African Journal of Biotechnology Vol. 5 (19), pp. 1819-1823, 2 October 2006
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2006 Academic Journals

*Full Length Research Paper*

# Continuous removal and recovery of lead by alginate beads, free and alginate-immobilized *Chlorella vulgaris*

**M. S. Abdel Hameed**

Botany Department, Faculty of Science, Beni Suef University, Egypt. E-mail: drmohamedsa@yahoo.com.

Accepted 1 September, 2006

**This study examines the possibility of using *Chlorella vulgaris* cells in repeated lead adsorption/desorption cycles. Alginate beads and immobilized with algal cells were more effective and suitable than free cells. Consistently high lead removal (>90%) and recovery (about 100%) were achieved. Lead adsorption was mainly via the alginate matrix and minor contribution was made by algae. Free cells were difficult to handle and give inconsistent lead removal and recovery throughout the experiment**.

**Key words:** Lead removal, recovery, immobilization, algae.

## INTRODUCTION

Pollution of the natural environment by heavy metals has become a serious problem in some industrialized countries (Inthorn et al., 1996). The release of large quantities of heavy metals from industries into the environment has resulted in a number of environmental problems. Heavy metals enter the environment through wastewater streams from industrial processes such as electroplating, plastics manufacturing, mining and metallurgical processes (Yu and Kaewsarn, 1999).

Heavy metal pollution of waterbodies due to indiscriminate disposal of industrial and domestic wastes threatens all kinds of inhabiting organisms (De Filippis and Pallaghy, 1994; Nagase et al., 1997). Therefore, it is necessary to alleviate heavy metal burden of wastewaters before discharging them into waterways. At present, a number of different technologies exist for treating heavy metals bearing streams, such as chemical precipitation, adsorption, solvent extraction, ion exchange and membrane separation (Eccles, 1999). However, these methods have several disadvantages, such as incomplete metal removal, expensive equipment and monitoring system requirements, high reagent or energy requirements and generation of toxic sludge or other waste products that require disposal. Further, they may be ineffective or extremely expensive when metal concentration in wastewater is in the range 10-100 mg $l^{-1}$ (Mehata and Gaur, 2005). The use of biological processes for the treatment of metal enriched wastewaters can overcome some of the limitations of physical and chemical treatments and provide a means for cost- effective removal of metals. A great deal of interest has recently been generated using different kinds of inexpensive biomass for adsorbing and removing heavy metals from wastewater (Volesky and Holan, 1995). In this context, accumulation of metals by microorganisms, including algae, has been known for a few decades, but has received increased attention only in recent years because of its potential for application in environmental protection or recovery of precious or strategic metals (Tsezos, 1985, 1986; Volesky, 1987; Malik, 2004). Algal biomass cannot be used directly in a standard sorption process. It is generally of small particle size and low strength and density, which can limit the choice of a suitable reactor and make biomass or effluent separation difficult (Tsezos, 1986).

Immobilized biomass has the potential to provide a simple technology to remove and recover heavy metals from wastewater, and is suitable for reuse (Rai and Mallick, 1992). This study aimed to investigate the continuous removal and recovery of lead using free (live or killed) algal cells or immobilized alginate beads (with live or dead cells or blank beads).

## MATERIALS AND METHODS

### Organism and culture media

*Chlorella vulgaris* Beyernick was isolated from the River Nile at Beni Suef (Egypt). The alga was grown in commercial Bristol medium (Starr, 1960) at 27±1ºC, and were illuminated at a light intensity of

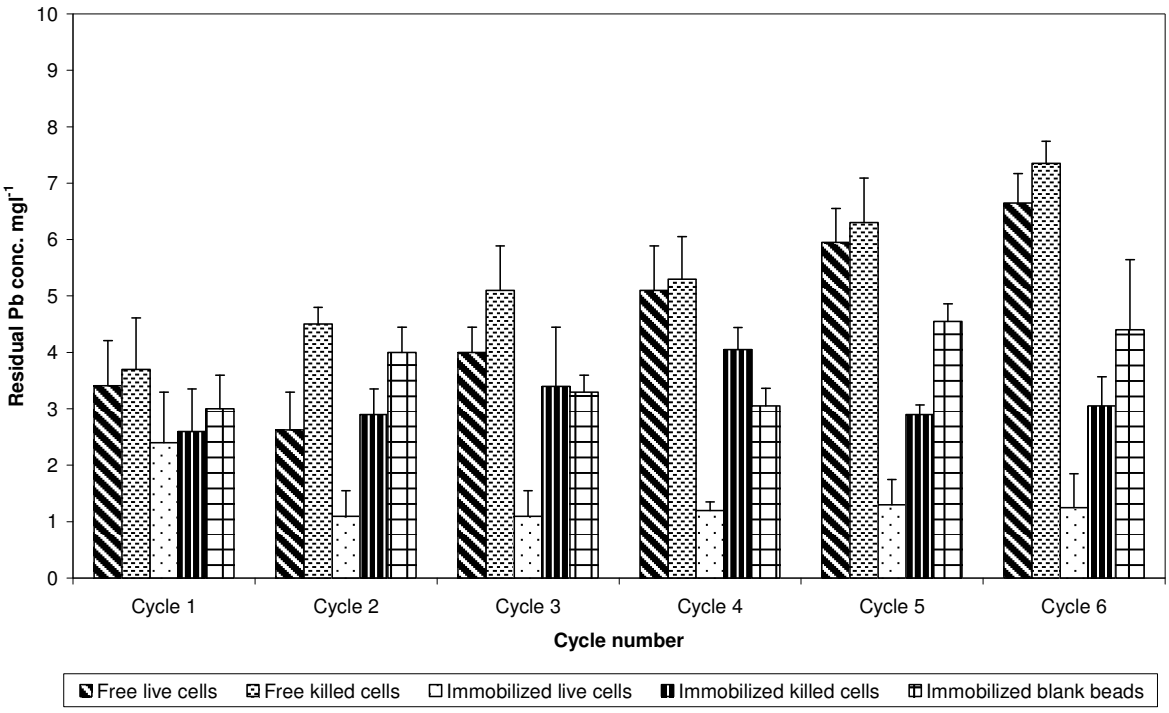


**Figure 1.** Residual lead concentrations in solutions during the six adsorption/elution cycles (mean ± standard deviation bar of three replicates).

75 µmol $s^{-1}$ $m^{-2}$ with a 16 h light/8 h dark photoperiod for 10 days, then harvested by centrifugation (2500 rpm for 10 min).

### Bead formation and immobilization of algae

The algal cells were either killed by heat in the autoclave (labeled as killed cells) or kept alive (named as live cells). The treated algal cells were mixed with equal volumes of 4% sodium alginate (Sigma, UK), and the alginate mixture was then dropped in a 2.5% $CaCl_2$ solution. The beads were then rinsed in deionozed water and stored at 4ºC prior to use. Each algal bead (approximately 2.5 mm in diameter) had an algal density of 3 X $10^6$ cells $bead^{-1}$. Similar procedures were employed except the algal cell suspension was replaced by an equal volume deionized water to obtain alginate blank beads (beads without algae).

### Adsorption/desorption cycles

Each cycle consisted of an adsorption and elution phase with the cell : lead solution ratio (3 X $10^7$ cells : 1 ml) kept constant through the cycles. The Pb solution was prepared by diluting standard Pb solution to the desired concentration (50 mg/L). The freshly prepared solution was used for each cycle. A total of 15 flasks (150 ml), each containing 40 ml freshly prepared lead solution (pH 6). Free algal cells, either killed or live, was added to these flasks and the cell density was kept at 3 X $10^7$ cells $ml^{-1}$ lead solution. Four hundred alginate algal beads, prepared from either killed or live cells and alginate blank beads as control were also placed in lead solution to give a density of 10 beads $ml^{-1}$. All treatments were in triplicates. The flasks were shaken for 2 h and lead solution was separated from the free algal cells by centrifugation (2500 rpm for 10 min). The supernatant was collected and analysed for residual lead concentration using Perkin-Elmer (model 2380) atomic absorption flame spectrophotometer. The alginate beads could easily be separated by pouring the mixture through a strainer. The algal mass and the beads were rinsed separately in 40 ml of deioniozed water. Removal of lead from algal biomass or beads was achieved by eluting with 0.1 M $HNO_3$ for 15 min, and the liquid was collected. The algal masses or beads were then rinsed again to remove any residual acidity. To monitor the cellular lead content, cells were digested according to Inthorn et al. (2002) and the Pb concentration obtained as above.

Cell viability of immobilized live cells was tested by dissolving 10 alginate beads in 1 ml sterilized 0.2 M sodium citrate solution and a series of dilutions was made using sterile Bristol media. 1 ml of free live cells was taken to assess cell viability, and a series of dilutions was made. Samples of 0.1 ml from each dilution were plated out dropwise, in triplicate, on Bristol agar plates. The plates were incubated at light/dark cycle (16/8 h) for 7 days. The colonies formed were then counted under the microscope.

### Digestion of the algae

The lead content of the alga was determined after digestion of the washed and dried material for 15 min in a boiling mixture of conc. $HNO_3$ and HCl (1:1, v/v) (Fathi et al., 2005).

## RESULTS

Immobilized *C. vulgaris* have been investigated for its potential use for the removal and recovery of lead. Data in Figure 1 revealed that free cells, immobilized cells and blank beads were all able to remove lead from solution with different efficiencies. The percentage of lead remo-

**Table 1.** Removal efficiency, expressed in terms of the percentage lead adsorbed of different treatments in 6 cycles (mean ± standard deviation of three replicates are shown).

| Cycle | Free live cells | Free killed cells | Immobilized live beads | Immobilized killed beads | Immobilized blank beads |
|---|---|---|---|---|---|
| Cycle 1 | 88.6±2.7 | 87.6±3.05 | 92±3 | 91.3±2.51 | 90±2 |
| Cycle 2 | 91.2±2.25 | 85±1 | 96.3±1.52 | 90.3±1.52 | 86.6±1.52 |
| Cycle 3 | 86.6±1.52 | 83±2.64 | 96.3±1.52 | 88.6±3.51 | 89±1 |
| Cycle 4 | 83±2.64 | 82.3±2.51 | 96±0.5 | 86.5±1.32 | 89.8±1.04 |
| Cycle 5 | 80.1±2.02 | 79±2.64 | 95.6±1.52 | 90.3±0.57 | 84.8±1.04 |
| Cycle 6 | 77.8±1.75 | 75.5±1.32 | 95.8±2.02 | 89.8±1.75 | 85.3±4.16 |

**Table 2.** Eluted lead concentrations (mg $l^{-1}$) of different treatments in 6 cycles (mean ± standard deviations of three replicates are shown).

| Cycle | Free live cells | Free killed cells | Immobilized live beads | Immobilized killed beads | Immobilized blank beads |
|---|---|---|---|---|---|
| Cycle 1 | 22.8±1.04 | 20.4±1.05 | 25.4±0.51 | 23.5±1.32 | 23.5±2.78 |
| Cycle 2 | 20.9±1.86 | 16.6±0.57 | 27.6±1.52 | 26.1±2.75 | 25.1±1.89 |
| Cycle 3 | 18.5±1.32 | 17.2±1.75 | 28.6±0.57 | 25.2±0.95 | 26.3±0.57 |
| Cycle 4 | 18.1±0.9 | 17.3±0.76 | 28.3±0.57 | 25.6±1.15 | 25.1±0.96 |
| Cycle 5 | 16.3±1.75 | 15.9±3.5 | 28.6±0.36 | 26.2±1.7 | 25.2±0.72 |
| Cycle 6 | 15.8±0.47 | 15.1±0.9 | 28.7±0.26 | 26.3±1.52 | 25.3±0.79 |

val in each of these treatments also varied over different cycles. The immobilized algae and the alginate blank beads significantly remove more lead than free cells ($p \leq 0.01$ according to one way analysis of variance). The algal biomass alone was not as effective as the alginate beads in removing lead from the solution except in the second cycle (Table 1). The removal efficiency of the free live cells was 88% in the first cycle, which increased to 91 in the second cycle, and then there was gradual decrease to reach 77% in the last cycle.

There was gradual decrease in the removal efficiency of lead by free killed cells from cycle one to cycle six. The immobilized live cells recorded 92% Pb removal in the first cycle and increased in the following cycles to 96% approximately. The lead removal by the immobilized blank beads fluctuated between 85 and 90% in the different cycles.

## Elution of lead

The amount of lead eluted from the free live and killed algal cells were significantly lower than that eluted from the immobilized algal beads and alginate blank beads (Table 2). The amount of lead eluted from free cells was always incomplete and the eluted Pb level was around 15% lower than the amount of adsorbed lead in the first cycle. The eluted lead level decreased consistently to record more than 30% in the following cycles (Figure 2).

Different pattern was monitored in the alginate beads treatments. The elution in cycle 1 was 92% in the immobilized live cells, 85% in the immobilized killed cells and 86% in the immobilized blank beads. In the subsequent cycles the elution efficiency was around 98% (Figure 2)

## Viability of the live cells

The number of live free algal cells expressed as colony forming unit/ml recorded continuous loss of cells during each cycle. The initial count was 3 X $10^7$ cells $ml^{-1}$ which dropped to 2.2 X $10^7$ cells $ml^{-1}$ at the end of the first cycle (more than 25% of the cells were killed). The loss continued in the subsequent cycles and only 9.9 X $10^6$ cells $ml^{-1}$ persisted throughout the course of the experiment (33% of the initial inoculum). The situation was different in case of immobilized live cells as the recorded reduction in the total count was less than that of free cells. At the end of first cycle the reduction was about 2% only. The live algal cells within the beads persisted throughout the experiment with only 25% loss. At the end of the six cycles the algal count reached 2.25 X $10^7$ cells $ml^{-1}$ (2.25 X $10^6$ cells $bead^{-1}$). No colonies were observed in cultures containing dead cells and blank beads.

## Cellular lead content

The cellular lead content of the free live cells increased by 8.5% more than the control after the first cycle. Seco-

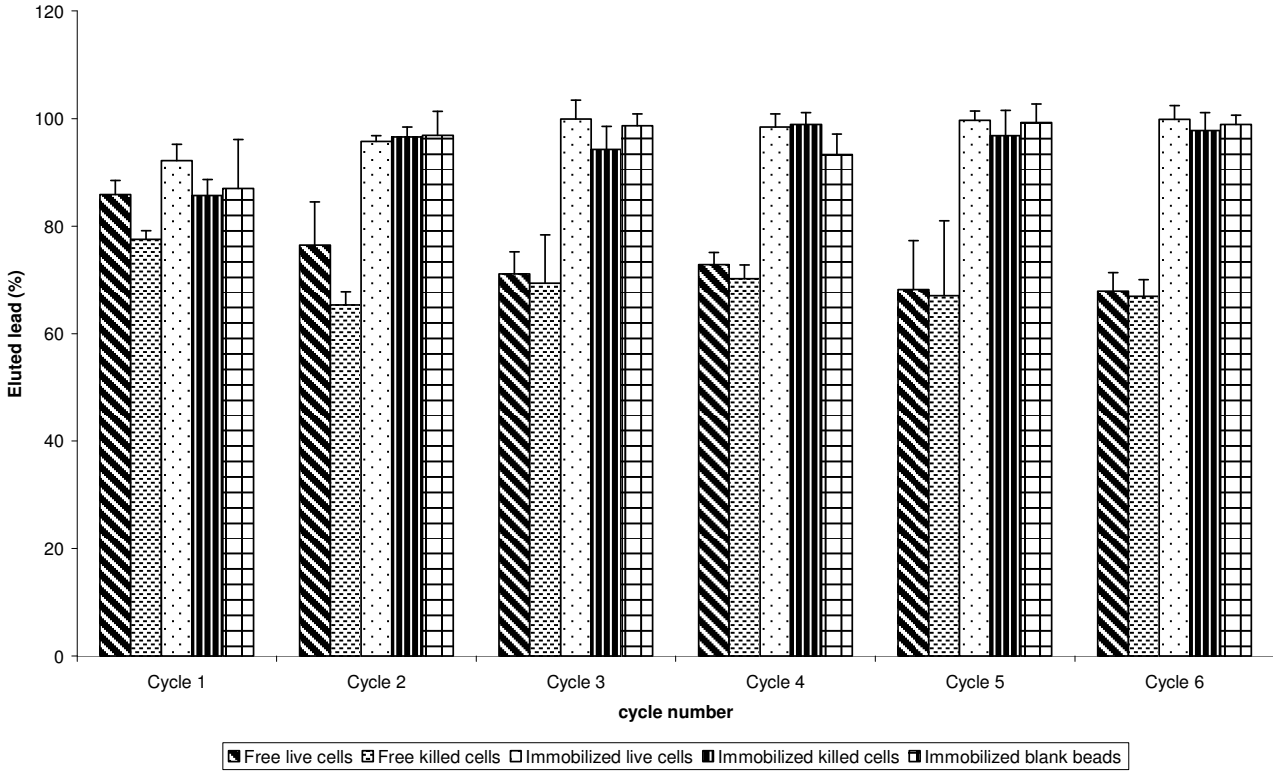


**Figure 2.** Elution efficiency, expressed in terms of lead eluted as a percentage of the total lead adsorbed, of different treatments in 6 cycles (mean ± standard deviation bar of three replicates).

nd and third cycle recorded slight increase and reached 13%, then remains nearly constant. No increase in cellular lead content was recorded in case of the immobilized live cells throughout the course of the experiment.

## DISCUSSION

Heavy metals such as lead are persistent in the environment and can accumulate in food chain, exhibiting toxic effects. Biosorption is considered a reliable, efficient, and low-cost technique for metal removal from wastewater (Balaria et al., 2005). The green alga *C. vulgaris* is often used to study adsorption of heavy metals (Aksu and Dönmez, 2006; Ruangsomboon and Wongrat, 2006). In this study, free live *C. vulgaris* cells adsorbed significantly more Pb than free dead cells in the first two cycles, and no significant difference was found thereafter, as most of the algal cells were killed during the first two cycles due to the lead toxicity and the increase in the cellular lead content. In comparison to live cells, the metal sorption capacity of dead cells may be greater, equivalent or less (Ozer, et al., 2000). Greene and Bedell (1990) mentioned that *C. vulgaris* cells, killed by heat, accumulated greater amounts of uranium (VI) than living cells. Tam et al. (1998a) found that the killed cells performed better than the initially live cells in copper removal and recovery.

The results in the present study showed that the repeated adsorption/elution cycles using the alginate immobilized algal cells are effective. The lead removal efficiency improved after the first cycle and remained consistently high throughout the course of the experiment. The data also revealed the efficiency of the immobilized beads over the free cells. Generally, it is reported (Ting et al., 1989) that the uptake of metal ions can be divided into two stages: rapid and slow stage. In the rapid stage, the metal ions are adsorbed onto the surface of the microorganism. In the slow stage, the metal ions transport across the cell membrane into the cytoplasm. Acid treatment during the first elution could remove contaminated metals previously bound to the alginate and cells, freeing up more binding sites for the next adsorption cycle and raising the efficiency (Mclean, et al., 1994; Wilhelmi and Duncan, 1996).

Immobilization generally tends to increase metal accumulation by biomass (Darnall et al., 1986; Aksu et al., 1998). Immobilized cells accumulate more metals than free cells due to (i) enhanced photosynthetic capacity (Khummongkol et al., 1982), and (ii) increased cell wall permeability (Brouers et al., 1989). Immobilization of living biomass also provides protection to cells from metal toxicity (Bozeman et al., 1989), which

agrees with our findings as most of the immobilized algal cells remained alive throughout the course of the experiment comparing to the free living cells and no difference monitored in the cellular lead content of the immobilized live cells.

Results obtained in this study clearly show that the adsorption of lead by the alginate blank beads was only slightly less than that of the immobilized algal beads, which means that the algal cells made minor contribution to the lead removal and that the alginate matrix was adsorbing most of the lead. Crist et al. (1994) recorded that the metal sorption capacity of calcium alginate powder was higher than that of *Chlorella*. Tam et al. (1998b) found that the algal cells only contributed 12 to 14% of copper removal while alginate accounted for >85% copper adsorption.

In conclusion, the alginate beads (with or without algae) can be used effectively to remove and recover lead from solution in successive cycles. The alginate matrix contributed significantly in adsorbing lead than the algal biomass, with little enhancement effects from the biomass either live or killed. On the other hand, the free cells were not suitable for repeated use.

**REFERENCES**


Aksu Z, Egretli G, Kutsal A (1998). A comparative study of copper (II) biosorption on Ca-alginate, agarose and immobilized *C. vulgaris* in a packed-bed column. Process biochem., 33: 393-400.

Asku Z, Donmez G (2006). Binary biosorption of cadmium(II) and nickel(II) onto dried *Chlorella vul*garis: Co-ion effect on mono-component isotherm parameters. Process Biochem. 41(4): 860-868

Balaria A, Schiewer S, Trainor T (2005). Biosorption of Pb(II) onto citrus pectin: Effect of process parameter on metal binding equilibrium and kinetics. World Water Congress. Impacts of Global Climate Changes. World Water and Environmental Resources Congress 2005. Raymond Walton-Editor, May 15-19, 2005 Anchorage, Alaska, USA.

Bozeman J, Koopman B, Bitton G (1989). Toxicity Testing Using Immobilized Algae. Aquat.Toxicol. 14(4): 345-352.

Brouers M, de Jong H, Shi DJ, Hall DO (1989). Immobilized cells: an appraisal of the methods and applications of cell immobilization techniques. In: Algal and Cyanobacterial Biotechnology (R.C. Cresswell, Rees, T.A.V. and Shah, N., Eds.) Longman Scientific and Technical, London 272-293.

Crist RH, Martin JR, Carr D, Watson JR, Clarke HJ, Crist DR (1994). Interaction of metals and protons with algae. 4. Ion exchange vs. adsorption models and a reassessment of scatchard plots; Ion-exchange rates and equilibria compared with calcium alginate, Environ. Sci. Technol. 28: 1859-1866.

Darnall DW, Greene B, Henzl MT, Hosea M, McPherson RA, Sneddon J, Alexander MD (1986). Selective recovery of gold and other metal ions from an algal biomass. Environ. Sci. Technol., 20: 206-208.

De Filippis LF, Pallaghy CK (1994). Heavy metals: sources and biological effects. In: RAI, L.C.; GAUR, J.P. and SOEDER, C.J. eds. Advances in Limnology Series: Algae and Water Pollution, E. Scheizerbartsche Press, Stuttgart, pp. 31-77.

Eccles H. (1999). Treatment of metal-contaminated wastes: why select a biological process? Trends in Biotechnol., 17(12): 462-465.

Fathi AA, Zaki FT, Ibraheim HA (2005). Response of tolerant and wild type strains of *Chlorella vulgaris* to copper with special references to copper uptake system. Protistol. 4(1): 73-78.

Greene B, Bedell GW (1990). Algal gels or immobilized algae for metal recovery. In: Introduction to applied phycology, (ed.) I. Akatsuka. SPB Academic Publishing by The Hague, The Netherlands, pp. 137-149.

Inthorn D, Nagase H, Isaji Y, Hirata K, Miyamoto K (1996). Removal of cadmium from aqueous solutions by the filamentous cyanobacterium Tolypothrix tenuis. J. fermentation and Bioengineering. 82(6): 580-584.

Inthorn D, Sidtitoon N, Silapanuntakul S, Incharoensakdi A (2002). Sorption of mercury, cadmium and lead by microalgae. Scienceasia, 28: 253-261.

Khummongkol D, Canterford G S, Fryer C (1982). Accumulation of heavy metals in unicellular algae Biotechnol. Bioeng. 24(12): 2643 – 2660.

Malik A (2004). Metal bioremediation through growing cells. Environ. Int. 30: 261-278

McLean RJC, Campbell AM, Khu PT, Persaud AT, Bickerton LE, Beauchemin D (1994). Repeated use of Bacillus subtilis cell walls for copper binding. World J. Microbiol. Biotechnol. 10: 472 - 474.

Mehata SK, Gaur JP (2005). Use of Algae for Removing Heavy Metal Ions From Wastewater: Progress and Prospects. Critical reviews in biotechnol., 25(3): 113-152.

Nagase H, Inthorn D, Isaji Y, Oda A, Hirata K, Miyamoto K (1997). Selective cadmium removal from hard water using NaOH-treated cells of the cyanobacterium Tolypothrix tenuis. J. fermentation and Bioeng. 84(2): 580-584

Özer D, Ayla Özer A, Dursun G (2000). Investigation of zinc(II) adsorption on *Cladophora crispata* in a two-staged reactor. J. Chem. Technol. Biotechnol. 75(5): 410-416.

Rai LC, Mallick N (1992). Removal and assessment of toxicity of Cu and Fe to *Anabaena doliolum* and *Chlorella vulgaris* using free and immobilized cells. World J Microbiol Biotechnol. 8: 110-114.

Ruangsomboon S, Wongrat L (2006). Bioaccumulation of cadmium in an experimental aquatic food chain involving phytoplankton (*Chlorella vulgaris*), zooplankton (*Moina macrocopa*), and the predatory catfish *Clarias macrocephalus* and *C. gariepinus*. Aquatic Toxicol., 78(1): 15-20.

Starr RC (1960).The Culture Collection of Algae at Indiana University Amer J. Botany, 47(1): 67-86.

Tam NFY, Wong YS, Simpson CG (1998a). Repeated removal of copper by alginate beads and the enhancement by microalgae. Biotechnol. Techniq. 12(3): 187-190.

Tam NFY, Wong YS, Simpson CG (1998b). Removal of copper by free and immobilised microalga, *Chlorella vulgaris*. In: Wastewater Treatment with Algae, (Eds. Wong Y. S. and N.F.Y. Tam), Environmental Intelligence Unity, Springer-Verlag , Berlin , pp. 17-36.

Ting YP, Lawson F, Prince IG (1989). Uptake of cadmium and zinc by the alga *Chlorella vulgaris* :Part I. Individual ion species. Biotech. And Bioeng. 34:990-996.

Tsezos M (1985). The selective extraction of metals from solution by microorganisms. *Can. Metall. Q.*, 24: 141-149.

Tsezos M (1986)." Immobilization of Ions by Naturally Occuring Materials as Alternatives to Ion Exchange Resins " Immobilisation of Ions by Biosorption " Ellis Horwood Publishers , Editors H. Eccles , S. Hont, London, UK,.

Volesky B (1987). Biosorbents for metal recovery. Trends Biotechnol. 5: 96-101.

Volesky B, Holan ZR (1995) Biosorption of heavy metals. Biotechnol. Progress, 11(3): 235-250

Wilhelmi BS, Duncan JR (1996) Reusability of immobilised *Saccharomyces cerevisiae* with successive adsorption-desorption cycles, Biotechnol. Let. 18: 531-536.

Yu Q, Kaewsarn P (1999). Fixed bed study for copper (II) removal from aqueous solutions by marine algae Durvillaea potatorum. Environ. Technol. 20: 1005-1008.