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Valentyna Motronenko[1]
Luidmyla Ruzhynska[2]
Vitalii Chumak[3]
Oleksandr Galkin[4]

EVALUATION OF MECHANICAL AGITATION EFFECT ON MICROSCOPIC FILAMENTOUS FUNGI CULTURING EFFICACY

EVALUATION OF MECHANICAL AGITATION EFFECT ON MICROSCOPIC FILAMENTOUS FUNGI CULTURING EFFICACY

[1,2,4]National Technical University of Ukraine "Kyiv Polytechnic Institute"
37, Peremohy Av., Kyiv, 03056, Ukraine
[3]National Aviation University
1, Kosmonavta Komarova Av., Kyiv, 03680, Ukraine
E-mails: [1]motronenko_valya@i.ua; [2]ruzhli@ukr.net; [3]chumak.vl@gmail.com; [4]alexfbt@mail.ua

Abstract

Goal: *to review the existing literature about the effects of agitation modes on viability and productivity of filamentous fungi in submerged cultivation conditions in order to select the cultivation mode with the resulting maximum product yield for different types of micromycetes. To find relationship between agitation speed, cell morphological structure of the filamentous fungi and their ability to biomass accumulation and synthesis of metabolites.* ***Methods:*** *A number of articles describing the agitation modes used in laboratory fermenters with mechanical agitation devices were analysed. The experiments were carried out for the selected agitator type by variation of agitation device rotation frequency in the apparatus. Measurements of accumulated biomass and synthesized metabolites was carried out after a specified period of time throughout the period biosynthesis .****Results:*** *The analysis showed that mechanical agitation intensity in the submerged cultivation plays an important role in productivity and morphological structure of the filamentous fungi. The recommended average stirrer rotation frequency is 120-180 rpm. A direct relationship between agitation speed between cell structure and level of synthesized metabolites of micromicetes was found but studies which would describe the mechanism of agitation of the cells of filamentous fungi were not revealed.*

Keywords: agitation intensity; filamentous fungi; mechanical agitation; morphological structure; submerged cultivation.

1. Statement of the problem

Nowadays it is difficult to imagine biotechnological industry without the use of submerged microbial cultivation in liquid medium. Year by year, this method becomes more and more widely used in cultivation of filamentous fungi (basidiomycetes), which are the producers of antibiotics, organic acids, etc. [1].

Agitation effect on microorganisms plays an important role in submerged cultivation, therefore agitation mode has to be selected to assure maximum final product yield, In this case the microbial cells receives the necessary quantities of micro- and macronutrients as well as oxygen contained in the nutrient medium, and the high cell viability is maintained [1-2].

The agitation for submerged cultivation of filamentous fungi is of the high importance as it ensures the selection of their optimal cultivation regimen which is a rather complicated task due morphological structure, in particular, mycelium branching. Submerged cultivation method has several advantages comparing to the surface one. In particular, mechanical agitation and continuous aeration create favorable conditions for the access of all microbial cells to nutrients and oxygen assuring equally favorable conditions for growth and accumulation of metabolic products [1].

2. Study goal

The study was aimed at the comparison of the effect of agitation modes based on the existing literature and selection of optimal agitation speed used in industrial and laboratory conditions during submerged cultivation of filamentous fungi. Whenever possible, investigation of the mechanism of agitation effect in submerged cultivation conditions on morphological structure of filamentous fungi cells, on their viability and productivity was studied. Identification of specific morphological structure of cells in various conditions of biotechnological synthesis, in particular, in surface cultivation and submerged cultivation with and without mechanical agitation was carried out.

3. Review of literature

The performed literature review shows that agitation intensity has essential effects on filamentous fungi growth and biomass accumulation capability. Depending on fungal morphological particulars, agitation rotation frequency varies between 50 and 900 rpm (see table 1) [3-14]. The average rotation frequency of the stirring device in filamentous fungi submerged cultivation is within the range of 120-180 rpm.

Table 1. Agitation conditions for the producer species used [3-17]

No.	Producer	Stirrer rotation speed, rpm
1	*Aspergillus awamori* - 460	48-300
2	*Fusarium sambucinum*	600-900
3	*Ganoderma lucidum*	50
4	*Beauveria bassiana*	180
5	*Eremothecium ashbyi Guilliermond* F340	150
6	*Laetiporus sulphureus ma Polyporus*	120-150
7	*Schirophyllum commune*	180-190
8	*Grifola frondosa*	60-70
9	*Laetiporus sulphureus*	120
10	*Trichoderma virens* BKMF-1117	200
11	*Chlorella vulgaris* C-1	180
12	*Aspergillus niger* A12	400-700
13	*Aspergillus niger* NRRL-3	200-800
14	*Thermomyces lanuginosus* RT9	100-300

1.3 l BioFlo 110 devices (manufactured in USA) with open-type double deck turbine agitator, of 55 mm in diameter, were used for investigation of agitation intensity effect on *Fusarium sambucinum* growth [4]. The curve in Figure 1 shows that the most intensive biomass accumulation occurs at 700 rpm, which is in conformity of linear agitation speed 2 m/s. The further increase of agitation speed results in higher biomass accumulation activity and active sporogenesis [4].

Ustinnikov B.A. et all [3] presented the relevant relation of enzyme produced by *Aspergillus awamori* for biosynthesis of glucoamylase. The experiments were carried out in industrial conditions with use of two 5 m^3 fermenters, equipped with single-deck turbine agitators of 600 mm in diameter (the apparatus diameter is 2000 mm), bubbler, and 4 defoaming baffles, in parallel. The rotation frequencies of agitator shaft varied between 48 and 300 rpm. The curve in Fig. 2 shows that the maximum enzyme yield is observed at specific mechanical power 6 kW/m^3. The further increase of agitation intensity results in decreased synthesis due to mechanical damage of fungal mycelium. Besides, it has been established that upon agitation intensity less than 2 kW/m^3 the fungal mycelium did not

receive sufficient quantity of oxygen for active biomass accumulation and enzyme synthesis [3].

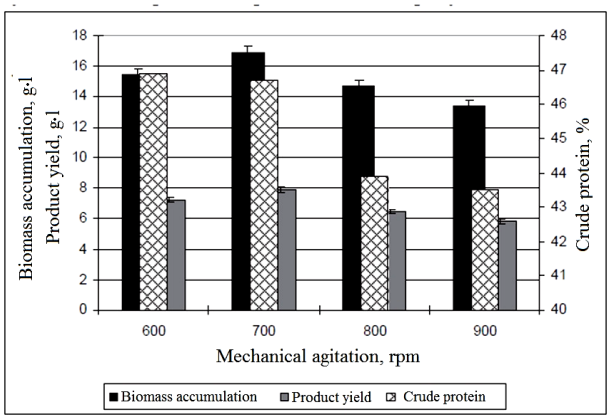

Fig. 1. The effect of agitation intensity on *Fusarium sambucinum* growth [4]

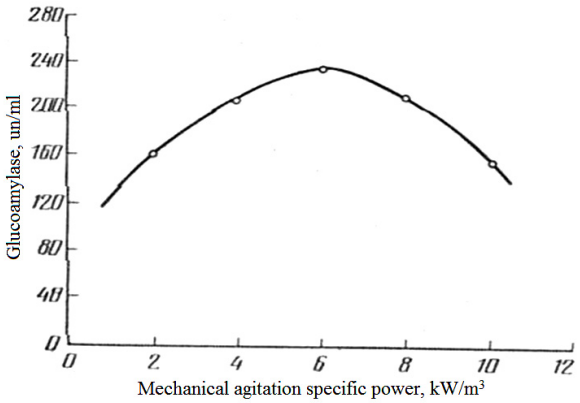

Fig. 2. The interrelation between glucoamylase biosynthesis by *Aspergillus awamori* and mechanical agitation specific power [3]

It has been also established that agitation intensity affects the change of culture fluid viscosity, (see Figure 3). It probably happens because the agitation mode affects first of all mycelium structure, i.e. its morphological characteristics. As appeared, the viscosity decreased in 30-80 hours since the beginning of fermentation. This period is in conformity to formation of extremely branched mycelium hyphae: the lower agitation intensity is, the more such colonies are formed. Upon the increase of agitation intensity viscosity increases, and the trend to shortened hyphae formation is observed. Besides, the same curve shows that the increase of specific mechanical power used for agitation results in the lower increase of viscosity [3].

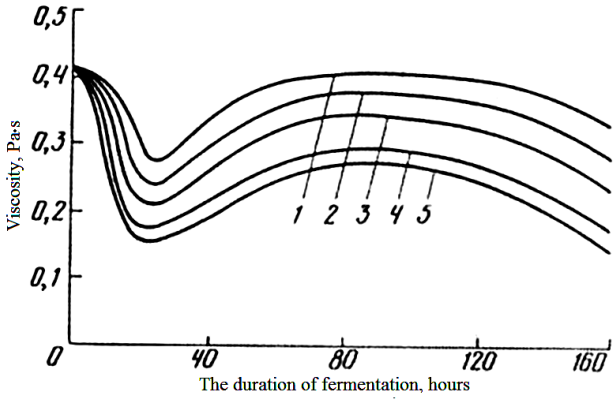

Fig. 3. Culture fluid viscosity versus agitation intensity [3]: the digits in Fig.3 indicate mechanical agitation specific power: 1 – 2 kW/m^3; 2 – 3 kW/m^3; 3 – 4 kW/m^3; 4 – 5 kW/m^3; 5 – 6 kW/m^3

During the deep cultivation of *Aspergillus niger* to obtain enzymes endohlyukonazy and ksylazy the experiments were conducted for two agitation speeds - 400 rpm and 700 rpm. In previously untreated sugar cane medium maximum biomass accumulation and synthesis enzymes were observed at a frequency of rotation of the agitators device 400 rpm with the use of mixed mode of cultivation (sequential alternation of superficial and deep methods), and at 700 rpm no signs of fungal growth was observed (see Figure 4). But on the treated sugar cane medium the results were quite opposite, showing that the maximum increase in biomass synthesis and endohlyukonazy and ksylazy was observed when speed agitators device was 700 rpm (see Figure 5) [15].

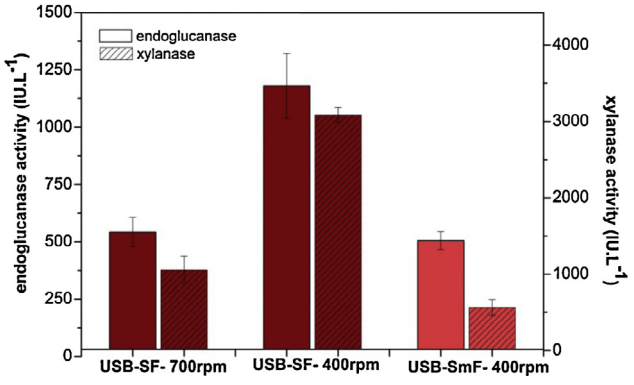

Fig. 4. The dependence of activity of synthesis enzymes of the fungus *Aspergillus niger* on not nutrient medium with crude sugarcane [15]: USB - no nutrient medium for refined sugar cane; SF - consistent use of superficial and deep cultivation; SmF - the usual deep cultivation

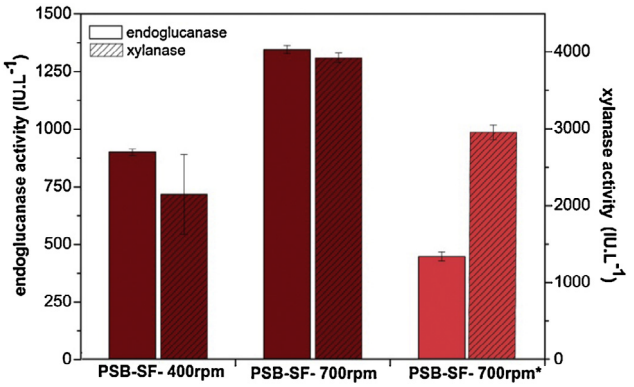

Fig. 5. The dependence activity of synthesis enzymes the fungus Aspergillus niger on nutrient medium with crude sugarcane: PSB – nutrient medium for refined sugar cane; SF - consistent use of superficial and deep cultivation;; * – cultivation without pH contro

When cultured strain of another *Aspergillus niger*, synthesizing hlyukooksydazu (deep method) agitation speeds of 200, 500 and 800 rpm were investigated. The maximum biomass accumulation was observed at 200 rpm and amounted to 30 g/l at 500 rpm, this figure dropped to 25 g/l, and at 800 rpm decreased twofold up to 15 g/l. But the maximal accumulation of enzyme hlyukooksydazy happened at agitation speed mixing device 500 rpm and was 800 mg/l. At higher agitation speeds (800 rpm) in the cultivation of nakypylosya only 600 mg/l enzyme accumulated and at 200 rpm, this figure fell twofold and was 300 rpm. It is necessary to note that upon the 200 and 500 rpm accumulation of enzyme originally occurred within the cell, but after a while, it was found in the culture fluid [16].

The experiments for the agitation effects on the synthesis of the fungus *Thermomyces lanuginosus* enzyme ksylazy were performed at rotation frequency mixing device 100, 200 and 300 rpm. The maximum output of the enzyme and biomass accumulation observed at 200 rpm. At 100 rpm biomass accumulation is less than in the previous case. This is due to lack of oxygen that enters into the cells of the fungus. Reduced the concentration of biomass is observed with increasing agitation speed of 300 rpm. In this case, the oxygen supply was at an appropriate level, but there have been mechanical damage of mushroom cells [17].

Kiseleva O.V. et all [5] compares the conditions of cultivation and morphological properties of *Laetiporus sulphureus* (sulphur polypore) depending on its cultivation conditions. Surface cultivation on wort agar lasted 7 days, and the submerged cultivation in bioreactors with or without mechanical agitation and with aeration for 48-72 hours. Microscopic experiments show that the mycelium in surface culture is comprised of long septate hyphae with simple branching, without formation of mycelial buckles (Fig. 4) [5].

In submerged conditions, the mycelium is capable of forming thick-walled chlamydospores (Fig. 5). Various morphological structures are present in culture fluid – colonies with flat center and peripheral mycelium, ball- and pear-like masses, filiform fragments. The biomass accumulation occurs faster in the submerged cultivation [5]. The submerged cultivation and the absence of mechanical agitation (Fig. 6, 7) result in formation of distinctly fragmented separately located structures with branched margins due to peripheral mycelial build-up [5].

Under submerged cultivation and mechanical agitation B (Fig. 8, 9), the mycelium is fragmented into relatively small segments preserving the ability to propagation and biomass accumulation; besides, considerable accumulation of spore material can be seen with the focused beam microscope. From this it can be concluded that it is expedient way of growing seeds for further fermentation. [3]

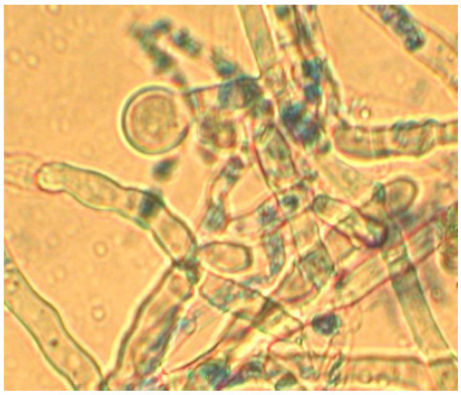

Fig. 4. Optical microphotograph of *Laetiporus sulphureus* fungi upon surface cultivation [5]

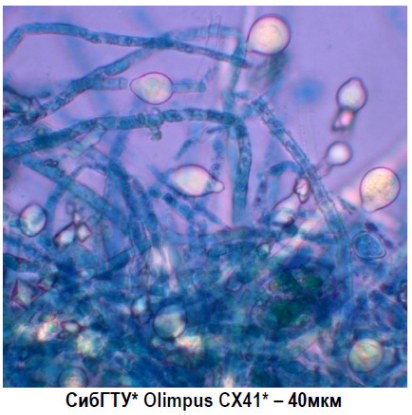

Fig.5. Optical microphotograph *Laetiporus sulphureus* fungi upon submerged cultivation [5]

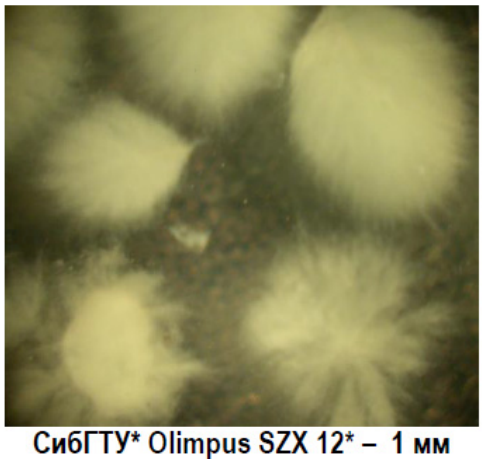

Fig. 6. Optical microphotograph of *Laetiporus sulphureus* fungi cultivated without mechanical agitation (glubules with flat center and peripheral mycelium) [5]

Fig. 7. Optical microphotograph of *Laetiporus sulphureus* fungi without mechanical agitation (biomass after cultivation) [5]

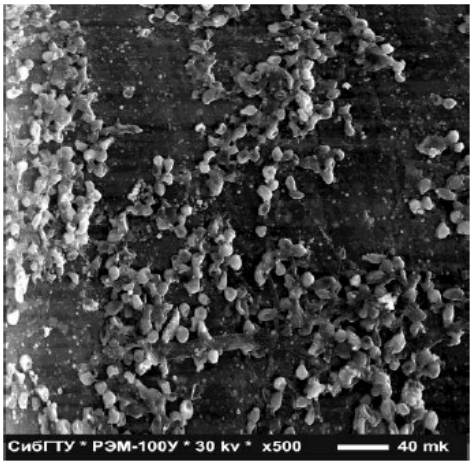

Fig. 8. Optical microphotograph of *Laetiporus sulphureus* upon submerged cultivation with mechanical agitation (magnification 500x) [3]

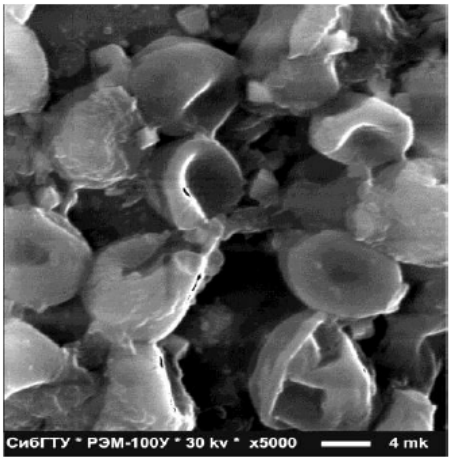

Fig. 9. Optical microphotograph of *Laetiporus sulphureus* upon submerged cultivation with mechanical agitation (magnification 5000x) [3]

4. Conclusion

Comparison of the studies of Ukrainian and international scientists on the effects of agitation growth and development of micromicetes showed that selection of mechanical agitation intensity for culturing depends on the fungus strains selected for experiments. The graphic presentation of nature of this influence has a nonlinear form. It was shown that rotation frequency of mixing device in the biosynthesis varies between 50 and 700 rpm, and is recommended 120-180 rpm for practical use in industry.

The detailed review of results of Kiseleva O.V. et all [5] may lead to the conclusion that cultivation method has essential effect on filamentous fungi morphological structure; the fungi cultivated in submerged culture with presence of mechanical agitation are capable of accumulation of spore material in higher amounts.

Reviewing the literature in this area of research shows the lack of studies aimed at investigation of the mechanism of impact on cells of mycelium fungus of various mixing devices, namely, their rotation frequencies and impeller design. Well studied impact of these devices will simplify the selection of optimal culture conditions and will affect the ability of microorganisms to accumulate biomass and metabolite synthesis. Thus, it is important to investigate the mechanisms of influence of mechanical agitation on the cells of microorganisms upon stress conditions which cells undergo and build a mathematical model of hydrodynamic processes in the fermenter.

5. References

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В.В. Мотроненко[1], Л.І. Ружинська[2], В.Л. Чумак[3], О.Ю. Галкін[4]

Аналіз впливу механічного перемішування на ефективність культивування мікроскопічних міцеліальних грибів

[1,2,4]Національний технічний університет України "Київський політехнічний інститут", пр. Перемоги, 37, м. Київ, 03056, Україна
[3]Національний авіаційний університет, пр. Космонавта Комарова, 1, м. Київ, 03680, Україна
E-mails: [1]motronenko_valya@i.ua; [2]ruzhli@ukr.net; [3]chumak.vl@gmail.com; [4]alexfbt@mail.ua

Мета: За існуючими літературними даними провести порівняльний аналіз впливу режимів перемішування на життєздатність та продуктивність міцеліальних грибів при глибинному культивуванні. А також підібрати режим культивування при якому вихід кінцевого продукту буде максимальним. **Методи:** У розглянутих статтях проводили дослідження режимів перемішування в лабораторних ферментерах з механічними перемішуючими пристроями. Досліди проводили для обраного типу мішалки, змінюючи частоту обертання перемішуючого пристрою в апараті. **Результати:** З проведеного аналізу видно, що важливу роль при глибинному культивуванні відіграє інтенсивність механічного перемішування. В середньому, кількість обертів мішалки становить 120-180 об/хв.

Ключові слова: глибинне культивування, інтенсивність перемішування, механічне перемішування, міцеліальні гриби, морфологічна будова.

В.В. Мотроненко[1], Л.И. Ружинскя[2], В.Л. Чумак[3], А.Ю. Галкин[4] **Анализ влияния механического перемешивания на эффективность культивирования микроскопических мицелиальных грибов**

[1,2,4]Национальный технический университет Украины "Киевский политехнический институт", пр. Победы, 37, г. Киев, 03056, Украина
[3] Национальный авиационный университет, просп. Космонавта Комарова,1, Киев, Украина, 03680
E-mails: [1]motronenko_valya@i.ua; [2]ruzhli@ukr.net; [3]chumak.vl@gmail.com; [4]alexfbt@mail.ua

Цель: За существующими литературными данными провести сравнительный анализ влияния режимов перемешивания на жизнеспособность и производительность мицелиальных грибов при глубинном культивировании. А также подобрать режим культивирования при котором выход конечного продукта будет максимальным. **Методы:** В рассмотренных статьях проводили исследования режимов перемешивания в лабораторных ферментерах с механическими перемешивающими устройствами. Опыты проводили для выбранного типа мешалки, изменяя частоту вращения перемешивающего устройства в аппарате. **Результаты:** С проведенного анализа видно, что важную роль при глубинном культивировании, важная роль принадлежит интенсивности механического перемешивания. В среднем, количество оборотов мешалки составляет 120-180 об/мин.

Ключевые слова: глубинное культивирование, интенсивность перемешивания, механическое перемешивание, мицелиальные грибы, морфологическое строение.

Motronenko Valentyna. Postgraduate Student.
Department of Industrial Biotechnology, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Kyiv Polytechnic Institute", Kyiv, Ukraine
Education: National Technical University of Ukraine "Kyiv Polytechnic Institute", 2012.
Research area: study heat and mass transfer processes in the submerged cultivation of microorganisms.
E-mail: motronenko_valya@i.ua.

Ruzhynska Luidmyla. PhD, Associate Professor.
Department of Bioengineering and Biotechnics, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Kyiv Polytechnic Institute", Kyiv, Ukraine
Education: National Technical University of Ukraine "Kyiv Polytechnic Institute", 1973.
Research area: mathematical modeling of heat and mass transfer processes, pharmaceutical and biotechnology industries.
E-mail: ruzhli@ukr.net.

Chumak Vitalii. Doctor of Chemical Sciences, Professor.
National Aviation University, Kyiv, Ukraine.
Education: National Technical University of Ukraine "Kyiv Polytechnic Institute",
Research area: physico-chemical basis of chemical and bioengineering.
E-mail: chumak.vl@gmail.com.

Galkin Oleksandr. Doctor of Biological Sciences, Associate Professor.
Department of Industrial Biotechnology, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Kyiv Polytechnic Institute", Kyiv, Ukraine
Education: National Technical University of Ukraine "Kyiv Polytechnic Institute", 2004.
Research area: industrial and pharmaceutical biotechnology.
E-mail: alexfbt@mail.ua.