker incubator at 120 rpm and the samples were taken at different time intervals i.e. 24, 48, 72 hours to study the amount of biosorption.

8. Effect of Culture age on Biosorption of Lead by Strain Pb6

To study the effect of Culture age on Biosorption, TGE Broth media was prepared and inoculated with isolates PB6 and was incubated on a rotary shaker at 120 rpm. The samples were taken out at different time intervals i.e. 24, 48, 72 Hours to study the amount of biosorption.

9. Identification of bacterial isolate by biochemical tests

Biochemical tests which are carried out to identify the bacterial isolate were; Gram stain, Acid fast stain, Motility test, MR-VP test, Indole test, Starch hydrolysis test, Citrate utilization test, Catalase test, Oxidase test, Triple sugar iron agar test and Gelatin liquefaction test.

RESULT AND DISCUSSION

1. Collection of soil sample

The soil sample was collected from the Bellandur Lake which is highly polluted by heavy metals like zinc, lead, cadmium, etc. The soil sample was collected from different locations around the area surrounding the Bellandur Lake. The soil sample was used for serial dilution to isolate lead resistant bacteria.

2. Selection of lead resistant bacteria

Different concentrations of the serially diluted sample were inoculated on TGEA media containing a different concentration of lead **(50 mg, 100 mg, 150 mg, 200 mg, 300 mg, 400 mg and 500 mg/50 ml).** 10^{-4} and 10^{-5} concentrations were selected for inoculation and found that good growth was observed on plates containing 50mg and 100mg of lead. Out of many colonies, four

strains which showed the very good growth were selected and were further sub-cultured to maintain the pure cultures on agar slants. The strains were lebelled as Pb3, Pb4, Pb6, and Pb10. Pb3, Pb4 andPb6 showed luxurious growth at 50 mg and Pb6 showed luxurious growth even at 100 mg/ml concentration.

3. Lead biosorption studies using atomic absorption spectroscopy

The atomic absorption spectroscopy analysis revealed that strain PB6 showed the highest biosorption of 252.22 mg/l of lead compared to the three isolates (Table 6). Hence, the PB6 isolate was selected for further studies to increase the efficiency of biosorption using parameters such as the effect of different pH, different temperature, and different culture age.

Table 6: Lead biosorption by different bacterial isolates

Strain No.	**Concentration** (mg)	**Absorption (**mg/l)
PB3	50	107.34
PB4	50	186.37
PB6	50	**252.22**
PB10	50	219.12

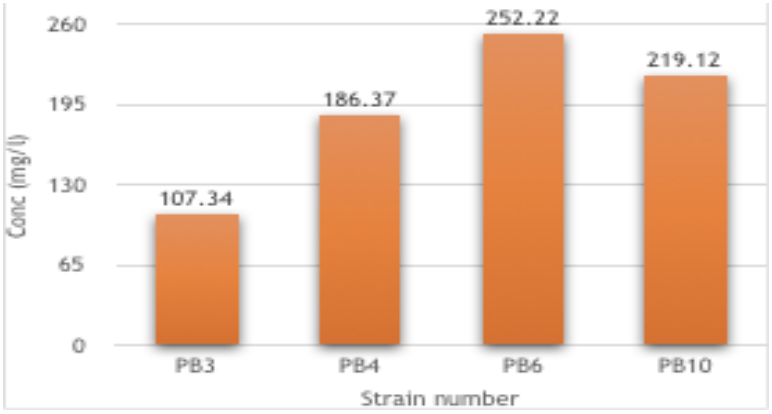

Figure 3: The graph showing biosorption efficiency of different bacterial isolates

4 **Effect of pH on biosorption of Pb by PB6 strain**

The atomic absorption spectroscopy analysis showed that at pH 5.7, after 24 hours incubation, the isolate PB6 showed maximum biosorption of 581.37l mg/l of lead as compared to other pH and incubation periods (Table 7).

Table 7: Effect of different pH on biosorption of lead by PB6 isolate

pH	**Concentration**(mg)	**absorption/Culture Age(mg/lt)**		
		24hr	**48hr**	**72hr**
5.7	50	**581.376**	263.689	126.586
6.2	50	292.266	8.1405	128.748
7.2	50	107.683	214.9605	55.6095
7.7	50	476.858	187.004	418.231

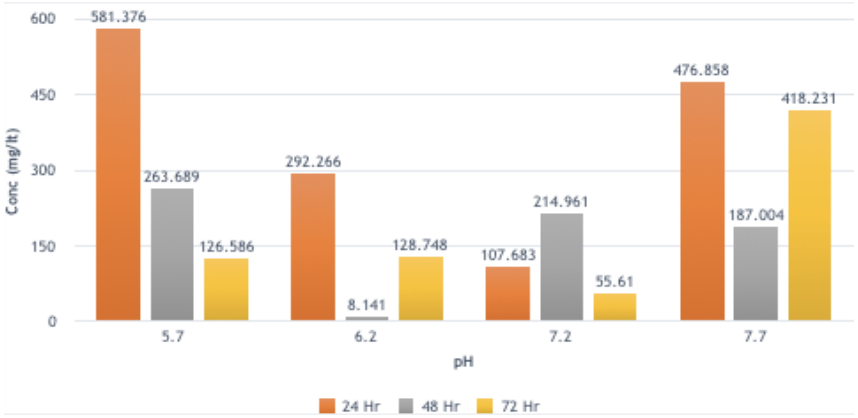

Figure 4: The graph showing effect of different pH on biosorption of lead by PB6 isolate

5. Effect of temperature on biosorption of Pb by PB 6 Isolates

The atomic absorption spectroscopy analysis showed that at 37 ^{0}C after 72 hours of incubation, isolate PB6 showed maximum biosorption of 550.16 mg/l as compared to other temperatures and incubation periods (Table 8).

Table 8: Effect of temperature on biosorption of lead by PB6 isolate

Temperature (^{o}c)	Concentration (mg)	Absorption (mg/lt)		
		24hrs	48hrs	72hrs
33	50	58.508	175.19	87.387
35	50	300.185	189.588	264.086
37	50	300.055	272.0605	**550.165**
39	50	179.1075	77.102	375.893

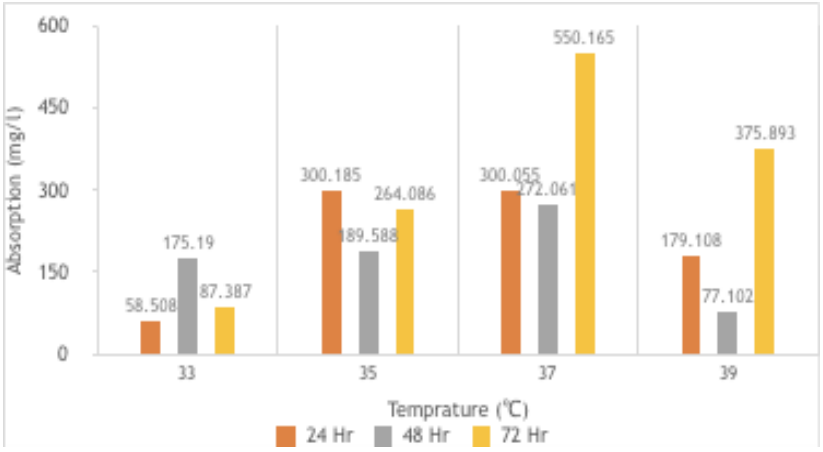

Figure 5: The graph showing effect of different temperature on biosorption of Pb by PB 6 Isolates

6. Effect of culture age on biosorption of Pb by PB 6 Isolates

The atomic absorption spectroscopy analysis showed that after 72 hours, the PB6 isolated showed maximum biosorption of 550.16 mg/l when compared to other culture age (Table 9)

Table 9: Effect of culture age on biosorption of lead by PB6 isolate.

Culture age	Concentration	Absorption(mg/lt)
24hrs	50mg	300.055
48hrs	50mg	272.0605
72hrs	50mg	**550.165**

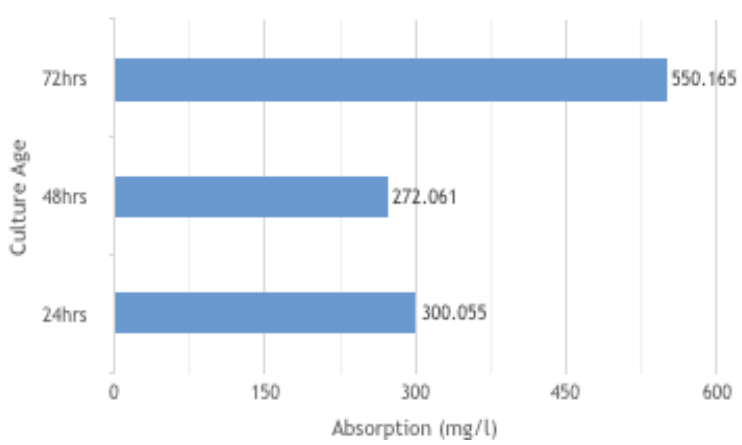

Figure 6: The graph showing effect of culture age on biosorption of Pb by PB 6 Isolates

7. Identification of strain

The results of the various biochemical test (Table 10) were compared with the "Bergey's Manual of Determinative Bacteriology" identification flow charts and available results, the PB6 bacterial isolate may belong to either Streptococcus spp. or Enterococcus spp.

Table 10: Details of biochemical tests to detect the genus of the PB6 isolate.

Sl No.	Biochemical test	Indication	Inference
1	Gram stain	purple appearance	Gram positive cocci
2	Acid fast stain	Blue colour	Non acid-fast bacteria
3	MR-VP test	Red colour	Positive
4	Indole test	Brown colour	Negative
5	Starch hydrolysis test	Clear zone of hydrolysis	Positive
6	Catalase test	No Bubbles	Negative
7	Oxidase test	Purple colour	Positive
8	Citrate utilization test	No colour change from green to blue	Negative

Identification flow charts

Bergey's Manual of Determinative Bacteriology

All of the unknowns will fall into the following groups in Bergey's Manual of Determinative Bacteriology (The pink book on the shelf in the laboratory).

GROUP 4

Description: Gram Negative, Aerobic/Microaerophilic rods, and cocci
Key differences are: pigments/fluorescent, motility, growth requirements, denitrification, morphology, and Oxidase, read Genera descriptions
Examples: *Acinetobacter, Pseudomonas, Beijerinckia, Acetobacter*

GROUP 5

Description: Facultatively Anaerobic Gram negative rods
Key differences are: growth factors, morph., gram rxn., Oxidase rxn., read Genera descriptions
Examples: Family Enterobacteriaceae and Vibrionaceae

GROUP 17

Description: Gram-Positive Cocci
Key differences are: oxygen requirements, morph., growth requirements (45°C and supplements), read Genera descriptions
Examples: *Micrococcus, Staphylococcus, Streptococcus, Enterococcus, Lactococcus, Aerococcus*

GROUP 18

Description: Endospore-Forming Gram positive rods and cocci
Key differences are: oxygen requirements, motility, and morph, Catalase **Examples:** *Bacillus, Clostridium*

GROUP 19

Description: Regular, Nonsporlating Gram positive rods **Key differences are:** morph., oxygen require, Catalase **Examples:** *Lactobacillus, Listeria*

GROUP 20

Description: Irregular, Nonsporlating Gram-positive rods
Key differences are Catalase, motility, morph., read Genera descriptions **Examples:** *Actinomyces, Corynebacterium, Arthrobacter, Propionibacterium*

GROUP 21

Description: Weakly Gram-Positive Nonsporlating Acid Fast Slender Rods
Key differences are: acid fast, growth
Examples: *Mycobacterium*

DISCUSSION

In the present study, we mainly focused on the collection of soil sample contaminated with heavy metals, screening for lead resistant bacteria, the effect of pH, and temperature and culture age on increasing efficiency of biosorption of lead and identification of isolate by the different biochemical test.

The soil sample was collected from the Bellandur Lake which is highly polluted by heavy metals like zinc, lead, cadmium, etc. The soil sample was collected from different locations around the area surrounding the Bellandur lake. The soil sample was used for serial dilution to isolate lead resistant bacteria. Out of many colonies, four strains which showed the very good growth were selected and were further sub-cultured to maintain the pure cultures on agar slants. The strains were lebelled as Pb3, Pb4, Pb6, and Pb10.The atomic absorption spectroscopy analysis revealed that strain PB6 showed the highest biosorption of 252.22 mg/l of lead compared to other three strains. Hence, PB6 was selected for further studies to increase their efficiency of biosorption using parameters such as the effect of different pH, different temperature, and different culture age etc.

The atomic absorption spectroscopy analysis showed that at pH 5.7, after 24 hours incubation, the isolate PB6 showed maximum biosorption of 581.37l mg/l of lead as compared to other pH and incubation periods. A temperature of 37 ^{0}C after 72 hours of incubation, isolate PB6 showed maximum biosorption of 550.16 as compared to other temperatures and incubation periods. With regard to the culture age, the atomic absorption spectroscopy analysis showed that 72 hours of incubation showed maximum biosorption of 550.16 mg/l.

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