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EFFECT OF LINEAR ALKYL BENZENE SULFONATE ON Cu^{2+} REMOVAL BY *SPIRULINA PLATENSIS* STRAIN (FACHB-834)¹

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The removal efficiency of Cu^{2+} by *Spirulina platensis* (strain FACHB-834), in viable and heat-inactivated forms, was investigated in the presence and absence of linear alkylbenzene sulfonate (LAS). When the initial Cu^{2+} concentration was in the range of $0.5\text{--}1.5 \text{ mg} \cdot \text{L}^{-1}$, a slight increase in growth rate of FACHB-834 was observed. In contrast, when Cu^{2+} or LAS concentrations were at or higher than 2.0 or $6.0 \text{ mg} \cdot \text{L}^{-1}$, respectively, the growth of FACHB-834 was inhibited and displayed yellowing and fragmentation of filaments. The presence of LAS improved Cu^{2+} removal by $\sim 20\%$, and accelerated attainment of Cu^{2+} retention equilibrium. For the $2\text{--}6 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatments, retention equilibrium occurred within 2 d and showed maximum Cu^{2+} removal of $1.83 \text{ mg} \cdot \text{L}^{-1}$. In the presence of LAS, the ratio of extracellular bound Cu^{2+} to intracellular Cu^{2+} taken up by the cells was lower ($1.05\text{--}2.26$) than corresponding ratios ($2.46\text{--}7.85$) in the absence of LAS. The percentages of extracellular bound Cu^{2+} to total Cu^{2+} removal (both bound and taken up by cells) in the presence of LAS ranged from 51.2% to 69.3% , which was lower than their corresponding percentages ($71.1\%\text{--}88.7\%$) in the absence of LAS. LAS promoted biologically active transport of the extracellular bound form of Cu^{2+} into the cell. In contrast, the addition of LAS did not increase the maximum removal efficiency of Cu^{2+} ($61.4\% \pm 5.6\%$) by heat-inactivated cells compared to that of living cells ($59.6\% \pm 6.0\%$). These results provide a theoretical foundation for designing bioremediation strategies using FACHB-

834 for use in surface waters contaminated by both heavy metals and LAS.

Key index words: Cu^{2+} ; extracellular bound; FACHB-834; heat-inactivated cells; intracellular uptake; linear alkylbenzene sulfonate; removal efficiency

Abbreviations: DAE, days after exposure; LAS, Linear alkylbenzene sulfonates; OD_{560} , optical density at 560 nm; R, removal efficiency

Heavy metal pollution in aquatic environments is of great global concern due to increasing accumulation and toxicity of these pollutants in the food chain and their continued persistence in the environment (Dudka and Miller 1999). Classical technologies applied for removal of toxic metals from aqueous solutions, such as ion exchange, chemical precipitation, membrane processing and adsorption, are often inefficient or expensive, especially when heavy metals are present at low concentrations (Gupta and Rastogi 2008a). Consequently, to maintain the safe use of environmental resources, it is important to devise efficient and low cost biochemical methods to sequester and remove these toxic elements from the environment.

In recent decades, cyanobacteria, primary producers at the base of the aquatic food chain, have received increased attention for their potential application in bioremediation and recovery of precious or strategic metals from the environment (Debelius et al. 2009). Several cyanobacteria species are very effective in adsorbing heavy metals from aqueous solutions because of their high metal binding affinity (Chong et al. 2000). Therefore, they are often used as biosorbents for recovery of precious

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metals (Mata et al. 2009) and for removal of toxic metals (Seka et al. 2008). The major challenge in biosorption studies is to select the most promising types of biosorbents from a large pool of readily available and inexpensive biomaterials (Kratovichil and Volesky 1998). The potential for metal sorption by *Spirulina* (family Oscillatoriaceae) is of great interest because of the inherent advantages associated with its mass cultivation (Gong et al. 2001, Chen and Pan 2005).

Recently, *Spirulina platensis* has been frequently used for bioremediation of metals because of its strong sorption properties and composition of bioactive substances (Aneja et al. 2010). *S. platensis* cells are known to accumulate metals passively by physical adsorption and actively by bioaccumulation (Khoshmanesh et al. 1996) due to the presence of polysaccharides, proteins and/or lipids on the surface of their cell wall, which contain charged functional groups (e.g., amino, hydroxyl, carboxyl and sulfate) (Gupta and Rastogi 2008a). Bioremoval of metal ions using *S. platensis* is affected by several factors, including the specific surface properties of the microorganism and the biomass concentration, as well as the physicochemical properties of the solution, including pH and metal ion concentration (Aksu and Donmez 2006). In addition, both viable and inactivated FACHB-834 (*S. platensis*) cells have been used to remove toxic metals from solutions (Abu Al-Rub et al. 2004), although inactivated cells may be more desirable as an adsorbent for industrial applications since they are not affected by metal toxicity (Chu and Hashim 2004). However, dead *Spirulina* sp. biomass has a maximum sorption for Cu^{2+} , Cr^{3+} , and Ni^{2+} of 130, 167, and 515 $\text{mg} \cdot \text{g}^{-1}$, respectively, while for live cells maximum sorption values were 389, 304, and 1,378 $\text{mg} \cdot \text{g}^{-1}$, respectively (Doshi et al. 2007). The metabolic activities of live cells facilitate the uptake and accumulation of metal ions, while the dead cells just serve as an adsorbent. These results suggest that both living and dead *Spirulina* sp. can function as a biosorption material for heavy metals (Doshi et al. 2007).

Most previous studies have focused on the removal efficiency of metal ions by *S. platensis*, while there is little information about metal removal efficiency under conditions of combined organic and heavy metal pollution. Linear alkylbenzene sulfonates (LAS) are a group of anionic surfactants employed in the formulation of laundry and cleaning products with a global production of 4 million metric tons (Wu et al. 2010). Concentrations of LAS have been detected as environmental contaminants in many countries (Holt et al. 2003, Morales-Munoz et al. 2004) at concentrations ranging from 0.01 to 10 $\text{mg} \cdot \text{L}^{-1}$ (generally concentrations are below 0.5 $\text{mg} \cdot \text{L}^{-1}$). In rural areas in China, where clothes are often cleaned in rivers, LAS pollution is introduced directly into river systems (Wu et al. 2010). Under such circumstances, organisms such as

S. platensis are simultaneously exposed to LAS and heavy metals (Gordon et al. 2008). As a polar surfactant, LAS acts by nonspecific denaturation of various macromolecules (e.g., proteins). Additionally, LAS can affect the activity of surface molecular groups related to sorption, and thus can change the ability of an adsorbent to remove toxic metals from an aqueous environment. Meng et al. (2012) found that LAS enhanced the biosorption of Zn^{2+} , while Zn^{2+} can also enhance LAS biodegradation (Sanz et al. 2003). Additionally, surfactants such as sodium dodecyl sulfate and cetyltrimethylammonium bromide may increase the solubility and mass transfer of hydrophobic organic compounds, leading to enhanced bioremediation efficiency in soil or water mediums (Seo and Bishop 2007, Seo et al. 2009). However, there is a paucity of data concerning the effects of LAS or other surfactants on metal removal efficiency by *S. platensis*.

The major goal of this research was to investigate removal efficiency of Cu^{2+} by *S. platensis* strain FACHB-834 in the presence of LAS by studying: (i) growth of FACHB-834 in the presence of Cu^{2+} or LAS, (ii) the effect of LAS on removal efficiency of Cu^{2+} , (iii) the effect of LAS on Cu^{2+} extracellular sorption and intracellular uptake, and (iv) Cu^{2+} removal efficiency by heat-inactivated forms of FACHB-834 in the presence of LAS. This study provides information to guide remediation efforts for surface waters contaminated with heavy metals and LAS using *Spirulina*.

MATERIALS AND METHODS

Cultivation of FACHB-834 and sample preparation. The unialgal FACHB-834 was purchased from the Chinese Freshwater Algal Library, Institute of Hydrobiology, Chinese Academy of Sciences (Wuhan, China). FACHB-834 was isolated from Dongying Salt Marshes, China as suspensible and spiral filaments of *Spirulina*. The culture medium was Zarrouk liquid medium, which consisted of NaHCO_3 : 16.8 $\text{g} \cdot \text{L}^{-1}$, NaNO_3 : 2.5 $\text{g} \cdot \text{L}^{-1}$, NaCl : 1.0 $\text{g} \cdot \text{L}^{-1}$, K_2HPO_4 : 1.0 $\text{g} \cdot \text{L}^{-1}$, K_2SO_4 : 1.0 $\text{g} \cdot \text{L}^{-1}$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$: 0.04 $\text{g} \cdot \text{L}^{-1}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$: 0.04 $\text{g} \cdot \text{L}^{-1}$, EDTA: 0.08 $\text{g} \cdot \text{L}^{-1}$, A5: 1 $\text{mg} \cdot \text{L}^{-1}$ and B6: 1 $\text{mg} \cdot \text{L}^{-1}$. The initial pH of the medium was adjusted to 8.0 using NaOH (Radmann et al. 2007).

FACHB-834 was cultivated in 150 mL of Zarrouk medium at $25^\circ\text{C} \pm 1^\circ\text{C}$, constant light from fluorescent lamps (illuminance 37.5 $\mu\text{mol photons} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) with a dark:light cycle of 12:12 h, and continuous mixing in a thermostatically controlled shaker in 250 mL flasks. In the Cu^{2+} removal experiments, a Cu^{2+} stock solution (100 $\text{mg} \cdot \text{L}^{-1}$) prepared from $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ was added to the growth medium to obtain working concentrations of 0.0, 0.5, 1.0, 1.5 and 2.0 $\text{mg} \cdot \text{L}^{-1}$.

The FACHB-834 culture sample in its logarithmic growth phase was centrifuged at 5,357g for 15 min, and the supernatant was discarded. The pelleted FACHB-834 cells were washed twice with sterile Milli-Q water for inoculation into the test growth medium. The initial FACHB-834 density was adjusted to 0.1 optical density at 560 nm (OD_{560}). Each treatment was prepared in triplicate and evaluated over a period of 8 d. FACHB-834 samples were collected on day 0.5, 1, 2, 4, 6 and 8 for evaluation of Cu^{2+} removal efficiency.

Chemicals and reagents. All chemicals were of analytical grade and solutions were prepared using Milli-Q water. LAS (95% purity) was supplied by Shanghai Shengzhong Fine Chemical Co., Ltd., Shanghai, China.

Medium-dissolved, intracellular-bound, and extracellular uptake Cu^{2+} concentrations. In the culturing process, the OD_{560} of FACHB-834 cells in Zarrouk medium was measured each day. At the end of the culturing period (8 d), the cells were centrifuged at $5,357g$ for 15 min and rinsed using Milli-Q water. The washing process was repeated three times to remove the culture medium and non-adsorbed Cu^{2+} . The concentrated cells were dried until the weight no longer changed, and then the biomass of cells was weighed. Finally, the linear relationship between weight (B) and density (OD_{560}) was established as:

$$B(\text{g} \cdot \text{L}^{-1}) = 0.0497 + 0.0653 \times \text{OD}_{560} \quad (1)$$

The Cu^{2+} concentration was measured as described by Ma et al. (2003) with minor changes. Dissolved Cu^{2+} concentrations were measured at 0.5, 1, 2, 4, 6 and 8 days after exposure (DAE). The spent FACHB-834 culture medium was generated by pelleting the cells by centrifugation at $5,357g$ for 15 min; the supernatant solutions (4 mL each) were used for determination of dissolved Cu^{2+} (C_{dis}). Eight microliters of concentrated HNO_3 was added to the spent medium, which was then analyzed by atom absorption spectrometry (Hitachi Z-5000; Ibaraki-ken, Japan) for Cu^{2+} concentration based on absorbance at 324 nm. At 8 DAE, the intracellular and extracellular Cu^{2+} concentrations were determined as reported by Zhou et al. (2012). The culture (10 mL) was centrifuged at $5,357g$ for 15 min, the supernatant was discarded and the pelleted FACHB-834 cells were re-suspended in 5 mL of 0.02 M EDTA and shaken for ~1 min to remove the Cu^{2+} adsorbed to the cell surface. The sample solution was then centrifuged for an additional 15 min at $5,357g$, and 8 μL of concentrated HNO_3 was added to the supernatant solution (4 mL), which was then used for the determination of the extracellular Cu^{2+} concentration (C_{ec} at $\mu\text{g} \cdot \text{g}^{-1}$ DW). The FACHB-834 cells were left to dry and then acid-digested with 0.5 mL of concentrated HNO_3 at 90°C overnight. The acid-treated sample was diluted to 5 mL with Milli-Q water and analyzed for intracellular Cu^{2+} concentrations (C_{ic} at $\mu\text{g} \cdot \text{g}^{-1}$ DW) by atomic absorption spectrometry. The extracellular bound (C_{ecb}) and intracellular uptake (C_{icu}) of Cu^{2+} was calculated as:

$$C_{\text{ecb}} = (C_{\text{ini}} - C_{\text{dis}}) \times C_{\text{ec}} = (C_{\text{ec}} + C_{\text{ic}}) \quad (2)$$

$$C_{\text{ecb}} = (C_{\text{ini}} - C_{\text{dis}}) \times C_{\text{ic}} = (C_{\text{ec}} + C_{\text{ic}}) \quad (3)$$

where C_{ini} , C_{dis} , C_{ec} , and C_{ic} represent the initial concentration of Cu^{2+} , the dissolved concentration of Cu^{2+} in growth medium, the detected extracellular Cu^{2+} concentration ($\mu\text{g} \cdot \text{g}^{-1}$ DW), and the detected intracellular Cu^{2+} concentration ($\mu\text{g} \cdot \text{g}^{-1}$ DW), respectively.

The removal efficiency (R, in percent) by FACHB-834 biomass was calculated using equation (4) as described by Ajjabi and Chouba (2009):

$$R(\%) = (C_{\text{ini}} - C_{\text{fin}}) \times 100 = C_{\text{ini}} \quad (4)$$

where R is the dissolved metal removal (in percent) and C_{ini} and C_{fin} are the initial and final concentrations of Cu^{2+} in the solution, respectively.

Removal of Cu^{2+} by heat-inactivated cells. As reported by Monteiro et al. (2011), with minor modifications, FACHB-834 cells were inactivated by heating at 100°C for 24 h. The

removal efficiency by the heat-inactivated cells was determined by exposing FACHB-834 to LAS (0 or $3.0 \text{ mg} \cdot \text{L}^{-1}$) and a Cu^{2+} concentration of $2.0 \text{ mg} \cdot \text{L}^{-1}$. Each treatment was performed in triplicate in a water bath at 25°C and stirred at 100 rpm. Samples were collected at 0.5, 1, 2, 4, 6 and 8 d, and then centrifuged at $5,357g$ for 15 min at 4°C . Pre-treatment procedures and analytical methods for Cu^{2+} quantification were performed as described above.

Statistical analysis. Data analysis was performed using Origin 8.0 software (OriginLab, Northampton, MA, USA). One-way ANOVA was employed to compare differences between means using the Dunnett or the non-parametric Jonkheere–Terpstra test in SPSS 16.0 (SPSS, Chicago, IL, USA) as a post-hoc test to assess differences between treatment and control groups. The Fisher's Least Significant Difference test was applied to evaluate differences among treatment groups using a significance level of $P < 0.05$. Data were reported as mean \pm SD unless otherwise stated.

RESULTS AND DISCUSSION

Growth of FACHB-834. The effects of LAS and Cu^{2+} on growth of FACHB-834 are shown in Figures 1 and 2, respectively. Compared with the control medium, initial LAS concentrations in the range of 0.5 – $3.0 \text{ mg} \cdot \text{L}^{-1}$ showed no obvious growth differences during 8 d of culturing. However, when LAS concentrations were 6.0 and $10.0 \text{ mg} \cdot \text{L}^{-1}$, the growth of FACHB-834 was markedly inhibited. The computed EC_{50} of LAS for FACHB-834 was $6.52 \text{ mg} \cdot \text{L}^{-1}$, which was in agreement with previously reported results of $6.0 \text{ mg} \cdot \text{L}^{-1}$ for *S. platensis* (Meng et al. 2012). After 8 d of exposure, the OD_{560} value for the control group was 1.595, while it was 0.945 and 0.725 (Fig. 1) in the 6.0 and $10.0 \text{ mg} \cdot \text{L}^{-1}$ LAS treatment groups, respectively. This represents a 41%–55% reduction of OD_{560} values compared to the control (Fig. 1). With initial Cu^{2+} concentrations in the range 0.5 – $1.5 \text{ mg} \cdot \text{L}^{-1}$ there was a slight increase in FACHB-834 growth rates compared to the control. In contrast, FACHB-

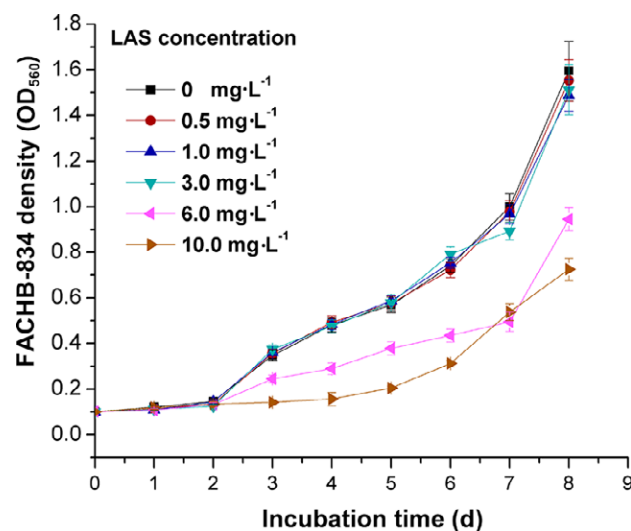


Fig. 1. The effect of LAS on growth of FACHB-834.

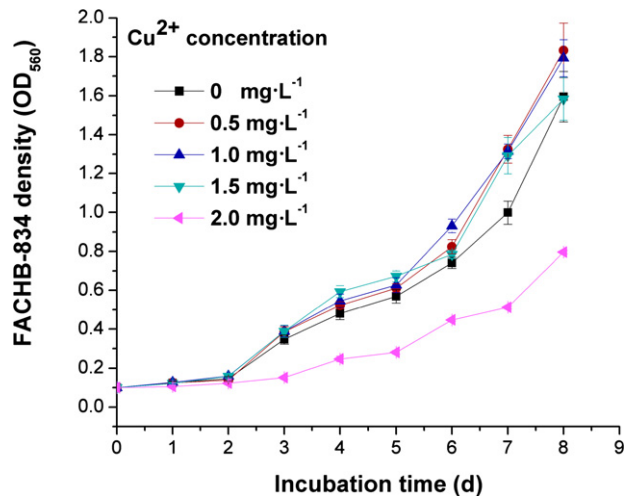


FIG. 2. The effect of Cu^{2+} on growth of FACHB-834.

834 growth rates were reduced at a Cu^{2+} concentration of $2.0 \text{ mg} \cdot \text{L}^{-1}$ (Fig. 2). At the end of the incubation, the OD_{560} value for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment was 0.796, approximately half of the OD_{560} value for the control group (Fig. 2). The calculated EC_{50} of Cu^{2+} for FACHB-834 was $1.87 \text{ mg} \cdot \text{L}^{-1}$. The stressed growth of FACHB-834 was visually apparent by yellowing and fragmentation of filaments in the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment.

Previous studies reported that *Synechocystis aquatilis* showed a decrease in growth with increasing Cu^{2+} concentrations at initial exposures of 0.1 – $0.5 \text{ mg} \cdot \text{L}^{-1}$ (Deniz et al. 2011), suggesting that different cyanobacteria have markedly different responses to Cu^{2+} stress. The effects of metal ions on cyanobacteria growth depended on the species and the metal concentration in the medium (Folgar et al. 2009). The reduction in growth could be due to inhibition of normal cell division by the metal, as reported for *Padina boeagesennt* exposed to Cu^{2+} and *Anabaena flosaquae* exposed to Cu^{2+} and Cd^{2+} (Surosz and Palinska 2004). The decrease in the rate of cell division caused by Cu^{2+} was primarily attributed to metal binding to sulfhydryl groups, which are important for regulating plant cell division. Debelius et al. (2009) found that the presence of high amounts of transitional metals, such as Cu^{2+} , stimulated free radical formation in *Spirulina* sp., which had a prominent deleterious effect on cell growth.

Cu^{2+} removal by FACHB-834 in the presence and absence of LAS. The kinetic profiles of Cu^{2+} removal by FACHB-834 are shown in Figure 3a and b in the absence and presence of LAS, respectively. In the absence of LAS, the Cu^{2+} removal efficiency increased rapidly during the first 2 d, with the exception of the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment, and thereafter remained nearly constant from 2 to 8 DAE (Fig. 3a). At the end of experimental period, FACHB-834 removed 64.2%–72.3% of Cu^{2+} (Fig. 3a)

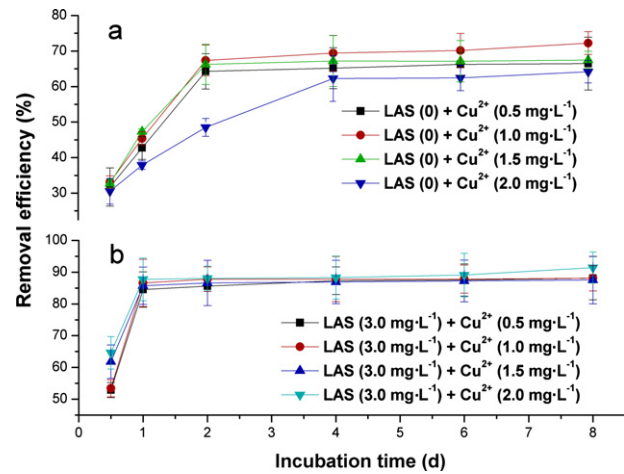


FIG. 3. (a) The removal efficiency of Cu^{2+} by viable FACHB-834 in the absence of LAS; (b) The removal efficiency of Cu^{2+} by viable FACHB-834 in the presence of LAS.

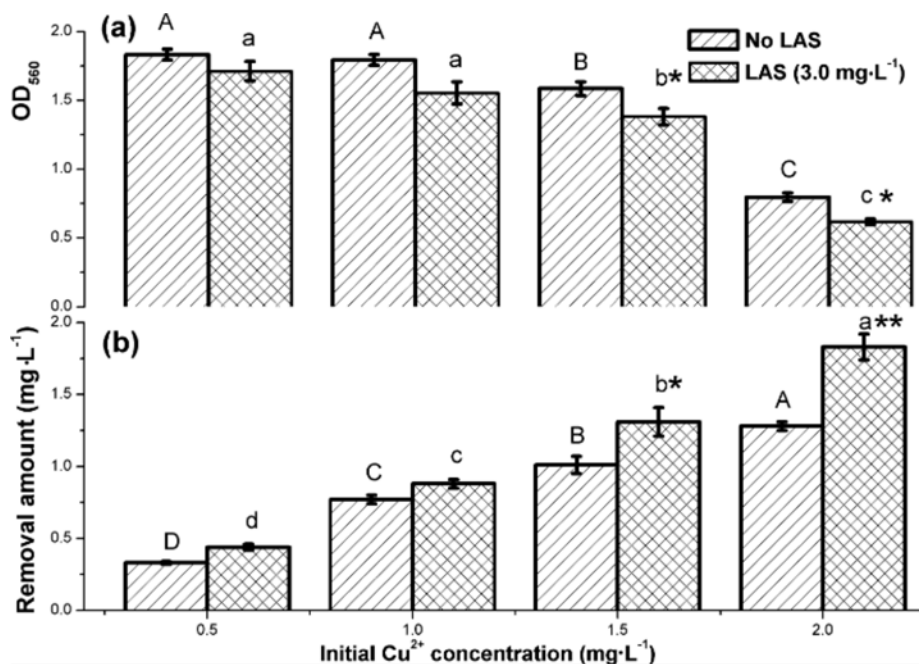
or 0.33 – $1.28 \text{ mg} \cdot \text{L}^{-1}$ of Cu^{2+} for the four treatments (Table 1). As shown in Figure 4b, the maximum removal amount ($1.28 \text{ mg} \cdot \text{L}^{-1}$) for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment was delayed ~ 2 d (at 4 DAE) compared to the maximum removal for the other treatments at 2 DAE. This lag for maximum removal might be due to the growth-inhibiting effect of $2 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} on *S. platensis*.

The removal efficiency was improved for each treatment in the presence of LAS ($3.0 \text{ mg} \cdot \text{L}^{-1}$). Equilibrium was established between adsorbed/absorbed Cu^{2+} and Cu^{2+} free in solution after 1 d with a maximum Cu^{2+} removal of 84.6%–87.7% (Fig. 3b). The above results demonstrate that the presence of LAS significantly increased Cu^{2+} removal efficiency by $\sim 20\%$ as compared with the absence of LAS, and in addition accelerated attainment of equilibrium. At 8 DAE, FACHB-834 adsorbed or absorbed up to 0.44 – $1.83 \text{ mg} \cdot \text{L}^{-1}$ of Cu^{2+} for the four treatments in the presence of LAS (Table 1 and Fig. 4b). Especially for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment, there was no delay in the time required for maximum removal. The extracellular bound and intracellular uptake amount for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ Cu^{2+} treatment in the presence of LAS reached highs of 1.27 and $0.56 \text{ mg} \cdot \text{L}^{-1}$, respectively. These results demonstrated that LAS pro-

TABLE 1. Removal efficiency and amount of Cu^{2+} at 8-DAE by FACHB-834.

Cu^{2+}	LAS ($0 \text{ mg} \cdot \text{L}^{-1}$)		LAS ($3 \text{ mg} \cdot \text{L}^{-1}$)	
	Removal efficiency of Cu^{2+} (%)	Removal amount of Cu^{2+} ($\text{mg} \cdot \text{L}^{-1}$)	Removal efficiency of Cu^{2+} (%)	Removal amount of Cu^{2+} ($\text{mg} \cdot \text{L}^{-1}$)
0.5	66.52 ± 7.45	0.33 ± 0.03	88.73 ± 6.81	0.44 ± 0.04
1.0	77.34 ± 3.21	0.77 ± 0.05	88.15 ± 3.98	0.88 ± 0.06
1.5	67.56 ± 2.45	1.01 ± 0.05	87.56 ± 7.48	1.31 ± 0.07
2.0	64.28 ± 3.17	1.28 ± 0.07	91.52 ± 4.89	1.83 ± 0.08

FIG. 4. (a) OD_{560} of FACHB-834 at 8 DAE (no LAS or addition of LAS); (b) The removal amount of Cu^{2+} at 8 DAE by viable FACHB-834 (no LAS or addition of LAS). Notes: uppercase letters indicate significant differences (ANOVA: $F_{3,8} = 298.546$, $P < 0.05$) for no LAS treatment; different lowercase letters indicate significant differences (ANOVA: $F_{3,8} = 410.261$, $P < 0.05$) for $3.0 \text{ mg} \cdot \text{L}^{-1}$ LAS treatment; "*" or "**" denote a significant difference at $P < 0.05$ or $P < 0.01$, respectively; $n = 3$ for all treatments; (4) "*" or "**" denote a significant difference at $P < 0.05$ or $P < 0.01$, respectively, between the same uppercase and lowercase letters (A and a, B and b, C and c).



moted sorption or uptake of metal ions by FACHB-834 cells. Moreover, irrespective of LAS concentrations, the Cu^{2+} removal amounts increased with increasing initial Cu^{2+} concentrations. The biosorption mainly consists of physical adsorption and chemical absorption. Previous studies suggest that the mechanism of metal ion uptake by *S. platensis* involved metal ion uptake and biosorption (e.g., sorption and chemical complexation; Li and Guo 2006). Meng et al. (2012) found that LAS enhanced maximum Zn^{2+} uptake capacity by *S. platensis* by increasing metal bioavailability. In this investigation, a similar increase in Cu^{2+} bioavailability to FACHB-834 was suggested in the presence of LAS.

Extracellular bound and intracellular uptake Cu^{2+} concentrations in the absence of LAS. In the absence of LAS, although the OD_{560} values of FACHB-834 were decreased from 1.832 to 0.796 with increasing initial concentrations of Cu^{2+} from 0.5 to $2.0 \text{ mg} \cdot \text{L}^{-1}$ (Fig. 4a), both the dissolved (C_{dis}) and extracellular bound (C_{ecb}) Cu^{2+} concentrations showed a significant increasing trend, and the intercellular uptake (C_{icu}) Cu^{2+} concentrations were also increased, except for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ treatment (Table 2). The C_{ecb} values increased from 0.23 to $1.14 \text{ mg} \cdot \text{L}^{-1}$, an increase of ~4-fold. The ratio of C_{ecb} to C_{icu} varied between 2.46 and 7.85, and the percentages of C_{ecb} to total biosorption (C_{tbs} , $C_{\text{ecb}} + C_{\text{icu}}$) ranged from 71.1% to 88.7%, which indicated that the extracellular bound Cu^{2+} in FACHB-834 accounted for the highest percentage of total Cu^{2+} retention. These results are consistent with previous studies of freshwater *Spirulina subspicatus* (Zhou et al. 2012) and marine *Dunaliella salina* (Folgar et al. 2009). Zhou et al. (2012) reported that extracellular Cu^{2+} contents were 50.8%–60.9% and

TABLE 2. Extracellular bound and intracellular uptake Cu^{2+} by FACHB-834 (no LAS) at 8 DAE.

C_{ini} ($\text{mg} \cdot \text{L}^{-1}$)	OD_{560}	C_{dis} ($\text{mg} \cdot \text{L}^{-1}$)	C_{ecb} ($\text{mg} \cdot \text{L}^{-1}$)	C_{icu} ($\text{mg} \cdot \text{L}^{-1}$)	$C_{\text{ecb}}/C_{\text{icu}}$	$C_{\text{ecb}}/C_{\text{tbs}}$
0.5	1.832	0.17 ± 0.03	0.234 ^a	0.095 ^a	2.46	71.1
1.0	1.793	0.28 ± 0.04	0.563 ^b	0.156 ^b	3.59	78.2
1.5	1.584	0.48 ± 0.07	0.799 ^b	0.220 ^b	3.63	78.4
2.0	0.796	0.72 ± 0.13	1.135 ^c	0.144 ^b	7.85	88.7

C_{dis} , C_{ecb} and C_{icu} indicate dissolved Cu^{2+} concentration in medium, concentration of extracellular bound Cu^{2+} uptake and concentration of intercellular Cu^{2+} uptake, respectively. C_{tbs} indicates total biosorption of Cu^{2+} ($C_{\text{ecb}} + C_{\text{icu}}$). The different lowercase letters indicate the significant level (ANOVA: $F_{3,8} = 182.433$, $P < 0.05$ for C_{ecb} , and ANOVA: $F_{3,8} = 126.457$, $P < 0.05$ for C_{icu} , respectively).

44.6%–53.8% for *Chlorella pyrenoidosa* and *Scenedesmus obliquus*, respectively. The $C_{\text{ecb}}/C_{\text{icu}}$ values of Cu^{2+} for *C. pyrenoidosa* (2.64–10.07) were slightly higher than the corresponding ratios for *S. obliquus* (1.47–4.22). The relationship of growth speed with photosynthetic pigment for *C. pyrenoidosa* and *S. obliquus* was attributed to binding of metals on the microalgal cell wall, not to metal binding at intracellular active sites as previously reported by Ma et al. (2003). Thus, extracellular bound metal concentrations are a good indicator of removal efficiency for *S. platensis*. Results from this and previous studies indicate that non-metabolic extracellular metal binding plays an important role in metal removal by *S. platensis*.

Extracellular bound and intracellular uptake Cu^{2+} concentrations in the presence of LAS. In the presence of LAS ($3.0 \text{ mg} \cdot \text{L}^{-1}$), the OD_{560} values of FACHB-834 decreased from 1.712 to 0.617 with increasing

TABLE 3. Extracellular and intracellular Cu^{2+} by FACHB-834 (LAS) at 8 DAE.

C_{ini} ($\text{mg} \cdot \text{L}^{-1}$)	OD_{560}	C_{dis} ($\text{mg} \cdot \text{L}^{-1}$)	C_{ecb} ($\text{mg} \cdot \text{L}^{-1}$)	C_{icu} ($\text{mg} \cdot \text{L}^{-1}$)	$C_{\text{ecb}}/C_{\text{icu}}$	$C_{\text{ecb}}/C_{\text{tbs}}$
0.5	1.71	0.06 ± 0.02	0.23^{a}	0.21^{a}	1.05	51.26
1.0	1.55	0.11 ± 0.03	0.55^{ab}	0.34^{ab}	1.59	61.44
1.5	1.38	0.19 ± 0.03	0.86^{b}	0.44^{bc}	1.99	66.64
2.0	0.62	0.17 ± 0.04	1.27^{c}	0.56^{c}	2.26	69.32

The initial LAS concentration is $3.0 \text{ mg} \cdot \text{L}^{-1}$. C_{dis} , C_{ecb} and C_{icu} indicate dissolved Cu^{2+} concentration in medium, concentration of extracellular bound Cu^{2+} and concentration of intercellular uptake Cu^{2+} , respectively. C_{tbs} indicates total biosorption of Cu^{2+} ($C_{\text{ecb}} + C_{\text{icu}}$). The different lowercase letters indicate the significant level (ANOVA: $F_{3,8} = 82.782$, $P < 0.05$ for C_{ecb} and ANOVA: $F_{3,8} = 96.345$, $P < 0.05$ for C_{icu} , respectively).

initial concentrations of Cu^{2+} from 0.5 to $2.0 \text{ mg} \cdot \text{L}^{-1}$ (Fig. 4a). Although the OD_{560} was decreased to 0.617 for the $2.0 \text{ mg} \cdot \text{L}^{-1}$ treatment, the average C_{dis} for Cu^{2+} was only $0.17 \text{ mg} \cdot \text{L}^{-1}$, indicating that more than 90% of retained Cu^{2+} was extracellularly bound or intracellularly uptaken. These data demonstrate that *S. platensis* had a very high biosorption capacity for Cu^{2+} . The ratio of C_{ecb} to C_{icu} varied between 1.05 and 2.26, and the percentage of C_{ecb} to total biosorption (C_{tbs} , $C_{\text{ecb}} + C_{\text{icu}}$) ranged from 51.2% to 69.3%. The total biosorption values were lower than their corresponding ratios (71.1%–88.7%) in the absence of LAS (Table 3). This result indicates that LAS promotes the uptake of Cu^{2+} into intracellular tissues. The cell wall, composed mainly by carbohydrates and proteins, is the first barrier in the intracellular uptake of metal ions. Extracellular components in the cell wall may have a larger quantity of binding sites available for metals than the cytoplasm (Gupta et al. 2006). Metal retained by sorption onto the cell surface may be released via desorption by chemical agents, such as synthetic surfactants, which may enhance transfer efficiency of metal ions from extracellular sorption sites for intracellular uptake (Belokobylsky et al. 2004, Gupta and Rastogi 2008b). The above results indicate that LAS can efficiently release Cu^{2+} adsorbed onto the cell surface to promote its intracellular uptake.

Removal of Cu^{2+} by heat-inactivated FACHB-834. The removal efficiency of Cu^{2+} by heat-inactivated FACHB-834 cells was investigated using Cu^{2+} ($2.0 \text{ mg} \cdot \text{L}^{-1}$) and combined Cu^{2+} ($2.0 \text{ mg} \cdot \text{L}^{-1}$) and LAS ($3.0 \text{ mg} \cdot \text{L}^{-1}$) additions. After addition of the inactivated biomass, the concentration of Cu^{2+} in solution dropped rapidly within the time interval of 12–24 h. The maximum removal efficiency (~60%) was observed at 1 DAE (Fig. 5), and remained nearly constant over the remaining 2–8 DAE. In addition, no significant difference in Cu^{2+} removal efficiency was observed in the presence and absence of LAS. This fast disappearance of Cu^{2+} from solution suggests that the metal removal by thermally inactivated cells occurs exclusively by sorption onto the FACHB-834 cell surface (i.e., independent of metabolism processes). It has been reported that the sorption of heavy metal ions by *S. platensis*

follows a two-step mechanism where the metal ion is physically or chemically retained on the surface of the *S. platensis* cell before being taken up biologically into the cells (Kojima and Lee 2001). The first step (known as passive uptake) occurs rapidly, while the second biological step, active transport, take more time to complete. Since the *S. platensis* in this study was dried and biological functions were no longer active, the sorption could only take place on the cell surface (Aneja et al. 2010). Monteiro et al. (2011) also observed metal retention by heat-inactivated *S. platensis* cells, and found that physical sorption of the metal via binding to the functional groups of cell wall polysaccharides was the most likely mechanism accounting for metal uptake. The uptake of Cu^{2+} by live *Spirulina* sp. was found to follow a second-order rate equation, while Cu^{2+} adsorbed on inactivated *Spirulina* sp. follow pseudo first-order kinetics. In the case of metal sorption by inactivated *Spirulina* sp., reaction with phosphate, hydroxyl, and carboxylate groups play the primary role (Flouty and Estephane 2012). The higher metal sorption by live *Spirulina* sp. may be traced to metal transporters through the cell, thereby all the functional groups of intercellular biopolymer matrix

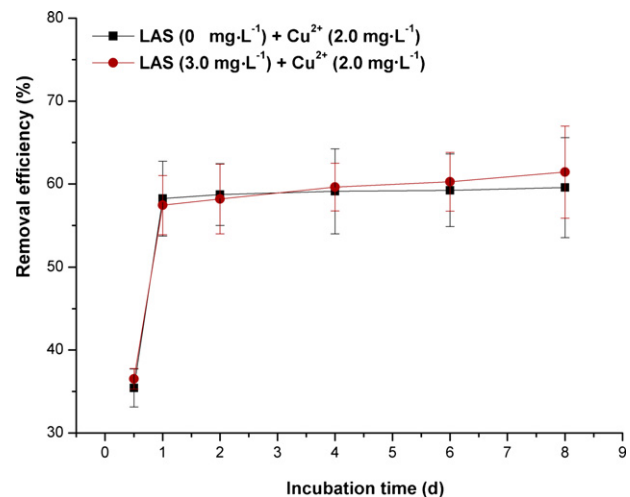


FIG. 5. The effect of LAS on Cu^{2+} removal efficiency by heat-inactivated FACHB-834.

take part in the sorption process (Doshi et al. 2007). Therefore, both viable and inactivated *S. platensis* cells can be used to remove toxic metals from solution, although inactivated cells are more efficient and cost-effective for industrial applications as they are not influenced by metal toxicity (Chu and Hashim 2004).

In the presence of LAS, the maximum Cu^{2+} removal efficiency ($61.4\% \pm 5.6\%$), under combined addition of LAS ($3.0 \text{ mg} \cdot \text{L}^{-1}$) and Cu^{2+} ($2.0 \text{ mg} \cdot \text{L}^{-1}$), by heat-inactivated cells was similar to removal efficiency in the absence of LAS ($59.6\% \pm 6.0\%$; Fig. 5). This is consistent with reports that the surfactant LAS can promote extracellular to intracellular transfer of Cu^{2+} in biologically active *S. platensis* (Monteiro et al. 2011), and relieve its toxicity effects to a certain extent. This metal transfer promoting role of LAS was not active in the heat-inactivated *S. platensis*, which resulted in the decreased uptake and Cu^{2+} removal efficiency compared to living cells. Such an observation is probably attributed to disruption of structural components in the cell walls owing to the heating/drying process, which leads to protein denaturation (Chu and Hashim 2004), and is thus responsible for the decrease in the number of the functional sites available to interact with Cu^{2+} (as occurs in living cells). In contrast, Flouty and Estephane (2012) proposed that both viable and non-viable biomass of *Chlamydomonas reinhardtii* exhibited the ability to biosorb and bioaccumulate Cu^{2+} and Pb^{2+} , with dead microalgal cells showing higher removal efficiencies than living cells and intracellular accumulation of metal ions was limited compared to living cells. These results also indicate that heat-inactivated FACHB-834 cells are a good adsorbing matrix for Cu^{2+} , although to a lesser degree than living cells. This form of biomass may consequently be a promising material for bioremediation of wastewaters contaminated jointly with toxic metals and LAS, since it does not require *S. platensis* survival.

CONCLUSIONS

Low LAS ($<3.0 \text{ mg} \cdot \text{L}^{-1}$) or Cu^{2+} ($<1.5 \text{ mg} \cdot \text{L}^{-1}$) concentrations had no effect or a small growth-promoting effect on FACHB-834 cells. In contrast, high LAS ($\geq 6.0 \text{ mg} \cdot \text{L}^{-1}$) or Cu^{2+} ($\geq 2.0 \text{ mg} \cdot \text{L}^{-1}$) concentrations lead to significant growth inhibition of FACHB-834 cells, with visually apparent yellowing and fragmentation of filaments. The FACHB-834 EC_{50} values for LAS and Cu^{2+} were 6.52 and $1.87 \text{ mg} \cdot \text{L}^{-1}$, respectively. LAS increased Cu^{2+} removal efficiency by $\sim 20\%$ at 8 DAE, and accelerated attainment of biosorption equilibrium. The maximum Cu^{2+} removal was $1.83 \text{ mg} \cdot \text{L}^{-1}$ for the combined LAS ($3.0 \text{ mg} \cdot \text{L}^{-1}$) and Cu^{2+} ($2.0 \text{ mg} \cdot \text{L}^{-1}$) treatment. LAS promoted biologically active transport of Cu^{2+} from extracellular bound forms to enhance intracellular uptake, result-

ing in a decrease in the percentage of extracellular bound to total biosorption from 71.1%–88.7% (no LAS) to 51.2%–69.3% ($3 \text{ mg} \cdot \text{L}^{-1}$ LAS). The extracellular bound metal concentration is a good indicator for removal efficiency, and non-metabolic extracellular binding plays an important role in metal retention by *S. platensis*. In contrast, LAS did not increase the maximum Cu^{2+} removal efficiency by heat-inactivated FACHB-834 cells because disruption of structural components and protein denaturation occurred in the heating/drying process. These results provide a basis for designing bioremediation strategies using FACHB-834 for use in surface waters contaminated by both heavy metals and LAS.

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