Cadmium and Copper Absorption Mediated by a Poly(vinyl alcohol)-*b*-Polyacrylonitrile Based Micelle/*Trichosporon cutaneum* Cell System

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ABSTRACT: The micelles of a recently synthesized copolymer of poly(vinyl alcohol)-*b*-polyacrylonitrile added to the growth solution of the filamentous yeast *Trichosporon cutaneum* strain R57 led to the formation of a binary system consisting of micelles and cells. The resulting micelle/cell system was studied as a model for the removal of toxic concentrations of heavy-metal ions (cadmium and copper) from aqueous solutions. The ion-removal efficiency mediated by this system was higher than for free-floating cells. The copper-removal efficiency from the solution reached a level of 65% after 24 h of cultivation, whereas the cadmium-removal efficiency reached 62% after 6 h of growth. For comparison, the free-floating cells removed 42% of copper and only 38% of cadmium from the solutions. The effects of surface interactions between the cells and polymer micelles on the biosorption capacity of the cells are discussed in the article. © 2010 Wiley Periodicals, Inc. J Appl Polym Sci 116: 2970–2975, 2010

Key words: biological applications of polymers; block copolymers; micelles

INTRODUCTION

The environmental impact and buildup of heavy metals on the ecosystem has been a cause of great concern in recent years.[1,2] Despite the fact that many metals, including Cu, Zn, Mn, and Co, are essential for microbial growth at low concentrations, many others have no essential biological function. For instance, As, Cd, Pb, Sn, and Cr are extremely toxic, even at low concentrations.[3] All of these ions can exhibit varying degrees of toxicity toward living cells, because they damage molecules through their ability to interact with the thiol groups of proteins, which blocks and inactivates their functions. The ions of cadmium and copper are commonly known as environmental pollutants because they are extremely toxic for any biological system when present in higher concentrations. Cadmium and copper ions usually exert their toxic effects by interacting with the molecules of the cell plasma membrane. Thus, they can change the membrane permeability and disturb cell–ion relations (mainly causing release of K ions from the cytoplasm or inhibiting enzyme activities[4]) when these cations are presented in the cell.

Metal ions, including cadmium, lead, mercury, and copper, although they are biocidal for most organisms, can be tolerated by some microorganisms. These organisms have evolved mechanisms of tolerance and the ability to detoxify some harmful ions. Cell detoxification strategies include mechanisms of metal sequestration or/and precipitation;[2,4] these lead to lower ion concentrations around the cells. Unlike organic contaminants, heavy metals cannot be destroyed chemically. The conventional methods used for metal-ion removal from industrial wastewaters include chemical precipitation, electrochemical treatment, ion-exchange processes, membrane separation, and evaporation.[5,6] However, some of the conventional technologies are ineffective because they create a sludge-disposal problem after treatment and can be expensive.[5] Among the living organisms used for metal-remediation procedures, microorganisms occupy a leading place. They exhibit a number of metabolic-dependent or/and

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independent processes for the uptake and accumulation of heavy metals.[7,8] Fungi and yeasts are known as better metal-remedy factors because of their ability to grow at high metal concentrations in solutions with other adverse properties, such as low pH. They possess thick cell walls with high ion-binding capacities and vast accumulation abilities in their vacuoles, although, in some cases, they show ion-extrusion mechanisms.[3,9] Recently, it was shown that the strain R57 of *Trichosporon cutaneum* possesses good Cd and Cu accumulating abilities when grown in solutions containing metal concentrations as high as 5 mg/L for Cu and 112 mg/L for Cd ions. This strain can accumulate Cd ions up to 12.8 mg/g of dry biomass and Cu up to 1.2 mg/g of dry biomass of the cells. One can significantly improve the bioaccumulation ability of the strain by attaching the cells to different adsorbents.[10]

In this study, we aimed to demonstrate that the introduction of poly(vinyl alcohol) (PVOH)-*b*-polyacrylonitrile (PAN) nanosized micelles into a medium containing growing cells of strain R57 of *T. cutaneum* in aqueous solution would positively affect the removal efficiency of Cu and Cd.

EXPERIMENTAL

Materials

Dimethyl sulfoxide (DMSO), $CuSO_4$, and $CdSO_4$ were supplied by Merck (Darmstadt, Germany). $PVOH_{269}$-*b*-PAN_{215} block copolymer was prepared as reported elsewhere.[11]

Strain and media

The filamentous yeast strain *T. cutaneum* R57, registered by Ivanova et al.[12] and maintained in the culture collection of the Bulgarian National Bank of Industrial Microorganisms and Cell Cultures under N2414, was used in the investigations. The cultivation was carried out in a medium according to the procedure of Georgieva et al.[10] A sterile glucose solution (20 g/L) was sterilized separately and added to the growth medium.

Micellization of the $PVOH_{269}$-*b*-PAN_{215} block copolymer in water

The preparation of PVOH-*b*-PAN based micelles was reported in detail in ref. 13. Briefly, a $PVAc_{269}$-*b*-PAN_{215} block copolymer (1 g) was dissolved in DMSO (5 mL) and added to a solution of potassium hydroxide (4.5 g) in methanol (50 mL, pure for analysis). The molar amount of KOH relative to the vinyl acetate (VAc) units was 9/1. After 48 h under stirring at room temperature, the PVOH-*b*-PAN copolymer was collected, washed with ethyl acetate, and dried *in vacuo* at 50°C. A degree of hydrolysis of 95% was calculated by ^{1}H-NMR spectra; the peak areas in the range 1.6–2.1 ppm corresponded to the CH_2 and CH_3 protons in the PAN and PVAc units, respectively; the peak area in the range 4.6–5.0 ppm corresponded to the CH protons in the PVAc units for the original PVAc-*b*-PAN block copolymers; those in the range 1.6–2.1 ppm corresponded to the CH_2 protons of PAN; and the peak areas in the range 3.5–4.0 were due to the CH protons of the PVOH units for the final PVOH-*b*-PAN copolymers.

Then, 1 mL of the PVOH-*b*-PAN copolymer solution in DMSO [$PVOH_{269}$-*b*-PAN_{215} copolymer concentration (c) = 10 mg/mL] was added to 9 mL of Milli-Q water (Millipore Corporation, USA) at flow rate of 7.93 mL/h under stirring. The solutions were stirred for 24 h. Dialysis against Milli-Q water for 48 h was performed to eliminate DMSO.

Cultivation conditions

Starter cultures were prepared by the loop inoculation of 100 mL of liquid medium and incubation for 18 h on a rotary shaker (180 rpm at 30°C). For experimental cultures, 100 mL of medium was inoculated with 5 mL of the starter culture (to an initial biomass concentration of approximately 0.1 mg of dry weight/mL) and incubated on the shaker for 72 h at 30°C. At the 6th h of the strain cultivation, 2 mL of a water solution of PVOH-*b*-PAN based micelles (c = 1 mg/mL) were added to 100 mL of the culture medium. The cadmium and copper ions were supplied to the cultivated strain at the 24th h of strain cultivation. The cadmium was applied in the form of $CdSO_4$ at a concentration of 89.6 mg of Cd/L (0.8 m*M* $CdSO_4$). The copper was applied in the form of $CuSO_4{\cdot}5H_2O$ at a concentration of 3.175 mg of Cu/L (0.05 m*M* $CuSO_4{\cdot}5H_2O$).

Different doses of yeast biomass between 200 and 1000 mg/L were applied to determine the removal efficiency of Cu and Cd ions. The micelle concentration was as described previously.

Analysis

Dynamic light scattering (DLS)

DLS measurements were performed on a Malvern CGS-3 apparatus (Malvern Instruments Ltd., Worcestershire, UK) equipped with a He–Ne laser with a wavelength of 632,8 nm. The temperature was set to 25°C, and the angle of measurements was 90°.

Transmission electron microscopy (TEM) and scanning electron microscopy (SEM) observations

TEM pictures were recorded on a Philips CM 100 microscope (Philips, Eindhoven, Netherlands) equipped

with a Gatan 673 charged coupling device camera and transferred to a computer equipped with Kontron KS 100 (Kontrone Elektronik GmbH, Edring, Germany) software. Samples were prepared by the deposition of a drop of micellar solution on Formvar-coated copper grids and drying in air.

SEM observation was carried out on a JEOL JSM-5510, JEOL JFC-1200 fine coater (JEOL Ltd, Tokyo, Japan).

Atomic absorption analysis

The determinations of the copper- and cadmium-ion uptakes by *T. cutaneum* R57 were done with an atomic absorption spectrophotometer (PerkinElmer, Windsor, Berkshire, England). After the desired incubation period, the aqueous phases were separated by centrifugation from the biosorbent, and the concentration of each metal ion in this phase was measured with atomic absorption analysis.

RESULTS AND DISCUSSION

The selective hydrolysis of the PVAc sequence of the PVAc-*b*-PAN block copolymer into PVOH-*b*-PAN was carried out in a methanol/DMSO mixture by potassium hydroxide at room temperature for 48 h. Micellization then occurred by the dissolution of the amphiphilic PVOH-*b*-PAN copolymers in DMSO (a good solvent for both blocks), followed by the slow addition of the dissolved copolymer in water. Micelles consisting of a PAN core and a PVOH shell with an average hydrodynamic diameter of 80 nm were then formed, as measured by DLS [Fig. 1(a)]. The morphology of the micelles was examined by TEM. Spherical micelles with an average diameter of 50–60 nm were clearly observed [Fig. 1(b)]. The difference in the diameter of the micelles measured by DLS and TEM was due to the PVOH chains forming the shell of the micelles because, in a solution, they were more extended in contrast to their dried state, where they were collapsed.

Furthermore, the addition of an aqueous solution of PVOH-*b*-PAN micelles into medium containing yeast cells led to the formation of a binary system consisting of polymer micelles and cells. Figure 2(a,b) shows the SEM images of the free cells and the micelle/cell binary system, respectively, where free micelles and micelles deposited on the yeast cells are clearly shown. To demonstrate the positive effect of the formed binary system on the removal efficiency of the heavy metals, the accumulations of copper and cadmium ions on the micelles, free cells, and micelle/cell system were monitored after 2, 6, and 24 h of cultivation of the strain (Tables I and II). The Cu-removal efficiency for the micelle/cell system was found to be maximal after the 24 h of cultivation (65%). The process of copper sorption on the binary system revealed that the residual ion concentration in the solution after the treatment was 1.08 mg of Cu/L compared to 3.175 mg/L for the starting solution. For the sake of comparison, free micelles ($c = 1$ mg/mL) showed a maximum copper removal of 37%, and the residual amount of ions in the medium was 2.00 mg of Cu/L, although the free cells showed a copper-removal efficiency of 42% and a residual ion concentration in the medium of 1.85 mg of Cu/L (Table I).

Meanwhile, the experiments with cadmium showed that the micelle/cell system had a maximum efficiency of cadmium removal equal to 62%, but this value was found at an earlier stage, after 6 h of cultivation, whereas the free micelles, incubated with cadmium, showed only 15% cadmium removal (Table II).

The effect of the yeast biomass on the amount of Cu^{2+}- and Cd^{2+}-ion removal was also studied by

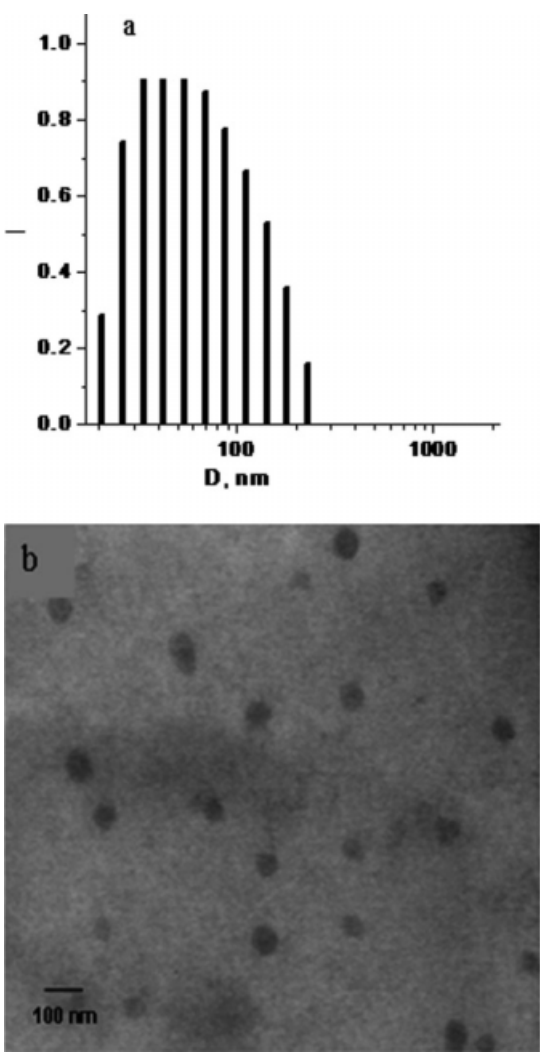

Figure 1 (a) DLS measurement and (b) TEM image of the PVOH-*b*-PAN micelles.

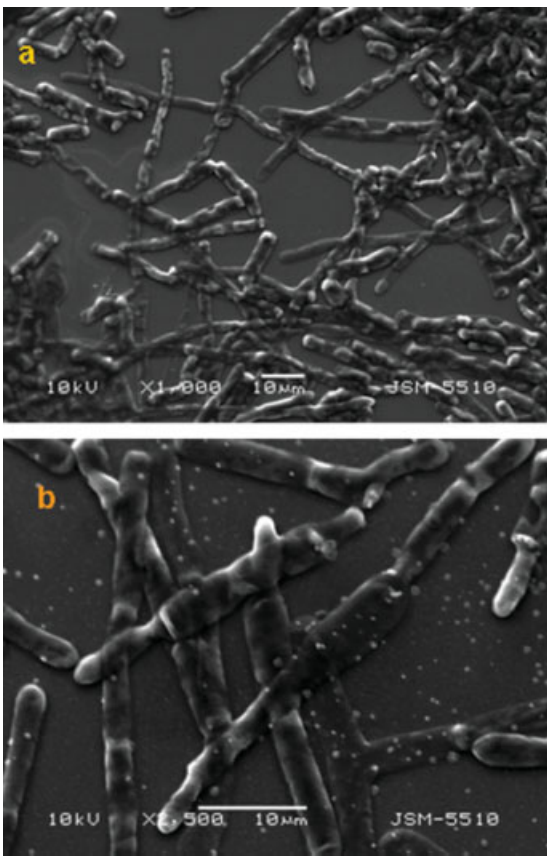

Figure 2 SEM images of the (a) free *T. cutaneum* R57 cells and (b) micelle/cell system after 2 h of cultivation. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the application of different doses of biomass with free cells and micelle/cell systems ranging from 200 to 1000 mg/L after 24 h of cultivation of the strain [Fig. 3(a,b)]. We expected that the increased amount of biomass would result in an enhanced number of adsorption sites and thus lead to better Cu^{2+}- and Cd^{2+}-ion removal efficiencies. For the yeast biomass consisting of free cells, the maximum biosorptions for Cu^{2+} and Cd^{2+} ions were reached at 800 mg/L [Fig. 3(a)]. However, when the biomass concentration was increased above this value, the cation removal declined, and the desorption of both cations prevailed; this indicated that the binding sites were saturated. In comparison, the micelle/cell system influenced the equilibrium between the adsorption and desorption processes. The biosorption for Cu^{2+} and Cd^{2+} ions increased above the value of 800 mg/L [Fig. 3(a,b)]. The reason for the increased efficiency of adsorption was due to changed adsorption sites on the cell wall surfaces,[4,5] which was probably driven by certain deposition of the micelles on the cells as a result of additional hydrogen bonds appearing between the hydroxyl groups (—OH) of the PVOH shell of the micelles and the carboxylic groups (—COOH) or hydroxyl groups (—OH) arising from the yeast cell wall. Thus, the introduction of micelles to the yeast cells may have led to an increase in the surface-specific area of the cells that allowed an increase in the amount of adsorbed metal. It has also been shown that the physical treatment can affect the binding capacity of cells.[4,14] Because the deposition of the micelles onto the yeast cells could account for physical interactions, effects on the ion-exchange properties of the cell wall occurred.

These results clearly indicate that the introduction of PVOH-*b*-PAN micelles into yeast cells significantly improved the Cu^{2+}- and Cd^{2+}-removal efficiencies of the strain because of an increasing number of adsorption sites instead of an increased concentration of biomass.

The absorption capacity of the cells during the growth was better than that for the preparations with free cells or micelles alone incubated with the ions. This mechanism could be regarded as the most probable because the existing thick cell wall of the cells ensured enough free space for water and ion passive movement into this space. In addition, yeast cell walls have been shown to have properties of good ion exchangers. This is due to the availability of a significant amount of polar groups in the cell walls, which are able to coordinate with metal cations. These can be SH—, —OH, CN—, COO—, —OCH_3, other hydrophilic groups that are usually part of polysaccharide molecules such as glucans and manans, and some protein molecules

TABLE I
Removal Efficiency of the Strain R57 Grown in the Medium Containing Toxic Levels of Copper Ions

	Free cells		Micelles		Micelle/cell system	
Incubation time (h)	Residual amount of Cu in the medium (mg of Cu/L)	Cu removal efficiency from the cultural medium (%)	Residual amount of Cu in the medium (mg of Cu/L)	Cu removal efficiency from the cultural medium (%)	Residual amount of Cu in the medium (mg of Cu/L)	Cu removal efficiency from the cultural medium (%)
2	1.90 ± 0.34	40	2.00 ± 0.35	37	1.38 ± 0.15	56
6	1.90 ± 0.34	40	1.93 ± 0.31	39	1.29 ± 0.12	59
24	1.85 ± 0.21	42	2.00 ± 0.35	37	1.08 ± 0.10	65

The data are presented as the means of three replicates plus or minus the standard deviations.

TABLE II
Removal Efficiency of the R57 Strain Grown in the Medium Containing Toxic Levels of Cadmium Ions

	Free cells		Micelles		Micelle/cell system	
Incubation time (h)	Residual amount of Cd in the medium (mg of Cd/L)	Cd removal efficiency from the cultural medium (%)	Residual amount of Cd in the medium (mg of Cd/L)	Cd removal efficiency from the cultural medium (%)	Residual amount of Cd in the medium (mg of Cd/L)	Cd removal efficiency from the cultural medium (%)
2	57 ± 1.37	36	76 ± 1.45	15	44 ± 1.15	50
6	55 ± 1.34	38	76 ± 1.45	15	34 ± 1.10	62
24	60 ± 1.40	33	80 ± 2.42	10	52 ± 1.32	42

The data are presented as the means of three replicates plus or minus the standard deviations.

incorporated into the cell walls of yeasts.[4] On the other hand, the presence of —OH— and —CN groups arising from the PVOH shell and PAN core of the micelles, which were also capable of complexing with the Cu and Cd ions in the medium, led to improved ion removal. Uptake and accumulation of some quantity of harmful ions into the cell vacuoles could not be excluded either. This ability of cells to transfer heavy-metal ions into vacuoles was supported by mechanisms involving the participation of some P-type proton pumps incorporated in the plasma membrane of cells.[15] They act as transport mechanisms, which include the uptake and excretion of ions. Thus, the cells can tolerate higher concentrations of heavy-metal ions floating around the cells.[7] All of these mechanisms are found in the group of yeast and can refer to the phenomena described previously.[8] Obviously, the introduction of micelles into the cells of strain R57 in aqueous solution can be used successfully for the improvement of the bioremediation capacity of the studied strain.

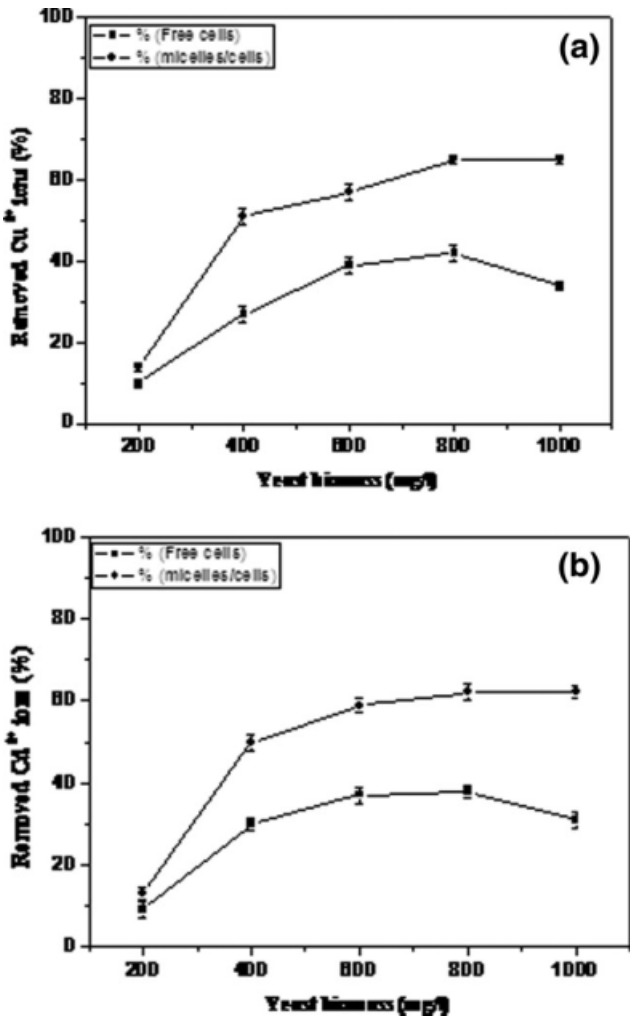

Figure 3 (a) Copper and (b) cadmium biosorption kinetics on the free cells and micelle/cell system.

CONCLUSIONS

The positive effect of the introduction of PVOH-*b*-PAN nanosized micelles into a medium containing growing cells of strain R57 of *T. cutaneum* in aqueous solution on the removal efficiency of Cu and Cd was successful demonstrated. The micelle/yeast system possessed better efficiency for copper and cadmium removal compared to the free cells and micelles. Maximum efficiencies for copper and cadmium removal at 24 and 6 h, respectively, were established. The removal of such harmful substances from effluents and wastewaters by microbe-based technologies may provide an alternative or additional means of metal recovery for economic reasons and environmental protection.

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