Water Air Soil Pollut (2014) 225:1834
DOI 10.1007/s11270-013-1834-4

Heavy Metal Biosorption by *Rhizopus* Sp. Biomass Immobilized on Textiles

Pedro Ferreira Gomes · Patrik R. Lennartsson · Nils-Krister Persson · Mohammad J. Taherzadeh

Received: 4 September 2013 / Accepted: 3 December 2013 / Published online: 5 January 2014

Abstract Pollution by heavy metals is at present one of the major environmental concerns. In the present study, the potential of the filamentous zygomycete fungus *Rhizopus* sp. to absorb/adsorb metal ions from solution was investigated. With the aim to develop a feasible process, the fungus was immobilized on 10 different textile materials during the cultivation. All immobilized biosorbents reduced the Cu^{2+} concentrations initially from 20 to 3.1–5.6 mg/l within 150 min, with the exception of the biomass immobilized on wool, which reduced the Cu^{2+} level to 10.2 mg/l. The immobilized biomass (with the exception of wool) fitted well into a pseudo-second-order model. The uptake of copper showed a slight dependence on initial metal concentration. By reapplying immobilized *Rhizopus* sp. to a solution containing a low concentration of Cu^{2+} after going through a first step of biosorption, a decrease of the concentration to below 2 mg/l was accomplished, meeting the stipulated level for Cu^{2+} in human drinking water. Immobilization of fungal biomass in a cushion was also successfully applied in the biosorption process. The positive results obtained in a two-step biosorption indicate that a sequential arrangement could be the foundation for a commercial product.

Keywords Biosorption · Copper · Immobilization · *Rhizopus* · Textile material · Zygomycetes

P. F. Gomes · P. R. Lennartsson (✉) · M. J. Taherzadeh
School of Engineering, University of Borås,
501 90 Borås, Sweden
e-mail: patrik.lennartsson@hb.se

P. F. Gomes
School of Engineering, Biological Engineering Center—IBQF, University of Minho,
4700 Braga, Portugal

N.-K. Persson
The Swedish School of Textiles, University of Borås,
501 90 Borås, Sweden

1 Introduction

Heavy metals polluting water are a major environmental concern. The metals may originate from mining, smelting, metallurgy, and electroplating or from fertilizers in agriculture and emission from leather shoes and traffic (Wang and Chen 2009; Aslan 2009). Heavy metals include for instance copper, lead, arsenic, zinc, chromium, and mercury. Heavy metal pollution is not solely an environmental problem; it is also a human health issue (Wang and Chen 2009). A too high intake of copper results for instance in gastrointestinal diseases, which has motivated the World Health Organisation (WHO) to recommend 2 mg/l as the limit of copper in drinking water (WHO 2004). Consequently, heavy metals should be removed from water to prevent release into the environment and to prevent human exposure to these metals through the drinking water.

Several methods have been developed for removal of heavy metals from water, and these comprise chemical, physical, and biological methods (Wang and Chen 2009). Particularly, biological methods have received significant attention in recent years (Wang and Chen

2009), as they show low costs and are adequately efficient. The biological methods in question are generally founded on non-directed physicochemical interaction between the metals and either dead or living biomass and is commonly referred to as biosorption (Gadd 1993). For practical reasons, dead biomass seems more advantageous, as it can be obtained as a by-product from fermentation, no nutrient supplementation is required, and regeneration of the biomass is a relatively simple procedure (Gadd 1993). A large variety of biomass has been studied, such as waste sludge cake (Sarioglu et al. 2009), sawdust (Yu et al. 2000), cone biomass of *Thuja orientalis* (Nuhoglu and Oguz 2003), and chitosanaceous materials (Hu et al. 2004). Fungal biomass has also received significant attention (Srivastava and Hasan 2011; Dursun et al. 2003; Yan and Viraraghavan 2003; Mullen et al. 1992) and has for instance been investigated for removal of oil from water (Srinivasan and Viraraghavan 2010). In general, fungi belonging to the genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium*, *Rhizopus*, *Saccharomyces*, and *Trichoderma* have been shown to biosorb metals (Viraraghavan and Srinivasan 2011), although the list is probably limited to the species investigated thus far. Research on fungal biosorption was recently reviewed by Viraraghavan and Srinivasan (2011). The research is still predominantly on a basic level, and the perspective of applications is so far limited. Among the various fungi, zygomycetes have emerged as promising top candidates. Zygomycetes have the benefit of being fast growing and able to grow in a wide range of temperatures and environments (Dijksterhuis and Samson 2006), the latter including lignocellulosic hydrolysates and waste streams (Taherzadeh et al. 2003). Some strains of zygomycetes are edible and are used for production of human food, such as Indonesian tempe, and can thus be considered safe from a human perspective (Wikandari et al. 2012).

A key part in accomplishing biosorption, particularly when dead biomass is employed, is immobilization of the biomass (Wang and Chen 2009). The procedure has to date entailed packed columns in which biosorbents are being immobilized as granules, using matrices as silica, sodium, or calcium alginate, polysulfonate, polyacrylamide, and polyurethane (Vijayaraghavan and Yun 2008). Using a porous media to which the fungal biomass is attached might be an attractive alternative as it significantly simplifies the biomass collection. Textile would be a suitable material for this purpose, as it is a large area system, produced at low cost with high precision and repeatability. Textiles can be composed of a variety of synthetic (polyester, polyolefin, etc.) and natural (wool, jute, etc.) fibers and exhibit a spectrum of surface geometries providing different affinity capacities for fungal growth. Fabrics can be produced in a variety of shapes with different degrees of porosity, enabling optimization of fungal growth, attachment, and water penetration.

The aim of the present study was to explore the potential of textile media for immobilization of zygomycetes biomass to be used for heavy metal absorption. The immobilization capability was studied by examining the attachments of zygomycetes to different textile media. The study furthermore investigated the possibility of immobilizing zygomycete biomass within cushions.

2 Materials and Methods

2.1 Fungal Strain

The zygomycete *Rhizopus* sp. CCUG 61146 (Culture Collection University of Gothenburg, Sweden; previously identified as RM3 (Wikandari et al. 2012) and originally isolated from Indonesian starter cultures for tempe production) was maintained on potato dextrose agar (PDA) containing 20 g/l glucose, 15 g/l agar, and 4 g/l potato extract and was stored at 4 °C. Spores for inoculation were prepared through 3–5 days of incubation on fresh PDA plates at 30 °C. The spores were harvested by flooding the plates with 20 ml sterile distilled water.

2.2 Textile Media

Ten different types (three knitted, seven woven) of textile fabrics were studied (see Table 1). The fabrics were produced by using the E12 gauge Mayer Relanit (Karl Mayer GmbH, Germany) circular knitting machine, the E18 Camber Velknit NS circular knitting machine, and the 12 shaft Dornier GWN 8 (Lindauer DORNIER GmbH, Germany) weaving machine, as specified in Table 1.

To remove spinning oils and other substances introduced during the manufacturing procedure, the fabrics were washed for 90 min at 60 °C in 16 l of tap water, using a standard Wascator washing machine

Table 1 Type, fabric, and yarn of the utilized textile media and type of machine

Type	Fabric	Yarn	Machine
PP1	Polypropylene-based knitted single jersey	Viafil dtex 78 Nm 32/2	E18 gauge Camber Velknit
PP2	Polypropylene-based knitted single jersey	Viafil dtex 110 Nm 32/2	E12 gauge Mayer Relanit
PP3	Polypropylene-based knitted single jersey	Nm 60/1	E18 gauge Camber Velknit
PA4	Polyamide-based weave Nm 14; 12 threads/cm	Nm 14	12 shaft Dornier GWN 8
PES5	Polyester-based satin weave; 12 threads/cm	Nm 40/2	12 shaft Dornier GWN 8
PES6	Polyester-based plain weave; 12 threads/cm	Nm 40/2	12 shaft Dornier GWN 8
PES7	Polyester-based honeycomb weave; 12 threads/cm	Nm 40/2	12 shaft Dornier GWN 8
WO8	Wool-based plain weave; 12 threads/cm	Nm 28/1	12 shaft Dornier GWN 8
JU9	Jute-based weft-dominated satin weave; 12 threads/cm	Weft: jute Nm 8/1, warp: polyester Nm 40/2	12 shaft Dornier GWN 8
JU10	Jute-based honeycomb weave; 12 threads/cm	Weft: jute Nm 8/1, warp: polyester Nm 40/2	12 shaft Dornier GWN 8

(Electrolux, horizontal rotating drum) and adding 50 ml of washing powder without optical whiteners. The fabrics were subsequently cut into 2×2 cm squares, dried for 3 h in an oven at 105 °C, and then weighed, followed by autoclavation for 20 min at 121 °C.

2.3 Fungal Cultivation and Preparation

Fungal cultivations were carried out in cotton-plugged 250-ml Erlenmeyer flasks, each flask with one textile square and 100 ml cultivation medium, the latter made up of 30 g/l glucose, 5.0 g/l yeast extract, 7.5 g/l $(NH_4)_2SO_4$, 3.5 g/l KH_2PO_4, 1.0 g/l $CaCl_2$ $2H_2O$, 0.75 g/l $MgSO_4$ $7H_2O$, 1 ml/l sterile filtered vitamin solution, and 10 ml/l trace metal solution (Sues et al. 2005). All solutions (excluding the one containing vitamins) were autoclaved for 20 min at 121 °C min. All cultures were inoculated by adding 5×10^5 spores/ml and cultivated for 48 h at 30 °C in 125 rpm shaking water baths. The textile-immobilized biomass was harvested with a sieve, soaked with 0.2 M NaOH for 30 min, and washed with ultrapure water until pH reached ≤8. The biomass was subsequently autoclaved for 30 min at 121 °C and then dried for 24 h in an oven at 60 °C (Yan and Viraraghavan 2003).

2.4 Biosorption

The biosorption experiments (using the dried biomass immobilized on the 10 different textile media) were conducted in 250-ml Erlenmeyer flasks, placed in water baths shaking at 125 rpm at 20 °C. The flasks contained 150 ml 20 mg/l Cu^{2+}, added as $CuSO_4$, with pH adjusted to 5.0, using 0.1 M HNO_3 and/or 0.1 M NaOH. No major changes to pH occurred during the experiments. Samples were taken after 0, 5, 10, 15, 20, 25, 40, 60, 100, and 150 min. Textiles without fungal biomass served as control. Furthermore, biosorption at different initial Cu^{2+} concentrations (25, 35, and 45 mg/l) was evaluated for biomass immobilized on polyamide weave (PA4) and polyester honeycomb weaves (PES7).

A two-step biosorption process, using biomass immobilized on jute honeycomb weave (JU10), was evaluated at an initial Cu^{2+} concentration of 20 mg/l. Samples were taken at the same intervals as above, with an additional point after 200 min. After the 200 min, the biomass/textile complex was replaced with a new one, followed by sampling at the same intervals, 0–200 min.

A water-permeable cushion for the immobilization process was also evaluated for biosorption capacity. Biomass of the desired type, either as powder or immobilized on PES7, was covered with a non-woven polypropylene membrane (Fitesa Sweden AB, Norrköping) of a type commonly used as surface layer in hygiene products. Sealing was carried out with a water-resistant tape (3M), forming a cushion of 20×20 cm. Biosorption was measured following the single-step protocol above, employing an initial Cu^{2+} concentration of 20 mg/l.

2.5 Analytical Methods

All samples were immediately centrifuged for 6 min at 10,000×*g*. The supernatants were analysed for Cu^{2+} using AAS (Atomic Absorption Spectrometer 3100,

PerkinElmer Inc., Waltham, MA, USA) with an air/acetylene flame.

Inoculum concentration of spores was determined by counting in a Buerker's counting chamber (depth 0.1 mm), using a light microscope. The spores were counted in 40 E-squares, each with a volume of 1/250 μl.

2.6 Rate Equation and Statistical Analysis

All experiments were carried out in duplicates, and the reported intervals represent 95 % confidence intervals, based on pooled standard deviations, unless otherwise noted. The software package Minitab® was used for all statistical evaluations, using a one-way general linear model.

Biosorption was modelled by means of a pseudo-second-order expression (Ho 2006):

$$\frac{dq_t}{dt} = k(q_e - q_t)^2 \quad (1)$$

yielding

$$q_t = \frac{q_e^2 kt}{1 + q_e kt} \quad (2)$$

which can be rearranged into the linear form

$$\frac{t}{q_t} = \frac{1}{kq_e^2} + \frac{1}{q_e}t \quad (3)$$

where k is the rate constant in grams/(milligrams per minute), q_e (milligrams per minute) is the amount of Cu^{2+} biosorbed per mass biosorbent at equilibrium, and q_t (milligrams per gram) is the amount of Cu^{2+} biosorbed per mass biosorbent after time t (min). The constants were used as response variables, and differences between materials were evaluated by using a one-way general linear model.

3 Results and Discussion

3.1 One-Step Biosorption

Exploring the ability of the zygomycetes fungus *Rhizopus* sp. to attach and remain immobilized on 10 different textile media (Table 1) revealed that the fungus attached and remained immobilized on all polyester, wool, and jute-based textiles. Attachment to polypropylene and polyamide was less regular and required the fungus to first germinate on the textile material soaked in medium.

Biosorption with immobilized *Rhizopus* sp. biomass significantly reduced the Cu^{2+} concentrations in the solution from an initial concentration of 20 to 3.1–5.6 mg/l within 150 min (Table 2), with one exception (p=0.000). Biomass immobilized on wool WO8 halved the Cu^{2+} concentration to 10.2 mg/l. The pseudo-second-order rate expression revealed Cu^{2+} uptakes of 4.5–7.2 mg/g biomass at equilibrium, except for PES5, which absorbed 14.8 mg/g (Table 2). With the exception of WO8, the Cu^{2+} biosorptions fitted very well into the model (Table 2). The linearized versions of the pseudo-second-order rate model of *Rhizopus* sp. being immobilized on PES7 and WO8, or as biomass powder, are depicted for one of the experimental replicates in Fig. 1. The statistical analysis of the rate constants in the model disclosed significant (p=0.000) differences in biosorption rates. Biomass powder and the JU10 accomplished the fastest removals of Cu^{2+} from the solution, while the remaining rate constants were distinctly lower (Table 2).

None of the control materials (textile without biomass) caused any measurable reduction of Cu^{2+} concentration. It was thus concluded that the reduction of Cu^{2+} concentration in the solutions was due to the presence of fungal biomass. The efficacy of Cu^{2+} biosorption capacity of the fungal biomass is most likely dependent on accessible surface area, seeing that the lowest final heavy metal concentration was observed in the solution, where biomass was present in the form of powder (see Table 2). The textile PES5 showed similar capability of Cu^{2+} absorption as the other textile materials (Table 2) in spite of significantly less biomass being attached, although having similar surface area as the other textiles. The increasing amount of biosorbed copper per biomass weight unit with decreasing concentration of biomass has previously been reported (Rome and Gadd 1987).

It may well be deduced that the choice of textile material has little or no effect on biosorption capacity, and the deciding factor should rather be whether or not attachment and immobilization of the fungal biomass occurs and is adequate.

3.2 Effect of Initial Cu^{2+} Concentration

No major difference in absorption by *Rhizopus* sp. immobilized on PA4 and PES7 was discerned when

Table 2 Characteristics of Cu^{2+} biosorption by *Rhizopus* sp. biomass, immobilized on different textile materials

Material	X^a (g)	$C_f{}^b$ (mg/l)	Q^c (mg/g)	$q_e{}^d$ (mg/g)	k^e (g/(mg min))	R^{2f}
PP1	0.32	3.5	5.3	6.6	0.0035	0.997
PP2	0.30	4.6	5.3	6.1	0.0068	0.998
PP3	0.36	3.6	4.7	6.0	0.0038	0.996
PA4	0.35	4.4	5.0	6.7	0.0029	0.998
PES5	0.14	3.9	11.8	14.8	0.0015	0.995
PES6	0.30	4.6	5.1	7.2	0.0021	0.998
PES7	0.30	3.1	5.8	6.6	0.0069	0.997
WO8	0.28	10.2	3.6	4.5	0.0064	0.963
JU9	0.33	5.3	4.5	5.5	0.0061	0.998
JU10	0.32	5.6	4.6	5.1	0.0117	0.998
Powder	0.28	0.7	5.9	6.4	0.0164	0.999
Confidence interval	0.04	1.6	0.6	0.8	0.0019	

[a] Fungal biomass attached to textile media

[b] Final Cu^{2+} concentration in the solution after 150 min

[c] Amount of biosorbed Cu^{2+} per biosorbent mass unit after 150 min

[d] Biosorption per biosorbent mass unit at equilibrium, calculated from the pseudo-second-order equation

[e] Rate constant, calculated from the pseudo-second-order equation

[f] Average R^2 value of the linear trend line chosen for the linearized pseudo-second-order equation

using different initial concentrations (25, 35, and 45 mg/l), except for an upward shift of the Cu^{2+} concentration curve with increasing initial concentration (Fig. 2). However, although small, there was a significant (p=0.007) difference in the total amount of biosorbed Cu^{2+}; on average, 4.0 and 5.0 mg/l less Cu^{2+} (p=0.0224 and p=0.0076) was biosorbed from the initial concentration of 25 mg/l compared to 35 and 45 mg/l. The calculated uptake of Cu^{2+} at equilibrium, (Table 3) was similarly lower (p=0.045) at the initial concentration of 25 mg/l than at the higher concentrations. The rate constant, on the other hand, was higher at the lower initial concentration (p=0.001).

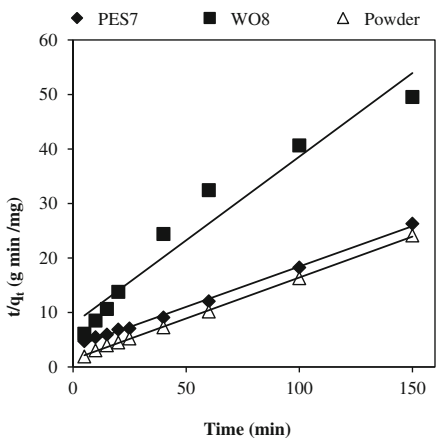

Fig. 1 One replicate each of the linearized version of the pseudo-second-order rate model used for *Rhizopus* sp., immobilized on WO8 and PES7, or as biomass powder, at an initial Cu^{2+} concentration of 20 mg/l. The latter two showed a good fit in contrast to the biomass immobilized on WO8

From an equilibrium perspective, it stands to reason that higher initial concentrations are followed by a slight increase in the masses biosorbed. Similar results have been reported by Rome and Gadd (1987) for *Rhizopus arrhizus*, *Penicillium italicum*, and *Cladosporium resinae*. A similar biosorption pattern has also been reported for different growth forms of *Mucor indicus* in the presence of different concentrations of Pb^{2+} (Javanbakht et al. 2011), for waste sludge cake at different concentrations of Cu^{2+} (Sarioglu et al. 2009), and for *Mucor hiemalis* at different concentrations of Cd^{2+} (Srivastava and Hasan 2011). Beolchini et al. (2003) reported increasing q_e values when the initial Cu^{2+} concentration was increased from 35 to 140 mg/l. This pattern thus appears to be consistent for different biosorbents and heavy metals and was retained also by *Rhizopus* sp. after immobilization on various textile materials.

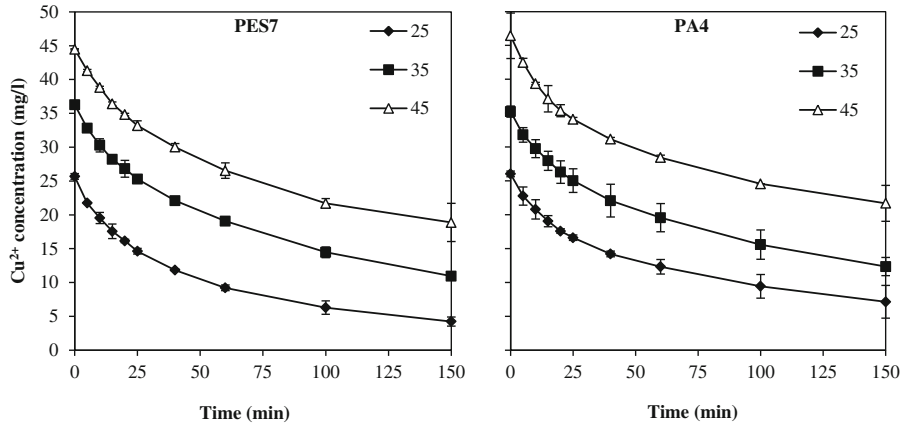

Fig. 2 Biosorption by *Rhizopus* sp. biomass immobilized on PES7 and PA4, at initial Cu^{2+} concentrations of 25, 35, or 45 mg/l. The *error bars* represent ±2 standard deviations

3.3 Two-Step Biosorption

Even though Cu^{2+} biosorption increased when the initial concentration was increased, the difference was not particularly large. It should thus be possible to further decrease the final Cu^{2+} concentration in the solution by replacing the biosorbent with a new one, using a two-step system. The experiments with *Rhizopus* sp. immobilized on JU10 and PA4 (Fig. 3) resulted in copper concentrations of 4.3 and 3.2±0.5 mg/l, respectively, at the end of the first step, and 0.44 and 1.0± 0.3 mg/l, respectively, at the end of the second step. Thus, 79 and 85±3 % of the Cu^{2+} was removed by the two textile materials in the first step and 89 and 69±9 % in the second step. Taking both steps into account, 98 and 95±1 % of the initial Cu^{2+} was removed at the end of the second step. The differences between JU10 and PA4 were statistically significant ($p<0.031$).

The amounts of biosorbed Cu^{2+} by JU10 and PA4 per biosorbent mass unit at equilibrium were calculated to 5.4 and 6.7±1.4 mg/g after the first step and 1.4±0.1 and 1.4±1.8 (±2 s)mg/g after the second step, respectively. The calculated reaction rate constants for the first step were 0.009 and 0.003±0.01 g/(mg min), respectively, and for the second step 0.011±0.008 and 0.012± 0.022 g/(mg min) (± 2 s), respectively.

The second step being more effective in terms of JU10 absorbing Cu^{2+} in comparison with the first step concurs well with the current literature (see Section 3.2). The lower degree of Cu^{2+} removal by PA4 during the second stage (69 %) in comparison with the first stage (85 %) may probably be explained by uneven

Table 3 Uptake of Cu^{2+} per biosorbent mass unit at equilibrium and rate constants. Initial Cu^{2+} concentration was 25, 35, or 45 mg/l

Material	C_i[a] (mg/l)	q_e[b] (mg/g)	k[c] (g/(mg min)	R^{2}[d]
PES7	25	7.9	0.0039	0.998
PES7	35	10.3	0.0021	0.993
PES7	45	11.2	0.0018	0.998
PA4	25	8.3	0.0033	0.997
PA4	35	9.6	0.0021	0.993
PA4	45	8.6	0.0027	0.999
Confidence interval		±1.6	±0.0005	

[a] Initial Cu^{2+} concentration

[b] Biosorption per biosorbent mass unit at equilibrium, calculated from the pseudo-second-order equation

[c] Rate constant, calculated from the pseudo-second-order equation

[d] Average R^2 value of the linear trend line chosen for the linearized pseudo-second-order equation

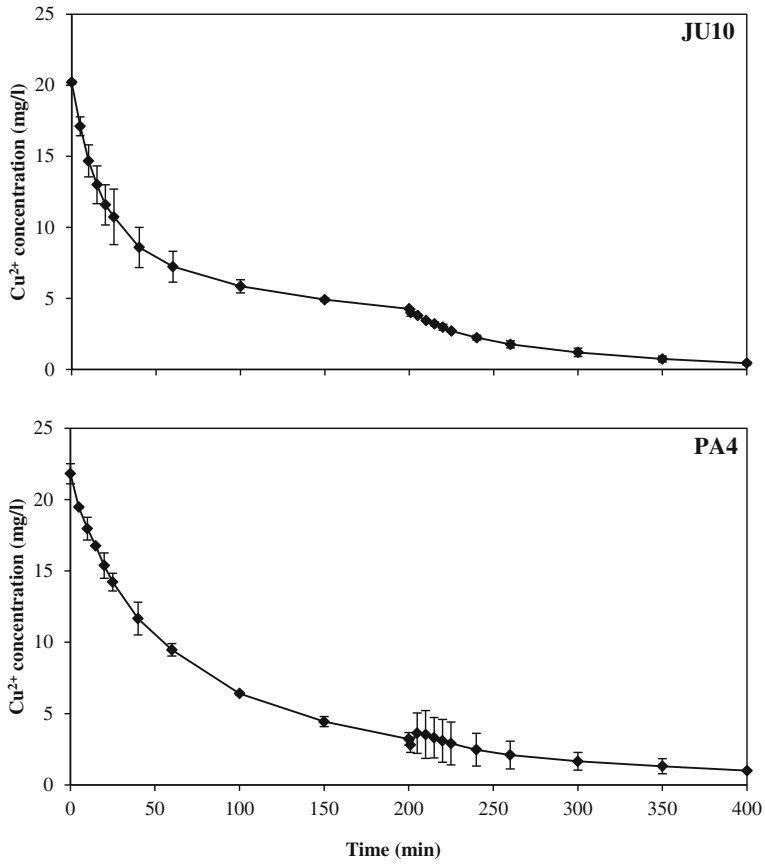

Fig. 3 Two-step biosorption of Cu^{2+} by *Rhizopus* sp. biomass immobilized on JU10 and PA4. The biomass was replaced after 200 min. The *error bars* represent ±2 standard deviations

attachment of the biomass to the textile (Section 3.1), resulting in less accessible surface area and also desorption during the removal of the first batch of biomass (Fig. 3: PA4).

WHO (2004) recommended a limit of 2 mg/l for copper in drinking water. Both two-step biosorption systems in the present study more than adequately met this requirement. Biosorption with *Rhizopus* sp. biomass immobilized on textiles can hence be considered to meet the most stringent demands for water purity, at least in terms of absence of copper ions. Biosorption has been proposed as a final treatment of water to remove the last remaining heavy metals from solution (Gadd 1993). The results suggest that a two-step biosorption system with *Rhizopus* sp. biomass immobilized on textile material holds the potential for such final treatment of drinking water.

3.4 Biosorption by Cushion Entrapped Biomass

Free powder biomass, not being immobilized on textile material, exhibited the greatest removal of Cu^{2+} from the solution (Table 2). On the other hand, free biomass needs to be easily collected after metal uptake. In order to achieve optimal uptake and avoid a collection step, a cushion was constructed, using a membrane of the same type as the one used for diapers. The cushion was filled with biomass, enabling contaminated water to enter the cushion but preventing powder biomass to escape into the solution. When saturated, the cushion can easily be removed from the cleaned water. When using the cushion system, applying *Rhizopus* sp. biomass in powder form, a final Cu^{2+} concentration of 2.7 mg/l was reached, while the PES7 complex resulted in 6.4± 2.2 mg/l (Fig. 4), a significant difference (p=0.036). Biosorption with powder and PES7, not using the cushion construction, resulted in significantly (p=0.003) lower levels of Cu^{2+} (2.4 and 3.4±1.6 mg/l, respectively) remaining in the solution.

The uptake of Cu^{2+} per biosorbent mass unit was 3.0 and 4.5±0.7 mg/g for the powder and PES7 cushion complexes, respectively, 2.9 and 1.3±0.7 mg/g less (respectively) than biosorption without the cushion, a highly significant difference (p=0.000). The calculated

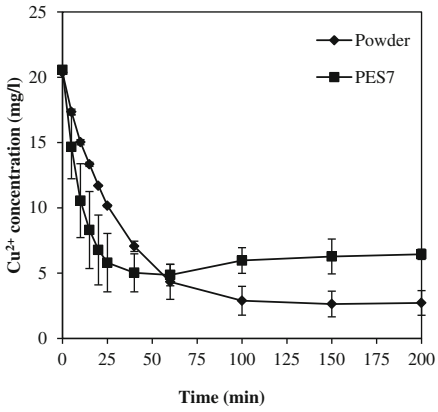

Fig. 4 Biosorption by *Rhizopus* sp. biomass in the form of powder placed in cushions and biomass immobilized on PES7. The *error bars* represent ±2 standard deviations

uptake of Cu^{2+} per biosorbent mass unit at equilibrium was 3.5 and 4.6±1.0 mg/g for the cushion entrapped powder and PES7, respectively (Fig. 5), 2.8 and 2.0± 0.6 mg/g less (respectively) than for the free biosorbents.

The calculated reaction rate constants of biosorption in a cushion were 0.011±0.002 g/(mg min) for the powder and −0.07±0.32 g/(mg min) (±2 s) for PES7. A large uncertainty in the values for PES7 made a direct comparison with the powder unfeasible. The uncertainty might be a result of large initial fluctuations in Cu^{2+} concentrations, and an apparent release after 100 min (Fig. 4), which also affected the linearized version of the pseudo-second-order reaction rate model, leading to a poorer fit (Fig. 5). A decrease in the reaction rate should not be disregarded. Although not statistically significant (p=0.0772), the biosorption process by the cushion entrapped powder biomass appeared to be slightly slower, and absorption rates have indeed been reported to decline after immobilization of the biosorbent. For instance, Wu and Yu (2007) reported a decreased rate of 2,4-dichlorophenol biosorption when *Phanerochaete chrysosporium* was immobilized in a polymer matrix, as compared to the biosorption rate of free biomass.

For practical reasons, since retention of the biosorbent, or separation of the biosorbent from the treated water, constitutes a necessary step, some type of immobilization of the biosorbent is usually considered a preferable measure. However, immobilization has two major drawbacks: mass transfer limitations and increased process costs (Vijayaraghavan and Yun 2008). Using cushions for immobilization of the biosorbent could improve the process economy. As shown in the present study, placing biomass in cushions, be it in the form of powder or attached to textile material during cultivation, holds a great potential for successful biosorption of metals from drinking water. In spite of a slightly slower biosorption process, and the cushion complex not being thoroughly efficient, the ease with which separation is accomplished would most likely outweigh these drawbacks.

4 Conclusions

The zygomycetes fungus *Rhizopus* sp. was successfully immobilized by cultivation on 10 different inexpensive textile materials. After treatment with NaOH, the

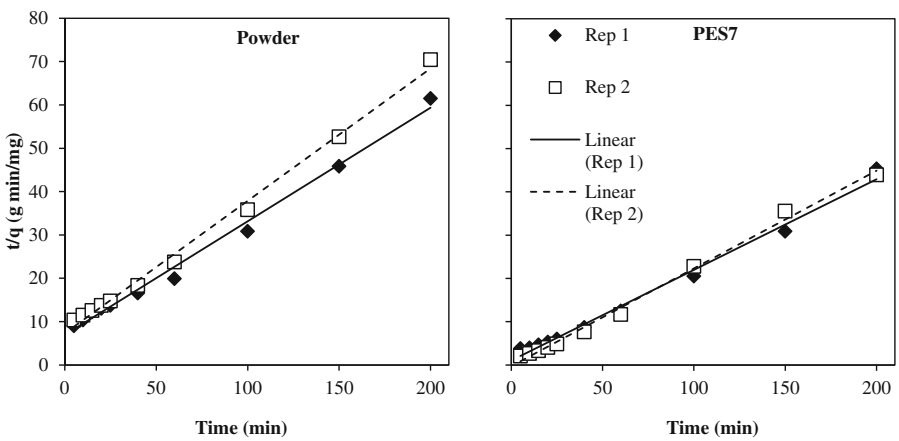

Fig. 5 Linearized version of the pseudo-second-order rate model used for testing biosorption of Cu^{2+} by *Rhizopus* sp. biomass in the form of powder placed in cushions and biomass immobilized on PES7

inactivated immobilized fungi effectively removed Cu^{2+} from the solution, although wool was not as effective as the other textiles. Furthermore, with the exception of wool, they all closely fitted into a pseudo-second-order reaction rate model. Increasing the initial Cu^{2+} concentration led to a small increase in removal of the heavy metal from the solution. By reapplying immobilized *Rhizopus* sp. to a solution containing a low concentration of Cu^{2+} after going through a first step of biosorption, the concentration of Cu^{2+} was successfully decreased to the level the WHO demands for drinking water. Immobilization by means of a cushion construction entrapping the fungal biomass was also successful when applied in the biosorption process. The positive results obtained in the two-step biosorption in the present study indicate that a sequential arrangement holds the potential of being the foundation for a real product. This could be realized either by water undergoing a serial passage through biomass-filled compartments or by utilizing the biomass-attached textile media being easily removable, foldable, possible to roll up, etc. With this approach, it would be fairly easy to manually, or automatically, exchange the biosorbent after a certain period of time, when the biosorption capacity of the biomass is exhausted.

Acknowledgments Fredrik Wennersten is acknowledged for his expertise regarding weaving and the Swedish Energy Agency for provided financial support.

References

Aslan, A. (2009). Determination of heavy metal toxicity of finished leather solid waste. *Bulletin of Environmental Contamination and Toxicology, 82*(5), 633–638. doi:10.1007/s00128-009-9656-1.

Beolchini, F., Pagnanelli, F., Reverberi, A. P., & Vegliò, F. (2003). Copper biosorption onto Rhizopus oligosporus: pH-edge tests and related kinetic and equilibrium modeling. *Industrial and Engineering Chemistry Research, 42*(20), 4881–4887. doi:10.1021/ie020829h.

Dijksterhuis, J., & Samson, R. A. (2006). Zygomycetes. In C. W. Blackburn (Ed.), *Food spoilage microorganisms* (pp. 415–436). Cambridge: Woodhead.

Dursun, A. Y., Uslu, G., Tepe, O., Cucï, Y., & Ekïz, H. İ. (2003). A comparative investigation on the bioaccumulation of heavy metal ions by growing *Rhizopus arrhizus* and *Aspergillus niger*. *Biochemical Engineering Journal, 15*(2), 87–92. doi:10.1016/S1369-703X(02)00187-0.

Gadd, G. M. (1993). Interactions of fungi with toxic metals. *New Phytologist, 124*(1), 25–60. doi:10.1111/j.1469-8137.1993.tb03796.x.

Ho, Y.-S. (2006). Review of second-order models for adsorption systems. *Journal of Hazardous Materials, 136*(3), 681–689. doi:10.1016/j.jhazmat.2005.12.043.

Hu, K.-J., Hu, J.-L., Ho, K.-P., & Yeung, K.-W. (2004). Screening of fungi for chitosan producers, and copper adsorption capacity of fungal chitosan and chitosanaceous materials. *Carbohydrate Polymers, 58*(1), 45–52. doi:10.1016/j.carbpol.2004.06.015.

Javanbakht, V., Zilouei, H., & Karimi, K. (2011). Lead biosorption by different morphologies of fungus *Mucor indicus*. *International Biodeterioration and Biodegradation, 65*(2), 294–300. doi:10.1016/j.ibiod.2010.11.015.

Mullen, M. D., Wolf, D. C., Beveridge, T. J., & Bailey, G. W. (1992). Sorption of heavy metals by the soil fungi *Aspergillus niger* and *Mucor rouxii*. *Soil Biology and Biochemistry, 24*(2), 129–135. doi:10.1016/0038-0717(92)90268-3.

Nuhoglu, Y., & Oguz, E. (2003). Removal of copper(II) from aqueous solutions by biosorption on the cone biomass of *Thuja orientalis*. *Process Biochemistry, 38*(11), 1627–1631. doi:10.1016/S0032-9592(03)00055-4.

Rome, L., & Gadd, G. (1987). Copper adsorption by *Rhizopus arrhizus*, *Cladosporium resinae* and *Penicillium italicum*. *Applied Microbiology and Biotechnology, 26*(1), 84–90. doi:10.1007/BF00282153.

Sarioglu, M., Güler, U. A., & Beyazit, N. (2009). Removal of copper from aqueous solutions using biosolids. *Desalination, 239*(1–3), 167–174. doi:10.1016/j.desal.2007.03.020.

Srinivasan, A., & Viraraghavan, T. (2010). Oil removal from water by fungal biomass: a factorial design analysis. *Journal of Hazardous Materials, 175*(1–3), 695–702. doi:10.1016/j.jhazmat.2009.10.065.

Srivastava, P., & Hasan, S. H. (2011). Biomass of *Mucor hiemalis* for the biosorption of cadmium from aqueous solutions: equilibrium and kinetic studies. *BioResources, 6*(4), 3656–3675.

Sues, A., Millati, R., Edebo, L., & Taherzadeh, M. J. (2005). Ethanol production from hexoses, pentoses, and dilute-acid hydrolyzate by *Mucor indicus*. *FEMS Yeast Research, 5*(6–7), 669–676. doi:10.1016/j.biombioe.2009.01.003.

Taherzadeh, M. J., Fox, M., Hjorth, H., & Edebo, L. (2003). Production of mycelium biomass and ethanol from paper pulp sulfite liquor by *Rhizopus oryzae*. *Bioresource Technology, 88*(3), 167–177. doi:10.1016/S0960-8524(03)00010-5.

Vijayaraghavan, K., & Yun, Y.-S. (2008). Bacterial biosorbents and biosorption. *Biotechnology Advances, 26*(3), 266–291. doi:10.1016/j.biotechadv.2008.02.002.

Viraraghavan, T., & Srinivasan, A. (2011). Fungal biosorption and biosorbents. In P. Kotrba, M. Mackova, & T. Macek (Eds.), *Microbial biosorption of metals*. New York: Springer.

WHO (2004). *Copper in drinking-water*. World Health Organization: WHO/SDE/WSH/03.04/88.

Wang, J., & Chen, C. (2009). Biosorbents for heavy metals removal and their future. *Biotechnology Advances, 27*(2), 195–226. doi:10.1016/j.biotechadv.2008.11.002.

Wikandari, R., Millati, R., Lennartsson, P. R., Harmayani, E., & Taherzadeh, M. J. (2012). Isolation and characterization of zygomycetes fungi from tempe for ethanol production and

biomass applications. *Applied Biochemistry and Biotechnology, 167*(6), 1501–1512. doi:10.1007/s12010-012-9587-x.

Wu, J., & Yu, H.-Q. (2007). Biosorption of 2,4-dichlorophenol by immobilized white-rot fungus *Phanerochaete chrysosporium* from aqueous solutions. *Bioresource Technology, 98*(2), 253–259. doi:10.1016/j.biortech.2006.01.018.

Yan, G., & Viraraghavan, T. (2003). Heavy-metal removal from aqueous solution by fungus *Mucor rouxii. Water Research, 37*(18), 4486–4496. doi:10.1016/S0043-1354(03)00409-3.

Yu, B., Zhang, Y., Shukla, A., Shukla, S. S., & Dorris, K. L. (2000). The removal of heavy metal from aqueous solutions by sawdust adsorption—removal of copper. *Journal of Hazardous Materials, 80*(1–3), 33–42. doi:10.1016/S0304-3894(00)00278-8.