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Economically Viable Fungal Production by Using Alternative Plant Material for the Removal of Heavy Metal

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ABSTRACT
Environmental pollution affects the quality of atmosphere, lithosphere and biosphere. Efforts have been made in the last two decades to reduce the pollution sources and remedy the polluted water sources. Water quality monitoring in developing countries is inadequate especially in stream water affected by urban effluent and runoff. The purpose of this study was to investigate heavy metal contaminants in sank stream water in Gwalior. The water sample from stream and industrial effluents that drain into the stream were collected and analyzed for the total elemental concentration using atomic absorption spectrophotometer. The result showed that:-the wastewater was highly encircled with copper above the maximum permissible limit. 2) the level of dissolved oxygen was below the maximum permissible limit, while biological oxygen demand was above the maximum permissible limit. All industrial effluents/wastewater were classified as strong. Factors analysis results reveal two sources of pollutants; multiple origin of copper and mixed origin or chemical phenomena of industrial and vehicular emission.

Key words: Environmental pollution, water quality, industrial effluents, multiple origins, Cu^{2+} metal

INTRODUCTION
Copper is a widespread in the environment and is derived from natural anthropogenic sources. Human widely use copper. For instance it is applied in the industries and in agriculture. The production of copper has lifted over the last decades. Due to this, copper quantities in the environment have increased. It is released into the environment by a large number of industrial operations, such as electrical equipment, construction, such as roofing and plumbing, industrial machinery, such as alloys and heat exchangers, mining, metal production, wood production and phosphate fertilizer production. Most of them discharge untreated wastewater into the environment. Several small scale electroplating units situated in the suburbs also do not have any effluent treatment facility and hence high loads of metals including Cu, Cr and Ni are discharged into surface waters. Copper becomes biologically important either, when it affects organism or is used by humans (Haygarth *et al*., 1991). However, when in excess copper is toxic as it generates reactive oxygen species via the fenton reaction, disrupts metal ions binding and homeostasis and bind macromolecules such as proteins inappropriate (Linder and Hazegh-Azam, 1996; Vulpe and Packman, 1995; Gadd *et al*., 2001; Medeiros *et al*., 1993). Copper is toxic to various member of food chain (e.g., plants, algae and humans) and generally toxic to heterotrophic bacteria in the aquatic environments (Lin and Lo, 1997; Nuhoglu *et al*., 2002; Andreazza *et al*., 2010; Iyer *et al*., 2005).

Copper is very rarely found in natural water. Any copper present normally originating from industrial effluent, seepage, water from refuse dumps, pesticides or corrosive water that has come into contact with fittings and pipes containing copper (Srinath *et al*., 2002; Stokes and Lindsay, 1979; Tsezos and Volesky, 1982). Copper containing effluent is produced in many metal processing industries for example in the manufacturing of printed circuits, fuel pipes, gutters, roofing sheets and pipes for fuel. About 1,400,000,000 pounds of copper were released into the environment by industries in 2000 (Kratochvil *et al*., 1998; Mao *et al*., 2004).

When copper ends up in soil it strongly attaches to organic matter and minerals (Brierley, 1990; Aldrich and Feng, 2000). As a result, it does not very far after release and it hardly ever enters groundwater. In surface water copper can travels great distances either suspended on sludge particles or as free ions. Copper does not break down in the environment and because of that it can accumulate in plants and animals on copper rich soil, only a limited numbers of plants have chance of survival (Spicer and Weber, 1991; Andreazza *et al*., 2011; Capone *et al*., 1983). That is why there is not much plant diversity near copper disposing factories. Due to the effects upon plants copper is a serious threat to the production of farmlands, depending upon the acidity of the soil and the presence of organic matter. Despite of this copper containing manures are still applied. Copper can interrupt the activity in soil, as it negatively influences the activity of micro-organism and earthworms (Alfrey, 1992; Szymanowski *et al*., 1990; Unz and Shuttleworth, 1996; White *et al*., 1997). The decomposition of organic matter may seriously slow down.

For ensuring the applicability of efficient strains in remote small-scale industries/units, it is essential that suitable locally available alternates to the commercial expensive growth media be worked out. Further, most of media components which have been used very commonly are of animal origin which is against the environmental ethics. Keeping this in view, there is a need to explore abundantly available plant-derived components as alternatives to animal based microbiological media. Recently, corn, sorghum, millet meal extract were used as an alternate culture supplements for *Aspergillus niger*, *Fusarium moniliforme*, *Penicillium* sp., *Cercospora* sp. (Adesemoye and Adedire, 2005). Fortification of the media containing mannitol or glucose, as a carbon source with Amaranthus seed meal (as a supplement providing growth factors) has been cited (Videira *et al*., 2002). Majority of such studies on alternative media have been reported for solidified growth medium, hence there is a large scope of research for alternative liquid growth medium or broth. Further, most of the existing investigations could lead to only functional supplements but not the complete substitute. It shall also be interesting to investigate whether the alternative media can solely support the growth and metal removal under stress conditions.

MATERIALS AND METHODS

Collection and characterization of industrial effluents

Cu (II) removal from industrial effluent: Industrial effluents were collected from two sources; Electroplating effluent from industry located in Malanpur and Banmore area, Gwalior, India and combined effluent treatment plant. Effluents were kept in ice during transportation and stored at 4°C. Then these were characterized by standard methods for pH, TDS, TSS, COD and heavy metals (APHA., 1989).

Heavy metal analysis: For analysis of heavy metal, samples were digested were with 1M HNO_3 and 1M HCl till a clear solution was obtained. The solution was then diluted and aspirated in air-acetylene flame in Atomic absorption spectrophotometer (An analyst A100 Perkins Elmer) at the specific wavelength of the metal to get the concentration range. Standards for respective metal to get concentration range. Standards for respective metals prepared using standard stock solution (10,000 mg L^{-1} Merck).

Characterization and identification of microbial isolates: All microbial isolates (bacterial as well as fungal) were picked from the plates and purified by repeated streaking and finally transferred to NA and PDA slants. Once a pure culture was obtained, all microbial isolates were initially identified on morphological and biochemical basis. Further, microscopic characterization was also done for selected resistant fungal strain.

Inoculums preparation: Fungal strain was grown on Potato Dextrose Agar (PDA) Slants by streaking method. The slants were kept in incubator at 30°C and 96 h. These slants were used to obtain spore suspension. Inoculums of 5% (v/v) of spore suspension were done by using Naubeur's chamber. For the experimental studies involving Cu (II) amendment during growth, an inoculums of 5% (v/v) of metal stressed spore suspension was used. Stressed spores were formed by inducing the metal stress at the time of spore production. Molten agar (PDA) slants was amended with 0.01 mg L^{-1} of Cu (II) before inoculating the spores.

Synthesis of various polymeric particles as carrier for biomass encapsulation

Preparation of Biopolymeric bead as adsorbent: Biopolymeric beads employed as adsorbent were prepared in two steps. In the first step, a known solution of mixture of sodium alginate (4.0 g) and gelatin (1.0 g) was added drop wise into a 0.05 M $CaCl_2$ solution with the help of a syringe and under constant stirring. The beads so produced were of almost identical spherical dimensions and allowed to harden by leaving them in $CaCl_2$ solution for 24 h and thereafter filtered and washed thrice with triple distilled water. The washed beads were further placed in as gluteraldehyde bath overnight and then filtered carefully and washed three water at room temperature and used as such. Beads were examined and tested in terms of size. The particle size distribution, determined by means of a sieving system, ranged from 0.5-0.9 mm. The surface of beads was examined using Dispersion microscope (Fig. 1). The weight drying show that the wet beads contain of 96.48% water. When left in water to swell the beads regained 2.35% of water (Bajpai and Tankhiwale, 2006).

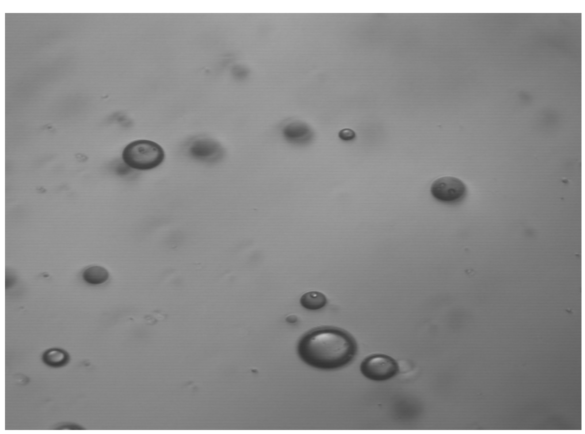

Fig. 1: Variation in size of biopolymeric bead at various initial concentration of Cu^{2+} ions

Immobilization of biomass: *Aspergillus lentulus* was immobilized by mixing sodium alginate, gelatin with above resting biomass in the ratio of 1:1:5. This mixtures were dropped in the form of beads of sodium alginate, gelatin covering the biomass. These beads were dipped into calcium chloride solution overnight. Finally beads of calcium alginate, gelatin entrapping the fungal biomass were obtained.

Effect of pH: Properties of adsorbant, pH and concentration of adsorbant and presence of co-ions solution affect the biosorption of metal ions from aqueous solution. Beads was contacted Cu^{2+} ions in separate solution (75 mL) at metal ions concentration of approximately 10 mg L^{-1} for 14 h with initial pH value of the solutions ranging from 3.0-8.0 (Fig. 2) show the effect of pH on biosorption of Cu^{2+} ions. At an initial pH biosorption occurred. A sharp increased in biosorption capacity took place in pH range 3.0-8.0. Above pH 6.0 biosorption of copper was to be relatively constant and still increase but to lesser extent.

Selection of plants for alternative medium and collection of plant material: A number of locally available plants and waste biomass were explored through literature search. Based on widespread availability, nitrogen-fixing ability, fast growth and ease of collection of the product, two plants (*Leucaena leucocephala* and *Sesbania* sp.) locally called as Subabool and Dhaincha, respectively, were selected. These plants were locally available especially in rural areas of northern India and can also grow on wasteland from Gwalior in the month of Feb 2013.

Preparation of plant materials for fungal growth: After washing of the collected plant materials, the clean seeds/pods were dried overnight at 40°C±2°C. These dried materials were grinded and made into powder form. To obtain the uniform size powder, grinded material was sieved (150 mesh). Both of these materials were characterized by proximate and ultimate analysis. Fine powder of *L. leucocephala* (7.5 g L^{-1}) and *Sesbania* sp. (15 g L^{-1}) was dissolved in distilled water.

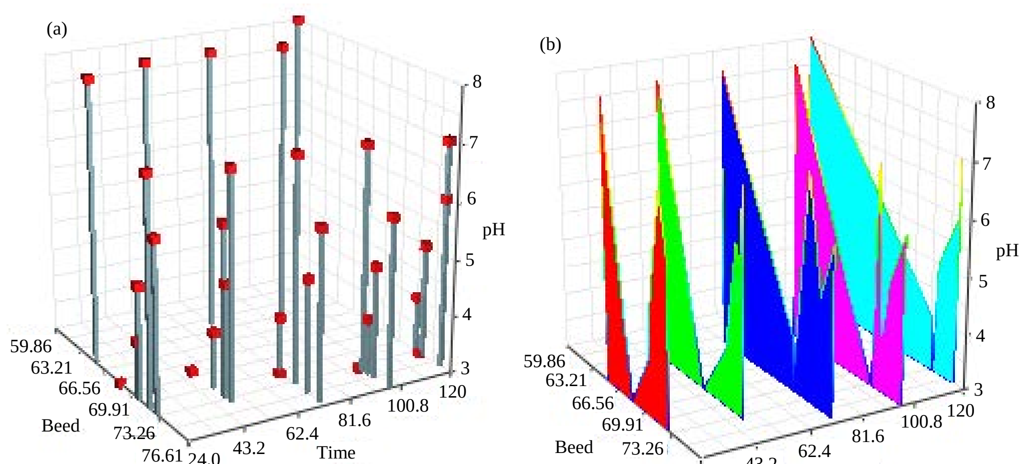

Fig. 2(a-b): (a) Represent absorption of Cu^{2+} at different pH at different time interval and (b) Where x-axis = no. of bead, y axis = Time, z-axis = pH. Represent absorption of Cu^{2+} at different pH at different time interval. By utilizing automated tools for three dimensional views of data

To increase the solubility of alternative media components in water, the mixture was heated for 2 min in microwave oven (1000 watt). The undissolved fraction was separated by centrifugation at 2000 rpm for 10 min. The final material obtained was used to study the growth and biomass production of fungal strain under normal conditions as well as in Cu (II) stress.

RESULTS AND DISCUSSION

Characterization of effluents: The data suggests that there is extensive increase in Copper removal, when pH is 3 at variant time intervals. The study suggests that as pH value at 4, 5, 6, 7 and 8 increase in the copper removal but as soon as it reaches 96 h there is simultaneous decrement in the removal values. The study conducted shows there is constant decrease in removal values at 120 h. As well, when the comparison is conducted between immobilized bead and pH values there is constant Increase in pH 3 but steadily the pH value starts decrementing as pH 4 is about to reach. Secondly the study also suggests that when attaining pH 4 the copper removal starts incrementing, then there is constant decrease while reaching pH 5, However, when the pH value has reached 5 there is increase in removal, while again there is decrement, while moving from pH 5-6. The study also suggests, when the pH value is 7 there is constant decrement in bead values till pH 8 has obtained.

The effluent collected from Effluent Treatment Plant (ETP) in Banmore industrial area and a small electroplating unit suited in Banmore, Morena and Gwalior, India. The characteristics of these effluents are shown in Table 1. As a result, although it was expected to have a mix of metallic contaminants, the concentrations of these contaminants were low. Thus, ETP effluents are voluminous with comparatively lower pollutant load. Nevertheless, this effluent contained heavy metals, such as Fe, Zn, Mn and Ni etc. but, the concentration of Cu (II) was found to be 249 mg L^{-1}. The analysis of electroplating effluent is revealed that it contained comparatively higher concentration of Cu (II) than ETP effluent. Other metals such as iron and nickel were also present (Machado *et al*., 2011). The organic load of both the effluent was very low (Machado *et al*., 2010, 2011; Javaid *et al*., 2011). The analysis of electroplating effluent is revealed that it contained comparatively higher concentrations of Cu (II) than CETP effluent. Other metal such as iron and nickel were also present. The organic load of the effluents was very low.

Increase in inoculum concentration leads to enhancement in sugar consumption rates shows in Fig. 3. The rate of Cu (II) removed by A. lentulus was also increased with increasing inoculum concentration. As the inoculum concentration was increased from 2.5-20%, the rate of Cu (II) removal almost doubled. Sen (2012) reported similar result with *Fusarium solani* that rate of Cr (VI) removal was increased, when inoculum concentration increased from 5-20%. An increase in

Table 1: Characterization of industrial effluents

Parameters	CETP	EETP
pH	3.20	2.80
TDS	7.00	3.30
COD	0.87	0.08
Cu (II)	67.00	249.00
Fe	148.00	1.33
Zn	180.00	0.50
Ni	5.00	0.50
Mn	69.30	20.90
Pb	1.60	0.40
Mg	98.50	12.80

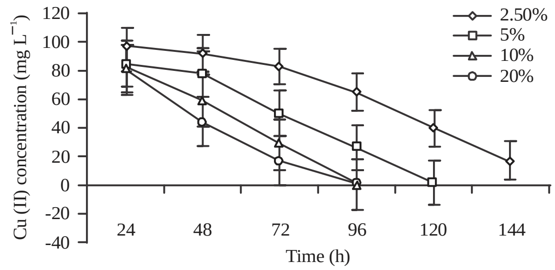

Fig. 3: Shows removal of Cu (II) by *Aspergillus lentulus* at different inoculum (2.5-20%) concentration

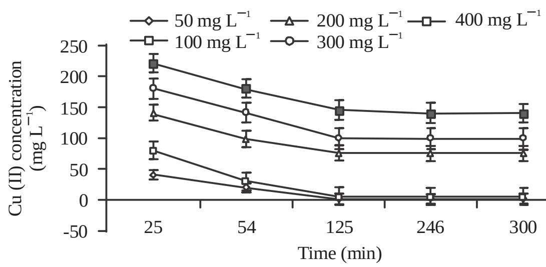

Fig. 4: Effect of initial copper concentration on copper biosorption using immobilized biomass of *Aspergillus lentulus* at pH-6 and biomass dose 4.0 g L^{-1}

rate of metal removal was found to be increased consequently with increase in inoculum concentration. The time of fungal growth decreased from 120-96 h with 20% inoculum concentration. Above studies using growing biomass demonstrate the remarkable tolerance of *A. lentulus* against Cu (II) as compared to widely reported bacterial and yeast strains.

Effect of initial Cu (II) ion concentration: Biosorption carried out with different initial Cu (II) ion concentration (50, 100, 200, 300 and 400 mg L^{-1}) was optimum at biomass concentration (dry wt) of 4.0 g L^{-1} at pH = 6 with increase in initial concentration of Cu (II) ion, specific uptake increased, may be due to an increase in electrostatic interactions involving sites of progressively lower affinity for metal ions but metal removal decreased Fig. 4. As the concentration of Cu (II) was increased from 50-100 mg L^{-1} the percentage removal increased but it decreased, when Cu (II) concentration increase from 100-400 mg L^{-1}. The equilibrium time for resting, autoclave killed and immobilized biomass remains unaffected.

Having established the need of alternative media worked for effluent treatment, experiments were set up with best alternative media worked out in the present. Based on the performance of alternative media components in Cu (II) removal from synthetic solutions, glucose and yeast extract (Composite media) were replaced by alternative media i.e., *L. leucocephala* and *Sesbania* sp. to find out whether Cu removal from actual effluent will be possible using these low cost materials instead of expensive yeast extract (Goplan *et al*., 1988; Evans and Rotar, 1987). The results shows in Fig. 5 indicate that Cu (II) removal in effluent supplemented with alternative media was at par with that in effluent supplemented with the complete composite media, although the time required for complete removal in the later case was 96 h as compared to 120 h in case of alternative media. Hence, alternative media components can be used for detoxification of the ETP effluent.

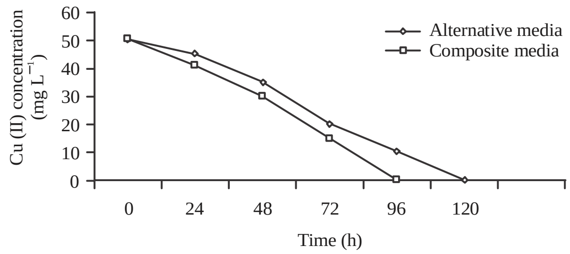

Fig. 5: Removal of Cu (II) from EFT effluent supplemented with alternative media vis-a vis composite media using *Aspergillus lentulus* biomass

CONCLUSION

For ensuring the utility of efficient strains in remote small-scale industries/units, it is essential that suitable locally available alternates to the commercial explore suitable replacements for carbon as well as nitrogen source, so that the media could be further simplified. Since, yeast extract is very expensive and difficult to procure, suitable alternatives to such conventional media components, were explored in order to facilitate the applicability of the treatment process in small-scale industrial units in rural areas. However, as the Cu (II) concentrations increased above 200 mg L^{-1}, growth as well as Cu removal was inhibited. These results indicate that above substitutes can effectively support the effluent treatment, if the Cu (II) concentrations are up to 100 mg L^{-1}.

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