

## Bioconversion of the fat-containing phase of effluents of fish processing enterprises into a feed additive

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**Abstract.** Currently, the branch of the Ukrainian food industry occupies a priority place among other spheres of the economy. However, the field of food production faces the problem of non-compliance with current requirements in the context of the environmental aspect. Among them is the aspect of waste processing, which is generated during the processing of fish raw materials, cleaning of sewage wells. A typical situation for fish processing enterprises is the disposal of waste and fatty effluents. Actual economic needs include the need for the production of protein feed additives, as well as solving environmental issues for the processing of fat-containing effluents from fish processing enterprises. Thus, the purpose of the article is to develop the technological basis of the aerobic bioconversion of the fat component of the effluents of fish processing enterprises into a protein feed additive. Therefore, a technology for utilization of the fat-containing phase of fish processing effluents based on a combination of physical and chemical effects and biological oxidation is proposed. It was established that the predominant organism of the autochthonous microflora of the waste is the fungus *Geotrichum sp.*, and also that the yeast *Yarrowia lipolytica*, selected for the substrate, is the most promising biodecomposer of fats. A basic technological scheme of bioconversion of the fat-containing phase of effluents of fish processing enterprises into yeast biomass for fodder purposes has been developed. The main chemical and microbiological indicators of the fat-containing phase of the effluents of fish processing enterprises were determined. It has been established that for the biodestruction of fatty effluents, it is appropriate to use both top-up and continuous methods of cultivation, which do not lead to radical changes in the quality of the obtained biomass. It was established that in the process of holding waste in a sludge collector (1-13 days) as a result of the vital activity of autochthonous microorganisms, the fat content decreases from 87 to 58

**Keywords:** technical ecology, protection technologies, waste management, normalization of the load on the environment, feed additive

### Article's History:

Received: 4.03.2021

Revised: 20.07.2021

Accepted: 26.08.2021

### Suggested Citation:

Madani, M., Shevchenko, R., & Hankonjich, O. (2021). Bioconversion of the fat-containing phase of effluents of fish processing enterprises into a feed additive. *Ukrainian Black Sea Region Agrarian Science*, 25(3), 54-65.

## INTRODUCTION

Currently, the food industry in terms of production occupies a leading position among the branches of the national economy. One of the main problems of food production is their non-compliance with modern environmental requirements, which can be solved by introducing low-waste technologies. For example, there is an urgent issue of processing waste from the fish processing complex, which is generated during the processing of fish raw materials, clarification of waste water in the grease trap and flotation device, and cleaning of sewage wells.

For fish processing enterprises, the main problems are the disposal of organic waste (blood, guts, scales, fish trimmings) and fatty effluents. Special attention should be paid to the fat component, since it accounts for the main share of the total amount of waste. Thus, the solid fish mass collected in sedimentation tanks contains at least 25-35% crude fat [1]. Thus, during the operation of fish processing enterprises, large amounts of fatty waste are inevitably generated, and their contamination with microflora leads to rapid decay with the formation of unpleasant odors. In addition, the high content of fats in wastewater complicates the functioning of treatment facilities of fish processing plants, as it contributes to the formation of deposits on the walls of pipes and tanks.

It should be noted that fat-containing waste varies in composition, which varies depending on the mode of operation of the fish processing enterprise, therefore most of the existing technologies can be applied only with a number of restrictions.

Existing technologies for the processing of fat waste can be divided into two types: physico-chemical and microbiological. The former require specific conditions, significant capital expenditures, are inefficient, and the demand for waste processing products is not high enough. Microbiological methods are characterized by high efficiency, but their significant drawback is that they do not obtain a final product, but only involve the disposal of waste. This significantly reduces their value from the point of view of increasing the profitability of the main production.

The microbial biomass produced as a result of the application of microbiological methods can be used as a valuable feed additive for farm animals and poultry. The latter is especially relevant for modern fodder production, as it allows more efficient use of raw materials and maximum productivity of livestock and poultry farming with the lowest costs.

Thus, modern economic needs include the need for the production of protein feed additives and the solution of environmental issues in the processing of fat-containing effluents of fish processing enterprises.

This allows us to consider research aimed at solving these problems as having great practical and social significance. Fish processing enterprises are faced with the problems of disposal of a number of different organic wastes. Among them, it is possible to single out fat-containing waste produced during fish processing, purification of wastewater in a grease trap and flotation device, cleaning of sewage wells [2-4]. The largest amount of fatty waste is produced during the purification of wastewater, which are diluted emulsions. The content of polluting fatty impurities in wastewater reaches 900 mg/L, while the permissible concentration should not exceed 50 mg/L. To achieve the desired result, the efficiency of wastewater treatment is about 95%. In addition, effluents from fish processing enterprises are characterized by unevenness of flow, changes in the concentration of pollutants during the day, fluctuations in pH values (6.5-8.5) [5-7].

The existing technological schemes of wastewater treatment provide for the periodic (1-2 times a day) removal of the fat sludge that floats to the sludge collector after passing through the grease trap. The volume of the sludge collector is calculated for the accumulation of waste for 3-5 days, then they are buried at the solid waste landfill [8].

The composition of the fatty phase of wastewater at fish processing enterprises can be heterogeneous and very complex [9], which is one of the main problems that arise when using such waste as raw materials.

Currently, a number of technologies are proposed that allow the utilization or processing of fat waste [10-18]. Two main directions of the proposed technologies can be distinguished: physico-chemical and biological.

Analysis of the literature shows that today there is a wide range of technologies based on physical and chemical effects on fatty waste. Some of them manage to obtain a product that can be used in further human economic activity [13].

Despite various researches by scientific groups, both in Ukraine and abroad, there are still no safe technologies for processing low-value fats, and no production has yet been established on an industrial scale. Therefore, low-value fats are destroyed as waste water treatment [15].

One of the modern trends in fat waste processing is the combination of physico-chemical influence and biological oxidation [16; 19]. As part of one of these technologies, it is proposed to use biological oxidation as an intermediate stage by using biological preparations containing a complex of lipolytic enzymes, as well as a live culture of microorganisms. This makes it possible to significantly shorten the terms of wastewater treatment

and at the same time reduce economic costs. This technology involves further oxidation of biomass with potassium permanganate and hydrogen peroxide [20].

It should be noted that a number of technological schemes provide for the purification of sewage from fatty impurities with preparations consisting of both monocultures and mixtures of microorganisms of the genus *Mucor*, *Bacillus*, *Pseudomonas*, etc. [21-23].

The disadvantage of the proposed technologies is the lack of a product that is of interest for further human activity. Thus, these methods allow only to reduce the toxicity of man-made waste, but not to process it.

Approaches to biosynthetic and enzymatic processing of animal fats into products with increased biological activity have already been developed. For example, there are technologies for the hydrolysis of fats in waste water from food industries using microbial lipases [20; 24; 25], as well as bovine pancreatic lipase. As a result of the enzymatic splitting of fat by animal lipases, the entire spectrum of fatty acids is released from the fat structure [26]. Thus, fat-containing additives with increased biological value for food compositions and microbiological environments can be obtained [27].

It should be noted that technologies are offered that allow for biological destruction both in aerobic conditions and through anaerobic fermentation.

Fermentation with lactic acid bacteria is a fairly widespread method of processing fat-containing waste from food enterprises. This method includes heat treatment of raw materials, enzymatic hydrolysis, and if necessary, it is suggested to additionally carry out filtration [28]. The disadvantage of this method is the need to carry out several stages, as well as the use of an enzyme preparation, which significantly increases the cost of the final product.

Anaerobic fermentation of waste in order to obtain biogas and other gaseous products is also proposed [29]. The residue formed during such waste processing can be used as fertilizer. Its composition depends on the composition of the raw material. Under favorable conditions for anaerobic fermentation, about 70% of organic substances are usually decomposed [30]. The main factor restraining the introduction of this technology at production enterprises, despite all the advantages, is significant capital costs [31].

There are also a number of technological schemes for the aerobic processing of fatty waste using yeast cultures, and a number of researchers recommend the moderately halophilic yeast *Yarrowia lipolytica* [32-36]. An analysis of the literature shows that *Y. lipolytica* yeast is successfully used in the treatment of fat-containing effluents of enterprises engaged in the processing of various types of vegetable

raw materials. For example, it has been proven that the strain *Y. lipolytica* 3589 reduces the chemical oxygen consumption (COD) of wastewater from palm oil production enterprises by 95% in two days [34], and the strain *Y. lipolytica* ATCC 20255 reduces the COD of wastewater from olive oil production by 80% in 24 hours. At the same time, the accumulation of yeast biomass was 22.45 g/l [37].

The possibilities of modern biotechnology make it possible to find an alternative way of using fat-containing effluents of fish processing enterprises, which affects not only the ecological aspects of production, but also the problem of feed protein deficiency. Thus, the ability of the thermophilic strain *Candida tropicalis* 928 and the above yeast *Y. lipolytica* to use fat as a carbon substrate has been established [38]. *Y. lipolytica* is recommended for the purification of wastewater from fat-containing pollutants in the case of using the target product as a feed additive.

The above allows us to conclude that fat-containing effluents of food enterprises can be used as a substrate for the cultivation of microorganisms – producers of fodder protein. An analysis of the literature proves that *Y. lipolytica* yeast is one of the cultures, the use of which can be effective in carrying out this process [36].

*The purpose of the article* is to develop the technological foundations of the aerobic bioconversion of the fat component of the effluents of fish processing enterprises into a protein feed additive. To achieve the goal, the following tasks are set:

1. To determine the chemical and microbiological indicators of the fat phase of the effluents of fish processing enterprises.
2. To isolate and characterize autochthonous microorganisms; choose microorganisms with high lipolytic activity, which are able to assimilate fats as the only source of carbon.
3. To study the processes of cultivation of destructive microorganisms on fatty substrates depending on the preliminary treatment of the substrate.
4. Investigate the influence of different modes of cultivation of microorganisms on the characteristics of the technological process and the quality of microbial biomass; conduct a comparative analysis of methods of concentration of the obtained biomass.
5. To develop a technological scheme for the processing of the fat-containing phase of the effluents of fish processing enterprises.

## MATERIALS AND METHODS

The main object of the study was the fatty effluents of the fish processing enterprise “RZK” Pivdenny LLC, whose production facilities are located in the city of Bilhorod-Dnistrovskyi, Odesa region. The chemical parameters of

the average waste sample (total fat, free fats, acid value of fat) were determined in accordance with DSTU 660:2019, DSTU ISO 7847:2006, DSTU EN 14105:2009. Microbiological characteristics, as well as isolation and research of autochthonous cultures of microorganisms were carried out using standard methods [39].

Microbial objects were strains of microorganisms with lipolytic activity: bacteria – *B. mesentericus*, *B. subtilis*, *Acinetobacter sp.*; mushrooms – *A. myxae*, *P. orysae*, yeast – *C. scottii*, *Y. lipolytica*. Cultivation was carried out in flasks with a volume of 250 ml (100 ml of medium) with stirring (150 rpm) and in a fermenter with a volume of 5 liters with a nutrient medium filling of 70% with stirring (250 rpm). Lipolytic activity was determined by the hydrolysis reaction of olive oil [40]. The content of crude protein, true protein, total carbohydrates, total fat, and nucleic acids in microbial biomass was determined in accordance with DSTU 8723:2017.

The results of the experiments were processed using the methods of mathematical statistics. The value of the reliability indicator was found by the Student's test with a confidence probability of  $P=0.95$  and the degree of freedom  $f=n-1$ . The permissible value of the relative error was considered to be its value, which does not exceed 5%.

## RESULTS AND DISCUSSION

Chemical and microbiological indicators of fatty effluents of fish processing enterprises. Since the composition of the fatty phase of the effluents can vary significantly as a result of the vital activity of autochthonous microflora, as well as depending on the quality of the processed raw materials, samples were taken during several cycles of the enterprise's operation from the sludge collector on 1, 5 and 13 days after receipt. The study of the chemical composition of fatty waste effluents of the same storage period (table 1) revealed minor differences in the mass fractions of the analyzed compounds in the composition of fatty raw materials, which indicates the same type of waste generated during the operation of the enterprise. However, clear changes in the composition of the fat mass during its aging in the sludge tank indicate the active development of autochthonous microflora, which consumes fat, reducing its share. At the same time, microbial biomass accumulates, as a result of which the content of crude protein and carbohydrates increases. The parallel decrease in the mass fraction of free fat and the acid number is probably associated with the consumption of the most easily digestible fats by the developing microflora, while the hard-to-digest fats remain unconsumed.

**Table 1.** Chemical indicators of the fat-containing phase of fish processing effluents with different periods of storage in the sludge collector

Waste storage, days	Total fat, %	Free-extracted fat, %	Acid number	Crude protein, %
1	86.9-88.4	82.2-84.3	190.1-219.4	12.5-13.9
5	70.3-76.6	63.3-74.6	131.4-140.3	17.5-21.0
13	58.3-61.4	46.1-48.3	118.8-124.6	19.0-22.1

**Notes:** \*- taking into account the spread of indicators of different samples

When studying the microbiological parameters of fat waste, high clogging (up to 108 cl/g) of waste was found (Table 2). With the increase.

During the storage period of waste, the number of bacteria decreases and the number of fungi increases.

**Table 2.** Microbiological parameters of the fat-containing phase of fish processing effluents with different periods of storage in the sludge collector

Waste storage, days	CFU, 10 <sup>6</sup> kl/h	
	bacteria	fungi
1	100.2	2.5
5	15.4	86.5
13	8.8	167.9

The study of the fungi isolate revealed a high activity of exolipase (120 units/mg ASB) and endolipase (68 units/mg ASB). Based on the results of determining morphological features and physiological properties, the isolate was classified as a yeast-like

fungus *Geotrichum sp.* Since the isolated microorganism was the dominant contaminant of the waste and had a high lipolytic activity, the necessary stage of further research was to find out the perspective of its use for the bioconversion of the fat phase of wastewater.

It was noted that other microorganisms (bacteria, molds), which are present in single quantities, are not capable of assimilating fats and, apparently, are allochthonous for this type of waste. The isolated bacteria, which were Gram-positive rods 1  $\mu\text{m}$  in size, capable of forming endospores without changing the shape of the cell, had relatively high rates of assimilation of the fat substrate. Despite the high exolipolytic activity, as well as the ability to assimilate fats as a carbon source, the growth rate and maximum accumulation of biomass in the isolated bacteria when growing on nutrient media with a fat waste content of 20 g/l did not exceed 0.1  $\text{h}^{-1}$  and 2.2 g/l respectively. In addition, the isolated bacteria were highly sensitive to changes in the pH of the environment and contamination by fungi, which does not allow considering them as promising destroyers of fatty waste.

Bioconversion of the fat-containing phase of fish processing effluents using a selected strain of the fungus *Geotrichum sp.* The growth of the fungal isolate

was studied in a laboratory fermenter on a medium containing fatty waste (20 g/l) with different periods of storage in a sludge collector (table 3).

The obtained results indicate that the strain *Geotrichum sp.* can be used for bioconversion of fat waste: the resulting biomass contained up to 45% protein. However, the process was long, had an insufficiently high growth rate of the fungus and a low biomass yield. This may be due to the limited ability of the isolated fungus to assimilate higher saturated carboxylic acids.

When studying different modes of mushroom cultivation based on quality indicators of the obtained biomass, a conclusion was made about the advantage of deep heterophase cultivation in a periodic mode. However, this regime is unprofitable in terms of economic indicators. Other modes of cultivation (with substrate feeding, continuous) led to a significant increase in the content of lipids and a decrease in the amount of protein in the final product.

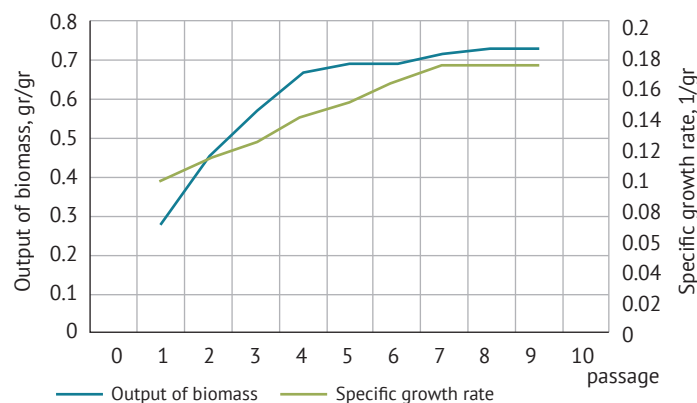


Figure 1. Change in the growth characteristics of yeast *Y. lipolytica* during selection with respect to the substrate

Table 3. Growth characteristics of *Geotrichum sp.* during cultivation on fat waste in periodic conditions

Characteristic	The term of waste storage in the sludge collector, days		
	1	5	13
Cultivation duration, hours	46	72	82
Growth rate, $\text{hour}^{-1}$	0.10	0.06	0.04
Maximum accumulation of biomass, g/l	13.5	9.1	6.4
Biomass output, g/g	0.68	0.46	0.37
Biomass composition, %			
- protein	44-45	28-29	19-20
- crude protein	53-55	34-35	23-25
- total fat	11.0-12.5	15.3-19.7	25.0-26.5

Since autochthonous cultures are not promising for the bioconversion of the fatty phase of effluents into a protein feed additive, there was a need to find a more effective microbial destructor of fats with a high degree of exolipase production. Exolipase activity and the content of protein substances in the biomass

were chosen as the main criteria for the selection of strains. The analysis of the results made it possible to conclude that the yeast *Y. lipolytica*, which has the highest lipolytic activity, provides a high yield of biomass – up to 0.6 g/g when cultivated on fatty waste and crude protein content – 38.3% of ASB (Table 4).

When growing *Y. lipolytica* in a laboratory fermenter in an environment containing fatty waste as a substrate (20 g/l), the duration of the process was 5 days, and the specific growth rate was 0.05 h<sup>-1</sup>. However, the

obtained biomass contained up to 21.5% lipids, which indicates incomplete consumption of the substrate and, therefore, the need to optimize the conditions of the process.

**Table 4.** Characteristics of lipase-producing microorganisms

Culture	Exolipase activity		Raw protein, %
	specific unit/mg ASB	general units/ml	
<i>Bacillus mesentericus</i>	0.53	2.9	25.4
<i>Bacillus subtilis</i>	0.73	6.7	28.3
<i>Acinetobacter sp.</i>	2.6	42.1	40.1
<i>Aspergillus oryzae</i>	34.2	4.6	31.5
<i>Penicillium oryzae</i>	0.7	5.1	29.7
<i>Candida scottii</i>	39.0	42.5	39.2
<i>Yarrowia lipolytica</i>	303.4	919.0	38.3

Therefore, the influence of the mineral composition of the nutrient medium and cultivation conditions (temperature, pH, aeration, "age" and concentration of fatty waste) on the lipolytic activity and biomass accumulation of yeast *Y. lipolytica* was further investigated. The best results were obtained under the following conditions: cultivation temperature 30-32°C; pH 5.0-5.5, fat substrate content 20 g/l. An increase in the concentration of the fat substrate over 20 g/l led to a significant decrease in the growth rate of yeast and the maximum accumulation of biomass, which is explained both by difficulties in aeration, as the content of dissolved oxygen in the nutrient medium decreases, and by the fact that part of it is spent on spontaneous oxidation processes of components fat. It was noted that a decrease in the growth characteristics of the yeast culture was observed when using fatty waste that was stored for more than a day. Therefore, only "fresh" waste should be used for an effective process.

In the course of the work, it was established that fat waste contains components that inhibit the growth of *Y. lipolytica* yeast. Since the inhibitory effect was higher in waste with longer storage periods and with high clogging, a conclusion was made about the microbial nature of the inhibitors. This negative effect can be eliminated by the selection of yeast in the direction of their adaptation to the stored fatty substrate. Breeding was carried out by means of multiple successive inoculations of yeast on media containing the waste of fatty effluents from fish processing. As a result, up to the 7th passage, the specific growth rate of microorganisms and the yield of biomass increased significantly to 0.8 h<sup>-1</sup> and 0.69 g/g, respectively (Fig. 1).

There is also an increase in the content of true protein from 30.6 to 38.9%. It was noted that using

an adapted yeast culture as inoculum, the required inoculation volume is reduced to 0.1% v/v. It has been proven that it is most expedient to use the culture in the phase of linear growth. However, when transplanting yeast adapted to fatty wastes to carbon-containing liquid/solid nutrient media, the previously achieved results were lost.

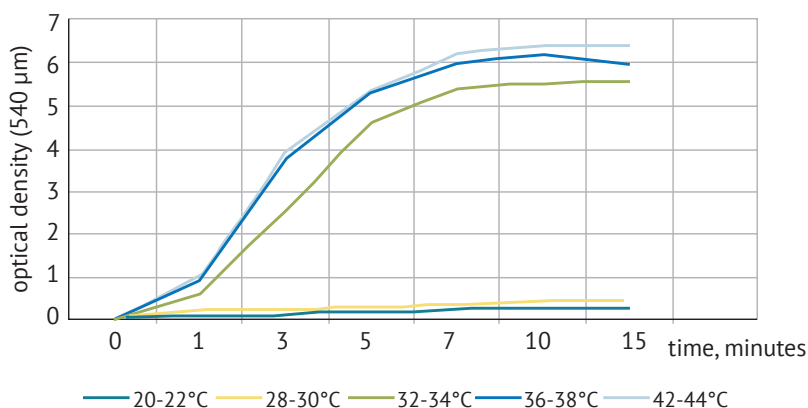
For effective bioconversion of the fat-containing phase of effluents, it is necessary to use the yeast strain *Y. lipolytica*, selected for the substrate, and the yeast should be stored only on fat-containing nutrient media.

Preliminary preparation of fat-containing nutrient medium. Since the process of assimilation of fats by microorganisms can be limited by the surface area of the substrate, the development of ways to increase the efficiency of bioconversion of the substrate by pretreatment of the medium is relevant.

Reducing the size of fat particles can be achieved by chemical or enzymatic hydrolysis, as well as by ultrasonication. In the course of research, alkaline hydrolysis of raw materials was carried out using sodium hydroxide (pH 8-13) followed by autoclaving at 0.5 and 1 atm. Pancreatic lipase (1,500 act units/g) was used for hydrolysis, while the concentration of the enzyme and the duration of treatment were changed. Ultrasonic disintegration was carried out in a laboratory ultrasonic bath with an operating frequency of 22 kHz, changing the temperature and duration of treatment. It was proven that both alkaline and enzymatic hydrolysis, although it allows to reduce the size of fat particles, but does not increase the efficiency of its bioconversion by yeast. In contrast to the described methods, ultrasonic dispersion of fat at the stage of preparation of raw materials had a significant effect on the growth characteristics of

yeast, since ultrasonic waves stimulate the processes of oxidation of fats [41; 42] and this contributes to the preliminary partial degradation of the substrate and increasing its bioavailability. The study of the

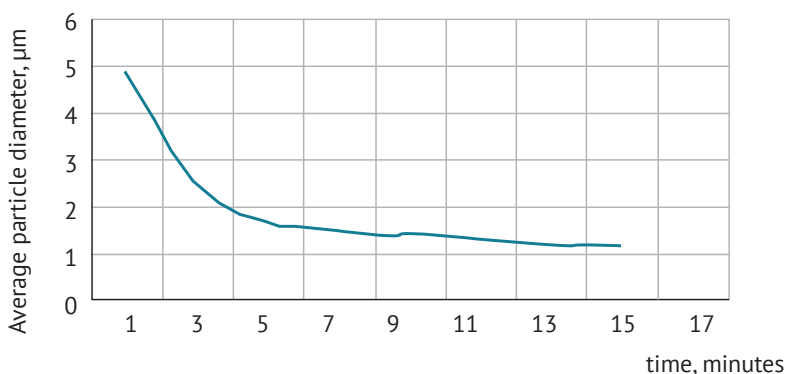
properties (optical density) of the obtained fat dispersion showed that the action of ultrasound at a temperature of 30°C and below allows to achieve the maximum degree of saturation of the system (Fig. 2)



**Figure 2.** Change in the optical density of the dispersion of fat waste (1 g/l) in the nutrient medium at different temperatures during ultrasound treatment

Microscopy of the resulting dispersion revealed a significant reduction in the size of fat particles. Ultrasonic treatment of a nutrient medium containing 20 g/l

fatty waste for 7 min resulted in a decrease in the average particle diameter to 1.5 μm. Longer action did not significantly affect the dispersion properties (Fig. 3).



**Figure 3.** Influence of the duration of ultrasonic treatment on the diameter of particles

The properties of the dispersion obtained by ultrasonic treatment in the optimal mode were affected by the concentration of fats: saturation of the dispersion was reached already at 5 g/l of fat, and when the concentration increased above 30 g/l, a decrease in optical density was observed. The latter may be due to the increased intensity of coagulation of microparticles that are formed at high concentrations of the dispersible substance. Optimization of the raw material pretreatment process in terms of all three parameters was carried out based on the results of *Y. lipolytica* yeast growth on it. According to the data in the table. 5, the mode of ultrasonic treatment of the medium containing 20-30 g/l of substrate at an initial temperature of 22-24°C for 7 minutes was optimal, during which the yield of biomass increased by 45%. In addition, an additional

positive effect of ultrasonic pretreatment of raw materials was revealed. During the cultivation of yeast on untreated fat-containing nutrient media in a laboratory fermenter, the substrate was unevenly distributed in the volume of the fermenter, stuck to the mixer blade and the lid of the apparatus. After ultrasonic treatment of the nutrient medium, these deficiencies were not found: the fat was in the culture liquid in the form of a dispersion and did not form dense deposits on the surfaces of the fermenter. As a result of preliminary processing of raw materials, the specific growth rate increased (from 0.18 to 0.20 h<sup>-1</sup>) and biomass accumulation (up to 19.8-28.5 g/l), which is significantly higher than previously achieved results. Therefore, ultrasonic pre-treatment of the fatty nutrient medium is a necessary technological stage of the fat bioconversion process.

**Table 5.** Biomass accumulation of *Y. lipolytica* at different initial concentrations of fat waste and modes of pretreatment of the nutrient medium

Initial substrate concentration, g/l	Preliminary treatment of the substrate ultrasound		Maximum accumulation of biomass, g/l	Biomass output, g/g
	duration, min	initial temperature, °C		
10		absent	8.14	0.81
10	7	22-24	12.56	1.26
20		absent	13.8	0.69
20	7	22-24	19.8	0.99
30		absent	19.28	0.64
30	7	22-24	28.50	0.95
50		absent	22.71	0.45
50	7	22-24	32.23	0.64
10	6	22-24	9.85	0.99
10	8	22-24	12.57	1.26
10	7	13-15	10.05	1.01
10	7	34-36	12.55	1.26

Study of the influence of cultivation regimes on the efficiency of the process of biodestruction of the fat-containing phase of effluents and the quality of biomass. Cultivation regimes were tested in a laboratory fermenter (volume 5 l, 70% filling).

Since the tasks of the research included not only the selection of conditions optimal for the biodegradation of fat-containing effluents, but also the further use of the obtained biomass as a feed

additive, it was necessary to conduct a comparative analysis not only of the process characteristics, but also of the quality of the obtained biomass. Several modes of cultivation were tested (Table 6). The obtained results allow us to conclude that for the biodestruction of fatty effluents, it is advisable to use both top-up and continuous methods of cultivation, which do not lead to significant changes in the quality of the obtained biomass.

**Table 6.** Characteristics of the process of bioconversion of the fat-containing phase of sewage (20 g/l) by yeast *Y. lipolytica* under different modes of cultivation

Characteristic	Modes of cultivation				
	periodic deep	deep with feeding on the substrate	Solid-phase	deep selection and filling	nonstop
Specific growth rate, h <sup>-1</sup>	0.26	0.14	0.01	0.24*	0.24**
Maximum accumulation of biomass, g/l	24.2	86.9	0.0028	16.5	16.5
Biomass composition, %					
white	43.5	35.7	less 1	42.7	41.4
total fat	6.7	18.3	93.6	7.2	7.9

**Notes:** \* – rate of sampling of culture fluid every hour; \*\* – flow rate of culture liquid

### Concentration and drying of biomass

Biomass concentration is one of the most expensive stages of feed protein production technology. We conducted a study of the effectiveness of a number of options for separating biomass from the culture liquid during yeast cultivation *Y. lipolytica* on fatty sewage waste. Such methods of biomass concentration as sedimentation, separation, filtration through fabric filters and flotation have been studied.

The conducted studies showed that a satisfactory degree of concentration is achieved when using sedimentation, which is the cheapest method of biomass separation, as a result of which after three hours the concentration of dry substances in the sediment is 15.8% by mass. At the same time, the decantate contains no more than 2 g/l of ASB, which allows it to be used in the amount of 70-80% in the recycling of culture liquid. It should be noted that the biomass

concentrate obtained in this way can be sent for drying without using additional methods of biomass concentration. It is most appropriate to use a belt dryer.

Technological scheme of bioconversion of the fat-containing phase of effluents of fish processing enterprises into yeast biomass for feed purposes. On the basis of the conducted research, we developed a basic technological scheme for the bioconversion of the fat-containing phase of effluents from fish processing enterprises into yeast biomass for feed purposes. The technological scheme provides for:

- use of microorganisms adapted to the substrate;
- preliminary treatment of the nutrient medium with ultrasound to disperse fat;
- fermentation stage in continuous or selective-topping-up mode;
- concentration of biomass by settling and drying.

The developed technology involves the selection of *Y. lipolytica* yeast culture with respect to the substrate (fatty effluents of fish processing) in the laboratory and subsequent cultivation in inoculators of various volumes. With a cell titer of at least 400 million/ml, the finished seed culture is transferred to the main apparatus with sterile air.

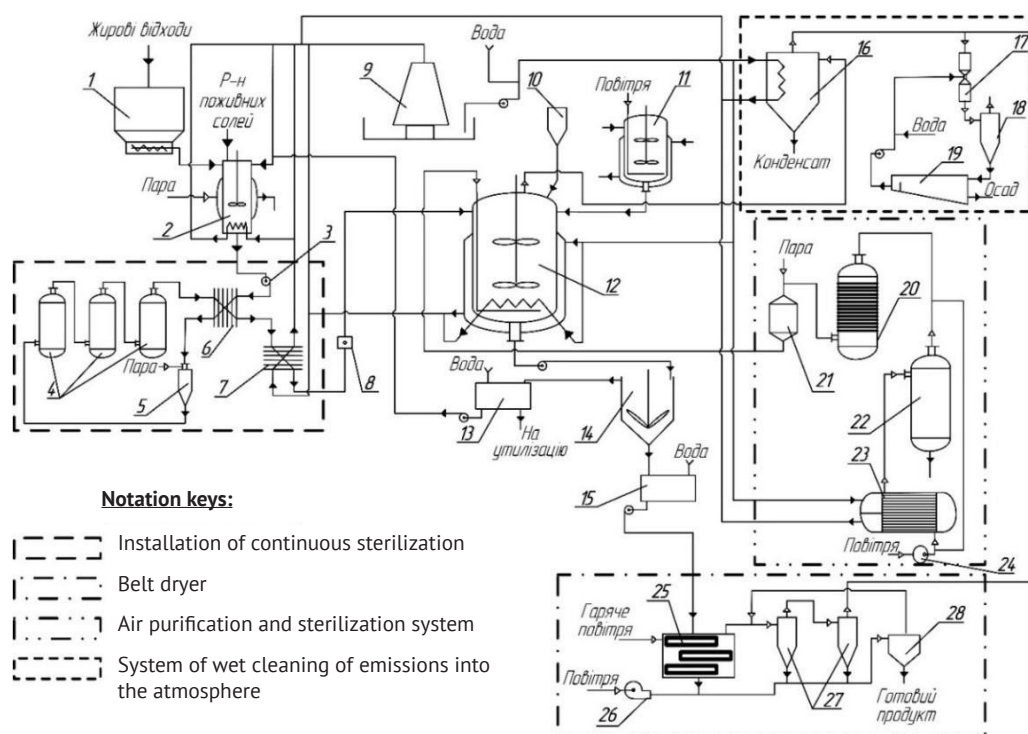
The main process is carried out in three parallel

devices, the volume of which is determined by the amount of waste. At the same time, the pH is maintained in the range of 5.0-5.5 by adding ammonia water (23-26%) through a meter-doser. The temperature in the fermenter is maintained at the level of 30°C by changing the flow rate of water supplied to the coils and the jacket.

Air for aeration of the seed fermenter is supplied by separate lines from the main blower through air intakes. The air is cleaned of dust on a coarse filter and compressed. Air enters the main sterilizing filter.

To prepare the nutrient medium, all the necessary components of mineral nutrition are supplied from the dispensers. A solution of nutrient salts and fat waste is prepared in temperature-controlled mixers and pumped through a recuperator heat exchanger into the sterilization unit, where it is sterilized. After that, the nutrient medium passes through a heat exchanger-recuperator and a refrigerator, where it is cooled to a temperature of 35°C, passes through an ultrasonic unit and is fed into the fermenter.

The yeast suspension enters the settling tank. The supernatant is fed into a container for clarified culture liquid (OKR), from where it is pumped by a pump into a container for recycling.



**Figure 4.** Basic technological scheme of bioconversion of the fat-containing phase of fish processing effluents into yeast biomass for fodder use: 1 – the container for the fat-containing phase of waste is open; 2 – mixer; 3 – centrifugal pump; 4 – holders; 5 – heater-sterilizer; 6 – heat exchanger-recuperator; 7 – heat exchanger-cooler; 8 – ultrasonic disperser; 9 – cooling tower; 10 – meter-dosier of ammonia water; 11 – inoculator; 12 – fermenter; 13 – an intermediate container for clarifying the culture liquid; 14 – sedimentation tank of continuous action; 15 – intermediate container for paste; 16 – heat exchanger; 17 – Venturi scrubber; 18 – cyclone; 19 – sump; 20 – coarse cleaning air filter; 21 – fine air filter; 22 – receiver; 23 – heat exchanger; 24 – compressor; 25 – belt dryer; 26 – blower; 27 – dryer cyclone; 28 – storage capacity.

The paste obtained in the settling process, with a content of 15.8% ASB, is sent to a belt dryer, where it is dried to a moisture content of 9-10%. Part of the yeast from the conical part of the dryer is fed by pneumatic transport through a pipe into the cyclone, where it is separated from the spent coolant, which enters the wet scrubber.

The resulting dried material enters the screw mixer, and from there into the automatic weighing dispenser, where it is packed in kraft bags, which are sent to the warehouse of finished products. A graphic representation of the technological scheme is presented in Figure 4.

## CONCLUSIONS

1. The main chemical and microbiological indicators of the fat-containing phase of the effluents of fish processing enterprises were determined. It was established that in the process of holding waste in a sludge collector (1-13 days) as a result of the vital activity of autochthonous microorganisms, the fat content decreases from 87 to 58%.

2. It was established that the dominant organism of the autochthonous microflora of the waste is *Geotrichum sp.* It has been established that *Yarrowia lipolytica* yeast is the most promising fat biodestructor.

3. A method of pretreatment of the fat-containing solid phase - ultrasonic - has been developed dispersion, as a result, the bioavailability of the substrate increases, the specific growth rate and the yield of microbial biomass increase by 11 and 30%, respectively.

4. Methods of seed preparation have been developed, which allow to increase the efficiency of the main process according to indicators: biomass yield by 10.1%; specific growth rate (from 0.20 to 0.26 h<sup>-1</sup>); protein content (by 16.7%). The methods include the selection (5-7 passages) of high-performance clones, which is carried out in the directions of increasing affinity to the substrate.

5. It was established that for the biodestruction of fatty effluents, it is expedient to use both top-up and continuous methods of cultivation, which do not lead to significant changes in the quality of the obtained biomass.

6. Based on the results of complex step-by-step studies of the bioconversion by *Y. lipolytica* yeast of the fat-containing solid phase of fatty effluents of fish processing enterprises into a protein feed additive, a technological scheme of continuous production was developed.

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## **Біоконверсія жировмісної фази стоків рибопереробних підприємств у кормову добавку**

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Одеська національна академія харчових технологій

**Анотація.** Запропоновано технологію утилізації жировмісної фази стоків рибопереробки на основі комбінування фізико-хімічного впливу і біологічного окиснення. Установлено, що найбільш перспективним біодеконструктором жирів є дріжджі *Yarrowia lipolytica*, селекціоновані щодо субстрату. Розроблено принципову технологічну схему біоконверсії жировмісної фази стоків рибопереробних підприємств в дріжджову біомасу кормового призначення

**Ключові слова:** технічна екологія, технології захисту, поводження з відходами, нормування навантаження на довкілля, кормова добавка