How Does Lead (Pb^{2+}) at Low Concentrations Affect on *Spirulina (Arthrospira) platensis*

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ABSTRACT

***Spirulina (Arthrospira) platensis,* a blue-green photoautotrophic alga has widely been used for the production of high valued protein for human and animal feeding as well. The present study focused on bioaccumulation of Pb^{2+} and its effects on growth of *S. platensis*. The species was cultured in Zarrouk liquid medium with different concentrations of Pb^{2+} (1, 2, 3, 4 and 5 µg/mL) and incubated for ten days under optimal conditions. Accumulated amount of Pb^{2+} increased with increasing Pb^{2+} concentrations (1.218, 2.254, 2.673, 3.713 and 5.173 mg/g dry weight, respectively for 1, 2, 3, 4 and 5 µg/mL of Pb^{2+}, at 2 days of incubation) and decreased as incubation progressed. Bioconcentration factor (BCF) also reached to the peak (1218, 1127, 888, 928 and 1035 for 1, 2, 3, 4 and 5 µg/mL of Pb^{2+} respectively) at 2 days of incubation followed by a gradual reduction. No marked differences were found in growth measured as optical density at 560 nm. It could therefore be concluded that no remarkable inhibition on growth of *S. platensis* is shown when cultured in contaminated water with low concentrations of Pb^{2+}. Furthermore, the species sounds potential accumulator of Pb^{2+} and thus could be used in detoxifying heavy metal affected aquatic ecosystems.**

Key words: bioaccumulation, bioconcentration factor, Pb^{2+}, *Spirulina platensis*

INTRODUCTION

In response to escalating public concerns on increased frequency and magnitude of heavy metal pollution, several methodologies have been developed to assess the risks involving ecological functions and human health from exposure to industrial toxic discharges. Microorganisms have the ability to bioaccumulate most metals to some degree, although the extent of bioaccumulation varies depending on the bioavailability of the metal, the organism under consideration and concentrations to which they are exposed. It is therefore, apparent that understanding the bioaccumulation of heavy metals in commercially grown microalgal species is vital in predicting metal bioavailability and risks involving human health.

Microbial biomass-related technologies have also been tested for heavy metal removal from polluted water bodies (Volesky and Holan, 1995) as the conventional methods are expensive (Eccles, 1999). In order to discover their potential capabilities, a variety of species has been assessed (Volesky and Holan, 1995; Chang *et al*., 1997). The cell wall components of microorganisms like polysaccharides, proteins and lipids offer many functional groups which can bind metal ions (Ari *et al*., 1999). Furthermore, it has been found that microalgae to be very effective biosorbents, as they possess a large surface area and high binding affinity (Roy *et al*., 1993). Efficient regeneration and easy separation of biomass and effluent are considered as the other merits of these species (Hu and Reeves, 1997; Robinson, 1998).

Spirulina (*Arthrospira*) is cultured commercially in China (Zhang, 1998; Michael, 1999) and in some other parts of the world as this cyanobacterium has been proved to be a valuable source of food supplement not only for human but for other farm animals too (Lee, 1997; Li and Qi, 1997). The species has gained a high economic value (Cohen *et al*., 1995)

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particularly because it contains some fine compounds such as essential fatty acids and amino acids, antioxidant vitamins and minerals *etc*. at relatively high concentrations (Roughan, 1989). Components found in the cell wall of *Spirulina*, such as peptydoglycan, teichuronic acid, teichoic acid, polysaccharides and proteins (Schiewer and Wong, 2000) which display mainly carboxylic, hydroxyl and phosphate groups (Aksu, 2002; Markai *et al*., 2003) may give algal wall binding properties. Under this background, the present study focuses on bioaccumulation capacity of Pb^{2+} in *Spirulina platensis* and bioconcentration factor (BCF), which has extensively been used to evaluate parameters linked to sublethal effects of heavy metals in biota (Parametrix, 1995).

MATERIALS AND METHODS

Species and culture

Spirulina platensis (S_{6-1}), preserved in a laboratory at College of Marine Life Sciences, Ocean University of China, was used in this study. It was grown at 25 ± 2 ^{0}C in Zarrouk liquid medium (Parada *et al*., 1998), for 8-10 days under white fluorescent light (90 mmol photon $m^{-2}s^{-1}$) with 14 h illumination. At the exponential growth phase, culture was filtered through muslin cloth that had been previously washed with sterilized distilled water. The pellet was washed 3 times with Zarrouk medium and used for Pb^{2+} treatments.

Regents and analytical methods

De-ionized water obtained from a Millipore Milli-Q system was used to prepare all solutions. All chemicals were of analytical grade and used without further purification. Lead stock solution of 1,000 µg/mL was prepared using $Pb(NO_3)_2$ and all working solutions were prepared by properly diluting this stock solution. The Pb^{2+} concentrations in the medium were determined using an Atomic Absorption Spectrophotometer (PGENERAL, TAS-986). Measurements of optical density were performed using a Spectrophotometer (UV - 2102) and pH of the medium was measured with a pH/ISE meter (model 868). All measurements of weight were performed using a digital balance (Sartorins, BS 210 S).

Metal bioaccumulation experiments

All experiments were carried out in 150 ml flasks, which contained various initial concentrations of Pb^{2+} (1, 2, 3, 4 and 5 µg/mL) with 100 ml of Zarrouk medium. Suspensions were continuously homogenized in a rotary shaker at 100 rounds per minutes and cultured for 10 days from initial OD_{560} nm 0.09 under the conditions as described above. Controls consisted of 0 µg/mL Pb^{2+} where all the other experimental conditions were the same.

Aliquots of 5 ml from each suspension were withdrawn at intervals of 2, 4, 6, 8 and 10 d. The aliquots were centrifuged for 10 min at 10,000 rpm and supernatant was separated for determinations of total dissolved Pb^{2+} concentration in the medium. Accumulated amount (q) of Pb^{2+} (mg/g-dry weight) was calculated using the simple concentration difference method (Volesky and Holan, 1995).

$$Q = (C_0 - C_t)\ V / 1000.W$$

Where, Q is the accumulated amount (mg/g); C_0-Initial concentration of Pb^{2+} in the medium (mg/mL); C_t-Pb^{2+} concentration at given t time (mg/mL); V-Total volume of suspension (mL); W-Dry weight of *Spirulina* (g)

The ratio between the accumulated amount which included Pb^{2+} bound in cell-wall adhering precipitate plus accumulated Pb^{2+} in the cells and the Pb^{2+} concentration in solution was used to calculate Bioconcentration factor (BCF). Cells filtered through a muslin cloth were dried at 103 ^{0}C for 2 h and kept in a desiccator for 30 minutes before measure the dry weight. A linear relationship; dry weight (g/L) = 0.207 + 0.960 x OD_{560} nm (r = 0.9927) derived in the present study was used to estimate algal dry weight at relevant OD values

Growth studies

The microalgal growth was monitored by measuring the OD_{560} nm. All the procedures were performed under aseptic conditions and experiments were triplicated in order to confirm the results. One-way ANOVA and Duncan's multiple range tests (SPSS ver.10.0 software, Chicago, Illinois**,** USA) were used in analyzing the data. Mean differences were considered significant at $P< 0.05$.

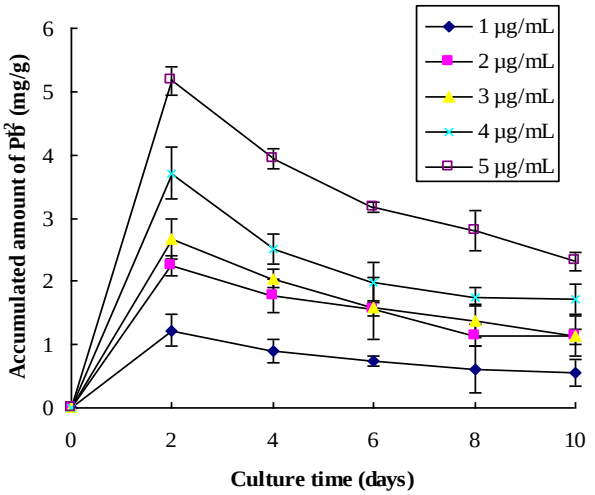

Figure 1: Accumulated amount (mean± SE) of Pb^{2+} (mg/g) in *S. platensis* grown in Zarrouk medium treated with different concentration of Pb^{2+} from 0 to 10 day culture period (n=3)

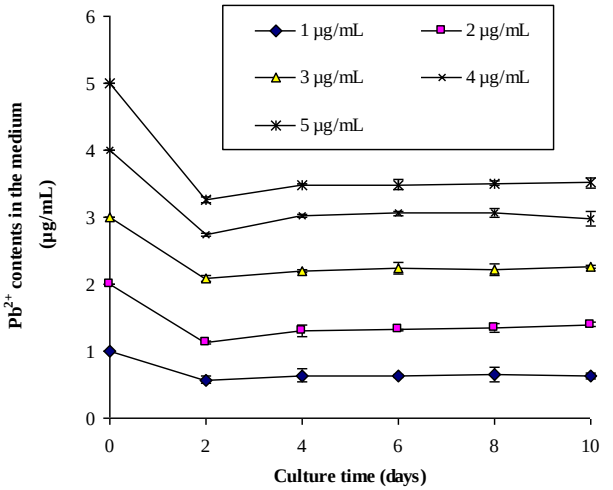

Figure 2: Residual concentration (mean ± SE) of Pb^{2+} (µg/mL) in the medium from 0 to 10 day culture period (n=3)

RESULTS

Accumulated amount of Pb^{2+}

Maximum accumulated amount of Pb^{2+} takes place within first 2 days of incubation followed by a gradual reduction for all the concentrations. Results further reveal that the accumulated amount increased with the increasing Pb^{2+} concentrations in the medium (Figure 1). Whereas a considerable reduction of the residual Pb^{2+} in the culture medium is shown during the first two days of incubation. However, Pb^{2+}concentrations in the medium are almost stable after 2 days of the incubation (Figure 2).

Bioconcentration factor (BCF) of Pb^{2+}

It is shown that the highest BCF reported at 2 days after the Pb^{2+} treatment followed by gradual reductions for all the concentrations (Figure 3). Furthermore, it reveals that BCF decreased as incubation progressed regardless the Pb^{2+} content in the medium

Effect of Pb^{2+} on growth of *S. platensis*

Growth expressed as OD_{560} nm, increases with low concentrations of Pb^{2+}. It is shown that 10, 5 and 4 % stimulation of growth in the cultures treated with 1, 2 and 3 µg/mL, respectively over that of the control, at 2 days of incubation. Higher concentrations, on the other hand, exert a slight inhibitory effect on algal growth showing 1 and 2 % reductions in cultures treated with 4 and 5 µg/mL, respectively, after 10 days of incubation. However, no growth increase is reported over 10 % (1 µg/mL, at 2 days of incubation) while the maximum growth reduction reported is 3 % (5 µg/mL, at 8 days of incubation) (Table 1).

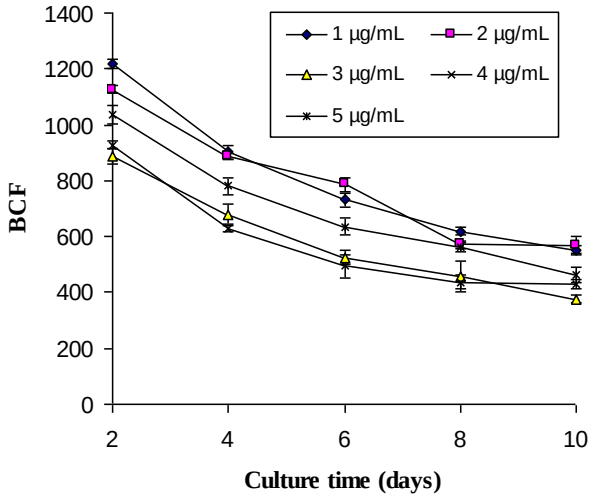

Figure 3: Changes in bioconcentration factor (BCF) of Pb^{2+} in *S. platensis* (S_{6-1}) cultured in Zarrouk medium treated with different concentration of Pb^{2+} from 0 to 10 day culture period (n=3)

DISCUSSION

Results of the present study reveal that *S. platensis* can accumulate detectable levels of Pb^{2+} from culture medium, which is in agreement with Slotton *et al*., (1989), who reported that when grown under contaminated conditions this species can contain detectable levels of Mercary and Lead. In the case of long-term accumulation of metals in microalgae, previous investigators (Kuyucak and Volesky, 1989; Knauer *et al*., 1997) have documented that the accumulation process start with fast passive adsorption followed by slow active uptake and de-sorption. It is therefore the highest accumulated amount of Pb^{2+} and BCF recorded in the present study during the first 2 days of incubation may be due to fast passive adsorption by *S. platensis*. In addition, as *S. platensis* can both adsorb and take up metals (Bender *et al*., 1994) the active absorption may also be contributed to the initial high accumulated amount and high BCF of the present results. The stress condition created by acumulated Pb^{2+} in microalgae may stimulate the secretion of organic ligands that can bind and exclude heavy metal irons from the cells could contribute to the reduction of BCF at the later stage of the incubation where the growth rate of algae was high. A similar phenomenon for Cu^{2+} absorption by three green microalgae including *Chlorella pyrenoidosa* was reported by Yan and Pan (2002) where BCF decreased at exponential growth stage. The present results of increased total Pb^{2+} accumulation by *S. platensis* cells with the increasing external Pb^{2+} concentration, is in agreement with Pawlik (2000) who obtained the similar results from *Stichococcus bacillaris* cells. Zhou *et al*. (1998) reported that the highest capacity for Cd^{2+} absorption by *S. platensis* was at pH of 6.7, followed by a reduction with increasing pH. Aksu and Kutsal (1991) reported that optimum pH for removal of Cu^{2+} by *S. platensis* was 6.7. We performed our experiment at pH 7 paying attention to minimize metal deposition and thus the observed accumulation capacity of Pb^{2+} by *Spirulina* cells might be lower than that of at low pH. However, if the species under consideration has shown a BCF of more than 1000, it would be ideal for removing heavy metals (Lamaia *et al*., 2005) and thus the present result of *S. platensis* with BCF around 1000 sounds potential accumulator for Pb^{2+}.

Previous investigations (El-Naggar *et al*., 1999) have found that lower concentrations of heavy metal (Co^{2+}) stimulate growth of microalgae (*Nostoc muscorum)*, followed by inhibition at higher concentrations, which is an agreement with the present results of positive influence of Pb^{2+} on growth of *S. platensis* at lower concentrations (1, 2 and 3 µg/mL). At low concentrations, substitution of Pb^{2+} for Zn^{2+} in some metabolloenzymes in vitro and in vivo may results in growth promotion as reported by El-Sheekh *et al*. (2003). It has extensively and long been studied the inhibition effects of heavy metals including lead on different physiological parameters and photosynthesis in algae (Navarro *et al*., 1997; Angadi and Mathad, 1998; Fargasova, 1998; Zeisler, 1998). Growth reductions at higher metal concentrations could be resulted from the inhibition of enzyme systems, photosynthesis, respiration, protein and nucleic acid synthesis. As demonstrated by Torzillo (1998) environmental stress affects the functioning of photosystem II (PS

Table 1: Effect of different concentrations of Pb^{2+} (µg/mL) on growth of *S. platensis* (S_{6-1}) as measured by optical density at 560 nm.

Pb^{2+} con. (µg/mL)	Growth measured as OD value at 560 nm				
	Culture time (days)				
	2	4	6	8	10
0	0.134±0.01	0.182±0.01	0.294±0.02	0.351±0.02	0.464±0.05
1	0.148±0.01	0.199±0.03	0.301±0.04	0.372±0.02	0.482±0.02
2	0.141±0.01	0.193±0.01	0.293±0.02	0.372±0.07	0.484±0.05
3	0.140±0.02	0.192±0.02	0.291±0.05	0.364±0.06	0.474±0.05
4	0.135±0.02	0.181±0.03	0.289±0.04	0.341±0.04	0.461±0.07
5	0.137±0.01	0.178±0.04	0.289±0.03	0.340±0.05	0.454±0.03
	NS	NS	NS	NS	NS

Initial mean OD value was measured as 0.09. Mean ± SE n = 3. , NS=Not significant

II) in *Spirulina* directly or indirectly causing growth reductions

In conclusions, no remarkable growth inhibition of *S. platensis* was shown with low concentrations of Pb^{2+}. Furthermore, the species sounds potential accumulator of Pb^{2+} and thus could be used in detoxifying metal contaminated water bodies.

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