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# Batch studies of copper bioremediation using *Bacillus pumilus* strain PD3 isolated from marine water

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**ABSTRACT**

Copper is one of the heavy metal which is essential but shows some toxic effects in high concentration. It has been found high copper uptakes lead to kidney, brain damage and death also. This study investigated copper degradation by a group of copper-resistant bacteria isolated from sea-water. Copper resistant bacteria, *Bacillus pumilus* Strain PD3 was isolated, which showed maximum copper-resistance at 150 mg $l^{-1}$ Cu (II).Using the isolated bacteria bioremediation experiments were performed, varying the copper concentration from 10-100 mg $l^{-1}$ and solution's pH 3-6 at different time intervals. 45-54% copper degradation was obtained at pH 6, 50 mg $l^{-1}$ and 47 hours. The result indicated that these isolated bacteria have a good potential in Copper degradation from aqueous solution.



## Introduction

Too much industrialization leads to environmental problem due to heavy metals (such as Cr, Cu, Pb, Zn etc.) presence in the industrial effluents [1-4]. Though copper is a trace elements but it has some negative impacts in human beings (such as kidney, brain, liver damage etc.) and on the environment in the higher concentration [5-7]. There are various old methods of copper (II) removal from wastewaters. But these old methods require high capital and operating costs, and have several disadvantages in removal also [8].

Environmental Microbiology is a popular field to degrade toxic organic pollutants, heavy metals using bacteria, fungi, algae etc.

Bioremediation is an effective process to reduce heavy metal pollution from the environment [9]. In bioremediation micro-organisms used to metabolize the heavy metal [1].

Biosorption is a cost-effective microbial process to reduce heavy metals from the aqueous solution [1, 10-22]. There are two types of biosorption- active biosorption using the live bacteria to degrade the metal metabolically and passive biosorption using the dead biomass to reduce heavy metals from solution by cell-surface adsorption methods.

Apart from micro-organisms there is also use of some agricultural peels, bark or other wastes etc. as biosorbents to remove heavy metals from the aqueous solution [23-25].

The objective of the present study was performed the bioremediation of copper varying the time, copper concentration and pH factors. The batch study was performed using the novel copper resistant bacteria *Bacillus pumilus* strain PD3, isolated from sea water of Pattaya. The bacteria is a gram-negative, rapidly growing bacteria. The batch study involves varying one independent factor while others maintain at a fixed level.

## Materials and methods:-

### Bacteria isolation and cultivation :-

Sea- Water was collected from pattaya of Thailand and serial dilution was followed upto $10^{-3}$ times. The nutrient agar plates containing various copper concentration was inoculated by the sea serial dilution's solution of $10^{-3}$.

These plates were incubated at 30°C for 24 hours. Maximum copper resistance of the bacteria was obtained at 150 mg $l^{-1}$ Cu (II) , using $CuSO_4$,$5H_2O$ in nutrient agar plate. Cu (II) concentration was varied from 10 - 150 mg $l^{-1}$ in the nutrient agar plates. Nutrient agar was obtained from Himedia.

### Characterization of the bacteria:-

The isolated bacteria was a gram-negative bacteria. On the basis of nucleotide homology and phylogenetic analysis the micro-organism was detected as *Bacillus pumilus* strain PD3.The bacterial 16s RNA sequence had been submitted to the GenBank under Accession Number: JQ809230 and had published also in the GenBank.

### Preparation of Metal solution:-

A stock solution of copper was prepared using copper sulfate pentahydrate ($CuSO_4$,$5H_2O$) dissolving in distilled water. 1000 mg $l^{-1}$ Cu (II) stock solution was prepared by diluting 3.93 g of $CuSO_4$, $5H_2O$ (obtained from Merck) in 1 liter distilled water. Using this stock different copper concentration solution was prepared.

### Bacterial growth-medium Preparation:-

Nutrient broth consists of different concentration of Cu solution & different pH was prepared for the copper reduction experiment. For pH adjustment 0.1(N) HCl and 0.1(N) NaOH solution was used and for pH calibration pH meter (ELICO) was used. After that media was autoclaved in 250 ml conical flasks containing 100 ml. Media.

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**Biosorption experiments**:-

The media was inoculated with the isolated bacteria maintaining aseptic condition in laminar-hood. Then the conical flasks were kept in rotary shaker incubator at 30°C, 120 rpm. After different time intervals sample was collected and centrifuged at 6000 rpm for 10 minutes. Supernatant fractions was analyzed for the remaining copper ions in the media after reduction, were determined spectrophotometrically at 460 nm by using sodium diethyl dithiocarbamate (obtained from Loba) as the complexing agent [26].Copper degradation percentage by *Bacillus pumilus* was obtained by using the formula-

$$\%Degradation = (C_0 - C_1)/C_0 * 100\% \quad \text{----------(1)}$$

Where, $C_0$ is initial copper concentration (mg $l^{-1}$) and $C_1$ is remaining copper concentration.

**Results and Discussions:-**

**Effect of initial copper concentration on bioremediation:-**

Bioremediation of copper got influenced by several factors such as initial pH, initial copper ion concentration, incubation time and temperature etc. The percentage of degradation was a function of the initial metal concentration. The increase in initial copper concentration resulted in increase in the capacity of metal degradation from 10 -100 mg $l^{-1}$. With increasing copper concentration, copper degradation increased upto 50 mg $l^{-1}$ and after that the degradation decreased with increasing copper concentration 50-100 mg $l^{-1}$ [Fig 1]. The %degradation was maximum i.e. 45.384% at 50 mg $l^{-1}$ copper concentration after 47 hours incubation time.

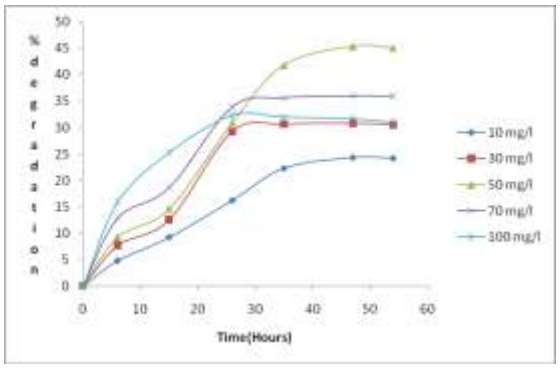


**Fig 1:- Graphical representation at different time vs. %reduction at different variation of concentration**

**Effect of initial pH on %reduction:-**

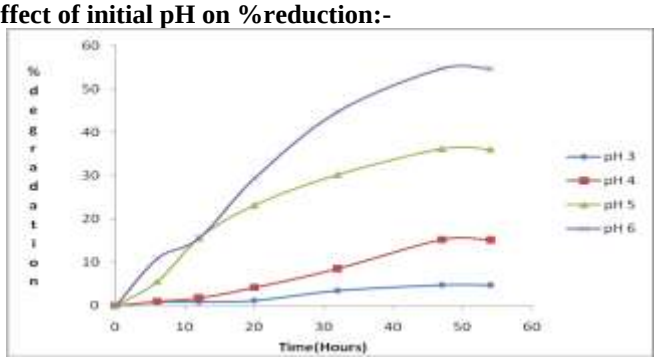


**Fig 2:- Graphical representation at different time vs. %degradation at different variation of pH.**

The batch study with varying pH was performed in 100 ml (50 mg $l^{-1}$) copper concentrated nutrient broth solution. Maximum removal (54.64%) was obtained at high pH, i.e. 6 (Fig 2). That means the bacterial growth is maximum at basic pH. It was observed that as the pH increased, the % removal of copper increased.

**Effect of time on % degradation:-**

Incubation time is an important factor in bioremediation study. As the time increased the bacterial growth increased and that resulted to increase in copper degradation. Fig 1,2 showed that with increasing time %degradation increased upto a certain time i.e. 47 hours, after that it reaches to the equilibrium [9]. That means bacteria goes to the death phase after 47 hours and the food in the media also gets finished.

**SEM Analysis:-**

Scanning electron microscopy analysis was performed to examine the surface of the biomass after biosorption (Fig 3). It was observed that the surface is very smooth. The rough surface area of biomass can help to increase the copper Biosorption (Fig 3). The SEM pictures were taken at 500X resolution using S-530, Hitachi Scanning Electron Microscope at an electron acceleration voltage of 25 kV.

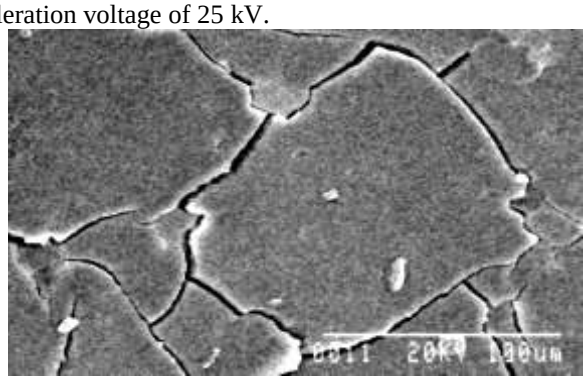

**Fig 3:- SEM picture of the micro-organism after biosorption at 500X resolution**

**Conclusion:-**

The bioremediation of Cu (II) ions were studied using isolated *Bacillus pumilus* strain PD3. From batch-study results it was observed that almost 45-54% of copper present in solution can be degraded by this species at pH 6, 50 mg $l^{-1}$ and 47 hours. So, this result indicated that the isolated bacteria may be used for effective degradation of Cu (II) ions present in wastewater.

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