

Technical Note
Influence of the hydration degree on copper removal
by *Spirulina platensis* biomass

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ABSTRACT

In the present work, hydrated biomass of *Spirulina platensis* has been used for copper removal from aqueous solutions. Biomass has firstly been characterised by potentiometric titration and the overall ionic content evaluated. Copper uptake experiments have been carried out using powdered dry biomass of this cyanobacterium, preliminarily hydrated for increasing time, namely 0, 24, 48, 72 and 96h. Titration experimental data suggest that biomass contains two main acidic groups and total specific negative charge of about 0.4meq/g biomass. The total amount of metal cations released by biomass indicates a metal adsorption capacity of 0.79meq/g. Adsorption tests have evidenced an increase in copper removal capacity of biomass induced by its pre-hydration, likely due to the fact that the contact with water was able to swell up dry biomass so as to expose to the outside a significant number of sites available for adsorption. A hydration time of 48h appears to be the best condition of *S. platensis* biomass pre-treatment for copper removal. © 2003 SDU. All rights reserved.

Keywords: *Spirulina platensis*; Adsorption; Copper removal; Hydration

1. INTRODUCTION

The presence of heavy metals in the environment over permitted levels is a severe public health problem (Beccari *et al.*, 1986). In particular, copper is widely present in the environment and has a relatively high added value. Besides its presence in natural deposits as ore and in industrial wastewater, it can contaminate drinking water in the case of corrosion of household copper pipes. Moreover, copper mining, smelting operations and municipal incineration may be further sources of contamination.

Many researchers have recently demonstrated the possibility of removing heavy metals from wastewater by means of biological organisms, among which algae, bacteria, fungi and yeasts (Gadd *et al.*, 1988; Siegel *et al.*, 1990; Volesky *et al.*, 1993; Kambe-Honjoh *et al.*, 1997). Among these, marine algae (Fourest and Volesky, 1996) represent a rich resource in the oceanic environment, are relatively cheap and particularly able to accumulate metals. Due to their high adsorption capacity, they are largely employed for heavy metal removal (Yang and Volesky, 1999). In particular, the alga *Spirulina platensis* grows very well in seawater or in highly saline waters (Vonshak, 1997). The abundance of this microalga during summer makes it largely available in dried form, mainly due to the high temperature that favours water evaporation. In this way, such a biomass can easily be recovered and utilised for metal adsorption or other applications (Ciferri and Tiboni, 1985; Cohen *et al.*, 1987; Dillon *et al.*, 1995).

Physico-chemical parameters such as temperature and pH are reported to have a strong influence on adsorption of metal ions, especially when using living biomass (Vegliò and

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Beolchini, 1997; Siegel *et al.*, 1990; Solisio *et al.*, 2000), whereas the effectiveness of dried biomass as biocatalyst is expected to depend mainly on the hydration degree. For this reason, the present work aims at determining the effect of preliminary hydration of powdered dried biomass of *S. platensis* on its copper uptake capacity.

2. EXPERIMENTAL

2.1. Sorbent preparation

Spirulina platensis UTEX 1926 was obtained from the University of Texas Culture Collection. Cells were grown batch-wise in outdoor cultivation ponds filled with the medium of Schlösser (1982). Each pond, having a surface area of 0.15m² and a water depth of 5cm, was kept at T=30°C and continuously illuminated by 8 fluorescent lamps (40W) located at about 40cm over its surface.

After one-month cultivation, a layer of microalga formed on the surface of the tanks. It was collected, rinsed three times by deionised water and dried at room temperature for 24h and subsequently at 80°C in oven for 12h. It was then ground and passed through a sieve of 60-100mesh (150µm) and stored in desiccator.

2.2. Biomass characterisation

The ionic exchange properties of *S. platensis* biomass were studied in order to determine the nature and the adsorption capacity of this sorbent. For this purpose, a known amount of dried biomass (0.82g) was submitted to four acidic treatments with 50ml 0.1N HCl for 30min on a shaker at 200rpm, at room temperature. The washing waters were analysed for the alkaline and alkaline earth metal content. The harvested biomass after acidic treatment (protonated biomass) was titrated with 0.1N NaOH in order to highlight the type and eventually quantify the functional groups on cell surface. Each NaOH addition was performed on the shaker and, after reaching the equilibrium, the suspension pH was measured.

2.3. Copper adsorption tests

Powdered biomass (200mg) was put in contact with 100ml of a 0.5g/l copper sulphate solution, corresponding to 0.2g/l Cu, in 250ml Erlenmeyer flasks. Magnetic stirrer provided mixing. Samples were withdrawn from the solution after increasing contact times up to reach the sorption equilibrium and analysed for metal content by atomic absorption spectroscopy (Perkin-Elmer, mod. 5000). The metal uptake was determined as difference of metal concentration in the sample and in the starting solution. pH changed only slightly during the adsorption tests, ranging from 7.5 to 8.5. Copper uptake experiments were carried out using dry biomass of *S. platensis*, preliminarily hydrated for increasing time, namely 0, 24, 48, 72 and 96h.

Additional tests of copper adsorption were also carried out at constant biomass level (2g/l) and variable starting copper concentration (0.1–0.4g/l) and hydration time (0–48h) to obtain the Langmuir isotherms.

3. RESULTS AND DISCUSSION

3.1. Biomass characterisation

Figure 1 shows the titration curve of *Spirulina platensis*, from which it is possible to point out three inflexion points. The observed shifting from the standard acid/base titration curve is due to the buffering capacity of some functional groups abundant in the cell wall of this species.

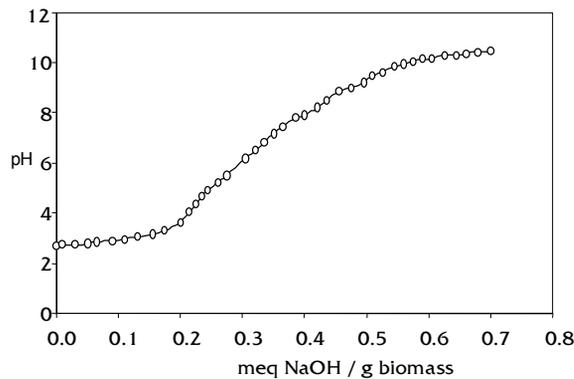


Figure 1. Potentiometric titration of *S. platensis* biomass

The first two inflexion points take place around pH 5.2 and 7.8, that is relatively close to the pK_a of carboxylic ($pK_a \approx 4.8$) and phosphoric groups ($pK_{a2} \approx 7.2$), respectively, the little difference being likely due to their link to the glycidic and/or aminoacidic structure of peptidoglycan (Tomaselli, 1997). The third inflexion point at more alkaline conditions (pH ≈ 9.0) can reasonably be ascribed to the dissociation of both lateral and terminal aminic groups of the aminoacids present in the cell wall. Considering that only the first two groups, when dissociated, can bind metal ions, a total acidic site content of about 0.4 meq/g biomass has been estimated.

The overall amount of metal cations released by biomass during the four acidic washings indicates a total metal adsorption capacity of 0.79 meq/g (Table 1). The difference between this value and the above content of acidic sites (0.4 meq/g) points out that the overall adsorption capacity of biomass is much higher than the one suggested by the titration curve; therefore, about one half of the metal ions could be adsorbed onto the cell wall by non-ionic bonds (charge-dipole interactions).

Table 1
 Alkaline and alkaline earth metals content of dried biomass of *S. platensis*

Washing	Ca ²⁺ (mg)	Mg ²⁺ (mg)	Na ⁺ (mg)	K ⁺ (mg)
1	3.36	0.735	2.73	0.910
2	0.640	0.181	1.73	0.411
3	0.570	0.155	1.08	0.193
4	0.161	0.0451	0.790	0.0479
Total ionic content (mg)	4.73	1.12	6.33	1.56
Specific ionic content (meq/g)	0.29	0.11	0.34	0.049

3.2. Adsorption tests

Figure 2 shows the time behaviour of Cu²⁺ adsorption by dry biomass of *S. platensis*, preliminarily hydrated for increasing times. It is evident that the curves referring to hydration time (t_h) of 0–24h are very close and evidence a good adsorption capacity of biomass ($Y \approx 80\%$), although it was lower than the excellent removal ($Y \approx 98\%$) observed with biomass hydrated for longer time (48–96h). From these results it can be deduced that $t_h = 48h$ is the minimum time necessary to obtain a nearly total Cu²⁺ removal.

Figure 3 shows the isotherms obtained fitting the equilibrium data of adsorption tests performed at constant biomass level (2g/l) and variable hydration time (0–48h) and starting copper concentration (0.1–0.4g/l) by the linear form of Langmuir equation:

$$\frac{1}{q} = \frac{1}{q_{\max}} + \frac{1}{q_{\max} \cdot b \cdot C_{\text{eq}}}$$

where q is biosorbent capacity, q_{\max} its maximum theoretical value, C_{eq} copper concentration at the equilibrium and b the adsorption affinity of biomass for copper. The values of Table 2

demonstrate that both parameters increase with increasing the hydration time of the sorbent, thus confirming the significant role of water on the adsorption capacity of *S. platensis* biomass.

The increase in the Cu^{2+} removal capacity of biomass induced by its pre-hydration can be due to the biomass swell provoked by its contact with water. The resulting exposure to the outside of a significant number of the above dissociable and polar active sites could have made them available for the electrostatic interaction with Cu^{2+} .

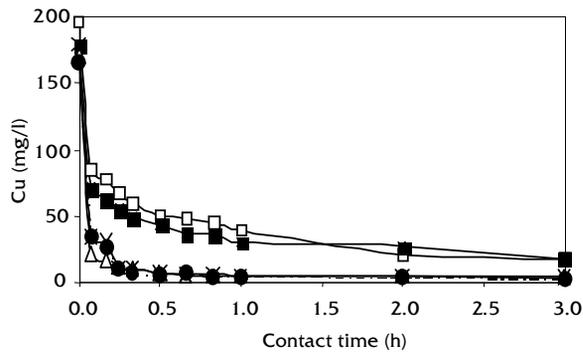


Figure 2. Time behaviour of copper removal in aqueous solution using *S. platensis* biomass pre-hydrated for increasing time. t_h (h): (□) 0, (■) 24, (△) 48, (●) 72, (×) 96.

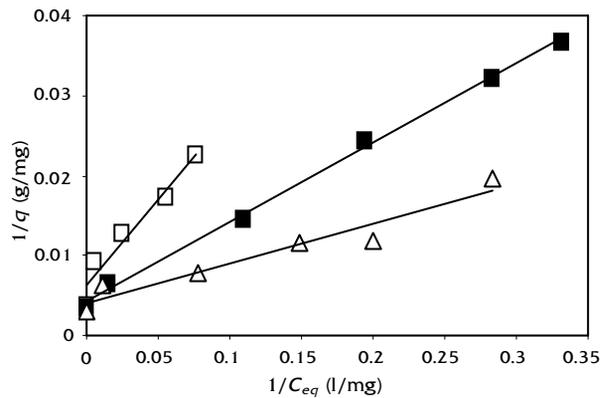


Figure 3. Langmuir isotherms of copper adsorption by dried biomass of *S. platensis* hydrated for variable time (h) : (□) 0; (■) 24; (△) 48

Table 2

Influence of the hydration time of *S. platensis* biomass on the Langmuir isotherm parameters

t_h (h)	q_{max} (mg/g)	b (l/mg)	r^2
0	161	0.029	0.9435
24	232	0.043	0.9976
48	250	0.080	0.9296

In addition, the dissociated carboxylic and phosphoric groups could have contributed to biomass particles dispersion, thus preventing their agglomeration. So, by pre-hydration of dry biomass, it is possible to reach two simultaneous goals: an increase in the specific surface of the adsorbing particles as well as a more effective removal of metal ions by both electrostatic and polar attractions.

Figure 4 shows the simultaneous effects of the time of biomass hydration and the contact time (t_c) on the capacity of *S. platensis* biomass in copper removal (q). It is noteworthy that, at $t_h < 48\text{h}$, a quite long contact time (t_c up to 3h) is required to reach a satisfactory removal capacity ($> 80\text{mg Cu}^{2+}/\text{g biomass}$). On the contrary, biomass pre-hydrated for long ($t_h=72-96\text{h}$) is able to immediately ensure ($t_c=0.25\text{h}$) even better results ($84\text{mg Cu}^{2+}/\text{g biomass}$).

Table 3 lists the values of the specific rate of copper adsorption, k_{ads} , estimated at the equilibrium by the equation of Lagergreen (1898). An increase in the biomass hydration time up to 48h brought about a clear increase in the adsorption rate, whereas it remained practically constant at higher t_h values.

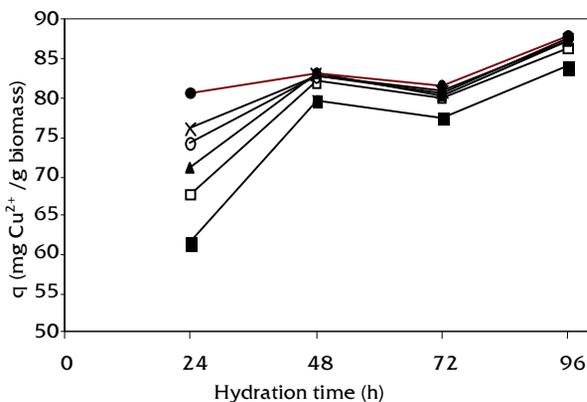


Figure 4. Dependence of Cu^{2+} adsorption capacity of *S. platensis* biomass (q) on the hydration time, at variable contact time. t_c (h): (■) 0.25, (□) 0.50, (▲) 0.83, (○) 1.0, (×) 2.0, (●) 3.0

Table 3

Specific rate of copper adsorption, k_{ads} , estimated by the Lagergreen's equation for *S. platensis* biomass hydrated for increasing time

t_h (h)	0	24	48	72	96
k_{ads} (h^{-1})	1.6	3.5	7.7	7.3	7.5

This result seems to confirm that a hydration time of 48h is the best condition of *S. platensis* biomass pre-treatment for copper removal.

4. CONCLUSIONS

In this study, *Spirulina platensis* biomass submitted to preliminary hydration was tested as a copper biosorbent and its performance was evaluated as a function of the biomass hydration time as well as the metal contact time. Biomass characterised by potentiometric titration demonstrated an excellent ability in copper removal, likely associated either to acidic (carboxylic and phosphoric) groups or to polar functional groups. The experimental results presented in this work point out a hydration time of 48h as the most favourable pre-treatment condition for copper removal using this kind of biomass.

REFERENCES

- Beccari, M., Di Pinto, A. C., Marani, D., Santori, M., Tiravanti, G., I metalli nelle acque: origine, distribuzione, metodi di rimozione. In Istituto di Ricerca sulle Acque, Quaderni, 1986, **71**, CNR, Rome.
- Ciferri, O., Tiboni, O., The biochemistry and industrial potential of *Spirulina*. Annual Review of Microbiology, 1985, **39**, 503-526.
- Cohen, Z.V., Vonshak, A., Richmond, A., Fatty acid composition of *Spirulina* strains grown under various environmental conditions. Phytochemistry, 1987, **26**, 2255-2258.
- Dillon, J.C., Phuc, A.P., Dubacq, J.P., Nutritional value of the alga *Spirulina*. World Review of Nutrition and Dietetics, 1995, **77**, 32-46.
- Fourest, E., Volesky, B., Contribution of sulphonate groups and alginate to heavy metal biosorption by the dry biomass of *Sargassum fluitans*. Environmental Science and Technology, 1996, **30**, 277-282.
- Gadd, G.M., White, C., De Rome, L., Heavy metal and radionuclide uptake by fungi and yeast. In Biohydrometallurgy, Norris, P.R., Kelly, D.P. (Eds.), Chippenham, Wilts, UK, 1988.

- Kambe-Honjoh, H., Sugawara, A., Yoda, K., Kitamoto, K., Yamasaki, M., Isolation and characterisation of nickel-accumulating yeast. *Applied Microbiology and Biotechnology*, 1997, **48**, 373-378.
- Lagergreen, S., About the theory of so-called adsorption of soluble substances. *Kungliga Svenska Vetenskapsakademiens Handlingar*, 1898, **24**(4), 1-39.
- Schlösser, U.G., Sammlung von Algenkulturen. *Ber. Deutsch Bot. Ges.*, 1982, **95**, 181-276.
- Siegel, S.M., Galun, M., Siegel, B.Z., Filamentous fungi as metal biosorbents: a review. *Water, Air and Soil Pollution*, 1990, **53**, 335-344.
- Solisio, C., Lodi, A., Converti, A., Del Borghi, M., The effect of acid pre-treatment on the biosorption of chromium(III) by *Sphaerotilus natans* from industrial wastewater. *Water Research*, 2000, **34**, 3171-3178.
- Tomaselli, L., Morphology, ultrastructure and taxonomy of *Arthrospira (Spirulina) maxima* and *Arthrospira (Spirulina) platensis*. In *Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology*. Vonshak, A. (Ed.), Taylor & Francis, London, 1997, 1-15.
- Vegliò, F., Beolchini, F., Removal of metals by biosorption: a review. *Hydrometallurgy*, 1997, **44**, 301-316.
- Volesky, B., May, H., Holan, Z. R., Cadmium biosorption by *Saccharomyces cerevisiae*. *Biotechnology and Bioengineering*, 1993, **41**, 826-829.
- Vonshak, A., *Spirulina: growth, physiology and biochemistry*. In *Spirulina platensis (Arthrospira): physiology, cell-biology and biotechnology*. Vonshak, A. (Ed.), Taylor & Francis, London, 1997, 43-65.
- Yang, J., Volesky, B., Biosorption of uranium on *Sargassum* biomass. *Water Research*, 1999, **33**, 3357-3363.