International Journal of Biological Macromolecules 137 (2019) 960–965

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: http://www.elsevier.com/locate/ijbiomac

Fungal (1 → 3)-α-D-glucans as a new kind of biosorbent for heavy metals

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ARTICLE INFO

Article history:
Received 21 March 2019
Received in revised form 26 June 2019
Accepted 4 July 2019
Available online 5 July 2019

Keywords:
Shiitake
Polysaccharide
(1 → 3)-α-D-glucans
Heavy metals
Biosorption

ABSTRACT

Although the fungal ability to bind heavy metals has been known for many years, it was not determined which component is responsible for this process. The aim of the study was to isolate (1 → 3)-α-D-glucans from various fungi, select the most efficient compound in the biosorption of heavy metals, and determine its characteristics. The best α-glucan for treatment of aqueous solutions from heavy metals (Ni^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}) is the polymer isolated from Shiitake, especially its SH 37 variety. Using various techniques, it was confirmed that the studied polysaccharide consists mainly of glucose molecules (75.9%), i.e. glucose α-anomers linked by (1 → 3)-linkage (86%). The well-developed surface, low crystallinity, and the large number of –OH groups provide this polymer with good sorption properties. Probably, the main mechanism of metal uptake is metabolism-independent process that depends on the content and spatial structure of the glucans present in their cell wall.

1. Introduction

Since 1907, there have been literature reports on the chemical composition and ability of fungi to accumulate metals [1]. However, credible results were achieved with the development of instrumental techniques and analytical methods only after 1970 [2–5]. The research on metal accumulation involved higher fungi [4,6] and filamentous fungi [7,8]. On the one hand, fungi contain magnesium, potassium, and selenium [6,9] which are essential elements required in the human diet. On the other hand, fungi accumulate toxic metals, e.g. cadmium, lead, and mercury [9].

The total global production of cultivated edible mushrooms exceeded 4×10^6 metric tons in 1991 and 6×10^6 t in 1997, while in 2009, it was estimated at 22×10^6 t [10–12]. In 2001–2002, the United States alone produced nearly $4{\cdot}10^6$ t of mushrooms [10]. *Agaricus bisporus, Lentinus edodes, Pleurotus* spp., *Auricularia* spp., *Volvariella volvacea, Flammulina velutipes*, and *Tremella fuciformis* are the seven most important cultivated mushrooms. In China, the main emphasis is placed on L. *edodes* and *Pleurotus* spp., while *A. bisporus* occupies the fourth place among all cultivated mushrooms. Global trade in mushroom in 2013 was worth 63 billion US $ [11]. Such a large production of mushrooms is associated with the generation of large quantities of totally untreated waste. However, they can be a valuable raw material for further processing. For instance, some authors have stated that the mycelium or fruiting body (live or dead) and spent mushroom substrate (SMS) can be used for the preparation of an effective biosorbent of heavy metals [13,14].

In recent years, bioremediation and its various varieties is an increasingly popular method of removing heavy metals using biological material [15–17]. Extracellular polymeric substances can be used for this process, including β-1,3-glucan. These type of polymers are cheap, environmentally friendly and biodegradable materials [15]. These natural polymers can be used as a biosorbent due to the high carbon content, the availability of heavy metal binding sites through specific groups (hydroxyl groups in the case of polysaccharides), and the porous surface. The advantage of using fungal polysaccharide is the management of waste material from mushroom production, which is carried out on a significant scale, as described above. In addition, ffungal glucans with good sorption properties are also insoluble in water; hence, they can be used as a sorbent in aqueous solutions. The disadvantage of this type of fungal sorbent is the low efficiency of α-glucan isolation from the fungal mass.

We propose a new biosorbent derived from biological waste as a source of (1 → 3)-α-D-glucans, which can be used for the biosorption of heavy metals from aqueous solutions. The high accumulation potential alkali-soluble fractions of α-glucans isolated from fungi are some of the advantages facilitating their application as new biosorbents. We screened (1 → 3)-α-D-glucans isolated from filamentous and higher fungi to assess their potential to remove metal ions: nickel, cadmium,

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https://doi.org/10.1016/j.ijbiomac.2019.07.036
0141-8130/

zinc, and lead from aqueous solutions. Based on these tests the glucan from L. *edodes*, in our opinion the best sorbent, was chosen and physico-chemically characterized.

2. Material and methods

2.1. Fungi and culture conditions

Four *Aspergillus* and two *Penicillium* species were used in the experiment, i.e. *A. awamori* DIM 3375 (Department of Industrial Microbiology, Maria Curie-Skłodowska University, Lublin, Poland), *A. nidulans* CIM 415 and *A. wentii* CIM 449 (Collection of Industrial Microorganisms, Warsaw, Poland), *A. oryzae* CBS 133.52 (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), *P. lanosum* DIM 110 and *P. notatum* DIM 102 (Department of Industrial Microbiology, Maria Curie-Skłodowska University, Lublin, Poland). The microorganisms were maintained on potato dextrose agar slants at 4 °C. The composition of the medium and the growth conditions were as described by Hasegawa et al. [18].

The fruiting bodies of *Boletus edulis* (Bull.) and *Piptoporus betulinus* (Bull.: Fr.) P. Karst. were harvested in the summer of 2011 in Poznań surroundings, Poland. The carpophores of *Ganoderma lucidum* (Fr.) Karst., *Hericium erinaceus* (Bull.) Pers., *Lentinus edodes* (Berk.) Pegler cv. "SH 37" (Department of Vegetable Crops of Poznań University of Life Sciences, Poland); "Sylvan 4080"; "Mycelia 3790", *Pleurotus citrinopileatus* Sing., *P. djamor* (Rumph. ex Fr.) Boedijn, *P. eryngii* (DC.) Quél., *P. ostreatus* (Jacq.) P. Kumm., and *P. precoce* (Fr.) Quel. were purchased from controlled cultivation carried out at the Department of Vegetable Crops of Poznań University of Life Sciences. Voucher specimens are deposited in the Department of Industrial Microbiology, Maria Curie-Skłodowska University, Lublin, Poland [18].

2.2. Isolation of the alkali-soluble polysaccharides from fungi

The cell wall was extracted according to the procedure described by Wiater et al. [19]. Table 1 shows the efficiencies of alkali-soluble polysaccharides isolation from individual fungal species.

2.3. Chemicals

Stock metal ion solutions of Ni(II), Cd(II), Zn(II), and Pb(II) ions (1000 mg·dm^{-3} each) were obtained by dissolution of the following salts: $Ni(NO_3)_2 6H_2O$, $Cd(NO_3)_2 4H_2O$, $ZnSO_4 6H_2O$, and $Pb(NO_3)_2$ (POCH, Gliwice, Poland), in redistilled water. A nitric acid (HNO_3 36%) (POCH, Gliwice, Poland) and NaOH (Merck, Darmstadt, Germany) were used for adjustment of the pH of the solutions.

2.4. Sorption experiment

The sorption capacity of the studied (1 → 3)-α-D-glucans for Ni^{2+}, Cd^{2+}, Zn^{2+}, and Pb^{2+} was determined using single metal systems at an initial pH 5 (regulated by NaOH and HNO_3). The initial concentrations of each of the studied ions were 100 mg·dm^{-3}. 0.1 g of each glucan was mixed with 10 cm^{-3} solutions of the analysed heavy metals. The solutions were shaken (250 rpm) at room temperature (22 ± 2 °C) for 24 h. Then, the solutions were centrifuged at a speed of $12{\cdot}10^3$ min^{-1} and the concentration of heavy metals were measured in the supernatant using an ICP-OES (Thermo Scientific iCAP Series 6500). All the experiments were carried out in three repetitions and mean values were used in the analysis of data.

2.5. Microscopic methods

2.5.1. SEM

The topographies and morphologies of the polymer surface were examined with an FEI Quanta 3D FEG at a magnification range from 250× to 3500×. Before measurement, the samples were covered with Pd—Au to eliminate the adverse electrostatic effects. Surface images of the (1 → 3)-α-D-glucans were obtained with the high vacuum secondary electron detection method.

2.5.2. Optical microscopy

Detailed information regarding the optical microscope analysis is presented in SI.

Table 1
Biosorption capacity of fungi α-(1 → 3)-glucans to heavy metals.[a]

Species	Type	α-(1 → 3)-glucan (dry matter) (%)	MRE* (%)				MRE* (μg h^{-1} mg^{-1})			
			Ni^{2+}	Cd^{2+}	Zn^{2+}	Pb^{2+}	Ni^{2+}	Cd^{2+}	Zn^{2+}	Pb^{2+}
Vegetative mycelium										
Aspergillus awamori		6.0	3.8	4.8	3.6	8.2	1.5	1.9	1.8	2.9
Aspergillus nidulans		9.4	6.8	14	14	37	2.7	5.5	6.8	14
Aspergillus oryzae		10	5.0	13	16	39	2.0	5.0	7.9	14
Aspergillus wentii		6.5	5.1	9.2	10	28	2.0	3.7	4.9	9.9
Penicillium lanosum		6.9	15	20	19	38	6.2	8.2	9.6	14
Penicillium notatum		9.0	7.8	7.6	3.8	13	2.7	2.9	1.8	4.6
Fruiting bodies										
Boletus edulis		2.2	40	79	46	96	18	33	18	36
Ganoderma lucidum		6.1	23	45	23	90	9.4	19	8.8	33
Hericium erinaceus		5.0	5.5	15	5.0	28	2.2	6.3	1.9	10
Pleurotus citrinopileatus		2.7	2.7	10	4.8	21	1.1	4.3	1.6	7.8
Pleurotus djamor		3.3	1.9	13	4.3	24	1.1	5.3	1.6	8.8
Pleurotus eryngii		2.6	7.0	3.8	0	13	2.8	1.6	0	4.8
Pleurotus ostreatus		4.3	9.7	13	9.2	36	3.7	5.1	4.3	14
Pleurotus precoce		4.7	0	16	9.6	28	0	8.1	5.7	17
Piptoporus betulinus		8.0	27	11	29	91	11	4.5	12	34
Lentinus edodes (Lentinula)		9.0	22	38	23	72	8.8	16	8.4	27
Lentinus edodes	Mycelia 3790 A	–	11	24	4.2	51	9.2	18	3.1	35
	Mycelia 3790 B	–	11	22	9.2	51	8.4	17	6.8	37
	Sylvan 4080 A	–	8.9	18	5.0	49	7.4	15	3.9	34
	Sylvan 4080 B	–	13	27	15	54	11	21	11	37
	SH 37 A	–	13	25	14	50	9.9	19	9.9	33
	SH 37 B	–	13	23	12	48	10	18	0.30	34

[a] MRE: Metal removal efficiency; A: waste type; B: consumption type.

2.6. XRD

Detailed information regarding X-ray diffraction analysis is presented in SI.

2.7. FT-Raman and FT-IR

FT-Raman spectra were obtained on an FT-Raman (NXR FTRaman) for a Nicolet 8700 FT-IR using an InGaAs detector (Thermo Scientific). The samples were illuminated using an Nd: YVO4 excitation laser operating at 1064 nm. The maximum laser power was 2 W. The spectra were recorded over the range of 100–4000 cm^{-1}. The FT-IR spectra were obtained using Nicolet 8700 spectrometer (Thermo Scientific) for Fig. 2. Powdered (1 → 3)-α-D-glucans preparation was placed on ATR crystal. Spectra were recorded in the range of 400–4000 cm^{-1}.

2.8. Methylation analysis

Monosugars were liberated from the polysaccharides by hydrolysis using 2 M trifluoroacetic acid (100 °C, 4 h). Next, they were converted into alditol acetates by reduction with $NaBD_4$ and peracetylation, as was described elsewhere [20]. The absolute configuration of the monosugars was established by analysis of peracetylated R-(−)-2-butyl glycosides, according to the method described by Gerwig and co-workers [21]. The sugar derivatives were analysed by GC–MS.

2.9. NMR

1D and 2D NMR experiments were recorded in a Me_2SO-d_6 solution at 80 °C (353 K) using a Varian Inova plus 500 spectrometer (operating frequencies: 499.81 MHz for ^1H NMR and 125.69 MHz for ^{13}C NMR) and applying standard Varian software. The following two-dimensional NMR experiments were performed: (homonuclear: ^1H,^1H DQF-COSY, TOCSY, NOESY, and heteronuclear ^1H,^{13}C HSQC). A mixing time of 100 and 150 ms was used in TOCSY and NOESY experiments, respectively. The ^1H and ^{13}C resonances were measured relative to the methyl group signal of Me_2SO-d_6 (δ_H 2.50 and δ_C 39.50).

2.10. Statistical analysis

The values from all tests are the means of three separate experiments ± standard deviation. Tukey's HSD test (STATISTICA 8.0, StatSoft, Inc., USA) was used for the determination of statistical differences with the significance denoted at $p < 0.05$.

3. Results and discussion

3.1. Sorption experiments

The total content of alkali-soluble of (1 → 3)-α-D-glucans ranged from 2.2 to 10 g/100 g on dry weight (DW)[1] basis of the crude polysaccharide extract. Interestingly, the (1 → 3)-α-D-glucans content of extracted polysaccharides expressed on DW basis of vegetative mycelium was significantly higher than the amounts of α-glucans isolated from fruiting bodies [20].

Table 1 also shows the sorption capacity of α-glucan materials isolated from the different species of the fungi tested for binding to four metals (Ni^{2+}, Cd^{2+}, Zn^{2+}, Pb^{2+}). The polysaccharides extracted from *G. lucidum, Boletus edulis*, and L. *edodes* (Shiitake) seem to be the best in terms of heavy metal adsorption.

However, for economic reasons, *L. edodes* was selected due to the complicated, multi-stage, and time-consuming cultivation processes of the other two species. Choosing the best biosorbent, the efficiency of glucan isolation was taken into consideration. In addition, the Shiitake mushroom is the third most common fungus in the world, but its production waste has not been subjected to treatment so far. The next stage of screening involved both the consumable and waste types of this fungus. Eventually, the L. *edodes* SH 37 variety was chosen (Table 1).

3.2. Microscopic methods

The SEM analysis showed the complex surface morphology of the polysaccharide from *Lentinus edodes*. The SEM image series (Fig. 1) documents the petal polysaccharide morphology. The subtle structure of biopolymer chains is also visible. Bayramoğlu and Arıca [22] studied the surface morphology of dried live fungal pellets from L. *edodes* using SEM. They reported that these pellets had a fibrous and porous surface structure, likewise the polysaccharide studied by us. This structure influences the total surface area, which can increase the efficiency of heavy metal sorption. Qian et al. [23] studied alkali-soluble polysaccharides of L. *edodes* and reported that the polysaccharides had a smooth surface and a clearly regular shape, i.e. these were round particles. Probably, these different results are related to the use of different extraction methods. The results obtained with SEM are confirmed by the images from the optical microscope (Fig. S1), which shows a structure like the flake with an inhomogeneous surface.

3.3. XRD

X-ray diffraction was used to define the structure of the fungal polysaccharide [23]. The XRD patterns of α-glucans extracted from *Lentinus edodes* are shown in Fig. S2. There are three peaks: the first broad peak around 9° and two peaks around 18° and 21°. These results are similar to previous studies of polysaccharides extracted from L. *edodes* [23,24]. In the literature, peaks around 2θ at 16° and 22.5° are characteristic of cellulose I [25]; therefore, the studied material resembles slightly the structure of this polymer. Similar to our study Ifuku et al. [26] have reported that in the case of *Hypsizygus marmoreus* the X-ray diffractogram contains crystal patterns of cellulose. Generally, the crystallization capacity of this type of polysaccharide is poor, which can be seen as bun-shaped X-ray diffraction curves [24] but the presence of the first broad peak is consistent with small and imperfect crystallites. The crystallinity index of the fungal polysaccharide sample was 8.5%. Aranaz et al. [27] have shown that another natural polymer chitin with a low crystallinity degree has a high capacity for heavy metal removal. Thus, the low crystallinity may determine the binding capacity of heavy metals by the polymer. In contrast, Jelmsa and Kreger [28] reported that (1 → 3)-α-D-glucans may have the ability to crystallize and can exist in three crystal forms (I, II, III). These forms depend on the type of fungus and the tissue in which they occur.

3.4. FT-IR and FT-Raman

The FT-IR and FT-Raman spectra of (1 → 3)-α-D-glucan from *Lentinus edodes* ranged from 4000 to 100 cm^{-1}, as shown in Fig. 2. These spectroscopy techniques were used to confirm the presence of specific groups as well as the position of glycosidic linkages and their anomeric configuration in glucan. Two spectral areas are important for the structural characterization of glucan: the "anomeric region" (750–950 cm^{-1}) and the "sugar region" (900–1200 cm^{-1}) [29]. Characteristic bands for a polysaccharide with α-1,3-linkages are present at 930, 840, and 820 cm^{-1} [30]. Moreover, a peak at 1140 cm^{-1} corresponds to α-1,4-glycoside bonds [30,31]; hence, this type of polysaccharide is present in this preparation as well. There is a broad intense peak at 3300 cm^{-1} characteristic of O—H stretching vibrations; therefore, it can be concluded that there are hydroxyl groups in the polysaccharide structure [23]. There is a clear peak at 2900 cm^{-1} characteristic for C—H stretching vibrations and a broad band at 900–1200 cm^{-1} for C—O and C—C stretching and C-OH bending vibrations [32,33]. The

[1] *Abbreviation*: DW, dry weight

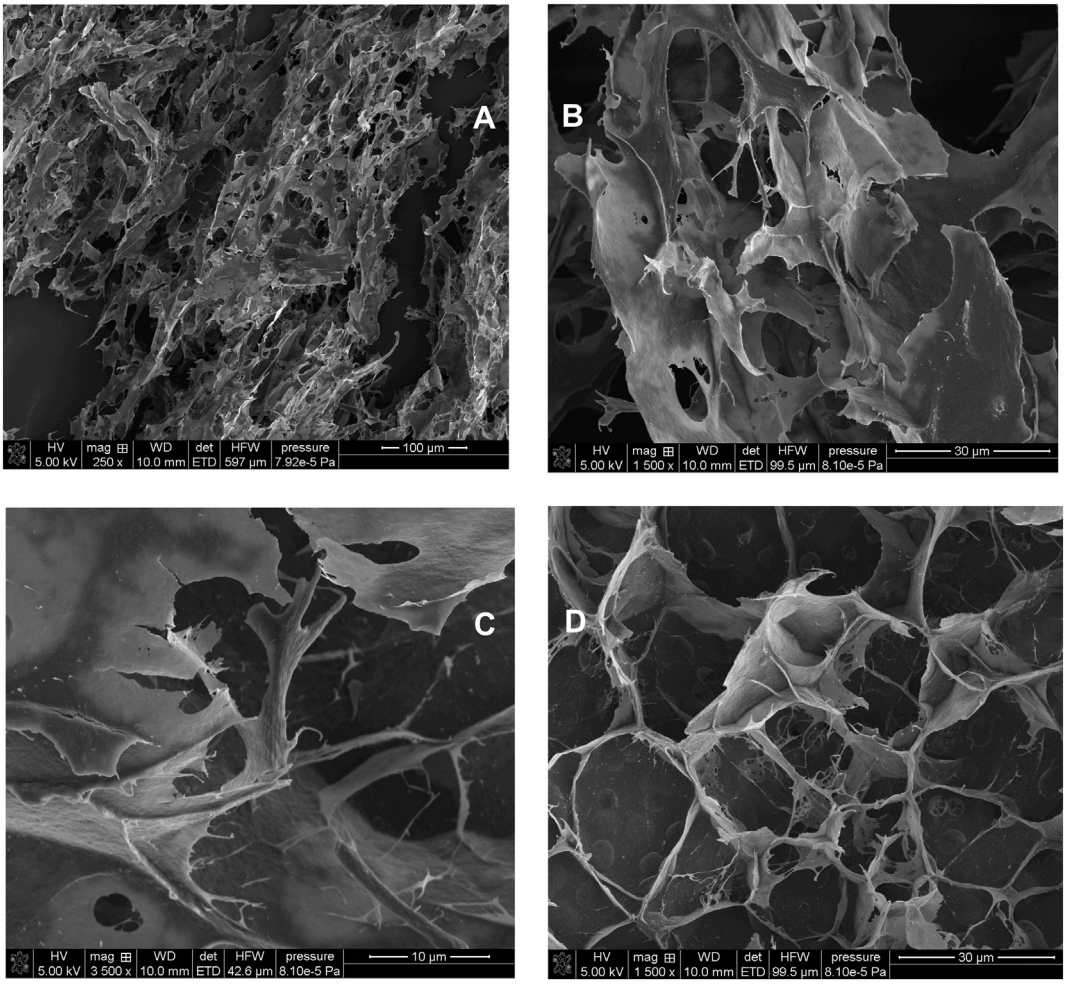

Fig. 1. SEM photographs of the α -(1 → 3)-glucan (A-D) of L. *edodes* at 250–3500 × magnification.

most important peaks obtained with the FT-IR and Raman techniques overlap; hence, the presented results are reliable.

Further, the FT-IR analysis of α-glucan (*L. edodes*) was performed after the biosorption with Pb^{2+} ions and the results were compared with the native glucan (Fig. S3). The changes observed in the spectrum were shifted from 3282 and 2914 to 3314 and 2950, respectively. These bands are characteristic of the stretching vibrations of the –OH group. Secondly, a shift of the peak from 1635 (–OH from adsorbed H2O vibration) to 1653 cm^{-1} and from 1015 to 1024 cm^{-1} was noticed. The differences between both spectra show that the adsorption of Pb^{2+} ions occurs through the hydroxyl groups [34]. Analogous spectra of glucan with sorbed Ni^{2+}, Cd^{2+}, and Zn^{2+} ions were obtained (not shown). The analysed heavy metal ions are complexed on the surface by oxygen-containing groups.

3.5. Methylation analysis

The monosaccharide analysis proved that the studied alkali-soluble polysaccharide contained two hexoses: glucose (75.9%) and mannose (19.5%) and a small amount of pentose (4.5%) identified as xylose. Linkage analysis showed that (1 → 3) linked Gl*cp* is the major chain constituent (86.5%) in addition to (1 → 4)-linked Gl*cp* (5.3%) and terminal glucose (8.2%). Bao et al. [35] isolated three polysaccharide fractions from *Ganoderma lucidum*. The polysaccharide material shows that it does not consist of glucose exclusively but there are also other polysaccharides, for example mannose or rhamnose, and the individual units combined different types of glycosidic bonds [35]. In summary, these results indicate that the studied fraction is mainly a glucose polymer or glucan. However, it is impossible to isolate polysaccharide fractions from biological material that contain only one type of polysaccharide and one type of bonds between them.

3.6. NMR

The 1D and 2D ^{1}H NMR (DQF-COSY, TOCSY, NOESY) and 2D ^{1}H—^{13}C NMR (HSQC) experiments were performed to determine the structure of the investigated preparation as well as the anomeric configuration of glucose (the major sugar component) residues. The NMR data are listed in Table 2. Two spin systems (marked by A and B) both characteristic of α-D-glucose were identified. Therefore, it can be claimed that two types of α-D-glucose-based polymers were constituents of the test material. The major fraction, represented by the A spin system, was (1 → 3)-α-D-glucan (86%). It is accompanied by a six-fold smaller amount of an amylose-like polymer (14%, B spin system). The HSQC spectrum (Fig. S4) contained only signals attributed to correlations between directly bounded carbon and proton nuclei, whereas the ^{1}H NMR and ^{1}H—^{1}H 2D NMR-correlated spectra contained hydroxyl proton signals as well (not shown). These signals were present because of the hygroscopic nature of both the solvent (DMSO d_6) and the glucan preparation. It was difficult to avoid the presence of residual water (δ at 3.14 ppm) absorbed from the surrounding environment. Three resonances (at 4.22, 4.51, 4.91 ppm) could be assigned to protons from the hydroxyl groups of (1 → 3)-α-D-glucan and other three small signals (at 4.32, 4.62, 4.98) from the (1 → 4)-linked α-D-glucose polymer. The first group of signals were assigned to OH-6, OH-2, and OH-4 of the glucose ring of (1 → 3)-α-D-glucan and another three signals to OH-6, OH-

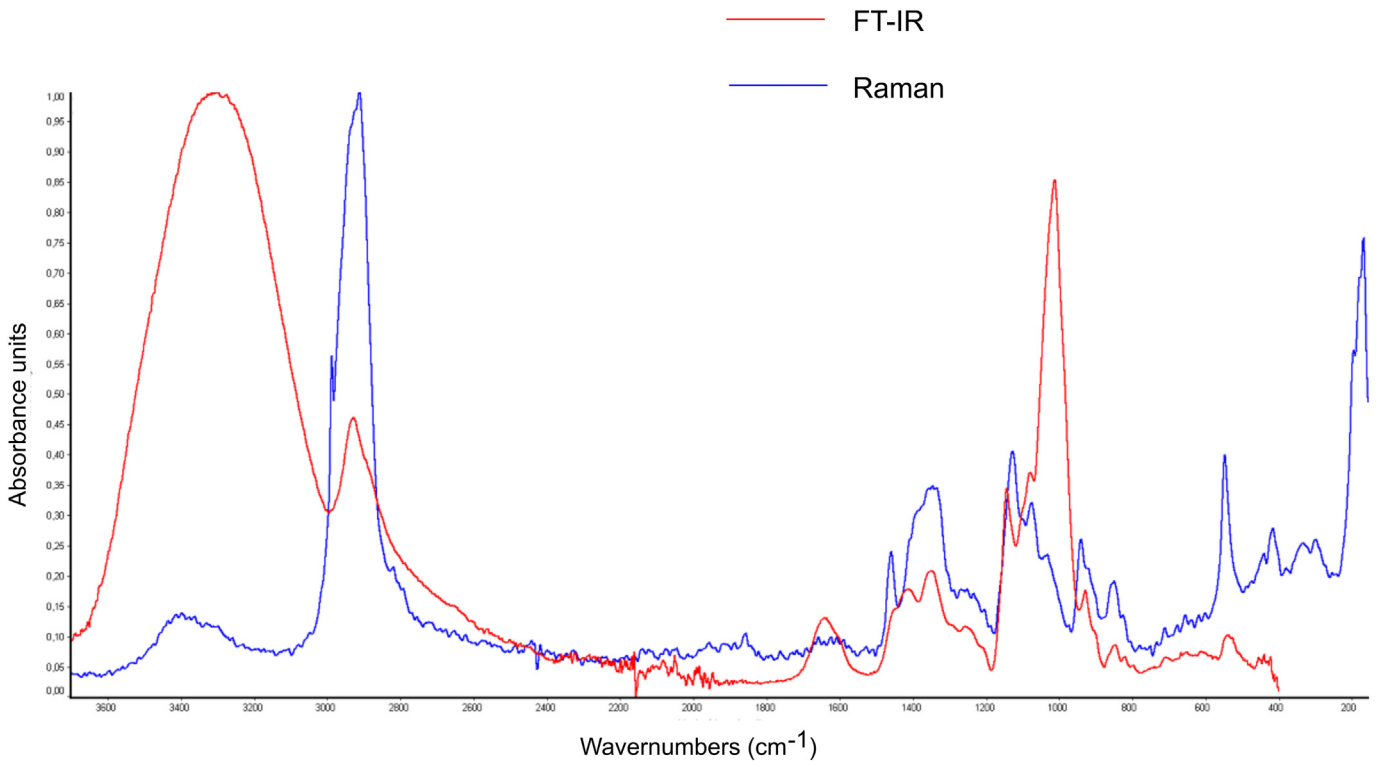

Fig. 2. Comparison of FT-IR (red line) and FT-Raman spectra (blue line) of sample α-(1 → 3)-glucan of L. *edodes*.

2, and OH-3 of the glucose ring of (1 → 4)-α-D-glucan, respectively. In the case of the main component of the preparation, the absence of an OH-3 signal in the ^{1}H NMR spectrum together with a downfield shift of C-3 (83.6 ppm, Table 2) indicated that the glucose residues were (1 → 3)-linked within the main polymer. The low field anomeric proton signal at 5.12 ppm and anomeric carbon at 100.1 ppm as well as the small value of the $^{3}J_{H1,H2}$ coupling constant (c.a. 3.0 Hz) are typical for the α-anomer of glucose.

Based only on NMR spectroscopic data, it is difficult to conclude whether the two components are chemically bonded to form one large water-insoluble polymer or are a *co*-separated mixture of polymers (using the alkaline extraction procedure) from the fungal (*L. edodes*) tissue. Expected signals corresponding to branching glucose and to terminal glucose were not detected by the NMR spectroscopy (i.e. the amounts of the residues were below the 0.5% detection limit of the method). Another glucan from L. *edodes* was reported [36], which contained a main chain of (1 → 4) and (1 → 3)-linked glucopyranose residues. This water-soluble glucan had (1 → 3)-β-D-glucan having two (1 → 6)-β-glucopyranoside branches for every five (1 → 3)-β-glucopyranoside linear linkages was the main structure [37].

4. Conclusion

The fungal (1 → 3)-α-D-glucan isolated from Shiitake has good sorption properties because of well-developed surface, low crystallinity and a large number of hydroxyls on the surface. Probably the main mechanism of metal uptake by fungi is the metabolism-independent process taking place through the formation of bonds with oxygen-containing surface groups of cell-wall polysaccharides. Given the ease of obtaining this biological waste material, α-glucan from Shiitake can be used as a new heavy metal biosorbent for the treatment of aqueous solutions. To the best of our knowledge, (1 → 3)-α-D-glucan isolated from L. *edodoes* has many advantageous features, including water-insolubility as well as the low cost and availability of raw biosorbents.

Table 2
The assignments of ^{1}H and ^{13}C NMR spectra and corresponding coupling constants of alkali-soluble polysaccharides from *Lentinus edodes*.

Spin system (% of residue)	Signals										
	H-1	H-2	H-3	H-4	H-5	H-6'	H-6	OH-2	OH-3	OH-4	OH-6
	C-1	C-2	C-3	C-4	C-5	C-6					
	$J_{1,2}$ [Hz]										
A → 3)-α-D-Glc(1 → (86%)[b]	5.12	3.47	3.69	3.49	3.89	3.57	3.70	4.46	nd[a]	4.86	4.17
	100.1	71.0	83.6	70.4	72.5	61.1		–	–	–	–
	<3 Hz										
B → 4)-α-D-Glc(1 → (14%)[b]	5.18	3.37	3.75	3.63	3.46	3.51	3.63	4.62	4.98	nd[a]	4.32
	100.4	72.3	73.3	83.7	70.5	61.4		–	–	–	–
	<3 Hz										

[a] nd - not exist in this polymer.
[b] Percentage of the polymer determined on the basis of surface areas of anomeric signals (^{1}H NMR spectrum).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijbiomac.2019.07.036.

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