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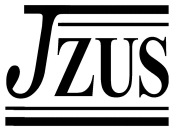

Bioremediation potential of spirulina: toxicity and biosorption studies of lead

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Abstract: This study examines the possibility of using live spirulina to biologically remove aqueous lead of low concentration (below 50 mg/L) from wastewater. The spirulina cells were first immersed for seven days in five wastewater samples containing lead of different concentrations, and the growth rate was determined by light at wavelength of 560 nm. The 72 h-EC_{50} (72 h medium effective concentration) was estimated to be 11.46 mg/L (lead). Afterwards, the lead adsorption by live spirulina cells was conducted. It was observed that at the initial stage (0–12 min) the adsorption rate was so rapid that 74% of the metal was biologically adsorbed. The maximum biosorption capacity of live spirulina was estimated to be 0.62 mg lead per 10^5 alga cells.

Key words: Bioadsorption, Bioremediation, Spirulina, Lead
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INTRODUCTION

Environmental contamination by toxic metals is a serious problem worldwide due to their incremental accumulation in the food chain and continued persistence in the ecosystem. Conventional technologies, such as ion exchange or lime precipitation, are often ineffective and/or expensive, particularly for the removal of heavy metal ions at low concentrations (below 50 mg/L). Furthermore, most of these techniques are based on physical displacement or chemical replacement, generating yet another problem in the form of toxic sludge, the disposal of which adds further burden on the techno-economic feasibility of the treatment process. In view of this, the development of new techniques is necessary to meet the environmental standards at affordable costs.

Biotechnology has been investigated as an alternative method for treating the metal-containing wastewater of low concentrations. In response to heavy metals, microorganisms have evolved various measures via processes such as transport across the cell membrane, biosorption to cell walls and entrapment in extracellular capsules, precipitation, complexation and oxidation-reduction reactions. It has been proved that they are capable of adsorbing heavy metals from aqueous solutions, especially for the metal concentration below 50 mg/L (Lu and Wilkins, 1995). The metal-binding capacities of several biological materials have been identified to be very high, including marine algae, fungi and yeasts. It was reported that these microorganisms can accumulate a wide range of metal species.

Most of the studies dealing with biological removal of metals used dead biomass. However, recently, it was reported that the live *Aspergillus niger* cells exhibits higher Ni biosorption capacity than dead biomass, probably due to intracellular Ni uptake (Kapoor *et al*., 1999). The aim of this study is to examine the possibility of using live spirulina to biologically remove lead of low concentration (below 50 mg/L) wastewater. The toxicity and biosorption of lead to live spirulina cultures were investigated. Both factors are very important in developing spirulina for the treatment of wastewater containing heavy metals.

MATERIALS AND METHODS

Biosorbent material

Spirulina was supplied by the Institute of Botany, Zhejiang University. The alga seed was first centrifugated and then stored in liquid medium (details are in Table 1) for 7 d at 20~26 °C under light generated by a 40 W white fluorescent lamp. The quantity of living biomass used for the biosorption studies varied from 1×10^5 to 5×10^5 cells/ml.

Table 1 Composition of growth medium

Constituents	Concentration (g/L)
EDTA	0.08
$CaCl_2\cdot2H_2O$	0.04
NaCl	1.00
$NaNO_3$	2.50
$NaHCO_3$	16.80
$FeSO_4\cdot7H_2O$	0.01
$MgSO_4\cdot7H_2O$	0.20
K_2SO_4	1.00
K_2HPO_4	0.50

Acute toxicity tests

Growth inhibition was achieved by adding log phase cells to five solutions containing aqueous lead of 1, 2, 4, 10 and 20 mg/L. The volume of each solution was 100 ml. The growth rate was determined every 24 h and EC_{50} was evaluated by probit analysis.

Lead sorption experiments

The Pb solutions were prepared by diluting standard Pb solution to the desired concentrations. The freshly diluted solutions were used for each biosorption study. The sorption experiments were conducted in 250 ml flasks containing 100 ml of lead solutions with initial concentrations ranging from 10 to 50 mg/L. During the adsorption process, the flasks were agitated on a shaker for 48 h under ambient temperature (25±2 °C). At the designed period of 5, 15, 20, 30, 60, 100, 120 and 150 min, 5, 12 and 24 h, 10 ml of the solution were collected for analysis. To determine the concentration of the remaining metal ions, the spirulina in the sample solutions was removed by filtration and the filtrate was analysed to measure the lead concentration spectrophotometrically.

Data analysis

The Langmuir isotherm model below is commonly used to describe the sorption of metals onto microbial surface.

$$q = q_{\max} bc/(1+bc) \tag{1}$$

where c is the final lead concentration (mg/L), q is the metal uptake (mg/10^5 cells), b is the sorption binding constant (L/mg), $q_{\max}$ is the saturation capacity (mg/10^5 cells).

The linear form of Eq.(1) is:

$$1/q = 1/(q_{\max} bc) + 1/q_{\max} \tag{2}$$

from the slope and intercept of a $1/q$ vs $1/c$ linear plot such that $q_{\max}$=intercept^{-1} and b=intercept/slope.

RESULTS AND DISCUSSIONS

Toxicity of lead on spirulina growth

To investigate spirulina's tolerance of Pb^{2+}, the living cells were cultivated in solution containing Pb^{2+} ions with various concentrations. The growth curves of spirulina are shown in Fig.1. The results indicated that the growth inhibition increased at higher aqueous lead concentration. In the presence of lowest Pb^{2+} concentration (1.0 mg/L), only a slight inhibition of cell growth occurred. By contrast, the highest concentration (20 mg/L) caused a large number of cells to die at first (0–1 d), but the cell growth recovered afterwards. Such responses of live and growing cells to high metal concentration are similar to the pure biological adsorption process using dead/treated biomass. In this study, the EC_{50} at 72 h was estimated to be 11.46 mg/L.

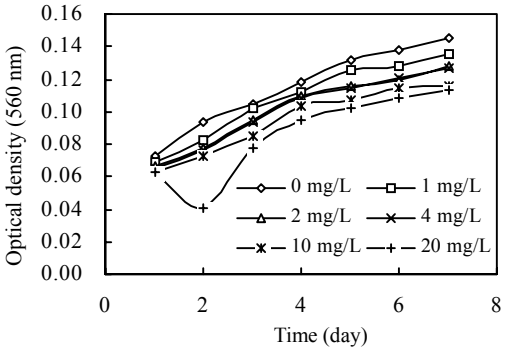


Fig.1 Growth of spirulina in solutions containing aqueous lead of various initial concentrations

Such finding also shows that spirulina has high tolerance to lead with EC_{50} higher than that of some species previously reported. The EC_{50} of *Navicula incerta* and *Nitzchia clasterium* are 3.01×10^{-6} and 0.48×10^{-6}, respectively. While the EC_{50} of a green alga, *Chlorococcum* sp. is 2.5×10^{-6} to 3.0×10^{-6} (Trevors *et al*., 1986). The resistance of spirulina to lead suggests its suitability for lead treatment.

Biological adsorption of lead

1. Time-dependent biosorption

The dependence of lead biosorption by living spirulina on time is shown in Fig.2. 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 microorganism. In the 'slow' stage, the metal ions transport across the cell membrane into the cytoplasm. Swift and Forciniti (1997) investigated different lead uptake mechanisms in various subcellular region of cyanobacteria and *Anabaena cylindrical*. They noticed that lead phosphate precipitated on the cell wall and inside the cell. Their results confirmed a very fast uptake in the cell envelope and then a longer uptake period inside the cell. In this study, rapid biosorption was observed at the beginning (0–12 min, with 74% of metal adsorbed), then reached the equilibrium within 24 h with 95% of metal ions adsorbed. Rangsayatorn *et al*.(2004) and Horikoshi *et al*.(1979) reported that cadmium was rapidly adsorbed by *S. platensis* during the first 5 min and by *C. regularis* within 6 min, respectively. Such rapid uptake of heavy metals by living cells is very significant when the cells are used in bioremediation process.

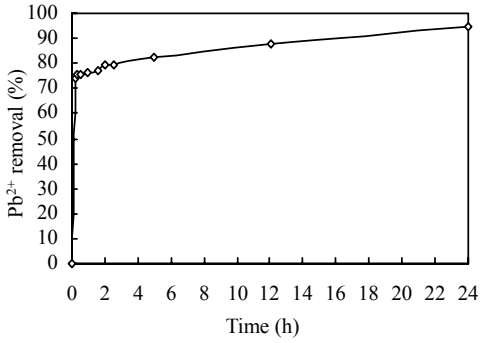


Fig.2 Time-dependence of lead biosorption by spirulina (Pb^{2+}: 30 mg/L)

2. Effect of spirulina concentration on lead biosorption

The effect of spirulina concentration on the lead adsorption rate is shown in Fig.3. The living biomass concentration varied from 1×10^5 to 5×10^5 cells/ml. The results demonstrated that the spirulina concentration considerably affected the metal removal rate. With the increased biomass concentration, the amount of adsorbed lead increased. However, the maximum adsorption rate estimated at equilibrium stage (mg Pb^{2+} removed per 10^5 alga cells) decreased at higher cell concentration. This is because at higher biomass concentration, the cells can provide more space for the adsorption process to take place.

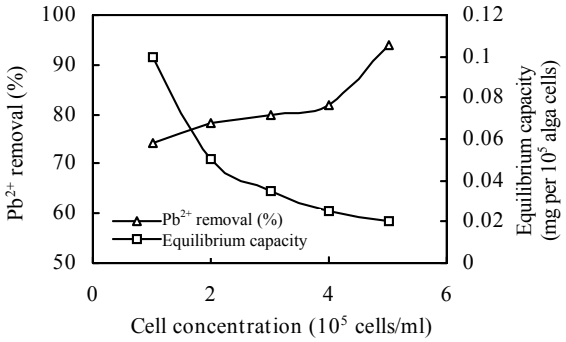
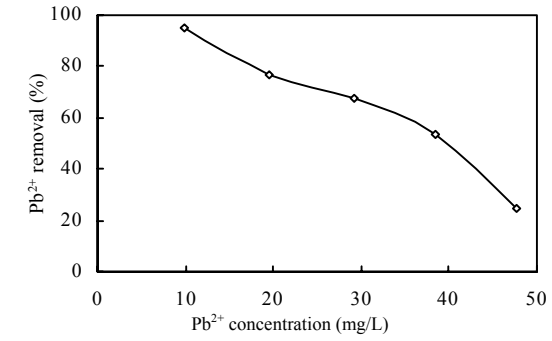


Fig.3 The effect of spirulina concentration on Pb^{2+} removal and maximum adsorption capacity (Pb^{2+}: 30 mg/L, temperature: 25 °C, agitation speed: 166 rpm)

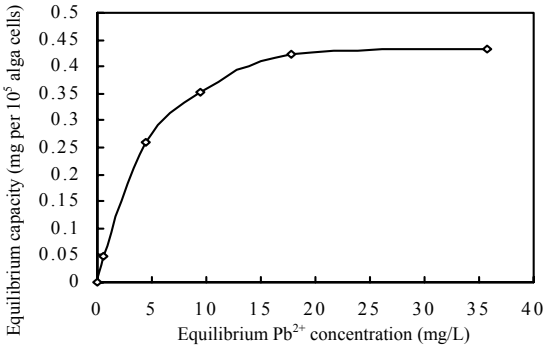
3. Effect of initial Pb^{2+} concentration on biosorption

The effect of initial Pb^{2+} concentration on biosorption is shown in Fig.4. The initial Pb^{2+} concentration remarkably influenced the metal adsorption rate at equilibrium stage. It was found that as Pb^{2+} concentration was reduced to below 10 mg/L, the Pb biosorption rate reached 95%. However, when the Pb^{2+} concentration was above 30 mg/L, the Pb^{2+} removal rate decreased. Such decline in lead removal rate is probably caused by the saturation of some adsorption sites. The shape of the adsorption isotherm was also important, and a steep isotherm from the origin at a low residual concentration of the sorbate was highly desirable because it indicated the high affinity of the biosorbent (Volesky, 1990). When the equilibrium Pb^{2+} concentration was increased from 0 to 35 mg/L approximately, the loading capacity increased from 0 to 0.44 mg per 10^5 alga cells after 24 h of adsorption. The maximum adsorption capacity and

the adsorption constant were calculated to be 0.62 mg per 10^5 alga cells and 0.135, respectively. The positive correlation between the maximum adsorption capacities of biosorbents and the Pb^{2+} concentration may be due to the higher collision between metal ions and biosorbents.



(a)



(b)

Fig.4 Lead removal rate as a function of Pb^{2+} concentration (a) and the adsorption isotherm of spirulina (b), initial cell concentration: 5×10^5 cells/ml, temperature: 25 °C, agitation speed: 166 rpm, time: 24 h

CONCLUSION

This study led to the conclusion that spirulina's rapid lead adsorption rate and high lead adsorption capacity made them well suited for the removal of lead in wastewater. In addition, living cells of spirulina were found to have high tolerance to lead and can be regarded as an attractive adsorbate option for the biosorption of heavy metal contaminant. However, there are still many uncertainties associated with the development of treating wastewater by living algae and more future work is necessary.

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