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Carlo Solisio[a]; Alessandra Lodi[a]; Attilio Converti[a]; Marco Del Borghi[a]
[a] Department of Chemical and Process Engineering "G. B. Bonino", Faculty of Engineering, University of Genoa, Genoa, Italy

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Influence of Temperature on Cadmium Removal by *Sphaerotilus natans* from Acidic Solutions

Carlo Solisio, Alessandra Lodi, Attilio Converti,* and Marco Del Borghi

Department of Chemical and Process Engineering ''G. B. Bonino,'' Faculty of Engineering, University of Genoa, Genoa, Italy

ABSTRACT

A culture of *Sphaerotilus natans* (NCIMB 11196) was used for cadmium removal from acidic solutions, simulating the composition of industrial wastewaters. Tests were carried out at temperatures increasing from 15 up to 40°C, to check the actual possibility of utilizing a biological system to remove this heavy metal from water as well as to shed light on the phenomenon responsible for its uptake. The highest values of the specific growth rate of this microorganism ($\mu_{max} = 0.11$ to $0.13\ h^{-1}$) and cadmium removal rate ($k_r = 0.15\ h^{-1}$) were obtained within 25 to 30°C. Under these conditions, biomass was able to increase the pH of the medium from 4.0 up to 7.0 to 7.8. The data of μ_{max} and k_r collected at different temperatures were finally used to estimate, according to Arrhenius, the

*Correspondence: Attilio Converti, Department of Chemical and Process Engineering ''G. B. Bonino,'' Faculty of Engineering, University of Genoa, via Opera Pia 15, Genoa 16145, Italy; Fax: +39-010-3532586; E-mail: converti@unige.it.

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thermodynamic parameters of cell growth and cadmium removal as well as of the related thermal inactivations. On the basis of these results, cadmium seemed to be removed by *S. natans* following a mechanism controlled by cell growth, implying the quick electrostatic attraction of ions to the negative charges present on the cell surface.

Key Words: Cadmium biosorption; *Sphaerotilus natans*; Acidic solutions; Batch tests; Temperature; Thermodynamics.

INTRODUCTION

Many metal recycling or processing industries, as well as surface treatment companies, produce wastewaters highly contaminated by heavy metals, which can constitute a source of strong pollution, if released into the environment without previous treatment. Different processes, mostly based on conventional technologies, such as chemical precipitation; coagulation, reduction, and membrane processes; ion exchange; and adsorption are used for the treatment of these wastewaters.[1,2] However, most of them suffer from significant disadvantages, among which are unpredictable and incomplete metal ions removal, high energy and reagent demands, relatively high capital and running costs, and generation of toxic sludge.[3] In particular, chemical precipitation and ion exchange become inefficient and economically unfeasible when the concentration of heavy metal ions is relatively low (from 1 to 100 mg L^{-1}).[4,5]

Therefore, the requirement of economic and effective methods for metal removal at low concentration has been stimulating the development of innovative treatment technologies. The feasibility has been demonstrated of employing either inorganic recycling materials, such as sand, kaolin, peat, dolomite,[6–10] or organic material (living or dead biomass) as sorbents for metal removal from wastewater.[8,11–14]

Biosorption is emerging as a potential option for heavy metal removal; it consists in the accumulation of heavy metals either using microorganisms (mainly bacteria and fungi) and photosynthetic life (such as algae and aquatic and emergent plants). In shallow bodies of water (1 to 5 m), having low concentrations of heavy metals (1 to 20 mg L^{-1}), biosorption can be very effective.[15] Previous studies suggested that biosorption occurs in a manner similar to ion exchange when inactivated cells are used.[16,17] Metal uptake capacity has been ascribed to the protein–lipopolysaccharide sheath of some filamentous microorganisms[16,18] or to metal precipitation as hydroxide within the microcrystalline cell wall.[19,20] On the other hand, in living cells, the preliminary external adsorption may be followed by a

metabolism-dependent uptake step in which the metal is transported into the cells.[19,21] The main advantages of biosorbents are competitive performance, selectivity for heavy metals, possibility of metal recovery and sorbent regeneration, use of well-known process equipments, and no sludge generation,[3] while the major disadvantage is the toxic effect of metals on the living organism.[22]

In recent years, the consumption pattern of cadmium has increasingly shifted away from the traditional market areas of pigments, stabilizers, and coating, to rapidly growing applications in Ni-Cd batteries, which constitute 70% of total cadmium employment.[23] For these reasons, cadmium emissions are increasing in air, water, and soil and there may be considerable transfer between these three environment compartments after initial deposition. High levels may accumulate in sedimentary rocks; marine phosphates and phosphorites have been reported to contain cadmium at considerable levels.[23] Weathering and erosion of rocks result in the transport by rivers of large quantities of this metal; in addition, effluents containing cadmium and coming from phosphate fertilizers, nonferrous metals production and iron and steel industry, are further causes of pollution.

The filamentous bacterium *Sphaerotilus natans*, being naturally present in sewage sludge and polluted waters,[24] is demonstrated to be one of the best microorganisms to remove metals from wastewaters.[21,25] In particular, the pH influence on metal removal has been investigated to simulate the composition of common industrial wastewaters[26] as well as to shed light on the mechanism of this process.

The present work aimed at evaluating the ability of this microorganism to remove cadmium as well as studying the influence of temperature on the removal rate and yield. It was subdivided into two parts: in the former, cell growth kinetics was studied at temperatures increasing from 15°C up to 40°C, to establish the optimum temperature for growth under acidic conditions (starting pH = 4.0); in the latter, biomass, previously grown at different temperatures, was employed in batch tests of cadmium removal.

MATERIALS AND METHODS

Biomass Cultivation

The strain *Sphaerotilus natans* (NCIMB 11196) was purchased from National Collection of Industrial Marine Bacteria Ldt. (Aberdeen, Scotland). The cells were grown for 36 to 48 h at different temperatures in a medium containing 1.5 g L^{-1} peptone and 1.5 g L^{-1} yeast extract in tap water. The medium pH was adjusted to 4.0 by means of sulfuric acid, to

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simulate the acidic conditions of an industrial effluent.[26] At the end of the exponential phase, biomass was harvested by centrifugation at 5000 rpm, resuspended in water, and used as inoculum (0.30 g L^{-1}) for batch tests either of biomass growth or metal removal.

Operating Conditions

Batch adsorption tests were carried out at pH = 4.0 in a 3.0 L-fermenter (Applikon, Z61103CT04, Schiedam, The Netherlands), containing 1.0 L of medium, stirred at 150 rpm and aerated at flow-rate of 0.5 L h^{-1}. To avoid inhibition of cell growth, the selected starting cadmium concentration (45 mg L^{-1}) was ensured by addition of cadmium sulfate rather than chloride. Such a relatively low concentration was chosen as the optimal compromise between the necessities of maximizing, on the one hand, the metal concentration in the solution and, on the other hand, the microbial removal efficiency. In fact, microbial removal of metal ions was demonstrated to be more effective at low metal concentrations.[27] The pH was let to vary without any control during metal uptake to simulate the actual conditions of a possible real-scale application. Batch runs were performed in triplicate at different temperatures, namely 15, 20, 25, 30, 35, and 40°C. The experimental error was always less than ± 6%.

Analytical Procedures

Samples (5 mL) were withdrawn at regular time intervals (8 h) and filtered through Millipore filters with 0.45-μm pore diameter. Biomass concentration was determined as the difference between the suspended solid content obtained by dry weight after washing with distilled water and the metal content of cells determined after acidic digestion.[25] Cadmium concentration in the filtrate was determined by an atomic absorption spectrophotometer (Perkin Elmer, mod. 5000, Norwalk, CT).

RESULTS AND DISCUSSION

Batch Tests of *Sphaerotilus natans* Growth at Different Temperatures

The influence of temperature on *S. natans* growth was studied in the first part of this work at a constant inoculum level ($X_o = 0.30$ g L^{-1}). Table 1 lists the values of the main cultivation and kinetic parameters obtained from batch runs carried out at different temperatures ($15 \leq T \leq 40$°C). In

Table 1. Cultivation and kinetic parameters of *S. natans* growth at different temperatures. $X_o = 0.30$ g L^{-1}.

T (°C)	μ_{max} (h^{-1})	K_s (g L^{-1})	pH	t (h)	X_f (g L^{-1})
15	0.040	0.18	7.0	50	0.65
20	0.052	0.21	7.2	47	0.76
25	0.11	0.22	7.6	45	1.6
30	0.13	0.20	7.8	40	1.7
35	0.066	0.19	7.3	45	0.86
40	0.049	0.18	7.1	50	0.81

T = temperature; μ_{max} = maximum specific growth rate; K_s = saturation constant of Monod equation; pH = final pH at the end of cultivations; t = time duration of cultivations; and X_f = final biomass concentration.

particular, the maximum specific growth rate (μ_{max}) and the saturation constant (K_s) of the Monod equation were estimated according to Lineweaver–Burk from the experimental data of biomass concentration.

Final biomass concentration and cultivation time were notably influenced by temperature, both showing optimal values around 30°C ($X_f = 1.7$ g L^{-1}; $t = 40$ h) and pointing out growth slowing down either at higher or lower temperatures. As already observed,[25] pH gradually increased during growth from 4.0 up to 7.0 to 7.8 within 40 to 55 h, likely due to an active microbial mechanism. The peculiar microbial activity responsible for such a pH increase during growth in metal ion-free solution was explained on the basis of an exchange of protons from the solution with light metal ions (sodium, calcium, and magnesium) present in the biomass.[28]

The highest μ_{max} values (0.11 to 0.13 h^{-1}) were obtained at 25 to 30°C, after an incubation time only of 20 h, which suggests that the microorganism was able, within this temperature range, to promptly neutralize the environment. At lower (15 to 20°C) and higher (35 to 40°C) temperatures, μ_{max} was one order of magnitude lower. On the other hand, no significant K_s variation is evident, which suggests that temperature does not affect the substrate affinity for biomass.

Batch Tests of Cadmium Removal at Different Temperatures

Biomass grown at different temperatures (ranging from 15 to 40°C) was recovered and employed in successive tests of cadmium removal, performed at the same temperature as the growth. The progressive decrease in cadmium concentration is plotted vs time in Figure 1. In all tests, except

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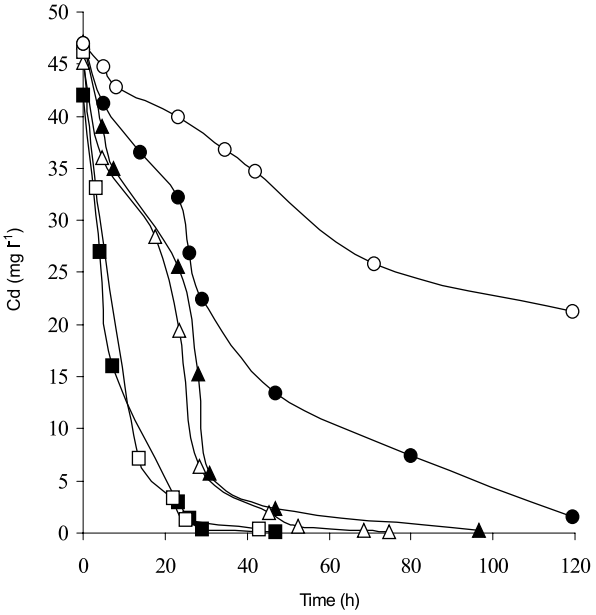

Figure 1. Results of batch tests of cadmium removal from acidic solutions. Temperature (°C): (○) 15; (●) 20; (□) 25; (■) 30; (△) 35; (▲) 40.

that performed at 15°C, cadmium was nearly completely removed, but the time necessary to get this result largely depended on temperature.

Comparing the abatement curves of this figure with those of pH variation (Figure 2), one can observe that the medium was subjected to progressive alkalinization like that observed during growth in metal ion-free solution. Such a pH increase during sorption can be alternately ascribed to dissolution of cytoplasmic components of biomass[16] or to alkaline metabolite release.[25] The metabolization of peptone and yeast extract to sustain growth during these tests could have led, in the present work, to ammonia release and partially contributed to such a pH increase. Whatever the reason may be for this behavior, cadmium already started to be removed and biomass started to grow under mildly acidic conditions ($5.5 < pH < 6.0$). In fact, for tests carried out at 25 to 30°C, the maximum removal yield was 0.98 after only 24 h; after this time, pH gradually increased up to neutrality, thereby favoring effective biomass growth (see Figure 3). On the other hand, the tests performed at higher temperatures (35 to 40°C) not only lasted a much longer time (70 to 90 h) to ensure comparable removal efficiency, but also showed much lower growth in the first 20 h because the

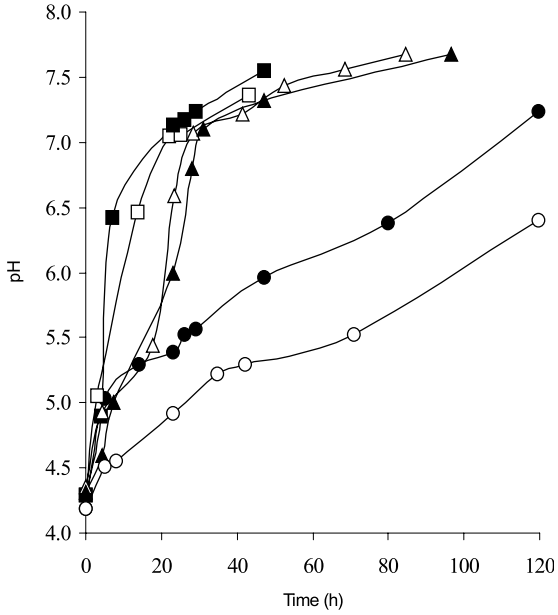

Figure 2. pH variation during batch tests of cadmium removal from acidic solutions. Temperature (°C): (○) 15; (●) 20; (□) 25; (■) 30; (△) 35; (▲) 40.

conditions were still very acidic. The yield of cadmium removal was quite unsatisfactory at lower temperatures (15 to 20°C) and no less than 120 h were necessary to reach neutrality at 20°C. But the most interesting finding is that, regardless of temperature, biomass started to grow with an acceptable rate only after achievement of pH = 7.0.

On the whole, these results suggest that cadmium was quickly and effectively removed by biomass at the beginning of every test, with rate depending on temperature. After neutrality achievement, lasting a time strongly influenced by temperature, cells were able to grow abundantly and to provide new biomass for further removal. Previous work suggested that such a peculiar behavior could be due to more than one active mechanism involved in metal uptake.[25] Likewise in other gram-negative species possessing multiple resistances to heavy metals, specific proteins could be organized in multimeric aggregates in the outer membrane, forming transmembrane pores notoriously suitable for metal uptake.[29] Besides, two types of acidic sites were identified on the cell wall of *S. natans*, corresponding to carboxylic and phosphate groups, respectively;[30] therefore, cadmium removal could be due partially to the presence of these

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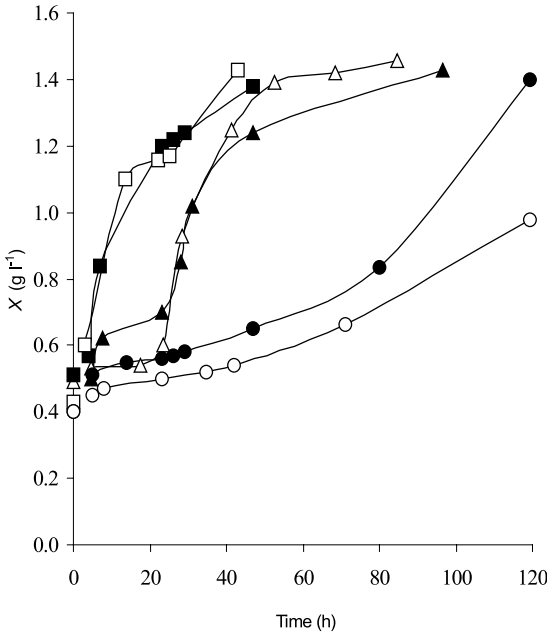

Figure 3. Biomass growth during batch tests of cadmium removal from acidic water. Temperature (°C): (○) 15; (●) 20; (□) 25; (■) 30; (△) 35; (▲) 40.

functional groups in relation to the pH solution. While at acidic pH these sites are protonated and the adsorption is ineffective, under alkaline conditions, their dissociation leads to an increase in the negative charge density onto the cell surface, thus promoting biosorption.

The results of cadmium removal at different temperatures have been worked out by the Lagergreen equation:

$$\log(q_e - q) = \log q_e - \frac{k_r}{2.303} \cdot t \tag{1}$$

where q_e and q are the amounts of adsorbed metal per unit cell mass at the equilibrium and after a contact time t, respectively, while k_r is the adsorption rate constant.

The values of k_r, listed in Table 2, confirm that the most favorable temperatures for metal uptake are within 25 to 30°C. This kinetic parameter progressively decreased out of this range. This behavior could be explained with a combination of physical adsorption and biological uptake. In particular, the progressive unexpected decrease of k_r for $T < 25$°C is consistent with a biological phenomenon, which would be oppositely

Table 2. Parameters of the Lagergreen model estimated at variable temperature for cadmium removal by *S. natans*.

T (°C)	k_r (h^{-1})
15	0.0125
20	0.0287
25	0.146
30	0.145
35	0.0898
40	0.0667

T = temperature; k_r = specific cadmium removal rate.

influenced by temperature with respect to adsorption. As recently reported for *Pseudomonas* sp., such a biological phenomenon could be due to a slow intracellular uptake through the membrane following external binding to the cell.[3] However, because of cadmium toxicity and the filamentous morphology of *S. natans*, we prefer to believe that the biological uptake could interest the protein-lipopolysaccharide sheath rather than the cell wall.

To explain the peculiar effect of temperature evidenced by the above kinetic approach, the equilibrium biosorption data of cadmium removal tests have been worked out by the Freundlich model. The dependences either of the adsorbing capacity of biomass (K_f) or of the adsorbing affinity (n) on temperature, evidenced in Table 3, confirm the above temperature range (25 to 30°C) as the optimum for metal uptake.

Table 3. Parameters of the Freundlich model estimated at different temperatures for cadmium removal by *S. natans*.

T (°C)	K_f (h^{-1})	n	r^2
20	0.0567	0.5627	0.993
25	0.7824	0.9287	0.998
30	0.8109	0.9158	0.997
35	0.5408	0.8551	0.999
40	0.5125	0.8206	0.997

T = temperature; K_f = adsorbing capacity of the Freundlich model; n = adsorbing affinity of the Freundlich model; and r^2 = determination coefficient.

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The results of Cd/biomass ratio, detected after a given contact time (24 h), are plotted in Figure 4 vs. the temperature at which each test was carried out (curve a). Such a ratio progressively increased up to 25 to 30°C, whereas it rapidly fell beyond this threshold, in agreement with the values of k_r listed in Table 2. However, since the pH solution was quite different after 24 h in the different tests (see Figure 2), the removal capacity of biomass may well largely be affected by pH variation. To properly illustrate the temperature effect without any pH interference, the same parameter has also been estimated at a given pH (6.0) and plotted in Figure 4 (curve b). As can be seen, the biomass capacity of removing cadmium estimated at a given pH was not significantly affected by temperature. This means that cadmium uptake was kinetically controlled and that any study on possible influence of temperature should deal with kinetic rather than equilibrium parameters, as discussed in the following.

Thermodynamic Parameters of Biomass Growth and Cadmium Biosorption

The values of μ_{max} and k_r have been plotted according to Arrhenius in semilog plots vs the reciprocal temperature to estimate the thermodynamic parameters of both biomass growth and cadmium biosorption (Figure 5).

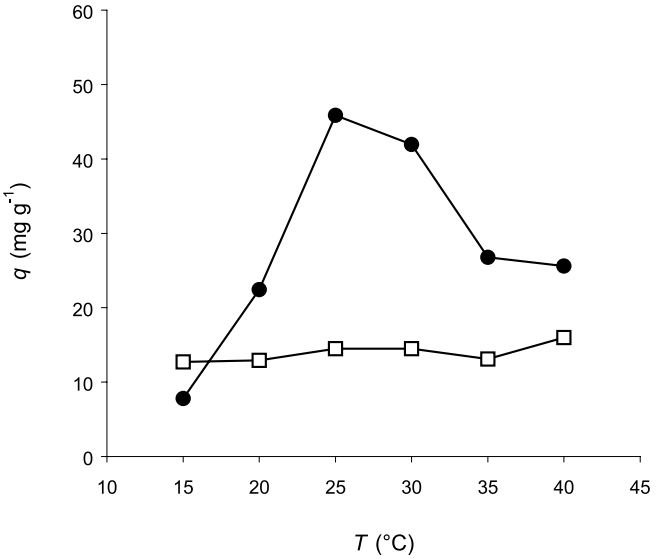

Figure 4. Temperature dependence of cadmium/biomass ratio calculated (a) after 24 h (●) of contact time and (b) at pH = 6.0 (□).

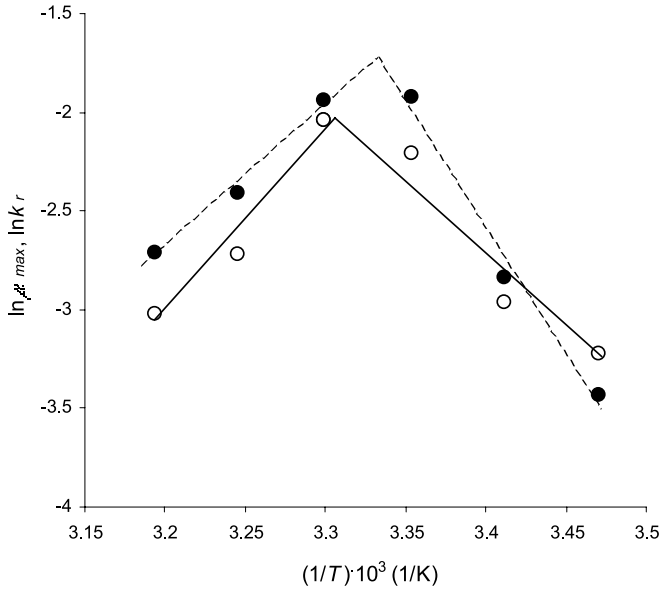

Figure 5. Arrhenius plots for the estimation of thermodynamic parameters of (○) *S. natans* growth and (●) cadmium removal.

Based on the hypothesis that the phenomenon controlling any biological activity, including cell growth, could have an enzymatic origin,[31,32] the temperature influence on bioprocess kinetics can be described, according to the formalism of Roels,[33] by the relationship:

$$v_i = \frac{A_i \exp(-\Delta h_i^*/RT)}{1 + B_i \exp(-\Delta h_i^{\circ}{}_D/RT)} \tag{2}$$

where v_i is the kinetic parameter influenced by temperature, A_i is the Arrhenius pre-exponential factor linked to the activation entropy, Δh_i^* the related activation enthalpies, B_i and $\Delta h_i^{\circ}{}_D$ the entropic and enthalpic contributions of thermal inactivation equilibrium, R the ideal gas constant, and T the absolute temperature.

So, at low temperature, the positive effect of temperature on the activated state formation would prevail according to the Arrhenius equation, whereas at high temperature, the system behavior would be dominated by the equilibrium of thermal inactivation. These situations were described by the two straight lines of Figure 5. From the slopes and intercepts on the ordinate axis the values of the thermodynamic parameters listed in Table 4 were estimated for both biomass growth and cadmium removal.

Table 4. Thermodynamic parameters of *S. natans* growth and cadmium removal.

	Δh (kJ mol^{-1})	Δs (kJ mol^{-1} K^{-1})
Cell growth	62.3	−0.12
Thermal inactivation of growth	139.4	0.46
Cd removal	107.4	0.85
Thermal inactivation of Cd removal	168.7	0.56

Δh = activation enthalpy or equilibrium enthalpy variation; Δs = activation entropy or equilibrium entropy variation.

The activation enthalpy of *S. natans* growth ($\Delta h_g^* = 62.3$ kJ mol^{-1}) compares reasonably with those (34 to 80 kJ mol^{-1}) estimated for several bioprocesses[31,34,35] and free enzymes.[33] On the other hand, that of cadmium removal ($\Delta h_r^* = 107.4$ kJ mol^{-1}) is considerably higher, which means that this removal takes place with more difficulty with respect to growth. Comparing the trends of Figure 5 and the data of Table 4, it seems that cadmium removal by *S. natans* is controlled by cell growth. On the contrary, physical adsorption appeared to be the controlling phenomenon with *Chlorella vulgaris*, which showed a negative biosorption activation enthalpy (−8.0 kJ mol^{-1}).[36]

The phenomena responsible for thermal inactivation of both cell growth and cadmium removal are characterized by $\Delta h_g^{\circ}{}_D = 139.4$ kJ mol^{-1} and $\Delta h_r^{\circ}{}_D = 168.7$ kJ mol^{-1}, respectively. These enthalpy variations are remarkably higher than the above Δh_g^* and Δh_r^* values, which means that both inactivation equilibria were favored by a temperature increase more than cell growth and cadmium removal, thus the overall rates declined above 27 to 29°C. As expected, both $\Delta h_g^{\circ}{}_D$ and Δh_r° were much lower than the values reported for microbial death (210 to 628 kJ mol^{-1})[31,34,37] and enzyme irreversible denaturation (285 to 550 kJ mol^{-1}),[37] but they were in reasonable agreement with thermal inactivation of other bioprocesses (176 to 200 kJ mol^{-1})[32,35] and most enzymatic systems (160 to 235 kJ mol^{-1}).[33,38,39]

This comparison seems to confirm the biological nature of the cadmium removal by *S. natans* and suggests that cell growth could be the limiting phenomenon. The removal rate decrease observed at high temperature could be due to the reversible inactivation of an enzyme that limits the growth of this microorganism. The cadmium removal would then be the result of its quick electrostatic interaction with negative charges present on the sheath surface.

The activation entropy of cell growth ($\Delta s_g^* = -0.12$ kJ mol^{-1} K^{-1}) compares with those of enzymatic reactions,[32] in accordance with the above

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hypothesis that the phenomenon limiting the growth could be an enzymatic reaction. On the contrary, the activation entropy of cadmium removal ($\Delta s_g^* = 0.85$ kJ mol^{-1} K^{-1}) appears to be consistent with the above-mentioned mechanism of electrostatic attraction kinetically controlled by the growth. The entropy variations of thermal inactivation of growth and cadmium removal ($\Delta s_g^\circ{}_D = 0.46$ kJ mol^{-1} K^{-1} and $\Delta s_r^\circ{}_D = 0.56$ kJ mol^{-1} K^{-1}) are both positive, like those estimated for many enzymatic processes.[32]

CONCLUSION

An acidic solution containing cadmium at relatively low concentration (45 mg L^{-1}) has been submitted to batch removal tests to study the uptake capacity of *S. natans* at variable temperature ($15 < T < 40$°C). During these tests, biomass was able to favorably modify the environmental conditions, increasing the pH from 4.0 up to 7.0 to 7.5. At the optimum temperature range (25 to 30°C), cadmium was removed nearly completely after a relatively short time (20 to 24 h). At higher (35 to 40°C) and lower (15 to 20°C) temperatures, biomass grew more slowly and consequently, showed a considerable decrease in its removal capacity. In particular at $T > 30$°C, a much longer time was necessary to reach a removal yield comparable to the one obtained at optimum temperature, while no less than 120 h were necessary to reach a satisfactory removal (about 0.93) at $T = 20$°C.

The results obtained in this work suggest that cadmium was quickly and effectively removed by biomass at the beginning of every test, with the rate depending on temperature. When the system reached neutrality, biomass grew rapidly, furnishing new substrate for metal uptake. The rate of cadmium removal estimated by means of the Lagergreen equation reached a maximum ($k_r = 0.15$ h^{-1}) at the same optimum temperature for growth (25 to 30°C), which demonstrates that biomass growth, pH increase, and metal removal rate are strongly related one another.

Using the data of maximum specific growth rate and k_r, the thermodynamic parameters of cell growth and cadmium removal, as well as those of the related thermal inactivations, have been estimated and used to formulate a hypothesis on possible mechanism of cadmium removal. It seems that *S. natans* is able to remove this metal following a mechanism controlled by cell growth, implying its quick electrostatic attraction by negative charges on the sheath surface.

Microscopic examination of cell morphology and quantitative determination of viable cells will be used in future work to allow for better understanding of this mechanism.

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