

## INFLUENCE OF HYDRODYNAMIC PARAMETERS ON THE SYNTHESIS OF TARGET METABOLITES AND THE DEGREE OF DISINTEGRATION DURING THE SUBMERGED CULTIVATION OF MICROMYCETES

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### ABSTRACT

One of the limiting parameters of the mycelial fungi submerged cultivation for the synthesis of metabolites used for medical, food and industrial purposes is the hydrodynamic conditions of submerged cultivation.

The effect of mechanical stirring rate on the yield of metabolites was investigated on the example of cultivation of mycelial fungi *Aspergillus awamori* – glucoamylase producer. It was found that the maximum yield of the enzyme was observed at the stirring speed of turbine impeller amounting to 60-120 rpm. As the stirring speed increased, the amount of synthesized metabolite decreased, which is a consequence of the destruction of the mycelial cells. The connection between the decrease in glucoamylase synthesis and the rejuvenation of the culture caused by its recovery from fragments of the destroyed mycelium and the subsequent increase in the amount of biomass was shown. At the same time, the degree of destruction was shown by the increase in the amount of nucleic acids in the filtrate.

The relationship between the degree of destruction of mycelial cells, determined by the amount of nucleic acids in the filtrate under extreme conditions of ultrasonic disintegration, and the viability of the culture was also demonstrated. The amount of viable cells increased at first (until the saturation of the culture fluid with nucleic acids), and then began to gradually decrease. The obtained results can serve as a starting point for further studies of the possibility of stimulating the primary accumulation of biomass of micromycetes by ultrasound.

**Keywords:** mycelial fungi, synthesis of metabolites, submerged cultivation, degree of disintegration, stirring, ultrasound

### INTRODUCTION

Mycelial fungi are one of the most common producers in biotechnology. They are widely used for the production of antibiotics, organic acids, enzymes, etc. (Grimmet *et al.*, 2005). One of the main advantages of this group of producers is their ability to release metabolic products into the culture broth. Also, they are undemanding to the culture media, cultivation conditions, except for mechanical influence (Müller *et al.*, 2021).

The influence of hydrodynamic conditions on mycelial fungal cells during submerged cultivation is one of the limiting factors of the biosynthesis of target products (Silva-Santisteban *et al.*, 2005; Lutsenko *et al.*, 2017; Gu *et al.*, 2018; Abdella *et al.*, 2020). This is due to the influence of heat and mass transfer, in particular aeration and mechanical stirring, on the metabolism of the producer, and the possible violation of the integrity of the mycelium, which is a consequence of the above processes, which causes a delay in the development stage of the culture in which the accumulation of the target product occurs (Abdella *et al.*, 2020; Lv *et al.*, 2017; Shin *et al.*, 2017). Most often, the impact due to mechanical disintegration is created in the processes of external and internal friction of solids and liquids, which are characterized by high flow velocities in the contact areas, in particular at the points of contact of the stirrer with cells (Kudriavtsev *et al.*, 1988). That is why the process requires a more detailed study both theoretically and experimentally.

There are a number of studies that confirm that at high intensities of hydrodynamic processes in the fermenter, mycelial cells can change their shape and size, which leads to a decrease in their growth rate and reduction of the amount of synthesized metabolites. In some cases, this result is associated with shear stresses that occur in cells in the case of significant mechanical impacts (Silva-Santisteban *et al.*, 2005; Gu *et al.*, 2018; Abdella *et al.*, 2020; Lv *et al.*, 2017; Shin *et al.*, 2017; Serbov *et al.*, 2019; Motronenko *et al.*, 2017). However, the regularities of the influence of the hydrodynamic parameters of the cultivation process in the presence of mechanical stirring and the degree of destruction of producer cells, their ability to accumulate biomass and synthesis of primary and secondary metabolites have not been precisely investigated.

Considering the importance of intensifying the production of target biological products by such important producers as micromycetes, the investigation of the influence of stirring speed in the fermenter, namely, the stirring rate by mechanical stirring devices during submerged cultivation of filamentous fungi on the degree of destruction, accumulation of biomass and biosynthetic ability of producers is a topical issue.

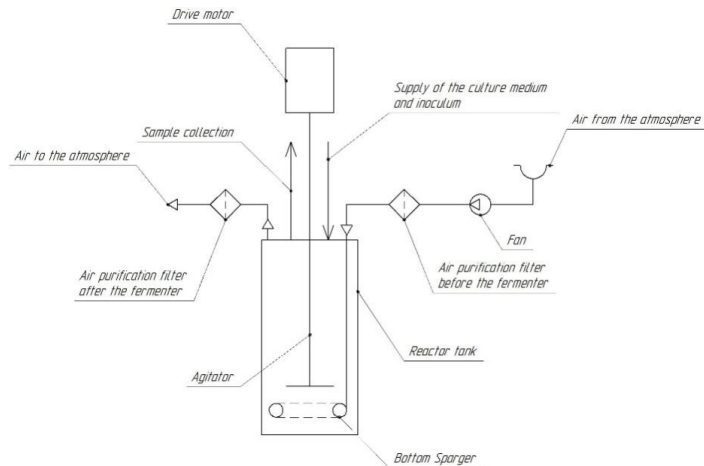
Therefore, the purpose of our work was to study the patterns of the influence of intensive hydrodynamic processes on the during the fermentation of micromycetes on the degree of cell destruction in the process of cultivation and synthesis of metabolic products. To achieve this goal, the degree of destruction was assessed by the amount of nucleic acids in the supernatant of the culture broth, and the synthesis of metabolic products was assessed by the activity of the enzyme glucoamylase.

### MATERIAL AND METHODS

Experiments on the effect of hydrodynamic parameters during submerged cultivation of micromycetes were carried out on the example of the strain *Aspergillus awamori* 120/177 – a producer of the enzyme glucoamylase. For modelling the process, a fermenter (Fig. 1) with mechanical stirring with a volume of 1.7 l equipped with a turbine stirrer was used, into which a 7-day culture (culture was washed from the surface with distilled water), of inoculum grown in test tubes on slant agar (with a calculation: inoculum from 1 test tube per 200 ml of culture medium) and 1 L of culture medium (liquid Czapek medium with starch) were added the filling factor of the fermenter is 0.7. The type of stirrer was chosen based on the standard recommendations for the use of stirrers of different types depending on the properties of the liquid in the fermenter (Serbov *et al.*, 2019; Motronenko *et al.*, 2017; Motronenko *et al.*, 2020) and known technologies of micromycetes submerged cultivation (Grimm *et al.*, 2005).

Cultivation was carried out for seven days at a constant temperature of 28 °C with aeration with compressed air supplied at a excess pressure of 70 mm Hg (9.33·10<sup>3</sup> MPa) in three repetitions for four different stirring rates: 60, 120, 240 and 480 rpm. The range of studied stirring rates was chosen based on studies conducted by various authors working in this field (Motronenko *et al.*, 2017) and were identified as the optimal parameters for cultivating this type of producers in previous works

(Serbov *et al.*, 2019). In addition to the optimal hydrodynamic parameters of fermentation, which according to literature sources were determined in the range from 120 to 240 rpm, the producer was cultivated with stirring rate 2 times less and higher than optimal. The level of aeration, temperature in the fermenter and the initial amount of culture medium and inoculum were the same for all experiments. As a comparison, we used a culture grown in conical flasks, which was shaken with an orbital stirring rate of 120 rpm (450 ml of culture medium and inoculum from 2 tubes were added to a 0.75 l flask), and the culture grown without stirring.



**Figure 1** Setup for the study of hydrodynamic parameters during submerged cultivation of filamentous fungi

Cultivation was carried out for 7 days - the recommended duration of cultivation of the producer strain. Starting from the second day, culture broth samples were taken every 24 hours to determine the amount of metabolite synthesized (by glucoamylase activity of the culture broth filtrate), the degree of destruction of the producer culture (by the amount of nucleic acids in the filtrate) and the amount of accumulated biomass.

Every 24 hours, samples with a volume of 20 ml of the culture broth were taken sterile. The taken sample was filtered on blue ribbon filters until filtering is complete. In the sediment the amount of accumulated biomass was determined, in the filtrate - the degree of destruction of mycelium cells and the activity of the synthesized enzyme. All tests were carried out in three repetitions.

The amount of accumulated biomass was determined by drying the filtered biomass (together with a pre-weighed filter) to a constant weight at a temperature of 105 °C. The dried samples were cooled to room temperature in a desiccator and weighed on laboratory scales TVE 2,1-0,01.

The activity of the synthesized enzyme glucoamylase in the culture broth filtrate was determined by the polarimetric method (Volkov *et al.*, 2015; Polygalina *et al.*, 2003). 1 ml was taken from the filtrate and diluted in 20 ml of distilled water to obtain a working solution of the culture broth filtrate. 25 ml of 2% maltose solution was poured into two 50 ml tubes each. Maintained in a thermostat or water bath at a temperature of 50 °C for 20 minutes. 5 ml of working solution of the culture broth filtrate was added to the contents of the tubes, the tubes were quickly shaken and the mixture was kept at 50°C for 15 min on the stopwatch. Then, to stop the reaction of the enzyme with the substrate, 1 ml of 5 mol/L hydrochloric acid solution was added to each tube and cooled to 20 °C. For the control sample to 25 ml of maltose solution first was added 1 ml of hydrochloric acid solution with a concentration of 5 mol/l, and then 5 ml of the working solution of the culture broth filtrate. The contents of the control tube were stirred and kept at 50 °C for 15 min on the stopwatch. The obtained working solutions and control were poured into a polarimetric cuvette with a length of 200 mm, placed in a polarimeter SU-5 and at 20 °C the angle of rotation of the plane-polarized light was determined. Glucoamylase activity (GAU, u/l) was estimated by the following formula (1):

$$GAU = \frac{13.94(P_{contr} - P_{exp})}{a} \quad (1)$$

where *a* - the amount of starting enzyme material:  $a = \frac{1.5}{20} = 0.25$  ml, 13,94 - is

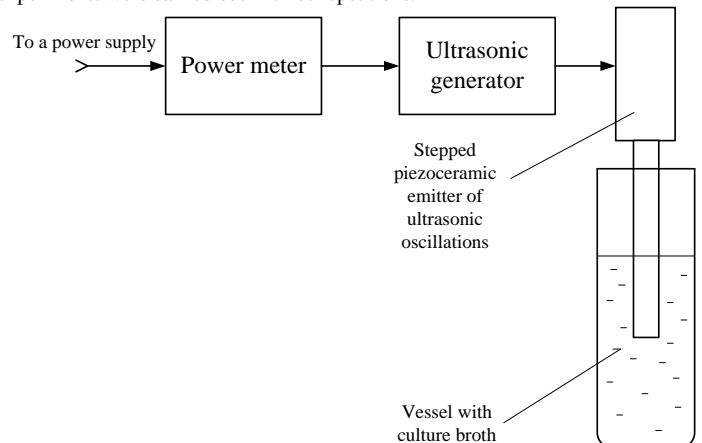
the conversion factor that takes into account the amount of glucose corresponding to 1 ° S, the number of solutions used for testing, the reaction time and others. The final result was the arithmetic mean between the three determinations, the difference between which did not exceed 5% of the arithmetic mean. To convert the units of activity obtained by the polarimetric method into the unit of the standard glucose oxidase method, the conversion factor *K* = 1.76 obtained experimentally was used.

The degree of destruction of producer cells was determined by the amount of nucleic acids in the culture broth filtrate by the spectrophotometric method (Spirin *et al.*, 1958; Nikolaenko *et al.*, 2005; Solecki *et al.*, 2015; Galkin *et al.*, 2018). To determine the amount of nucleic acids 1 ml of the culture broth filtrate was taken in a separate tube and 10 ml of 0.5 N solution of perchloric acid was added to it. The resulting mixture was boiled for 20 minutes in a water bath and then cooled to room temperature. The cooled hydrolyzed solution was poured into a 1 cm<sup>2</sup> cuvette. The light absorption parameters for the wavelengths of 270 nm and 290 nm were measured on a SF-46 spectrophotometer (0.5 N perchloric acid solution boiled under the same conditions was used as a control). The amount of nucleic acids (NA, g/l) was determined by the following formula (2):

$$NA = \frac{10.3(A_{270} - A_{290})P}{0.19} \quad (2)$$

where *P* – dilution: *P* = 10 , 0,19 – coefficient of phosphorus extinction in nucleic acids at a concentration of 1 mg / l; 10.3 - the average conversion factor of the phosphorus content to the concentration of nucleic acids, based on its theoretical content in nucleic acids.

As an auxiliary control of the complete disintegration of the mycelium, the treatment of the culture broth with ultrasound with a speed of 29.4 kHz and a power of 70 W in the range of exposure from 30 s to 15 min was used. The scheme of the ultrasonic generator is shown in fig. 2. It consists of an ultrasonic generator, a piezoelectric meter and a power meter. The generator creates electrical oscillations that excite the piezoelectric emitter, which is structurally a Langevin element with a stepped speed transformer. The piezoelectric emitter converts the energy of electric oscillations into the energy of acoustic oscillations of ultrasonic speed. Acoustic oscillations when passing through a liquid excite cavitation, which destroys the mycelium. The electrical power meter is used to control the stability of the acoustic oscillation power. As a control for mechanically intact cell culture, we used culture broth that had not been sonicated. For the experiment, a 4-day culture broth was used, which was grown in conical flasks with shaking on an orbital shaker at a rate of 120 rpm. The grown culture was placed in 50 ml test tubes, 15 ml in each. The treated culture broth was filtered through a blue-ribbon filter and the amount of nucleic acids was determined as described above. The experiments were carried out in three repetitions.



**Figure 2** Scheme of an ultrasonic setup for studying the degree of destruction of mycelial fungi

To study the viability of cells after sonication, model experiments were performed using 4-day culture broth grown in conical flasks, which were shaken at an orbital stirring rate of 120 rpm. The culture broth was sterile poured into 50 ml tubes, 15 ml in each, and sonicated at a speed of 29.4 kHz and a power of 70 W (using the setup shown in Fig. 2) in the range of exposure from 30 s to 5 min. Cell viability was determined by inoculating the culture on Czapek agar medium with glucose in Petri dishes. Before plating, the culture broth was diluted 10 times. The experiments were carried out in three repetitions.

**RESULTS AND DISCUSSION**

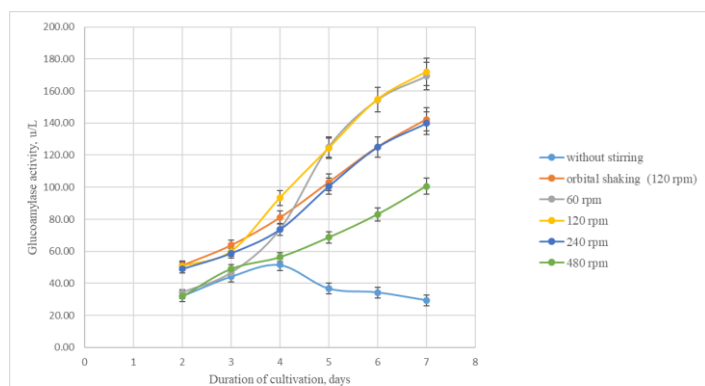
**Investigation of the effect of the stirring rate of the stirring device on the biosynthetic properties of micromycetes**

The obtained results of the biosynthetic activity of the producer *Aspergillus awamori* in terms of glucoamylase activity, the amount of nucleic acids in the filtrate and the amount of accumulated biomass are presented in Table 1, Fig. 3-5.

**Table 1** Investigation of the influence of hydrodynamic parameters of cultivation on the biosynthetic activity of mycelial fungi *Aspergillus awamori*

Duration of cultivation, days	Stirring rate, rpm	Glucoamylase activity, u/l	The amount of nucleic acids·10 <sup>3</sup> , g/l	The amount of accumulated biomass, g/l
2	without stirring	31.90±1.60	traces	9.5±0.48
	orbital shaking (120)	51.22±2.56	4.47±0.22	11.0±0.55
	60	34.35±1.72	traces	8.0±0.40
	120	50.70±2.54	traces	8.0±0.40
	240	49.07±2.45	traces	8.5±0.33
	480	31.90±1.60	8.22±0.41	5.5±0.28
3	without stirring	44.16±2.21	traces	7.0±0.35
	orbital shaking (120)	63.80±3.19	5.07±0.25	14.5±0.73
	60	46.62±2.33	traces	9.0±0.45
	120	59.88±2.99	traces	10.0±0.50
	240	58.88±2.94	traces	12.0±0.60
	480	49.07±2.45	10.03±0.50	7.0±0.35
4	without stirring	51.52±2.58	traces	5.0±0.25
	orbital shaking (120)	81.00±4.05	5.37±0.27	18.0±0.90
	60	73.60±3.68	traces	10.5±0.53
	120	93.23±4.66	traces	12.0±0.60
	240	73.60±3.68	traces	14.5±0.73
	480	56.43±2.82	14.03±0.70	10.5±0.53
5	without stirring	36.80±1.84	3.51±	4.0±0.20
	orbital shaking (120)	103.04±5.15	5.96±	20.0±1.00
	60	125.13±6.26	traces	14.0±0.70
	120	124.31±6.22	traces	15.5±0.78
	240	100.50±5.03	traces	16.5±0.83
	480	68.70±3.44	21.17±1.06	16.0±0.80
6	without stirring	34.35±1.72	5.21±0.26	3.5±0.18
	orbital shaking (120)	125.13±6.26	6.56±0.33	23.5±1.18
	60	154.57±7.73	0.87±0.04	14.5±0.73
	120	154.66±7.73	1.79±0.09	16.5±0.83
	240	124.94±6.25	2.34±0.12	15.0±0.75
	480	83.06±4.15	25.27±1.26	17.5±0.88
7	without stirring	29.44±1.47	13.42±0.67	3.0±0.15
	orbital shaking (120)	142.30±7.12	10.31±0.52	24.0±1.20
	60	169.29±8.46	1.94±0.10	15.0±0.75
	120	171.93±8.60	3.79±0.19	17.5±0.88
	240	139.85±6.99	6.86±0.34	19.5±0.98
	480	100.60±5.03	30.71±1.54	14.0±0.70

As a result of the conducted experiments, it was found that the synthesis of the target metabolite (enzyme glucoamylase) was most active in the range of rotation rate of the stirrer at 60–120 rpm (Fig. 3), and the level of glucoamylase activity on the last day of cultivation was within the margin of error. It should be noted that in the first days of cultivation at 60 rpm, the level of metabolite synthesis was lower than in the same period of 120 rpm. This, on the one hand, confirms the need for mass transfer for the active growth of biomass at the initial stages of biosynthesis. On the other hand, with the accumulation of a larger amount of biomass, the likelihood of its damage during stirring increases, which causes rejuvenation of the culture and a delay in the biosynthesis of the target product.



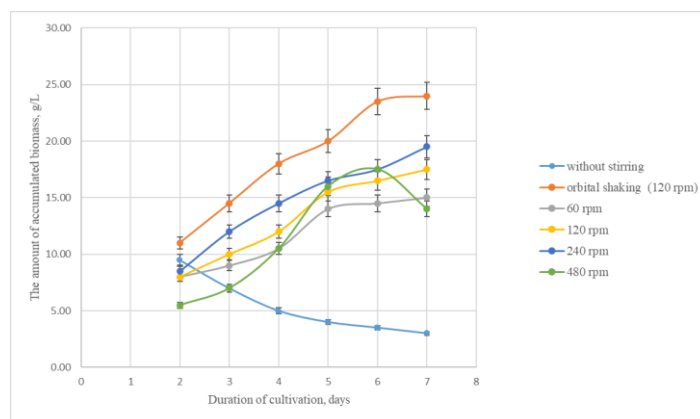
**Figure 3** Graph of the dependence of the amount of synthesized glucoamylase by mycelial fungi *Aspergillus awamori* on the duration of cultivation for different stirring rates

Thus, the assumption made in our previous works (Serbov et al., 2019) regarding the negative impact of hydrodynamic regime intensification (above 120 rpm) was confirmed. In the case of increasing the stirring rate to 240 rpm, the enzymatic activity of the culture broth on day 7 was lower by 18.7% compared with the

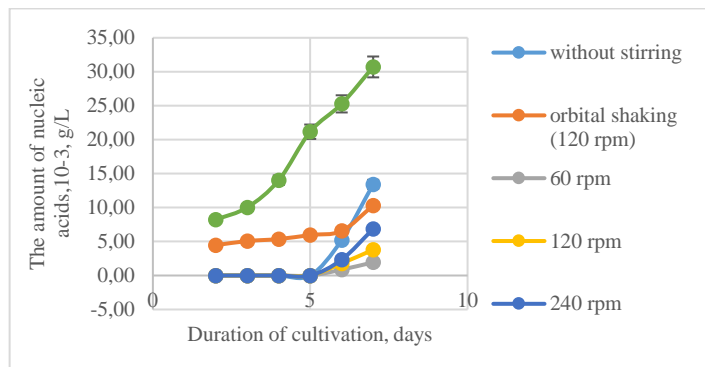
activity at 120 rpm, and at a stirring rate of 480 rpm the level of glucoamylase biosynthesis decreased by 41.5%.

It should be noted that the activity of the culture broth after cultivation under stirring conditions on an orbital shaker at a rate of 120 rpm on the 7th day was also worse than in the fermenter with a turbine stirrer and stirring rate of 240 rpm. It can be assumed that the cultivation conditions on the orbital shaker also cause mechanical damage to the micromycetes, and greater than at the same stirring rates in the fermenter. However, the biomass accumulation curve does not repeat the glucoamylase activity curve, which indicates the additional influence of other factors, such as the degree of aeration.

Under conditions without stirring, the culture was not predictably developed, although some increase in activity was observed up to the 4th day, reaching the maximum value. Subsequent cultivation showed a decrease in the amount of glucoamylase in the culture fluid, which may be associated with degradation of the culture. This is confirmed by a decrease in the amount of accumulated biomass (Fig. 4) and the release of nucleic acids into the culture broth (Fig. 5). Thus, we can say that the intensity of metabolites synthesis by micromycetes depends on the intensity of stirring and decreases significantly with its increase above 120 rpm.



**Figure 4** Graph of the dependence of the amount of biomass accumulated by mycelial fungi *Aspergillus awamori* on the duration of cultivation for different stirring rates



**Figure 5** Graph of the dependence of the amount of nucleic acids in the culture fluid during the cultivation of mycelial fungi *Aspergillus awamori* depending on the duration of cultivation for different stirring rates

The reason for this result is damage of the mycelium, which causes its rejuvenation and delay in the production of the target enzyme, a significant increase of which is observed after 4-6 days of cultivation (Fig. 4). Indeed, the amount of accumulated biomass did not depend on the stirring rates: close (within the margin of error) values for all studied stirring rates were determined.

It should be noted that at stirring rates above 480 rpm on the 7th day a decrease in the amount of biomass was observed, which may indicate depletion of the culture and its degradation, namely the ability of damaged mycelium to recover at these parameters. The exception was the experiment without stirring: the culture did not develop, but degraded - the amount of biomass decreased. This can be explained by the uneven supply of nutrients and oxygen to the producer cells in the absence of intensive mass transfer.

Another method used to determine the damage was the evaluation of the amount of nucleic acids in the culture broth filtrate. It was considered, that the greater the amount of nucleic acids in the culture broth filtrate, the greater the degree of destruction of the fungal mycelium. Moreover, with an increase of the stirring speed of the stirring device, the amount of nucleic acids also increased significantly (Fig. 5). The same effect was observed in the case of cultivation without stirring, i.e. without aeration of the medium and intensive mass transfer, the culture degraded, lysis took place, and the genetic material was secreted outside the cell. It should be noted that up to the 5th day of cultivation, the amount of nucleic acids in stirring conditions of 60, 120 and 240 rpm was at the margin of error, increasing up to the 7th day in proportion to the increase of the stirring rate. This is due to the fact that the damaged mycelium became the growing point for the new cells. In the later stages of growth, the culture did not have optimal conditions for rapid recovery, probably due to increased cell concentration and accumulation of metabolic products. In experiments without stirring, the degradation of biomass occurred after the 2nd day, which was reflected in the curve of the increase in nucleic acids in the filtrate. At a stirring rate of 480 rpm, an exponential increase in amount nucleic acids was observed from the 2nd day of cultivation, which may also indicate significant damage to the mycelium.

The biomass accumulation curve (Fig. 4) confirms this conclusion, as it shows that the recovery and rejuvenation of the culture were much worse than under stirring conditions at a rate of 240 rpm and shaking on an orbital shaker. After the 6th day, there was a sharp decrease in the amount of biomass, which could be predicted by the amount of nucleic acids in the filtrate after the 4th day. The degradation of culture was noticeable even visually.

The amount of nucleic acids in the case of cultivation in flasks on an orbital shaker was also noticeably higher than with all stirring modes, except for the stirring rate of 480 rpm. This is generally consistent with the data on biomass accumulation and glucoamylase synthesis, taking into account the relationship between mycelium destruction and culture rejuvenation and the synthesis of glucoamylase, which occurs at the stationary growth phase.

Thus, based on the obtained results, we can conclude that the intensification of hydrodynamic conditions is necessary for the normal growth of micromycetes culture. However, with the increase of stirring rate, partial destruction of cells occurs, which correlates with the amount of nucleic acids in the filtrate of the culture broth. With the rejuvenation of biomass, which occurs due to the regeneration of damaged fragments (to a certain degree of destruction and the period of cultivation in the batch cultivation without renewal of the culture medium), the amount of accumulated biomass increases. However, this does not increase the ability of producers to synthesize the target metabolite - glucoamylase, because the active synthesis of enzymes by micromycetes occurs in the stationary phase of growth.

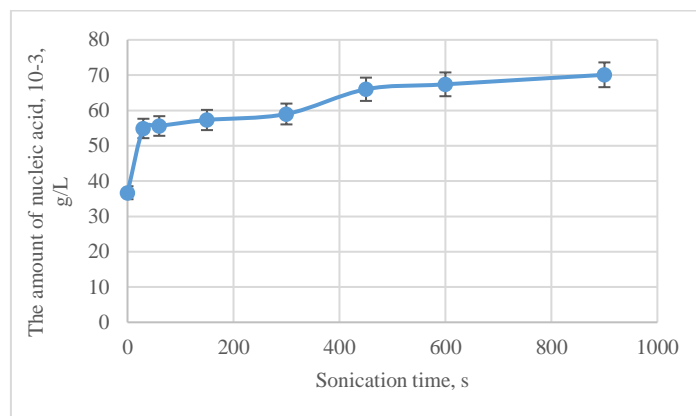
Therefore, in the case of submerged cultivation of mycelial fungi to obtain target metabolites, it is important to develop such stirring devices that would enhance

mass transfer, but not cause damage to the mycelium, which together with the search for optimal stirring speed will provide maximum yield.

#### Investigation of the degree of disintegration of mycelial fungi under the influence of ultrasound

Given the results obtained in the previous experiment, the hydrodynamic regime significantly affects the accumulation of the target enzyme, causing the destruction of the mycelium, which correlates with the presence of nucleic acids in the filtrate, the need for investigation of the critical parameters of the mechanical effect on the cells of micromycetes (up to 100% destruction of cells) arose. Ultrasound is considered to be one of the most widely used methods for the disintegration of cells of microorganisms (Kudriavtsev et al., 1988; Galkin et al., 2017; Golembiovskaya et al., 2019; Grigorieva et al., 2019), which prompted us to use this method in this stage of research. This study aimed to determine the universal relationship between the amount of nucleic acids in the culture broth filtrate and the degree of destruction evaluated by counting the colonies of mycelial fungi *Aspergillus awamori* grown on a solid medium. Spirin's spectrometric method is quite fast and simple, so its use would greatly simplify the procedure for determining the state of the culture in terms of cell integrity.

From the results obtained, shown in Fig. 6, it can be seen that already in the first 30 s of sonication the maximum value of the nucleic acid content in the culture broth filtrate was determined, which gradually increased within the margin of error with an increase in the period of ultrasonic disintegration. The obtained results showed that the destruction of cells is several times higher even after the minimum treatment time compared to the influence of mechanical stirring, but with increasing ultrasonic disintegration time to 15 min (900 s), the amount of nucleic acids in the disintegrant supernatant did not reach saturation.



**Figure 6** Graph of the dependence of the amount of nucleic acids in the filtrate during submerged cultivation of mycelial fungi *Aspergillus awamori* depending on the duration of exposure to ultrasound

It is known that the disintegration of the mycelium does not lead to the death of the culture (Abdella et al., 2020), and our previous studies have shown that under conditions of sufficient nutrition with the intensification of hydrodynamic conditions, the amount of biomass increased.

Further studies were performed to verify the claim that the production of the target enzyme was delayed due to the rejuvenation of the micromycetes culture caused by the partial destruction of the mycelium with the increase of stirring rate. To confirm this thesis and to establish the relationship between the number of nucleic acids in the filtrate, the degree of destruction and the number of viable cells, their number was determined after different durations of sonication.

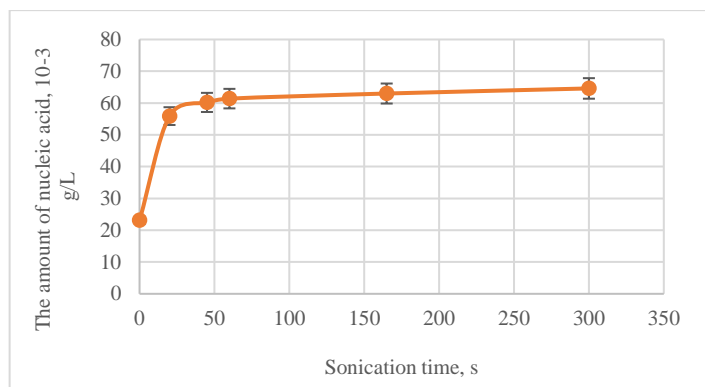
The first stage of the experiments was performed similarly to the previous ones. The results of the experiment are presented in table 2. To compare the results with the previous experiment, the amount of nucleic acids in the culture fluid was determined by the spectrophotometric method, which obtained similar results (the deviation between the obtained data was within the margin of error), presented in Fig. 7.

**Table 2** Investigation of the effect of the sonication time on the viability of cells of mycelial fungi *Aspergillus awamori*.

Sonication time, s	The amount of nucleic acid 10 <sup>3</sup> , g/l	Number of viable colonies in a Petri dish, pcs
0	23.1±1.16	37±1.16
20	55.9±2.80	345±2.80
45	60.2±3.01	260±3.01
60	61.4±3.07	208±3.07
165	63.0±3.15	174±3.15
300	64.6±3.23	118±3.23

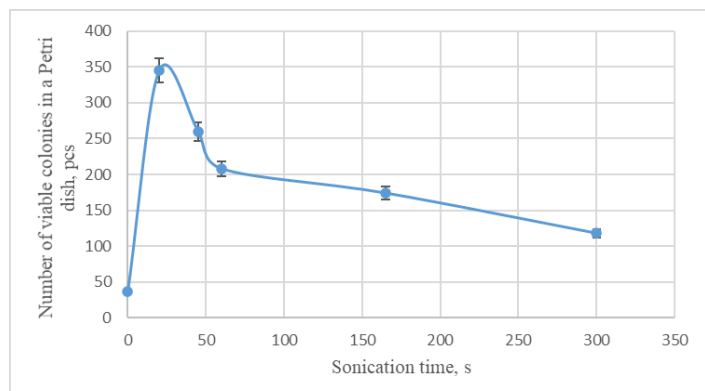


As can be seen from the presented data, after 20 s of ultrasonic disintegration, the amount of nucleic acid in the filtrate, which was determined by the reevaluation of the amount of nucleic phosphorus, reached almost maximum values. At the same time, the number of viable mycelial fragments that formed colonies was maximum at this sonication time. Based on this, it can be argued that the fragments of the mycelium in favorable conditions restore viability. As the treatment time increased, it is likely that such fragments were further destroyed, resulting in a reduction in colonies grown on a solid nutrient medium.



**Figure 7** Graph of the dependence of the amount of nucleic acids in the culture broth filtrate during the submerged cultivation of mycelial fungi *Aspergillus awamori* depending on the sonication time

As you can see from the graph shown in Fig. 8, after 20 s of exposure, the number of colonies formed from mycelial fragments increases sharply (almost 10 times), and then begins to decline. After 60 s of sonication, the decrease in the number of viable cells in the culture fluid ceases to occur rapidly but does not stop completely. This confirms the known data on the effects of ultrasound on the cells of microorganisms (Kudriavtsev et al., 1988; Nikolaenko et al., 2007; Marynchenko et al., 2019).



**Figure 8** Graph of the dependence of the number of viable cells of mycelial fungi *Aspergillus awamori* in the culture broth on the sonication time

The obtained results suggest that the slight effect of ultrasound on the cells of mycelial fungi in the process of submerged cultivation can be used as a stimulating factor to intensify the accumulation of biomass.

## CONCLUSION

As a result of experiments, we found that the best biosynthetic properties of mycelial fungi are observed at a stirring rate of 60-120 rpm (glucoamylase activity in the culture broth reaches 154.6-154.7 u/l), with an increase of stirring rate the amount of synthesized metabolite decreases by 41.5% (glucoamylase activity is reduced to 100.6 u/l). Thus, we can conclude that to obtain the best results of biosynthetic activity of micromycetes hydrodynamic conditions should be carefully selected, in particular the stirring rate, because of the destruction of the mycelium, which was detected by the presence of nucleic acids in the culture broth filtrate (at the rate of 60 rpm the amount of nucleic acids was 1.94 g/l, and when increasing the rate to 480 rpm it increases 15.8 times to 30.71 g/l), causes a delay in the stationary phase of culture growth, and hence the delay in the intensity of glucoamylase accumulation in the culture fluid.

At the same time, it was found that even with significant and prolonged mechanical effects on the cells of mycelial fungi, in particular ultrasonic disintegration, there was no complete destruction (maximum exposure time was 15 min), and the number of viable colonies, although declining, did not reach zero. Therefore, the complete destruction of mycelial fungi under the studied parameters of ultrasonic

treatment could not be achieved, and at a critical value of sonication time saturation of the filtrate with nucleic acids was observed.

Since the presence of nucleic acids in the filtrate satisfactorily describes the state of the culture in terms of mechanical damage to cells, the degree of destruction of micromycetes indirectly, but more accurately than by inoculation on Petri dishes, can be determined by the amount of nucleic acids in the submerged. Also, the results obtained suggest that short-term ultrasonic treatment at the early stages of batch cultivation can have a stimulating effect on the accumulation of micromycetes biomass, which can be used for industrial purposes.

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