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Bioremediation of Copper(II) Using Microoraganisms and Agro-Wastes.

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Abstract

One of the most serious problems of environmental pollution is caused by metals. In the present study, various bio-sorbents were used to carried out for the adsorption of Cu(II) by using microorganisms isolated from soil samples that was collected from two different contaminated sites of copper and agro-wastes such as groundnut shells, sawdust, orange peels, cotton, coconut husk and sugarcane bagasse for the removal of Cu(II) from aqueous copper solution. The effect of metal ion concentration, biomass concentration, pH, contact time, particle size, pre-treated biomass were investigated.The experiment was performed using solvent extraction method. The Experimental results showed that Groundnut shells, Saw dust andFungi gave better efficiency in removal of metal as compared to other tested biomasses i.e. more than 50% bio-sorption. Determination of Cu(II) was done spectrophotometrically at 560nm and all biomasses were characterized using FTIR. As the method is efficient and cost effective for environmental metal pollution removal, it can be scaled up and used for the removal of copper from contaminated sites.

Keywords: bio-sorption, bio-sorbent.

Introduction

Pollution is the introduction of contaminants into a natural environment that causes instability, disorder, harm or discomfort to the ecosystems. Bio-sorption is a property of certain types of inactive, dead, microbial biomass to bind a concentrate metals from even very dilute aqueous solutions. Bio-sorption is a physico-chemical and metabolically independent process based on variety of mechanisms including absorption, adsorption, ion exchange, surface complexation and precipitation. Bioremediation is gaining significant attention due to its economical and eco-friendly nature. The study was aimed to provide a pragmatic view of the process involved in bioremediation dealing with the copper contaminated water using microorganisms and agro-wastes.

Sources of metal pollution

Metal pollution mainly arises from the effluents of industrial units. Some of the common industrial units releasing toxic metal into environment include rubber industries, plastic industries, metal refineries, pesticides Irrigation by effluents released from paper mills and fertilizer factories are adding various alkalis, ammonia, and cyanides into water sources. The wastes from the dyes and pigment industries, film and photography, galvanometric, metal cleaning, electroplating, leather and mining industries contain considerable amount of metal ions. In contrast to herbicides, pesticides and other potential toxicants which undergo break down, albeit extremely slowly, metals cannot be eliminated from a water body and thus persist in sediments where these are slowly released into the water.

Copper as pollutant

Copper is a reddish metal with a face-centred cubic crystalline structure. It reflects red and orange light and absorbs other frequencies in the visible spectrum, due to its band structure, so it as a nice reddish color. It is malleable, ductile, and an extremely good conductor of both heat and electricity. It is softer than zinc and can be polished to a bright finish. It is found in group Ib of the periodic table, together with silver and gold. Copper has low chemical re-activity. In moist air it slowly forms a greenish surface film called patina; this coating protects the metal from further attack.

Major advantages of bio-sorption over conventional treatment methods

Low operating cost, High efficiency, Minimization of chemical and biological sludge. No additional nutritional requirement. Regeneration of bio-sorbent.Possibility of valuable metal recovery. Resins are hard ligands and are less effective in adsorbing minute quantities of metals when compared to soft ligands of biological origin. Microbial biomass required for bio-sorption may be available as a fermentation waste product or specifically grown one, using cheap substrates. Bio-sorption processes may serve as polishing system to existing processes.

Materials and Method

Chemicals/ Media

1) Stock solution of Cu^{2+}.
2) 25% Citric acid.
3) Ammonia solution.
4) 4% EDTA.
5) 0.2% SDDC.
6) n-Butyl Acetate .
7) 10% H_2SO_4.
8) Nutrient Broth.
9) Potato dextrose Broth.
10) Agar powder.

Sample Collection

Soil sample was collected from two different metal contaminated sites at Rakhial and Odhav, Ahmedabad for isolation of microorganisms and different Agro-wastes like groundnut shells, Saw dust, Orange peel, Coconut husk, Cotton, and Sugarcane bagasse were collected and dried for three days under sunlight. Materials were ground into appropriate particle size using mortar and pestle and then sieved through different BIS standard mesh sieves (BIS18, BIS52, and BIS100)

Isolation

1gm of soil sample was added to distilled water and dilutions were made. Sterile NA plates and Sterile PDA plates were inoculated according to dilutions. PDA plates were incubated for 7 days at room temperature and NA plates were incubated at $37^{\pm O}$C for 24 to 48 hours. The plates were observed for growth and isolates were preserved.

Activated culture of bacteria and fungi were inoculated in 100ml sterile NB and sterile PDB for biomasses respectively and were kept on shaker for two or more days. The biomasses were then collected, filtered, weighed and dried for 2 or more days. The dried biomasses were then grounded using mortar and pestle and further used for experiments.

Screening

The dried biomasses of microorganisms and all the agro-waste materials were screened on the basis of copper (Cu^{2+}) sorption in definite time (24 h).

Experiment

Adsorption studies were performed in 250mL erlenmeyer flask by addition of adsorbent (Groundnut shells) to 50mL copper solutions. The pH was adjusted with 1N H_2SO_4 and 1N NaOH before addition of adsorbents. In all the experiments, the flasks were shaken at 150 rpm on a rotary shaker. Contact time was 2h except for the screening studies and contact time experiments. At different interval of time, the samples were withdrawn and filtered. The residual metal ion in the filtrate was extracted using solvent extraction method and was determined spectrophotometrically. Control system(blank) was prepared without adding the biomass.

The effect of metal ion concentration, biomass concentration, pH, contact time, particle size,pre-treated biomass were investigated. Effect of metal ion concentration was checked using different metal concentrations (10ppm-500ppm/50mL) while other experimental conditions were kept constant (amount of bio-sorbent 25mg/50mL for microbial biomass and 200/50mL for agro-waste, pH 5.0,).To determine the effect of bio-sorbent concentration on adsorption of copper ion (Cu^{2+}), 15mg-55mg microbial biomass and 100-500mg/50mL agro-waste adsorbent was used and other experimental conditions were fixed (copper solution concentration 50ppm&100ppm/50mL, pH 5.0). The effect of pH on the copper adsorption was studied at different pH values (2.0-6.0), at constant copper concentration (50ppm/ 50mL), amount of bio-sorbent (25mg&200mg/50mL). To optimize the contact time, it was varied from 0 minute to 120 minute while other experimental conditions were kept constant (copper concentration 50ppm/50mL, bio-sorbent concentration 25mg&200mg/50mL).

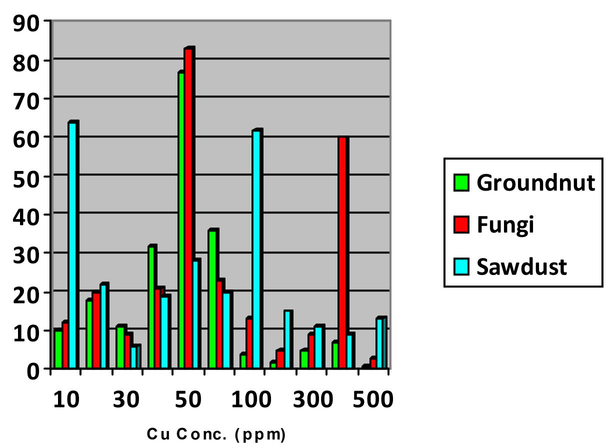
Solvent Extraction Method

Working solution of cupric sulphate pentahydrate was prepared from the stock solution by diluting it to get desired concentration and biomass was added to it. 1.0mLfrom the experimental system was added into the breaker.10.0mL distilled water was then added.Then 5.0mL of 25% aqueous citric acid solution was added to it. It was rendered slightly alkaline with ammonia solution, alternatively adjusted to pH 8.5.Then it was added with 15mL of 4% EDTA solution and was transfered to a separatory funnel.0.2% aqueous SDDC solution was added and then shaken well for 45 second or given 90 strokes, a yellow brown colour developed in the solution.Further 10.0mL of n-butyl acetate was added in to the funnel and shaken for 45 second again or given 90 strokes. Allowed the phases to get separated, the lower aqueous layer was removed.10.0mL of 10% of sulfuric acid was added and shaken for 15 second or given 15 strokes. Organic phase was allowed to get separated and the lower aqueous layer was discarded.The optical density was determined at 560nm spectrophotometrically.

Results and Discussion

1. Effect of Copper ion concentration

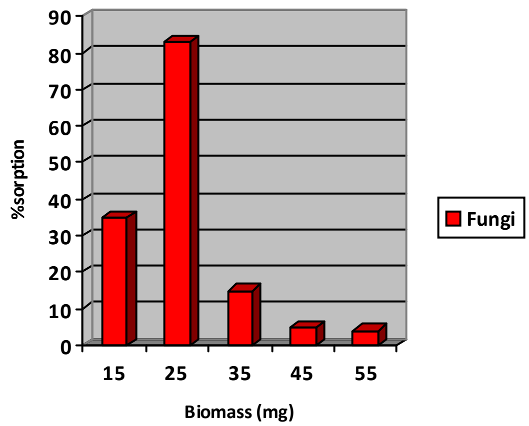
Effect of metal ion concentration was studied on different biomasses (Fungi, Groundnut, Saw dust). Sorption efficiencies initially increased with increasing Cu(II) concentration and gave best sorprtion at 50 ppm[For fungi(83%) and ground nut(77%)] and at 10 ppm [For saw dust(64%)]. Sorption decreased with increasing copper concentrations as the equilibrium level has reached.



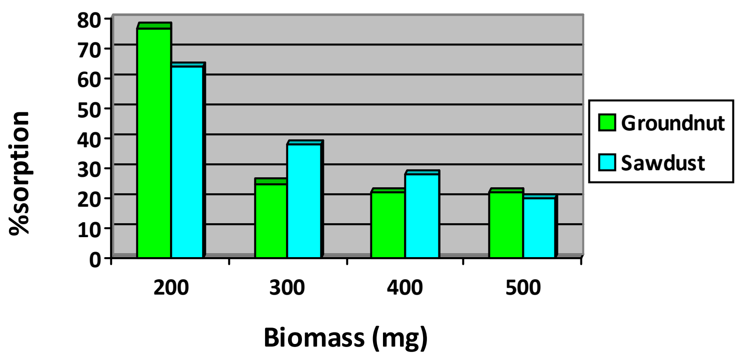
Graph 1: Effect of copper ion concentration

2. Effect of biosorbent concentration

The removal of Cu(II) ions from aqueous solution was significantly dependent on the amount of adsorbent. The results are represented in the graph. It can be seen that the sorption increases upto a certain limit and then it decreases. The increase in the biosorption with the biosorbent concentration can be attributed to greater surface area and the availability of more biosorption sites. . At biosorbent concentration greater than 200mg (For groundnut and saw dust) and 0.25 mg (For fungi), the surface Cu(II) concentration and the solution Cu(II) concentration come to equilibrium with each other. However, the biosorption capacity decreased with increase in biosorbent concentration. This may be attributed to overlapping or aggregation ofbiosorbent sites resulting in decrease in total biosorbent surface area available to Cu(II) ions.

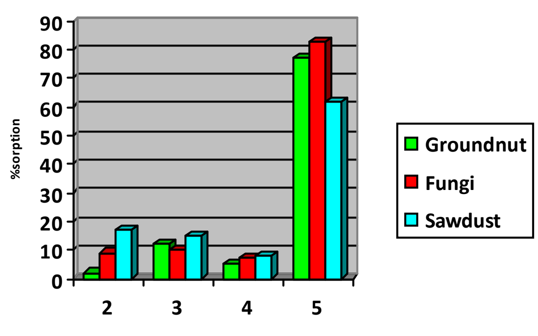


Graph 2.1 : Effect of pH on fungi

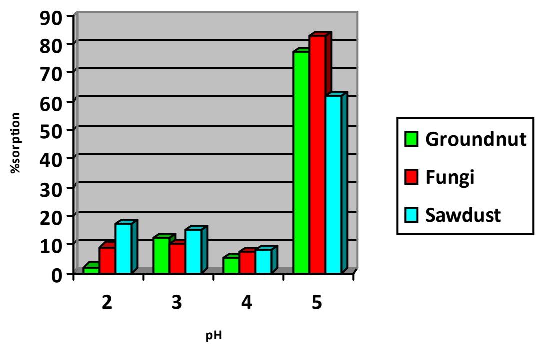


Graph 2.2: Effect of pH on (i) groundnut and (ii) Sawdust

3. Effect of pH



Results of the experiments showed that biosorption was low at strong acidic medium and adsorption capacity increased with increasing pH values, until a certain pH was reached. Biosorption was increased between pH 2.0 and 6.0. Results are presented in the graph(Graph 4). At the optimal pH 5.0, 77% Cu(II) was removed bu groundnut shells; 84% by fungi and 645 by saw dust. At pH value higher than pH 5.0 copper ion in the solution get precipitated and thus the sorption decreased.



Graph 3: Effect of p

Conclusion

Hence, from this study, it may be concluded that the fungi, groundnut shells and sawdust are more helpful for sorption of copper from aqueous copper solution as compared to all other biomasses and successful studies on all biomasses could be beneficial. The bio-sorption is rapid and is achieved at small time interval within an hour of experiment. Many problems arise from copper contaminated water. Therefore, an economically viable and easy method for bio-sorption of copper from aqueous solution is highly desirable. This study can be applied for removal of Cu from waste water and this study is fairly helpful in developing a wastewater treatment plant of Cu from the waste water by using the microorganisms and cheap agro-wastes materials which will be economically easy to carry out.

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