Preparation of a surface molecular-imprinted adsorbent for Ni^{2+} based on *Penicillium chrysogenum*

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Abstract: A new chitosan molecular-imprinted adsorbent was prepared from the mycelium of waste biomass. The results showed that an adsorbent using *Penicillium chrysogenum* mycelium as the core material was better than one derived from peanut coat. The adsorption capacity of the surface-imprinted adsorbent for Ni^{2+} was enhanced by increasing the chitosan concentration in the imprinting process. Epichlorohydrin was better than glutaraldehyde as a cross-linking agent; the optimal imprinted Ni^{2+} concentration for preparing the surface-imprinted adsorbent was 2 mg (Ni^{2+}) g^{-1} of mycelium. The adsorption capacity of the surface-imprinted adsorbent was 42 mg g^{-1} (at 200 mg dm^{-3} initial metal ions concentration) and twice that of the mycelium adsorbent. The surface-imprinted adsorbent can be reused for up to 15 cycles without loss of adsorption capacity.
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Keywords: adsorption capacity; chitosan; heavy metal ion; mycelium; surface molecular imprinting; wastewater treatment

1 INTRODUCTION

Heavy metal ions such as Cu^{2+}, Ni^{2+}, Cr^{3+} and Zn^{2+} contaminate the wastestreams from many industries such as electro-plating, battery manufacture, dyeing, etc and, because of toxicity to human and other forms of life, are serious pollutants.[1] The currently used methods for removal of heavy metal ions from industrial effluents appear to be inadequate and expensive and often create secondary pollutant problems such as metal-bearing sludges. The use of waste biomass to adsorb heavy metal ions from industrial wastewater has received attention over the last two decades[2–4] and has shown that using waste biomass as a biosorbent for removing toxic heavy metal ions is competitive and cheap. However the direct use of waste biomass for wastewater treatment is difficult due to limited reusability (only three to five cycles) and low adsorption capacity (10–40 mg g^{-1}).

Chitosan has many –NH$_2$ and –OH groups that can chelate heavy metal ions, providing high adsorption capacity and good selectivity.[5–7] When chitosan is used for industrial wastewater treatment, the presence of flakes or chitosan powder may give rise to high-pressure problems; also the use of dilute acids is precluded since they dissolve chitosan. Consequently, treatment costs are high, limiting the value of chitosan as a biosorbent for the removal of heavy metal ions in large-scale wastewater treatment.

Using the molecular imprinting method introduced by Ekberg and Mosbach,[8] an imprinted chitosan resin prepared by Tan *et al*[9] showed chemical and physical stability and could be reused up to ten times without loss of adsorption capacity. However, the cost of the template chitosan resin was high and the inter-diffusion resistance through the pores of the adsorbent reduced the adsorption capacity for heavy metal ions. A new molecular-imprinted adsorbent, based on the surface molecular imprinting technique of *Penicillium chrysogenum* mycelium from waste biomass, has been described but its preparation was not optimised.[7] The surface-imprinted adsorbent has high uptake, low cost and the adsorbed metal ions were

easily desorbed compared with the mycelium biomass alone and a surface-coated adsorbent. In this paper, the optimised preparation of the surface molecular-imprinted adsorbent on *P chrysogenum* mycelium is described.

2 MATERIALS AND METHODS
2.1 Materials
Chitosan was obtained from shrimp shells with 90% degree deacetylation and provided by Haidebei Biochemical Engineering Company of Jinan (Shandong Province). Epichlorohydrin and $NiSO_4.6H_2O$ and other chemicals used were of analytical grade and obtained from the Chemical Reagent Company of Beijing.

Mycelium adsorbent obtained from waste *P chrysogenum* biomass was provided by the Dongchen Biochemical Engineering Company of Dongying (Shandong Province).

2.2 Pretreatment of the mycelium adsorbent
The waste *P chrysogenum* mycelium adsorbent was washed with deionised water until the pH value of the washing was 7.0, then dried at 60 °C for 4–6 h. Finally the dry adsorbent was sized by an 80-mesh sieve and stored in a sealed bottle for further use.

2.3 Preparation of the surface-coated adsorbent
The preparation process was as follows. Chitosan (0.1g dry weight) was dissolved in $10 \, cm^3$ 2.5% (v/v) acetic acid solution. The mixture was stirred until all chitosan was dissolved. Then, $0.5 \, cm^3$ epichlorohydrin as cross-linking agent was added to the mixture and

allowed to react for 12 h at room temperature. Then 2g (dry weight) of mycelium was added to the cross-linking reaction mixture and the suspension was stirred for 10 min. Finally, the surface-coated adsorbent was filtered using filter paper and dried at 60 °C for 4–6 h. The dry adsorbent was sized by an 80-mesh sieve and stored in a sealed bottle for further use.

2.4 Preparation of the surface-imprinted adsorbent
$NiSO_4.6H_2O$ was dissolved in $2 \, cm^3$ dilute acetic acid solution (2.5%v/v) to give an Ni^{2+} solution of $2 \, mg \, cm^{-3}$. Then 0.1g chitosan (dry weight) was dissolved in this solution, 2g mycelium (dry weight) was then added to the above solution, and the mixture was stirred for 10 min. Cross-linking using epichlorohydrin was carried out as described in section 2.3.

Finally, the Ni^{2+} imprinted in the adsorbent was removed by treating with a solution of ethylenediamine tetraacetic acid (EDTA) containing $0.2 \, g \, dm^{-3}$ for 12 h. Regeneration was carried out by washing the adsorbent with $0.2 \, mol \, dm^{-3}$ NaOH for 2 h by resuspension and shaking. The surface-imprinted adsorbent was filtered using filter paper and dried at 60 °C (see Fig 1). The dry adsorbent was sized by an 80-mesh sieve and stored in a sealed bottle for further use.

2.5 Adsorption of metal ions
An aqueous solution containing the prescribed Ni^{2+} concentration (metal ion concentration $200 \, mg \, dm^{-3}$) was prepared. The pH of the solution was adjusted with $1 \, mol \, dm^{-3}$ NaOH or $1 \, mol \, dm^{-3}$ HCl to the prescribed values of 2.0, 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0. The dry adsorbent (0.15g) was added to a glass bottle
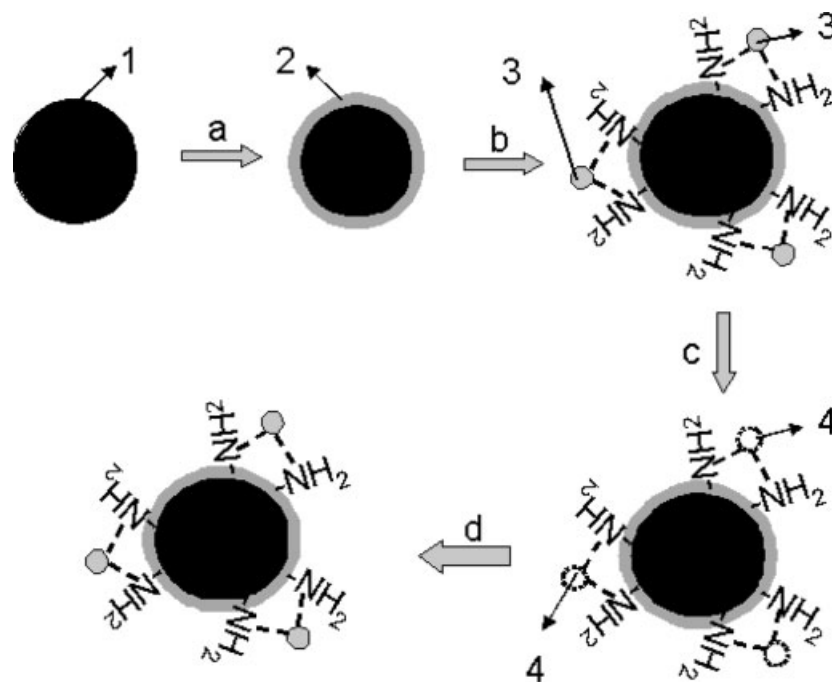

Figure 1. Schematic illustration of the surface-imprinted adsorbent. 1, Mycelium core; 2, chitosan coat; 3, metal ion; 4, specific space of metal ion; a, coated; b, imprinted; c, desorbed; d, adsorbed.

containing 50 cm^3 of Ni^{2+} solution (200 mg dm^{-3}). The mixture was stirred at 40 °C for 8–14 h. The uptake (Q) of the metal ion can be calculated according to the following equation:

$$Q = \frac{(C_0 - C_{eq})V}{W} \text{(mg g}^{-1})$$

where C_0 and C_{eq} are the initial and equilibrium concentrations of metal ion (mg dm^{-3}), respectively, W is the dry weight of the adsorbent (g) and V is the volume of solution (dm^3).

2.6 Metal ion analysis

The concentration of Ni^{2+} was determined by an adsorption spectrophotometer according to the method of Lao and Ceng.[10]

2.7 Surface structure of adsorbent

The morphology of adsorbent was assessed using a Scanning Electron Microscope (SEM) (S-250MK, made by Cambridge Company, Cambridge, UK).

3 RESULTS AND DISCUSSION
3.1 The effect of the core material on the surface-coated adsorbent

P chrysogenum mycelium and peanut coat were selected as core material for the preparation of surface-coated adsorbents. With the peanut coat as the core material, the uptake for Ni^{2+} of the surface-coated adsorbent was only 20.1 mg g^{-1}, an enhancement 10–40% compared with that of untreated peanut coat (see Fig 2). Using *P chrysogenum* mycelium as the core material, the adsorption capacity of the surface-coated adsorbent was 30.6 mg g^{-1}, an increase of 50–70% over that of the untreated mycelium adsorbent. The adsorption capacity for Ni^{2+} of the surface-imprinted mycelium adsorbent was 40–45 mg g^{-1} (at 200 mg (Ni^{2+})dm^{-3}), twice that of the untreated mycelium adsorbent (Fig 3).

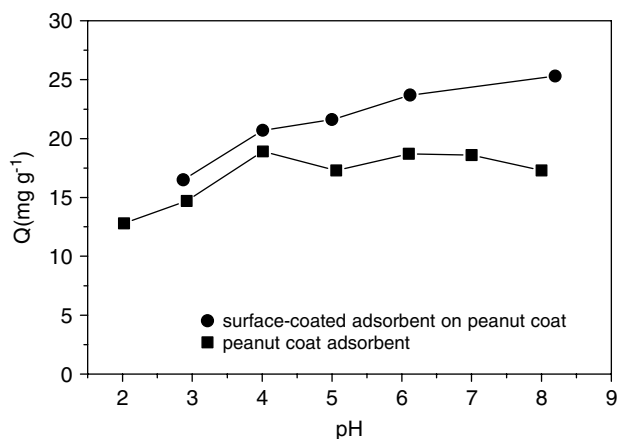**Figure 2.** Effect of pH on the surface-coated adsorbent of peanut coat. The initial metal ion concentration in the solution was 200 mg dm^{-3}.
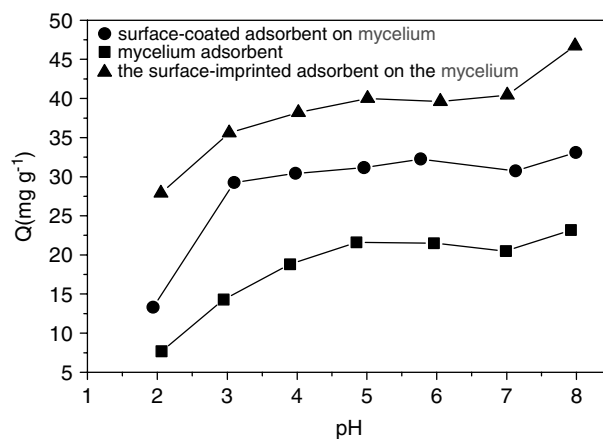
Figure 3. Effect of pH on the surface-coated adsorbent of mycelium. The initial metal ion concentration in the solution was 200 mg dm^{-3}.

The reason for the improved performance is that the mycelium adsorbent has also many −NH$_2$ and −OH groups that can chelate heavy metal ions. When the chitosan coating is not uniform due to shedding or cracking, the adsorption of heavy metal ions onto the mycelium adsorbent core may predominate. In contrast, the peanut coat lacks such functional groups for adsorbing heavy metal ions, but is loose and has a large specific surface area (Figs 4a and 5a), such that physical adsorption of heavy metal ions predominates. Coating with chitosan reduces the specific adsorption of the peanut coat for a given concentration of chitosan. The chitosan coating on the peanut coat is thinner than that on the *P chrysogenum* mycelium (Figs 4b and 5b) because of the large specific surface area of the former. Thus, with the reduced specific surface area, the contribution of the chitosan coat to adsorption capacity was offset by the reduction of physical adsorption. Consequently, *P chrysogenum* mycelium was selected as the core material for the present work.

## 3.2 Influence of chitosan concentration on the adsorption capacity

The adsorption capacity, thickness of the surface coat and reusability of adsorbent are all influenced by the concentration of chitosan used in preparation of the adsorbent.

As seen in Table 1, the adsorption capacity of the surface-coated adsorbent gradually increased with increasing concentration of chitosan. At a chitosan concentration of 0.02 g g^{-1} biomass (dry), the uptake of the surface-coated adsorbent was not measurably different from that of untreated biomass.
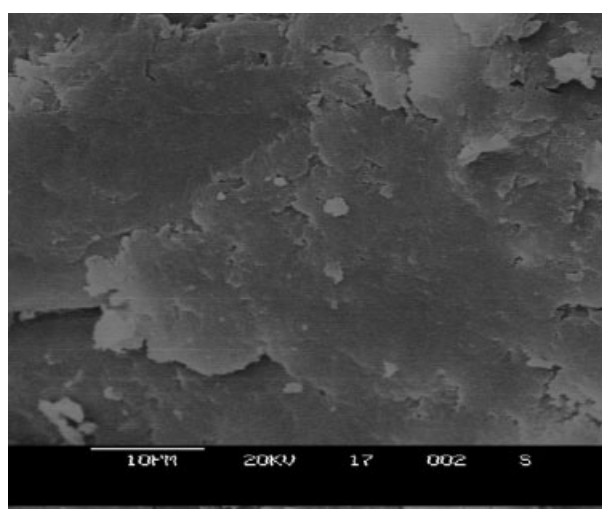
Table 1. The influence of chitosan concentration on the adsorption capacity for Ni^{2+a}

g chitosan g^{-1} biomass:	0	0.02	0.025	0.033	0.05	0.1
Uptake, Q(mg g^{-1}):		20	21.3	24.4	30	30.7	34.7

[a] The initial Ni^{2+} ion concentration was 200 mg dm^{-3}, the volume of solution was 50 cm^3, the initial pH was 6.0–7.0.
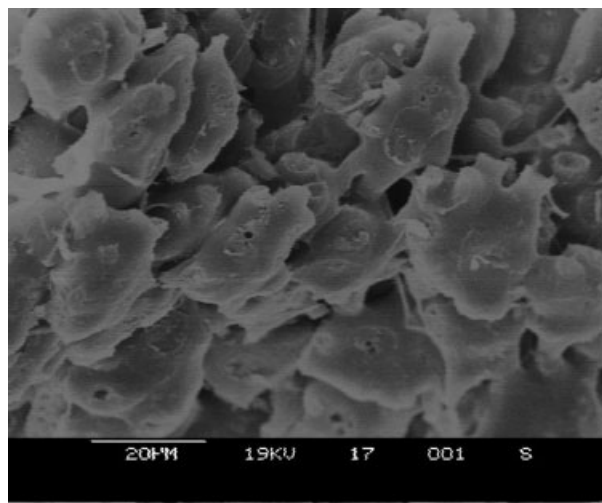
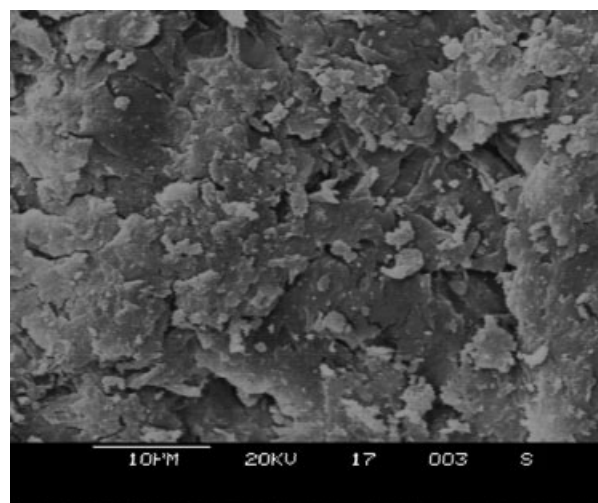**Figure 4.** SEM of peanut coat and the surface-coated adsorbent on peanut coat. a, peanut coat; b, the surface-coated adsorbent on peanut coat.

Figure 5. SEM of the mycelium and the surface-coated adsorbent on the mycelium. a, mycelium coat; b, the surface-coated adsorbent on the mycelium.

At concentrations of chitosan greater than $0.033\,g\,g^{-1}$ biomass, the adsorbent capacity of the surface-coated adsorbent increased 50–75% to $34.7\,mg\,g^{-1}$. For this study, $0.05\,g$ chitosan g^{-1} biomass was used to prepare both the surface-coated adsorbent and the surface-imprinted adsorbent.
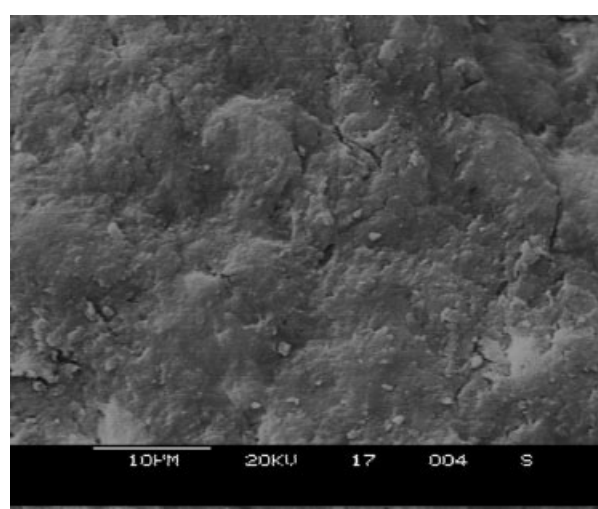
SEM showed (Fig 6), that the surface-imprinted adsorbent was roughly spherical, and had a smoother surface than the untreated mycelium adsorbent. At the same time, the surface-imprinted adsorbent showed superior mechanical properties to the mycelium adsorbent and the untreated mycelium adsorbent appeared much less physically compact.

3.3 Influence of cross-linking agent
Glutaraldehyde and epichlorohydrin were assessed as cross-linking agents for increasing the stability of chitosan in the acid solution. Glutaraldehyde is often used for cross-linking of chitosan. However, here the adsorption capacity for heavy metal ions decreased greatly following reaction with glutaraldehyde, probably because the cross-linking agent reacted mainly with $-NH_2$ groups, so reducing their availability as metal ion-binding functional groups (Fig 7).

Epichlorohydrin was superior as a cross-linking agent; the adsorption capacity of the cross-linked adsorbent was 94% of that of the surface-coated adsorbent without cross-linking. The reason for this is probably that, although epichlorohydrin chelates functional groups ($-NH_2$ and $-OH$), on chitosan, denying their availability for metal ion binding, the cross-linker contributes functional $-OH$ groups that can bind heavy metal ions. Consequently, epichlorohydrin was selected as the preferred cross-linking agent.

3.4 Influence of imprinted Ni^{2+} concentration in preparation on adsorption capacity
When selected heavy metal ions were reversibly bound on the chitosan coating in the preparation of an adsorbent and subsequently desorbed, the imprinting
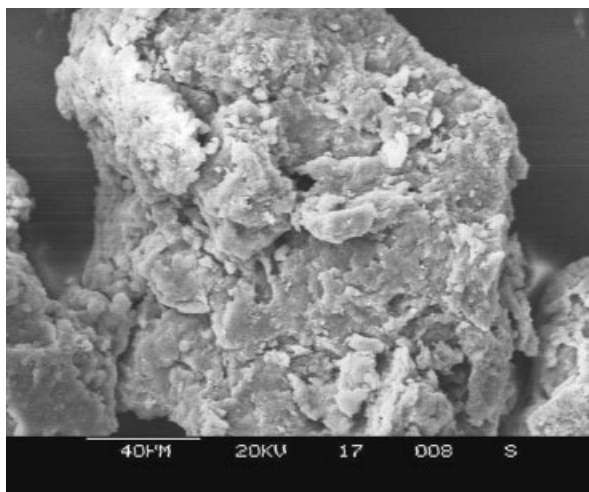
(a)
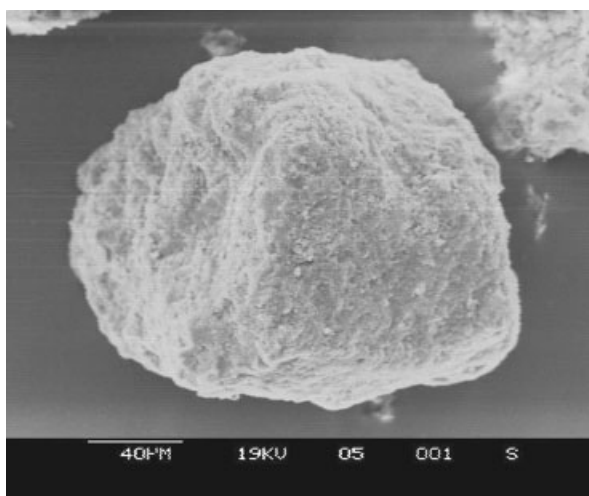
(b)

Figure 6. SEM of mycelium and the surface-imprinted adsorbent. a, mycelium; b, the surface-imprinted adsorbent.
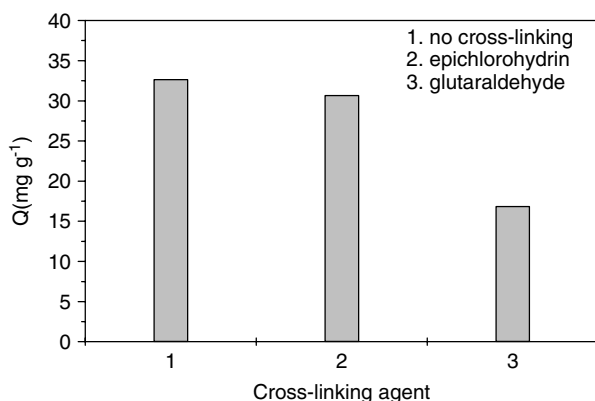

Figure 7. Influence of cross-linking agent. The initial Ni^{2+} ion concentration in the solution was $200\,mg\,dm^{-3}$. The volume of solution was $50\,cm^3$. The initial pH in the solution was 6.0–7.0.

site, or specific space, of the imprinted metal ion was retained on the surface coat of the adsorbent, so enhancing the selectivity for the heavy metal ions.

When Ni^{2+} was used as the imprinted metal ion, the adsorption capacity for Ni^{2+} increased
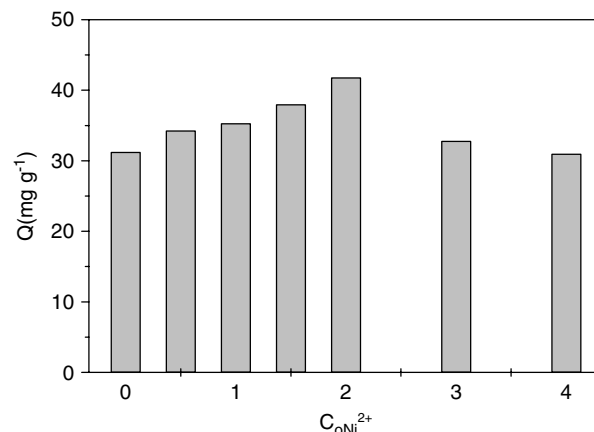
Figure 8. The influence of imprinting Ni^{2+} concentration in preparation on uptake. The initial Ni^{2+} ion concentration in solution was $200\,mg\,dm^{-3}$. The initial pH was 6.0–7.0.

considerably compared with that of the surface-coated adsorbent. The imprinted Ni^{2+} concentration used in preparation of the adsorbent had an optimal value of $2\,mg\,(Ni^{2+})g^{-1}$ biomass (Fig 8). The adsorption capacity of the surface-imprinted adsorbent for Ni^{2+} increased with increasing Ni^{2+} concentration for imprinting up to $2\,mg\,(Ni^{2+})g^{-1}$ biomass. The reason for this is probably that, when higher imprinted Ni^{2+} concentrations were used, more functional groups ($-NH_2$) were protected and more imprinting sites were retained. On the contrary, at Ni^{2+} concentrations for imprinting greater than $2\,mg\,(Ni^{2+})g^{-1}$ biomass, the adsorbent capacity of the surface-imprinted adsorbent decreased because the higher Ni^{2+} concentration affected the chitosan's solubility in the dilute acid solution, so the chitosan coating could not be formed. Consequently, $2\,mg\,(Ni^{2+})g^{-1}$ (biomass) was selected as the optimal Ni^{2+} concentration for imprinting.

3.5 Adsorption–regeneration batch

The surface-imprinted adsorbent had increased adsorption capacity and could be reused for up to 15 times without loss of performance, as shown in Fig 9. The untreated mycelium adsorbent could be reused up to 10 times, but its adsorption capacity was less than that of the surface-imprinted adsorbent.

4 CONCLUSION

A new surface molecular imprinting adsorbent on the waste biomass from the penicillin industry can considerably increase adsorption capacity for selected heavy metal ions. *P chrysogenum* mycelium was superior to peanut coat as the core material for the surface-imprinted adsorbent. The adsorption capacity of the surface-imprinted adsorbent for Ni^{2+} was almost twice that of untreated mycelium and reached $40–45\,mg\,g^{-1}$ (for an initial metal ion concentration of $200\,mg\,dm^{-3}$). The adsorption capacity of the surface-imprinted adsorbent for Ni^{2+} increased with
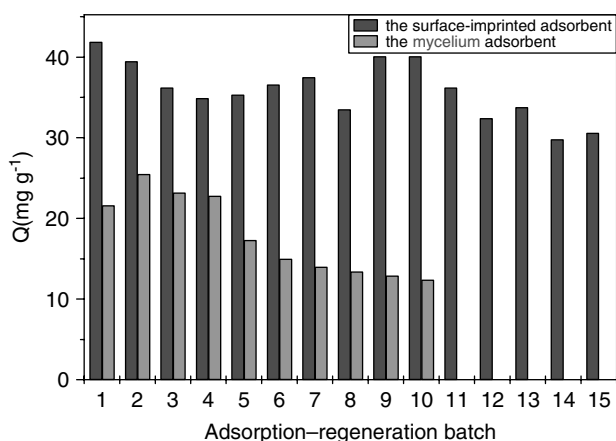
Figure 9. Reuse batches of the surface-imprinted adsorbent. The initial Ni^{2+} ion concentration was 200 mg dm^{-3}. The initial pH was 6.0–7.0.

increasing concentration of chitosan used in its preparation. Epichlorohydrin was the preferred cross-linking agent. The optimal Ni^{2+} concentration for imprinting was 2 mg (Ni^{2+})g^{-1} biomass. The surface-imprinted adsorbent combines the advantages of the mycelium adsorbent with that of the imprinted chitosan adsorbent, giving good adsorption capacity and stability that allows the reuse of the adsorbent for up to 15 cycles. These properties may offer cost benefits for the treatment of industrial wastewaters contaminated with heavy metal ions.

ACKNOWLEDGEMENTS

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