Biosorption of Lead (Pb^{2+}) by using *Chlorella vulgaris*

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***Abstract*—**Algae are interested for carbon fixation from green house gas for waste water treatments and it has the high potential for production of biodiesel. The adsorption of heavy metals onto the surface of the algae is alternative technique for current wastewater treatment systems. Thus, this study was conducted to determine the biosorption characteristic of algae with lead. In this study, the cultivation of algae *Chlorella vulgaris* in closed pond with different amount of carbonated water (25, 50, 75, 100, 200 and 300 ml of soda per 1 L of culture) was studied along the biosorbent collection and found that the additional carbonated water shortens the accelerated growth phase of the algae cultivation by 24 hours. Algae biomass were harvested with ferric chloride coagulation and dried at 60°C for 1 day. Acid treatment of the biosorbent was also conducted with 0.2 M H_2SO_4 (1 L of acid per 1 g of algae biomass). The size of the selected algae biosorbent is less than 75 µm. In biosorption, optimum pH of the adsorption (6, 5, 4, 3) and kinetic experiment for optimum adsorption time (5, 15, 30, 90, 180, and 360 mins) were investigated. The resulting samples were analyzed with Atomic Absorption Spectrophotometer (AAS). The optimum pH for the biosorption is 6 with 99.4% removal efficiency. The kinetic of the biosorption of Pb^{2+} by *Chlorella vulgaris* followed the pseudo-second order model. The values of parameters k_2, q_e and correlation coefficient R^2 were 0.186×10^{-3}, 250 mg/g and 0.997 respectively. The initial Pb concentration also varied 51.79, 103.59, 207.19, 310.78 and 414.38 mg/L for determination of the adsorption isotherm of heavy metal on algae. The biosorption isotherm of lead on *C. vulgaris* follows Langmuir isotherm model**.**

***Keywords*—**algae cultivation, biosorption, *chlorella vulgaris*, heavy metal, lead removal.

I. Introduction

MICROALGAE, which means including prokaryotic photosynthetic microorganisms such as cyanobacteria, are organisms that play a key role in aquatic ecosystems. It is estimated that around 40% of global photosynthesis is performed by microalgae. Microalgae form the basis of most of trophic aquatic chain. The use of microalgae in biotechnology has been increased in recent years, these organisms being implicated in food, cosmetic, aquaculture and pharmaceutical industries [1].

Heavy metals are among the major concerns in wastewater treatment. Heavy metals are often derived from heavy industry, such as electroplating and battery factories. The treatment of this type of wastewater involves high cost techniques such as ion exchange, evaporation, precipitation, membrane separation etc. However, these common techniques are too expensive to treat low levels of heavy metal in waste water. Therefore a low cost biosorption process using algae as an adsorbent has lately been introduced as an alternative [2].

Another issue from burning of fossil fuels is the ever-increasing carbon dioxide (CO_2) emission, whose trend will continue with the fast pace of modern industry development if a feasible energy source replacement could not be found. Algae, which can assimilate CO_2 photoautotrophically or mixtrophically, is a perfect candidate for CO_2 sequestration and greenhouse gas reduction. The concept of using wastewater as a medium and source of nutrients for algae production has the benefit of serving both environmental and energy-related needs. Compared to the conventional wastewater treatment process, which introduces activated sludge, a biological floc, to degrade organic carbonaceous matter to CO_2, algae can assimilate organic pollutants into cellular constituents such as lipid and carbohydrate, thus achieving pollutant reduction in a more environment-friendly way [3].

Metal pollution is one of the most important environmental problems today. Three kinds of metals are of concern, including toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclide (such as U, Th, Ra, Am, etc.) Metals can be distinguished from other toxic pollutants, since are non-biodegradable and can accumulate in the living tissues, thus becoming concentrated throughout the food chain [4]. This work is to study the removal efficiency of lead from water by the algae *Chlorella vulgaris*. The most common sources of lead are lead-based paint, contaminated soil, household dust, drinking water, lead-glazed pottery and the effluents of industrial wastewaters.

Using algae for wastewater treatment offers some interesting advantages over conventional wastewater treatment. Advantages of algae wastewater treatment are:

- Cost effective
- Low energy requirement
- Reductions in sludge formation

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- Green house gas emission reduction
- Production of useful algal biomass [5].

Biosorption is a physiochemical process that occurs naturally in certain biomass which allows it to passively concentrate and bind contaminants onto its cellular structure. Though using biomass in environmental cleanup has been in practice for a while, scientists and engineers are hoping this phenomenon will provide an economical alternative for removing toxic heavy metals from industrial wastewater and aid in environmental remediation.

II. Experimental Methods

A. Cultivation of Algae

Chlorella vulgaris in lag phase used in the experiments was inoculated in Cornway Medium. It was cultivated outdoors in growth media containing ammonium sulfate NH_4SO_4 (0.16 g/l), Urea $CO(NH_2)_2$ (0.016 g/l), T-super P_2O_5 (0.016 g/l) made up in tap water. The pH was maintained near 5.5 with aeration.

The first inoculation was done in 3 flat bottomed flasks (1 L). Then it was transferred to two 5 L plastic bottles. When the desired density of algae was reached, the algae from 2 bottles were cultivated in the 40 L glass tank. The algae were cultured for 1 week per batch. The cells were harvested by coagulation with ferric chloride and filtration.

B. Effect of Carbonated water concentration on Cultivation

During cultivation of algae, CO_2 is needed for the photosynthesis process of the algae. The effect of the carbonated water (soda) concentration cultivation of *Chlorella vulgaris* was also determined. The amount of the carbonated water was varied from 0 to 300 ml. The other nutrients were same as above cultivation. The samples were taken out daily and counted the cells numbers with Neubauer hemocytometer on the microscope.

C. Preparation of Biosorbent

The harvested biomass was extensively washed two times with water (1 L of de-ionized water per 1 g of biomass) to get rid of medium remnants. Then it was dried in the oven (at 60°C) overnight and pulverized. Acid pre-treatment was then done by washing the sorbent with 0.2 M solution of H_2SO_4 (1 L of acid per 1 g of biomass for 90 minutes), washing twice again by de-ionized water, filtering with filter paper and drying 24 hours at constant temperature of 60°C. After drying, the algae biomass was pulverized and separated the different sizes of algae by sieves shaker.

D. Determination of Effect of pH on Lead Biosorption

A series of experiments, with 207.2 mg/L (1 mmol/L) Pb solutions, were conducted under different pH to investigate the effect of pH on the adsorption. The pH was first adjusted to a designed value, from 3.0 to 6.0 with H_2SO_4 or NaOH. Then it was measured hourly and maintained steady throughout the experiment. The resulting solution was analyzed with Atomic Absorption Spectroscopy, AAS, at Universities' Research Centre, University of Yangon.

E. Kinetic Experiment

Kinetic studies provide information relating to time required for the establishment of sorption equilibrium. They also give information about the reactant species that govern the rate of the reaction and up to what order. Various models can describe the transient behavior of a batch adsorption process. Most of these have been reported as pseudo-first-order and some as pseudo-second- order kinetic processes.

Kinetic experiments were performed in continuously stirred beakers containing 500 ml metal solutions at initial concentrations 207.2 mg/L and 0.5 g of biosorbent at room temperature (around 30°C). Samples of 50 ml solution were withdrawn at scheduled time intervals and analyzed for residual Pb concentration by the Atomic Absorption Spectroscopy (AAS).

F. Biosorption Isotherm

To determine the adsorption isotherm, the initial concentration of the metal solution must be varied at a constant contact time. For equilibrium studies, the concentrations of Pb solutions will be varied from 51.79 to 414.38 mg/L and biosorbent dosage was 2 g/L. The pH will adjust to 6.0 using H_2SO_4 or NaOH hourly throughout the experiment. The mixtures will be agitated on a magnetic stirrer for 6 hr at constant temperature and then set still 12 hr. Then the biosorbent will be filtered through an acid-cleaned filter paper.

III. Results And Discussions

For the effect of the carbonated water concentration on cultivation of *Chlorella vulgaris*, the resulting growth curves are as shown in Fig. 1 and Fig. 2 according to the initial concentration of the algae (0.95 and 1.25 × 10^9 cells respectively).

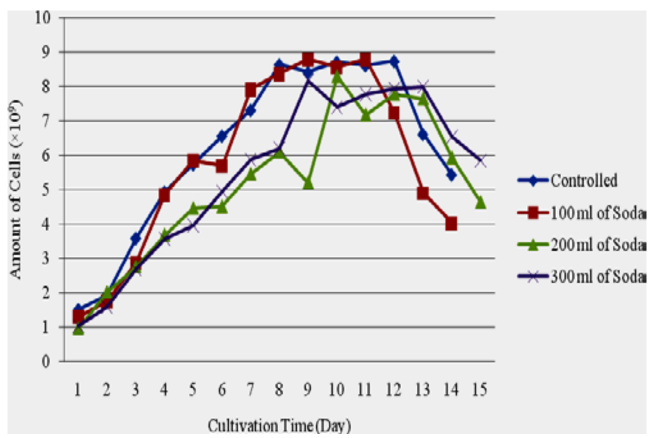

Fig. 1 The Algae Growth Curves with and without High Carbonated Water

According to the curves in Fig. 1, the cultures of without additional CO_2 and with 100 ml of additional CO_2 give similar growth curve and the higher carbonated water concentrations on cultivation give unfavourable growth curve because of the inhibition effect on algae growth. The lag phase and accelerated growth phase is found in day 1 and 2. The exponential growth phase is from day 2 to 7. In day 8, it shows the decelerated growth phase and the stationary phase is day 9 to day 11 or 12. Then, the death phase is found on day 13 onwards.

In Fig. 2, the lag phase of second batch cultivation is in day 1 and the accelerated growth phase is in day 2 to 3 and found that the additional carbonated water shortens the accelerated growth phase of the algae cultivation by 24 hours. The exponential growth phase is from day 3 to 9 and the decelerated growth phase was in day 10. The stationary phase is stable only in culture without soda and it is from day 10 to 13 until the cells are tend to decrease in death phase. According to the curves, when lower carbonated water is used, it could help to get the highest cells faster but no distinct stationary phase is appeared which can be noted that no additional CO_2 is necessary for optimizing the *Chlorella vulgaris* cultivation.

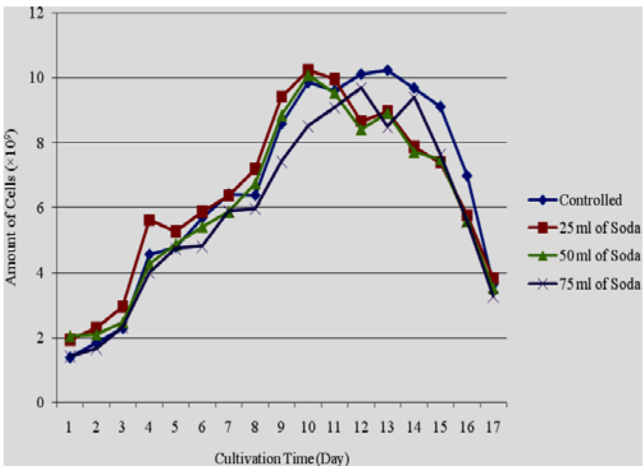

Fig. The Algae Growth Curves with and without Low Carbonated Water

TABLE I
SCREEN ANALYSIS OF ACID-TREATED ALGAE POWDER

Size Range	Weight %
>300 um	20.74
300 um – 212 um	13.57
212 um – 150 um	12.44
150 um – 100 um	10.87
100 um – 75 um	10.16
<75 um	32.21

Screen analysis of the acid-treated algae powder is shown in Table I. In adsorption process, the smaller particle size adsorbent is attractive because it can give larger internal surface area for good adsorption. So, the particle size of less than 75 µm of acid-treated algae powder was use as biosorbent.

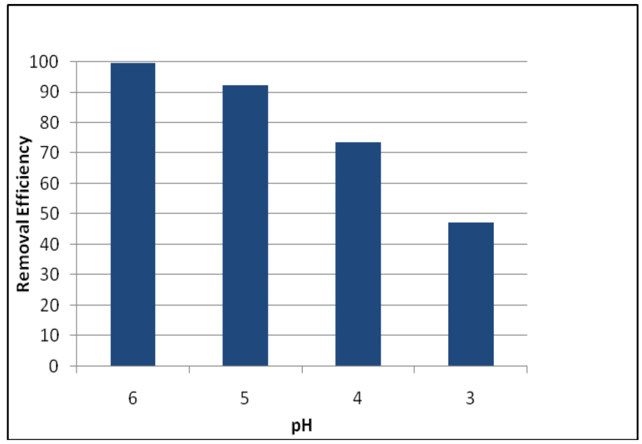

Fig. 3 Pb removal efficiencies by Chlorella vulgaris at different pH

According to the analytical data, the optimum pH for the Pb adsorption is at 6 with 99.4% removal efficiency. In Fig. 3, It is found that the greater the pH the higher the removal efficiency of adsorption.

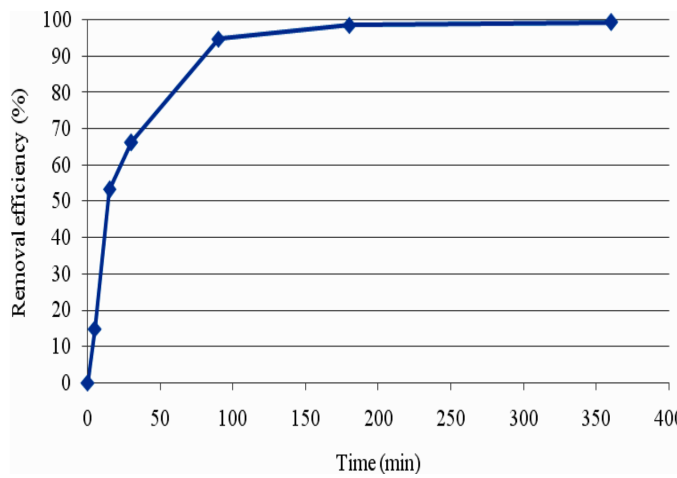

Fig. 4 Adsorption rate of Lead on biosorbent

Biosorption rate is shown in Fig 4. According to the figure, the adsorption of lead on the algae biosorbent is fast in first 30 mins and the rate is then slow as approching the equilibrium.

A number of models have been developed to describe the kinetics of heavy metal biosorption. The pseudo-second order kinetic model based on the sorption capacity of solid phase can be used in this case assuming that measured concentrations are equal to cell surface concentrations. The pseudo-second order kinetic rate equation is expressed as:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \qquad (1)$$

Integrating for the boundary condition conditions $q_t = 0$ at $t = 0$ and q_t at time t, the form of linearization of pseudo-second order model is obtained:

$$\frac{t}{q_t} = \frac{1}{k_2 q_e^2} + \frac{t}{q_e} \qquad (2)$$

where k_2 is the second order biosorption rate constants (g/mg min−1), q_e and q_t are the amounts of adsorbed metal ions on the biosorbent at the equilibrium and at any time t, respectively. The kinetic data was analyzed in term of the pseudo-second order.

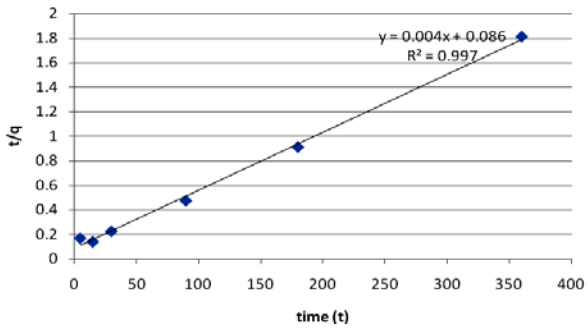

Fig. 5 Plot of linearization of pseudo-second order model

The plots of t/q_t versus t is shown in Fig. 5. The values of parameters k_2, q_e and correlation coefficient R^2 were 0.186×10^{-3}, 250 mg/g and 0.997 respectively. So, it is suggested that the sorption of Pb^{2+} onto *Chlorella vulgaris* followed the pseudo-second order model very well.

The capacity of an adsorbent can be described by equilibrium sorption isotherm, which is characterized by certain constants whose values express the surface properties and affinity of the adsorbent. The Langmuir isotherm is probably the most widely applied adsorption isotherm. A basic assumption of this model is that adsorption takes place at specific homogeneous sites within the adsorbent. It is based on the assumption that all the adsorption sites have equal affinity for molecules of the adsorbate and there is no transmigration of the adsorbate in the plane of the surface. It is presented by the following equation:

$$q_e = \frac{bq_m C_e}{1+bC_e} \quad (3)$$

where q_m is the maximum metal uptake capacities (mg/g); q_e is the amounts of the metal ions adsorbed at equilibrium (mg/g); C_e is the equilibrium concentration of metal ions (mg/L); b is the equilibrium constant. The linear form of the Langmuir equation is:

$$\frac{1}{q_t} = \frac{1}{q_m} + \frac{1}{bq_m}\frac{1}{C_e} \quad (4)$$

The Freundlich isotherm is an empirical isotherm that can be used for non-ideal adsorption, which is commonly presented as:

$$q = kC^{\frac{1}{n}} \quad (5)$$

Where q is the amounts of the metal ions adsorbed, k and n are empirical constants for each adsorbent-adsorbate pair at a given temperature. The linear form of the Freundlich equation is given as:

$$\log q = \log k + \frac{1}{n}\log C \quad (6)$$

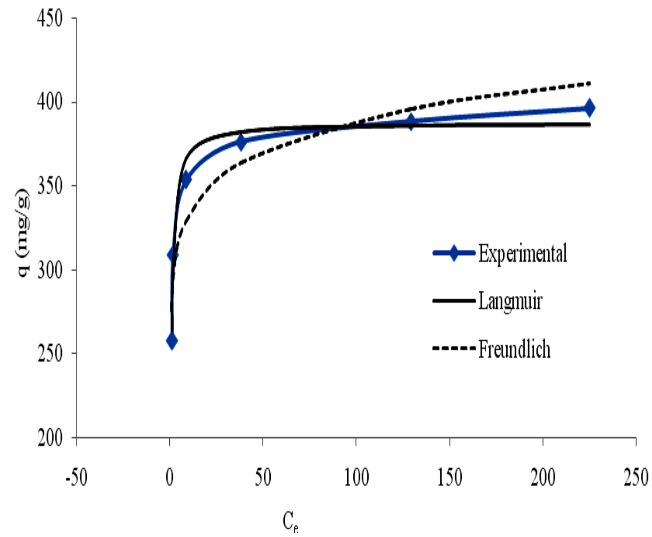

Fig. 6 Isotherms of Lead Biosorption

The biosorption isotherm of Pb^{2+} by *C. vulgaris* was compared with Langmuir and Freundlich isotherm models, as shown in Fig. 6. The plot of linearization of the two models are shown in the Fig. 7 and Fig. 8.

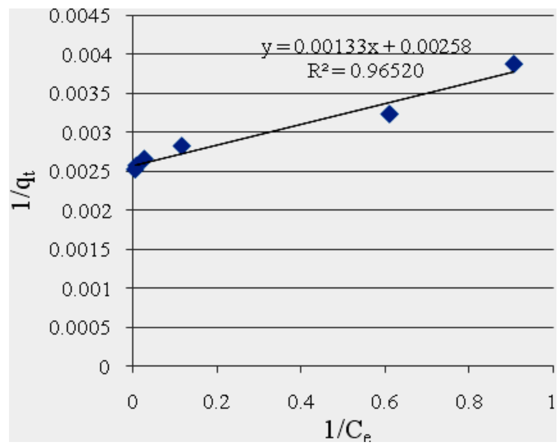

Fig. 7 Plot of Linearization of Langmuir Model

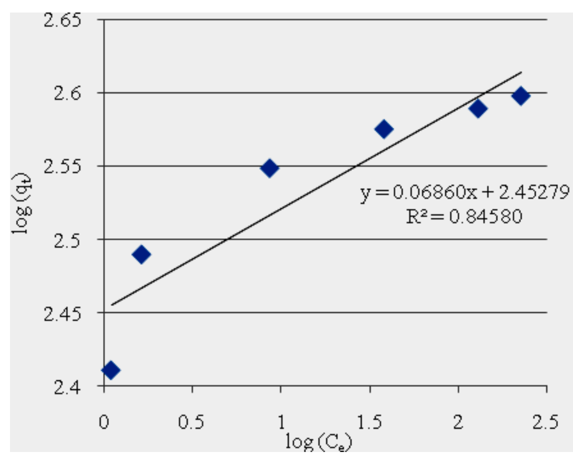

Fig. 8 Plot of Linearization of Freundlich Model

According to the R^2 value, it can be said the biosorption isotherm of lead on *C. vulgaris* is well described as Langmuir isotherm model.

IV. Conclusion

This study indicated that green algae *Chlorella vulgaris*, which could cultivate at low cost, can be possible to use as an efficient biosorbent material for removal of Pb from wastewater. In cultivation of algae, it is found that the additional carbonated water shortens the accelerated growth phase of the algae cultivation by 24 hours, the exponential growth phase was reached in day 3 – 9 from starting point and cultivation time of 7 days is suitable for large scale cultivation. It was found that no carbonated water was necessary to optimize the cultivation of *Chlorella vulgaris.* The size less than 75 um of the prepared biosorbent was used in biosorption. The optimum pH for biosorption is at 6 with the removal efficiency of 99.4 %. The pseudo-second order kinetic model is suitable for describing the biosorption. The biosorption isotherm of lead on *C. vulgaris* is well described as Langmuir isotherm model.

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