Chemistry, Technology and Application of Substances

Vol. 6, No. 2, 2023

ТЕХНОЛОГІЯ БРОДІННЯ, БІОТЕХНОЛОГІЯ

A. O. Vikhrova[1], M. S. Kurka[1], Izabela Jasicka-Misiak[2], I. R. Buchkevych[1]

[1] Lviv Polytechnic National University,
Department of Biologically Active Compounds, Pharmacy and Biotechnology

[2] University of Opole, Opole, Poland

anastasiia.vikhrova.bt.2019@lpnu.ua

FUNDAMENTALS OF PROBIOTIC PRODUCTION TECHNOLOGY BASED ON *LACTOBACILLUS ACIDOPHILUS* STRAINS

https://doi.org/10.23939/ctas2023.02.102

Designed and engineered the production of a probiotic preparation based on antibiotic-resistant strains of *Lactobacillus acidophilus*, which is beneficial to use in conjunction with antibiotic therapy for the treatment of various types of infections to maintain normal intestinal microflora. Provided qualitative characteristics of the raw materials, producer microorganisms, and the final product. On the basis of the material balance of cultivation agreed with the design capacity, technological equipment of appropriate parameters was selected, a block diagram of the technological line and a technological scheme of probiotic production were developed. Implementation of the proposed production of a probiotic preparation based on *Lactobacillus acidophilus* will allow to expand the range of domestic probiotic dietary supplements.

Key words: *Lactobacillus acidophilus*; cultivation; growth medium; sterilization; fermenter; technological scheme.

Introduction

The life of modern individuals is more diverse than ever before, yet it is also complicated by external factors that can directly impact their health. Since the mid-20th century, humanity has made tremendous progress in the pursuit of knowledge, exploration, and practical activities. However, the fast pace of life, often leading to stressful situations that exert a toll on the human body, the availability of a variety of food and especially medications, have brought about drawbacks that were initially overlooked. Working beyond normal limits, lack of quality rest without the interference of modern technologies, sedentary lifestyles, diets predominantly consisting of processed food, self-prescribed treatments without medical intervention, or even ignoring health problems – all of these have resulted in negative consequences. As a result, practically every person has encountered dysbiosis or other gastrointestinal disorders in their life. Dysbiosis most commonly arises from antibiotic usage or improper diet and a sedentary lifestyle.

Ignoring a problem like dysbiosis is both dangerous and ineffective because the problem will not disappear without timely treatment prescribed by qualified doctors. Dysbiosis directly affects not only the functioning of the gastrointestinal tract but also the overall functioning of the body. The intestinal microflora is extremely important for normal digestion of food and the proper metabolism of compounds, including the intake of medications. Only a healthy state of the microflora, known as normaflora, can ensure an efficient and proper process of food digestion and the biotransformation of substances.

Considering the significant prevalence of infections affecting almost all systems of the body,

ranging from respiratory to urinary and reproductive tracts, people are increasingly using antibiotic drugs for treatment. These antibiotics are widely available in pharmacies. However, besides their effectiveness in combating harmful microorganisms, they can also have a negative impact and harm the normal flora of the human intestine. To prevent such an impact, a qualified doctor may prescribe the patient special accompanying probiotic preparations that can improve the condition of the microflora and preserve its microbiological composition. Probiotics contain microorganisms, most of which are bacteria similar to the beneficial bacteria naturally found in the human intestine. Relevance of the Study: in connection with the frequent and widespread use of antibiotic therapy for the treatment of infections, there is a need for accompanying antibiotic-resistant drugs to maintain the normal intestinal microflora. Medicines created on the basis of so-called "good bacteria", in particular *Lactobacillus* bacteria, which are one of the most famous and used bacteria, have demonstrated their effectiveness. This paper examines the production project - a probiotic preparation based on strains of "good bacteria" *Lactobacillus acidophilus*, the production of which has not yet been offered on the Ukrainian market.

The aim of this work – development of a preparative, cost-effective method for the production of a probiotic preparation based on Lactobacillus acidophilus bacteria and the stages necessary for its introduction into production. Determine the norms of consumption of raw materials and cultures of Lactobacillus Acidophilus for the industrial production of a dietary supplement. At the same time, it is necessary to carry out material, technological, thermal calculations and develop a basic technological scheme for the production of a probiotic preparation based on Lactobacillus acidophilus strains.

Materials and research methods

1. Justification of the current state

The human gastrointestinal tract is home to approximately 85 % beneficial microorganisms and 15 % pathogenic microflora. This balance is crucial for food digestion and regulation of metabolic processes in the body.

Normoflora (microflora in a normal state) refers to the qualitative and quantitative ratio of various microbial populations in individual organs and systems, which maintains the necessary biochemical, metabolic, and immunological balance for maintaining human health. The most important function of microflora is its contribution to the development of the body's resistance to various diseases and prevention of colonization by foreign microorganisms.

Disruption of the microflora leads to dysbiosis, which can be caused by host-specific factors such as genetic background, health status (infections, inflammation), and lifestyle habits, as well as environmental factors such as diet (high sugar content, low fiber content), xenobiotics (antibiotics, other medications), and personal hygiene. Fatigue, weak immunity, and poor condition of the skin, nails, and hair are the main signs of dysbiosis [1].

In the modern world, probiotics are often prescribed by doctors as dietary supplements to help restore the balance of microflora in the treatment of dysbiosis.

Probiotics contain microorganisms, the majority of which are bacteria, similar to the beneficial bacteria naturally found in the human gut [2–4]. Probiotics have been extensively studied for various gastrointestinal disorders. The most studied types include *Lactobacillus*, *Bifidobacterium*, and *Saccharomyces.* Probiotics play an important role in maintaining immune balance in the gastrointestinal tract through direct interaction with immune cells. The effectiveness of probiotics may depend on the specific strain, dosage, and the condition being treated, while the duration of therapy depends on clinical indications. There is strong evidence supporting the effectiveness of probiotics in acute infectious diarrhea, antibiotic-associated diarrhea, *Clostridium* difficile-associated diarrhea, hepatic encephalopathy, ulcerative colitis, irritable bowel syndrome, functional gastrointestinal disorders, and necrotizing enterocolitis. Conversely, there is evidence that probiotics are ineffective in acute pancreatitis and Crohn's disease [5].

The use of bacteria of the genus *Lactobacillus*, particularly the species *Lactobacillus acidophilus*, is

quite relevant today. Acidophilus bacteria are naturally present in the human body and can be found in the mouth, stomach, intestines, lungs, vagina, and urinary tract. The human body utilizes acidophilus (a fermented dairy product obtained from cow's milk through fermentation by acidophilus bacteria) to break down food and absorb nutrients.

Acidophilus is considered a "good" bacterium. It is necessary for supporting the immune system and the digestive system. *Acidophilus* helps maintain an acidic environment in the body, which prevents the growth of harmful bacteria that can lead to dysbiosis.

Today, there are several preparations available in Ukraine that contain acidophilus bacteria. For example, there is a product called "Lactiale Synbiotic (probiotic + prebiotic)" manufactured by JSC "Pharmak" in Ukraine. The product is available in capsules, and the manufacturers claim that it promotes the restoration of the intestinal microflora balance when disrupted due to antibiotic treatment, changes in climate during travel, and the influence of adverse environmental factors [6].

Another preparation available on the Ukrainian pharmaceutical market is "Probiotiks Imuno Nova", manufactured by Kharkiv Pharmaceutical Factory LLC, Ukraine. The product is in the form of tablets and is intended to support the balance of intestinal microflora and its restoration after disruption caused by antibiotic treatment. The manufacturer also claims that this probiotic has general strengthening properties and supports the body's immune system, helping it overcome the negative impact of antibiotics [7].

2. Characteristics of raw material biosynthesis

The production of the proposed probiotic preparation based on strains of Lactobacillus acidophilus is an analogue of the product "Lactofor" by Ananta Medicare Ltd (India), which contains acidophilus bacteria Lactobacillus acidophilus. In this project, it is an oral suspension powder packaged in sachets, with the active composition of lactitol – 10 g, *Lactobacillus acidophilus* – 12 million CFU, simethicone – 40 mg, folic acid – 300 mcg, vitamin B_{12} – 100 mcg.

2.1 Characteristics of biosynthesis of starting materials

Lactitol

Lactitol (4-O-β-D-galactopyranosyl-sorbitol) is a synthetic sugar alcohol derived from lactose. It is not metabolized or absorbed in the small intestine and promotes the colonization of bifidobacteria and lactobacilli in the colon [8].

Lactitol is chemically obtained by hydrogenation of lactose using metallic catalysts at high pressures and temperatures, which result in the conversion of a portion of glucose in lactose to sorbitol. Lactitol is not found in nature and is industrially produced through catalytic hydrogenation of lactose. The reaction temperature is an important variable that affects the yield of catalytic hydrogenation of lactose and typically ranges from 110 to 150 °C, while the pressure of gaseous hydrogen ranges from 20 to 70 bar.

Transition metals such as Ni, Pd, or Ru are used as catalysts at concentrations of 1.5 to 10 % based on carbon or aluminum oxide. These metals facilitate the dissociation of H_2 molecules, resulting in reactive hydrogen molecules. These unstable species react with the carbonyl group of lactose when both are adsorbed on the catalyst surface. The primary product of the reaction is lactitol with a reaction yield of over 90 %, and a significant amount of lactulose, 1.7–1.9 %, is also found. Lactitol can be hydrolyzed and further hydrogenated, leading to the formation of sorbitol and galactitol (yielding 4.6–4.8 %). After the completion of the reaction, the catalyst is separated by filtration, and the lactitol suspension is purified using ion-exchange resins. The purified suspension is evaporated to obtain lactitol syrup, which is then crystallized, centrifuged, and dried.

Therefore, lactitol is chemically synthesized through the hydrogenation of lactose using metallic catalysts under high pressure and temperature conditions.

The yield typically exceeds 90 %, and the process has been optimized to achieve a yield close to 100 % with high productivity by using ruthenium/carbon and sponge nickel catalysts at temperatures ranging from 110 °C to 120 °C and hydrogen pressure above 50 bar.

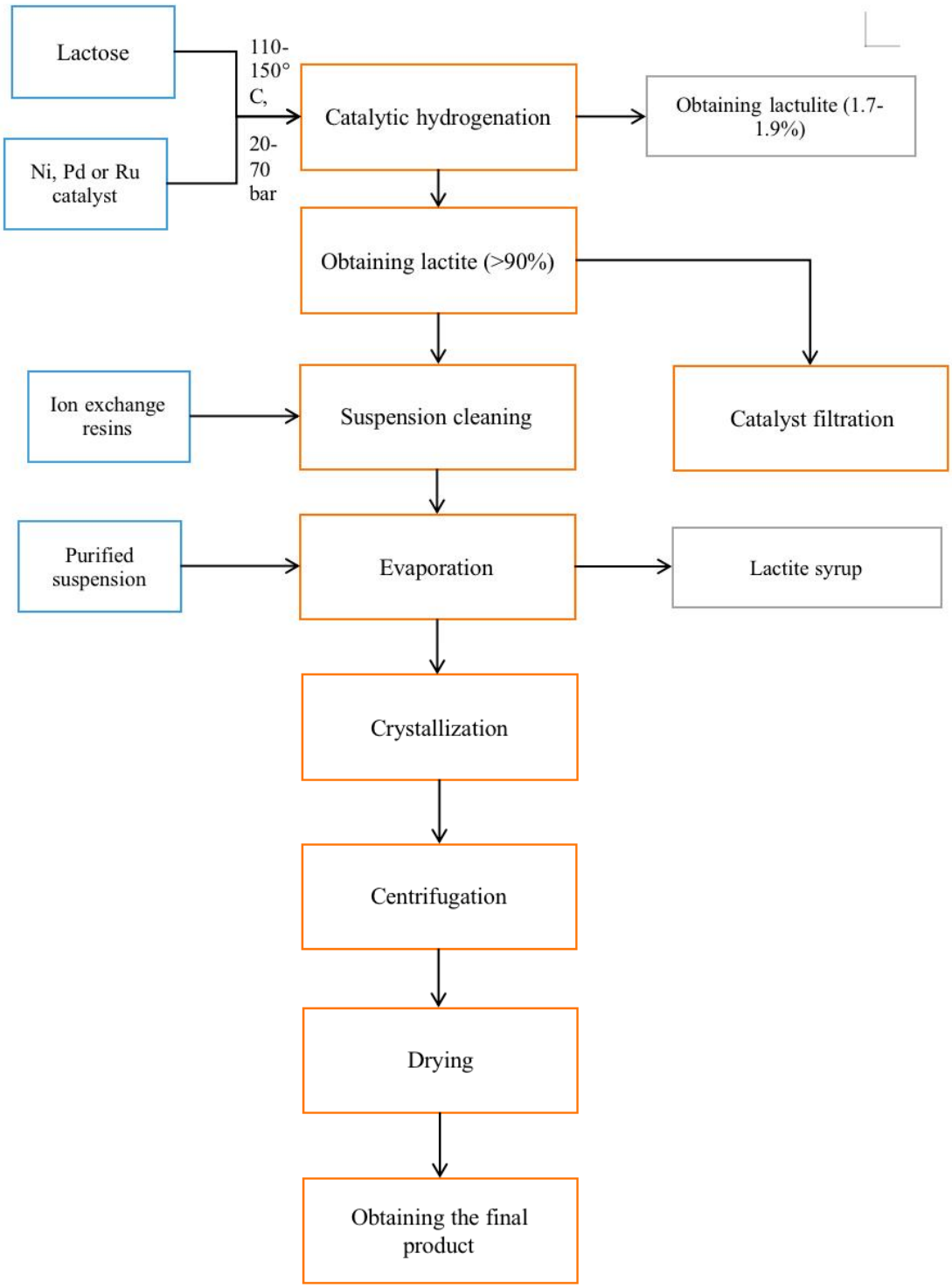

Fig. 1. Technological stages of the Lactitol production process

Lactitol is sold as a concentrated syrup and crystalline powder in the form of lactitol monohydrate. The main producers of lactitol are Purac Biochem (Netherlands), Danisco (Denmark), and Mitsubishi Shoji Foodtech Co (Japan). The low price of lactitol compared to other prebiotics or prebiotic candidates makes it an attractive and beneficial product derived from lactose. However, for its more challenging applications, intensive purification will be required, which can significantly increase its cost. Derivatives of lactitol, such as lactitol oligosaccharides and lactitol fatty acid esters, have been proposed as

valuable products for food and detergent applications, respectively, but their technological impact is still questionable [9].

Simethicone

Simethicone relieves discomfort caused by the presence of excess gas in the gastrointestinal tract. Recently, it has been actively used for endurance athletes to reduce gastrointestinal symptoms associated with physical exercise. Simethicone functions as a nonsystemic surfactant, reducing the surface tension of gas bubbles in the gastrointestinal tract. This action leads to the merging and dispersion of gas bubbles, allowing them to be eliminated from the gastrointestinal tract as flatulence or belching. Simethicone causes the accumulation of gas bubbles that pass more easily through the upper or lower parts of the GI tract. Simethicone does not suppress conditions such as lactose intolerance or the side effects of medications that increase gas bubble formation in the gastrointestinal tract [10].

The industrial synthesis of simethicone typically takes place in a special reactor at a temperature of 275 °C. The preparation method of simethicone involves the following steps:

1. Simethicone is heated to 50–85 °C in an isolating environment and mixed with silicon dioxide. The isolation effect helps in the uniform distribution of silicon dioxide.

2. The solution obtained in step (1) is combined and homogenized in a high-pressure homogenizer (50–100 MPa, holding time: 20–60 minutes), after which the environment is sealed to obtain the final product.

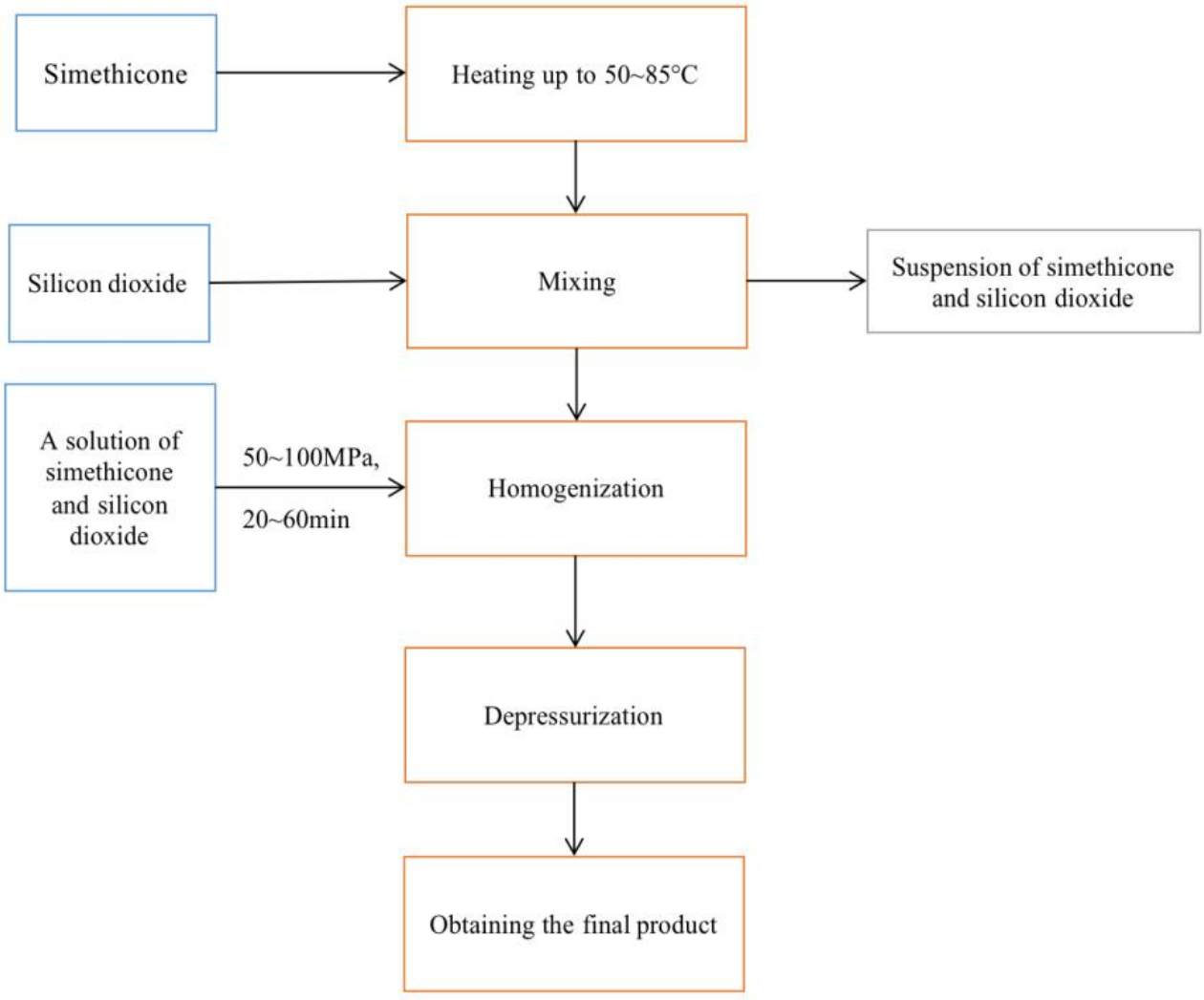

Fig. 2. Technological stages of the Simethicone production process

In the industry, a high-pressure homogenizer is often used, which uniformly disperses the silicon dioxide. It can make the material in a liquid state undergo suspension under the effect of extremely high voltage (up to 400 MPa) as it passes through the high-pressure homogenization chamber with specially designed internal properties, such as a specifically designed geometric shape at high speed. Under the influence of the carrier fluid, the material can be mechanically processed, such as high-speed shearing, high-frequency oscillation, cavitation, convection currents, and the corresponding thermal effect simultaneously. The mechanical force and chemical effects induced in this way can induce changes in the macromolecular physics, chemistry, texture, and other properties, ultimately achieving uniformity. This equipment is widely used for the preparation of nanocapsules and nanoparticles in the pharmaceutical industry [11].

Folic Acid

Folic acid, also known as vitamin B_9, is necessary for the body to synthesize purines, pyrimidines, and methionine before they are incorporated into DNA or protein.

One of the beneficial roles of folic acid is its ability to lower the level of homocysteine in cases of neural tube defects [12].

In the industry, folic acid is obtained through the following method: initially, p-nitrobenzoyl chloride and sodium glutamate are used to obtain the intermediate product N-p-aminobenzoylglutamic acid; then, methylcyanooacetate, guanidine nitrate, and sodium methoxide are used to obtain the intermediate product 6-hydroxy-2,4,5-triaminopyrimidine sulfate; finally, two types of intermediate products are utilized to prepare crude folic acid, which is subsequently purified to obtain pure folic acid [13].

Vitamin B_{12}

Methylcobalamin and 5-deoxyadenosylcobalamin are the metabolically active forms of vitamin B_{12}. [14–15]. Vitamin B_{12} is necessary for the development, myelination, and functioning of the central nervous system, formation of healthy red blood cells, and DNA synthesis.

Two main families of microorganisms are typically used for the biosynthesis of vitamin B_{12}: several strains of *Propionibacterium freudenreichii* and strains related to *Pseudomonas denitrificans*.

P. denitrificans is a Gram-negative bacterium that utilizes an aerobic biosynthetic pathway for vitamin B_{12} production. Despite the lack of a generally recognized as safe (GRAS) status, *P. denitrificans* is currently the primary producer of vitamin B_{12} used by industrial manufacturers such as Sanofi in Europe or Huarong Pharmacy Corporation in China.

The reductive process of vitamin B_{12} is a well-described process that has remained largely unchanged on an industrial scale over the past decades. It involves several stages of separation and purification of the culture broth (including extraction, filtration, and adsorption processes), which influence the overall process yield and feasibility. The classical downstream processing begins with biomass concentration to reduce the volume significantly, typically achieved through centrifugation.

In any case, all types of corrinoids are extracted by incubation at a temperature of 80–120 °C and pH 6.5–8.5 for 10–30 minutes. Cyanidation can be performed during the extraction process or after the initial stages of filtration and adsorption. In both cases, various corrinoids are converted to cyanocobalamin (CNCbl) by adding cyanide or potassium thiocyanate. This process is typically carried out in the presence of sodium nitrite and heat.

Subsequently, the CNCbl solution is purified using one or several filtration processes (microfiltration and/or nanofiltration) and adsorption. If the produced cobalamin is intended for animal feed, the vitamin solution is often treated with zinc chloride and precipitated using organic solvents such as acetone to obtain the final product. When higher purity is required, such as for pharmaceutical purposes, further adsorption steps with different resins (e.g., aluminum oxide) are often necessary.

After the purification of vitamin B_{12}, it can undergo various post-modifications for use as a food additive or an oral pharmaceutical formulation to enhance its bioavailability [14].

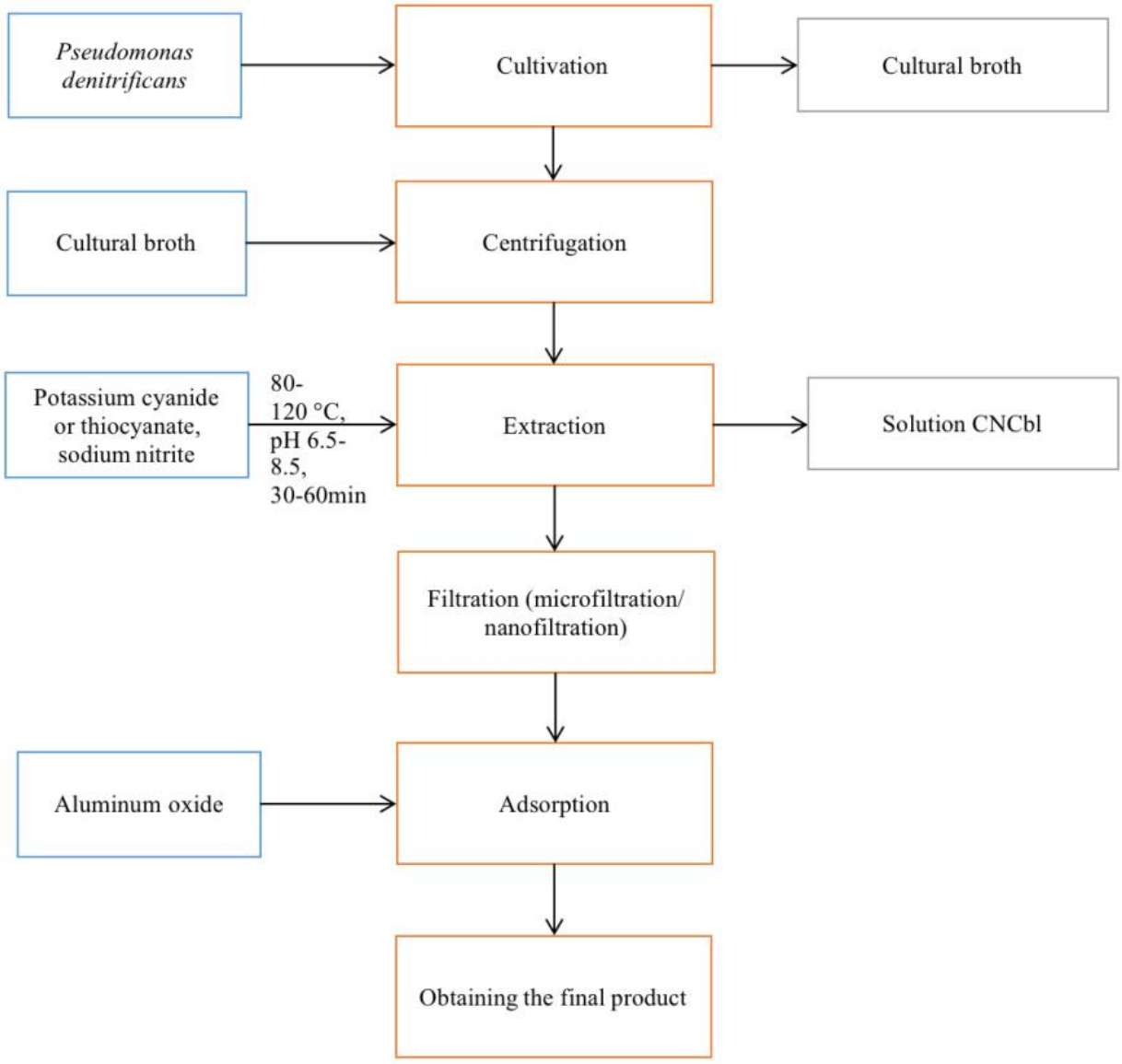

Fig. 3. Technological stages of the vitamin B_{12} production process

Sodium benzoate

Sodium benzoate (known as E211 in European nomenclature) is the salt of benzoic acid. It is used as a preservative in food at strictly defined doses. It inhibits the growth of bacteria, yeasts, and molds. The Food and Drug Administration (FDA) has approved sodium benzoate as the first of all food preservatives.

Sodium benzoate is a granular substance that has a spherical shape with a diameter of 1.5–2 mm. The production process of granulated sodium benzoate involves several stages. First, benzoic acid and sodium hydroxide are placed in a neutralization tank where a neutralization reaction takes place. The pH value is carefully controlled within the range of 7.5–8.0, and the reaction temperature is maintained at 95–98 °C for a duration of 30–40 minutes. A concentrated solution of sodium benzoate is then created, and a decolorizing gas is added to the solution. The solution is subsequently filtered under a pressure of 0.3–0.4 MPa to obtain a pure sodium benzoate solution.

The mass ratio of benzoic acid to sodium hydroxide is maintained at 3:0.8–1.5. After filtration, the sodium benzoate solution undergoes a granulation process, followed by drying and packaging. Granulation involves transferring the filtered sodium benzoate solution to a tower equipped with 4–6

nozzles that evenly distribute liquid sodium. Simultaneously, air is supplied through these nozzles into the tower. The internal temperature in the tower is controlled at 145–150 °C, and the air flow rate at the inlet is maintained at 6–8 m^3/min. The control pressure at the inlet is set at 0.3–0.4 MPa.

In the tower, sodium crystal grains are placed, and the drying process takes place in an oven at a temperature of 150–155 °C. Granulated sodium benzoate is convenient to use, reduces the labor involved, and does not solidify during transportation and storage [16].

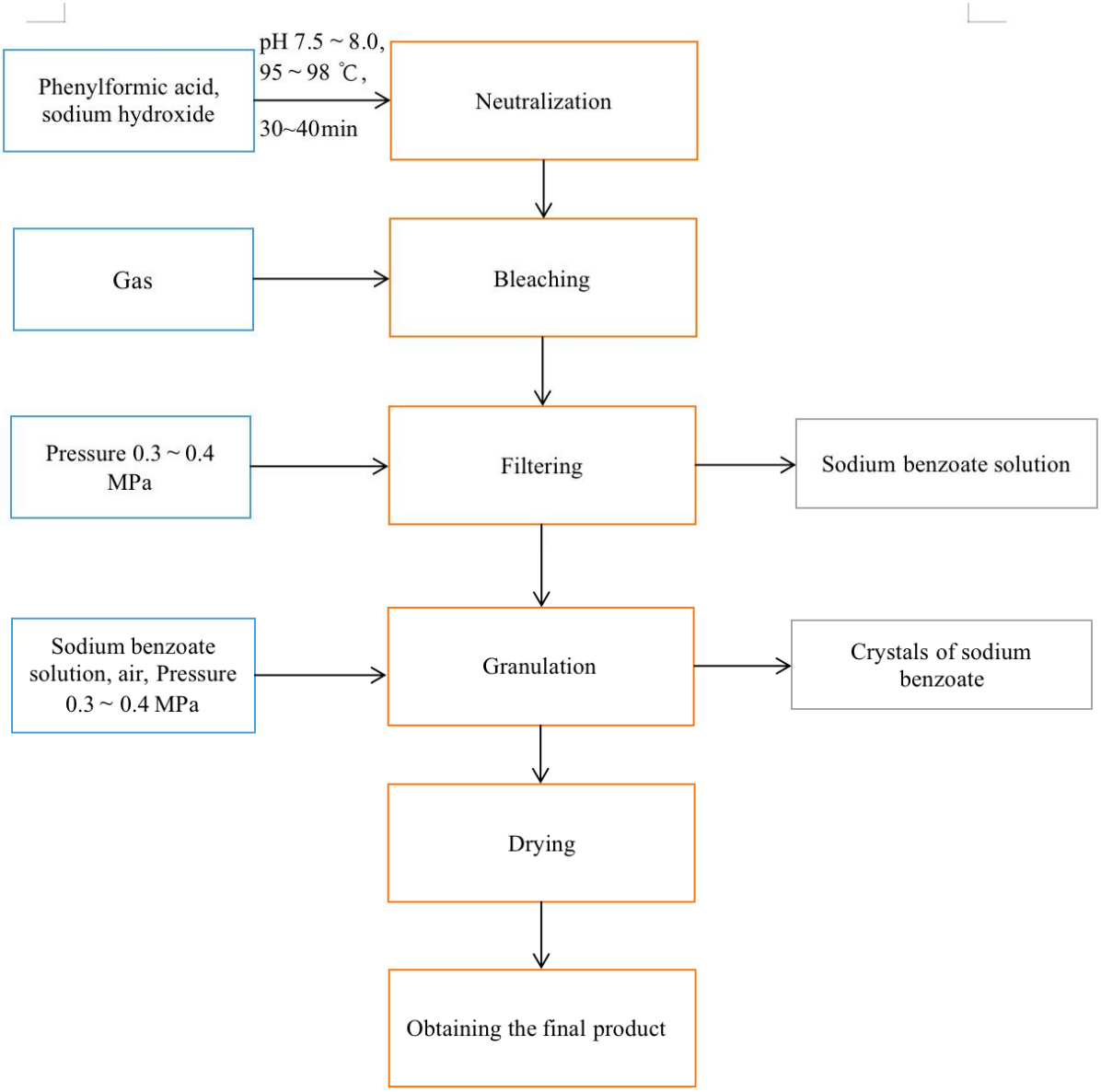

Fig. 4. Technological stages of the Sodium benzoate production process

2.2. Characterization of L. acidophilus biosynthesis for the production of a probiotic preparation

Given the biosynthetic pathways encoded in its genome, *L. acidophilus* is auxotrophic for 14 amino acids and is unable to synthesize several cofactors and vitamins, including riboflavin, vitamin B_6, nicotinate, nicotinamide, biotin, and folate. An example of this deficit in anabolic capacity is the need to use nutrient-rich media such as DeMann, Rogosa, and Sharp (MRS) agar for conventional culture.

The table below shows the biochemical characteristics of *L. acidophilus*.

Table 1

Biochemical characteristics of *Lactobacillus acidophilus*:

No.	Characteristics	L. acidophilus
	Capsule	None
2	Form.	Wand
3	Gram staining	Gram-positive
4	Catalase	Negative (–)
5	Oxidase	Negative (–)
6	Citrate	Negative (–)
7	Methyl red	Negative (–)
8	Voges-Proskauer reaction	Negative (+)
9	Glucose oxidative enzyme test	Oxidizing
10	Coagulase	Negative (–)
11	Deoxyribonuclease	Negative (–)
12	Urease	Negative (–)
13	Gas	Negative (–)
14	H_2S	Negative (–)
15	Hemolysis	β-hemolytic
16	Mobility	Some strains are motile with a single flagellum
17	Nitrate reductase	Negative (–)
18	Hydrolysis of gelatin	Negative (–)
19	Pigmentation	Negative (–)
20	Indole	Negative (–)
21	Disputes.	Does not form

Table 2

Fermentation and substrate characteristics of *Lactobacillus acidophilus*

No.	Substrate	L. acidophilus
	Arabinose	Positive (+)
2	Cellobiose	Positive (+)
3	Fructose	Positive (+)
4	Galactose	Positive (+)
5	Glucose	Positive (+) Bonded homoenzymatic
6	Glycerol	Positive (+)
7	Glycogen	Positive (+)
8	Inulin	Negative (–)
9	Lactose	Positive (+)
10	Maltose	Positive (+)
11	Manitol	Negative (–)
12	Mannose	Positive (+)
13	Pyruvate	Negative (–)
14	Raffinose	Positive (+)
15	Ribose	Negative (–)
16	Sorbitol	Negative (–)
17	Starch	Positive (+)
18	Sucrose	Positive (+)
19	Xylose	Positive (+)

Table 3

Enzymatic reactions of *Lactobacillus acidophilus*

No.	Enzyme	L. acidophilus
	Acetone	Negative (–)
2	Acetate utilization	Positive (+)
3	β-Haloxidase	Positive (+)
4	Hydrolysis of casein	Negative (–)
5	Lactase	Positive (+)
6	Lysine	Negative (–)
7	Phenylalanine deaminase	Negative (–)

Bacteria in general need a suitable biochemical and biophysical environment in order to grow. The biochemical medium is available as a culture medium that contains the appropriate amount of nutrients [17]. *Lactobacillus is a* group of picky bacteria that requires a rich, complex culture medium for normal growth. In addition to carbohydrates (simple sugars such as dextrose, sucrose, maltose or lactose), *Lactobacillus* cultivation media usually contain various nitrogen sources (such as peptone, yeast extract, beef extract or whey protein), minerals (mainly Mn^{2+} and Mg^{2+}), and buffering agents (such as sodium acetate and dinitroglycerophosphate). Simple carbohydrates or sugars are the main sources of carbon and energy for bacterial growth. Dextrose is the sugar most commonly used for bacterial growth, while lactose, the main sugar in dairy products, is hydrolyzed relatively slowly and is one of the least utilized forms of sugar by Lactobacillus strains.

Most *Lactobacillus require a* wide range of growth factors. Nitrogen sources such as peptones, beef extract and yeast extract are sources of amino acids, peptides, nucleic acid derivatives, minerals and vitamins. Peptides are most commonly obtained by enzymatic digestion or acid hydrolysis of natural products such as animal tissue, milk, plants, or microbial cultures. In the dairy industry, milk, skimmed milk powder, whey protein and reconstituted whey are more commonly used as sources of peptides and amino acids [18]. More than one type of nitrogen source is usually included in the *Lactobacillus* medium. Manganese sulfate ($MnSO_4$ – $5H_2O$) and magnesium sulfate ($MgSO_4$ – $7H_2$ O) are also commonly included in *Lactobacillus* cultivation media in trace amounts.

Species belonging to the genus *Lactobacillus* are among the most widely used probiotics in animal

nutrition, which are defined as living microorganisms that provide health benefits to the host when administered in the proper dose [19]. Probiotics have been proven to have a beneficial effect on people suffering from gastrointestinal disorders such as infectious diarrhea, inflammatory bowel disease, celiac disease and food allergies, and many others Moreover, scientists have recently described the positive effect of *L. acidophilus* probiotic strains, in the presence of inulin and fructooligosaccharides, on elderly patients with reduced cardiovascular and insulin-resistant risk factors associated with metabolic syndrome. In addition, these beneficial microorganisms are used as an alternative to antibiotic growth promoters (AGPs), which were banned in the European Union in 2006, leading to an increase in livestock infections. By 2024, the probiotics market is expected to be worth USD 74 billion, more than double the 2015 figure of USD 35 billion [20].

The growing demand for the use of *L. acidophilus* in industry has led to the need to obtain high densities of these bacteria at low cultivation costs. *L. acidophilus is* characterized by a high nutritional requirement due to its poor ability to synthesize amino acids and B vitamins. Therefore, their cultivation must be carried out in a saturated medium containing fermentable carbohydrates, nucleic and amino acids, B-complex vitamins, and various minerals. In addition, *L. acidophilus* bacteria predominantly utilize peptides to meet their nitrogen requirement. For the purposes of LAB cultivation, on a laboratory scale, meat or yeast extract is commonly used as a nitrogen source; however, these components contribute to the high cost of the commonly used de Man, Rogosa and Sharp (MRS) medium.

The increasing need for the use of *Lactobacillus* bacteria in industry and the growing market for probiotics have led to the search for new cost-effective fermentation media from which high yields of these bacteria can be obtained. Alternatively, the approach is to use food or agricultural by-products that can support the growth of bacteria due to their components.

Among all possible components, cereals and flours are used worldwide; therefore, their use as a medium for *L. acidophilus* can be accessible, simple and cheap. Studies have shown that a mixture of wheat, barley, corn and rye flours in combination with distilled water is a good medium for the efficient growth of *L. acidophilus*.

Cereals are mainly composed of carbohydrates, which make up up to 75 % of the dry weight of the grain; however, they are also a good source of protein, while lipids make up a small part of their nutrients (1.7 to 7.0 % of dry weight). In addition, grains contain significant amounts of vitamins E and B vitamins, as well as minerals such as zinc and magnesium, which are essential for *L. acidophilus* growth. Despite a significant decrease in the nutritional value of cereals during the milling process, which is defined as grinding grains into flour or meal, their bioavailability increases [21].

Wheat flour is a powder made from milled wheat that is used for human consumption. Wheat varieties are called “soft” or “weak” if their gluten content is low, and are called “durum” or “strong” if they have a high gluten content. Hard flour, or bread flour, has a high gluten content (12–14 % gluten). Soft flour has a relatively low gluten content, so bread has a finer, crumbly texture.

In terms of the parts of the grain (grass fruit) used in flour - the endosperm or protein/starch part, the germ or protein/fat/vitamin-rich part, and the bran or fiber – there are three general types of flour. White flour is made only from the endosperm. Brown flour contains a portion of the germ and bran of the grain, while whole wheat or whole grain flour is made from the whole grain, including the bran, endosperm, and germ. Germ flour is made from the endosperm and germ, excluding the bran [22].

Barley flour is a flour made from dried and ground barley. Barley flour is used to make barley bread. There are two general types of barley flour: coarse and fine. To produce coarse barley flour, barley groats are milled, and for fine barley flour, pearl barley is milled. In addition, patent barley flour is a finer barley flour that is milled to a greater extent than fine barley flour [23].

Corn starch is a starch derived from corn kernels. The starch is obtained from the kernel endosperm. Cornstarch is a common food ingredient that is often used to thicken sauces or soups, as well as to make corn syrup and other sugars. Corn starch is versatile, easily modified and widely used in industry, for example in adhesives, paper products, as an anti-stick agent and in textile production. It is

also used in medicine, for example, to provide glucose to people with glycogen storage disease.

Like many other products in the form of dust, it can be dangerous in large quantities due to its flammability. When mixed with a liquid, corn starch can turn into a non-Newtonian liquid [24].

Rye flour is obtained by grinding rye grain. It is made from ground rye berries, also known as whole rye kernels. Rye berries are small, hard, starchy grains similar to nuts that are ground to make rye flour. Before being milled, the hard, inedible outer part of the rye berry is removed – the hull. Different varieties of flour are produced with different degrees of purification (removal of the outer bran) and grinding [25].

Rye flour can be white to dark brown in color. The color of rye flour is the result of the milling process.

Results and discussion

The following technological stages were adopted to design the industrial scale-up of the chemical-pharmaceutical production of a probiotic preparation based on *Lactobacillus Acidophilus* strains:

- preparation of the culture medium;
- sterilization of the culture medium;
- inoculation;
- fermentation;
- centrifugation;
- lyophilization;
- mixing of drug components.

For this project, material calculations were made [26], which at each stage allowed us to determine the consumption of raw materials for the production of 1 kg of finished product. The production capacity of a probiotic preparation based on *Lactobacillus Acidophilus* strains was assumed to be 100 tons per year. As a result of the material calculations, the consumption rates for the industrial production of the substance of the probiotic preparation based on *Lactobacillus Acidophilus* strains were determined (Table 4).

The choice of technological equipment was made on the basis of technological calculations [14–16] (Table 5), which resulted in determining the required number of each type of main and auxiliary equipment, its volume (length), the material from which it should be made, and its main dimensions. To carry out the technological process, the main and auxiliary equipment must be made of stainless steel, for example, popular brands for the chemical and pharmaceutical industry AISI 304 or 316. The main dimensions of the equipment were selected from the catalogs of the relevant equipment [14], and the principle of combining their work in the technological process is shown in Fig. 9 and Fig. 10.

Table 4

Raw material costs

No.	Substance.	Unit of measurement	Standard consumption per 1 kg
1	Wheat flour	kg	0.062
2	Rye flour	kg	0.016
3	Corn flour	kg	0.031
4	Barley flour	kg	0.047
5	Water.	m^3	0.233
6	Ammonium hydroxide	m^3	0.015
7	Lactylol	kg	0.91
8	Simethicone	kg	0.003
9	Folic acid	kg	0.018
10	Cyanobalamin	kg	0.00013
11	Sodium benzoate	kg	0.0005

Table 5

Specification of technological equipment

Designation	Name and purpose	Quantity.	Technical characteristics	Dimensions
R-1	Reactor for the preparation of culture medium	1	The VEE type tank with a volume of 6.3 m^3 It is equipped with a stirrer for better mixing of components	*D*=1800 mm, *L*=1880 mm
UNS-5	Continuous sterilization unit for sterilization of culture media	1	The working capacity of the line is 1.31 m^3/h, the maximum capacity is 5 m/h^3 The holder is a 0.17 m coil^3	Refrigerator: 700×9000. The holder: *D*=1000 mm; *L*=1000 mm
I-1	Inoculant for accumulation of bacterial culture inoculum	1	VEE type tank with a volume of 0.063 m^3. It is equipped with a stirrer, a bubbler and a shell	*D*=400 mm, *L*=665 mm
F-1	Bioreactor for cultivation of bacterial culture	1	VEE type tank with a volume of 8 m^3. It is equipped with a stirrer, bubbler and shell	*D*=1600 mm, *L*=3450 mm
PF	Air filters for sterilizing air for aeration	2	Deep filters designed by Gidromedprom	*D*=700 mm, *L*=1500 mm
BC-1	Buffer vessel for storing culture liquid between fermentation and centrifugation steps	1	The VEE type tank with a volume of 6.3 m^3	*D*=1800 mm, *L*=1880 mm
FC-1	Filter type centrifuge for separation of biomass from native solution	1	FGN-200 type centrifuge with a working capacity of 1.25 m^3	$D_б$ = 2000 mm, *L*=910 mm, filtering surface 5.72 m^2, *n*=10 with^{-1}
FD-1	Freeze drying chamber for lyophilization of wet biomass sludge	1	It is equipped with a refrigeration machine, pump and coolant tank	*D*= 1500 mm, *L*=1100 mm
BM-1	Drum mixer with a diagonal axis for mixing drug components	1	VEE type vessel with a volume of 25 m^3. The reactor is equipped with a stirrer and a jacket	*D*=2770 mm, *L*=4150 mm

Thus, the basic technological scheme for the production of a probiotic preparation based on *Lactobacillus Acidophilus* strains is shown in Fig. 5. It includes the implementation of the following seven technological stages, which are preceded by the mandatory preparatory stages of sanitary preparation of production (DR1) and preparation of raw materials (DR2) (Fig. 5).

Flow chart of production of a probiotic preparation based on Lactobacillus Acidophilus strains

At the first stage, the culture medium is produced. The R-1 mixing reactor is sterilized by supplying water vapor into the apparatus. The required amount of distilled water for the preparation of the culture medium is fed into the apparatus tank through the meter. Turn on the apparatus stirrer (anchor stirrer, since the medium is quite viscous) and manually load the dry components of the medium: wheat flour, barley flour, corn flour, rye flour, ammonium hydroxide. The components of the medium are mixed well to dissolve the components in water. The finished culture medium is fed to the UNS-5 line for sterilization by the P-1 pump. After discharging the medium, pipeline water is fed into the R-1 reactor, the reactor is washed with soapy water or an acid or alkali solution, and the solution is drained into the sewage line leading to the production treatment plant. The condensate formed during steam sterilization of the device is fed through a condensation pot into the production return water line.

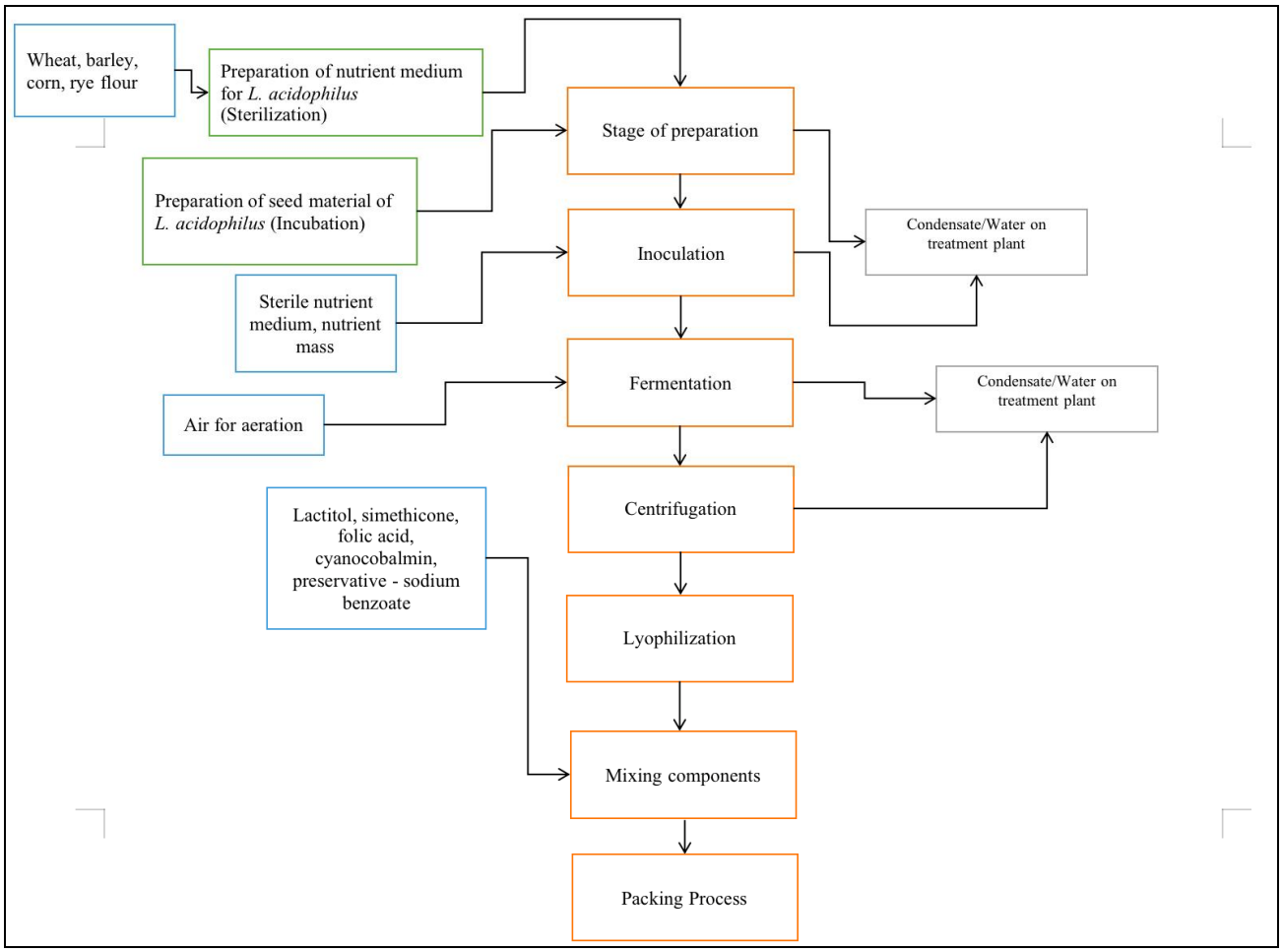

Fig. 5. Technological stages of the production process of a probiotic preparation based on Lactobacillus Acidophilus strains

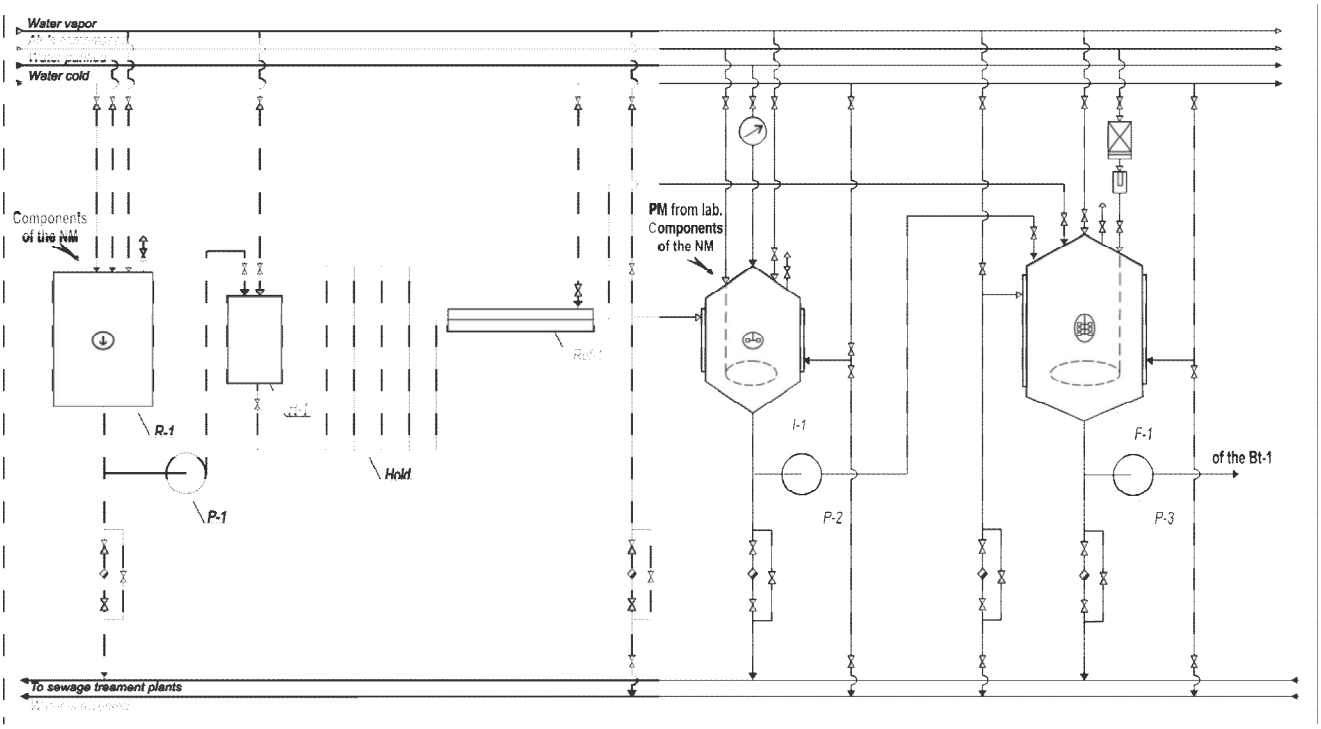

Fig. 6. Schematic flowchart of production of a probiotic preparation based on Lactobacillus acidophilus strains (part 1)

At the second stage, the culture medium is sterilized. The culture medium is fed by pump P-1 from R-1 to the heater of line UNS-5, to which the water vapor supply is connected, the medium is heated to the sterilization temperature, i. e. 121 °C. The heated medium is fed to the holder of line UNS-5, in which the medium is kept at the sterilization temperature for 7–8 minutes to ensure the required level of sterility of the medium. After incubation, the medium is fed into the refrigerator of the UNS-5 line, to which a cold water line is connected. After cooling, the sterile culture medium is fed to the pre-sterilized fermenter F-1 by pump P-2.

At the third stage, the inoculum is obtained Before use, the *Lactobacillus acidophilus* culture is stored in suspension in MRS broth in a freezer at –80 °C. In the laboratory, the inoculum of the culture is accumulated in an amount of 0.46 l. The culture is incubated at 37 °C for 24 hours. Before use, inoculant I-1 is sterilized by supplying water vapor into the apparatus. The required amount of the prepared culture medium is loaded into the apparatus, which is sterilized by supplying water vapor to the inoculator shell. After sterilization, the medium is cooled by supplying cold water to the inoculator shell, after which the inoculum from the laboratory is manually loaded. Stirring is turned on, air is supplied through the bubbler for aeration of the culture and the fermentation process is carried out for 48 hours, the fermentation temperature is maintained at 37 °C by supplying cold water to the reactor shell. Upon completion of the process, the seed in the amount of 48.267 kg is fed to the fermenter F-1 by pump P-3. After unloading the seed, the inoculant is washed by supplying water from the factory pipeline, which is sent to the production wastewater treatment plant after washing. Water and condensate from the inoculant shell are sent to the return water line.

Fermentation takes place at the fourth stage. The F-1 fermenter is sterilized by supplying water vapor inside the apparatus. After sterilization, the fermenter is cooled by supplying cold water to the shell of the apparatus. Sterile culture medium is loaded from the UNS-5 line, and seed is loaded from the I-1 inoculant using the P-2 pump. Stirring is switched on, air is supplied through the bubbler for aeration of the culture. The air for aeration is collected from the atmosphere in the cleanest possible place from a height of 8–10 meters, after which it is passed through coarse filters. Then the air is compressed using a turbocharger to a pressure of 0.32 MPa. The compressed air is fed to a line of deep air filters PF, where it is sterilized. Before being fed to the bubbler, the air passes through individual fermenter filters for additional sterilization.

The fermentation process lasts for 48 hours, the fermentation temperature is maintained at 37 °C by supplying cold water to the reactor shell. After completion of the fermentation cycle, the culture liquid is discharged by the P-3 pump into the buffer tank BC-1 for storage between stages. Washing of the fermenter is carried out similarly to the inoculator. Water and condensate from the inoculant shell are sent to the return water line.

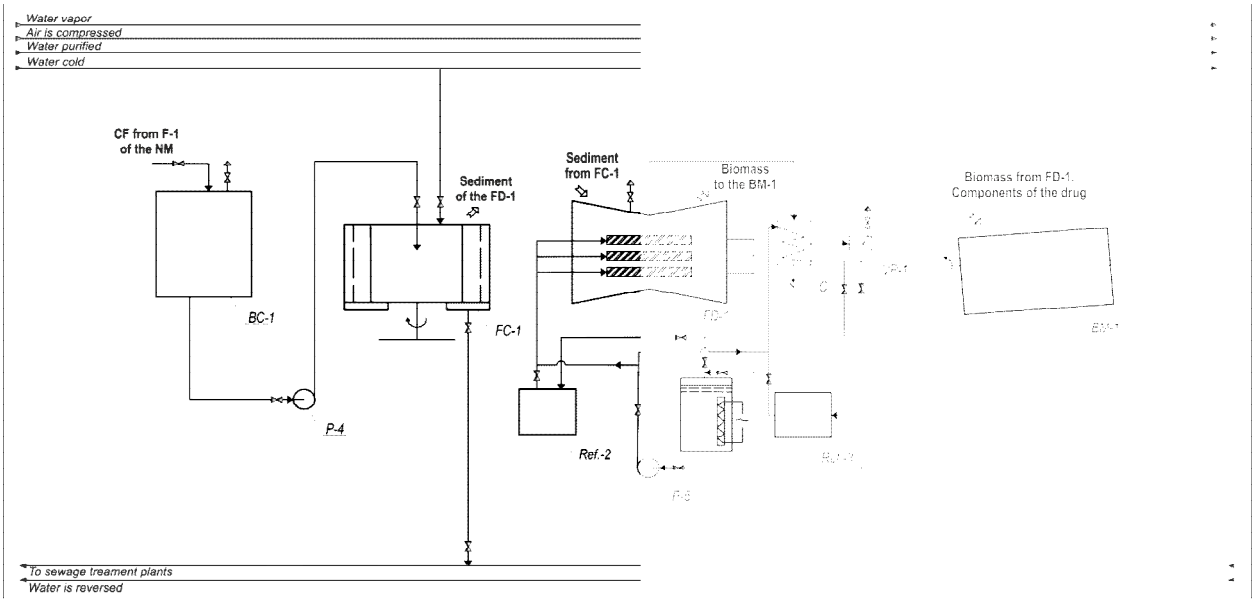

Fig. 7. Schematic flow chart of production of a probiotic preparation based on Lactobacillus acidophilus strains (part 2)

At stage V, the biomass is separated from the native solution. A suspension of culture liquid is loaded into the centrifuge with the P-4 pump from the BC-1. The centrifuge electric drive is switched on, which drives the cylindrical centrifuge body to rotate. The suspension is fed into the centrifuge body from above. Under the action of centrifugal force, solid particles of biomass settle on the inner side walls of the centrifuge body, and the filtrate is discharged from the centrifuge from the bottom and sent to the wastewater treatment plant. As the biomass sludge accumulates, the centrifuge is turned off and the sludge is manually discharged. The process cycle is repeated to process the entire slurry. After the biomass sludge is discharged, the centrifuge is washed with tap water, which is sent to the production wastewater treatment plant.

At the VI stage, the biomass is lyophilized. The wet biomass sludge is manually poured into the cuvettes of the sublimation chamber. The freeze-drying chamber is sealed and the Ref.-2 refrigeration machine is turned on, which supplies the coolant (brine) to the plates on which the cuvettes with the material are installed. The biomaterial is frozen to the state of ice. After freezing the material, the vacuum pump VP-1 creates a deep vacuum in the chamber. Brine with a temperature higher than the temperature of the frozen material is passed through the plates on which the cuvettes with the material are installed, as a result of which thermal energy is transferred to the material and water sublimation occurs. The evaporated moisture enters the condenser C, which is equipped with a heat exchanger, where steam condenses into a layer of ice. The ice is defrosted with a heated coolant and sent to the production wastewater treatment plant. Upon completion of the lyophilization cycle, the vacuum pump is turned off and the pressure in the chamber is set to atmospheric pressure. The biomaterial is thawed by the supply of warm coolant, which is unloaded from the cuvettes and sent to the BM-1 mixer.

At the VII stage, the components of the preparation are mixed. The required amount of *lyophilized* biomass of *Lactobacillus acidophilus* (797.45 kg) is loaded into the BM-1 mixer and the remaining components are added: lactitol (11425.00 kg), simethicone (31.40 kg), folic acid (228.50 kg), cyanobalamin (1.60 kg), and sodium benzoate (6.27 kg). The components are thoroughly mixed, and the finished product is unloaded from the container and sent to the packaging stage.

Conclusions

As a result of the conducted work, a project for the production technology of a high-yield of a probiotic preparation based on *Lactobacillus acidophilus* strains on an industrial scale has been proposed. For this production, a process consisting of five technological stages has been proposed. The evaluation of the raw material requirements for producing 1 kg of the probiotic preparation based on *Lactobacillus acidophilus* strains has been performed. The quantity and technological parameters of the main and auxiliary equipment have been determined, the process of ensuring thermal regimes has been evaluated, and a conceptual technological production scheme has been proposed. The proposed production of the probiotic preparation will expand the range of high-quality domestic products of this range on the market and will allow the domestic manufacturer to enter the world market with this product.

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A. O. Vikhrova, M. S. Kurka, Izabela Jasicka-Misiak, I. R. Buchkevych

А. О. Віхрова[1], М. С. Курка[1], Ізабела Ясицька-Місяк[2], І. Р. Бучкевич[1]
[1] Національний університет "Львівська політехніка",
кафедра технології біологічно активних сполук, фармації та біолтехнології
[2] Опольський університет, Ополе, Польща

ТЕХНОЛОГІЯ ВИРОБНИЦТВА ПРОБІОТИЧНОГО ПРЕПАРАТУ НА ОСНОВІ ШТАМІВ *LACTOBACILLUS ACIDOPHILUS*

Розроблено та спроєктовано виробництво пробіотичного препарату на основі антибіотикорезистентних штамів *Lactobacillus acidophilus,* який доцільно використовувати разом із антибіотикотерапією під час лікування різноманітних інфекцій для підтримання мікрофлори кишечника в нормі. Наведено якісну характеристику сировини, мікроорганізмів-продуцентів та кінцевого продукту. На основі матеріального балансу культивування, узгодженого із проєктною потужністю, підібрано технологічне обладнання відповідних параметрів, розроблено структурну технологічну схему виробництва пробіотика. Впровадження запропонованого виробництва пробіотичного препарату на основі *Lactobacillus acidophilus* дасть змогу розширити асортимент вітчизняних пробіотичних БАД на світовому ринку.

Ключові слова: *Lactobacillus acidophilus*; культивування; поживне середовище; стерилізація; ферментер; технологічна схема.