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METHODS OF CONTROLLING STRUCTURAL-MECHANICAL AND DISPERSED CHARACTERISTICS OF POWDERED BACTERIAL PREPARATIONS

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Subject. *Analysis of structural-mechanical and technological characteristics of powders of a number of bacterial preparations that were obtained by spray drying.* ***Purpose.*** *To investigate the influence of physicochemical, rheological properties of biosuspensions and their drying conditions on the structural, mechanical and dispersion characteristics of powders of a number of bacterial preparations.* ***Methods.*** *The objects of the study were biosuspensions of bacterial preparations, such as Lactin-K, Bifacyl, and BPS-44R, consisting of bacterial cell biomass and protective medium (PM) components. Food-grade soluble substances, including skimmed milk powder (SMP), sugar, and gelatin, were used as the PM. To obtain the powder form of the bacterial preparations, laboratory and industrial spray dryers equipped with various types of spraying devices were used.* ***Results****. The studies have shown that powders have a high level of dispersion: the particle size range is 2–40 µm, and the average particle size is 6-18 µm; powders are hygroscopic, lack sufficient flowability, and have a low bulk density of 360-550 kg/m³. The study investigated the influence of the following factors: the temperature of the biosuspension before feeding into the spray chamber, the weight ratio of biomass to protective medium in the biosuspension, and the drying temperature regime on the average particle size of the powder. Microstructural analysis established that the use of a pneumatic nozzle promotes the production of a powder with an average particle size 1.8 times smaller than that obtained using a centrifugal disc sprayer (model Ts-18). When studying the effect of different disc device types, it was found that dispersion with a high-speed disc (TsV-1.5–36, n=36,000 rpm) also contributes to a reduction in particle size by approximately 2 times compared to using a disc with a lower rotation speed (Ts-18, n=18,000 rpm).* ***Scope of the results application.*** *The research results can be used to determine methods that contribute to improving the qualitative characteristics of bacterial preparation powders obtained by the spray drying method.*
Keywords: *bacterial preparations, spray drying, dispersion, bulk density, microstructural analysis*

МЕТОДИ УПРАВЛІННЯ СТРУКТУРНО-МЕХАНІЧНИМИ ХАРАКТЕРИСТИКАМИ ТА ДИСПЕРСНІСТЮ ПОРОШКІВ БАКТЕРІАЛЬНИХ ПРЕПАРАТІВ

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Предмет. *Аналіз структурно-механічних та технологічних характеристик порошків низки бактеріальних препаратів, отриманих методом розпилювального сушіння.* ***Мета***. *Дослідити вплив фізико-хімічних, реологічних властивостей біосуспензій та умов їх сушіння на структурно-механічні та дисперсні характеристики порошків низки бактеріальних препаратів.* ***Методи***. *Об'єктом досліджень були біосуспензії бактеріальних препаратів: Лактин-К, Біфацил, БПС-44Р, що складаються з біомаси бактеріальних клітин та компонентів захисного середовища (ЗС). Як ЗС використовують харчові розчинні речовини: сухе знежирене молоко (СЗМ), цукор, желатин. Для отримання порошкової форми бактеріальних препаратів використовували лабораторну та промислові розпилювальні сушарки, які комплектуються різними типами розпилювальних пристроїв.* ***Результати***. *Дослідженнями встановлено, що порошки мають високий рівень дисперсності: діапазон розмірів частинок становить – 2…40 мкм, а середній розмір часток – 6…18 мкм; порошки є гігроскопічними, недостатньо сипучими, мають низьку насипну щільність – 360…550 кг/м^3. Досліджено вплив таких факторів, як температура біосуспензії перед подачею в розпилювальну камеру; співвідношення кількості (за масою) біомаси та захисного середовища в біосуспензії та температурного режиму сушіння на середній розмір часток порошку. Мікроструктурним аналізом було встановлено, що використання пневмофорсунки сприяє отриманню порошку, середній розмір частинок якого у 1,8 рази менший, ніж порошку, отриманого при використанні відцентрового дискового розпилювача Ц-18. При дослідженні впливу типорозміру дискових пристроїв встановлено, що диспергування швидкообертовим диском (ЦВ-1,5-36, n=36000 об/хв), також сприяє зменшенню розміру частинок порошку у 2 рази у порівнянні з використанням диску з меншим числом обертів (Ц-18, n=18000 об/хв).* ***Сфера застосування результатів.*** *Результати досліджень можуть бути використані для визначення методів, що сприяють покращенню якісних характеристик порошків бактеріальних препаратів, отриманих методом розпилювального сушіння.*

Ключові слова: *бактеріальні препарати, розпилювальне сушіння, дисперсність, насипна щільність, мікроструктурний аналіз*

Problem statement. The production of bacterial preparations is a highly relevant field due to its wide application in pharmaceuticals, the food industry, agriculture etc. These preparations facilitate the production of vital medicines, improve food quality, and enhance agricultural efficiency. In Ukraine, there is a growing interest in developing domestic preparations based on bacterial complexes (lactic acid bacteria, bifidobacteria, spore-forming bacteria, etc.), which allows for reduced dependence on expensive imported analogues. In this regard, in addition to ensuring the required qualitative indicators (according to Technical Specifications), bacterial preparations must have a low production cost.

The dry form of bacterial preparations is currently the standard. Its main advantages include: long shelf life (from 1 to 5 years); resistance to environmental conditions; stability of composition; convenience of transportation; precise dosing; and high viability after reconstitution.

The main methods for drying bacterial preparations are freeze-drying (lyophilization) and spray drying, which ensure the preservation of microorganism viability.

In lyophilization, moisture is removed by ice sublimation under vacuum at low temperatures. This method provides the highest cell viability, preserves the original structure, allows the preparation to reconstitute instantly when dissolved in water, and ensures product stability during long-term storage [1–4]. However, this method is too time-consuming (ranging from several hours to days), has low productivity (suitable for small batches), and is energy-intensive and expensive due to equipment costs and process time; moreover, the final material requires grinding. This leads to an increase in the cost of the powder forms of the preparations. Furthermore, it should be noted that lyophilization can result in the loss of cell viability due to factors such as the formation of intracellular ice crystals and membrane damage caused by high concentrations of internal solutes [5].

The principle of spray drying consists in spraying a suspension into a chamber with hot air, leading to instant moisture evaporation. The main advantages of this method are speed (the process takes seconds), cost-effectiveness (it is less energy-intensive and cheaper compared to lyophilization), scalability (industrial-scale production), and the formation of fine particles [6–11].

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The disadvantages of this method include thermal impact (high temperatures can reduce the viability of certain microorganisms), a shorter shelf life compared to freeze-drying, and slower dissolution in water. However, to minimize these drawbacks, special protective media (protectors) or process intensification are often used. Therefore, for certain types of bacterial preparations, it is advisable to use the spray dehydration method [12,13].

In the dehydration of various materials, significant attention is paid to preserving product quality and improving its structural-mechanical properties and dispersion composition [14–16].

The dispersion composition of a powder product (particle size and distribution) has a significant impact on its hygroscopicity and solubility. Bulk density and flowability are also critical product characteristics, as they determine transportability during production and subsequent use, the suitability for granulation and tableting, and also influence the production cost [17–19].

Materials and Methods. The studies were conducted using bacterial preparations, the characteristics and compositions of which are presented in Table 1.

Table 1

Characteristics and composition of bacterial preparations

Name of the preparation	Biosuspension components (biomass + protective medium)		Dry matter (DM) content in the biosuspension, C_o,%	Biosuspension pH
	Bacterial cell cultures (BM)	Protective medium		
Lactin-K (veterinary preparation)	*Lactobacterium salivarius, Lactobacterium fermentum* (vegetative cells)	SMP + sugar.	17.3	5.5–6.0
Bifacyl (therapeutic and prophylactic preparation)	*Bifidobacterium adolescent; Lactobacterium acidophilus* (vegetative cells)	SMP + sugar + gelatin	9.5 14.0	4.7
BPS-44R veterinary preparation)	*Bacillus subtillis 44R* (spore-forming bacteria)	SMP.	12.5–23	6.5–6.8

For the study of the spray drying process of bacterial preparation biosuspensions, laboratory and industrial dryers (models RTs-1.2, RTs-1.3, and SUM-1.5) were used.

In the production and application of powdered materials, a series of mechanical processes are carried out, which in our case include transportation, separation, unloading, and storage. During these processes, it is necessary to take into account the structural-mechanical properties of the powder. Deficiencies in these properties of the powdered product lead to disruptions in the technological regime, deterioration of product quality, sticking and clogging of equipment, as well as to product loss.

During the studies, we determined the following characteristics: bulk density, dynamic angle of repose, particle size distribution, and degree of dispersity. The bulk density indicates the mass per unit volume of the dry product and depends on the density of individual particles as well as their packing arrangement within a unit volume. The dynamic angle of repose is also related to the density of the powder particles and indicates the product's flowability. Furthermore, this indicator is essential for designing bins and cyclone devices. To determine these indicators, methods regulated by the State Pharmacopoeia of Ukraine (SPhU), which are based on the European Pharmacopoeia (Ph. Eur.) – specifically SPhU sections 2.9.34 and 2.9.36 [19] – were applied. The final moisture content was determined by the gravimetric method: the sample is weighed, dried in an oven (at 102–105°C) to a constant weight, and then reweighed. The moisture content is calculated by the difference in mass before and after drying. When determining the final moisture content, bulk density, and angle of repose, the result was taken as the arithmetic mean of six measurements, ensuring that the difference between individual determinations did not exceed 3%.

To determine the dispersion composition of the powdered materials, a microscopic method was used, which allows for observing particle shape, the surface of particles and aggregates, and obtaining data on the maximum and minimum particle sizes. An "MBI 3U 4.2" microscope was used, and microphotography of individual powder samples was simultaneously performed using a Sony Cyber-Shot DSC-W160 digital camera.

Results and Discussion. The bacterial preparation in liquid form is a high-moisture biosuspension containing bacterial cells, their metabolites, residues of the nutrient medium, and a protective medium. The initial dry matter concentration of the biomass itself ranges from 3.5% to 10%.

Parameters such as the initial dry matter concentration of the biosuspension (C_o) and its viscosity before feeding into the dryer, the composition of the protective medium, the weight ratio of biomass to protective medium in the biosuspension, the drying temperature regime, and the design features of the spraying devices used, allow us to control the qualitative, structural-mechanical, and dispersion characteristics of the powdered product.

The bulk density of the bacterial preparation powders under study ranges from 360 to 550 kg/m³, the average particle size varies within 7–18 μm, and the final moisture content is 4.2–7.8%.

Table 2 presents the research results. The introduction of protective medium components into the biomass and its adjusted composition allows not only for preserving the viability of bacterial cells during drying but also for increasing the product concentration before drying from 9.5% to 23%, improving the drying process and the qualitative characteristics of the powdered product. For instance, the intensification of the drying process by increasing the inlet air temperature (for the Lactin-K preparation with identical composition and biosuspension ratio) allowed for a reduction in the final moisture content from 7.8% to 4.7% and an increase in bulk density to 540 kg/m³ while maintaining a high activity titer. For the Bifacyl preparation, the adjusted composition of the protective medium itself (reducing the amount of sugars and gelatin, which exhibit adhesive properties during drying) and the biosuspension as a whole (weight ratio of biomass to PM) also enabled intensification of the spray drying process, achieving a final moisture content of 4.5%, a bulk density of 550 kg/m³, and an increased activity titer of up to $1 \cdot 10^{10}$ CFU/g.

For the BPS-44R preparation, it can be noted that the bulk density of the powders obtained under equal drying temperature regime is influenced by the initial dry matter concentration of the biosuspension, which depends on the weight ratio of biomass to the protective medium.

It is known that the efficiency of particle separation in cyclone devices after spray drying depends on their size and density. The fine fraction of the powder is poorly captured in cyclones, leading to product loss with the exhaust drying agent. A correlation exists between the particle density values and the bulk density of the powder.

The average particle size of the powders depends on the design characteristics of the dryers, the initial concentration of the biosuspension before drying, the drying temperature regimes, and other factors. Particle formation during drying is evidently influenced by the size of the bacterial cells, which varies for different bacterial species. For instance, under identical drying temperature regimes and nearly equal dry matter concentrations of the biosuspensions, the powdered product containing vegetative cells exhibits a larger average particle size than the powder containing spore cells.

The angle of repose for almost all obtained powdered products is 30–40°, indicating insufficient flowability of the powder and the possibility of its accumulation in the lower part of the chamber and the air duct leading from the drying chamber. Considering the abovementioned, the following objectives were set:

- to investigate the influence of the rheological properties of the liquid product and drying conditions on the structural-mechanical and dispersion characteristics of the resulting bacterial preparation powders;

- to investigate the influence of the type of spraying device on the dispersion characteristics of bacterial preparation powders.

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Table 2

Structural-mechanical and dispersion characteristics of powdered bacterial preparations

Preparation	Weight ratio of biosuspension components, (%)	Initial dry matter concentration of the biosuspension C_o, %	Drying temperature		Final powder water content W_f, %	Bulk density ρ_b, kg/m^3	Angle of repose, deg.	Particle size range, μm	Average powder particle size δ_{avg}, μm	Dryer type	Powder activity titer, CFU/g
			T_{in}, °C	T_{out}, °C							
Lactin-K	BM (45%) SMP (28%) Sugar (27%)	17.3	115±5 160±5	60±3 70±3	7.8 4.7	500 520-540	40 35	4-40	10...11 16…18	SUM-1.5 RTs-1.3	$22\cdot10^{10}$ $25\cdot10^{10}$
Bifacyl	BM (18%) SMP (22%) Sugar (30%) Gelatin (30%)	9.5	130±5	80±5	6.3	475	38	2-35	10	RTs-1.3	$1\cdot10^{8}$
	BM (50%) SMP (23%) Sugar (23%) Gelatin (4%)	14.0	160±5	80±5	4.5	550	30	2-30	14	RTs-1,2	$1\cdot10^{10}$
BPS-44R	BM (30%) SMP (70%)	12.5	160±5	80±5	4.8	360	42	2-30	10	RTs-1.3	$7\cdot10^{9}$
	BM (50%) SMP (50%)	18.9	160±5	80±5	4.5	380	40	2-30	12		$4\cdot10^{10}$
	BM (40%) SMP (60%)	23.0	175±5	80±5	4.2	400	40	2-40	16		$4\cdot10^{10}$

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The size of the droplets (and, consequently, the powder particles), with constant geometric and flow characteristics of the spraying device, is determined by the physical properties of the liquid: density, surface tension, and viscosity. Studies by the authors [20–22] have shown that when the dry matter content of the biosuspension increases, its density and surface tension change by no more than 10%, while the maximum and average values of these indicators, depending on the product temperature, differ by no more than 5%.

The rheological properties of the BPS-44R preparation biosuspension of various compositions were studied (measured with a VPZh-1 glass capillary viscometer). The graphical dependencies (Fig. 1) show that at T_p=24°C, the kinematic viscosity coefficient of the biosuspension with a dry matter concentration of 12.5%, which includes non-concentrated biomass (C_o^{bm}=4.2%), is ν=2,3·10^{-6} m²/s. For the biosuspension with a dry matter concentration of 18.9%, including concentrated biomass (C_o^{bm}=10.0%), it is ν=4.0·10^{-6} m²/s. The kinematic viscosity coefficient for the biosuspension with a dry matter concentration of 23.0% is ν=4.9·10^{-6} m²/s.

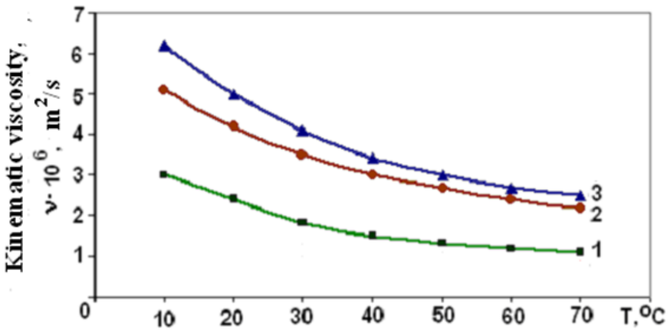

Fig. 1. Dependence of the kinematic viscosity coefficient on temperature:
1 – "BPS-44R" preparation biosuspension: Co=12.5%, (Cobm=4.2%; BM:SMP – 30:70%);
2 – "BPS-44R" preparation biosuspension: Co=18.9%, (Cobm=10.0%; BM:SMP – 50:50%);
3 –"BPS-44R" preparation biosuspension: Co=23.0%, (Cobm=10.0%; BM:SMP – 40:60%)

The viscosity of the BPS-44R biosuspension changes by 50% or more, depending on the biomass dry matter content and the amount of the protective medium component (SMP) being added. Thus, the main physical parameter affecting the size of the dispersed particles is the viscosity of the biosuspension.

Based on this, the influence of the initial biosuspension temperature before feeding onto the dryer disc, the amount of SMP, and the drying temperature regimes on the average particle size of the resulting powder was studied. The research was conducted using an RTs-1.3 spray drying unit, with a Ts-18 disc sprayer featuring the following characteristics: d_d= 0.12 m; n= 18000 rpm. The research results are presented in Table 3.

Table 3

Dispersion characteristics of BPS-44R preparation powders obtained under various thermal and technological conditions

No.	BM: SMP ratio, %	Suspension dry matter content,%	Suspension temperature at dryer inlet, T_{susp}, °C	ν·10^6, m^2/s	Drying temperature regime		Morphological and dispersion characteristics of the powder
					T_{in}, °C	T_{out} °C	
1.	50:50	18.9	25	4.0	160	80	Particle size range: 2-30 μm. **δ_{avg}≈10 μm;** 80% of the total particle mass: 9-12 μm.
2.	50:50	18.9	10	4.8	160	80	Particle size range: 2-30 μm; **δ_{avg}≈12 μm.** 80% of the total particle mass: 10-14 μm
3.	40:60	23.0	10	5.8	175	80	Particle size range: 4-35 μm; **δ_{avg}≈16 μm.** 80% of the total particle mass: 14-18 μm

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As shown in Table 3, with a constant dry matter content in the biosuspension C_o = 18.9%, the average particle size of the powder is influenced by the temperature at which the suspension enters the dryer. Under identical spray drying conditions (T_{in} = 160°C; T_{out} = 80°C; air flow rate Q_{air} = 180 kg/h) and a BM:SMP ratio of 50:50%, the average particle size was larger for the biosuspension that was fed into the dryer at a temperature of 10°C (δ_{avg} = 12 μm). When the suspension was fed at 25°C, the average particle size was 10 μm. During the spray drying of a biosuspension with the following characteristics: dry matter content – 23%; BM:SMP weight ratio – 40:60%; T_{susp} = 10°C; and drying agent parameters: T_{in} = 175°C; T_{out} = 80°C; air flow rate Q_{air} = 165 kg/h, the average particle size of the powder sample was 16 μm.

Studies have established that using a larger amount of protective medium (in this case, skimmed milk powder, SMP), cooling the biosuspension to 10°C before drying, and conducting the spray drying process at elevated drying agent temperatures contributed to obtaining a powder with an average particle size 1.6 times larger than when drying a biosuspension at T_{susp} = 25°C, with a 50:50% component ratio and an inlet air temperature of 160°C.

The powders of all samples consist of particles with a smooth surface and a shape close to spherical (Fig. 2). The particle size range is from 2 to 35 μm. As follows from the presented data, certain methods can increase the average particle size of the powder; however, particle size analysis of the polydisperse system shows that 20–30% of the particles have sizes within 2–15 μm. Therefore, it is necessary to perform a calculated efficiency analysis of the cyclones used in the drying plant configurations.

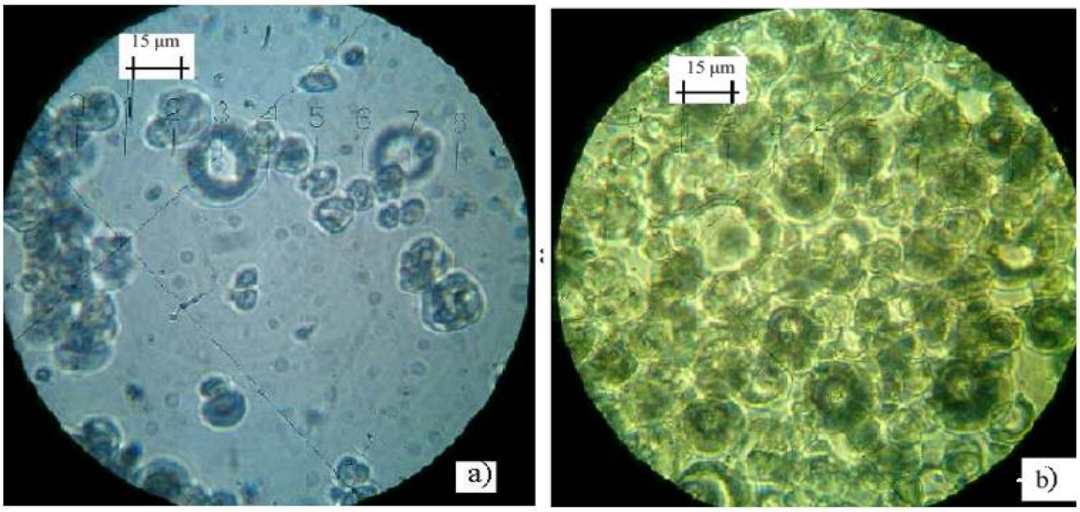

Fig. 2. Micrographs of BPS-44R powder samples (referring to Table 3):
a) exp. 1; b) exp. 3.

The spray drying units RTs-1.2, RTs-1.3, and SUM-1.5 are equipped with various types of spraying systems. The impact of sprayer design features on the dispersity of the resulting powders was investigated. Microstructural analysis revealed that the use of a pneumatic nozzle facilitates the production of powder with a mean particle size of 11 μm, which is 1.8 times smaller than that obtained using the Ts-18 centrifugal disc sprayer (Table 4). Furthermore, investigating the effect of disc sprayer specifications showed that high-speed disc dispersion (TsV-1.5-36, n = 36000 rpm) results in an approximately twofold reduction in particle size compared to a disc with lower rotational speed (Ts-18, n = 18000 rpm) (Table 5).

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Table 4

Dispersion characteristics of Laktin-K powder (C_0 = 17.3%) obtained using various types of spraying devices

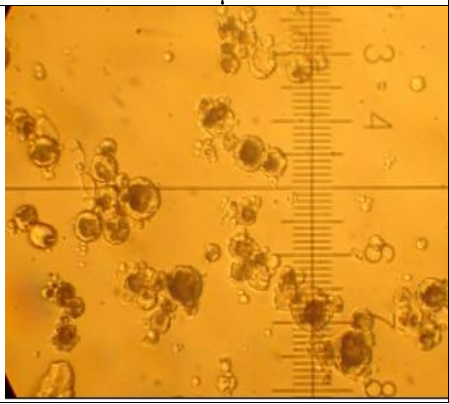
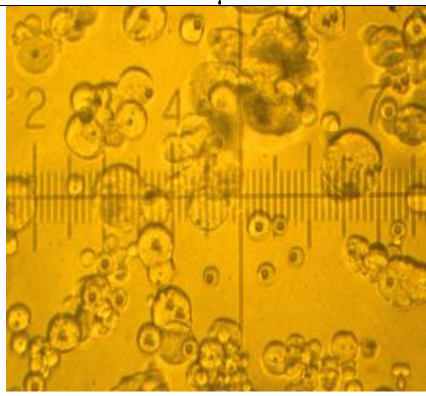
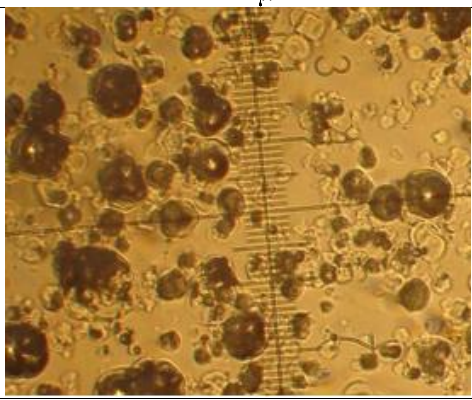
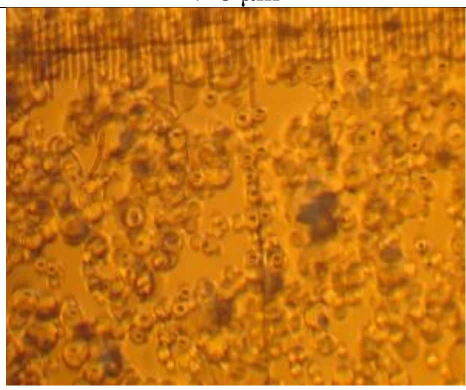
Type and characteristics of the spraying device	Pneumatic nozzle: - pressure - *P* = 0.3–0.5 MPa; - air flow rate: 0.4 m³/kg of solution	Centrifugal disc sprayer Ts-18: d_d = 0.120 m; n = 18000 rpm.
Drying regime	T_{in}=160 °C; T_{out} =70 °C; Q_{air}=680 kg/h	
Average particle size	11 μm	18 μm
Micrograph of the sample; 1 scale division = 4 μm		

Table 5

Dispersion characteristics of Byfatsyl preparation powder (C_o= 16.6%) obtained using centrifugal disc sprayers of various specifications under drying conditions of T_{in}=160 °C; T_{out} =80 °C

Type and characteristics of the spraying device	Centrifugal disc sprayer Ts-18 d_d= 0.120 m; *n* = 18000 rpm	Centrifugal disc sprayer TsV-36 d_d=0.078 m; *n*=36000 rpm
Average particle size	12-14 μm	7-8 μm
Micrograph of the sample; 1 scale division = 4 μm		

Conclusions. The dispersion and structural-mechanical characteristics of bacterial preparation powders obtained by high-temperature spray dehydration, as well as the influence of several factors on their variations, were investigated.

It was established that the powders exhibit a high level of dispersity, with particle sizes ranging from 2 to 40 μm and an average particle size of δ_{avg} = 6–18 μm. The powders are hygroscopic, possess insufficient flowability, and have a low bulk density ranging from 360 to 550 kg/m³. For the BPS-44R preparation, it was found that cooling the biosuspension to T_{susp}=10°C prior to spray dehydration and introducing a protective medium (SPM) at a weight ratio of 60% to the cell biomass allows for an increase in the average particle size of the powder product during disc spraying.

The degree of influence of the spraying method and the centrifugal disc sprayer specifications on the dispersion characteristics of the resulting powders was determined. Based on the powder microstructure research, the expediency of using a Ts-18 centrifugal disc sprayer with the following parameters: d_d = 0.120 m; n = 18000 rpm for spray drying of this class of products was demonstrated.

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