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Production and Isolation of Chitosan from *Aspergillus terreus* and Application in Tin(II) Adsorption

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ABSTRACT: Fed-batch fermentation was used for biomass and fungal chitosan production by *Aspergillus terreus* (BCRC 32068) grown in a potato dextrose agar medium. The polysaccharides were extracted by an alkali–acid treatment, and structural investigations by X-ray diffraction, Fourier transform infrared analysis, and viscosity and thermal analysis were done. A high level of chitosan was extracted from *A. terreus;* this implied that it was feasible to produce chitosan from industrial waste mycelia. Fungal chitosan derived from *A. terreus* showed the highest adsorption capacity for Sn(II). The order of Sn(II) adsorption capacity for these chitosanaceous materials was Fungal chitosan > Chitin > Biomass. Fungal chitosan derived from *A. terreus* was well correlated with Langmuir's isotherm model. The maximum capacity for Sn(II) sorption deduced from the use of the Langmuir isotherm equation was 303 mg/g; this was significantly higher than that of *A. terreus*. Fungal chitosan is an easy and cost-effective material for the abatement of pollution. © 2014 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 40436.

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INTRODUCTION

Heavy-metal pollution inside the water process has changed into a serious terror right now and in many ecological problems, as they are non-eco-friendly and thereby consistent.[1] Metals can certainly consistently continue to display characteristics in addition to accruing through foodstuffs. This poses a serious danger to the surroundings and to general public health.[2]

Traditional engineering pertaining to the treatment of most of these contaminants usually include reverse osmosis, electrodialysis, ultrafiltration, ion exchange, chemical precipitation, adsorption, and phytoremediation.[3] Nevertheless, the main disadvantages of those techniques consist of a high price, incomplete metal removal, high reagent concentration, and even high energy needs and the generation of sludge generation that needs to be disposed.[2]

Chitosan is known as the best heavy-metal adsorptive for the everyday polymers known as chelating polymers. It is produced commercially by the deacetylation of chitin, which is the structural element in the exoskeleton of crustaceans and cell walls of fungi. The traditional industrial source of chitin is shellfish waste from shrimp, crab, and lobster processing. Nevertheless, problems including seasonality, confined manufacturing locations, item variability, and higher processing costs linked to the chemical transformation of chitin to chitosan may actually restrict the possible industrial acceptance of the polymer. Moreover, the removal process and the variability of the source materials bring about inconsistent physicochemical characteristics in developed chitosan.[4] As a result of these disparities, fungi has long been thought of as a replacement source of chitosan simply because it is even more associated with persistent physicochemical properties. Filamentous fungus tend to be used in the fermentation processes for the manufacturing of a lot of items, such as primary metabolites, antibiotics, industrials enzymes, and proteins.[5] During these processes, many tons of fungi biomass are produced each and every year and burned off or disposed of improperly. The use of waste mycelia to produce valuable products, which include chitosan/chitin and glucan, has been considered a resolution to the solid waste disposal problem.[6] The extraction of chitosan from fungal sources is considered to have a number of advantages over crustacean sources:

1. The raw material is consistent in composition and available all year round.
2. The raw material can be totally free of heavy-metal toxins.
3. The demineralization stage is not necessary in the production of chitosan from fungal mycelia.

Furthermore, chitosan can be produced through fungal mycelia developed in submerged fermentation with industrial byproducts and wastes such as soybean deposits, sweet potato–shochu wastewater, and corn deposits.[7] Because of the ability of fungi to grow on industrial byproducts and waste, this will also eliminate environmental pollution concerns.

The inward development of fermentation technologies have actually shown that the large-scale culturing of an organism that has chitosan is usually a desirable approach to the production of this polymer. Many investigations have been performed on the production of chitosan through simple fermentation with different strains, including *Absidia coerulea, Absidia fusca, Absidia glauca, Absidia repens, Choanephora cucurbitarum, Mucor rouxii, Gongronella butleri, Phycomyces blakesleeanus* and *Absidia blakesleeanus*.[8] Fungi similar to *Gongronella* spp., *Absidia* spp., *Aspergillus* spp., and *Rhizopus* spp. belong to the class Zygomycetes and can be used as set sources in the production of chitosan with a more consistent level of quality.[9] Furthermore, fungal chitosan also exhibits a better collection capacity than crustacean chitosan.[8] This kind of chelating ability is mainly due to its high content of associated amino groups and also abundant hydroxyl groups. Therefore, chitosan is a nice substitute for the removal of hazardous metals and radioactive isotopes through contaminated waters and for the recovery of noble metals.

Generally, fungi from Zygomycetes class has higher levels of chitin and chitosan within their cell walls in comparison with other classes of fungi.[10] *Aspergillus terreus* biomass, produced with fermentation procedures to acquire nutrients and supplementary metabolites, has actually been acknowledged as an aggressive copper biosorbent.2 Dias et al.[11] also reported that heavy metals can be removed by an *A. terreus* strain immobilized in a polyurethane matrix. Sun et al.[12] demonstrated that the adsorption capacities of *A. terreus* biomass on a loofa sponge were 247.2, 37.7, and 23.8 mg/g for Pb(II), Hg(II), and Cd(II), respectively. Cerino-Córdova et al.[13] indicated that lead removal was highly affected by the *A. terreus* dose, pH, and interactions between the pH with the other factors. The use of *A. terreus* biomass as an adsorbent for metal ions has been investigated. However, there are no data on *A. terreus* fungal chitosan as an adsorbent or a chelator, and this material has not been investigated comparatively.

In this study, we report on *A. terreus* as a possible producer of fungal chitosan for use as an adsorbent. Large amounts of *A. terreus* BCRC 32068 were cultivated by fed-batch fermentation, and high levels of chitosan were extracted from its mycelia. With Sn(II) used as a model, the adsorption kinetics and adsorption isotherms of the fungal materials for Sn(II) were evaluated.

EXPERIMENTAL

Strain and Materials

The strain used in this study was *A. terreus* BCRC 32068, which was obtained from the Food Industry Research and Development Institute, HsinChu, Taiwan. Commercial chitosan [weight-average molecular weight = 940 KDa, degree of deacetylation (DD) = 98%] was obtained from Sigma. The potato dextrose agar was scratched with several lines and with a solid, inclined surface as the pure culture.

Cultivation of the Strains

Precultivation was performed with potato dextrose broth at a temperature of 28°C for 7 days, where a concentration of 2140 spores/mL was inoculated into the main culture. The composition of the main culture for the batch fermentation included 13.3 g/L KH_2PO_4, 4.0 g/L $(NH_4)_2HPO_4$, 1.2 g/L $MgSO_4{\cdot}7H_2O$, and 27.5 g/L glucose, of which $MgSO_4{\cdot}7H_2O$ and glucose needed to be separately sterilized. After inoculation, the flasks were shaken in an incubator shaker (120 rpm) at 28°C for 24 h.

Fed-Fermentation Conditions

Fermentors with a nominal volume of 3 L and a working volume of 1 L were used. The growth medium contained the following components per liter: 275 g of glucose, 4 g $(NH4)_2HPO_4$, 133 g of KH_2PO_4, and 12 g $MgSO_4{\cdot}7H_2O$. After sterilization, filtered sterile trace mineral solution was added. In all of the runs, the pH was maintained at 5.0 with NH_3. The temperature was maintained at 28°C, and the air flow rate was controlled at 1 L/min. The agitation rate was controlled at 400 rpm. During the fed-batch fermentation, when the glucose was completely consumed, the metabolites from the fungal cells increased, and thus, the fungal cells entered the death phase and could not continue to grow. This served as the basis for the start of the fed-batch fermentation. The fed-batch fermentation method allowed teh microorganisms grow through the addition of fresh medium intermittently or continuously until the fermentation process reached a maximum cell density or the work volume of the fermentation tank reached its limit. That is, when the fungal cells entered the stationary phase, additional glucose at 300 g/L was added. No fungal wall growth was evident in any of the fermentations. The biomass was expressed by dry fungus weight; that is, the fermentation liquid was filtered by a 0.45-μm filter membrane and then placed in an oven at 80°C until the weight became constant.

Chitosan Extraction

The method used for chitosan extraction in this study followed the procedures described by White et al.[14] This extraction protocol included an alkaline treatment, which was followed by an acid treatment. In the optimization of the extraction protocol, the dried fungal biomass was subjected to modified alkaline treatment followed by the acid treatment method of extraction. Meanwhile, in the acid treatment, the fungal biomass was subjected to the standard alkaline treatment before the modified acid treatment. Briefly, dried mats of *A. terreus* were homogenized and then autoclaved in 1.0*N* sodium hydroxide for 20 min at 121°C and 15 lb./in.^{2}. The ratio of the wet biomass to the sodium hydroxide solution was 1:40. The biomass suspension was collected and filtered through a Buchner funnel *in vacuo* with Whatman number 40 filter paper. The extracted biomass wall material was then washed and dried.

The fungal chitosan from 2 g of dried *A. terreus* was isolated according to the following process: deproteinization with 2*N* sodium hydroxide solution (20:1 v/w, 121°C, and 15 min), separation of the alkali-insoluble fraction by centrifugation (3400 rpm and 10 min), extraction of chitosan from the alkali-insoluble

fraction under reflux (2% acetic acid, 40:l v/w, and overnight), separation of crude chitin by centrifugation (10,000 rpm and 15 min), and precipitation of the chitosan from the extract at a pH of greater than 11, as adjusted with a 6*N* NaOH solution. Crude chitin and fungal chitosan were washed in a coarse sintered-glass funnel (G-4) with water, ethanol, and acetone and air-dried at 20°C.

Fungal Chitosan Characterization

Determination of DD. DD was determined by the first-derivative UV spectrophotometry method.[15] Briefly, 0.01 g of the fungal chitosan sample was dissolved in 10 mL of a 0.15*M* acetic acid solution, and then, deionized water was added to a volume of 1000 mL. This sample was scanned at UV wavelengths of 190–250 nm to measure its first-derivative adsorption data. The data were then compared with the calibration curve to obtain the *N*-acetyl glucosamine concentration, and the chitosan DD (%) was calculated by the following equation:[16]

$$\mathrm{DD}\ (\%) = 100 - \left[\frac{\frac{A}{204}}{\frac{(W-A)}{161} + \frac{A}{204}}\right] \times 100\% \qquad (1)$$

where A is the N-Acetyl glucosamine concentration, which is obtained by the fungal chitosan deacetylation calibration curve, and W is the concentration of the fungal chitosan solution.

Determination of the Molecular Weight of the Fungal Chitosan. The intrinsic viscosity of the fungal chitosan was determined with an Ubbelohde capillary viscometer in a 0.2*M* acetic acid/0.1*M* sodium acetate solution, and the viscosity-average molecular weight was calculated by the Mark–Houwink equation[17] with k and α values of 1.424×10^{-3} and 0.96, respectively. k and α are Mark-Houwink constants that depend upon the type of polymer, solvent, and the temperature of the viscosity determinations.

Thermal Analysis. Thermogravimetric analysis (TGA) was carried out with a Shimadzu model 50WS thermal analysis instrument. An accurately weighed (10 mg) fungal chitosan sample and a commercial chitosan sample were placed in an aluminum cup and sealed. The experiment consisted of heating the samples from 25 to 400°C under the continuous flow of dry nitrogen gas at a heating rate of 10°C/min.

X-Ray Diffraction (XRD) Analysis. The crystal structure of the fungal and commercial chitosan were determined with a Rigaku D/max3 Vx X-ray diffractometer (Tokyo, Japan) at a scanning rate of 4°/min at 2θ values from 5 to 100°.

IR Spectrum. The IR spectrum of fungal chitosan was used to monitor chitosan extraction by its comparison with the standard spectrum of commercial chitosan. Finally, the uptake of Sn(II) by amine groups (—NH_2) on chitosan was also investigated in the range 400–4000 cm^{-1}. IR spectra were recorded with KBr discs on a Nicolet Fourier transform infrared (FTIR) spectrophotometer 750 equipped with OMNIC FTIR software.

Adsorption Experiments. A stock solution (1000 ppm) of Sn(II) was prepared with a tin standard solution (Panreac, European Union). The stock solution was then diluted to give a standard solution of appropriate concentration. Batch experiments were conducted in 250-mL beakers and equilibrated with a magnetic stirrer. Then, 100-mL aliquots of these standard solutions were placed in 250-mL beakers, and the biomass, fungal chitosan. and commercial chitosan powder were added. In the adsorption equilibrium experiments, 0.025 g of biosorbent and 100 mL of tin solution buffered at pH 5 containing 1000 ppm of Sn(II) ions were used. The tin-ion-uptake experiments were conducted at pH 5 with the addition of sodium hydroxide as required.

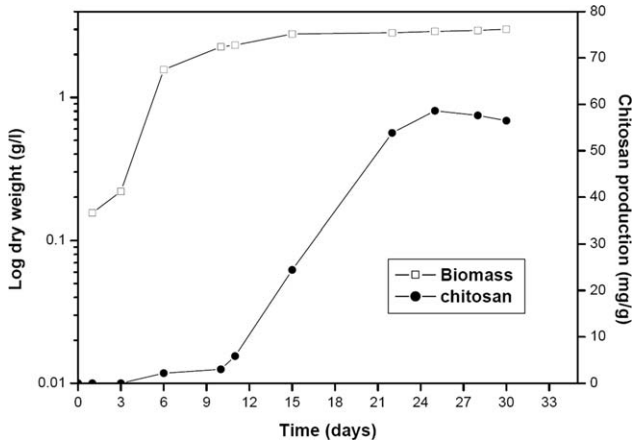

Figure 1. (□) *A. terreus* growth and (○) chitosan production.

The system was maintained under shaking at 28°C until the adsorption equilibrium was reached. After equilibration, the chromium standard solutions were filtered through papers and quantified by a GBC Scientific Equipment SensAA flame atomic absorption spectrophotometer (Melbourne, Australia). The adsorption capacities of the Sn(II) taken up by the biosorbent in each flask were determined with the following mass balance equation:

$$q = \frac{C_0 - C}{X} \qquad (2)$$

where q is the amount in milligrams of metal ions per gram of biosorbent, C_0 is the initial metal ionic concentration (mg/L), C is the residual metal ion concentration (mg/L), and X is the concentration of biosorbent (g/L).

For batch kinetic studies, the biosorbent and 100 mL of Sn(II) solutions with initial concentrations of 1000 ppm were placed in 250-mL beakers. The biosorbent (0.025 g) was placed in 250 mL of the Sn(II) solution with shaking for 24 h at 260 rpm. Isotherm studies were conducted with a constant weight and various initial concentrations of tin ions in the range 5–100 ppm.

RESULTS AND DISCUSSION

Growth Profile of *A. terreus*

The growth rate of *A. terreus* is shown in Figure 1. *A. terreus* grew very slowly with a maximal biomass of 2.8 mg/L after 15 days of cultivation. Thus, the extracted amount of chitosan was very low (0.4 mg). Therefore, fed-batch fermentation was required to enhance the volume production rate of the fungal cells to obtain more chitosan. Figure 1 shows that the biomass production increased within 15 days of growth and reached a statistically significant higher production of biomass in 25 days of cultivation with an average dry weight corresponding to

Table I. Amount of Chitosan Produced by the Different Fungi

Microorganism	Biomass (g/L)	Chitosan produced (mg/g of biomass)	Chitosan content (%)	Reference
Aspergillus niger TISTR3245	9	107	11	18
Mucor circinelloides	20.7	64	6.4	18
Lentinus edodes number 1	1.4	33	3.3	18
Cunninghamella elegans	24.3	66	6.6	20
Zygosaccharomyces rouxii TISTR5058	4.4	36	3.6	18
Canduda albicans TISTR5239	1.8	44	4.4	18
Cunninghamella bertholletiae	7.7	128	12.8	20
A. terreus BCRC 32068	2.9	58	5.8	This study

2.9 g/L. These results were compared with those in the literature, as verified in Table I. Figure 1 also shows the extracted chitosan yield after each 3-day period from *A. terreus,* which was grown in potato dextrose broth for 30 days of cultivation. The best yields of the polysaccharides were obtained after 25 days of culturing for chitosan (59 mg/g or 5.9%). Similar results were reported by Pochanavanich and Suntornsuk,[18] who studied four species of filamentous fungi and observed that *A. niger* TISTR3245 was the best chitosan-producing strain, with a yield of approximately 11.0% of chitosan per dry weight of mycelia.

Chitosan production stabilized after 25 days of culturing. The higher chitosan yields at 25 days of growth suggested that during the initial growth, chitin was less crystalline and was thus more susceptible to chitin deacetylase. The chitosan formed by this enzyme prevailed under acid pH. According to Amorim et al.,[19] the optimum pH for chitin deacetylase activity from Zygomycetes is 4.5. During *A. terreus* growth, the pH of the potato dextrose broth medium stabilized at around 5 after 30 days if cultivation (5 ± 0.5) and showed a low metabolic interchange between the medium substrate uptake and the release of ions from the cells. The data in this study were in agreement with Pochanavanich and Suntornsuk,[18] Amorim et al.,[19] Stamford et al.,[20] and Nadarajah et al.,[21] who stated that chitosan production by microorganisms was strongly dependent on the culturing conditions, including the cultivation time. The decline in the quantity of extractable chitosan observed in the time–culture curve might have been due to physiological changes within the cell wall from the fungi. Chitosan is produced in the fungal cell wall by the deacetylation of its precursor, nascent chitin. The ratio associated with free chitosan substances is fairly high throughout the exponential stage because of active growth. When the culturing comes into the stationary growth stage, more chitosan can be anchored to the cell wall within the zygomycetes and binds to chitin along with polysaccharides, so extraction is much harder.[21]

Characterization of the Fungal Chitosan

The characterization of fungal chitosan obtained from *A. terreus*, fungal chitosan-Sn(II) complex and commercial chitosan by FTIR spectroscopy (Figure 2) was similar to that reported in the literature.[22] From Figure 2, we observed that the FTIR spectrum of fungal chitosan was similar to that of commercial chitosan. The spectrum of the fungal chitosan exhibited absorption bands around 1655 and 1590 cm^{-1}, which represented the amide I and amide II bands, respectively. There were bands at 1420, 1380, and 1320 cm^{-1}, in addition to the usual C—H aliphatic band at 2880 cm^{-1}. The OH and NH_2 overlapping bands were found around 3310–3450 cm^{-1}. A reordering of the hydrogen linking of fungal chitosan was observed in the central band at approximately 3450 and 3305 cm^{-1}. This may have been due to the axial deformation of OH, which appeared to

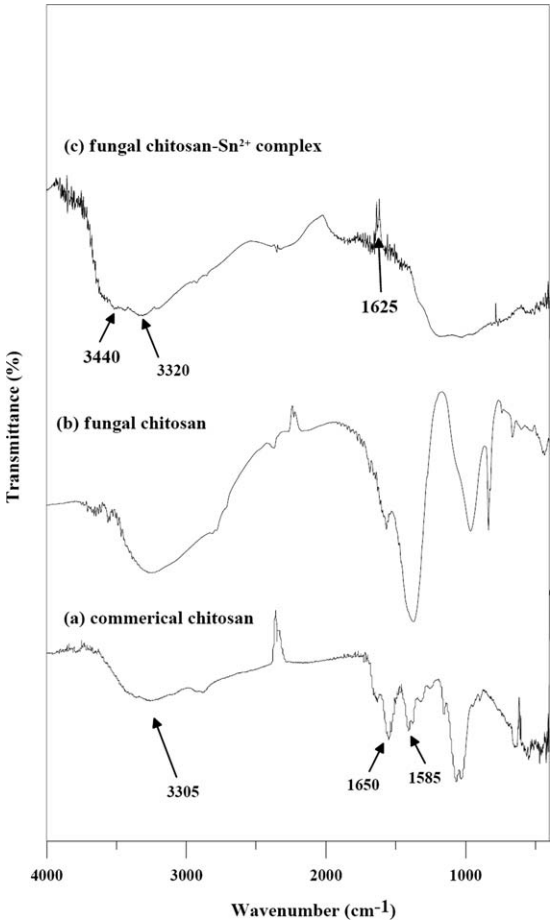

Figure 2. FTIR spectra of the (a) commercial chitosan, (b) fungal chitosan, and (c) fungal chitosan–Sn(II) complex.

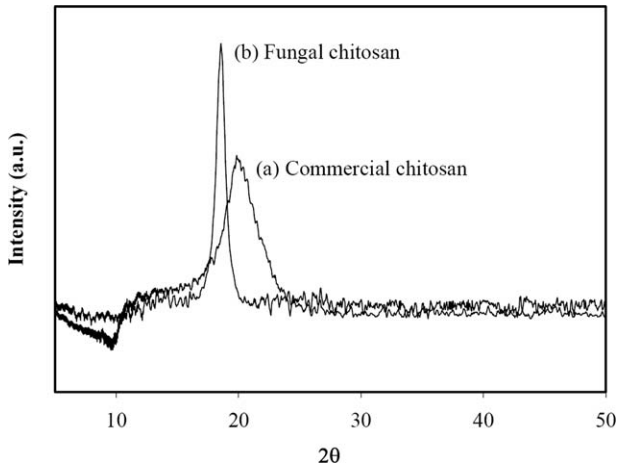

Figure 3. XRD patterns of the (a) commercial chitosan and (b) fungal chitosan.

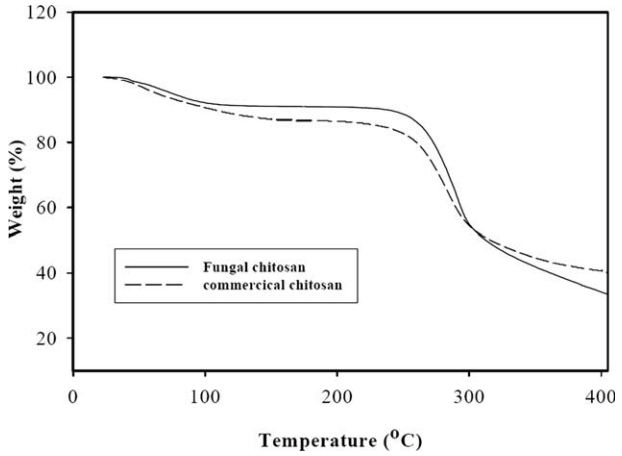

Figure 4. TGA curve of the commercial chitosan and fungal chitosan under an N_2 atmosphere.

overlap the band of the axial deformation of NH and indicated an intermolecular hydrogen linking formation.[23] However, in the chitosan–metal complexes, a brand-new band around 1625–1635 cm^{-1} showed up. This band corresponded to the in-plane bending of N—H, which also appeared as a shoulder around 1605 cm^{-1}. This detected redshift occurred because of the interaction of chitosan with the metal ions.[24] The wide peak at 3310–3450 cm^{-1}, corresponding to the stretching vibration of the —NH_2 group and —OH group, was split into two bands at 3320 and 3440 cm^{-1}. This indicated that —NH_2, chemical groups or —OH groups took part in the complexation. Most of these changes within the FTIR spectra may have been related to the interaction between the functional groups of the fungal chitosan and tin ions throughout the adsorption process.[25]

The XRD patterns (Figure 3) of the commercial chitosan and fungal chitosan showed diffraction angles ranging from 5 to 50°. They had similar characteristic peaks at 10 and 20°; this indicated that the extracted product from *A. terreus* was chitosan. However, the intensity of this peak was different and was less than the peak of fungal chitosan around 20°. This showed that the commercial chitosan had a lower crystallinity.

Figure 4 shows the TGA curves of the commercial chitosan and fungal chitosan under an N_2 atmosphere between 25 and 400°C. From the TGA curve, we observed that both of them degraded in three stages. The first degradation stage could be explained as the loss of water. The second stage, which began at 170°C, was due to the decomposition temperature of this polymer with a carbonized residue formation, and the third weight loss point was at 335°C, which corresponded to the start of the consumption of the carbonized material. These results were in agreement with those of Liu et al.[26]

DD is an important parameter associated with the physicochemical properties of chitosan because it is linked directly to the chitosan cationic properties.[17] In this study, fungal chitosan obtained from *A. terreus* grown on potato dextrose agar medium presented an 83% DD. That result was in accordance with the results of other research,[20] in which the DD of chitosan from fungi was between 80 and 90%. The average viscometric molecular weight of the fungal chitosan from *A. terreus* obtained in this study was 1.5×10^4 g/mol, which was considered relatively low. However, the viscosity of the fungal chitosan was 15 CP, which was considered relatively high. Given a low molecular weight, the polycationic characteristic of fungal chitosan can be used together with its solubility in physiological pH ranges and with a lower antigene effect. On this basis, low-molecular-weight fungal chitosan could be used as a potential drug carrier. Gene therapy research is aiming for nonviral gene-delivery systems, for which this chitosan showed promising results in former studies.[27] These results were compared with those in the literature, as verified in Table II.

Sorption Kinetics for the Tin Ions

In our preliminary study, the effect of the pH on the adsorption of tin ions by chitosan was studied (as shown in Figure 5). The adsorption capacity (Q) of tin reached a maximum at pH 5

Table II. Properties of the Chitosan Produced by Different Fungi

Source of chitosan	DD (%)	Molecular weight (Da)	Viscosity (cP)	Reference
Crab shell (Sigma)	97.9 ± 0.9	9.4×10^5	372.7	18
Aspergillus niger TISTR3245	90.0 ± 2.1	1.4×10^5	6.2	18
Lentinus edodes no. 1	86.5 ± 2.2	1.9×10^5	5.8	18
Zygosaccharomyces rouxii TISTR5058	85.1 ± 1.1	2.7×10^4	3.3	18
Candida albicans TISTR5239	83.8 ± 0.8	1.1×10^5	3.1	18
A. terreus BCRC32068	83.0 ± 0.1	1.55×10^4	15	This study

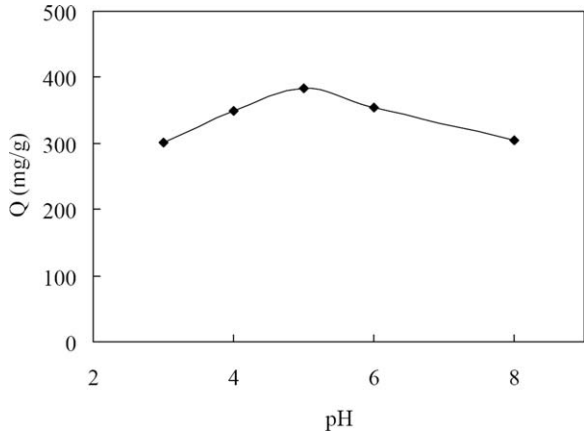

Figure 5. Effect of the pH on the adsorption of tin ions by the chitosan. Q is defined as adsorption capacity.

and decreased at lower and higher pH values. According to Rangel-Mendez et al.'s study,[28] a low pH favors the protonation of amino sites, which results in a reversal of charge and greatly diminishes the metal chelating ability of chitosan. Heavy-metal cations are completely released under extremely acidic conditions, although at higher pH values beyond 6.5, the precipitation of metal hydroxide occurs simultaneously with the sorption of metal ions, which affects the sorption by the chitosan. The result of our preliminary study was similar to that of Rangel-Mendez et al.'s study. Therefore, the tin-ion-uptake experiments were conducted directly at pH 5.

The results of the adsorption kinetics for biomass, chitin, fungal chitosan, and commercial chitosan are shown in Figure 6. Tin uptake on chitin and biomass had a low sorption capacity to 127 and 58.5 mg/g, respectively. The equilibrium concentration of biomass and chitin were easily achieved after 45 min. The fungal chitosan and commercial chitosan with 15–840 min of contact were quite close, and 120 min was sufficient for achieving equilibrium in these materials. For fungal chitosan, the adsorption process occurred rapidly, as a high sorption capacity for tin of about

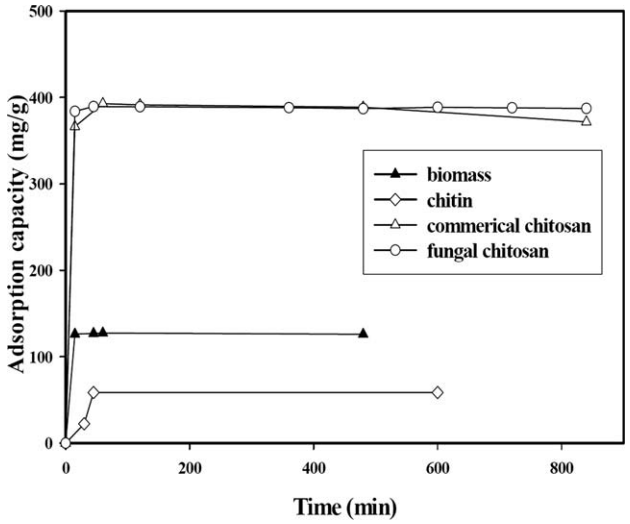

Figure 6. Adsorption kinetics for the (▲) biomass, (◇) chitin, (○) fungal chitosan, and (△) commercial chitosan.

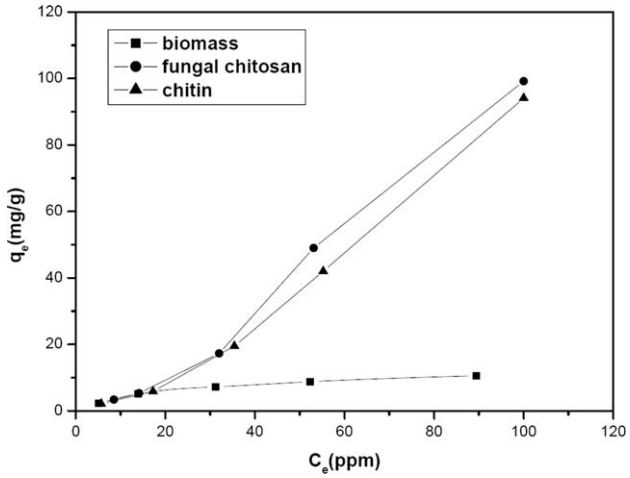

Figure 7. Langmuir isotherm for tin adsorption on the different chitosanaceous materials.

383 mg/g was achieved after 15 min of contact, and then the equilibrium between the solid and liquid phase was achieved after 2 h. In all cases, the tin adsorption was more sensitive and intensive for fungal chitosan, even in a solution of low tin concentration. On the basis of the kinetic results, a 2-h contact time was used in the examination of the tin adsorption isotherm.

Equilibrium Isotherm and Tin Collection Capacity

The tin uptake under equilibrium concentrations for the biomass, chitin, and fungal chitosan are shown in Figure 7 with their Langmuir isotherms. There was a rise in the tin uptake with increasing equilibrium concentration until the maximum tin uptake was reached. Fungal chitosan and chitin had higher adsorption capacities under all equilibrium concentrations. However, the biomass had a much lower collection capacity. The maximum tin uptake calculated by the Langmuir method were as follows: 302 mg/g for fungal chitosan > 83 mg/g for chitin > 13.3 mg/g for biomass.

The Langmuir model [eq. (3)] was used to describe the data obtained for the equilibrium of Sn adsorbed onto the biomass, chitin, and fungal chitosan:

$$q_e = q_{\max} \frac{K_L C_e}{1 + K_L C_e} \tag{3}$$

where C_e is the equilibrium concentration of tin ions (ppm), q_e is the amount of tin ions sorbed per unit weight of absorbable material at equilibrium or the equilibrium capacity obtained from the experiment (mg/g), $q_{\max}$ is the maximum amount adsorbed on a monolayer (mg/g), and K_L is the Langmuir sorption equilibrium constant (mL/mg) and is a measure of the energy of sorption. The Freundlich isotherm model is based on the assumption of an exponentially decaying adsorption site energy distribution. It is applied to describe a heterogeneous system characterized by a dimensionless heterogeneity factor (n). The Freundlich model is expressed as follows:

$$q_e = K_F C_e^{1/n} \tag{4}$$

where K_F is the Freundlich isotherm constant.

Many experts have exerted that the nonlinear method is known as a better way to determine the isotherm parameters.

Table III. Isotherm Parameters Obtained with the Nonlinear Method for the Adsorption of Sn(II) on Different Materials

	Langmuir			Freundlich		
Material	K_L	q_m (mg/g)	Correlation coefficient	K_F	n	Correlation coefficient
Biomass	0.04404	13.27328	0.999499	1.373982	2.19357	0.988765
Chitin	0.005321	82.99972	0.99943	0.378916	1.063924	0.998168
Fungal chitosan	0.001545	301.7669	0.996068	0.076216	0.732034	0.994902

The trial-and-error process, which does apply to computer operations, was accustomed to compare the very best fit from the two isotherms with an optimization routine to maximize the coefficient associated with determination (r^2) between the experimental data and isotherms in the solver add-in with Microsoft Excel.[29,30] r^2 was calculated as follows:

$$r^2 = \frac{\sum (q_m - \overline{q_e})^2}{\sum (q_m - \overline{q_e})^2 + \sum (q_m - \overline{q_e})} \quad (5)$$

where q_m is the equilibrium capacity obtained from the isotherm model and $\overline{q}_e$ is the average q_e.

Listed in Table III are the K_L and K_F values for the adsorption of Sn(II) ions on different chitosanaceous materials with a working pH of 5.0 and a working temperature of 28°C. Error estimation of the Langmuir and Freundlich equations showed that the Langmuir model was more accurate in describing the tin adsorption isotherm than Freundlich model, except for the biomass, for which the Langmuir equation was slightly better. However, the equation for biomass had the lowest accuracy. The Langmuir equation was developed on the basis that the adsorbent is assumed to have a homogeneous surface and that the interaction between adsorbed molecules is negligible. This did not describe the adsorption data as accurately as the Freundlich equation, which is an empirical model for an adsorption isotherm. This was supported by these observations.

The essential features of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter (R_L), which is used to predict whether an adsorption system is favorable or unfavorable. R_L is defined by eq. (6):[31]

$$R_L = \frac{1}{1 + K_L C_0} \quad (6)$$

where C_0 is the initial tin concentration (ppm). Table IV lists the calculated results. On the basis of the effect of R_L on the isotherm shape, the R_L values were in the range $0 < R_L < 1$; this indicated that the adsorption of Sn(II) on the fungal chitosan was favorable. Thus, the fungal chitosan was a favorable adsorbent.

Table IV. R_L Values Based on the Langmuir Equation

C_0 (mg/L)[a]	R_L values
5	0.992335
15	0.977353
35	0.948706
55	0.92169
100	0.866192

[a] For tin ions.

CONCLUSIONS

The use of fed-batch fermentation gave high-quality chitosan by *A. terreus*. This technique provides an economical and efficient route, suitable for direct scaling up to large-scale industrial production for high-quality chitosans. The fungal chitosan derived from *A. terreus* had a much higher collection ability for tin ions under all equilibrium concentrations. The maximum tin uptake of the fungal chitosan in a tin solution at pH 5 and 28°C was 319 mg/g. The maximum tin uptakes calculated by the Langmuir method were 302 mg/g for fungal chitosan > 83 mg/g for chitin > 13.3 mg/g for biomass. The equilibrium sorption data were satisfactorily fitted with the Freundlich and Langmuir equations. The calculated values of R_L from K_L confirmed the favorable sorption of Sn(II) onto the fungal chitosan. These biosorbed ions could be eluted, and the fungal chitosan could be regenerated and used again. Thus, this represents an easy and cost-effective technology for the abatement of pollution.

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