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Biosorption of Heavy Metals in Petroleum Effluent using Species of *Bacillus* and *Staphylococcus*

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Abstract: Industrialization has led to introduction of heavy metals in the environmental. Heavy metals are known to persist in the environment and become a risk for organisms. Microorganisms are present in industrial effluent. They have adopted different strategies to cope up with the harmful effect of these metals. These can be metabolism dependent or independent. Once such strategy is biosorption which is binding of metal ions with metal binding proteins present on the cell wall. Biosorption is exhibited by bacteria. Different factors affects the rate of biosorption which includes temperature, pH, nature of biosorbent, surface area to volume ratio, concentration of biomass, initial metal ion concentration and metal affinity to biosorbent. Desorption agent should using carefully selected to prevent of physical parameters of the biosorbent. Biosorption of chromium and copper was analysed using atomic absorption spectrometry. Wherein, *Bacillus* species is found to be more efficient in biosorption of heavy metals than *Staphylococcus* species.

Keywords: Biosorption , Cr reduction, Chromium resistance bacteria, *Bacillus spp* and *Staphylococcus spp* , Atomic Absorption Spectrometry.

I. INTRODUCTION

Heavy metals are natural components of the Earth's crust. They cannot be degraded or destroyed. To a small extent they enter our bodies via food, drinking water and air. As trace elements, some heavy metals are essential to maintain the metabolism of the human body. However, at higher concentrations they can lead to poisoning. Heavy metal poisoning could result, for instance, from drinking-water contamination , high ambient air concentrations near emission sources, or intake via the food chain.

Heavy metals are dangerous because they tend to bioaccumulate. Bioaccumulation means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical's concentration in the environment. Compounds accumulate in living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted.

Heavy metals can enter a water supply by industrial and consumer waste, or even from acidic rain breaking down soils and releasing heavy metals into streams, lakes, rivers, and groundwater.

II. PETROLEUM REFINERY

Petroleum refinery is an industrial process plant where crude oil is transformed and refined into more useful products such as petroleum naphtha, gasoline, diesel fuel, asphalt base, heating oil, kerosene, liquefied petroleum gas, jet fuel and fuel oils. Petrochemicals feed stock like ethylene and propylene can also be produced directly by cracking crude oil without the need of using refined products of crude oil such as naphtha. Oil refineries are typically large, sprawling industrial complexes with extensive piping running throughout, carrying streams of fluids between large chemical processing units, such as distillation columns. In many ways, oil refineries use much of the technology of, and can be thought of, as types of chemical plants.

The crude oil feedstock has typically been processed by an oil production plant. There is usually an oil depot at or near an oil refinery for the storage of incoming crude oil feedstock as well as bulk liquid products.

Petroleum refineries are very large industrial complexes that involve many different processing units and auxiliary facilities such as utility units and storage tanks. Each refinery has its own unique arrangement and combination of refining processes largely determined by the refinery location, desired products and economic considerations.

An oil refinery is considered an essential part of the downstream side of the petroleum industry.

III. MATERIALS AND METHODS

A. Sample Collection Site

The effluent was collected from MLF industry Chennai. About 1 liter of effluent were collected using sterile plastic container. The collected effluent were stored at 4°C until use.



B. Isolation of Microorganism From Soil

The sample was serially diluted with sterile distilled water. About 1 ml of waste water effluent was dissolved in 100ml of sterile distilled water. The different concentration was made upto 10^{-1} to 10^{-5} . 100 μ l of each dilution was spread on to nutrient agar plates. Preparation of glycerol stocks and preservation of the microorganism isolated different colonies form different nutrient agar plates.

C. Preliminary Identification of Isolates

1) *Gram staining*: Bacterial smear were prepared, air dry and heat fixed the smear. The smear was flooded with crystal violet stain and allowed to stain for 2 minutes. The slide was rinsed with running tap water. Gram's iodine solution was added to the sample for 1 minutes. The slide was rinsed with running tap water and rinsed with decolourize for 3-5 seconds. And saffarin stain were added to the glass slide for 2 minutes. And examined under 10X magnification.

D. Biochemical Tests Performed

Biochemical tests were performed for the microorganisms isolated.

- 1) *Catalase test*: A drop of 3% hydrogen peroxide was added on a clean slide. And a speck of a colony from the agar plate was added to the sample and it was assessed for the release of bubbles.
- 2) *Oxidase test* : With the help of glass rod pick up a colony from the culture plate. A colony from an agar plate was rubbed over the oxidase reagent paper and observed for the development of purple colour with in 10 seconds indicates the test to be positive. Negative control does not show any change in colour.
- 3) *Triple sugar iron agar test* : Sterilize the inoculating loop the medium was incubated with the sample by streaking on slant and piercing into butt. The medium was incubated at 37°C for 24 hours.
- 4) *Urease test*: A small quantity of the culture was streaked on the urease agar on the slant in the test tubes. The tube was incubated at 37°C overnight.
- 5) *Indole test*: Transfer a small quantity of the pure growth on nutrient agar into the peptone water using the inoculation loop. Mix the content by gently rolling the tubes between the palms. Incubate the tube at 37°C for 24 hours. A few drops of Kovac's reagent was added.
- 6) *Citrate utilization test*: A small quantity of the pure growth on nutrient agar in the inoculation loop and streak it on the Simon's citrate agar medium on the slant in the test tube. Incubate the tube at 37°C overnight.
- 7) *Nitrate test*: The ingredient was weighed dissolved, dispensed into test tubes and autoclaved at 121°C for 15 mints. The test organism were inoculated and incubated at 37°C for 24 hours. After incubation and 1ml of reagent A and B.
- 8) *Methyl red test* : Methyl red broth was prepared and sterilized. The test organism was inoculated and incubated at 37°C for 24 hours. Methyl red reagent was added after incubation.
- 9) *Voges – Proskeur test* : Voges-proskeur broth was prepared. The test organism was inoculated and incubated at 37°C for 24 hours. After incubation 0.2 ml of VP reagent A and B was added.

E. Morphological Characterization

Colony characterization of Gram staining were performed for identification of potential isolate of bacteria. Culture characteristics of colony such as margin, size, shape, type of colony nature of colony.

F. Estimation Of Chromium Using Atomic Absorption Spectrometry

In atomic spectroscopy the sample normally is found in one of two forms : solid or liquid. The liquid phase seems to be the easiest form in which to handle the sample, but some requirement for filtration is required. However, the inherent lack of sensitivity of many spectroscopic techniques and the need to carry out determinations at lower and lower levels means that invariably some form of pre-concentrations is required. If the sample is in a solid form, the normal requirement is to convert it into the liquid form although it is possible to analyse solids directly by using atomic spectroscopy, but this is not the preferred approach. The principal objectives of sample preparation for residue analysis are dissolution of the analyse in a suitable solvent, isolation of the analyse of interest from as many interfering compounds as possible, and pre-concentration.

G. Sample Procedure for AAS

1) For determination of growth of isolated bacteria, conical flasks containing 5ml nutrient broth were taken in three sets, autoclaved and inoculate with overnight culture the isolate. And the flasks were incubated at 30°C for 24 hours in shaker.

- 2) The isolated bacterial culture were inoculated in nutrient broth along with the petroleum effluent sample in 250ml conical flask and incubate for 24,48,72 hours at 36°C in a shaker at 440 rpm.
- 3) After 24 hours 10ml of culture were measured in the sterile centrifuge tubes.
- 4) And the tubes were centrifuged at 5000 rpm for 10 minutes. After it gets over , separate the supernatant and pellet in each sterile tubes.
- 5) The steps were followed for 48 and 72 hours. And the sample were absorbed in AAS.

IV. RESULTS

The petroleum effluent sample were collected in industrial and the effluent was in pale yellow and odourless and the turbidity was clearance and the pH range was 5 and maintained under 26°C.

A. Biochemical Characterization of Isolates

Biochemical test plays a vital role in identifying and characterizing the microbial species. From the characterization of the 3 isolates obtained from the soil sample shows the isolate1 was found to be *staphylococcus* and isolate 2 and 3 was found to be *Bacillus* species from the different biochemical tests.

Table 1:Biochemical results:

TEST NAME	ISOLATE 1	ISOLATE 2	ISOLATE 3
GRAM STAINING	NEGATIVE	POSITIVE	POSITIVE
CATALASE	POSITIVE	POSITIVE	POSITIVE
OXIDASE	POSITIVE	POSITIVE	POSITIVE
CITRATE	POSITIVE	POSITIVE	POSITIVE
UREASE	POSITIVE	POSITIVE	POSITIVE
INDOLE	NEGATIVE	NEGATIVE	NEGATIVE
METHYL RED	NEGATIVE	NEGATIVE	NEGATIVE
VOGES-PROSKAUER	NEGATIVE	NEGATIVE	NEGATIVE
NITRATE	POSITIVE	NEGATIVE	POSITIVE
TSI	POSITIVE	POSITIVE	POSITIVE

B. Result for AAS

The AAS results were analyzed that the chromium level has reduced in 72 hours analysis before and after culture. In 72 hours analysis *Bacillus* species has an high yield than *staphylococcus* species.

Fig 1 Histogram Showing Chromium Biosorption In 24hours

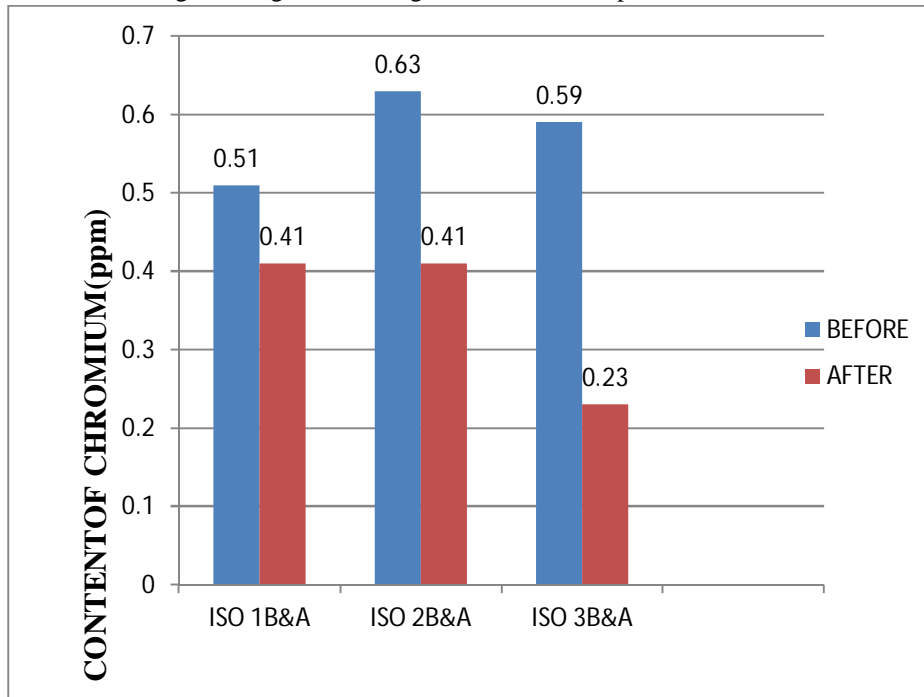


Fig 2: Histogram Showing Chromium Biosorption In 48hours

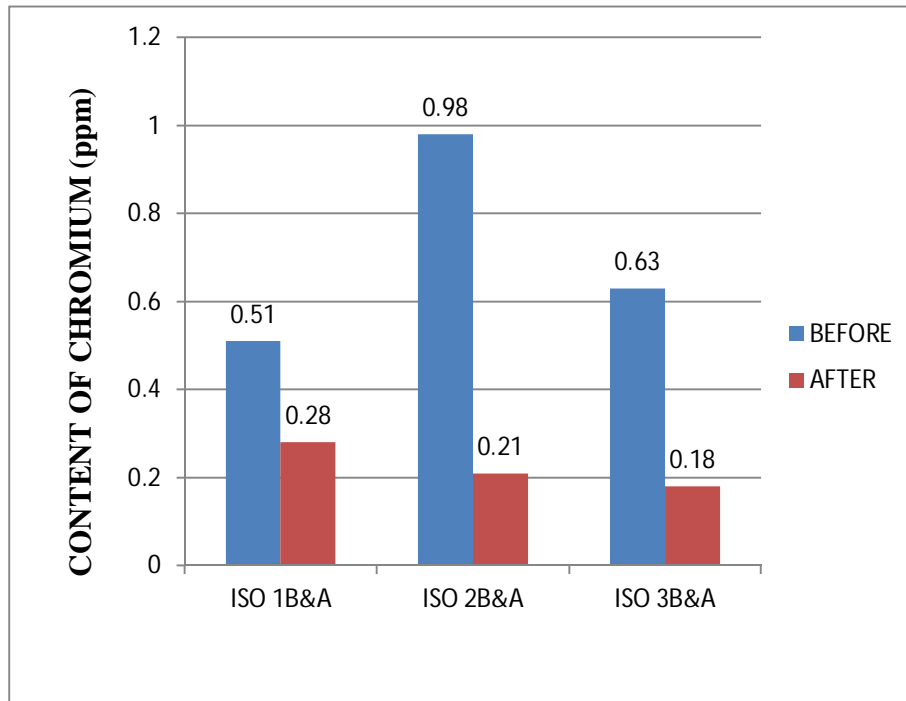
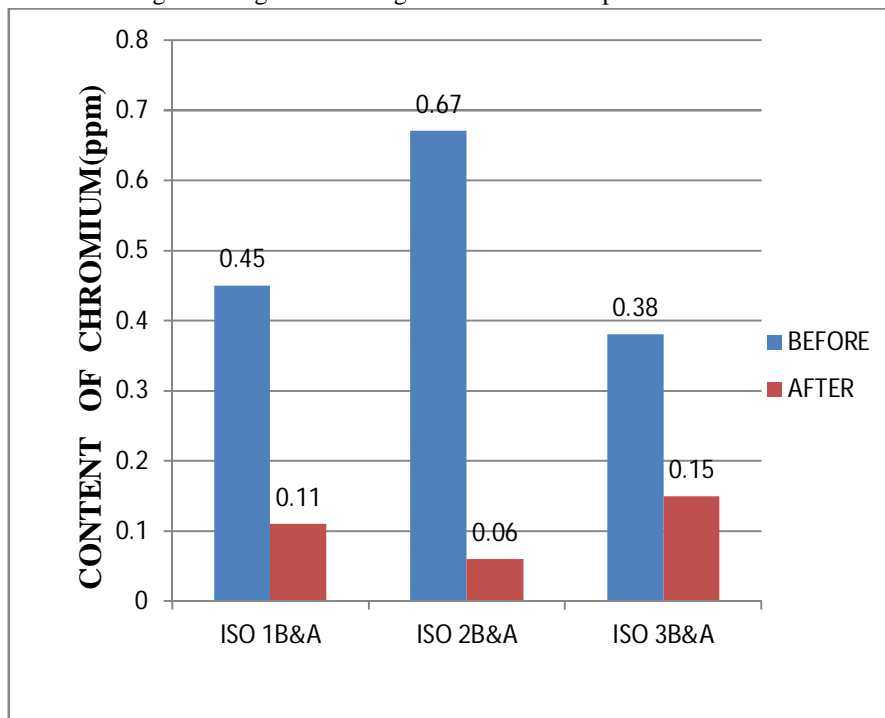


Fig 3: Histogram Showing Chromium Biosorption In 72hours



The AAS results were analyzed that the copper level has reduced in 72 hours analysis before and after culture. In 72 hours analysis *Bacillus* species has an high yield than *staphylococcus* species.

Fig 4: Histogram Showing Copper Biosorption In 24hour

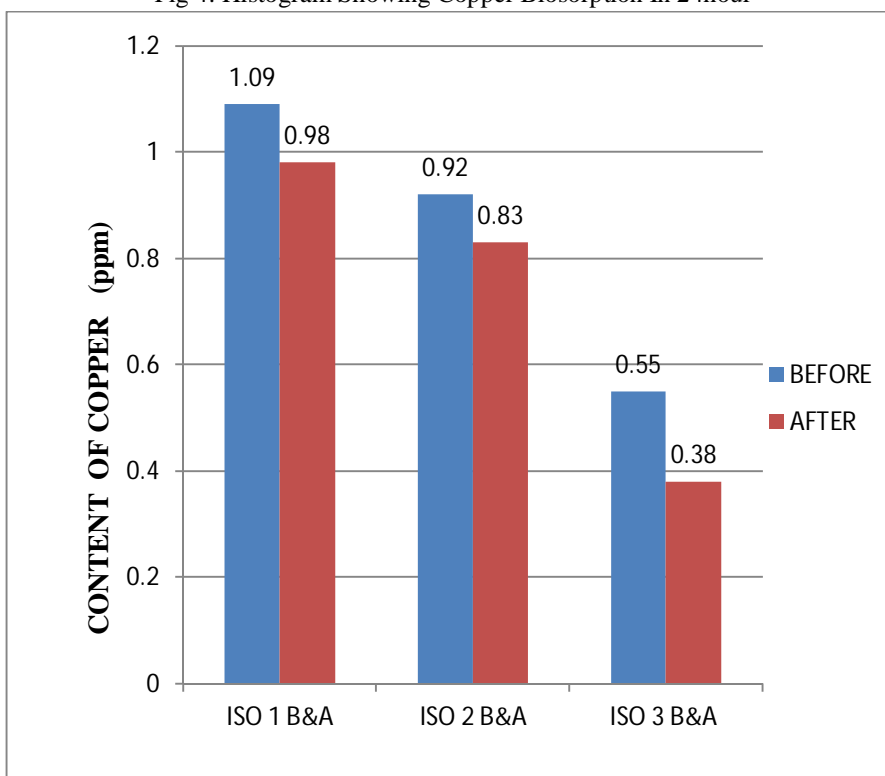


Fig 5: Histogram Showing Copper Biosorption In 48 Hours

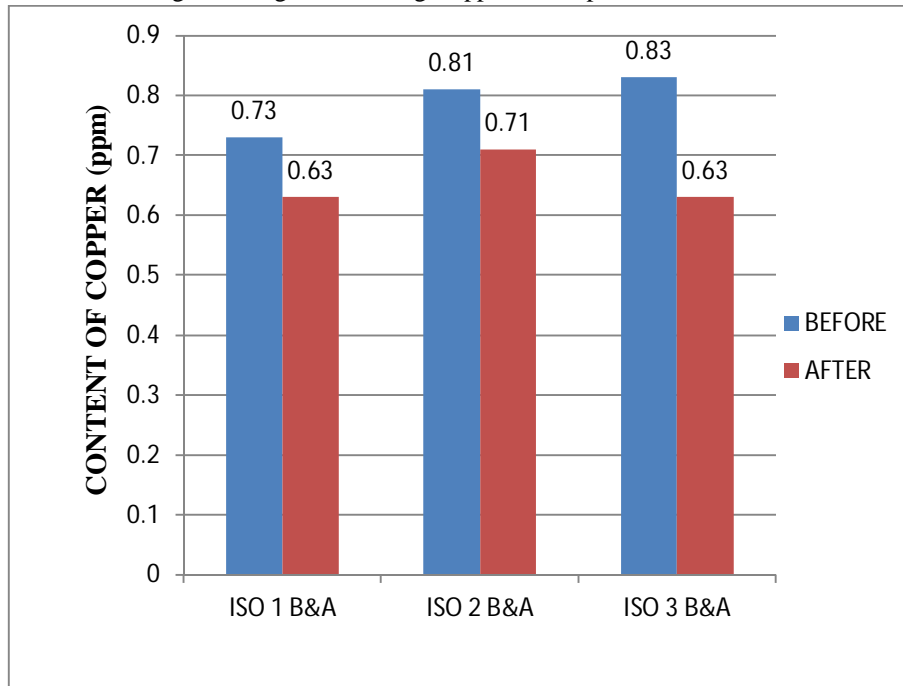
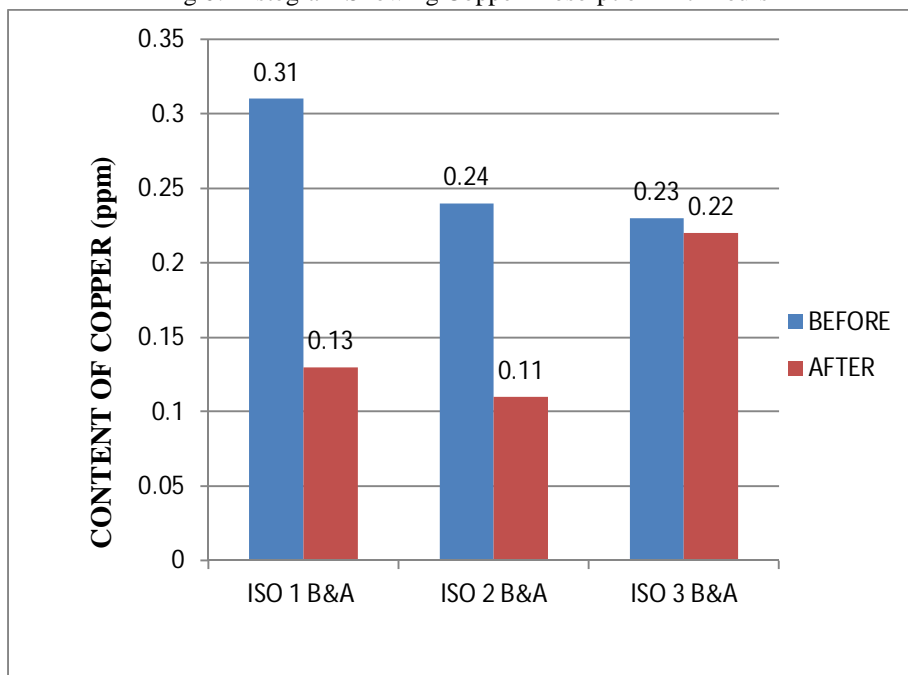


Fig 6: Histogram Showing Copper Biosorption In 72hours



V. DISCUSSION

In general, potential microorganisms especially bacterial species can remove heavy metals from solution by biosorption. A variety of mechanisms exist for the removal of heavy metals from aqueous solution by bacteria and higher plants. The bacterial species were isolate from the soil that found to be *Bacillus* species and *Staphylococcus* species. *Bacillus* reduce chromium than *Staphylococcus* And the petroleum effluent has heavy metals which are very toxic to the environmental.

The bacterial species high yield of reduction the heavy metals in petroleum effluent.



Biosorption largely involves physical adsorption followed by chemical bondage. Chromium resistant bacteria have been isolated from petroleum effluent. During the present investigation *Bacillus spp* and *Staphylococcus spp* both were found to be highly resistance to chromium.

Bacillus spp showed maximum resistance against chromium heavy metals.

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