Investigation of Heavy Metal Removal by *Sargassum* spp from Industrial Wastewater

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***Abstract*—In the present study, the batch removal of metallic ions from wastewater using *Sargassum* spp. (brown marine algae) and the protonated algae prepared from it was examined. The two effluents of wastewater were collected around the direct municipal wastewater pipe at Naungnipin village and industrial zone (2) at Mandalay. The physicochemical characteristics of two discharge sites were studied. In this research, the removal of Pb^{2+}, Cd^{2+} and Zn^{2+} ions in semi synthetic wastewater were studied by using the two biomasses in lab scale. For Pb^{2+} ions, greater percentage removal was determined for the two biomasses. But the reducible activities of Zn^{2+} ions on two biomasses were not found. The sorption capacities of two adsorbents were analyzed before and after the experiment using Atomic Absorption Spectrophotometer (AAS). The functional groups present in marine algae was identified by using FT-IR.**

***Keywords*— adsorption capacity, Atomic Absorption Spectrophotometer, functional group, FT-IR, *Sargassum* spp.**

I. Introduction

Biosorption may be defined as the removal of substances from solution by biological material. Biosorption is a physico-chemical process and includes such mechanisms as absorption, adsorption, ion exchange, surface complexation and precipitation [1].

The biosorption rate depends on the type of the process. According to literature, biosorption can be divided into two main processes: adsorption of the ions on cell surface and bioaccumulation within the cell [2].

There are many types of biosorbents which can be used in the biosorption process. In this process, a wide range of microbial biomass types was investigated. These include archaea, bacteria, cyanobacteria, algae (including macroalgae, i.e. seaweeds) and fungi, the latter including filamentous form as well as unicellular yeast, fruiting bodies (mushrooms, brackets, etc) and lichens[3].

Gram-positive bacterial cell walls contain Peptidoglycan carboxyl groups which are the main binding sites for metal cations with phosphate groups contributing significantly in and polysaccharides are the other bacterial metal-binding components [5].

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Gram-negative species [4]. Proteinaceous S-layers, and sheaths largely composed of polymeric materials including proteins and polysaccharides are the other bacterial metal-binding components [5].

Fungal cell walls are complex macromolecular structures predominantly consisting of chitins, glucans, mannans and proteins, but also containing other polysaccharides, lipids and pigments, e.g. melanin. [6]. But heavy metals have dangers effect on biological treatment, because they have toxic effect on microorganism, e.g. bacteria [7].

Seaweeds are one of the types of macroscopic biomass known for their metal-sorbing potential. In several ocean locations, marine macroalgae are abundant and of very fast growth, which allows them to be easily collected in large quantities [8].

In brown seaweeds, the capacity of removing several metallic species is attributed to the biochemical constitution of their cellular wall, which is basically composed by three types of biopolymers: alginate, fucoidan and cellulose, which might provide several functional groups as binding sites (amino, carboxyl, sulfates) [9].

In this study, brown algae were easily collected from the Myanmar costal region and its metal binding capacities were determined by AAS and FT-IR techniques. The aim of this research is to reduce heavy metal from industrial wastewater.

II. Materials and Methods

A. Sample Collection of seaweed

Fresh brown seaweed drifts containing of *Sargassum trichophyllum, S. salicifoloides, S. kasyotenese, S.tenerrimun, S. carpophyllum, S. duplicatum, S. ilicifolium, S.cristaefolium, S. plagiophyllum,S. swartzii, S .polycystum* were collected from Shwe Thaung Yan Beach in Ayeyawady region. The wet seaweeds were cleaned from impurities materials with fresh water. After washing the seaweed biomass, it was sun-dried for 2-3days.

B. Preparation of sorbents

The biomass was necessary to eliminate the soluble component such as alginate and the light metals Ca^{2+},Mg^{2+},etc. in the biomass by protonating it with 0.1 N HCl (10 g biomass/L). After five hours of contacting with acid, the biomass was rinsed with deionized water to remove the excess acid. Then the biomass was dried in an oven at 40-60 °C overnight.

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C. *Site selection of industries wastewater*

The effluent samples of industrial wastewater were collected from the municipal directed effluent pipe at Naungnipin village and near Truck Bus Terminal on 67th street, Pyigyitagon Township, Mandalay Region, Myanmar as shown in figure 1.

(a)Sample 1 *(b) Sample 2*

Fig. 2 Collection sites

D. *Characterization of wastewater*

The physiochemical characteristics of effluent 1 and effluent 2 were determined in Table 1.

E. *Adsorption capacities of algae*

The brown algae, *Sargassum spp* can be used in wastewater treatment of industries. These experiments were carried out in 250 ml conical flasks containing 1 g of dried biomass and 100 ml industrial wastewater and 100 ppm of each metal was added to this solution. These flasks were placed on shaker incubator with constant shaking at 150 rpm and 28˚C. After the seperation of used biosorbent by using filter papers the residual concentration of each metal in industrial wastewater were determined using atomic absorption spectrophotometer.

The percentage of adsorbed metal ions per gram of dead biomass was caculated by using the following equation;

$$reduction\% = \frac{Initial\ amount - Final\ amount}{Final\ amount} \times 100$$

The adsorption capacities were determined by the simple concentration different methods. When the initial and final metal concentration, C_0 and C_i, respectively, were determined by AAS, the metal ion binding q was calculated from the mass balance as follows

$$q = \frac{(C_0 - C_i)V}{W}$$

where V is the solution volume and W is the mass of sorbent.

F. *Fourier Transform Infrared Spectroscopy (FT-IR)*

In order to identify functional groups in the brown algae, the FR-IR was used. The information on the nature of the bands present on the surface of the algae before and after the biosorption process can be known by using FT-IR techniques.

III. RESULTS AND DISCUSSION

A. *Characterization of discharge wastewater*

Both the effluents 1 and 2 of industrial wastewaters were analyzed their physiochemical parameters. According to the data showed in Table 1, the effluent 1 and effluent 2 increased in the value TS, TDS and TSS. In the data analysis, the BOD and COD values of effluent 1 were higher number than effluent 2. The effluent 1 should be treated because this value was a little more than the limit values of EPA's standards. The effluent 1 was lower number in the dissolved oxygen level than the effluent 2. According to the AAS data, the heavy metals content of these effluents were not as high as the EPA's typical wastewater standard. But heavy metal content accumulated in the discharge sites can be lead to the toxicity condition. Thus, the wastewater treatment was to be required.

TABLE I. PHYSICAL AND CHEMICAL PROPERTIES OF INDUSTRIAL EFFLUENT 1 & 2

No.	Samples / Contaminants	Sample1	Sample 2
1	Solids, total(TS)	3530 mg/l	1290 mg/l
2	Dissolved, total (TDS)	1686 mg/l	1294 mg/l
3	Settleable solids (TSS)	1815 mg/l	1330 mg/l
4	BOD	1350	44
5	COD	3500	146
6	DO	0.10	0.04
7	pH	5	7.6
8	$CdSO_4$	0.041 mg/l	0.076 mg/l
9	$Pb(NO_3)_2$	0.029 mg/l	0.040 mg/l
10	$Zn(SO_4)$	0.028 mg/l	0.021 mg/l

B. *Identification of functional groups present in Sargassum spp.*

The results of FTIR spectrum are shown in figure 2 that different functional groups were detected on the surface of the algal biomass. The band at 1029 cm^{-1} was attributed to alcohol groups. The band at 1228/1236 cm^{-1} was attributed to sulphate (SO_3) groups present in the fucoidan biopolymer. The 1408 and 1612 cm^{-1} bands were indentified to carboxylic (COOH) groups present in the alginate biopolymer.

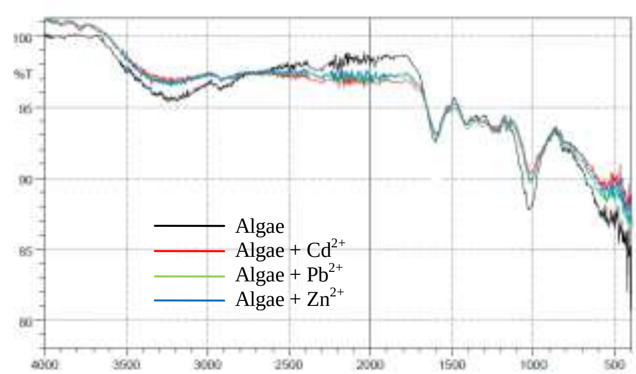

Figure.2 Fourier Transform Infrared (FT-IR) Spectra of *Sargassum spp.* and Saturated with Cd^{2+}, Pb^{2+} and Zn^{2+}.

C. Identification of functional groups present in protonated Sargassum spp.

The functional groups of protonated *Sargassum spp*. are shown in figure 3. According to the spectra results, the functional groups of protonated algae were as same as the functional groups of natural surface algae. The peaks detected in spectra were indentified at 1018 cm^{-1} (alcohol groups), 1231 cm^{-1} (sulphate groups, SO_3), 1408 $cm^{-1}$and 1602 cm^{-1} (carboxylic groups, COOH). The broad peak value at 3178cm^{-1} was indentified amino ($-NH_2$) groups.

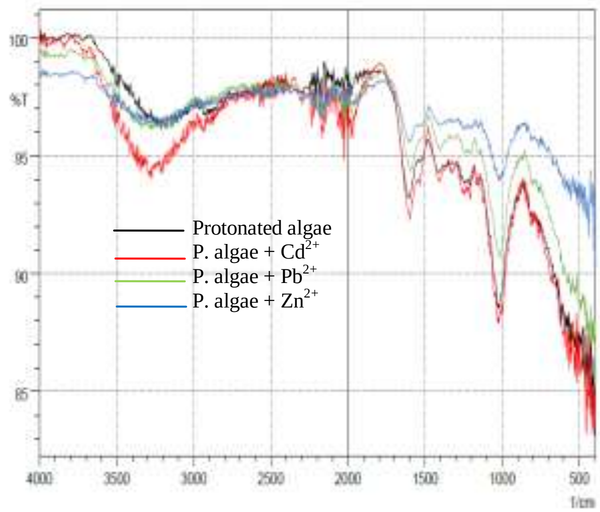

Figure 3. Fourier Transform Infrared (FT-IR) Spectra of Protonated *Sargassum spp*. and Saturated with Cd^{2+}, Pb^{2+} and Zn^{2+}

D. Changes after the bioadsorption of different metallic ions by Sargassum spp

The spectra obtained for the brown seaweed algae *Sargassum spp* before and after the biosorption of different metallic ions are shown in figure 3. The change suffered by the bands after the biosorption of metallic ions by these algae is shown in Table 2.

Shifts in the 1020 and 3190 bands can be seen, which represent the alcohol group in the alginate biopolymer. It can be observed that these groups are participating in the biosorption process of all metallic ions, since the ions cause the shifting of this band.

By analysing the shift in 1228 cm^{-1} band, its disappearance after the biosorption of Cd^{2+} and 1249 and 1217 after the bioadsorption of the remaining ions such as Pb^{2+} and Zn^{2+} can be observed, indicating that the SO_3 groups, present in the fucoidan biopolymer are involved in the bioadsorption process of these ions.

The 1409 cm^{-1} disappeared in the presence of Cd^{2+} ions, and were shifted to Pb^{2+} and Zn^{2+}, indicating the importance the carboxylic groups present in the alginate biopolymer.

The band at 1598 cm-1 cannot be seen in shifting, which represents amino groups present in proteins So the bands corresponding to alcohol, sulphate and carboxylic groups indicate the strong participation of these groups in the bioadsorption process.

TABLE II. FT-IR BANDS OF WASTEWATER TREATED WITH *SARGASSUM SPP*.

Sargassum **(cm^{-1})**	**After removal of metallic ions**		
	Cd^{2+} (cm^{-1})	**Pb^{2+} (cm^{-1})**	**Zn^{2+} (cm^{-1})**
1020	1026	1014	1031
1228	-	1249	1217
1409	-	1408	1408
1598	1598	1598	1598
3190	3248	3234	3234

E. Changes after the bioadsorption of different metallic ions by protonated Sargassum spp.

The spectra obtained for the protonaed algae before and after the biosorption of different metallic ions are shown in figure4. The change suffered by the bands after the biosorption of metallic ions by these algae is described in Table 3.

Shifts can be observed in the bands of 1018 and 3178 cm^{-1} which represent the alcohol groups in the alginate biopolymer. The band at 1018 cm^{-1} presented a more significant shifting in the presence of Cd^{2+}. The bands at 3178 cm^{-1} presented a significant shifting in the presence of Cd^{2+}, Pb^{2+} and Zn^{2+} ions, indicating the importance of the alcohol group in removing metallic ions. The bands corresponding to the mannuronic and guluronic groups forming the alginate biopolymer were shifted after the ion bioadsorption process, showing the effective participation of these acids in the bioadsorption process.

TABLE III. FT-IR BANDS OF WASTEWATER TREATED WITH PROTONATED *SARGASSUM SPP*.

Protonated. *Sargassum* (cm^{-1})	**After removal of metallic ions**		
	Cd^{2+} (cm^{-1})	**Pb^{2+} (cm^{-1})**	**Zn^{2+} (cm^{-1})**
1018	999	1022	1022
1231	1236	1228	-
1408	1408	1408	1408
1602	1602	1585	1604
3178	3282	3199	3253

F. Bioadsorption capacities of Sargassum spp. and protonated algae

The metal uptake capacities of two adsorbents in the solution including different metallic ions are shown in figure 4. In the comparative adsorption capacities of both biomasses, the *Sargassum spp.* had more bioadsorption capacities than the protonated algae when Cd^{2+} ion was present in the solution. For the Pb^{2+} ion, the protonated *Sargassum spp*. was slightly increased in metal sorption capacities than the dried biomasses of *Sargassum spp*. Among three metals, Zn^{2+} was not shown in the uptake capacities by the two biosorbents.

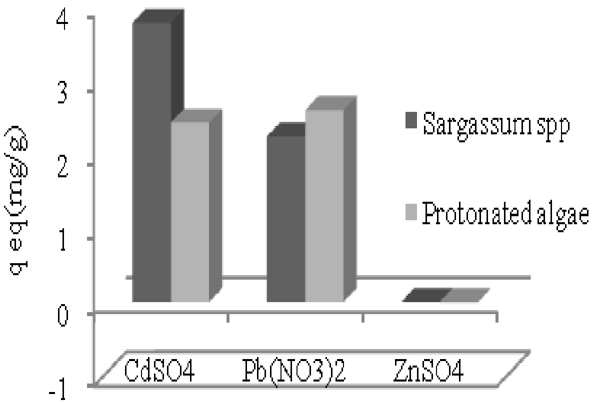

Figure. 4 Metal Uptake Capacities of Two Biomasses

IV. Conclusions

In this paper, it can be focused on the adsorption of Cd^{2+}, Pb^{2+} and Zn^{2+}ions in the industrial wastewater by the natural dried biomass of *Sargassum spp* and the protonated *Sargassum spp*.. Although the two algae biosorbents reduced the two metals, Cd^{2+} and Pb^{2+}, these biosorbents were not capable of the metal uptake when Zn^{2+} ion presents in the solution. Among them *Sargassum spp.* reduced Cd^{2+} was the highest adsorption capacity. All these experiments were made in the laboratory scale. From the FT-IR results, it can be known that the algae present the functional groups which can uptake heavy metal ions. The algae biosorbents are safe the time, cost and energy, high uptake capacity and suitable for aerobic and anaerobic effluent treatment units. Therefore, it can be concluded that the brown algae can be used as biosorbents in the appropriate bioreactors.

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References

[1] Gadd GM, Fungi and yeasts for metal binding in microbial Mineral Recovery, ed. by Ehrlich H and Brierley CL. McGraw-Hill, New York, pp.249-275 (1990).

[2] G.M. Gadd, "Heavy metal accumulation by bacteria and other microorganisms Experentia," Vol. 46, pp. 834-840, 1990.

[3] Munoz R, Alvarez MT, Munoz A, Terrazas E, Guieysse B and Mattiasson B, Sequential removal of heavymetals ions and organic pollutants using an algal-bacterial consortium. *Chemosphere* 63:903–911 (2006). https://doi.org/10.1016/j.chemosphere.2005.09.062

[4] Gadd GM, Fungi and yeasts for metal binding in microbial Mineral Recovery, ed. by Ehrlich H and Brierley CL. McGraw-Hill, New York, pp.249-275 (1990).

[5] G.M. Gadd, "Heavy metal accumulation by bacteria and other microorganisms Experentia," Vol. 46, pp. 834-840, 1990.

[6] Munoz R, Alvarez MT, Munoz A, Terrazas E, Guieysse B and Mattiasson B, Sequential removal of heavymetals ions and organic pollutants using an algal-bacterial consortium. *Chemosphere* 63:903–911 (2006). https://doi.org/10.1016/j.chemosphere.2005.09.062

[7] McLean JS, Lee J-U and Beveridge TJ, Interactions of bacteria and environmental metals, fine-grained mineral development, and bioremediation strategies, in *Interactions Between Soil Particles and Microorganisms*, ed. by Huang PM, Bollag J-M and Senesi N. John Wiley and Sons, New York, pp. 228–261 (2002).

[8] Davis TA, Mucci A and Volesky B, A review of the biochemistry of heavy metal biosorption by brown algae. *WaterRes* 37: pp. 4311–4330 (2003). https://doi.org/10.1016/S0043-1354(03)00293-8

[9] Kratochvil D and Volesky B. Advances in the Biosorption of Heavy Metals. *Trends Biotechnology*. 1998; 16: pp 291-300. https://doi.org/10.1016/S0167-7799(98)01218-9

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