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Accumulation of Cu by Microalgae Scenedesmus obliquus and Synechocystis sp. PCC 6803

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Abstract: *This study focused on the use of microbial potential for metal ion removal. Scenedesmus obliquus and Synechocystis sp. PCC 6803 were compared for accumulation of Cu in batch mode. During time course accumulation, S. obliquus accumulated Cu up to 124 μg mg^{-1} dry wt after 1h of incubation, whereas for Synechocystis sp. PCC 6803 it reached up to 64.9 μg mg^{-1} dry wt after 2h. Low biomass concentration were found more efficient in accumulating Cu with a value of 178 μg Cu mg^{-1} dry wt at 0.3 mgL^{-1} biomass concentrations of S. obliquus. However, for Synechocystis sp. PCC 6803 the maxima was reached at 0.6 mgL^{-1} of biomass concentration. Cu accumulation reached steady-state at a concentration of 1.5 μg mL^{-1} Cu in the external medium for Synechocystis sp. PCC 6803, whereas for S. Obliquus the steady-state was achieved at 2.5 μgmL^{-1} Cu. Maximum accumulation was observed between pH ranges of 5-7. Adsorption isotherm study depicted Freundlish model for S. obliquus, where as binding in Synechocystis sp. PCC 6803 followed the Langmuir model. As Freundlich principle indicates a reversible binding, which is regulated by van der Waals forces, it is obvious that the desorption/ recovery of the adsorbed metals could be much easier as compared to Langmuir binding, which is guided by chemical forces. Thus, this study suggests that the biomass of S. Obliquus hold immense potential for use as biosorbent in the removal/ recovery of heavy metal ions.*
Keywords: *Scenedesmus obliquus; Synechocystis sp. PCC 6803; Biosorption; Cu; Adsorption isotherms*

I. Introduction

Industrial and municipal wastewaters often contain metal ions. These metal ions, when present in higher quantity, can harm aquatic and human life. Current methods for such wastewater treatment include precipitation, coagulation/floatation, sedimentation, filtration, membrane processes, electrochemical techniques, ion exchange, and chemical reactions, but each of these methods has its merits and limitations in application. Some of the present methods of clean-up result in production of harmful by-products (Gardea-Torresdey et al., 1998). Environmental friendly processes need to be developed to clean-up the environment without creating harmful waste by-products. The adsorption process with activated carbon is attracted by many scientists because of its effectiveness for the removal of heavy metal ions at trace quantities. But the process has not been used extensively owing to its high cost (Selvi and Jeyanthi, 2004).

Now days, the application of microbial biosorption is emerging as a viable alternative. Biosorption exploits the ability of microbial and plant biomass to sequester heavy metal ions from aqueous solution by physicochemical mechanisms. Charged groups such as carboxylate and hydroxyl present in the biomass cell walls are responsible for the adsorption of metal ions (Gardea-Torresdey et al., 1990; Kaplan et al., 1987). The living microbes are obvious choice because of their unlimited capacities of cleaving organo-metallic complexes and accumulating other inorganic ions such as ammonium, nitrate and phosphate (Malik, 2004). Algae are native to the vast array of freshwater and marine environments, and can be grown in large quantities with relative ease (Wong et al., 2000). The metal biosorption involves a variety of mechanisms, which can differ quantitatively and qualitatively, in agreement with the species used, the origin of biomass, and several other factors (Volesky and Holan, 1995; Schiewer and Volesky, 1996).

Till date various investigators have explored the potential of microalgae including cyanobacteria for biosorption of heavy metals. Oliveira et al. (2011) have studied the potential of using *Sargassum* biomass from Brazil as a biosorbent for Sm (III) and Pr (III) from synthetic solutions and the results were promising for using it as a biosorbent. Chromium accumulation studied by Mohanty et al., 2006 by using water hyacinth (*Eichhornia crassipes*) as a biosorbent.

Aphanothece halophytica and *Chlorella sorokiniana* were also found to sorb and desorb Zn and Cd, respectively with 10mM EDTA and 0.1N HCl (Incharoensakdi and Kitjaharn, 2002; Akhtar et al., 2003). 0.1N HCl was also found highly effective in uranium desorption from *Microcystis aeruginosa* sample (Li et al., 2004). These findings reflect the possibility of using such organisms for removal/ recovery of harmful/ precious metals from the environment. In this report we assess the ability of two microalgae, *Synechocystis* sp. PCC 6803 and *Scenedesmus obliquus* for accumulation of Cu with special reference to the adsorption isotherms.

II. Materials And Methods

A. Organisms and growth conditions

The test organisms, *Scenedesmus obliquus* and *Synechocystis* sp. PCC 6803 were grown respectively in Chu-10 (Gerloff et al. 1950; pH 6.8) and BG-11 media (Stainier et al. 1971; pH 8.0) under 72 µmol photon m^{-2} s^{-1} PAR light intensity and a photoperiod of 14:10 h at 25 ± 1^0C. At the beginning of each metal uptake experiment, logarithmic phase cultures were diluted appropriately in the growth medium and supplemented with a designated amount of metal chloride solution. The cultures were then incubated under the standard growth conditions in a shaker at 50 rev min^{-1} without any gas purging. Growth was measured in terms of dry weight. Milli-Q water and acid-soaked glasswares were used for all experiments. All the reagents were of Merck grade.

B. Time-course study

Exponentially grown cells of *Scenedesmus obliquus* and *Synechocystis* sp. PCC 6803 were taken for the study. Cells in growth medium alone (no metal ion addition) and in growth medium containing 2µgmL^{-1} of Cu ion were incubated under standard growth condition in a shaker at 50 rev min^{-1} (replicates: 4). At timed intervals after the addition of metal, 3 ml portion of each culture was collected and subjected to centrifugation. Supernatant were collected and analyzed for residual metal concentration by Ion Analyzer (model 757 VA Computrace, Metrohm, Switzerland).

C. Effects of metal and biomass concentrations

Twenty-five milliliter growth medium containing various concentrations (0.5-4µgmL^{-1}, at an interval of 0.5µgmL^{-1}) of Cu were taken in 100 ml Erlenmeyer flasks. After introducing the test organisms, flasks were agitated in the shaker at 50 rev min^{-1} under continuous light for 1h in case of *S. obliquus* and 2h for *Synechocystis* sp. PCC 6803. 3 ml samples were withdrawn at the stipulated time. Samples were centrifuged and supernatant were collected to determine the residual metal content in aqueous phase. Similarly, to study the impact of biomass concentrations, different quantities of biomass were suspended in 25 ml of growth medium with 2 µgmL^{-1} Cu and incubated for the stipulated time. Residual metal content were determined as described above.

D. Effect of pH

To find out the impact of pH, growth medium containing test metal with different pH values were prepared with 1N NaOH or HCl ranging from 3.0 to 10.0 at an interval of 1.0, before introducing the cells into the medium.

E. Isotherm studies

The relation between the amount of metal adsorbed by an adsorbent and the concentration of the adsorbet at a constant temperature is called the adsorption isotherm. These mathematical models provide information on biosorption mechanisms and surface behaviour of biosorbent. Results were analysed following Freundlich and Langmuir isotherms.

III. Results And Discussion

A. Time-course study

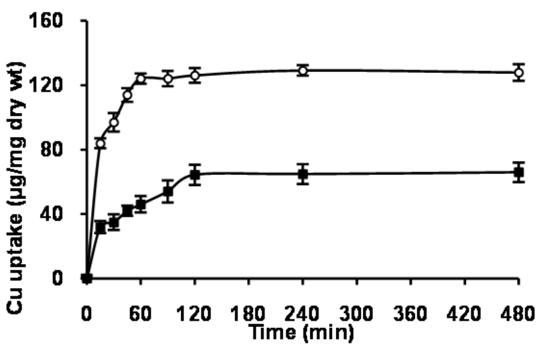

Fig. 1: Time-course accumulation of Cu by *S. obliquus* (○) and *Synechocystis* sp. PCC 6803 (■).

Fig. 1 compares the time-dependent accumulation of Cu by *Scenedesmus obliquus* and *Synechocystis* sp. PCC 6803. The time-course metal accumulation showed an initial very rapid i.e. about 70-80% of accumulation was completed within the first 30 min of initial contact of metal-bearing solution, and the level of accumulation for Cu achieved equilibrium within 60 min with a value of 124.2 ± 6.4 µg Cu mg^{-1} dry wt for *S. obliquus,* whereas for *Synechocystis* sp. PCC 6803 the equilibrium was recorded after 2h with the maxima of 64.9 ± 4.5. *Microcystis* cells immobilized by sodium alginate demonstrated an accumulation up to 108.5 ± 8.4 µg Cu mg^{-1} dry wt under packed-bed reactor operation (Pradhan and Rai, 2000). However, for dead biomass of *Microcystis* (field-isolated), *Microcystis* (laboratory-grown), *Spirogyra* and *Lemna* the accumulation values were respectively, 24.2, 22.9, 16.9 and 25.8 µg Cu mg^{-1} dry wt. (Singh et al., 2000). For living cells of *Chlorella vulgaris* an accumulation up to 35.3 ± 2.45 µg Cu mg^{-1} dry wt. was recorded under batch mode study (Mallick, 2003). This implies that *Scenedesmus obliquus* has a much greater potential for Cu accumulation.

B. Effect of biomass concentration

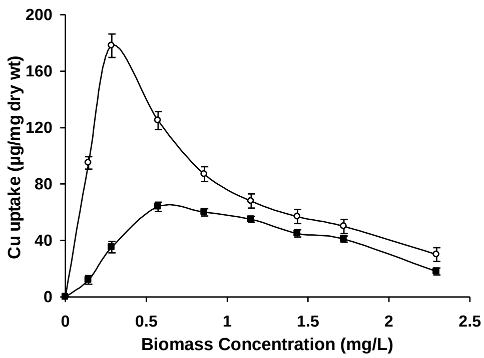

Fig. 2: Accumulation of Cu by *Scenedesmus obliquus* (○) and *Synechocystis* sp. PCC 6803 (■) at different biomass concentrations.

The biomass concentration was found to have profound impact on metal accumulation. The amount of Cu accumulated per unit weight was maximal at lower biomass concentrations and decreased with increasing amount of biomass, except for the cell concentrations up to 0.3 mgL^{-1} for *S. obliquus,* and 0.6 mgL^{-1} for *Synechocystis* sp. PCC 6803 (Fig. 2). *S. obliquus* was found to accumulate about 178.3 ± 7.4 µg Cu mg^{-1} dry wt at a biomass concentration of 0.3 mgL^{-1}, whereas the value decreased to 40.4 ± 4.2 µg Cu mg^{-1} dry wt at 2.4 mgL^{-1} of biomass. But for *Synechocystis* sp. PCC 6803 maximum accumulation of Cu was recorded at a biomass concentration of 0.6 mgL^{-1}. This reduced metal accumulation at higher biomass concentrations could be attributable to the electrostatic interactions because more cations are adsorbed on the cell when the cell distances are greater (Itoh et al., 1975), or it could also be likely that higher cell concentration might lead to formation of cell aggregates, thereby reducing the effective biosorption area (Aksu and Kutsal, 1998).

C. Impact of Cu ion concentration

Impact of Cu ion concentrations on accumulation by *S. obliquus* showed an initial rise with increasing Cu concentrations up to 2.5 µgm L^{-1} Cu, after which a steady-state was achieved (Fig. 3). In *Synechocystis* sp. PCC 6803 the steady state was however, observed at 1.5 µgmL^{-1} Cu, thus reflecting the availablability of lesser number of Cu binding sites in *Synechocystis* sp. PCC 6803 as compared to *S. obliquus.*

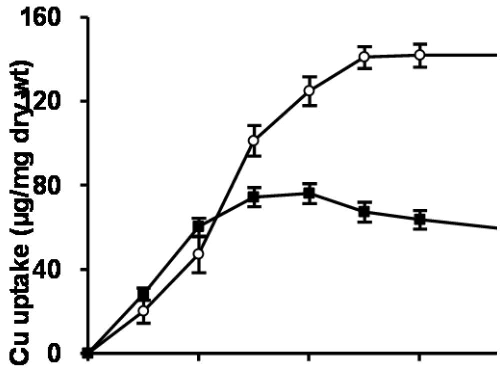

Fig. 3: Uptake of Cu per unit biomass of *Scenedesmus obliquus* (○) and *Synechocystis* sp. PCC 6803 at different metal concentrations.

D. Effect of pH

Accumulation of Cu by *S. obliquus* and *Synechocystis* sp. PCC 6803 was affected significantly by acidic pH ranging from pH 3 to 5 (Table 1). The maximum uptake was observed between pH ranges of 5-7. However, a decreasing trend was observed at higher pH. The observed low metal uptake at pH 3 and 4 could be owing to the strong competition from hydrogen ions for binding sites on the cell surface. Moreover, the role of carboxyl groups could not be ruled out. The acid dissociation constants (pKa) for carboxyl groups have been reported to be around 3-4 (Hunt, 1986). Thus as pH decreases, these groups become protonated and repel the positively charged ions. However, a decreasing trend at higher pH may be ascribed to metal-hydroxylation yielding metal-hydroxides or hydrated oxides, which leads to metal passivation (reference to Pourbaix diagram), thereby lowering the metal availability for accumulation.

Table 1: *Impact Of Ph On Cu Accumulation By S. Obliquus And Synechocystis* Sp. Pcc 6803

pH	Cu accumulation (µg Cu mg^{-1} dry wt)	
	S. obliquus	*Synechocystis* sp. PCC 6803
3.0	63.5 ± 3.8	nd
4.0	80.4 ± 2.7	16.3 ± 3.2
5.0	103.5 ± 5.9	50.8 ± 2.1
6.0	111.8 ± 3.2	59.3 ± 1.9
7.0	124.4 ± 4.1	68.2 ± 3.3
8.0	85.2 ± 3.9	44.5 ± 2.5
9.0	62.6 ± 2.9	27.1 ± 4.1
10.0	nd	29.2 ± 5.7

nd: not detected.
Values are mean ± SE

E. Adsorption isotherm study

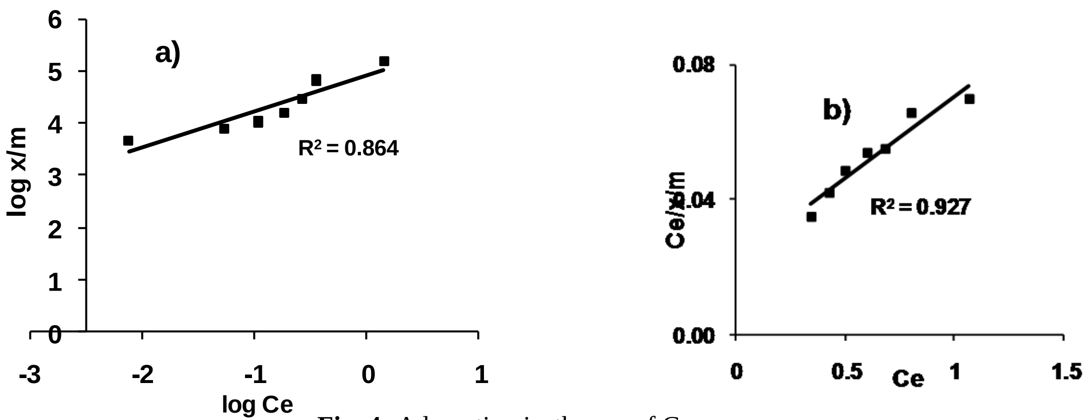

Fig. 4: Adsorption isotherms of Cu

a) Freundlich isotherm for *Scenedesmus obliquus, and*
b) Langmuir isotherm for *Synechocystis* sp. PCC 6803.

Isotherms for adsorption of Cu are illustrated in Fig.4. The binding data at equlibrium for *S. obliquus*, shown in Fig. 4, seemed to fit Freundlich isotherm. A log-log plot between the amount of Cu in the solution and in the biomass followed a straight line with a highly significant R^2 value (Fig. 4a). This isotherm suggests a multilayer sorption with heterogeneous energetic distribution of active sites having interaction between sorbed Cu ions. This physical adsorption is based on *van der Waals* forces and indicates that adsorption equlibrium is established rapidly and is generally reversible. As *van der Walls* adsorption is more a function of the adsorbate, the reduction in accumulation at alkaline and acidic pHs is ascribed to the low Cu ion availability, possibly due to formation of metal-hydroxides complexes at high pHs and competition with hydrogen ions at acidic pHs. In contrast, isotherm for Cu adsorption in *Synechocystis* sp. PCC 6803 followed the Langmuir equation (Fig. 4b), forming a unimolecular layer involving chemical forces. In chemisorption though the attachment is much stronger, the equlibrium is attained relatively slowly. Our results in Fig.1 also give support to this view as equlibrium was attained after 1h in *Scenedesmus*, whereas as 2h was required for *Synechocystis* to attain the equlibrium. Moreover, the lower accumulation potential as observed for the latter could be attributable to the monolayer binding as compared to the multilayer binding for *Scenedesmus*.

IV. Conclusion

This study concludes that the biomass of *S. obliquus* hold immense potential in accumulating Cu ions, and the accumulation potential is significantly higher than that of the immobilized *Microcystis* cells operated under packed-bed reactor, the value highest recorded for microbial species (Pradhan and Rai, 2000). Adsorption isotherm study depicted a multilayer binding for *S. obliquus.* As Freundlich isotherm indicates a reversible easy binding, which is regulated by *van der Waals* forces, it is obvious that the desorption/ recovery of the adsorbed metals could be much easier as compared to Langmuir/ BET binding, which is guided by chemical forces. Thus a suitable desorbing agent could desorb the adsorbed metals and the biomass could be subjected for repeated use as bioreactors. However, during the operation the pH and the biomass concentration should be taken into consideration for optimal performance of the algal reactor.

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