Electronic Journal of Biotechnology 30 (2017) 77–82

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

Research article

Effects of fermentation conditions on valuable products of ethanolic fungus *Mucor indicus*

Shabnam Sharifyazd [a], Keikhosro Karimi [a,b,*]

[a] Department of Chemical Engineering, Isfahan University of Technology, Isfahan 84156-83111, Iran
[b] Industrial Biotechnology Group, Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan 84156-83111, Iran

ARTICLE INFO

Article history:
Received 6 August 2017
Accepted 15 September 2017
Available online 22 September 2017

Keywords:
Biomass
Ethanol
Fungus lipids
Fungus morphology
Glucosamine
Glucose
Glycerol
Morphology
N-acetyl glucosamine
Oil
Zygomycetes

ABSTRACT

Background: Mucor indicus is a dimorphic fungus used in the production of ethanol, oil, protein, and glucosamine. It can ferment different pentoses and hexoses; however, the yields of products highly depend on the nutrients and cultivation conditions. In this study, the effects of different morphologic forms, cultivation time and temperature, presence or absence of oxygen, carbon sources, and concentration of nitrogen source on the products of *M. indicus* were investigated.

Results: The fungus with all morphologies produced high yields of ethanol, in the range of 0.32–0.43 g/g, on glucose. However, the fungus with filamentous morphology produced higher amounts of oil, protein, phosphate, and glucosamine together with ethanol, compared with other morphologies. A higher amount of oil (0.145 g/g biomass) was produced at 28°C, while the best temperature for protein and glucosamine production was 32 and 37°C, respectively. Although ethanol was produced at a higher yield (0.44 g/g) under anaerobic conditions compared with aerobic conditions (yield of 0.41 g/g), aerobic cultivation resulted in higher yields of protein (0.51 g/g biomass), glucosamine (0.16 g/g alkali insoluble material, AIM), and phosphate (0.11 g/g AIM).

Conclusions: It is not possible to have the maximum amounts of the products simultaneously. The fermentation conditions and composition of culture media determine the product yields. Carbon source type and the addition of nitrogen source are among the most influencing factors on the product yields. Moreover, all measured products were made with higher yields in cultivation on glucose, except glucosamine, which was produced with higher yields on xylose.

1. Introduction

Mucor indicus, also as known as *Mucor rouxii*, *Amylomyces rouxii, Chlamydomucor rouxii,* and *Mucor rouxianus*, is a Zygomycetes fungus, recognized in 1665 by Robert Hooke [1]. This dimorphic fungus was originally separated from traditional foods, e.g., tempeh and beers, such as channg (or jnard) from millet and barley in India and Nepal. It can grow on a variety of lignocellulosic sugars, including hexoses and pentoses. *M. indicus* is also capable of producing valuable products such as bioethanol, glucosamine, and polyunsaturated fatty acids, especially gamma-linolenic acid (omega 6) [1,2].

In terms of volume, market, and sustainability, ethanol is the most important product of biotechnology and considered the best substitute for fossil fuels. It can be produced with high yield and productivity by *M. indicus* [3,4]. In addition, the fungus cell wall contains a relatively high concentration of chitosan. Chitosan is a biocompatible, antimicrobial, non-toxic natural polymer. This linear polysaccharide is produced through the chemical deacetylation of *N*-acetyl glucosamine in the fungus. This low-cost biodegradable polymer has many applications, especially in the pharmaceutical, food, and agriculture industries [1,2,5,6,7]. Moreover, the main building block of chitosan is a highly valuable chemical, glucosamine. Glucosamine is required for healing skin injuries and osteoarthritis therapy and nowadays is widely used as a dietary supplement and for medical purposes [8,9].

Another important aspect of *M. indicus* is essential lipids production, which has commercial, pharmaceutical, and nutraceutical importance. These fatty acids include linolenic, linoleic, and oleic acids, which cannot be made in the human body and must be provided by food. Moreover, these lipids have a high potential for biodiesel synthesis [10,11,12]. Furthermore, linolenic acid has an important role in the pharmaceutical industry, especially in making drugs for treating illnesses such as atopic eczema, rheumatoid arthritis, fatty liver, kidney diseases, and multiple sclerosis [10,13].

* Corresponding author.
E-mail address: karimi@cc.iut.ac.ir (K. Karimi).
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.

https://doi.org/10.1016/j.ejbt.2017.09.003

Bioethanol, glucosamine, and oil production have been the subject of several separate studies in recent years by using different microorganisms. *M. indicus* showed a number of advantages, one of which is production of glucosamine and oil in addition to ethanol [2,14,15,16]. It was shown that ethanol and glucosamine yields by this fungus depend highly on the medium's composition [3,17]. However, limited information is available on oil production by this fungus [18,19]. To our knowledge, no data are available in the literature on the improvement of *M. indicus* cultivation, despite its many valuable products.

This study deals with efficient production of major metabolites (i.e., ethanol and glycerol), glucosamine, protein, and oil by *M. indicus*. Effects of different morphologies of *M. indicus*, cultivation time and temperature, presence or absence of oxygen, carbon sources and nitrogen sources of the products were investigated. Moreover, possible improvement in oil extraction was investigated by changing the number of extraction steps, period of sonication, and solvent volume.

2. Materials and methods

2.1. Organisms and growth conditions

M. indicus CCUG 22424 from the Culture Collection, University of Gotëborg, Sweden, was used as the fermenting microorganism in all experiments. The fungal spores were grown on slants containing (g/L) glucose, 40; agar, 16; and peptone, 10, at 32°C for 5 d. Then, the spore suspension was prepared by adding 10 mL of sterile distilled water to each slant and vigorously shaking with a tube shaker. Next, the fungal spores were germinated and grown using a specific amount of spores in 500-mL Erlenmeyer flasks with 250 mL of a liquid mixture containing (g/L) glucose, 50; $CaCl_2$, 1; $MgSO_4.7H_2O$, 0.75; $(NH_4)_2SO_4$, 7.5; and KH_2PO_4, 3.5. The flasks were incubated in a shaking incubator at 32°C and 130 rpm for 48 h. Adding a large amount of spores ($6\ (\pm 3) \times 10^6$ spores/mL) resulted in growing the fungus as purely yeast-like and mostly yeast-like morphology under anaerobic and aerobic conditions, respectively. Lower inoculum levels ($3\ (\pm 1) \times 10^4$ spores/mL) under aerobic conditions caused filamentous growth.

2.2. Biomass determination and lipid extraction

The fungal biomass was centrifuged (4000 rpm, 10 min), washed three times with distilled water, and freeze-dried (Alpha 1-2 LD., Christ, Osterode am Harz, Germany). Then, 1 g of dried biomass was mixed with 20 mL of hexane-ethanol (1:1) and sonicated in an ice ultrasonic bath (SONICA3200L ETH S3, Soltec, Milano, Italy) for 3 min. The solution was then incubated at 32°C with shaking speed of 130 rpm for 2 h. Afterward, the mixture was filtered (Whatman no. 40), 20 mL of fresh solvent was added, and then it was sonicated again in an ice bath. Next, all filtered solutions were mixed together and evaporated at 65°C by a vacuum evaporator. Finally, the oil's weight was determined gravimetrically [19]. Oil extraction of the samples was performed at the optimum conditions of extraction, a 3-stage process with 14-min sonication time and 80-mL solvent. Obtaining the optimum condition was based on preliminary experiments explained in detail in the Supplementary material Figure S1.

2.3. Analytical method

Ethanol and glycerol concentrations were analyzed by high-performance liquid chromatography (HPLC) equipped with UV and RI detectors (Jasco International Co., Tokyo, Japan) using an ion-exchange column (HPX-87H, Bio-Rad Laboratories, Hercules, USA) at 60°C with 0.6 mL/min eluent (5 mM H_2SO_4).

The protein content of the fungal biomass was determined by the Biuret method [20]. The amounts of glucosamine and *N*-acetyl glucosamine were analyzed using colorimetric and HPLC methods, as reported by Zamani et al. [17]. This method was based on a combination of a two-step sulfuric acid hydrolysis and nitrous acid degradation that produces acetic acid and 2,5-anhydromannose. The concentrations of acetic acid and 2,5-anhydromannose were measured by HPLC with an Aminex HPX-87H column (Bio-Rad Laboratories, Hercules, USA) at 60°C with 0.6 mL/min eluent of 5-mM H_2SO_4. The acetic acid was monitored by UV detector (Jasco International CO., Tokyo, Japan), while anhydromannose was measured by the chromatogram provided by RI detector (Jasco International CO., Tokyo, Japan).

The amount of phosphate was also measured by a spectrometric method according to European Standard ISO 6878 [21]. The method was based on measuring the absorbance of the blue complex obtained by mixing the biomass hydrolysate with ammonium molybdate reagent and ascorbic acid at 880 nm.

All experiments in this work were duplicated, and the averages of the two replications are presented. To analyze the data statistically, the analysis of variance (ANOVA) was performed at 5% level of significance ($P < 0.05$) to compare the means of two replications. The statistical analysis results are presented in Supplementary material (**Table S1-S11**).

3. Results

3.1. Effects of morphology

M. indicus was cultured in filamentous, mostly filamentous, mostly yeast-like, and purely yeast-like morphologies. Its morphology was induced by changing the size of the inoculum and the presence of oxygen in the medium. Ethanol, glycerol, and oil extraction yields of various morphologies of this fungus are presented in Fig. 1, while protein, glucosamine, and *N*-acetyl glucosamine contents are shown in Table 1.

M. indicus with filamentous morphology after 48-h cultivation produced 0.32 (g/g glucose consumed) ethanol and 0.032 (g/g glucose consumed) glycerol. In this morphology, the biomass contained 0.51 and 0.14 (g/g biomass) protein and oil, respectively. Furthermore, *N*-acetyl glucosamine, glucosamine and phosphate accounted for 0.21, 0.16, and 0.11 (g/g AIM), respectively.

After 48 h, compared to the filamentous morphology, the amounts of ethanol and glycerol increased in other morphologies, particularly in the purely yeast morphology (**Table S1**). On the other hand, the filamentous morphology contained the highest amounts of protein, *N*-acetyl glucosamine, and glucosamine (**Table S2**). Furthermore, phosphate yield was almost the same in filamentous and mostly filamentous morphologies, while it was higher in yeast-like and mostly yeast-like morphologies (**Table S2**). Moreover, changing the morphology showed significant effects on oil production yield (**Table S1**). The oil yield was considerably lower in the yeast-like and mostly yeast-like morphologies.

As the fungus with filamentous morphology under aerobic conditions produced higher yields of oil, protein, and glucosamine and comparable amounts of ethanol, this morphology was selected for further investigation.

3.2. Effects of cultivation time

The products of *M. indicus* after 24, 48, and 72 h were established (Table 2, Fig. 2). Increasing cultivation time resulted in the production of lower amounts of ethanol and glycerol (**Table S3**). Reduced yields were observed after consuming glucose in the media. Moreover, the amounts of protein, *N*-acetyl glucosamine, glucosamine, and phosphate did not significantly change with fermentation time (**Table S4** and Table 1b) and reached a maximum at 48 h. In addition,

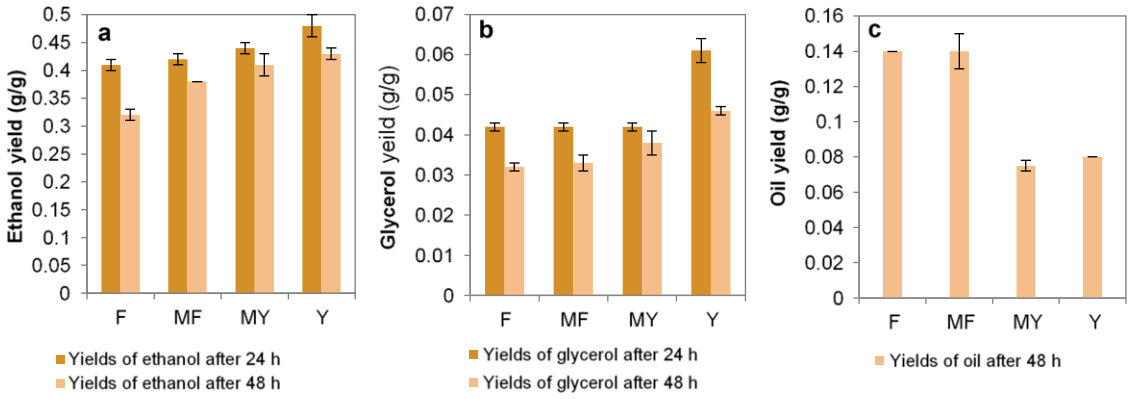

Fig. 1. Effects of *M. indicus* morphology on ethanol, glycerol, and oil production. "F," "MF," "MY," and "Y" are the abbreviation of filamentous, mostly filamentous, mostly yeast, and yeast, respectively. (Ethanol and glycerol yields are calculated as g/g consumed glucose and oil yields are calculated as g/g biomass).

oil production yield was 0.175, 0.14, and 0.155 (g/g biomass) after 24, 48, and 72-h cultivations, respectively (**Table S3**) (Fig. 2).

3.3. Effects of cultivation temperature

M. indicus fungus was cultured at 28, 32, and 37°C, and the products of fermentation have been summarized (Fig. 3, Table 3). Results showed that fermentation temperature affects both fungal growth and ethanol and glycerol production (**Table S5**). The highest amounts of ethanol and glycerol were detected in fermentation at 28°C (Fig. 3), and the highest protein yield was obtained at 32°C (**Table S6**) (Table 3). According to the results, the highest amount of glucosamine was obtained at 37°C, while the highest amounts of *N*-acetyl glucosamine and phosphate were detected at 28 and 32°C, respectively (**Table S6**).

The yield of oil production was in the range of 0.13–0.145 (g/g biomass). There was a minor decrease in the amount of oil with an increase in the temperature from 28 to 37°C (**Table S5**), and the highest amount was detected at 28°C.

3.4. Effects of oxygen

M. indicus consumed all glucose under anaerobic conditions and produced more ethanol and glycerol than under aerobic conditions (**Table S7**) (Table 4). The results show that the amount of protein decreases in anaerobic condition (**Table S7**). *N*-acetyl glucosamine, glucosamine, and phosphate yields were almost the same amount in all cases (**Table S7**). Furthermore, the absence of oxygen resulted in an increase in oil production yield from 0.14 to 0.15 (g/g biomass) under aerobic conditions (**Table S7**).

3.5. Effect of carbon source

M. indicus consumed xylose and produced ethanol. However, the ethanol yield was lower with cultivation on xylose (0.13 g/g sugar consumed) than on glucose (0.32 g/g sugar consumed) (**Table S8**). A similar trend was also observed for glycerol (**Table S8**) (Fig. 4). Moreover, change in carbon sources did not significantly affect the protein, glucosamine, *N*-acetyl glucosamine, and phosphate content (**Table S9**). Glucosamine production increased while *N*-acetyl glucosamine and phosphate production decreased in cultivation on xylose, compared with cultivation on glucose (Table 5). Furthermore, oil production yield declined when the fungus was cultivated on xylose (0.13 g/g biomass) instead of on glucose (0.14 g/g biomass) (**Table S8**) (Fig. 4).

3.6. Effect of nitrogen

Changing nitrogen source concentration created considerable effects on cultivation products (Fig. 5). Maximum ethanol and glycerol yields

Table 1
Protein and cell wall content of different morphologies of *Mucor indicus* biomass.

Morphology	Protein yield[a]	*N*-acetyl glucosamine yield[b]	Glucosamine yield[b]	Phosphate yield[b]
Filamentous	0.51 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.11 ± 0.01
Mostly filamentous	0.48 ± 0.01	0.19 ± 0.02	0.16 ± 0.01	0.12 ± 0.00
Mostly yeast-like	0.52 ± 0.00	0.15 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Yeast-like	0.46 ± 0.00	0.11 ± 0.01	0.12 ± 0.01	0.08 ± 0.01

[a] (g/g biomass).
[b] (g/g AIM).

Table 2
Effects of cultivation time on protein and cell wall contents of *Mucor indicus* with filamentous morphology under aerobic conditions.

Cultivation time (h)	Protein yield[a]	*N*-acetyl glucosamine yield[b]	Glucosamine yield[b]	Phosphate yield[b]
24	0.44 ± 0.02	0.19 ± 0.01	0.12 ± 0.01	0.09 ± 0.01
48	0.51 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.11 ± 0.01
72	0.49 ± 0.01	0.19 ± 0.01	0.11 ± 0.01	0.09 ± 0.01

[a] (g/g biomass).
[b] (g/g AIM).

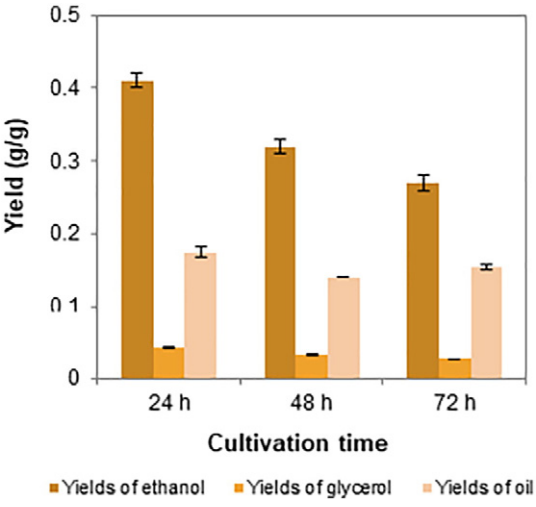

Fig. 2. Yields of ethanol, glycerol, and oil production after different cultivation times for filamentous morphology under aerobic conditions. (Ethanol and glycerol yields are calculated as g/g consumed glucose and oil yields are calculated as g/g biomass).

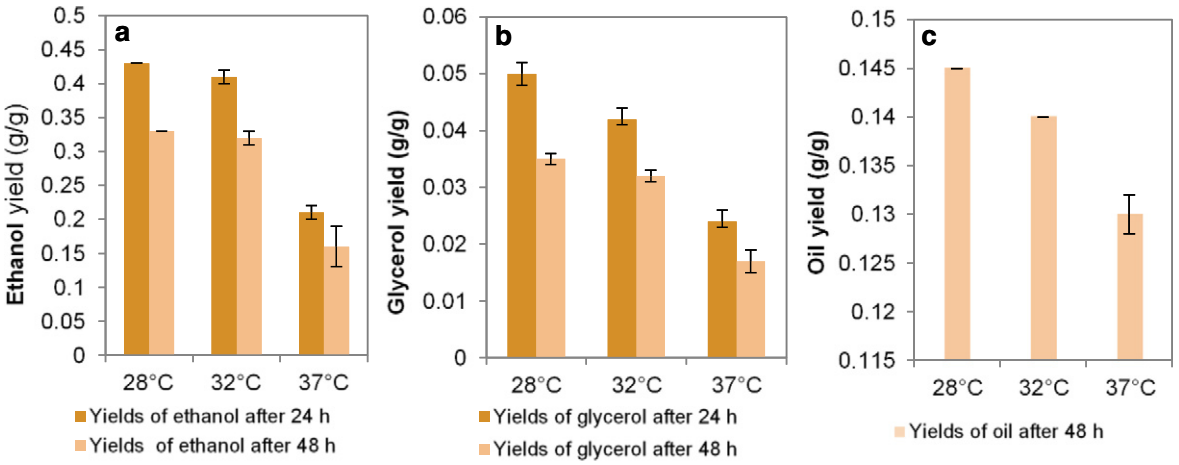

Fig. 3. Effects of cultivation temperature on ethanol, glycerol, and oil production yields by filamentous morphology under aerobic conditions. (Ethanol and glycerol yields are calculated as g/g consumed glucose and oil yields are calculated as g/g biomass).

were obtained at 7.5 (g/L) concentration of ammonium sulfate (**Table S10**). The amount of protein produced depended on the ammonium sulfate concentration, and the highest amount of protein was detected at 7.5 (g/L) ammonium sulfate (**Table S11**) (Table 6). However, *N*-acetyl glucosamine and glucosamine contents increased at 1 (g/L) ammonium sulfate and decreased at higher concentrations (**Table S11**). The change in ammonium sulfate concentration did not significantly affect the phosphate and oil contents (**Table S10–S11**). However, lower nitrogen concentration resulted in higher oil production yield (Fig. 5).

4. Discussion

M. indicus has been reported to produce bioethanol, glucosamine and oil (rich in gamma-linolenic acid) with high yields and productivities [1]. However, in previous studies, the effects of various parameters such as cultivation temperature have been investigated in one or two products of the fungus, while a number of parameters have remained uninvestigated.

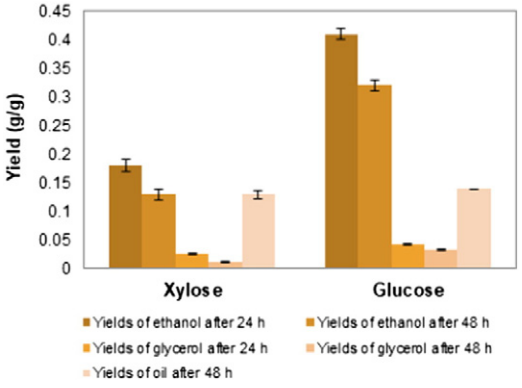

Fig. 4. Effects of carbon source on ethanol, glycerol, and oil production by filamentous morphology under aerobic conditions. (Ethanol and glycerol yields are calculated as g/g consumed sugar and oil yields are calculated as g/g biomass).

In this work, the effects of the most important influencing parameters on simultaneous production of major products of the fungus, i.e., ethanol, glucosamine, and oil, were investigated. These fermentation parameters are morphology, cultivation time and temperature, presence or absence of oxygen, type of carbon source, and concentration of nitrogen. All forms of this fungus can produce ethanol with high yield; however, yeast-like morphology produces higher ethanol yield than other morphologies. Furthermore, in yeast-like and mostly yeast-like morphology, *M. indicus* consumed sugar in a shorter time under both aerobic and anaerobic conditions. Higher concentration of the initial spores necessary to develop the yeast-like morphology caused more carbohydrate consumption. Furthermore, mass transfer is faster in yeast-like morphology than in filamentous morphology, in which diffusion inside the clumps and pellets is the limiting stage [1]. Moreover, differences in the cell wall

Table 3
Effects of cultivation temperature on protein content and cell wall composition of *Mucor indicus* filamentous morphology under aerobic conditions.

Cultivation temperature (°C)	Protein yield[a]	*N*-acetyl glucosamine yield[b]	Glucosamine yield[b]	Phosphate yield[b]
28	0.47 ± 0.00	0.21 ± 0.00	0.13 ± 0.00	0.10 ± 0.00
32	0.51 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.11 ± 0.01
37	0.43 ± 0.01	0.14 ± 0.01	0.28 ± 0.01	0.06 ± 0.00

[a] (g/g biomass).
[b] (g/g AIM).

Table 4
Mucor indicus products in cultivation under aerobic and anaerobic conditions.

Cultivation time (h)	Ethanol yield[a]		Glycerol yield[a]		Protein yield[b]	*N*-acetyl glucosamine yield[c]	Glucosamine yield[c]	Phosphate yield[c]	Oil yield[b]
	24	48	24	48	48	48	48	48	48
Aerobic condition	0.41 ± 0.01	0.32 ± 0.01	0.042 ± 0.001	0.032 ± 0.001	0.51 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.11 ± 0.01	0.14 ± 0.00
Anaerobic condition	0.44 ± 0.01	0.42 ± 0.01	0.045 ± 0.001	0.038 ± 0.001	0.42 ± 0.01	0.24 ± 0.00	0.13 ± 0.01	0.09 ± 0.00	0.15 ± 0.01

[a] (g/g glucose consumed).
[b] (g/g biomass).
[c] (g/g AIM).

Table 5
Effects of carbon source of protein content and cell wall composition of *Mucor indicus* with filamentous morphology under aerobic conditions.

Carbon source	Protein yield[a]	*N*-acetyl glucosamine yield[b]	Glucosamine yield[b]	Phosphate yield[b]
Xylose	0.48 ± 0.01	0.17 ± 0.01	0.22 ± 0.01	0.07 ± 0.00
Glucose	0.51 ± 0.01	0.21 ± 0.00	0.16 ± 0.01	0.11 ± 0.00

[a] (g/g biomass).
[b] (g/g AIM).

Table 6
Effects of ammonium sulfate concentration on protein content and cell wall composition of *Mucor indicus* filamentous morphology under aerobic conditions.

Ammonium sulfate concentration (g/L)	Protein yield [a]	*N*-acetyl glucosamine yield[b]	Glucosamine yield[b]	Phosphate yield[b]
0	0.43 ± 0.02	0.14 ± 0.03	0.22 ± 0.01	0.13 ± 0.01
1	0.48 ± 0.00	0.34 ± 0.02	0.37 ± 0.01	0.10 ± 0.01
4	0.5 ± 0.0	0.28 ± 0.02	0.17 ± 0.01	0.09 ± 0.01
7.5	0.51 ± 0.01	0.21 ± 0.02	0.16 ± 0.01	0.11 ± 0.01
10	0.48 ± 0.01	0.18 ± 0.01	0.15 ± 0.01	0.08 ± 0.01

[a] (g/g biomass).
[b] (g/g AIM).

structure of yeast and filamentous morphology affect the transportation of substrate and fermentation products. The cell wall of the yeast-like form is more diffusible, resulting in better mass transfer [1,2]. Protein analysis showed that the protein yields are almost the same for all morphologies, in line with previous studies [8,9]. However, *N*-acetyl glucosamine and glucosamine were found in higher amounts in the cell wall of the filamentous morphology than in others. This was also observed in previous research [2].

Morphology was the most effective parameter in oil production yield. Change in morphology from filamentous to yeast-like form caused drastic reduction in oil yield. Conditions at which the fungus grows in yeast-like morphology were the least favorable conditions for oil-producing enzymes; however, the reason is not yet determined [1].

After finishing consumption of glucose in the medium, the fungus started to consume the produced ethanol, resulting in lower ethanol yield. Evaporation of a part of the produced ethanol may also increase the ethanol consumption rate. Glycerol showed the same trend as ethanol in all cases. Change in cultivation time did not show significant change in protein, *N*-acetyl glucosamine, or glucosamine production and displayed the highest values after 48 h of cultivation. However, change in fermentation time significantly affected fatty acid production. The highest oil yield was detected after 24 h of cultivation. This is also similar to the results observed in solid-state fermentation by *M. indicus* [22].

M. indicus produced a higher amount of ethanol at 28°C than at 32°C and 37°C. Temperature had significant effects on cell wall components but not on protein content. The fungal cell wall had a higher amount of protein at 32°C than at 28°C and 37°C. Temperatures below 28°C also did not produce higher ethanol production yield [23]. At high temperature (37°C), *N*-acetyl glucosamine content decreased, whereas glucosamine content increased. *N*-acetyl glucosamine and glucosamine contents were the same at 28 and 32°C. There was a minor change in the oil content when the temperature was increased from 37 to 28°C. By decreasing the temperature to 28°C, oil production was increased. The increased oil content at low temperature could be due to the presence of desaturase genes, which receive signals in the cytoplasm membrane. These genes could cause the accumulation of acetyl CoA, which led to the fermentation of unsaturated long chain fatty acids. Moreover, the increase in unsaturated fatty acid could be caused by membrane fluid stability. The results demonstrate that the amounts of produced oil and biomass decrease at temperatures lower than 28°C. The membrane fluid stability of the fungus as an adaptive mechanism to cold environments causes a decrease in the amounts of oil and biomass [8,9,24].

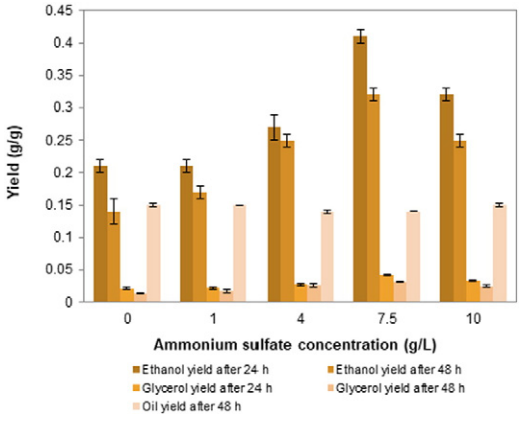

Fig. 5. Effects of ammonium sulfate concentration on ethanol, glycerol, and oil production using filamentous morphology under aerobic conditions. (Ethanol and glycerol yields are calculated as g/g consumed glucose and oil yields are calculated as g/g biomass).

M. indicus produced ethanol and glycerol with higher yield under anaerobic conditions than under aerobic conditions. Furthermore, the fungus cannot consume the produced ethanol under anaerobic conditions, resulting in higher ethanol yields [2]. Under anaerobic conditions, the fungal cells produced less protein, while *N*-acetyl glucosamine and glucosamine were almost constant and oil production increased. Absence of oxygen was found to induce a number of enzymes, including oil-producing enzymes [25].

One of the advantages of *M. indicus* compared with *S. cerevisiae* is its ability to consume xylose and produce ethanol. However, the fungus could uptake xylose only under aerobic conditions, and the yields of different products were significantly different in cultivation on xylose, compared to cultivation on glucose. In cultivation on xylose, protein, glucosamine, and *N*-acetyl glucosamine contents did not considerably change. Moreover, cultivation on xylose resulted in lower oil production, as compared with cultivation on glucose. This was also observed in the cultivation of other strains of zygomycetes, including *M. circinelloides, M. isabellina, M. plumbeus, M. vinacea,* and *Rhizopus oryzae* [26].

Optimization of *M. indicus* cultivation media to obtain the maximum ethanol yield and productivity has been investigated by Sues et al. [3]. Their results showed that nitrogen concentration in the form of ammonium sulfate could significantly increase biomass and ethanol yields; the highest yields were obtained at 7.5 (g/L) of ammonium sulfate. However, they did not study the effects of the nitrogen loading on the protein, oil, and cell wall concentrations. The present research results demonstrated that the maximum amount of protein was also produced at 7.5 (g/L) of ammonium sulfate. The maximum amount of *N*-acetyl glucosamine and glucosamine were obtained at 1 (g/L) ammonium sulfate. This might be due to the adverse effects of a high N/C ratio on *N*-acetyl glucosamine and glucosamine production. No significant change in oil production was detected by changing the ammonium sulfate concentration.

The effects of morphology and other parameters on the oil profile were not investigated in this study, and even the profile of fatty acids is not available in the literature. Moreover, the fungus behavior and its products have not been studied in pilot and commercial scales. These can be the subject of future studies.

5. Conclusions

Production of ethanol, glucosamine, and oil by *M. indicus* highly depends on fungal morphology, cultivation time and temperature, presence or absence of oxygen, and carbon and nitrogen source concentration. The highest ethanol production was obtained with yeast morphology cultivated at 28°C for 24 h under anaerobic conditions on glucose and 7.5 g/L ammonium sulfate. In addition, the best conditions for oil production are filamentous morphology cultivated at 28°C for 24 h under anaerobic conditions. Furthermore, the best conditions for glucosamine production are cultivation using mostly filamentous morphology at 37°C and relatively low concentration of ammonium sulfate concentration (e.g., 1 g/L). Further studies are necessary to investigate the effect of mentioned cultivation conditions and medium composition on the fatty acid profile and oil-producing enzymes.

Conflict of interest

None declared.

Financial support

This work was financially supported by the Research Institute for Biotechnology and Bioengineering, Isfahan University of Technology, Isfahan, Iran.

Acknowledgments

The authors are grateful to Mrs. Maryam Molaverdi and Dr. Safoora Mirmohamadsadeghi, Department of Chemical Engineering, Isfahan University of Technology, for scientific revising and Center of English Language, Isfahan University of Technology, for revising the language of this paper.

Supplementary data

https://doi.org/10.1016/j.ejbt.2017.09.003

References

[1] Karimi K, Zamani A. *Mucor indicus*: Biology and industrial application perspectives: a review. Biotechnol Adv 2013;31(4):466–81. https://doi.org/10.1016/j.biotechadv.2013.01.009.
[2] Sharifia M, Karimi K, Taherzadeh MJ. Production of ethanol by filamentous and yeast-like forms of *Mucor indicus* from fructose, glucose, sucrose, and molasses. J Ind Microbiol Biotechnol 2008;35(11):1253–9. https://doi.org/10.1007/s10295-008-0422-x.
[3] Sues A, Millati R, Edebo L, et al. Ethanol production from hexoses, pentoses, and dilute-acid hydrolyzate by *Mucor indicus*. FEMS Yeast Res 2005;5(6–7):669–76. https://doi.org/10.1016/j.femsyr.2004.10.013.
[4] Achinas S, Euverink GJW. Consolidated briefing of biochemical ethanol production from lignocellulosic biomass. Electron J Biotechnol 2016;23:44–53. https://doi.org/10.1016/j.ejbt.2016.07.006.
[5] Madihally SV. Processing chitosan for tissue regeneration. Curr Trends Polym Sci 2011;15:83–8.
[6] Ribeiro JCV, Vieira RS, Melo IM, et al. Versatility of chitosan-based biomaterials and their use as scaffolds for tissue regeneration. Sci World J 2017;2017:1–25. https://doi.org/10.1155/2017/8639898.
[7] Liu C, Zhang J, Jingli Yue Y, et al. One-pot synthesis of graphene-chitosan nanocomposite modified carbon paste electrode for selective determination of dopamine. Electron J Biotechnol 2014;17(4):183–8. https://doi.org/10.1016/j.ejbt.2014.04.013.
[8] Mohammadi M, Zamani A, Karimi K. Determination of glucosamine in fungal cell walls by high-performance liquid chromatography (HPLC). J Agric Food Chem 2012;60(42):10511–5. https://doi.org/10.1021/jf303488w.
[9] Mohammadi M, Zamani A, Karimi K. Effect of phosphate on glucosamine production by ethanolic fungus *Mucor indicus*. Appl Biochem Biotechnol 2013;171(6):1465–72. https://doi.org/10.1007/s12010-013-0440-7.
[10] Somashekar D, Venkateshwaran G, Sambaiah K, et al. Effect of culture conditions on lipid and gamma-linolenic acid production by mucoraceous fungi. Process Biochem 2003;38(12):1719–24. https://doi.org/10.1016/S0032-9592(02)00258-3.
[11] Kot AM, Blazejak S, Kurcz A, et al. Effect of initial pH of medium with potato wastewater and glycerol on protein, lipid and carotenoid biosynthesis by *Rhodotorula glutinis*. Electron J Biotechnol 2017;27:25–31. https://doi.org/10.1016/j.ejbt.2017.01.007.
[12] Fu CC, Su CH, Nair GR, et al. Estimation of fungal biomass and lipid production by morphological characteristics of *Mucor rouxii*. J Biosci Bioeng 2010;110(3):367–71. https://doi.org/10.1016/j.jbiosc.2010.04.003.
[13] Nettleton JA. Omega-3 fatty acids and health. 1rd ed. New York: Chapman and Hall; 1995.
[14] Lee SE, Kim YO, Choi WY, et al. Two- step process using immobilized *Saccharomyces cerevisiae* and *Pichia stipitis* for ethanol production from Ulva pertusa Kjellman hydrolysate. J Microbiol Biotechnol 2013;23(10):1434–44. https://doi.org/10.4014/jmb.1304.04014.
[15] Tang H, Hou J, Shen Y, et al. High β-glucosidase secretion in *Saccharomyces cerevisiae* improves the efficiency of cellulase hydrolysis and ethanol production in simultaneous saccharification and fermentation. J Microbiol Biotechnol 2013;23(11):1577–85. https://doi.org/10.4014/jmb.1305.05011.
[16] Millati R, Karimi K, Edebo L, et al. Ethanol production from xylose and wood hydrolyzate by *Mucor indicus* at different aeration rates. BioResources 2008;3(4):1020–9.
[17] Zamani A, Jeihanipour A, Edebo L, et al. Determination of glucosamine and *N*-acetyl glucosamine in fungal cell walls. J Agric Food Chem 2008;56(18):8314–8. https://doi.org/10.1021/jf801478j.
[18] Ruenwai R, Cheevadhanarak S, Laoteng K. Overexpression of acetyl-CoA carboxylase gene of *Mucor rouxii* enhanced fatty acid content in *Hansenula polymorpha*. Mol Biotechnol 2009;42(3):327–32. https://doi.org/10.1007/s12033-009-9155-y.
[19] Mitra D, Rasmussen ML, Chand P, et al. Value-added oil and animal feed production from corn-ethanol stillage using the oleaginous fungus *Mucor circinelloides*. Bioresour Technol 2012;107:368–75. https://doi.org/10.1016/j.biortech.2011.12.031.
[20] Verduyn C, Postma E, Scheffers WA, et al. Physiology of *Saccharomyces cerevisiae* in anaerobic glucose-limited chemostat cultures. J Gen Microbiol 1990;136:395–403. https://doi.org/10.1099/00221287-136-3-395.
[21] Naghdi M, Zamani A, Karimi K. A sulfuric-lactic acid process for efficient purification of fungal chitosan with intact molecular weight. Int J Biol Macromol 2014;63:158–62. https://doi.org/10.1016/j.ijbiomac.2013.10.042.
[22] Jangbua P, Laoteng K, Kitsubun P, et al. Gamma- linolenic acid production of *Mucor ruxii* by solid-state fermentation using agricultural by-products. Lett Appl Microbiol 2009;49(1):91–7. https://doi.org/10.1111/j.1472-765X.2009.02624.x.
[23] Torija MJ, Rozes N, Poblet M, et al. Effects of fermentation temperature on the strain population of *Saccharomyces cerevisiae*. Int J Food Microbiol 2003;80(1):47–53. https://doi.org/10.1016/S0168-1605(02)00144-7.
[24] Mamatha SS, Ravi R, Venkateswaran G. Medium optimization of gamma linolenic acid production in *Mucor rouxii* CFR-G15 using RSM. Food Bioproc Tech 2008;1(4):405–9. https://doi.org/10.1007/s11947-008-0103-9.
[25] Jeennor S, Laoteng K, Tanticharoen M, et al. Comparative fatty acid profiling of *Mucor rouxii* under different stress conditions. FEMS Microbiol Lett 2006;259(1):60–6. https://doi.org/10.1111/j.1574-6968.2006.00242.x.
[26] Zheng Y, Yu X, Zeng J, et al. Feasibility of filamentous fungi for biofuel production using hydrolysate from dilute sulfuric acid pretreatment of wheat straw. Biotechnol Biofuels 2012;5:1–10. https://doi.org/10.1186/1754-6834-5-50.