RESEARCH ARTICLE

BIODEGRADATION OF HEAVY METAL CONTAMINATED EFFLUENT USING OPTIMIZED ACTIVATED MICROORGANISM.

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Manuscript Info	*Abstract*
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	Heavy metal pollution represents an important environmental problem due to the toxic effects of metals and their invasion into the food chain leads to the serious ecological and health problems. Metal remediation through common physiochemical techniques is expensive and unsuitable in treating large contaminated area effectively. It employs the microorganisms capable of degrading toxic contaminants or have the ability to accumulate it in their cells. Bioremediation of heavy metal is an effective method of removing metals from a polluted area using organisms. The aim of this work is to study the ability of microorganisms in this bioremediation process, since microbial metal bioremediation is an effective approach for the elimination of heavy metals due to its low cost, high efficiency and eco-friendly nature. *Bacillus cereus* shows effective degradation of chromium than *Bacillus thuringienesis*at optimized condition.

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Introduction:-

More research has been conducted on heavy metal contamination in soils from various anthropogenic sources such as industrial wastes, automobile pollution, mining activity and agricultural practices etc. Heavy metal pollution such as nickel, chromium, zinc, cadmium, cobalt, mercury etc. are an major environmental problems owing to the toxic effects of metals and their incursion into the food chain leads to the grim environmental health problems such as they cause damage in nerves ,liver and bones , and also block functional groups of vital enzymes(Moore *et.al*.,1990). Among 65groups of heavy metals ,they are classified into criteria such as their cationic-hydroxide formation, specific gravity ,complex formation, hard-soft acids, bases and more recently association with eutrophication and environmental toxicity. The toxicity of metal ion was due to their capability to bind with protein molecules and prevent replication of DNA and thus subsequent cell division(Kar*et.al*.,1992) .Bioremediation is defined as the use of biological treatment systems to destroy or reduce the concentration of hazardous wastes from contaminated sites. Compared to other methods, bioremediation is a more promising and less expensive way for cleaning up contaminated soil and water. In bioremediation process , microorganisms use the contaminants as nutrient or energy sources. Bioremediation uses biological agents, mainly microorganisms, e.g :*Yeast, fungi* or *bacteria* to clean up contaminated soil and water(Kumar *et.al*., 2011). Potentially any microorganism or cell fraction that exposes negatively charged groups on its surface should have an affinity for metal cations (Ehrlich *et.al*.,1986). Biosorption also lacks specificity in metal binding(Baudet*et.al.,*1988) , the metals diffused into the cells during detoxification get bound to intracellular proteins or chelating before being incorporated into vacuoles and

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other intracellular sites. These process are often irreversible and ensure less risk of metal releasing back to the environment (Gekeler*et.al*.,1988). A majority of intracellular metals become bound to polyphosphate granules localized in and near the vacuoles or may also get detoxified via binding to specific low molecular weight proteins, namely metallothioneins and phytochelatin was indicated (Volesky*et.al*.,1992.The present study was focused on bioremediation of heavy metals (chromium) at various optimized conditions using bacteria as bioremediators.

Materials and Methods:-
Collection of samples:-
Effluent Samples were collected from electroplating industries in and around Coimbatore and Vellore, India. Samples were collected in sterile glass – screw cap tubes and preserved at 4^0c in refrigerator for further studies.

Isolation, Screening and Identification of bacteria:-
The heavy metal degrading bacteria were isolated from electroplating effluent by serial dilution technique on minimal salt agar medium with 0.1% concentration of chromium. The viable colony on minimal agar plate indicates that the organism use metals as nutrient and energy source. Those organisms were selected for further studies and the selected isolates were identified on the basis of Morphological and Biochemical analysis according to Bergey's Manual of systematic Bacteriology and 16s rRNA gene sequence.

Optimization:-
Various factors were optimized to achieve the highest degradation effect of heavy metal by the selected isolates of bacteria. Heavy metal degradation was optimized at various factors such as concentration of metals, pH, temperature, carbon source, nitrogen source.

FTIR analysis:-
UV Spectral analysis was done by using a UV - Vis Spectrophotometer. Fourier transform infrared spectroscopy (FTIR) analysis was carried out using Shimadzu 8400s Spectrophotometer in the mid - Infrared of 400 - 4000cm^{-1}

Result and Discussion:-
Isolation and screening:-
The isolates were confirmed through biochemical characterization and 16s RNA sequencing as *Bacillus cereus, Bacillus thuringiensis.*(Table1)

Table 1:- Biochemical characterization

S.No	Biochemical characteristic	*Bacillus cereus*	*Bacillus thuringiensis*
1.	Gram staining	Gram positive	Gram positive
2.	Shape	Rod	Rod
3.	Catalase test	Positive	Positive
4.	Oxidase test	Negative	Negative
5.	Indole test	Negative	Negative
6.	Methyl red test	Negative	Negative
7.	Vogespraskauer test	Positive	Positive
8.	Citrate utilization test	Negative	Negative
9.	Triple sugar ion test	Negative	Negative
10.	Urease test	Negative	Negative
11.	Nitrate test	Positive	Positive
12.	Carbohydrate fermentation test	No acid	No acid

Figure 1 (a)

(b)

**Figure 1:-** a) Phylogenetic tree of Bacillus cereus b) Bacillus *thuringiensis*

Optimization:-

The effective degradation of chromium and nickel was done with the high and low amount of pH, glucose , nitrogen and yeast extract. The values of the pH are the most important physical parameter influencing not only the site dissociation but also the solution of the heavy metal hydrolysis, complication by organic or inorganic ligands, redox reactions, precipitations, functional group activity in the biomass and the competition of metal ion.(Friis*et.al.,*1998). The value of pH was measured by using pH electrode. Here, the pH taken in the range from 2 to 10. At pH 8 the degradation is high while at pH 2 and pH 10 it shows maximum degradation.(Figure 1). At range of below pH 7 it shows degradation of about 65% whereas at pH 10, it shows degradation at 69.7%.


Figure 1: Optimization of chromium at pH 8
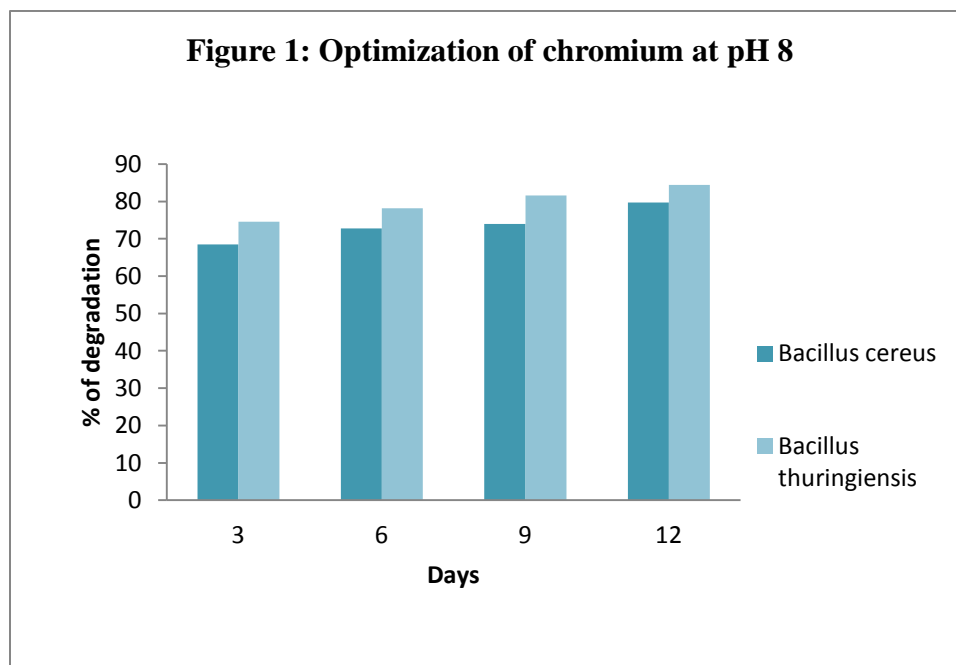
At various temperatures maximum degradation was observed at 35^0C whereas at minimum degradation was observed at other temperatures(25, 30,40 and 45^0C) (Figure 2). At range of below 30^0 C it shows degradation of about 56.8% whereas at above 40^0C , it shows degradation at 67.9%. Temperature plays an important role in the metabolic pathway it may affects the functions of microorganisms.


Figure 2: Optimization of chromium at 35^0 C
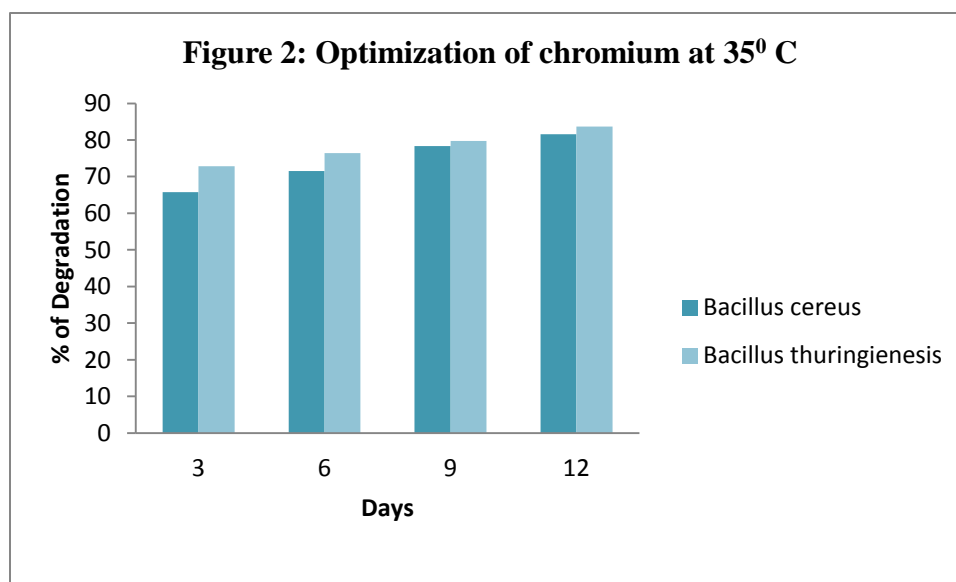
In Yeast extract the highly degradation of chromium was done at conc: 0.3% in *Bacillus cereus, Bacillus thuringiensis,* upto 81.7%, 79.3% respectively (Figure 3) and the degradation rate was below 64.5% at other concentrations(0.1, 0.2, 0.4 and 0.5%). Yeast extract acts as an extra energy source which helps to degrade the chromium.

Figure 3: Optimization of chromium at 0.3% of yeast extract
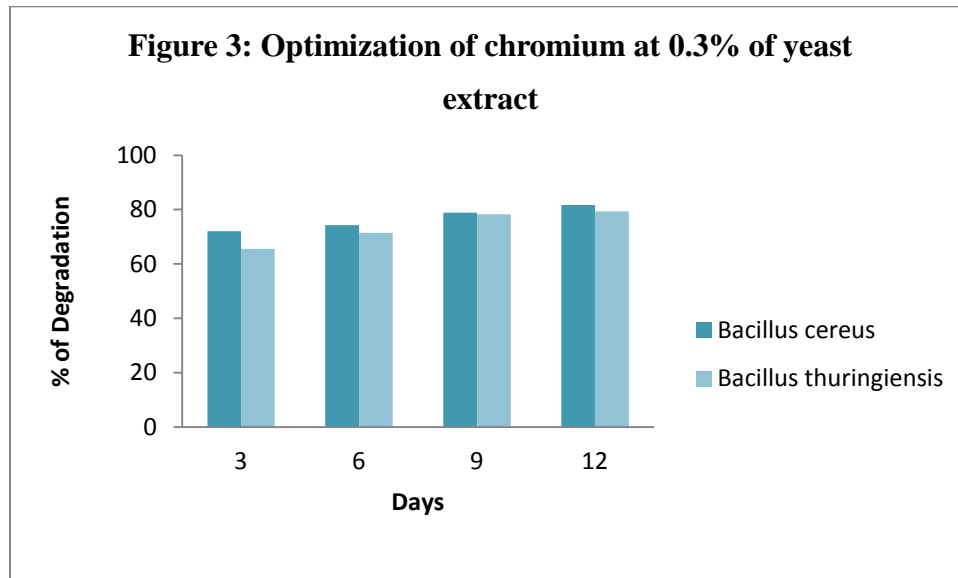
In Glucose the highly degradation of chromium was done at concentration of 0.1% in *Bacillus cereus, Bacillus thuringiensis*upto 82.1% ,78%(Figure 4).and the degradation rate was below 70.3% at other concentrations(0.2, 0.3, 0.4 and 0.5%)

Figure 4: Optimization of Chromium at 0.1% of Glucose
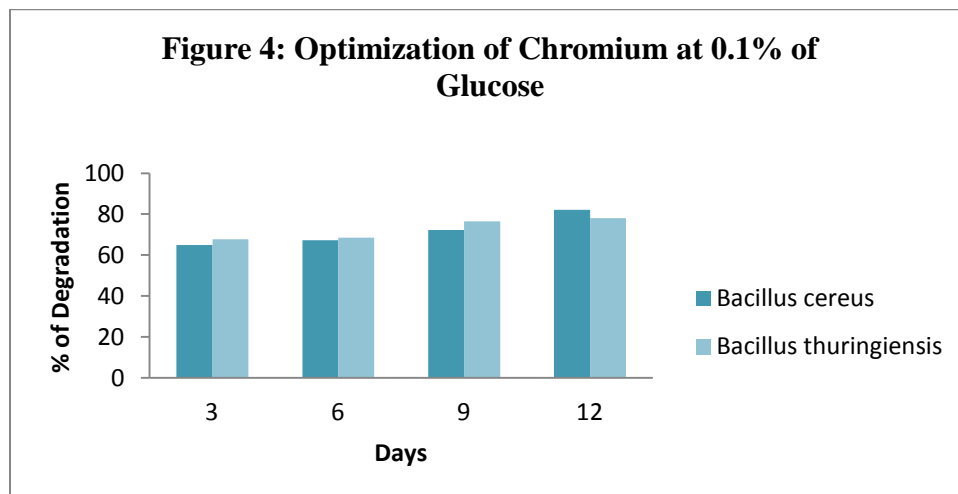
In ammonium sulphate the highly degradation of chromium was done at conc: 0.1 in *Bacillus cereus, Bacillus thuringiensis,* upto 82.4%,79.3%, (Figure 5).and the degradation rate was below 50% at other concentrations. (0.2, 0.3, 0.4 and 0.5%)

Figure 5: Optimization of chromium at 0.1% Ammonium sulphate
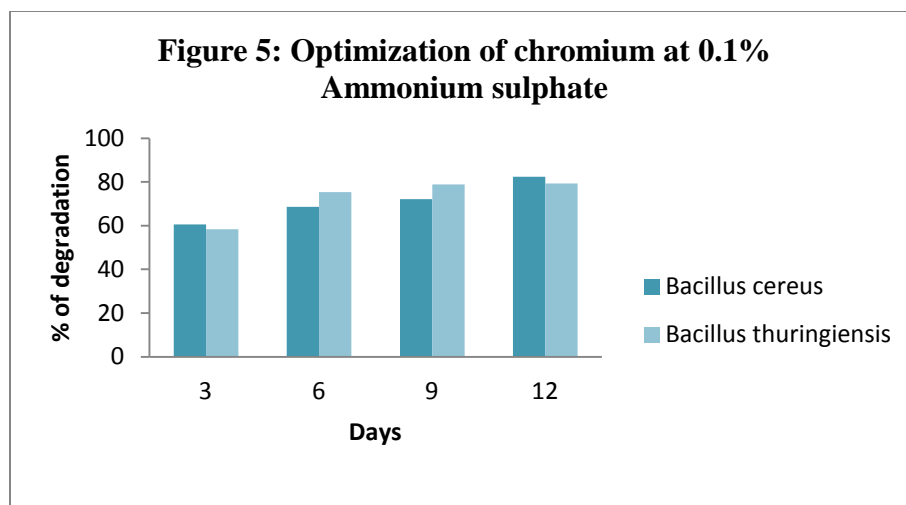
FTIR analysis:-

In C1 sample, IR spectroscopic study of the cell surface of *Bacillus cereus* was done to understand and interpret the mechanism involved in metal-ion binding. Shifting of the IR band to 3379.29 cm–1 with substantial decrease in intensity indicates the role of the amine group in metal binding as well as indicate the involvement of hydroxyl and amine stretching of the protein. Shift in the band to 2785.21 cm–1 can be assigned to the –CH stretching after metal binding. Decrease in the band intensity at 1643.35 cm–1 may be due to the amide group taking part in metal absorption. The shifting of the band to 2962.66 with a decrease in intensity corresponds to O–H bending after metal binding to the carboxylate ions. Shift of band at 1303.88 cm–1 may be due to the possible role of C–O polysaccharide in the biosorption process. Amorphous substances bound to the terminals of *Bacillus sp* were found, which were presumed to be precipitates of Cr compounds as a result of heavy metal reduction. The electronegative functional groups like hydroxyl, carboxyl and phosphate on the Gram-positive bacterial surface help in the binding of these reduced products.

In C2 sample, IR spectroscopic study of the cell surface of *Bacillus thuringiensis* was done to understand and interpret the mechanism involved in metal-ion binding. Shifting of the IR band to 3402.43 cm–1 with substantial decrease in intensity indicates the role of the amine group in metal binding sites. Shift in the band to 3294.42 cm–1 can be assigned to the –CH stretching after metal binding. Decrease in the band intensity at 1643.35 cm–1 may be due to the amide group taking part in metal absorption. Shift of band at 1651.07 cm–1 may be due to the possible role of C–O polysaccharide in the biosorption process. Amorphous substances bound to the terminals of *Bacillusthuringiensis* were found, which were presumed to be precipitates of Cr compounds as a result of heavy metal reduction. The electronegative functional groups like hydroxyl, carboxyl and phosphate on the Gram-positive bacterial surface help in the binding of these reduced products.

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