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# THE EFFECT OF ACID PRE-TREATMENT ON THE BIOSORPTION OF CHROMIUM(III) BY *SPHAEROTILUS NATANS* FROM INDUSTRIAL WASTEWATER

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**Abstract**—Living cells of a strain of *Sphaerotilus natans* are employed to remove Cr(III) from acid wastewater. Batch experiments carried out at starting acid conditions (pH 3.0–3.5) show that the pH progressively increases but the removing activity starts only when conditions closed to neutrality are reached. Studies carried out either at initial acid conditions or at standard conditions for this microorganism (pH 7.0) confirm that the biomass is able to grow also on acid medium, although the lag phase is longer than the one observed at standard conditions. This strain shows its maximum ability to remove Cr(III) at a biomass concentration of about 0.4 g $l^{-1}$. Tests carried out at both lower and higher biomass levels show lower yields, while the time necessary to reach the maximum removal considerably increases. Biomass previously adapted to acid conditions ensures a specific uptake of this metal of 120 mg $g^{-1}$, which is much higher than that reported in the literature for other microorganisms. Continuous tests in CSTR confirm the possibility of developing a biological treatment process for the continuous removal of Cr(III) from acid solutions. © 2000 Elsevier Science Ltd. All rights reserved

*Key words*—biosorption, *Sphaerotilus natans*, chromium(III), acid wastewater, batch tests, continuous tests

## INTRODUCTION

The treatment of an industrial wastewater can be particularly difficult in the presence of toxic compounds, so, before discharging, it has to be submitted to pre-treatments devoted to the reduction of the concentration of these pollutants. A special problem is constituted by the removal of heavy metals, with particular concern to chromium, that is largely present in effluents coming from many industrial processes, such as chromium leather tanning, chromium plating, metal cleaning and processing, wood preservation, and alloy preparation. Chromium is present in the effluents chiefly as hexavalent chromium, which is toxic and mutagenic for most organisms (Carson *et al*., 1986). In humans it causes irritation and corrosion of skin and respiratory tract and is suspected to be responsible for lung carcinoma. Chromate is also hazardous to fauna and flora in natural aquatic ecosystems (Runnels and Shepherd, 1992).

Recently, the possibility of eliminating chromate from wastewater has been studied, by means of chemical or biochemical processes (Komori *et al*., 1990) that reduce chromate to the less toxic form of chromium(III). Since chromium(III) is used as tanning agent in the leather industry (Macchi *et al*., 1991), large quantities of this metal are present in the exhausted liquid discharges and in the sludge coming from the tannery process. As mentioned in a previous work (Lodi *et al*., 1998), conventional technologies used for eliminating heavy metals from effluents present some limitations (Benjamin, 1983; Benjamin *et al*., 1996). In fact, heavy metals present at low concentration cannot be eliminated from wastewater by conventional treatments and the physico-chemical methods used to this purpose are too expensive.

For this reason, particular attention has been paid recently to the use of biological systems, which can constitute an actual alternative to the traditional treatments. Biosorption was successfully applied in the removal of several heavy metals (Volesky, 1986). Died cells (Chang and Hong, 1994; Chong and Volesky, 1996) or lyophilised biomass (Friis and Myers-Keith, 1986; Vegliò *et al*., 1998) are generally utilised as adsorbents for different metal ions, while living biomass is rarely employed because of metals toxicity (Jewell, 1994).

The mechanism of metal accumulation is not satisfactorily explained. Some workers attributed the

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metal uptake capacity to the protein–lipopolysaccharidic sheath of some filamentous micro-organisms; in this case, the phenomenon could be due to the polysaccharidic cell wall constituents as the primary sites of metal accumulation (Friis and Myers-Keith, 1986; Kuyakak and Volesky, 1989). In other systems, the metals precipitate as hydroxides within the micro-crystalline cell wall (Converti *et al.*, 1992; Hatch and Menawatt, 1978). In any case, the uptake process seems to be external to the cells also when living biomass is used, as in the present work.

The bacterium *Streptomyces noursei* (Mattuschka and Straube, 1993) and the alga *Halimeda opuntia* (Volesky, 1992) were successfully used for the removal of this metal at pH values around 4.0 and 5.5 but specific uptake never exceeded 40 mg $g^{-1}$.The bacterium *Sphaerotilus natans* utilised in this work has recently been tested with good results (Lodi *et al.*, 1998), its removal activity having been proved at relatively low pH (3.5–4.0) if compared with other species or strains. *S. natans* is a Gram negative filamentous bacterium, which is naturally present in sewage sludge and polluted waters in the form of superficial mucilage layer; in the outer membrane it contains a protein–lipopolysaccharidic sheath which is probably responsible for the binding of metal ions (Beveridge, 1984). In addition, it has subpolar flagella that give motility and possibility to strike ions. Probably because of a combination of these properties, *S. natans* is responsible for excellent metal removal activity, even under unfavourable conditions, like those tested in this work.

The aim of this study is to investigate on the actual possibility of removing chromium(III) from acid solutions simulating the composition of common industrial wastewater (Tiravanti *et al.*, 1997) as well as on the mechanism of this process in the presence of living cells of this micro-organism.

## MATERIALS AND METHODS

### *Micro-organism*

The cells of *Sphaerotilus natans* (NCIMB 11196) were purchased by National Collection of Industrial Marine Bacteria Ltd (Aberdeen, Scotland). They were grown for 36–48 h in shaken flasks containing 1.5 g $l^{-1}$ dry meat extract in tap water and kept at 28°C. The cells, harvested at the end of the exponential phase, were centrifuged at 5000 rpm, re-suspended in water and finally used as inoculum for batch growth tests at pH 3.0, 4.0, and 7.0. A starting biomass concentration of 0.3 g $l^{-1}$ was used in these tests.

Tests with biomass adapted to acid conditions (pH 3.0) were also done using, as inoculum, cells recovered (by the same procedure) during the stationary growth phase of a preceding test carried out at the same pH.

### *Feed solution*

Synthetic wastewater containing 1.5 g $l^{-1}$ of meat extract and variable amounts of $Cr_2(SO_4)_3$ was used in the batch experiments. Continuous tests were carried out using a fixed $Cr_2(SO_4)_3$ concentration (16.0 mg $l^{-1}$) at pH 3.5. Since the growth of *S. natans* is not affected by the presence of sulphates but is inhibited by chlorides (Hatch and Menawatt, 1978), a 5.0-M sulphuric acid solution has been used to ensure the acid pH (about 3.0–3.5) necessary to simulate the acid wastewater.

### *Operating conditions*

Batch tests were carried out at the selected pH in 1-l Erlenmeyer flasks kept at 28°C and stirred at 150 rpm. Two separate flasks were simultaneously used for each test; one was employed as control flask (blank), while the other was inoculated. According to the tests, different starting operating conditions were selected, in terms of chromium(III) concentration, biomass concentration, and pH. During all the runs the pH was let to vary without any control.

### *Analytical procedures*

Samples drawn from the flasks at regular time intervals were filtered through 0.45-$\mu$m Millipore filters in order to remove the suspended particles and then were analysed to determine the dissolved ion concentration by an atomic absorption spectrophotometer (model 5000 Perkin–Elmer). Total suspended solids (biomass plus adsorbed metal) were measured by dry weight, while the content of chromium(III) adsorbed onto the cells was determined by atomic absorption, after digestion in 10 M HCl at 70°C. Biomass concentration was then roughly estimated as the difference between the suspended solid content, previously determined, and the metal content of cells.

### *Continuous tests*

A modular Gallenkamp-500 series laboratory-CSTR, with 4.0-l working volume, was used for the continuous experiments. A peristaltic pump was used to feed the medium into the reactor. An aeration module provided aeration while efficient mixing was ensured by a mechanical stirrer. The temperature within the reactor was kept at 28°C by a temperature control module.

## RESULTS AND DISCUSSION

### *Preliminary growth tests*

Some preliminary tests were carried out to study the growth behaviour of the strain under conditions of increasing acidity without the metal. The values of the main parameters describing the growth at different starting pH values (Table 1) confirm that the fastest growth is obtained at pH = 7.0, that is under the best conditions suggested in the literature for this micro-organism (standard conditions). In order to enhance the ability of the micro-organism to grow at low pH, additional tests were also car-

Table 1. Lag phase duration and maximum growth rate of *S. natans* cells grown under different conditions of acidity. $X_o = 0.3$ g $l^{-1}$

| Starting pH | Lag phase duration (h) | $\mu_{max}$ ($h^{-1}$) |
|---|---|---|
| 7 | 20 | 0.0142 |
| 4 | 30 | 0.0112 |
| 3 | 40 | 0.0111 |
| 3[a] | 20 | 0.0140 |
| 3[b] | 17 | 0.0149 |

[a]Biomass once adapted to acid conditions.
[b]Biomass twice adapted to acid conditions.

ried out using biomass progressively adapted to acid conditions.

During all these tests, carried out without pH control, the pH continuously increased but the cell growth was nearly negligible until the pH reached a value of 6.0 (data not shown) and the biomass concentration reached its maximum value at pH $\approx$ 8.0. Also the substrate consumption was negligible during the lag phase, while it became significant only when pH exceeded this threshold value. For this reason the duration of the lag phase increased with decreasing the starting pH of the medium. This peculiar behaviour, never put in evidence up to now, could be related to a defence system which would imply the release of hardly-identifiable alkaline metabolites by this micro-organism. Only once the pH would achieve values approaching the optimal one for the growth (around the neutrality), could the cell become able to sustain an appreciable growth. On the contrary, in the case of biomass once adapted to acid conditions (pH = 3.0), the lag phase lasted about the same time as that observed at standard conditions and exactly one half the time taken by not adapted biomass under the same conditions. In addition, the cells previously adapted to acid conditions started to grow at a lower pH (5.0) and appeared to grow more rapidly than those growing under standard conditions, likely due to a progressive biomass adaptation to these stress conditions.

The lag phase present at pH 7.0 is the acclimation period usually experienced by a cell culture during its adaptation to a new culture medium. On the other hand, the lag phase observed at starting acid pH is likely due to a combination of this natural adaptation plus that consequent to a pH decrease with respect to the optimal conditions.

The growth kinetics at different starting pH values were studied only during the exponential phase using the well-known equation:

$$\ln\ X/X_i = \mu_{max}\ t \qquad (1)$$

where $X$ and $X_i$ are the biomass concentrations (g $l^{-1}$) at the start of the exponential phase and after a time $t$, respectively, while $\mu_{max}$ is the maximum specific growth rate ($h^{-1}$).

As the values of Table 1 show, the maximum specific growth rate is nearly the same at pH 4.0 and 3.0, while it is notably higher under standard growth conditions (pH = 7.0). At the same time, a progressive increase in the lag phase duration can be noticed for not adapted biomass when decreasing the starting pH from 7.0 to 3.0, while a preliminary adaptation to acid conditions halves its duration at pH 3.0.

On the other hand, a further adaptation cycle appeared unsuitable to provide either a significant improvement of the growth kinetics or a reduction of the lag phase duration.

*Tests at different starting metal and biomass concentrations*

The progressive decrease of chromium(III) concentration in the solution at different initial concentrations of both this metal and adapted biomass is evident in Figs 1 and 2, while the related equilibrium results are summarised in the upper part of Table 2. A comparison among these data shows that the highest uptake yield is achieved at an initial biomass concentration of about 0.4 g $l^{-1}$. In addition, considering the time ($t_f$) necessary to reach the equilibrium concentration ($C_{mf}$), it should be noticed that this situation is achieved more rapidly (only 25 h) during the tests carried out at $C_{mo} \approx$ 13–16 mg $l^{-1}$ and at an intermediate initial biomass concentration (0.40 g $l^{-1}$), while a longer time is necessary using both lower (0.27 g $l^{-1}$) and higher (0.85 g $l^{-1}$) initial biomass levels. During all these batch tests, done at a starting pH of 3.0, a remarkable increase of pH was also observed, with negligible dependence on both metal and biomass starting levels: the biomass growth always started at a pH close to 6.0 and the final pH ranged from 7.8 to 8.2.

The above strange behaviour can be associated to a decrease in cell uptake activity over a certain concentration threshold. While the reduced removal efficiency observed at the lowest concentration of adapted biomass can easily be ascribed to a decrease in the number of sites available for cation adsorption, the one detected at the highest cell concentration could be due to an increased fraction of cells grown within the sheath, which are not available for adsorption. This supposition seems to be confirmed by the formation of cells aggregates observed during these tests.

From the above results it can be deduced that the best biomass concentration for chromium(III) removal could be around 0.4 g $l^{-1}$. In order to confirm the actual utility of the acid pre-treatment of biomass, a further set of tests at initial biomass concentration of 0.4 g $l^{-1}$ has been carried out in the presence of starting chromium concentrations similar to those utilised in the previous tests (20–60 mg $l^{-1}$) but using biomass not previously adapted (lower part of Table 2).

While the results of these experiments, expressed in terms of yields and final equilibrium concentrations, compare well with the ones obtained with adapted biomass, the equilibrium time, $t_f$, appears to be one order of magnitude longer. Such experiments confirm the previously supposed advantage of using biomass previously adapted to acid conditions. Then, it is possible to conclude that utilising adapted biomass reduces the lag phase duration, without implying any relevant modification of cell activity.

A pH increase similar to that observed during the above growth runs was also observed during these

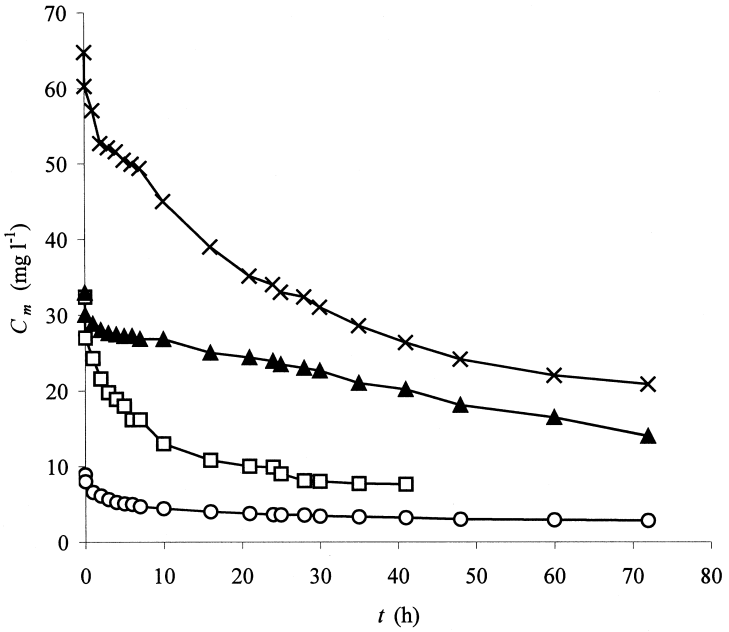


Fig. 1. Chromium(III) removal from aqueous solutions by living cells of *S. natans* at starting pH = 3.0. (x) $C_{mo} = 64.8$ mg l$^{-1}$, $X_o = 0.40$ g l$^{-1}$; (□) $C_{mo} = 32.9$ mg l$^{-1}$, $X_o = 0.40$ g l$^{-1}$; (▲) $C_{mo} = 32.4$ mg l$^{-1}$, $X_o = 0.25$ g l$^{-1}$; (○) $C_{mo} = 8.9$ mg l$^{-1}$, $X_o = 0.40$ g l$^{-1}$.

chromium(III) removal tests. A possible defence mechanism like that proposed by Scott and Palmer (1990) could support the previous one in the presence of the metal and could allow this microorganism to regenerate ATP. After $Cr^{3+}$ passive diffusion from the solution into the cell, this ion could be substituted by $H^+$, which is present in excess at low pH. This ionic exchange would be made possible thanks to a specific pump, which could regenerate ATP. As a consequence of $Cr^{3+}$ expulsion and $H^+$ depletion, the solution pH would increase and chromium(III) could then precipitate

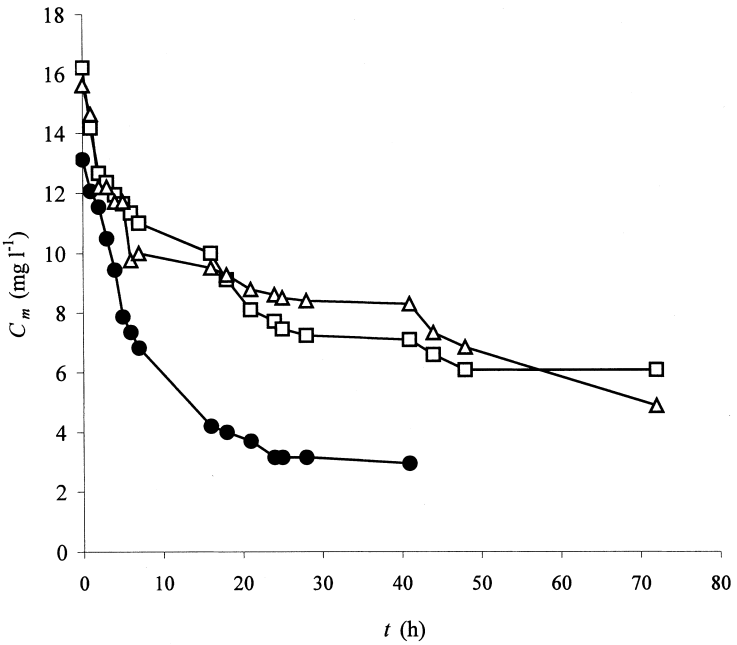


Fig. 2. Chromium(III) removal from aqueous solutions by living cells of *S. natans* at starting pH = 3.0. (△) $C_{mo} = 15.6$ mg l$^{-1}$, $X_o = 0.85$ g l$^{-1}$; (●) $C_{mo} = 13.1$ mg l$^{-1}$, $X_o = 0.40$ g l$^{-1}$; (□) $C_{mo} = 16.2$ mg l$^{-1}$, $X_o = 0.27$ g l$^{-1}$.

Table 2. Final yields of chromium removal, $\eta$, and equilibrium concentration, $C_{mf}$, obtained at different starting concentrations of both metal ($C_{mo}$) and biomass ($X_o$)

| | $C_{mo}$ (mg $l^{-1}$) | $\eta$ (%) | $C_{mf}$ (mg $l^{-1}$) | $t_f$ (h) | $X_o$ (g $l^{-1}$) |
|---|---|---|---|---|---|
| Adapted biomass | 64.7 | 67.8 | 20.8 | 72 | 0.40 |
| | 32.9 | 62.5 | 12.3 | 81 | 0.25 |
| | 32.4 | 75.0 | 8.1 | 28 | 0.40 |
| | 16.2 | 62.5 | 6.1 | 48 | 0.27 |
| | 15.6 | 68.7 | 4.9 | 40 | 0.85 |
| | 13.1 | 76.0 | 3.1 | 25 | 0.40 |
| | 8.9 | 68.4 | 2.8 | 22 | 0.40 |
| Not adapted biomass | 59.0 | 72.8 | 16.0 | 262 | 0.60 |
| | 32.4 | 71.0 | 9.4 | 250 | 0.40 |
| | 23.5 | 70.0 | 7.1 | 250 | 0.40 |

onto the sheath and the cell walls, mainly in the form of $Cr(OH)_3$. The resulting external layer would be able to avoid further metal penetration into the cell (Vegliò and Beolchini, 1997).

Chromium(III) uptake capability of *S. natans* has then been evaluated by the related isotherm (Fig. 3), assuming that biomass concentration did not vary considerably during every tests, i.e. considering biomass as a simple adsorbent (physical process). This supposition was confirmed by periodical check of biomass concentration during the batch runs. The trend in this Figure shows that specific uptake capability, $q$, increases, as it occurs for traditional adsorbent, with increasing final equilibrium concentration, $C_{mf}$. It can be seen that a threshold value for uptake capability has probably not been reached in this study because of the moderate initial chromium concentrations tested. Further tests are in program to verify not only the adsorption power of *S. natans* at higher metal concentration but also the possible occurrence of a related toxic effect of chromium. Anyhow, using biomass previously adapted to acid conditions, a specific uptake (120 mg $g^{-1}$) from 3 to 10 times higher than those reported in the literature for *Streptomyces noursei* (Mattuschka and Straube, 1993) and *Halimeda opuntia* (Volesky, 1992) has been obtained.

### *Kinetics of chromium(III) removal*

From the above results, the removal kinetics have been studied in order to obtain further information on the biosorption phenomenon. To this purpose, the kinetic approach followed by Hatch and Menawatt (1978) has been extended to Cr(III) uptake by microbial cells either adapted or not adapted to acid conditions.

As suggested by the above authors, and recently confirmed by our group (Lodi *et al.*, 1998), first-order kinetics cannot be justified by growth limitation (that would imply an increase in rate con-

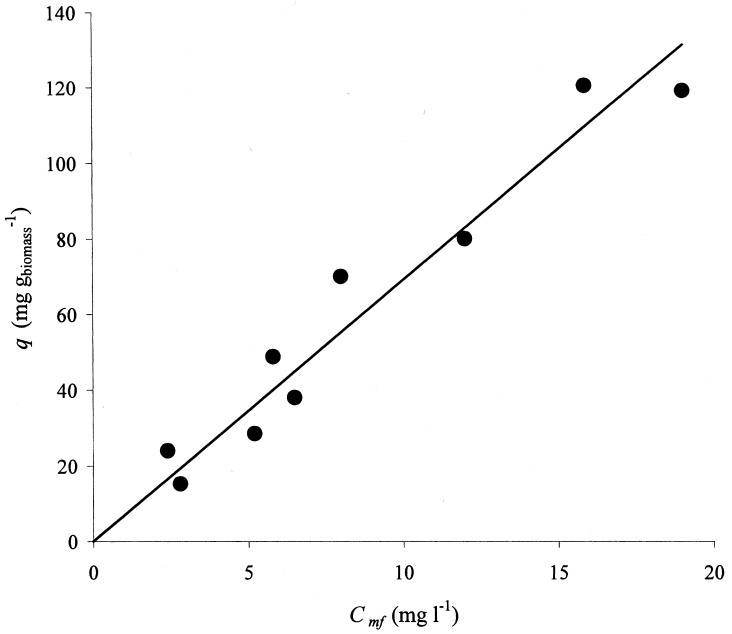


Fig. 3. Sorption isotherm of chromium(III) on living cells of *S. natans*.

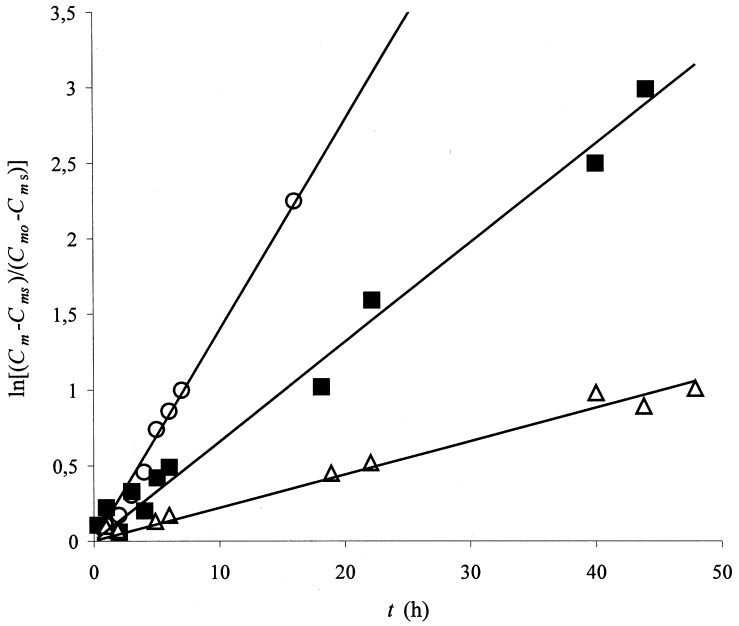


Fig. 4. Batch tests of chromium uptake by living cells of *S. natans*. $k_d$ and $C_{me}$ estimation by Eq. (5). $X_o$ (g $l^{-1}$): (△) 0.85; (○) 0.40; (■) 0.27.

stant with biomass growth), and then mass transfer limitation could be the only reasonable explanation of such a kinetic behaviour. So, mass transfer from an infinite medium to a spherical surface (*S. natans* clumps) can be described by the relationship:

$$-\frac{dC_m}{dt} = \frac{2\pi d_s D_m}{V_L}(C_m - C_{ms}) \quad (2)$$

where $V_L$ is the liquid volume, $C_m$ and $C_{ms}$ the metal concentrations in the solution and at the surface of *S. natans* clumps, $D_m$ the approximate effective molecular diffusivity, and $d_s$ the mean diameter of the clump. Integration of this equation gives:

$$\ln\left(\frac{C_m - C_{ms}}{C_{mo} - C_{ms}}\right) = -k_d t \quad (3)$$

where:

$$k_d = 2\pi d_s D_m / V_L \quad (4)$$

is the diffusion rate constant.

Observing the values of $C_{ms}$ reported in previous papers (Hatch and Menawatt, 1978; Lodi *et al.*, 1998), the metal concentration at the surface appears to be always negligible for metals not naturally utilised by the cells in metabolic processes, except for chromium. The above model has been modified in order to become able to describe metal removal from the solution also under conditions where the metal uptake is not complete and $C_m$ reaches an equilibrium concentration $C_{me} > 0$. In this case, Eq. (3) becomes:

$$\ln\left(\frac{C_m - C_{me}}{C_{mo} - C_{me}}\right) = -k_d t \quad (5)$$

By this equation, we have calculated the first-order kinetic constant, $k_d$, on the basis of the experimental data of $C_m$ and the time necessary for metal removal, $C_{me}$ being estimated, case by case, by linear regression (Fig. 4).

The average values of the first-order kinetic constants, $k_d$, calculated for tests carried out using micro-organisms both adapted and not adapted to acid conditions, are listed in Table 3. Firstly, it should be noticed that the order of magnitude of $k_d$ is the same as that observed in other studies for the removal of chromium and other xenobiotic metals by not adapted biomass (Hatch and Menawatt, 1978; Lodi *et al.*, 1998). In addition, when biomass adapted to acid conditions is used for chromium(III) removal, the kinetic constant is about one order of magnitude greater than in the presence of not adapted biomass. This influence of biomass adaptation to acid conditions on the diffusion con-

Table 3. First-order kinetic constant of metal uptake by living cells of *S. natans*

| | $C_{mo}$ (mg $l^{-1}$) | $k_d \cdot 10^3$ ($h^{-1}$) | $r^2$ |
|---|---|---|---|
| Adapted biomass | 8.9 | 90 | 0.95 |
| | 16.2 | 85 | 0.97 |
| | 32.4 | 31 | 0.99 |
| | 64.8 | 14 | 0.98 |
| Not adapted biomass | 23.5 | 8.7 | 0.96 |
| | 32.4 | 6.3 | 0.95 |
| | 59.0 | 1.4 | 0.98 |

stant suggests that it cannot be considered a purely biological process. In fact it can induce relevant effects on both cell wall and membrane consistency and, therefore, on the adsorption power of the cells. This result is obviously very important from the application point of view because an acceleration of metal removal would imply a reduction of plant size, if the phenomenon were to be exploited at industrial scale. Furthermore, the decrease of the kinetic constant observed at fixed biomass concentration with an increase in the starting metal concentration, could likely be due to a progressive saturation effect caused by chromium on the adsorbing biomass.

The values calculated for this parameter at given initial metal concentration ($C_{mo} = 13$–$16$ g $l^{-1}$), but using variable amount of biomass previously adapted to acid conditions (Table 4), show that also the kinetic constant reaches a maximum value at a biomass concentration of 0.4 g $l^{-1}$. As said above, up to a certain threshold, all biomass is available to contribute to the adsorption phenomenon, while, beyond this level, a decrease of the specific removal rate follows any further increase in biomass concentration, probably due to a reduction of the specific adsorption surface. Finally, the growth of biomass appeared to be nearly negligible during these tests, which confirms the validity of assuming a relatively constant biomass concentration during the equilibrium uptake tests previously discussed.

### *Continuous tests in CSTR*

Continuous tests have also been carried out in CSTR in order to gather the figures necessary for the design of a biological unit devoted to the improvement of chromium removal in an already existing wastewater treatment plant. The whole experimental schedule, which lasted about 150 days, included tests using a feed with constant composition ($C_{mo} = 16$ mg $l^{-1}$, pH = 3.5). The residence time was progressively reduced from 96 to 36 h in order to individuate the optimal conditions to obtain a desired yield of continuous metal removal.

The results of these runs, shown in Fig. 5, confirm the preliminary indications put in evidence from batch tests. Working at a dilution rate ($D$) of 0.010 $h^{-1}$, the average yield reached about 85% under steady-state conditions, while the pH in the solution kept nearly constant at 8.2 (Table 5). The biomass level in the reactor (starting value of 0.40 g $l^{-1}$), initially decreased because of the new acid conditions experienced, but, after one week of continuous working, reached a constant value of 0.45 g $l^{-1}$. After 30 days, the dilution rate was regulated at 0.017 $h^{-1}$ and the average yield slowly decreased to about 80%, while the pH reached a steady-state value of 7.8. Once again, biomass, after an initial decrease in concentration, started to grow and achieved values around 0.50 g $l^{-1}$, which is indicative of a reasonable capacity of *Sphaerotilus natans* cells to adapt themselves to acid conditions. This effect was confirmed by the highest value obtained for biomass concentration (0.64 g $l^{-1}$) at $D = 0.021$ $h^{-1}$. When the dilution rate was increased up to 0.026 $h^{-1}$, the removal yield became unacceptably low (about 10%). This result indicated for the system conditions close to the wash-out, which was fully experienced at a dilution rate of 0.028 $h^{-1}$, where biomass concentration at steady state was nearly negligible (0.04 g $l^{-1}$) and the pH close to the starting value (3.9).

Table 4. First-order kinetic constant of metal uptake by living cells of *S. natans* previously adapted to acid conditions ($C_{mo} = 13$–$16$ mg $l^{-1}$)[a]

| $X_o$ (g $l^{-1}$) | $k_d \cdot 10^3$ ($h^{-1}$) | $r^2$ |
|---|---|---|
| 0.27 | 63 | 0.98 |
| 0.40 | 85 | 0.97 |
| 0.85 | 45 | 0.92 |

[a]Values calculated at different starting biomass concentrations.

## CONCLUSIONS

The goal of this study has been to investigate the possibility of removing chromium(III) from acid solutions simulating the composition of common industrial wastewater and to study the mechanism of this process. Following the suggestions from a previous work, where *Sphaerotilus natans* was successfully employed for the removal of different xenobiotic metals, living cells of this filamentous bacterium have been used for both batch and continuous removals of chromium(III) from acid solutions.

The first indication from these tests was the capability of this micro-organism to grow and to remove chromium(III) also from solutions with a very low starting pH by gradually increasing the pH up to values close to 6.0. It was able to reach a maximum removal yield nearly coincident with the corresponding value obtained at standard conditions (pH = 7.0), although after a longer lag phase.

As regards to the chromium(III) biosorption, the maximum removal was about 75% at initial biomass and metal concentrations of 0.40 g $l^{-1}$ and 16–32 mg $l^{-1}$, respectively. At both lower and higher biomass levels, the yield decreased while the

Table 5. Experimental data of continuous tests of Cr(III) removal obtained by living cells of *S. natans* under steady-state conditions[a]

| $D$ ($h^{-1}$) | pH | $X$ (g $l^{-1}$) |
|---|---|---|
| 0.010 | 8.2 | 0.45 |
| 0.017 | 7.8 | 0.50 |
| 0.021 | 6.9 | 0.64 |
| 0.026 | 4.3 | 0.09 |
| 0.028 | 3.9 | 0.04 |

[a]$C_{mo} = 16$ mg $l^{-1}$; starting pH = 3.5.

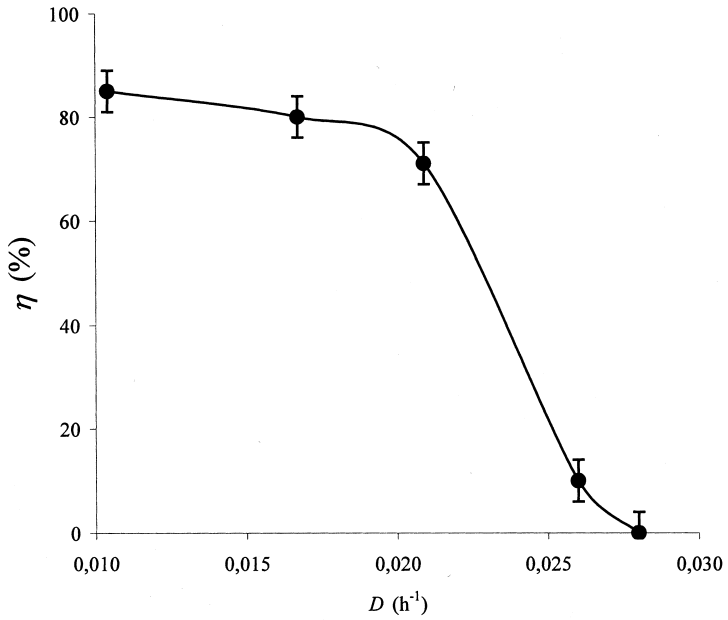


Fig. 5. Continuous tests of chromium(III) uptake in CSTR by living cells of *S. natans*. Dependence of the chromium removal yield on the dilution rate.

time necessary to reach the maximum removal considerably increased.

Continuous tests in CSTR have confirmed the possibility of developing a continuous biological treatment process able to remove chromium(III) with a yield around 75% at a residence time of 48 h.

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