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Characteristic Study of the Marine Algae *Sargassum sp*. on Metal Adsorption

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Abstract: Problem statement: Biomass of brown marine macro algae is a biological resource that is available in large quantities and can form a good base for the development of biosorbent material. **Approach:** Algae have been found to be potentially suitable biosorbents because of its cheap availability, both in fresh or salt water, relatively high surface area and high binding affinity. **Results:** The study considered the molecular status of the biomass such as DNA, Protein and Pigment analysis on the adsorption of metal from effluent. The Molecular studies showed that DNA, protein and chlorophyll content are greatly affected because of metal adsorption. **Conclusion/Recommendations:** The Active-site prediction showed the binding site of the metal on the biomass. Based on the results obtained it can be concluded that the biomass *Sargassum sp*. has higher affinity towards the adsorption of metals.

Key words: Brown algae, greatly affected, biomass sargassum, higher affinity, metal adsorption, results obtained, active-site prediction, chlorophyll content, agarose gel, marine macro, molecular studies, higher affinity

INTRODUCTION

Biomass of brown marine macro algae is a biological resource that is available in large quantities and can form a good base for the development of biosorbent material (Chu *et al*., 1997). The brown seaweed *Sargassum sp*. is mainly constituted by the polysaccharide alginate, usually calcium and sodium alginates; thus, with a high potential for the accumulation of heavy metals, as compared to other algal genera (Da Costa and De Franca, 1996). Those polysaccharides are produced due to the interaction between alginic acid and alkaline-earth elements from the seawater. Metal ion uptake by biomass is believed to occur through interactions with the cell walls. This is due to the presence of various functional groups such as carboxyl, amino, sulphate and hydroxyl groups, which can act as binding agents, include ionic interactions and complex formation between metal cations and ligands on the surface of the seaweeds.

MATERIALS AND METHODS

Sample collection: Fresh samples of brown marine macro alga, *Sargassum sp*. were collected from rocky seashores near Uvari, Tirunelveli District, Tamilnadu. The collected samples were rinsed with distilled water for the removal of external salts and sand and then with acetone solution.

Effluent: The effluent used was obtained from Electroplating Metal Finishers, SIPCO Industrial Estate, Chennai, Tamilnadu.

DNA isolation: To study the molecular status, the biomass was soaked in the effluent for 5 hrs. The DNA was isolated from fresh and metal adsorbed biomass. To isolate the DNA the biomass was ground with lysis buffer (2% Sarcosyl, 10mM EDTA (pH 8.0), 0.1M NaCl, 20mM Tris (pH 8.0), 2% PVP (pH 7.6). It was then incubated at room temperature for two hours followed by centrifugation at 10000 x g for 10 mins, 4°C. Proteinase K was added to the supernatant and incubated at 37°C for 1.5 hours. An equal volume of phenol was added, mixed and centrifuged at 10000 × g for 10 min, 4°C. The top phase was subsequently re-extracted twice with an equal volume of 24:1 chloroform: isoamylalcohol. The DNA was precipitated with 2 volumes of ice cold 95% ethanol and left at -

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20°C overnight. The DNA was washed with 70% ethanol, partially dried and redissolved in 40 µL TE buffer (0.01M Tris (pH 8.0), 0.001 M EDTA). The isolated genomic DNA is characterized through agarose gel electrophoresis (Ho *et al*., 1995).

Pigment analyses: The photosynthetic pigments of the brown algae are Chlorophyll, carotene and xanthophylls. The amount of chlorophyll and carotene present in the leaf was estimated by the method of Arnon. 1gram of fresh/metal adsorbed algae was weighed and homogenized in mortar and pestle with 10ml of water. It was then mixed with 4.5 mL of 80% acetone and was centrifuged at 8,000rpm for 5mins. The supernatant was collected and used for the measurement of optical density at different wavelength such as 645, 663, 638 and 490 nm respectively (Arnon, 1949).

Protein estimation: Proteins were extracted using the phenol extraction method (Wang *et al*., 2003). The tissue was homogenized with extraction buffer (1.5% PVP, 0.7M Sucrose, 0.1 M KCl, 0.5 M Tris-HCl (pH-7.5), 250Mm EDTA, 2% β-mercapto-ethanol) at 4°c for 20 mins. Then, an equal volume of Tris–HCl (pH 7.5) and saturated phenol was added and the mixture was rehomogenized for 20 min at 4°C. The mixture was centrifuged at 10,000g for 20 min and the upper phenol phase was removed. The lower phase was re extracted using the same volume of phenol as above. Proteins in the phenol phase were precipitated by addition of five volumes of 0.1 M ammonium acetate dissolved in methanol and incubated at 20°C for 3 hrs. The extracted protein was estimated by Lowry's method (Lowry and Lopaz, 1946).

3D Structure predictions of the brown algae: Protein structural prediction offers a theoretical alternative to experimental determination of structures. It is an efficient way to obtain structural information when experimental techniques are not successful. It has been shown that protein structures are more conserved than protein sequences.

Active site prediction: The active site of the protein is the binding site where catalysis occurs. The purpose of the Site Finder application is to calculate possible active sites in a receptor from the 3D atomic coordinates of the receptor. It helps to determine potential sites for ligand binding in docking calculations (Brindha *et al*., 2009).

RESULT AND DISCUSSION

The isolated DNA of both the fresh and metal adsorbed biomass samples were run in agarose gel electrophoresis and bands were observed under uv-transilluminator. Figure 1 shows no clear bands were observed in the metal adsorbed biomass. It represent that the metals may affect the DNA.

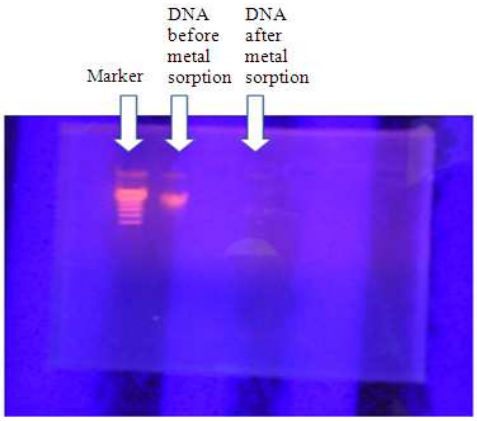

Fig. 1: Genomic DNA of *sargassum sp*. before and after metal adsorption

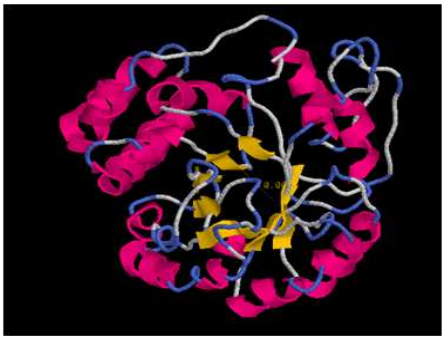

Fig. 2: 3D Structure of *sargassum sp*

Table 1: Protein estimation from the biomass *sargassum sp*

Type of extract	Absorbance at 620nm	Concentration of protein
Biomass before Metal adsorption	0.38	23
Biomass after Metal adsorption	0.30	17

Table 2: Pigment analysis

Type of chlorophyll	Before metal adsorption ($g\ l^{-1}$)	After metal adsorption ($g\ l^{-1}$)
Total chlorophyll content	0.020	0.015
Chlorophyll a	0.009	0.006
Chlorophyll b	0.0097	0.0062

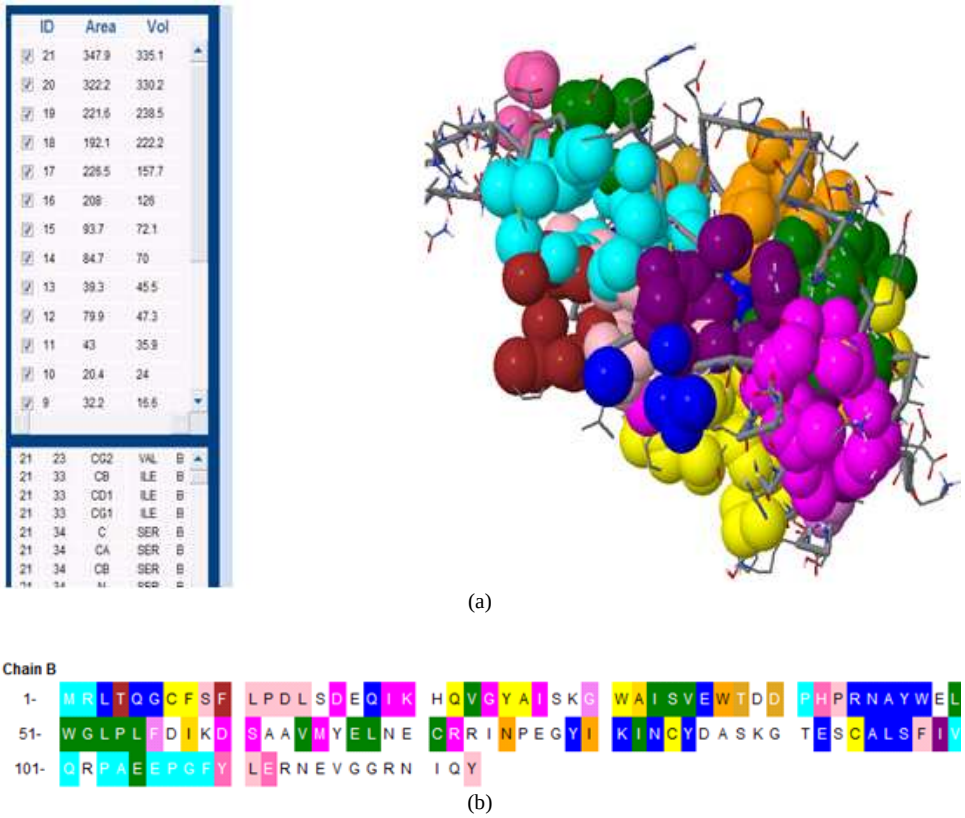

Fig. 3: Binding site of the algae

The chlorophyll and carotene test were undergone to check the amount of pigment present in both the fresh and metal adsorbed biomass and the results were tabulated and compared in Table 2. The pigment content in algae is a specific feature of each species. Its evaluation is essential as an indirect measure of cell growth, as well as a parameter to check the trophic level of waters. Chlorophyll a is usually the parameter used as the trophic indicator, mainly because the relationship between the content of this pigment and the amount of algal biomass is quite direct. Brown algae vary in coloration from olive-yellow to deep brown. The coloration is due to the accessory carotenoid pigment. The amount of pigment present in the metal adsorbed biomass is comparatively lower than the fresh algae. The result indicates that the adsorption of metal may affect photosynthetic process of the algae.

The extracted protein was estimated by Lowry's method and the concentrations were tabulated in Table 1. The result interprets that the protein concentration may reduce because of metal adsorption. Homology modeling is performed using the software MODELLER (www.salilab.org/modeller) and the energy minimization is done by swiss pdb viewer. Protein three-dimensional (3D) structure (i.e., the coordinates of all atoms) determines protein function. The predicted 3D structure of the *Sargassum sp*. is shown in Fig. 2. Active site of the protein is the binding site where catalysis occurs and this can be generated using Castp. Fig. 3 shows the binding site of the *Sargassum sp*.

CONCLUSION

Sargassum sp were selected for studying biosorption due to its originality and to assess the possibility of utilizing a waste biomass for heavy metal removal. Molecular studies showed that DNA, protein and chlorophyll content are greatly affected because of metal adsorption. Active-site prediction showed the binding site of the metal on the biomass. Based on the results obtained it can be concluded that the biomass *Sargassum sp*. has higher affinity towards the adsorption of metals.

REFERENCES

Arnon, D.I, 1949. Copper enzymes in isolated chloroplast, polyphenol oxidase in beta vulgaris. Plant Physiol., 2: 1-15. PMID: 16654194

Brindha, V., A. Saravanan and R. Manimekalai, 2009. Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from *terminalia arjuna*. Ind. J. Sci. Technol., 2: 22-26. http://indjst.org/archive/vol.2.issue.2/feb09brinda.pdf

Chu, K.H., M.A. Hashim, S.M. Phang and V.B. Samuel, 1997. Biosorption of cadmium by algal biomass: Adsorption and desorption characteristics. Water Sci. Technol., 35: 115-122. DOI: 10.1016/S0273-1223(97)00121-2

Da Costa, A.C.A. and F.P. De Franca, 1996. Biosorption of zinc, cadmium and copper by a brown seaweed (*Sargassum sp*.) in a continuous fixed-bed laboratory reactor. Bioseparation, 6: 335-341. http://www.ingentaconnect.com/content/klu/bios/1996/00000006/00000006/00136631

Ho, C.-L., S.-M. Phang and T. Pang, 1995. Molecular characterization of argassum polycystum and S.siliquosum by PCR using RAPD primers. J. Applied Phycol., 7: 33-41. DOI: 10.1007/BF00003547

Lowry, O.H. and J.A Lopaz, 1946. The determination of inorganic phosphate in the presence of labile phosphate ester. J. Biol. Chem., 162: 421-428. http://www.jbc.org/content/162/3/421.short

Wang, S.B., M. Sommerfeld and F. Chen, 2003. An optimized protocol for isolation of soluble proteins from microalgae for two-dimensional gel electrophoresis analysis. J. Applied Phycol., 15: 485-496. DOI: 10.1023/B:JAPH.0000004324.88897.b2