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Screening of fungi for chitosan producers, and copper adsorption capacity of fungal chitosan and chitosanaceous materials

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Abstract

Thirty-three fungal strains were screened for chitosan producers. Although chitosan is believed to occur only in *Mucorales* strains in the class of Zygomycetes, we found that chitosan was extractable from all the 33 strains from the four classes. High level of chitosan was extractable from some non-zygomycetes strains. *Absidia glauca*(+) was found to be a promising chitosan producer. High level of chitosan can be extracted from some common industrial fungi, implying that it is feasible to produce chitosan from industrial waste mycelia. Fungal chitosan derived from *A. glauca*(+) showed the highest adsorption capacity for Cu(II). The order of copper adsorption capacity for these chitinous/chitosanaceous materials is: fungal chitosan > alkali-insoluble materials (AIM) > crustacean chitosan > fungal biomass. The Langmuir and Freundlich constants for the Cu(II) adsorption isotherms were determined with Freundlich model providing a better description of the copper adsorption isotherms.
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Keywords: Fungal chitosan; Fungal alkali-insoluble materials; *Absidia glauca*; Copper adsorption; Isotherm

1. Introduction

In 1978, Allan et al. (Allan, Fox, & Kong, 1978) made a critical evaluation of the potential sources of chitin and chitosan, and pointed out that cultured fungi capable of synthesizing chitin alone or in association with chitosan will assume the major role in chitin and chitosan supply. White, Farina, and Fulton (1979) later developed a method for the lab-scale production and isolation of chitosan from hyphal wall of *Mucor rouxii*. Knorr and Klein (1986) used *M. rouxii* and *Phycomyces blakesleeanus* to produce chitosan, and reported that these two strains can convert commercial chitin and chitin from waste mycelia of *Aspergillus niger* into chitosan. Much research have been conducted for the production of chitosan by fermentation using different strains such as *Absidia coerulea* (Davoust & Hansson, 1992; McGahren, Perkinson, Growich, Leese, & Ellestad, 1984; Muzzarelli, Ilari, Tarsi, Dubini, & Xia, 1994; Rane & Hoover, 1993a,b), *Absidia fusca*, *Absidia glauca*, *Absidia repens* (Davoust & Hansson), *Choanephora cucurbitarum* (Muzzarelli, Tanfani, & Emanuelli, 1981), *M. rouxii* (Knorr & Klein; Muzzarelli et al.; Rane & Hoover; White et al.), *Gongronella butleri* (Rane & Hoover), *P. blakesleeanus* (Knorr & Klein; Muzzarelli et al.; Rane & Hoover) and *Absidia blakesleeanus* (Rane & Hoover). Recently, we have developed a rapid and non-degradable method for the extraction of high-quality fungal chitosan (Hu, Yeung, Ho, & Hu, 1999).

Chitosan is known to be the best heavy metal adsorbent for all polymers so far characterized as chelating polymers (Muzzarelli, 1973). Fungal chitosan even exhibits greater collection ability than crustacean chitosan. Such chelating ability is mainly due to its high content of amino groups and the abundant hydroxyl groups. Therefore, chitosan is well suitable for the removal of hazardous metals and radioactive isotopes from contaminated waters, and for the recovery of noble metals. Due to this unique property, chitosan is also used

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as chromatographic supports to separate trace metals in analytic chemistry, and in the metallurgical industry to separate and to purify metals (Inoue, Baba, & Yoshizuka, 1993).

The complexation between crustacean chitosan (Crustacean-Cs) and metal ions is well documented (Hauer, 1978; Masri & Randall, 1978; Muzzarelli, 1973, 1977; Roberts, 1992). The use of fungal biomass as an adsorbent for metal ions has also been investigated. However, there is little data on fungal chitosan (Fungal-Cs) as an adsorbent or a chelator, and these materials have not been investigated comparatively. The chelating ability of 'chitosan-like materials'/'chitosan–glucan complex'/'chitinous materials' from some fungi was studied (Miyoshi, Shimura, Watanabe, & Onodera, 1992; Muzzarelli, Tanfani, & Scarpini, 1980; Muzzarelli, Tanfani, Scarpini, & Tucci, 1980; Muzzarelli et al., 1981). Furthermore, the chelating ability of chitosan was evaluated using only a single concentration and a fixed volume of metal solutions, i.e. under just one equilibrium concentration. However, the uptake of metals onto adsorbent is dependent on the equilibrium concentrations of metal ions. There is no attempt to obtain the adsorption isotherms and the maximum uptake (Q_{max}) of fungal chitosan and of the fungal alkali-insoluble materials (AIM) Only crustacean chitosan was examined for its copper adsorption isotherm (McKay, Blair, & Findon, 1985).

In this paper, we report the screening of 33 fungi for possible producers of fungal chitosan that could be used in chitosan industry. We showed that high levels of chitosan could be extracted from some fungal mycelia. Using Cu(II) as a model, we have systematically investigated the adsorption kinetics and adsorption isotherms of different fungal materials, and found that fungal chitosan displayed higher capacity of metal adsorption than traditional crustacean chitosan.

2. Materials and methods

2.1. Chemicals and materials

The standard 1000 ppm copper solution was purchased from Fisons Scientific Equipment, Incorporating Griffin and George. Copper(II) sulphate 5-hydrate (Merck) was extra pure. The practical Sigma crab shell chitosan was used as purchased, and its degree of de-acetylation examined by IR method was 73.2%.

2.2. Strain and its cultivation

2.2.1. Strains

Thirty-three strains of fungi were used. There was one *Basidiomycetes*; there were nine Zygomycetes, five *Ascomycetes* and 18 *Deuteromycetes*. It is generally accepted that chitin is the only structural polymer of *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* (Ruiz-Herrera, 1978). Strains from these classes represent the chitin-containing fungi. Strains from *Zygomycetes* represent the chitosan-containing fungi. *A. niger*, *Ashbya gossypii* and *Penicillin chrysogenum* are the representatives of typical industrial fungi.

Of the 33 strains, eight strains were obtained from Agricultural Research Service, National Center for Agricultural Utilization Research, United States Department of Agriculture: *A. coerulea* (NRRL 1310), *G. butleri* (NRRL 1307), *Agaricus bisporus* (NRRL 2335), *Aspergillus oryzae* (NRRL 447), *Aspergillus terreus* (NRRL 680), *Aspergillus terricola* (NRRL 425), *Aspergillus usamii* (NRRL 363), and *Blakeslea trispora* (NRRL 1348); another eight were obtained from the American Typical Culture Collection: *A. gossypii* (ATCC 10895), *Aspergillus flavus* (ATCC 32592), *Cladosporium cladosporioides* (ATCC 20251), *Gibberella fujikuroi var. intermedia* (ATCC 42052), *M. rouxii* (ATCC 24905), *Rhizopus oryzae* (ATCC 46242), *Trichoderma viride* (ATCC 32098) and *Trichothecium roseum* (ATCC 8685). The remaining strains were from our own culture collection.

For the investigation of the chelating capacity of fungal chitosan, *A. glauca*(+) was used as the chitosan producer.

2.2.2. Cultivation of fungal strains

For the screening, fungal strains were maintained at 4 °C on YM (DIFCO) slant and transferred on a monthly basis. Seed cultures were prepared by incubating the fungi in a 250-ml Erlenmeyer flask containing 60 ml of YM broth (DIFCO), and cultured at 26 °C, at 200 rpm for 24 h. The culture media (PGY salt broth) consisted of 20 g glucose, 10 g peptone, 1 g yeast extract, 5 g ammonium sulfate, 1 g di-potassium hydrogen orthophosphate, 1 g sodium chloride, 5 g magnesium sulphate-7-hydrate, 0.1 g calcium chlorid-2-hydrate and 1 l of distilled water. The pH of PGY salt broth was adjusted to 4.5 using hydrochloric acid, and autoclaved at 121 °C for 15 min. Fifteen milliliter of 24-h seed culture was added into a 1-l flask containing 400 ml of PGY salt broth. The cultures (three duplicates) were grown at 26 °C, at 200 rpm until growth ceased.

For analysis of chelating, *A. glauca*(+) was grown in 1-l Erlenmeyer flasks containing 400 ml of broth media consisting of glucose 30 g l^{-1}, yeast extract 3 g l^{-1} and peptone 10 g l^{-1}, at 26 °C in a rotary shaker-incubator with a set speed of 200 rpm, for 72 h. It is well known that living microorganisms (bacterium, fungus and algae) can bio-accumulate metal ions from ambient waters. In order to minimize the possibility of any metal bioadsorption by the living organism during the cultivation of fungi prior to analysis of copper adsorption, no supplementary metals were added into the growth media.

2.3. Preparation of the fungal cell or fungal mycelia or fungal biomass

For screening experiment, the mycelia were harvested by filtrating through a 4-layer gauze and washing with

approximately 1 l of distilled water. The mycelial mat was pre-frozen at −70 °C, then lyophilized with a Heto FD8 freeze dryer, and weighed.

For analysis of chelating, fungal biomass was collected by filtering the 72-h-old cultures through a (2–4)-layer gauze strainer, washed with distilled-and-deionized water to neutrality. The mat fungal mycelia was squeezed to remove water, and finally lyophilized. The weight of freeze-dried mycelia (FDM) was used as the dry cell weight (DCW).

2.4. Preparation of fungal cell wall or alkali-insoluble material

The lyophilized mycelia were grounded with a mortar immediately after freeze–drying, and stored in conical flasks at 4 °C for future use. Forty parts of 1 N NaOH solution were mixed with 1 part of mycelia (*V*/*W*). The mycelia in NaOH solution were then homogenized in a Waring blender at high speed for 1 min. The slurry was then autoclaved at 121 °C for 15 min. The alkali-treated mycelia were centrifuged at 12,785*g* for 20 min. The supernatant was decanted. Distilled water was added into the centrifuge tube, and homogenized with the precipitate. The mixture was centrifuged again to pellet the AIM. The materials were pre-frozen in a −70 °C freezer then freeze-dried. The freeze-dried materials are referred to as AIM or cell wall materials.

2.5. Fungal chitosan

Chitosan in the screening was extracted from AIM by 1 N hydrochloric acid at 95 °C for 3 h in a flask fitted with a condenser. The ratio of HCl solution to AIM is 40 (*V*/*W*). The slurry was centrifuged at 39,191*g* for 20 min. The supernatant was transferred to a new tube and the pH of the supernatant was adjusted to around 12. It was centrifuged again and the supernatant was decanted. The pellet was re-suspended with distilled water and mixed with a homogenizer. The suspension was centrifuged again to pellet the chitosan. Chitosan was then subject to lyophilization with a Heto FD8 programmable freeze dryer.

The purified fungal chitosan for chelating analysis was prepared by using one of the two-step methods reported earlier (Hu et al., 1999). The fungal chitosan was extracted from AIM by autoclaving the AIM at a ratio of 1:100 to 2% acetic acid solution (W/V) at 121 °C for 15 min. Before autoclaving, the AIM suspension in acetic acid solution was mixed for 2 min with a Waring blender set at high speed. The autoclaved suspension was centrifuged at 11,325*g* at 20 °C for 25 min. The precipitate was discarded and the supernatant was adjusted to pH 12 to precipitate fungal chitosan; this fungal chitosan suspension was then centrifuged at 14,333*g* to collect the fungal chitosan. The fungal chitosan was washed four times with distilled-and-deionized water, and was recovered by centrifugation, and finally was dried by lyophilization. The degree of deacetylation of fungal chitosan obtained was 76.6%, similar to that of the Sigma crab chitosan (73.2%).

2.6. Infrared spectrum

Infrared spectrum of fungal chitosan was used to monitor the chitosan extraction by comparing it with the standard spectrum of Sigma chitosan. IR spectra were recorded using KBr disc on Nicolet FT-IR spectrophotometer 750 equipped with OMNIC FT-IR software. KBr was pre-grounded and desiccated at 500 °C for 12 h in a muffle furnace, and then stored in a vacuum oven set at 50 °C for future use. Immediately after lyophilization, chitosan was stored in a vacuum oven before the IR spectrum was analyzed.

2.7. The determination of the degree of acetylation of chitosan

The degree of acetylation of chitosan (DAC) was evaluated from IR spectrum because this method is simple, rapid and requires only a trace amount of chitosan. The IR spectrum recording procedure is the same as described above. The amide I band at 1655 cm^{-1} was used, and the hydroxyl group absorption band at 3450 cm^{-1} was used as an internal reference. The DAC was calculated by the formula: $DAC = A_{1655}/A_{3450} \times 100/1.33$ (Domard & Rinaudo, 1983; Moore & Roberts, 1980).

2.8. Determination of the copper adsorption capacity

Two hundred milligram (100 mg for fungal chitosan) of freeze-dried fungal chitosanaceous materials (fungal chitosan, AIM and FDM) and crustacean chitosan were added, respectively, into plastic (glass can absorb metal ions) bottles containing copper ($CuSO_4$) solutions of known concentrations and known volumes. The mixture was immediately homogenized for 2 min using an ULTRA-TURRAZ T25 homogenizer set at 20,500 rpm. Subsequently, the bottles containing the suspension was mounted in a shaker-incubator set at 26 °C, and were shaken at a speed of 200 rpm for the desired period of time. After that, the fungal material or crustacean chitosan was filtered out, and the filtrate was subjected to atomic absorption (AA) spectrophotometric analysis. The sample solutions were diluted to less than 20 ppm before AA analysis. A series of standard copper solutions of 2, 5, 10 and 20 ppm were prepared, and stored in plastic bottles for future use. Distilled-and-deionized water was used throughout the whole experiment. The degree of sorption was determined by measuring the concentrations of the metal ion in the aqueous phase before and after contact with the fungal materials or crustacean chitosan, and expressed as: metal uptake $q_e = (C_0 - C) \times (V/W)$, where C_0 and C are the initial and equilibrium (final) concentration of metal ions (ppm or mg l^{-1}), respectively; V is the volume of the sample (l), and W the dry weight of the adsorbent added (g).

The unit for q_e is mg adsorbate (metal) per g adsorbent (mg g^{-1}). A computerized Unicam atomic absorption spectrophotometer equipped with an automatic liquid handler (including sampler and washer) was used for all experiments. A hollow cathode lamp was used as the light source, and an air–acetylene flame was used.

3. Results

3.1. Productivity of chitosan and alkaline-insoluble materials

The results of the screening of fungi for chitosan producers were shown in Table 1. The top five strains for each parameter are highlighted by italicised.

Under the cultivation conditions, DCW of the strains ranges from 1585.3 mg l^{-1} (*C. cladosporioides*) to 10,209.8 mg l^{-1} (*A. bisporus*). Most of the cultures were harvested in 2–3 days. Some cultures were incubated longer because of a slower growth in PGY salt broth. The hourly mycelial productivity was therefore used to measure the rate of growth, which ranges from 19.8 mg h^{-1} (*C. cladosporioides*) to 209.3 mg h^{-1} (*R. oryzae*).

The amount of chitosan produced by the fungi examined ranges from 8 mg l^{-1} (*A. gossypii*) to 646 mg l^{-1} (*A. glauca*(+)). During the course of screening, it is difficult to cultivate the large number of strains under their respective optimal conditions. It is more suitable to evaluate the chitosan productivity using percentage chitosan. In this research, two types of percentage chitosan are examined—DCW percentage chitosan and AIM percentage chitosan.

Table 1
Amount of DCW, AIM and chitosan, and DA of chitosan isolated

Fungal strains	DCW (*A*) (mg l^{-1})	Culture time (h)	Hourly mycelial productivity (mg h^{-1})	AIM (*B*) (mg l^{-1})	Percentage AIM (*B*/*A*) (%)	Chitosan (*C*) (mg l^{-1})	Percentage chitosan (%)		DAC
							C/*A*	*C*/*B*	
A. glauca (+)	*8786.3*	48	*183*	*3329.3*	*37.9*	*646*	*7.4*	*19.4*	30.4
A. coerulea	7135.8	48	*148.7*	1994.3	16.7	89.3	1.3	4.5	32.3
A. bisporus	*10,209.8*	76	134.3	*2394*	23.5	93.5	0.9	3.9	24.1
A. gossypii	3069	48	63.9	588.5	19.2	8	0.3	1.4	
Aspergillus clavatus	5215.8	91	57.3	1202.8	23.1	49.3	0.9	4.1	50.5
A. flavus	5670.8	48	118.1	1444	25.5	113.5	2	7.7	*22.7*
A. nidulans	5148.5	48	107.3	879	17.1	*201.3*	*3.9*	*22.9*	30.6
A. niger	*9291.8*	48	*193.6*	*2629.5*	28.3	78.3	0.8	3.0	28.4
A. oryzae	6206.5	70	88.7	1334.3	21.5	69.8	1.1	5.2	24.3
A. terreus	7627.8	74	103.1	1498	19.6	36.3	0.5	2.4	24.7
A. terricola	4139	48	86.2	1322	*31.9*	141.3	*3.4*	10.7	26.6
A. usamii	6555.8	48	*136.6*	1341.3	20.5	85.5	1.3	6.4	*23.1*
B. trispora	6508.3	72	90.4	*2038.8*	31.3	91.8	1.4	4.5	27.8
B. cinerea	2653.8	92	28.8	281	10.6	51.5	1.9	*18.3*	31.1
Ceratocystis ips	5904.3	48	123	1164	19.7	141.8	2.4	12.2	39.9
Cladosporium cucumerinum	5104.3	48	106.3	1384.8	27.1	101.8	1.99	7.3	63.8
C. cladosporioides	1585.3	80	19.8	379.5	23.9	65.5	*4.1*	17.3	35.3
Epicoccum nigrum	4023.8	89	45.2	444	11	27.8	0.7	6.3	33.3
G. fujikuroi	7002.8	72	97.3	1512.5	21.6	87.8	1.3	5.8	30.4
Gliocladium catenulatum	6421.5	48	133.8	1424.3	22.2	101.5	1.6	7.1	68.6
G. butleri	5458	48	113.7	*2161.3*	*39.6*	75.8	1.4	3.5	24.2
Humicola grisea	5950.3	48	123.9	1185.8	19.9	57.8	1	4.9	36.2
Mucor hiemalis	5984.5	73	81.9	788.3	13.2	*142*	2.4	18	45
M. rouxii	5552.5	48	115.7	1117.5	20.1	*213.3*	*3.8*	*19.1*	32
Myrothecium verrucaria	4020	120	33.5	1143	28.4	53.5	1.3	4.7	30.7
P. chrysogenum	5484.3	48	114.3	1950	*35.6*	24	0.4	1.2	28.9
Penicillium digitatum	5919.3	148	40	1340.5	22.6	*174.5*	2.9	13	29.6
P. blakesleeanus(+)	4428	96	46.1	659.3	14.9	120.8	2.7	*18.3*	33.2
R. oryzae	*10,048*	48	*209.3*	2007.5	19.9	89.8	0.9	4.5	32.3
Rhizopus stolonifer(−)	3573.5	48	74.5	1216.5	*34*	69.5	1.9	5.7	39.1
Sclerotinia sclerotiorum	4810.5	219	22	1118.3	23.2	49	1	4.4	*24*
T. viride	*8102.8*	71	114	2036.8	25.1	70.3	0.9	3.5	*23.6*
T. roseum	4331.3	72	60.2	925.3	21.4	40.8	0.9	4.4	42.9

DCW, dry cell weight; AIM, alkali insoluble materials; DA, degree of acetylation; DAC, degree of acetylation of chitosan; A, DCW; B, AIM; C, chitosan.

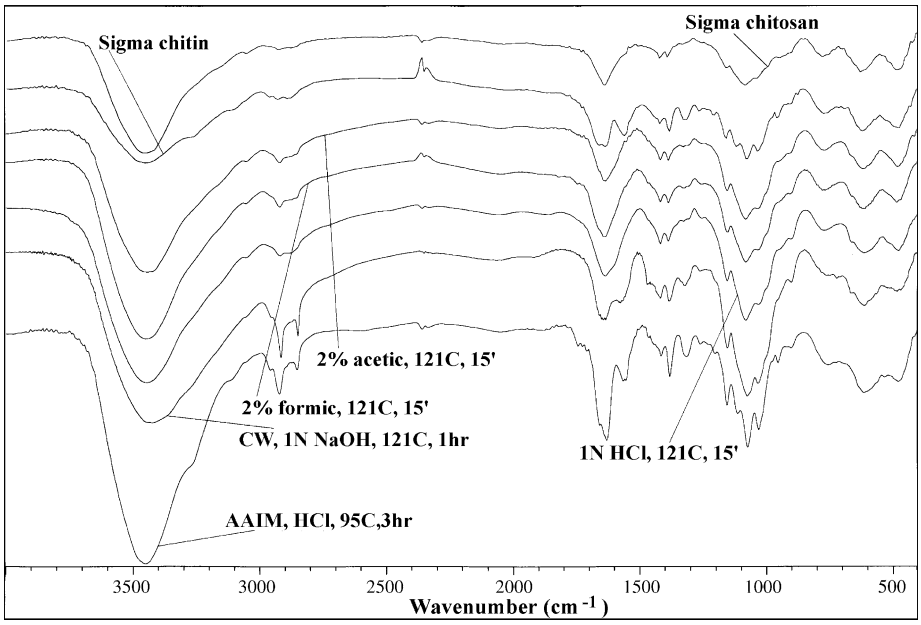

Fig. 1. Infrared spectra of chitin/chitosan and fungal chitinous materials.

DCW percentage chitosan ranges from 0.3% (*A. gossypii*) to 7.4% (*A. glauca*(+)), whereas AIM percentage chitosan varies from 1.2% (*P. chrysogenum*) to 22.9% (*Aspergillus nidulans*).

The amount of AIM in each fungus varied greatly with the percentage AIM ranging from 10.6% (*Botrytis cinerea*) to 39.6% (*G. butleri*).

We found that chitosan is extractable from all the 33 strains examined although only a trace amount of chitosan was obtained from some strains. Although it is reported that chitosan occurs in the cell wall of Mucorales strains of Zygomycetes (Muzzarelli et al., 1994), we found that chitosan is extractable from *Ascomycetes* strains (e.g. *A. gossypii* and *G. fujikuroi var.*), *Deuteromycetes* strains (e.g. *Aspergillus spp.*) and Basidiomycetes strains (e.g. *A. bisporus*) (see discussions below).

The *Mucorales* strain *A. glauca* is good resources for chitosan/chitin raw materials. According to Bartnicki-Garcia (1968), *A. glauca* belongs to the chitosan–chitin category. One conspicuous feature of this group is the absence of glucose polymers, i.e. glucans. Therefore, AIM could therefore be the mixtures of chitin and chitosan. The IR spectra (Fig. 1) support this assumption. The IR spectra for AIM or CW and alkali- and acid-insoluble materials (AAIM) from *A. glauca* are similar to that of Sigma crab-shell chitin. So, the *A. glauca* AIM and AAIM can be considered to be free of glucan, and virtually a mixture of chitin and chitosan. As a result, it can be assumed that AIM and AAIM from *A. glauca* should give a superior performance in removal of transition and post-transition metals. Such assumption is substantiated by the results of copper adsorption analysis below.

3.2. The kinetics of copper adsorption

The results of adsorption kinetics for fungal chitosan, AIM, FDM and crustacean chitosan are shown in Table 2. It can be seen that copper uptake on AIM, FDM and crustacean chitosan from 1 to 48 h contact are quite close, and 1 h is sufficient to achieve equilibrium for these materials. For fungal chitosan, there is a steady increase of copper uptake during the entire 48-h period but, as observed in practical fixed-bed operation, equilibrium between solid and liquid phase is rarely achieved (Sundstrom & Klei, 1979). The reason for the slower adsorption process for fungal chitosan is not clear, but the adsorption process occurs rapidly as 90.7% of Cu(II) was removed from

Table 2
The kinetics of copper adsorption on fungal chitosan and other chitosanaceous materials

Contact time (h)	Copper uptake (mg g^{-1})			
	Fungal-Cs	AIM	Crustacean-Cs	FDM
1	90.7	49.9	12.3	5.7
2	93.4	49.9	12.3	6.1
6	96.3	49.9	12.4	5.6
12	96.6	49.9	12.5	6.1
24	97.1	49.9	12.3	5.1
48	97.6	50.0	12.5	6.1

Fungal-Cs, fungal chitosan; Crustacean-Cs, crustacean chitosan; AIM, fungal alkali-insoluble materials; FDM, fungal freeze-dried mycelia. Mass of adsorbents, 200 mg except Fungal-Cs which was 100 mg; Initial copper concentration, for Fungal-Cs and AIM 100 ppm, for Crustacean-Cs and FDM 50 ppm; Volume of copper solution, 100 ml except Crustacean-Cs which is 50 ml; pH of copper solution, 5; Working temperature, 26 °C; Shaking rate, 200 rpm.

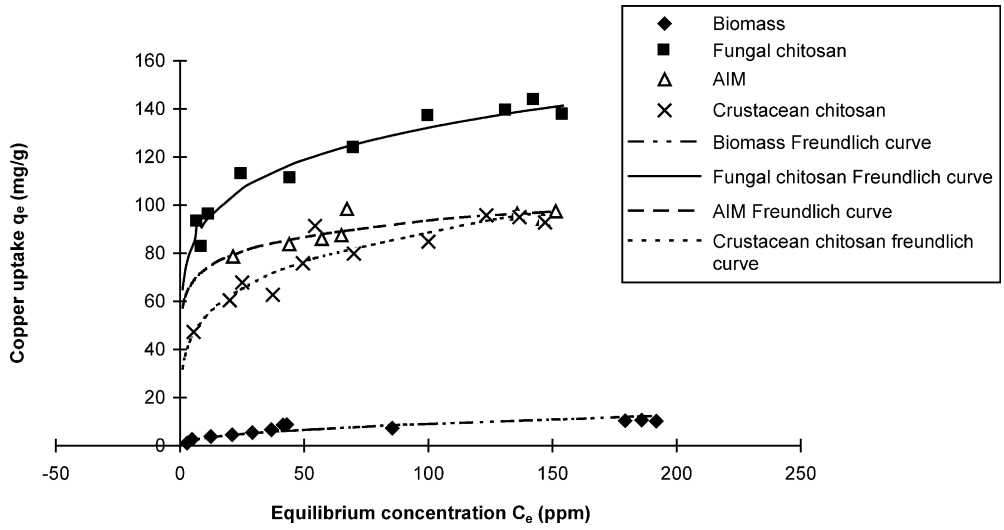

Fig. 2. Freundlich isotherm for copper adsorption on the different chitosanaceous materials.

the solution in the first hour. In all cases, the copper adsorption was accompanied by a development of blue color on chitosanaceous materials, and it was more sensitive and intensive for fungal chitosan, even in a solution of low copper concentration. Based on the kinetic result, 6-h contact time was used in the examination of copper adsorption isotherm.

3.3. Equilibrium isotherm and copper collection capacity

The copper uptakes (mg g^{-1}) under equilibrium concentrations for fungal chitosan, AIM, fungal biomass and crustacean chitosan are shown in Fig. 2 with their Freundlich isotherms. It can be seen that there is a rise of copper uptakes with an increase of equilibrium concentrations until the maximum copper uptakes are reached. Fungal chitosan has the highest adsorption capacity under all equilibrium concentrations, while AIM and crustacean chitosan have similar ability. However, FDM has a much lower collection capacity. The maximum copper uptakes calculated by Langmuir method are: fungal chitosan 135.2 mg g^{-1} > AIM 98.8 mg g^{-1} > crustacean chitosan 90.2 mg g^{-1} > fungal biomass 10.8 mg g^{-1}.

Listed in Table 3 are the Langmuir and Freundlich constants for the adsorption of Cu(II) ions on different chitosanaceous materials with a working pH of 5.0 and a working temperature of 26 °C. Error estimation of the Langmuir and Freundlich equations show that Freundlich model is more accurate to describe the copper adsorption isotherm than Langmuir model except that of fungal biomass for which Langmuir equation is slightly better. However, equation for fungal biomass has 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. It does not describe adsorption data as accurately as the Freundlich equation, which is an empirical model for adsorption isotherm (Snoeyink, 1990). This is supported by the present observations.

4. Discussion

Muzzarelli et al. (1981) treated the mycelia of *A. niger* with 30–40% NaOH aqueous solution to obtain AIM to which they referred as chitosan–glucan complex. They found that a number of transition- and post-transition-metal ions are chelated and collected by the chitosan–glucan complex with higher yields than by animal chitosan. They also found that immediate flocculation occurs upon mixing chitosan–glucan dispersions with alginate and polymolybdate solutions. From their observations as well as ours, it is reasonable to select high AIM-yielding fungus for practical use. By using AIM rather than chitosan to remove heavy metal, the cost can be reduced by omitting the acid treatment of AIM.

Muzzarelli et al. (1981) proposed that waste fungal mycelia from industrial fermentation could be used to produce chitosan as an alternative to the production of chitosan from crab and shrimp shells. This approach

Table 3
Langmuir and Freundlich constants for the Cu(II) adsorption of different chitinous materials

Materials	Langmuir constant		Freundlich constant		Equilibrium range (ppm)
	q_{max} (mg g^{-1})	b (l mg^{-1})	K_F	n	
Fungal chitosan	135.14	0.237	65.02	6.49	6.91–154.20
AIM	99.80	0.169	57.34	9.48	21.30–151.30
Crustacean-Cs	90.22	0.193	32.13	4.51	5.50–147.00
Biomass	10.79	0.50	1.08	2.16	2.83–191.90

Working temperature, 26 °C; pH of copper solutions, 5.0.

of chitosan production not only provides good qualities of the fungine product, but also save cost and is environmentally friendly. In our screening, we found that chitosan is extractable from some common industrial fungal mycelia which usually exist as waste in the fermentation industry. Such products include *A. niger* (A producer of citric and gluconic acids, β-glucanase, cellulase and glucose oxidase), *A. gossypii* (A producer of riboflavin), *P. chrysogenum* (A producer of penicillin), *Aspergillus spp.* (A producer of lipase, pentosanase and proteases) and *Mucor spp.* (A producer of rennin) (Wainwright, 1992). Waste mycelia from these industries can be used to produce chitosan, and as a result, the incineration of these fungal mycelia can be omitted.

Although *A. glauca*(+), *A. nidulans* and *M. rouxii* are of little or no industrial importance, they possess a high content of chitosan in their cell walls, and therefore are promising chitosan producers.

Fungi of *Ascomycetes*, *Basidiomycetes* and *Deuteromycetes* contain chitin as the principal structural component of their cell walls (Ruiz-Herrera, 1978), but the presence of native chitosan has not been reported for these fungi. According to Muzzarelli et al. (1980), concentrated sodium hydroxide can simultaneously deacetylate the chitinous fraction, dissolve the proteins, remove the soluble glucan, and hydrolyze the lipids. Their observations support the finding in the present experiment that chitosan is extractable from the chitinous, but native-chitosan-free strains of fungi. In the screening, we employed more severe conditions to extract chitosan, which can cause the deacetylation of native chitin in the fungal cell wall (Hu, 1997; Hu et al., 1999).

An exciting result is that the fungal AIM exhibited a high ability for copper collection, even higher than the commercial crab chitosan. This is in agreement with the experiments of Muzzarelli et al. (Muzzarelli, Tanfani, & Scarpini, 1980; Muzzarelli, Tanfani, Scarpini, & Tucci, 1980; Muzzarelli et al., 1981) who showed that the chitosan–glucan complex, obtained by treating fungal biomass with 30–40% (W/W) NaOH solution at 100 °C for 2–4 h, had higher adsorption ability for a number of transition and post-transition metal ions. This also means that acid treatment can be circumvented when the end product of the fungal chitinous materials is for the use of wastewater treatment. This results in cost reduction and avoids a second contamination to the environment during industrial production of chitosan. This result also suggests that fungal chitosan or AIM can replace the expensive animal chitosan when used as a metal ion adsorbent.

5. Conclusion

It has been found that chitosan is extractable not only from *Zygomycetes* fungi, but also from non-Zygomycetes fungi. Some industrial fungi do exhibit a high productivity of chitosan. *A. glauca*(+) is a promising chitosan producer. The fungal chitosan derived from *A. glauca*(+) mycelia had a much higher collection ability for copper ion under all equilibrium concentrations. The maximum copper uptake of fungal chitosan in a copper solution of pH 5 at 26 °C was 135.2 mg g^{-1}, an amount that corresponds to a 2:1 mole ratio of amine groups to copper ions, indicating that one copper ion might coordinate with two nitrogen atoms from two amine groups in the same chitosan chain or from two adjacent chains. The order for capacity of the copper collection was found to be: fungal chitosan > AIM > crustacean chitosan > fungal biomass.

The fungal alkali-insoluble-materials, derived by a single-alkali treatment under mild conditions compared with other workers, still had high copper collection capacity, even greater than that of commercial crab-shell chitosan. This finding shows that AIM has a great potential as a metal adsorbent to sequester toxic metals from contaminated waters in place of animal chitosan. Freundlich model provides a better description of the adsorption isotherms of fungal chitosan, AIM and crustacean chitosan than Langmuir model.

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