International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2013): 6.14 | Impact Factor (2013): 4.438

Effect of pH and Temperature on the Biosorption of Heavy Metals by *Bacillus licheniformis*

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Abstract: ***Among all the pollutants, heavy metals are most dangerous one as these are non –biodegradable and persist in environment. Human activities, such as mining operations and the discharge of industrial wastes, have resulted in accumulation of heavy metals in the environment. Removal of metals and their recovery is one of the major concerns in sewage and industrial effluent treatment. This paper attempts to present a brief summary of the role of bacterial biomass in heavy metal removal from aqueous solutions. Biosorption has emerged as a low-cost technological option for removal or recovery of base metals from aqueous wastes. Screened Bacillus licheniformis was evaluated for biosorption ability for copper and iron ions. Bacteria were grown at different pH (3, 4, 5, 6, 7 and 8) and temperature (30 ℃, 35 ℃, 40 ℃, 45 ℃, 50 ℃ and 55 ℃). It was found that bacteria were able to decontaminate with good efficacy for Fe and Cu ions at pH 8 with 92% and 93% respectively. For Fe ions 92% removal was seen at 30 ℃ and for Cu ions 94% of removal was seen at 45 ℃. This will be advantageous for effluent treatment plant to recover important heavy metals with low cost.***

Keywords: Biosorption, heavy metals, copper, iron, *Bacillus licheniformis.*

1. Introduction

Among all the environmental pollutions, pollution of water resources is a matter of great concern. Poor and developing countries are at high risk due to lack of waste water treatment technologies. Increasing contamination of aquatic sources with large number of pollutants is not only endangering the aquatic biota but creating a worldwide shortage of recreational waters. The water of aquatic systems gets polluted by domestic activities, mining activities, municipal wastes, modern agricultural practices, marine dumping, radioactive wastes, oil spillage, underground storage leakages and industries [8],[9]. Indiscriminate discharge of toxic chemicals through effluents from a wide range of industries (i.e. textile, steel, oil, tanneries, canneries, refineries, mines, fertilizers production units, detergent production units, electroplating units and sugar mills) into water bodies pollutes these resources and causes hazardous effects on flora and fauna.

Millions of gallons of water containing toxic heavy metals are generated annually from several metal processing industries and discharged into the water bodies which are creating large impact on the environment and public health [1]. Heavy metals usually form compounds that can be toxic, carcinogenic or `mutagenic, even at low concentration. Examples of heavy metals include Mercury (Hg), Cadmium (Cd), Arsenic (As), Chromium (Cr), Thallium (Tl), Lead (Pb), Copper (Cu), Zinc (Zn), Cobalt (Co), Nickel (Ni), and Iron (Fe)[1],[3],[8],[9]. These metals are classified in to three categories: toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.)[8].The increasing concern about the contamination of water bodies by heavy metals has stimulated a large number of researches to find possible ways to remove these toxic substances from the environment.

Various conventional methods are available for the removal of heavy metals from aqueous solution include chemical precipitation, adsorption process, electrochemical treatment, ion exchange, membrane treatment, crystallization [8],[9]. Concerning the cost, technical complexity and limitation of conventional methods make the alternative biological methods more appealing.

Biosorption is cheap and eco-friendly method that utilizes microbes to concentrate and to decontaminate water. Biomaterials like algae, fungi, bacteria and activated sludge have been tested as biosorbents for heavy metal removal [1]-[7], [10]-[12]. Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells [3],[8],[9].Various industries like electroplating, metal cleaning, metal processing, mining, coating, car and aeronautics produce waste water containing large quantity of Iron. The presence of Iron in water results in undesirable colour, odour and taste which makes water unfit for industry and domestic consumption [1],[4]-[7]. Presence of Copper ions also poses serious environmental and human health hazard due to their toxicity [1], [10]-[12].

The main aim of the present work was to investigate the potential of the *Bacillus licheniformis* to accumulate the heavy metals and to be used as bioremediating agent *in situ*. The effect of pH and temperature on metal removal was also studied. This study is an attempt to provide a multipurpose alternative for waste water treatment.

2. Review of Literature

The increasing concern about the contamination of water bodies by heavy metals has stimulated a large number of researches to find possible ways to remove these toxic substances from the environment. To overcome the heavy metal pollution researchers have used many methods to obtain the most optimum and cost effective method to remove heavy metals from water. The most abundant metal existing in wastewater are Chromium, Mercury, Lead, Zinc, Nickel, Arsenic, Copper, Magnesium. Some of the methods

employed, or studied, include precipitation, filtration, coagulation, ion-exchange, magnetic fields, fluidized bed reactor, ion flotation, reverse osmosis and adsorption. Hence, the disadvantages like incomplete metal removal, high reagent and energy requirement, generation of toxic sludge or other wastewaters that require careful disposal has made it imperative for a cost effective treatment method that is capable of removing heavy metals from aqueous effluent [8],[9]. Different materials may be used as adsorbents which collect, or adsorb, the heavy metals from the wastewater. Various biomaterials like bacteria [1]-[4],[10],[11], fungi [6],[7], algae [12], activated sludge [5] have been investigated in prior research.

3. Materials and Method

1.1 Bacteria and Media:

Bacterial species *Bacillus licheniformis* was isolated from soil sample collected from galvanize industry. Cells were cultured in nutrient broth: beef extract 3.0g/L, peptone 10.0g/L, sodium chloride 5.0g/L and final pH adjusted to 7.4-7.6. Strain was maintained by subculturing on nutrient agar. The culture was stored at 4^0C between transfers and subcultured before experimental use. Microscopic and biochemical tests were applied to this isolate according to Bergy's Manual of Systematic Bacteriology to determine the genus to which the isolate belong [2].

1.2 Metal Solution:

Heavy metals used in the study were Fe (III) and Cu (II) in the form of their respective metal solution. A synthetic liquid media (1% Tryptone, 0.5% Yeast extract and 0.5% NaCl) containing 15mg/L of each Fe and Cu ions was prepared from their respective stock solutions (1000mg/L). Chemicals used for preparing their stock solutions were ferric chloride and copper sulphate. Stock solutions were prepared using deionised water. Prior to addition, both the stock solution of metals and liquid media were autoclaved separately at 121^0C for 20min. All the additions were performed aseptically [1].

1.3 Effect of pH Biosorption:

Experiments of heavy metals biosorption were carried out in the batch mode in the Erlenmeyer flasks containing 50 ml of synthetic multi-element solution (15 mg% of each metal). Prior to addition of biosorbent inoculum to the solution the samples were adjusted to different pH viz. pH 3, 4, 5, 6, 7 and 8 using 1M HCl and 1M NaOH solutions [1]. Samples were inoculated with overnight grown culture of *Bacillus licheniformis* and incubated at 120 rpm for 48hrs at 30^0C for Fe ions and at 45^0C for Cu ions. After incubation period the cells were harvested by centrifugation for 30 min at 5000 rpm. The residual metal concentrations were measured by Atomic Adsorption Spectrophotometer (AAS).

1.4 Effect of Incubation Temperature on Biosorption:

A set of samples were prepared for Fe and Cu ions as described above and pH was adjusted to pH 8 for Fe ions and Cu ions. After inoculation, samples were incubated at 120 rpm for 48hrs at different temperature viz. 30^0C, 35^0C, 40^0C, 45^0C, 50^0C and 55^0C. After incubation the residual metal concentration were measured using AAS.

4. Result and Discussion

The present study indicates that microbes might be used to remove metal contamination. This is primarily accomplished by biosorption of metals or enzymatically catalysed changes in the metal redox state.

1.5 Identification of bacterial strain:

The organism isolated from soil sample was identified as *Bacillus licheniformis* according to Bergy's Manual of Systematic Bacteriology was used for the biosorption of heavy metals Iron and Copper. Effect of pH and incubation temperature was studied on removal efficacy of the isolated organism. Table 1 shows the biochemical test results for isolated bacterial strain.

Table 1: Morphological and biochemical characteristics of the isolated bacterial strain

Characterization test	*Bacterial strain*
Gram reaction/shape	Positive/rod
Spore	Positive
Catalase	Positive
Starch hydrolysis	Positive
MR test	Negative
VP test	Positive
Citrate utilisation test	Negative
Indole test	Negative
Urea hydrolysis test	Negative
Acid from sugar fermentation	
Glucose	Positive
Sucrose	Positive
Lactose	Negative
Mannitol	Positive
Maltose	Positive
Xylose	Positive
Probable identity	*Bacillus licheniformis*

4.2 Effect of pH:

The pH of the aqueous solution has been considered as one of the most important factors influencing the biosorption process, it influence not only the dissociation of functional groups on the active sites of the biosorbent but also the solution ion chemistry. Different metals show different pH optima for their biosorption.

Figure 1 shows % removal of heavy metals Iron and Copper by *Bacillus licheniformis* at pH- 3, 4, 5, 6, 7 and 8. The maximum biosorption for Iron and copper ions was found at pH- 8with removal efficacy of 92% and 93% respectively. This result suggested that the alkaline pH was optimum for biosorption of these heavy metals using *Bacillus licheniformis*.

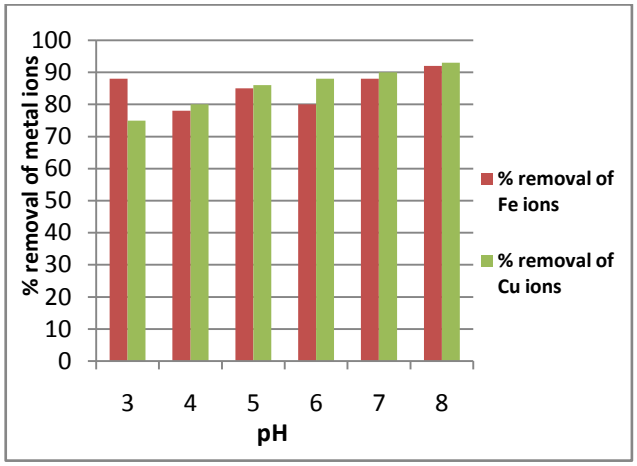

Figure 1: Effect of pH on biosorption by *Bacillus licheniformis*

4.3 Effect of Incubation Temperature:

Figure 2 shows the effect of incubation temperature on metal biosorption. Maximum % removal of metal ions was observed at 30^{0}C for Iron and 45^{0}C for Copper ions with efficacy of 92% and 94% respectively. The results showed higher metal removal efficacy of living biomass of *Bacillus licheniformis*, thereby suggesting its possible application in multiple metal removal in effluent treatment.

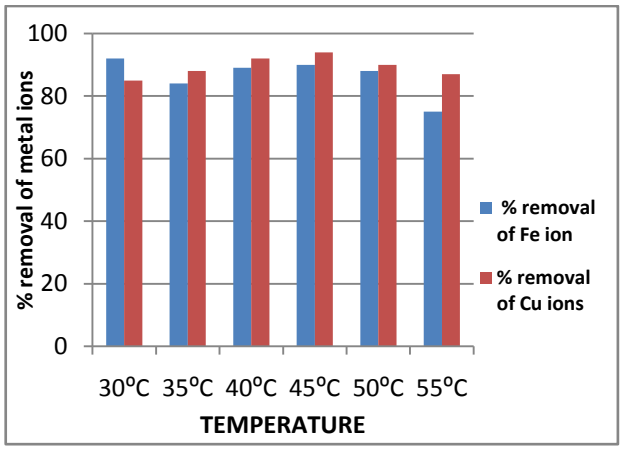

Figure 2: Effect of temperature on biosorption by *Bacillus licheniformis*

The cell wall of Gram positive bacteria are efficient metal chelators. The carboxyl group of the glutamic acid of peptidoglycan in Bacillus spp. was the major site of metal deposition. Teichoic and techuronic acids were important binding sites in Bacillus spp. [2],[8],[9].

The possible mechanism of Iron and Copper ion removal by *Bacillus licheniformis*, isolated from galvanize industry soil sample has been evaluated at different pH and temperature. The bacterium showed excellent ability to reduce Iron (92%) and Copper ions (93% & 94%) respectively. Hence the isolate has been identified as potential microbe for its ability to reduce heavy metals from the industrial wastewater.

5. Conclusion

Adsorption has been used to remove many pollutants from wastewater, whether seawater or industrial wastewater, purifying drinking water, or as a polishing phase at the end of sewage treatment. These pollutants include heavy metals, which is the focus of this work. Heavy metals are toxic and hazardous to humans, marine life and the water body in which it is contained. The metals studied in this work include Copper, and Iron, due to their abundance in water, in addition to their toxicity. The microbes play vital role in the biosorption of heavy metals. The present study demonstrated the use of *Bacillus licheniformis* in the wastewater treatment. The process would not only be economic but also eco-friendly and multipurpose as an alternative to conventional methods of biosorption of heavy metals. This catabolic potential of microbes is enormous and is advantageous to mankind for a cleaner and healthier environment through biosorption. However, further research is needed to establish the process with specific attention.

6. Future Aspect

Several biotechnological approaches seem to be established as a means of combating toxic metal pollution from industrial and other sources, although none are yet in widespread use. Several design preferences are beginning to emerge within the field with the processes currently in or near to practical operation mainly utilizing biosorption and or bio precipitation. Biosorption of heavy metals from aqueous solution is relatively newer technology for the treatment of waste water from various industries such as metal cleaning, plating baths, refineries, mining, electroplating, paper & pulp, paint, textile and tennaries.

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