

WASTE FUNGAL BIOMASS FROM BIOTECH INDUSTRY AS A SOURCE OF CHITOSAN

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Abstract

*The filamentous fungus *Mucor circinelloides* IBT-83 is an excellent producer of intracellular lipids. This makes it a promising candidate for large-scale cultivation for industrial oil production. However, this process generates significant amounts of defatted biomass, which can serve as a source of valuable compounds such as chitosan. It was shown that almost 100 g of chitosan (unpurified) can be extracted from 1 kg of defatted and dried mycelium of *M. circinelloides* IBT-83. Pre-dehydration of the mycelium by freeze-drying positively affects both the lipid extraction efficiency and the subsequent recovery of chitosan. The method developed in this study represents a promising approach for increased waste valorisation and supports the production of an industrially important biological product.*

Keywords: *chitosan, fungal biomass, *Mucor circinelloides**

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1. Introduction

Over 60% of biotechnology industries utilise fungi in various processes, including brewing, baking, food production, and pharmaceuticals. Thousands of tonnes of fungal biomass waste are generated annually, which is typically disposed of through landfilling or incineration for convenience [1]. Filamentous fungal biomass produced by fermentation is an excellent alternative source of high-quality protein, polysaccharides, glycoproteins and other valuable compounds such as lipids (including polyunsaturated fatty acids), vitamins and minerals. Several industrially applicable enzymes are isolated from fungal cells. In Europe, this biomass is used by many companies, including AB Enzymes, BASF, Bayer, DSM, DuPont, Novozymes and Roal Oy [2].

So far, the primary commercial sources of chitin have been crab and shrimp shells. Approximately 80,000 tonnes of chitin are extracted annually from these marine by-products [3]. This biopolymer is converted into its industrially valuable, water-soluble derivative, chitosan, through alkaline deacetylation. Due to the seasonal availability of marine crustacean shells, the use of filamentous fungi as an alternative source of chitosan presents a promising and sustainable option [1]. The cell walls of these microorganisms are composed of polysaccharides (such as chitin, chitosan, and glucan) and glycoproteins (including mannoproteins, galactoproteins, xylomannoproteins, and glucuronoproteins). Filamentous fungi, particularly those in the class Zygomycetes, are a rich source of chitin and chitosan [4, 5]. Within this class, the most efficient chitosan producers are found in the order Mucorales, specifically within two basal families: Cunninghamellaceae and Mucoraceae. The Cunninghamellaceae family includes several high-yielding genera, such as *Absidia*, *Cunninghamella*, and *Gongronella*, while the Mucoraceae family comprises genera such as *Mucor*, *Rhizomucor*, and *Rhizopus* [5].

The first microbiological approach to obtaining chitosan was developed by White, Foulton and Farin in 1979. They extracted this biopolymer from purified mycelial walls using hydrochloric acid, with yields ranging from 4 to 8% of the dry weight of the cell wall material [6]. Filamentous fungi cultivation has several advantages; additionally, it is independent of weather conditions, geographical location or season. These microorganisms exhibit a fast growth rate, significantly shortening the biomass production cycle. It is also possible to use cheap substances as culture medium components, e.g., waste products from various industries. Although the microbiological method of obtaining chitosan has existed for over 40 years, it has not yet been adopted on a large scale in industry. Several companies on the market commercialise chitosan-based products of fungal origin, such as Kitozyme, MycoDev Group Inc. and Chibio [5]. A literature review showed that fungal chitosan, due to its favourable properties such as a high degree of deacetylation and lower molecular weight, is suitable for various industrial applications, especially biomedical ones, including drug delivery systems and wound healing. Among the advantages of the microbiological approach is the possibility of obtaining chitosan with different properties by changing the species and culture conditions [5, 7].

Mucor circinelloides IBT-83, originating from the Institute of Molecular and Industrial Biotechnology of the Lodz University of Technology, is a high-yielding oleaginous strain [8, 9]. It also produces membrane-bound lipase, which is useful in the hydrolysis and synthesis of esters [8]. The crude enzyme preparation from defatted mycelium is a source of chitosanolytic enzymes and is used for hydrolysis, producing biologically active chitosan oligomers [10, 11]. Obtaining lipids based on the above-mentioned strain involves leaving large amounts of used defatted biomass, which can be a source of valuable substances, in addition to the above-mentioned enzymes. The study's objective

was to confirm the possibility of using the waste biomass of *M. circinelloides* IBT-83 strain (after lipid extraction) as a source of chitosan.

2. Materials and Methods

2.1. Materials

Corn steep liquor powder was purchased from Roquette (Belgium). Used cooking oil (rapeseed oil “Kujawski” from ZT “Kruszwica” S.A., Poland) was obtained from a local food service establishment. All other chemicals used in this study were of analytical grade.

2.2. Microorganism and Culture Conditions

The study used the strain of filamentous fungus *Mucor circinelloides* IBT-83 from the culture collection of the Institute of Molecular and Industrial Biotechnology of the Lodz University of Technology (GenBank database with accession numbers KR056084, KR056083). *M. circinelloides* IBT-83 was cultured in a 30 L Techfors-S fermenter (Infors HT, Switzerland) filled with 18 L of medium (3.7 (w/v) corn steep liquor powder, 2.7 (v/v) used cooking oil, pH 4.7). The strain was cultivated for 72 hours at 30°C with stirring at 100 rpm and aeration at 1 vvm, following the previously described methodology [12].

After cultivation, the mycelium was harvested by filtration (filter funnel with sintered disc G2) and washed with distilled water. The biomass was thoroughly squeezed to remove excess water and minimise moisture content, then divided into two portions. One portion was freeze-dried (−30°C, 0.37 mbar, Alpha 1-4 freeze dryer, Christ, Germany). The overall experimental scheme is shown in Figure 1, which also includes the steps of lipid and chitosan extraction from the obtained fungal biomass, as described in Sections 2.3 and 2.4, respectively.

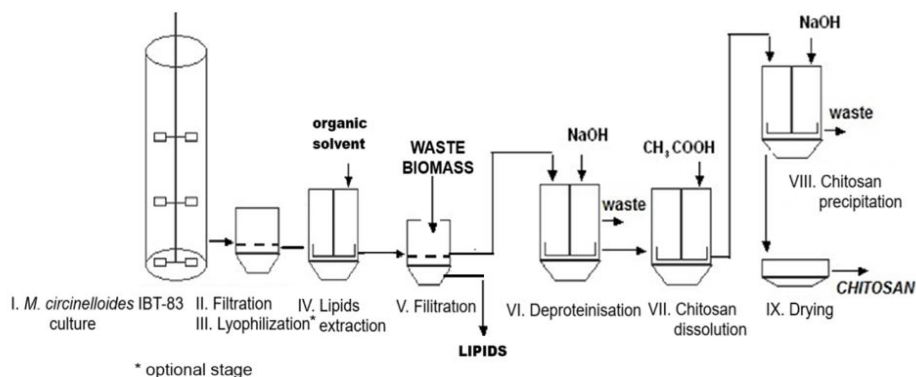


Figure 1. General scheme of the research: cultivation of *M. circinelloides* IBT-83, extraction of lipids and chitosan from filamentous fungal biomass.

2.3. Lipids Extraction From the Mycelium of *M. circinelloides* IBT-83

The fungal biomass was transferred to a Reactor-Ready system (5 L, Radleys, United Kingdom), and acetone or one of the following organic solvent mixtures was added: hexane:isopropanol (2:1, v/v); hexane:isopropanol (3:2, v/v) or hexane:isopropanol (1:2, v/v). For each gram of biomass (DW), 10 mL of the solvent or solvent mixture was used. Lipid extraction was carried out three times, with each extraction lasting 15 minutes at room temperature. The extracted lipids and residual biomass were subsequently dried at room temperature for 48 hours and weighed.

2.4. Chitosan Extraction From the Mycelium of *M. circinelloides* IBT-83

Chitosan extraction from *M. circinelloides* IBT-83 mycelium included the following stages:

- Alkaline treatment. 15 g of defatted and dried mycelium was suspended in 1 L of 1 M NaOH solution and heated at 100°C for 2 h, with mixing at 100 rpm. The alkali-insoluble fraction was centrifuged at 10,000 rpm for 10 min, washed with distilled water to neutral pH and centrifuged again.
- Acid treatment. The pellet was suspended in 5% acetic acid solution (1:40, w/v) at room temperature for 12 h, mixing at 100 rpm. The acid-insoluble component was removed by centrifugation at 10,000 rpm for 10 min. The chitosan contained in the supernatant was precipitated by adjusting the pH to 10 with 2 M NaOH, then filtered, washed first with ethanol and then repeatedly with distilled water until a neutral pH was achieved. The obtained chitosan was freeze-dried (−30°C, 0.37 mbar, Alpha 1-4 freeze dryer, Christ, Germany).

2.5. Determination of the Average Molecular Weight of Chitosan

The average viscosity molecular weight (Mv) of chitosan was calculated based on the intrinsic viscosity (η_i), determined at a temperature of $25 \pm 0.10^\circ\text{C}$. The viscosity was measured in an Ubbelohde dilution viscometer (Schott GmbH, type 53110/I) using a kinematic viscosity determination system (Schott CT52). Freeze-dried chitosan was used to determine the Mv. The chitosan was dissolved in a solvent system proposed by Roberts [13], consisting of 0.1 M sodium chloride, 0.2 M acetic acid, and 4.0 M urea. The detailed procedure has been described elsewhere [14].

2.6. Fourier Transform Infrared Spectroscopy Analysis

The chemical structure of fungal chitosan samples was determined by Fourier Transform Infrared Spectroscopy (FTIR) using a Thermo Scientific NICOLET 6700 FTIR spectrophotometer. Spectra were recorded at room temperature in the wavenumber range of $4000 - 400 \text{ cm}^{-1}$ using the Attenuated Total Reflectance (ATR) mode. Each spectrum represents the average of 32 scans collected at a resolution of 4 cm^{-1} . The chitosan degree of deacetylation (DD%) was calculated using the formula [15]:

$$\text{DD}\% = \left(1 - \frac{A_{1650}}{A_{3450} \cdot 1.33}\right) \cdot 100\% \quad (1)$$

where:

A_{1650} – absorbance of the band at 1650 cm^{-1} (amide I), analytical band;

A_{3450} – absorbance of the band at 3450 cm^{-1} (hydroxyl band), internal reference;

1.33 – the factor representing A_{1650}/A_{3450} ratio of fully N-acetylated chitin.

2.7 Statistical Analysis

Measurements were taken in triplicate, and the results were expressed as mean \pm standard deviation.

3. Results and Discussion

M. circinelloides IBT-83 produces oils and accumulates them inside the mycelium, both *de novo* (when sugars are the only carbon source) and *ex novo* (when lipids, e.g. oils, are the carbon source). This fungus, therefore, has significant potential for oil production in culture media based on various types of food industry waste [8, 9]. This approach could be used to produce microbial oil, for example, as a sustainable feedstock for biodiesel

production [8, 16, 17]. As a result of this process, large quantities of spent, defatted fungal biomass would be generated as a by-product, which could serve as a source of valuable substances such as chitosan.

Commonly known and applicable lipid extraction procedures, such as Bligh-Dyer [18] or Folch [19], use chloroform and methanol to improve the accessibility and solubility of polar lipids, improving the overall lipid extraction efficiency. Unfortunately, due to the toxicity of chloroform and methanol, various combinations of less toxic solvents such as hexane and short-chain alcohols are often used [17]. Therefore, the study used different organic solvents (hydrophilic and hydrophobic and their mixtures) for lipid extraction. It was determined how their use would affect the next stage of fungal biomass utilisation, i.e., chitosan extraction. Lipid recovery was performed using both water-containing and freeze-dried biomass. The presence of water may reduce the efficiency of solvent-based extraction, possibly due to limited mass transfer and emulsion formation [17]. The extraction procedure applied in this study yielded a crude chitosan complex that still contained various non-chitosan components and therefore requires further purification. The presence of pigments, residual lipids and proteins, and other impurities may adversely affect the final product's physicochemical properties, quality, and potential applications. Table 1 presents the yield of the chitosan complex obtained from *Mucor circinelloides* IBT-83 biomass, depending on the pretreatment method applied.

It has been found that the yield of chitosan complex separated from *M. circinelloides* IBT-83 mycelium was equal to 35 - 99 g/kg of waste, defatted and dried biomass (Table 1). The water in the mycelium obtained after the culture adversely affects the lipid extraction and the separation of chitosan. Initial dehydration of the mycelium by freeze-drying affected the extraction of lipids and, consequently, chitosan from the biomass. Our studies revealed that the mixture of hexane: isopropanol in a ratio of 3:2 v/v was the best for fungal oils extraction. Extraction of lipids with the mixture of these solvents in combination with pre-lyophilisation of biomass also gave the highest efficiency of chitosan separation, up to 98 g per 1 kg of defatted and dried biomass.

Among filamentous fungi, *Absidia coerulea* is recognised as one of the most efficient chitosan producers, with reported yields exceeding 300 g/kg of dry biomass [5, 20]. Within the genus *Mucor*, several species, including *M. circinelloides*, *M. rouxii*, *M. rouxianus*, *M. racemosus*, and *M. indicus*, have also been identified as promising chitosan producers. However, chitosan yields among *Mucor* species vary considerably, influenced by factors such as strain specificity, cultivation conditions, and the composition of the growth medium [5].

The study confirmed that the strain *Mucor circinelloides* IBT-83 exhibits a high chitosan production potential, reaching approximately 100 g/kg of defatted and dried biomass, making it a promising fungal producer of this biopolymer. In comparison, another strain of the same species, *M. circinelloides* UCP 050, achieved a lower chitosan yield of 64 g/kg in submerged fermentation using an economical yam bean-based medium. The Mv and DD values of the chitosan were $2.70 \cdot 10^4$ g/mol and 83%, respectively [21]. Chitosan obtained from UCP 050 showed efficacy in inhibiting the growth of the pathogenic fungi *Aspergillus niger* URM 5162 and *Rhizopus stolonifer* URM 3482 [22]. A slightly higher chitosan production efficiency of 112 g/kg was noted for *M. circinelloides* ZSKP. In the case of this strain, lipids were also produced simultaneously with chitosan [23]. There are limited scientific publications regarding the chitosan extraction from *M. circinelloides*.

Table 1. Effect of *M. circinellides* IBT-83 biomass pre-treatment on the amount of chitosan complex.

Type of solvent	FR	Lipids		Defatted and dried biomass [g/L]	Chitosan complex	
		[g/L]	[g/kg]		[g/L]	[g/kg]
Acetone	+	24.01 ± 1.68	600.34 ± 42.08	16.91 ± 0.91	0.68 ± 0.06	40.21 ± 5.65
	-	22.93 ± 1.11	573.17 ± 27.68	15.71 ± 0.57	0.72 ± 0.05	45.83 ± 1.69
Hexane: isopropanol (2:1, v/v)	+	26.19 ± 1.10	654.75 ± 27.56	13.90 ± 0.99	0.85 ± 0.04	61.15 ± 5.75
	-	22.71 ± 0.76	567.75 ± 19.05	15.60 ± 0.50	0.72 ± 0.05	46.15 ± 2.14
Hexane: isopropanol (3:2, v/v)	+	31.08 ± 1.55	777.08 ± 38.86	9.82 ± 0.70	0.97 ± 0.05	98.78 ± 8.94
	-	26.16 ± 1.58	653.92 ± 39.55	13.39 ± 0.59	0.85 ± 0.04	63.48 ± 5.47
Hexane: isopropanol (1:2, v/v)	+	23.74 ± 0.71	593.50 ± 17.66	16.90 ± 0.73	0.69 ± 0.07	40.83 ± 2.90
	-	20.35 ± 1.14	508.75 ± 28.38	18.81 ± 0.51	0.66 ± 0.07	35.09 ± 2.86

*Note. FR, biomass freeze-drying; [g/L], represents the mass [g] of lipids, defatted and dried biomass, or chitosan complex obtained per litre of culture; [g/kg], represents the mass [g] of lipids per kilogram of initial, dried biomass or mass [g] of chitosan complex obtained per kilogram of defatted and dried biomass.

A good producer of chitosan is the *M. indicus* strain. The study by Safaei *et al.* [24] showed the maximum chitosan yield of 51% of the cell walls. Other species, such as *M. rouxii* UCP 064 [25] and *M. subtilissimus* UCP 1262 [26], have also shown the ability to produce chitosan, but with lower yields: 62 g/kg and 32.41 g/kg of dry biomass, respectively. The deacetylation degrees for these chitosans were 85 and 80%.

To evaluate the physicochemical properties of chitosan extracted using a hexane:isopropanol mixture (3:2, v/v) from previously lyophilised *M. circinelloides* IBT-83 biomass, the degree of deacetylation (DD) and average molecular weight (Mv) were determined. The DD was assessed using Fourier-Transform Infrared Spectroscopy (FTIR), a widely accepted technique. This method relies on identifying and quantifying characteristic absorption bands corresponding to functional groups in the chitosan structure. Specifically, the ratio of the absorbance of the amide I band (approximately 1655 cm^{-1}) to that of the hydroxyl group band (around 3450 cm^{-1}) is commonly used to estimate the DD [27]. The obtained FTIR spectrum for the chitosan complex derived from *M. circinelloides* IBT-83 is presented in Figure 2.

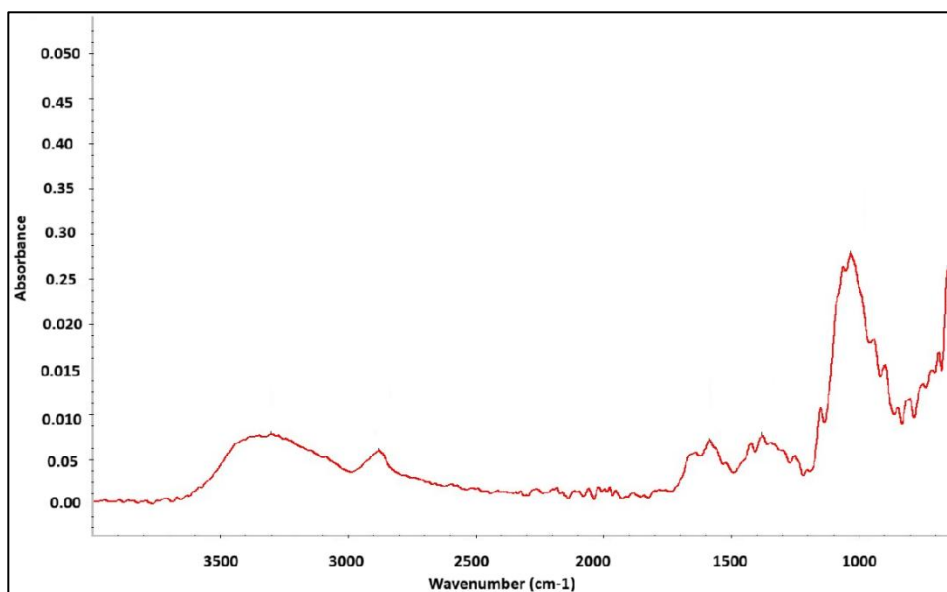


Figure 2. FTIR spectra of chitosan complex extracted from *M. circinelloides* IBT-83 biomass using hexane:isopropanol (3:2, v/v) (freeze-dried biomass).

The characteristic peaks of the tested sample are comparable to those observed in other studies of fungal chitosan [3, 28]. It is important to emphasise that the obtained chitosan requires additional purification to improve its quality; post-purification, the FTIR spectrum should exhibit higher resolution and more distinct peaks. These impurities (pigments, proteins, etc.) can affect the physicochemical properties of chitosan, such as DD, Mv and solubility, which are critical for its intended applications. Based on the FTIR analysis (Figure 2), the DD of the chitosan sample was calculated to be 68%, indicating a low level of deacetylation, which can significantly influence its solubility and biological activity. The obtained DD result should be confirmed in subsequent tests using other methods, e.g. potentiometric titration. FTIR analysis was presented only for this sample because it was considered the most thoroughly defatted.

Furthermore, the M_v determined using the viscometric method as described in Section 2.5 equals 188.7 ± 2.5 kDa, classifying the material as medium molecular weight chitosan. These parameters are essential for determining the potential applications of chitosan in biomedical, pharmaceutical, and environmental fields. It is worth noting that no issues were encountered with the complete dissolution of the chitosan in the solvent system used for analysis.

4. Conclusions

Mucor circinelloides IBT-83 has demonstrated great potential as a sustainable source for the biotechnological production of chitosan. In addition to its well-established ability to accumulate intracellular lipids, this filamentous fungus enables the efficient reuse of defatted biomass as a valuable source of chitosan. The high yield of approximately 100 g per kg of defatted and dried biomass, combined with the organism's capacity to grow on low-cost media and agro-industrial residues, highlights its suitability for industrial-scale applications.

Compared to traditional sources of chitosan, such as crustacean shells, fungal chitosan offers several key advantages: it can be produced under controlled fermentation conditions, its production supports the principles of the circular economy by utilising fermentation by-products, and it is independent of chitin production from shrimp or crab shells. Once purified, this chitosan can be modified to yield a polymer tailored to specific applications. Furthermore, fungal chitosan has been reported to exhibit improved solubility, enhanced antimicrobial activity and favourable molecular weight properties, making it particularly attractive for industrial applications [5].

The chitosan yield from *M. circinelloides* IBT-83 is competitive and potentially advantageous for industrial applications, particularly due to the efficient utilisation of defatted biomass as a feedstock. Importantly, this strain was cultivated on waste-derived substrates, specifically corn steep liquor and waste rapeseed oil, which positively impacts the process's economic and environmental aspects. However, the crude chitosan obtained still requires further purification to remove residual proteins, pigments, and other impurities that may affect its quality and functionality. Future research will focus on optimising the purification protocol and thoroughly characterising the bioactivity and physicochemical properties of the chitosan, which may expand its applicability across pharmaceutical, agricultural, and environmental sectors.

In conclusion, *M. circinelloides* IBT-83 provides an efficient, sustainable and industrially relevant platform for large-scale chitosan production, contributing to waste valorisation and developing high-value bioproducts.

5. References

- [1] Ghormade V, Pathan EK, Deshpande MV; (2017) Can fungi compete with marine sources for chitosan production? *Int J Biol Macromol* 104, 1415–1421. DOI: 10.1016/j.ijbiomac.2017.01.112
- [2] Meyer V, Basenko EY, Benz JP, Braus GH, Caddick MX, Csukai M, de Vries RP, Endy D, Frisvad JC, Gunde-Cimerman N, Haarmann T, Hadar Y, Hansen K, Johnson RI, Keller NP, Kraševc N, Mortensen UH, Perez R, Ram AFJ, Record E, Ross P, Shapaval V, Steiniger C, van den Brink H, van Munster J, Yarden O, Wösten HAB; (2020) Growing a circular economy with fungal biotechnology:

- A white paper. *Fungal Biol Biotechnol* 7(1), 5. **DOI:** 10.1186/s40694-020-00095-z
- [3] Tan YN, Lee PP, Chen WN; (2020) Dual extraction of crustacean and fungal chitosan from a single *Mucor circinelloides* fermentation. *Fermentation* 6(2), 40. **DOI:** 10.3390/fermentation6020040
- [4] Bartnicki-Garcia S; (1968) Cell wall chemistry, morphogenesis, and taxonomy of fungi. *Annu Rev Microbiol* 22(1), 87–108. **DOI:** 10.1146/annurev.mi.22.100168.000511
- [5] Crognale S, Russo C, Petruccioli M, D'Annibale A; (2022) Chitosan production by fungi: Current state of knowledge, future opportunities and constraints. *Fermentation* 8(2), 76. **DOI:** 10.3390/fermentation8020076
- [6] White SA, Farina PR, Fulton I; (1979) Production and isolation of chitosan from *Mucor rouxii*. *Appl Environ Microbiol* 38(2), 323–328. **DOI:** 10.1128/aem.38.2.323-328.1979
- [7] Arcidiacono S, Kaplan DL; (1992) Molecular weight distribution of chitosan isolated from *Mucor Rouxii* under different culture and processing conditions. *Biotechnol Bioeng* 39(3), 281–286. **DOI:** 10.1002/bit.260390305
- [8] Szczęsna-Antczak M, Struszczyk-Świta K, Rzyńska M, Szelał J, Stańczyk Ł, Antczak T; (2018) Oil accumulation and *in situ* trans/esterification by lipolytic fungal biomass. *Bioresour Technol* 265, 110–118. **DOI:** 10.1016/j.biortech.2018.05.094
- [9] Szczęsna-Antczak M, Antczak T, Piotrowicz-Wasiak M, Rzyńska M, Binkowska N, Bielecki S; (2006) Relationships between lipases and lipids in mycelia of two *Mucor* strains. *Enzyme Microb Technol* 39(6), 1214–1222. **DOI:** 10.1016/j.enzmictec.2006.03.008
- [10] Struszczyk K, Szczęsna-Antczak M, Walczak M, Pomianowska E, Antczak T; (2009) Isolation and purification of *Mucor circinelloides* intracellular chitosanolytic enzymes. *Carbohydr Polym* 78(1), 16–24. **DOI:** 10.1016/j.carbpol.2009.04.010
- [11] Struszczyk-Świta K, Kaczmarek MB, Antczak T, Marchut-Mikołajczyk O; (2024) Continuous production of chitooligosaccharides in a column reactor by the PUF-immobilized whole cell enzymes of *Mucor circinelloides* IBT-83. *Microb Cell Fact* 23(1), 258. **DOI:** 10.1186/s12934-024-02529-4
- [12] Struszczyk-Świta K; (2024) The use of enzymes from *Mucor circinelloides* IBT-83 in the synthesis of chitooligosaccharides – preliminary research. *Prog Chem Appl Chitin Deriv* 29, 270–275. **DOI:** 10.15259/PCACD.29.021
- [13] Roberts GAF; (1992) *Chitin Chemistry*. Macmillan, London.
- [14] Struszczyk K, Szczęsna-Antczak M, Pomianowska E, Stańczyk Ł, Wojciechowska J, Antczak T; (2010) Process of continuous production of oligoaminosaccharides in a column reactor. *Prog Chem Appl Chitin Deriv* 15, 177–188.
- [15] Domszy JG, Roberts GAF; (1985) Evaluation of infrared spectroscopic techniques for analysing chitosan. *Die Makromolekulare Chemie*, 186, 1671–1677. **DOI:** 10.1002/macp.1985.021860815
- [16] Carvalho AKF, Rivaldi JD, Barbosa JC, de Castro HF; (2015) Biosynthesis, characterization and enzymatic transesterification of single cell oil of *Mucor Circinelloides* – a sustainable pathway for biofuel production. *Bioresour Technol* 181, 47–53. **DOI:** 10.1016/j.biortech.2014.12.110

- [17] Dong T, Knoshaug EP, Pienkos PT, Laurens LML; (2016) Lipid recovery from wet oleaginous microbial biomass for biofuel production: A critical review. *Appl Energy* 177, 879–895. **DOI:** 10.1016/j.apenergy.2016.06.002
- [18] Bligh EG, Dyer WJ; (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol* 37(8), 911–917. **DOI:** 10.1139/o59-099
- [19] Folch J, Lees M, Stanley GHS; (1957) A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem* 226(1), 497–509. **DOI:** 10.1016/S0021-9258(18)64849-5
- [20] Jiang L, Pan S, Kim JM; (2011) Influence of nitrogen source on chitosan production carried out by *Absidia coerulea* CTCC AF 93105. *Carbohydr Polym* 86(1), 359–361. **DOI:** 10.1016/j.carbpol.2011.04.045
- [21] Fai AEC, Stamford TCM, Stamford-Arnaud TM, Santa-Cruz PD, Silva MCFD, Campos-Takaki GM, Stamford TLM; (2011) Physico-chemical characteristics and functional properties of chitin and chitosan produced by *Mucor circinelloides* using yam bean as substrate. *Molecules* 16(8), 7143–7154. **DOI:** 10.3390/molecules16087143
- [22] de Oliveira CEV, Magnani M, de Sales CV, de Souza Pontes AL, Campos-Takaki GM, Stamford TCM, de Souza EL; (2014) Effects of post-harvest treatment using chitosan from *Mucor Circinelloides* on fungal pathogenicity and quality of table grapes during storage. *Food Microbiol* 44, 211–219. **DOI:** 10.1016/j.fm.2014.06.007
- [23] Zininga JT, Puri AK, Govender A, Singh S, Permaul K; (2019) Concomitant production of chitosan and lipids from a newly isolated *Mucor Circinelloides* ZSKP for biodiesel production. *Bioresour Technol* 272, 545–551. **DOI:** 10.1016/j.biortech.2018.10.035
- [24] Safaei Z, Karimi K, Zamani A; (2016) Impact of phosphate, potassium, yeast extract, and trace metals on chitosan and metabolite production by *Mucor indicus*. *Int J Mol Sci* 17(9), 1429. **DOI:** 10.3390/ijms17091429
- [25] Bento RA, Stamford TLM, Campos-Takaki GM, Stamford TCM, de Souza EL; (2009) Potential of chitosan from *Mucor rouxii* UCP064 as alternative natural compound to inhibit *Listeria monocytogenes*. *Braz J Microbiol* 40(3), 583–9. **DOI:** 10.1590/S1517-838220090003000022
- [26] Amorim RVDS, Souza W, Fukushima K, Campos-Takaki GM; (2001) Faster chitosan production by Mucoralean strains in submerged culture. *Braz J Microbiol* 32, 20–23. **DOI:** 10.1590/S1517-83822001000100005
- [27] Sánchez-Machado DI, López-Cervantes J, Escárcega-Galaz AA, Campas-Baypoli ON, Martínez-Ibarra DM, Rascón-León S; (2024) Measurement of the degree of deacetylation in chitosan films by FTIR, ¹H NMR and UV spectrophotometry. *MethodsX* 12, 102583. **DOI:** 10.1016/j.mex.2024.102583
- [28] Chatterjee S, Adhya M, Guha AK, Chatterjee BP; (2005) Chitosan from *Mucor rouxii*: Production and physico-chemical characterization. *Process Biochem* 40(1), 395–400. **DOI:** 10.1016/j.procbio.2004.01.025