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INTENSIFICATION OF MICROBIAL EXOPOLYSACCHARIDE ETHAPOLAN BIOSYNTHESIS ON MIXTURE OF MOLASSES AND SUNFLOWER OIL

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The purpose of the research was to establish *Acinetobacter* sp. IMV B-7005 cultivation conditions, which provide the maximal synthesis of microbial exopolysaccharide ethapolan on a mixture of molasses and sunflower oil, and to explore the possibility of replacing refined oil in a mixture with molasses for waste one. On the basis of theoretical calculations of energy consumption for the synthesis of ethapolan and biomass, it was determined that the optimal molar ratio of the concentrations of energy-deficient (sucrose) and energy-excessive (sunflower oil) substrates in the mixture was 1.0:0.9. Experiments have shown that the highest values of exopolysaccharide synthesis were observed at the molar ratio of monosubstrates in mixture 1.0:1.1, which is as close as possible to the theoretically calculated one. It was shown that increasing concentration of molasses and refined oil in mixture from 1.0 to 1.5% was accompanied by increase in amount of synthesized exopolysaccharide and its synthesizing capacity in 1.2 and 1.3 times, respectively. The possibility of replacing refined oil in a mixture with molasses for various types of waste (after frying potatoes, meat, vegetables and mixed) was established. The maximum parameters of exopolysaccharide synthesis (concentration 14 g/l, synthesizing capacity 3.5 g exopolysaccharide /g biomass) were observed when using mixed waste oil for both inoculum obtaining and EPS biosynthesis. The obtained results testify to the possibility of development of universal technology for obtaining microbal exopolysaccharide ethapolan on a mixture of waste (molasses and waste oil) independent of the type and provider of waste oil.

Key words: microbial exopolysaccharides, synthesis intensification, mixture of substrates.

The microbial exopolysaccharides (EPS) are exogenous high-molecular products of microbial metabolism, which are biodegradable, non-toxic, and resistant to temperature, oxidative and mechanical destruction. Due to their abilities to form gels and emulsions and to change rheological characteristics of aqueous systems, EPS are widely used in various industries (food, oil, agricultural, etc.) [1–4].

It is assumed that the global marketof xanthan, one of the most famous EPS, in 2021 will be more than 1,25 billion US dollars [https://www.zionmarketresearch.com/report/xanthan-gum-market].

Despite such considerable implementation and more than 40 years of microbial EPS studies, in their biosynthesis, mostly expensive hydrocarbon substrates are used [1]. It should be noted that almost half of the final value of microbial synthesis products can be the cost of the carbon and energy source. This necessitated finding alternative cheap substrates for biosynthesis of polysaccharides, with the focus on toxic waste which should be, from an environmental point of view, safely disposed of. We recently published a review [5] summarizing recent data on the synthesis of microbial EPS on waste of various industries (food, agricultural, biodiesel production, etc.). In that paper we noted that there are only few reports on the biosynthesis of EPS on oil-containing substrates, and no reports on their synthesis on waste oils.

One of the advantages of the microbial polysaccharide ethapolan (produced by *Acinetobacter* sp. IMV B-7005), as compared to other well-known EPS, is the possibility of producing it on a wide variety of C_2–C_6

substrates (carbohydrates, ethanol, acetate, organic acids), as well as on refined and wasted sunflower oil [1, 5].

Using growth substrates mixture is one of the approaches to the intensification of microbial synthesis technologies. Previous studies [1, 6] showed the possibility of intensifying the synthesis of ethapolan on mixture of energy-excessive (ethanol and glucose, fumarate and glucose) and energy-deficient substrates (acetate and glucose). Subsequent studies have allowed replacing glucose in mixed C_2–C_6 substrates with molasses, a by-product of sugar production [1].

The main effectiveness criterion of mixed substrates using is ensuring the maximum full carbon conversion of both monosubstrates into EPS, that achieved at optimal molar ratio of their concentrations in the mixture. This requires the appropriate theoretical calculations, which are based on the energy needs determining of EPS synthesis and biomass on the energy- deficient substrate, with subsequent establishing the concentration of energy-excessive substrate. It will provide "coverage" of energy expenditures for this process.Then optimal molar ratio of monosubstrates will be confirmed by experimental researches. In our previous studies, this technique was used to intensify the synthesis of not only ethapolan [1] but also microbial surfactants [6–8].

The purpose of this work is to determine the cultivation conditions of *Acinetobacter* sp. IMV B-7005 for the maximum indicators of the ethapolan synthesis on a mixture of molasses and sunflower oil, as well as to investigate the possibility of replacing refined oil in a mixture with molasses on the waste oil.

Materials and Methods

Study objects. The study object is EPS-synthesizing strain *Acinetobacter* sp. 12S, deposited in the Depository of Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the number IMV B-7005.

The complex polysaccharide preparation ethapolan consists of one neutral and two acidic EPS, one of which is acylated (AP). The acylated and non-acylated (NAP) polysaccharides are identical in molar ratios of *D*-glucose, *D*-mannose, *D*-galactose, *L*-rhamnose, *D*-glucuronic acid and pyruvic acid (3:2:1:1:1:1) and the structure of repeated unit of the carbohydrate chain. The difference between those EPS is that the acylated polysaccharide contains fatty acids (C_{12}–C_{18}) [1].

Composition of medium and cultivation conditions. The IMV B-7005 strain was grown in such liquid mineral medium (g/l): 6.8 KH_2PO_4; 0.9 KOH; 0.4 $MgSO_4 \cdot 7\ H_2O$; 0.1 $CaCl_2 \cdot 2H_2O$; 0–0.2 NH_4NO_3; 0.001 $FeSO_4 \cdot 7\ H_2O$.

An additional 0.5% (v/v) of yeast autolysate was added to the medium, as well as the multivitamin complex "Complevit" at a concentration of 0.00085% (w/w by pantothenate).

As carbon and energy source, a mixture of molasses (1.0–2.0%, w/w by carbohydrates) and refined sunflower oil (0.4–2.0%, v/v) was used. In one experiment variant, the refined oil was replaced with various waste oils: after frying vegetables, after frying potatoes and meat (from McDonald's restaurant network, Kyiv), and mixed oil (after roasting meat, potatoes, onions, cheese, from "RockerPub", Kyiv).

Since the producer of the ethapolan does not assimilate sucrose, the molasses was pre-hydrolyzed: distilled water was added to the 100 g of molasses to a final volume of 200 ml, and 20 ml of 1N H_2SO_4 (to pH 4.0) were added to the obtained solution and sterilized at 112 °C for 30 min.

The culture in exponential growth phase, grown in a medium with monosubstrates, was used as inoculum: 0.5% refined and waste sunflower oil, and molasses (0.5% by carbohydrates). Concentration of inoculum was 10%.

Cultivation of the IMV B-7005 strain was carried out in flasks (750 ml) with 100 ml of medium in shaker (320 rpm) at 30 °C for 120 hours.

Growth and EPS synthesis indicators. Biomass concentration was determined by optical density of the cell suspension followed by the recalculation to absolutely dry biomass according to the calibration curve. The amount of synthesized ethapolan was determined gravimetrically. For this, 1.5–2.0 volumes of isopropanol were added to a certain volume of culture liquid (10–15 ml). EPS precipitate was washed with pure isopropanol and dried at room temperature for 24 hours. The EPS-synthesizing ability was calculated as the ratio of the EPS concentration to the concentration of biomass and expressed in g EPS/g biomass.

Determination of energy expenditures for the synthesis of biomass and EPS. Energy expenditures for the synthesis of ethapolan on sucrose were determined as described previously [1]. The energy, generated for the catabolism of linoleic and oleic acids, was calculated on the basis of information on fatty acids β-oxidation

[9], as well as data on the activity of Krebs cycle enzymes, glyoxylate cycle and gluconeogenesis of the strain *Acinetobacter* sp. IMV B-7005 [1].

All experiments were conducted in triplicates, the number of parallel determinations in experiments ranged from three to five. Statistical processing of experimental data was carried out as described earlier [7, 8]. Differences in average figures were considered significant for $P < 0.05$.

Results and Discussion

Calculation of molar ratio of molasses (sucrose) and refined sunflower oil for cultivation of Acinetobacter sp. IMV B-7005 on their mixture. The theoretical basis for the work was the concept of auxiliary substrate, developed by Babel in 1980s [10]. According to this concept, all substrates can be divided into energy-excessive and deficient, depending on the amount of energy generated by their catabolism to the central carbon precursor, phosphoglyceric acid (PGA).

If the amounts of ATP and reducing equivalents produced in the transformation of the substrate to PGA are sufficient for the synthesis of cellular components, such substrate is called energy-excessive. The substrates which must be partially oxidized to CO_2 in order to obtain the energy necessary for constructive metabolism are energy-deficient [10].

Cultivating microorganisms on a mixture of growth substrates helps avoiding unproductive carbon and energy consumption that occurs when using monosubstrates. It also improves the efficiency of substrate carbon transformation into biomass and intensifies the synthesis of secondary metabolites [1, 6], for several possible reasons: 1) one of the substrates is used solely as a source of carbon or energy; 2) simultaneous use of both substrates, both in energy and in constructive metabolism; 3) the expansion of "bottlenecks" of monosubstrate metabolism by introducing an "auxiliary substrate".

Finding the optimal substrate ratio requires: 1) calculating the energy of the biomass and exopolysaccharide synthesis on energy-deficient substrate; 2) determining the concentration of energy-excessive substrate, which adds to refill the carbon loss of energy-deficient substrate under its oxidation to CO_2 to obtain the energy necessary for constructive metabolism. Also it is necessary to know the pathways of growth substrate metabolisms, the structure of the repeated unit of the carbohydrate chain of the EPS, and the P/O ratio [1].

According to Babel's energy classification [10], glucose and fructose (included in the molasses sucrose) are energy-deficient substrates, and oil which contains higher fatty acids is an energy-excessive substrate.

It is known [9] that the major fatty acids comprising the oil are linoleic and oleic acids; others are minor components the content of which does not exceed 10%.

We followed such assumptions to calculate the optimal molar ratio of sucrose and sunflower oil concentrations for *Acinetobacter* sp. IMV B-7005 cultivation on their mixture, [1]:

- sucrose is mainly used in the synthesis of biomass and EPS, while the oil is used for energy needs;
- 50% of sucrose is catalyzed by glycolysis and 50% by the Entner-Doudoroff path (ED-pathway; 2-keto-3-deoxy-6-phosphogluconate (KDPG) is a key compound of the pathway);
- refined sunflower oil contains 50% linoleic and 50% oleic fatty acids;
- EPS ethapolan contains 50% of acylated polysaccharide;
- AP contains two fatty acid residues (lauric and palmitic);
- NADPH, formed in catabolism of sucrose and fatty acids, is a source of reducing equivalents that are oxidized to water through the respiratory chain;
- P / O ratio is 2.

Expenditures and generation of ATP during the synthesis of ethapolan on sucrose. In the process of ethapolan biosynthesis, ATP is spent on the synthesis of monosaccharides and fatty acids, and is generated during the synthesis of pyruvic acid (PA), glucuronic acid and acetyl-CoA [1].

These calculations were carried out similarly to determination of energy expenditures for the formation of EPS from glucose [1]. The only difference was that the expenditures, calculated per one mole of sucrose, are twice smaller than for glucose, since 1 mole of sucrose is formed by single moles of glucose and fructose.

Generally, 7.75 mol of sucrose are spent on the formation of a unit of acylated polysaccharide (4.0 mol for synthesis of monosaccharides and glucuronic acid, 0.25 mol for the formation of PA and 3.5 mol for fatty acids formation). The synthesis of non-acylated polysaccharide requires 4.25 mol of sucrose (4.0 mol for the synthesis of monosaccharides and glucuronic acid, 0.25 mol for the formation of PA).

Energy expenditures of biosynthesis of AP and NAP per one mole of sucrose are given in Table 1.

Table 1. **Energy expenditures of acylated and non-acylated polysaccharides synthesis from sucrose**

Catabolism pathway for sucrose	EPS	Consumption of sucrose for the synthesis of the EPS unit, mol	Consumption of energy, ATP mol		Generated energy, ATP mol	
			per synthesis of EPS unit	per mole of used sucrose	for synthesis of EPS unit	per mole of used sucrose
Glycolysis	AP	7.75	29	3.74	77	9.94
	NAP	4.25	17	4	7	1.65
	AP+NAP	12	46	3.83	84	7
ED-pathway	AP	7.75	29	3.74	69.5	8.97
	NAP	4.25	17	4	6.5	1.53
	AP+NAP	12	46	3.83	76	6.33

Thus, the energy generated in synthesis of the AP and NAP unit (AP + NAP) is:

7 – 3.83 = 3.17 mol ATP/mol of used sucrose (glycolysis);

6.33 – 3.83 = 2.5 mol ATP/mol of used sucrose (ED-pathway).

According to our assumption, 50% of sucrose is glycolized and 50% of sucrose is catabolized by ED-pathway. Consequently, the generation of energy in the process of EPS synthesis from sucrose is 2.84 moles ATP/mole of used sucrose.

Generation of energy in the catabolism of linoleic and oleic fatty acids. To determine the optimal concentration of energy-excessive substrate in the mixture, at first it is necessary to calculate the amount of energy its catabolism generated.

The transformation of the linoleic ($C_{17}H_{31}COOH$) and oleic ($C_{17}H_{33}COOH$) fatty acids into PGA proceeds in several stages [9] (Figure).

1. Activation of fatty acid and its conversion to the corresponding ether of coenzyme A with the help of the enzyme acyl-CoA synthetase. It should be noted that in that reaction AMP is released and energy of two macroergic bonds is spent.

2. Oxidation of CoA-ether in β-position, and its cleavage to acetyl-CoA and CoA-ester of fatty acid, reduced to two carbon atoms. It should be noted, that in this series of reactions, single moles of NAD and FAD are restored. Notably, FAD is not restored in areas with unsaturated bonds, since there is no need to form double bonds.

The amount of acetyl-CoA, FADN and NADH formed in the process of β-oxidation can be calculated as follows:

$$\text{Acetyl-CoA} = n/2,$$

where n is the number of carbon atoms in fatty acid;

$$\text{NADH} = n/2\text{-}1;$$

$$\text{FADH} = n/2\text{-}1\text{-}m,$$

where m is the number of unsaturated bonds in fatty acid.

3. Attracting acetyl-CoA to an incomplete Krebs cycle (the strain IMV B-7005 is characterized by low activity of 2-oxoglutarate dehydrogenase [1]) and glyoxylate cycle, which is accompanied by the restoration of FAD and NAD.

4. Formation of PGA in gluconeogenesis, in which NADN is formed and the energy of ATP is consumed. Note, that phosphoenolpyruvate synthetase is a key enzyme in gluconeogenesis in *Acinetobacter* sp. IMV B-7005 [1].

Considering the above information, the equation for the transformation of linoleic and oleic acids into PGA can be represented as:

$$C_{17}H_{31}COOH + 11\ ATP \rightarrow 4.5\ PGA + 17\ NADH + 10.5\ FADH_2 + 4.5\ CO_2; \quad (1)$$

$$C_{17}H_{33}COOH + 11\ ATP \rightarrow 4.5\ PGA + 17\ NADH + 11.5\ FADH_2 + 4.5\ CO_2. \quad (2)$$

At P / O = 2, the equations are thus:

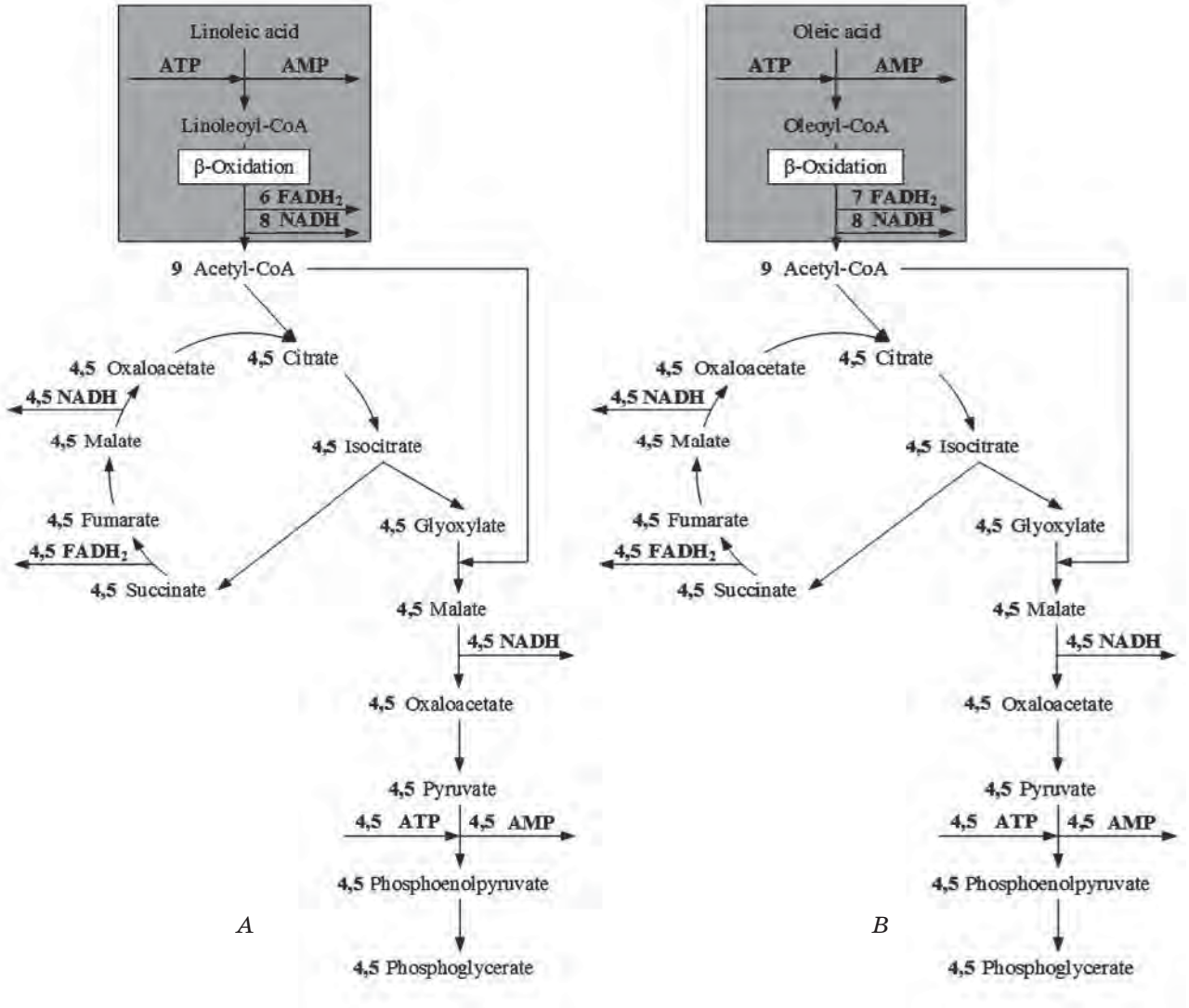
$$C_{17}H_{31}COOH \rightarrow 4.5\ PGA + 33.5\ ATP; \quad (3)$$

$$C_{17}H_{33}COOH \rightarrow 4.5\ PGA + 34.5\ ATP. \quad (4)$$

Taking into account the assumption that refined sunflower oil contains 50% of linoleic and 50% oleic acids, equations 3 and 4 can be represented as:

$$0.5\ C_{17}H_{31}COOH + 0.5\ C_{17}H_{35}COOH \rightarrow 4.5\ PGA + 34\ ATP. \quad (5)$$

Energy expenditures of biomass synthesis. Synthesis of biomass from PGA (using an



Transformation of linoleic (*A*) and oleic (*B*) fatty acids into PGA
(the literature data highlighted in gray)

ammonium nitrogen source) can be represented by the equation [6]:

$$4\ PGA + NH_3 + 29\ ATP + 5.5\ NAD(F)H \rightarrow \rightarrow 4.5\ (C_4H_8O_2N_1)_3, \quad (6)$$

where $(C_4H_8O_2N_1)_3$ is the formula of one biomass mole.

The overall transformation of sucrose to PGA is expressed by the following equations:

$$C_{12}H_{22}O_{11} \rightarrow 4\ PGA + 4\ NAD(F)N\ (glycolysis); \quad (7)$$

$$C_{12}H_{22}O_{11} + 4\ ATP \rightarrow 4\ PGA + 4\ NAD(F)H\ (ED\text{-}pathway). \quad (8)$$

Taking into account the assumption that 50% sucrose is glycolized, and 50% catalyzed by ED-pathway, equations 7 and 8 can be combined:

$$C_{12}H_{22}O_{11} + 2\ ATP \rightarrow 4\ PGA + 4\ NAD(H)H. \quad (9)$$

If P / O is 2, the equation 9 is thus:

$$C_{12}H_{22}O_{11} \rightarrow 4\ PGA + 6\ ATP. \quad (10)$$

Based on the equation for the synthesis of biomass from PGA (equation 6) and the sucrose catabolism equation for PGA (equation 10), it can be calculated that under cultivation on sucrose, the ATP needed for the synthesis of biomass (per molar sucrose) is 34 mol. Let us assume that this energy can be obtained from fatty acids of oil. Considering that 2.84 mol of ATP / mol of used carbohydrate are generated in the EPS synthesis from sucrose, it is necessary to obtain 34 – 2.84 = 31.16 mol of ATP out of the oil. From equation 5 it follows, that 0.9 mol of oil fatty acids is required to obtain such amount of energy.

Consequently, the molar ratio of sucrose and refined sunflower oil in the medium should be 1:0.9. For example, in 1% of molasses (carbohydrate mass, 10 g sucrose/l, or 0.03 mol/l), the oil concentration should be 0.027 mol/l or 7.6 g/l or 0.8% (v/v). Thus, the ratio of molasses (w/w, by carbohydrates) and refined sunflower oil (v/v) in the cultivator of the producer should be 1: 0.8.

Synthesis of ethapolan under Acinetobacter sp. IMV B-7005 cultivation on a mixture of molasses and refined sunflower oil.

At the next stage, ethapolan synthesis was investigated at different molar ratios of sucrose (molasses) and refined sunflower oil in the medium mixture.

Experiments have shown that the highest rates of ethapolan synthesis (8.8 g/l synthesized EPS, EPS- synthesizing ability of 2.8 g EPS/g biomass) were observed for the 1:1.1 molar ratio of monosubstrates in the mixture, maximally approximated to the theoretically calculated (1.0: 0.9) (Table 2).

It should be noted that the efficiency of technologies of microbial product synthesis on mixed substrates depends both on the molar ratio of monosubstrates in the mixture and on their concentration [6]. Hence, in subsequent experiments, the effect of various concentrations of molasses and sunflower oil in the mixture on the synthesis of ethapolan was investigated.

According to data in Table 3, an increase in concentrations of monosubstrates up to 1.5% was accompanied by an increase in the amount of synthesized EPS and EPS-synthesizing capacity in 1.2 and 1.3 times, respectively. In the case of further increase in the concentrations of molasses and oils, the ethapolan synthesis rates decreased.

Investigation of the possibility of refined oil replacing with the waste oil in a mixture with molasses. It was established that irrespective of the carbon source (molasses, different types of waste oil) nature in the inoculum medium, and type of fried oil mixed with molasses, the concentration of the synthesized polysaccharide (10–14 g/l) was same as with using refined substrate (10.0–12.5 g/l) (Table 4). However, economically, it is advisable to use waste oil for the inoculum production, since it does not require sterilization and is currently much cheaper than molasses. In addition, the use of processed oil for the preparation of inoculum and EPS biosynthesis supports the disposal of this toxic waste, and further reduces the cost price of the target product.

Given that virtually all establishments mix waste oils before disposing of for recycling (production of biodiesel, use in other biotechnological processes), at the next stage of work, the ethapolan synthesis was studied on the mixture of molasses and mixed waste oils.

Table 2. **Influence of sucrose (molasses) and refined sunflower oil molar ratio of concentrations on the ethapolan synthesis**

Molar ratio of monosubstrates	Concentration,%		EPS, g/l	EPS-synthesizing ability, g EPS/ g biomass
	molasses	oil		
1:0.5	1.0	0.4	5.65 ± 0.28*	2.18 ± 0.11*
1:0.7	1.0	0.6	6.19 ± 0.31*	2.22 ± 0.11*
1:0.9	1.0	0.8	7.16 ± 0.36 (control)	2.35 ± 0.12 (control)
1:1.1	1.0	1.0	8.81 ± 0.44*	2.77 ± 0.14*
1:1.3	1.0	1.2	7.89 ± 0.39*	2.06 ± 0.10*

Note. Inoculum was grown on refined oil. Here and after * — $P < 0.05$ compared to control.

Table 3. **Ethapolan synthesis depending on the concentrations of molasses and refined oil in the medium mixture**

Concentration of molasses and oil in the mixture,%	EPS, g/l	EPS-synthesizing ability, g EPS/ g biomass
Molasses, 1.0 + oil, 1.0	8.81 ± 0.44	2.77 ± 0.14
Molasses, 1.5 + oil, 1.5	10.09 ± 0.50*	3.60 ± 0.18*
Molasses, 2.0 + oil, 2.0	9.32 ± 0.47*	2.46 ± 0.12*

Note. Molar ratio of monosubstrates concentrations in the mixture 1.0 : 1.1.

Table 4. **Indicators of the ethapolan synthesis on molasses (1.5%) and sunflower oil (1.5%) mixture depending on the method of inoculum preparation**

Oil and molasses mixture	Substrate for inoculum growth	EPs, g / l	EPS-synthesizing ability, g EPS / g biomass
Refined oil	Refined oil	10,09 ± 0,50 (control)	3,60 ± 0,18 (control)
	Molasses	12.25 ± 0.61*	3.35 ± 0.17*
Oil after frying potato fries	Oil after frying potato fries	11.06 ± 0.55*	2.61 ± 0.13*
	Molasses	13.52 ± 0.68*	3.21 ± 0.16*
Oil after frying potato wedges	Oil after frying potato wedges	11.66 ± 0.58*	2.69 ± 0.13*
	Molasses	13.19 ± 0.66*	2.25 ± 0.11*
Oil after frying meat	Oil after frying meat	12.41 ± 0.62*	3.42 ± 0.17*
	Molasses	11.33 ± 0.57*	2.87 ± 0.14*
Oil after frying vegetables	Oil after frying vegetables	9.94 ± 0.50*	2.95 ± 0.15*
	Molasses	10.71 ± 0.54*	3.15 ± 0.16*
Mixed processed oil	Mixed processed oil	13.92 ± 0.70*	3.49 ± 0.17*
	Molasses	12.90 ± 0.65*	3.28 ± 0.16*

As evidenced by data from Table 4, using mixed fried oil for inoculum and biosynthesis of EPS, the ethapolan synthesis rates increased (EPS concentration 14 g/l, EPS-synthesis capability — 3.5 g EPS/g biomass) compared to other types of waste oils (after frying potatoes, meat, and vegetables).

Notably, the concentration of ethapolan in the process of *Acinetobacter* sp. IMV B-7005 cultivation on mixture of glucose (molasses) with ethanol, fumarate, acetate did not exceed 9–11 g/l [1].

Previously in review [5] we noted that there is limited data on the synthesis of microbial polysaccharides in various industrial waste (not oil-containing only). Recently, several new such reports appeared in the literature. For example, the strain *Komagataeibacter sucrofermentans* DSM 15973 in a medium containing 17 g/l technical glycerin (waste of biodiesel production) synthesized 13.3 g/l of cellulose [11]. Under *Rhizobium leguminosarum* ATCC 10004 cultivation in a medium with sewage waters after fish processing EPS concentration was 11 g/l [12]. However, in the available literature, we were not able to find information about the synthesis of microbial EPS on a mixture of industrial waste, although there are isolated data on the synthesis of other economically important products on such mixed substrates [13—16].

The lipid synthesis productivity of old and young cultures of heterotrophic *Chlorella protothecoides* in the medium with glucose and yeast extract was 2.07 and 1.61 g/l/day respectively. Using the mixture of beer-brewing waste and technical glycerin increased it to 2.12 and 1.81 g/l/day, respectively [13]. It was shown in [14] that using orange pulp and biodiesel production waste mixture for methane synthesis reduces the inhibitory effect of the components of those substrates and provides a proper balance of nutrients.

A similar approach was used by Louhasakul and Cheirsilp [15]. Mixing weakly acidic wastewater after the production of palm oil, and alkaline technical glycerin allowed to forgo titrating agents for keeping the pH at an optimal level, while the amount of lipids synthesized by *Yarrowia lipolytica* TISTR 5151 was 1.55 times higher than by the yeast cultivated on waste of palm oil production only [15].

Using glucose, xylose and arabinose as co-substrates for the cultivation of *Clostridium diolis* DSM 15410 on glycerol was accompanied by an increase in the yield of 1,3-propanediol (1,3-PD) by 28%, 19% and 18% respectively [16]. Also, under strain DSM 15410 cultivation on glycerol and a mixture of sugars (glucose, xylose, arabinose in a mass ratio of 1:1:1), 1,3-PD concentration increasing to 19% (up to 13.9 g/l) was observed, which points to the possibility of using a mixture of glycerol and lignocellulosic hydrolysates to produce 1,3-PD. This assumption was confirmed empirically: cultivation of *C. diolis* DSM 15410 on a mixture of technical glycerin and corn stalk hydrolysate was accompanied by the synthesis level of 1,3-PD of 42.9 g/l, which is 31% higher than in yeast growth on glycerol as monosubstrates [16].

Under *Leuconostoc mesenteroides* DSM 20343 cultivation on a medium containing a mixture of soy (or fava beans) flour with sucrose, the viscosity of the culture liquid was significantly increased

[17]. That indicated an increased synthesis of exopolysaccharides (glucan and fructan) compared to other metabolites (mannitol, lactic and acetic acid). This work is one of the few reports on the synthesis of EPS on substrates mixture, although the used substrates are not industrial waste.

It should be noted that researchers usually empirically determine both the concentration of substrates in the mixture, and the choice of monosubstrates. Our previous studies [1] on the intensification of the ethapolan synthesis and the results of this work, as well as data on the synthesis of microbial surfactants on a mixture of substrates [6—8], show that enhancing the carbon transformation of substrates mixture to the target product requires establishing an optimal molar ratio of monosubstrates concentrations in a mixture. That necessitates the prior theoretical calculations of energy needs of the biosynthesis process.

Also, the presented results demonstrate the possibility, firstly, of replacing refined oil in molasses with different types of processed oil (after frying potatoes, meat, vegetables and mixed) mixture for the synthesis of ethapolan. That allows not only to significantly reduce the cost price of the target product, but also to dispose of toxic waste oil. Secondly, the results show the possibility for developing a universal technology for the synthesis of this EPS on a mixture of wastes (molasses and waste oil), regardless on the type and the supplier of the processed oil.

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ІНТЕНСИФІКАЦІЯ БІОСИНТЕЗУ МІКРОБНОГО ЕКЗОПОЛІСАХАРИДУ ЕТАПОЛАНУ НА СУМІШІ МЕЛЯСИ ТА СОНЯШНИКОВОЇ ОЛІЇ

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Метою роботи було встановити умови культивування *Acinetobacter* sp. IMB B-7005, які забезпечували би максимальні показники синтезу мікробного екзополісахариду етаполану на суміші меляси та соняшникової олії, а також дослідити можливість заміни рафінованої олії в суміші з мелясою на відпрацьовану.

На основі теоретичних розрахунків енерговитрат на синтез етаполану та біомаси визначено, що оптимальне молярне співвідношення концентрацій енергетично дефіцитного (сахароза) та надлишкового (соняшникова олія) субстратів у суміші становить 1,0:0,9. Експерименти показали, що найвищі показники синтезу екзополісахариду етаполану спостерігалися за молярного співвідношення моносубстратів у суміші 1,0:1,1, максимально наближеного до теоретично розрахованого. Підвищення концентрації меляси та рафінованої олії у суміші з 1,0 до 1,5% супроводжувалося збільшенням кількості синтезованого екзополісахариду етаполану та його синтезувальної здатності в 1,2 і 1,3 раза відповідно. Встановлено можливість заміни рафінованої олії в суміші з мелясою на різні типи відпрацьованої (після смаження картоплі, м'яса, овочів та змішану). Найвищі показники синтезу екзополісахариду етаполану (концентрація 14 г/л, синтезувальна здатність 3,5 г екзополісахариду етаполану/г біомаси) спостерігалися за умови використання змішаної відпрацьованої олії як для одержання посівного матеріалу, так і біосинтезу екзополісахариду етаполану. Одержані результати засвідчують можливість створення універсальної технології одержання мікробного екзополісахариду етаполану на суміші відходів (меляси та відпрацьованої олії), незалежно від типу та постачальника відпрацьованої олії.

Ключові слова: мікробні екзополісахариди, інтенсифікація синтезу, суміш субстратів.

ИНТЕНСИФИКАЦИЯ БИОСИНТЕЗА МИКРОБНОГО ЭКЗОПОЛИСАХАРИДА ЭТАПОЛАНА НА СМЕСИ МЕЛАССЫ И ПОДСОЛНЕЧНОГО МАСЛА

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Целью работы было установление условий культивирования *Acinetobacter* sp. ИМВ B-7005, обеспечивающих максимальные показатели синтеза микробного экзополисахарида этаполана на смеси мелассы и подсолнечного масла, а также исследование возможности замены рафинированного масла в смеси с мелассой на отработанное. На основе теоретических расчетов энергозатрат на синтез этаполана и биомассы определено, что оптимальное молярное соотношение концентраций энергетически дефицитного (сахароза) и избыточного (подсолнечное масло) субстратов в смеси составляет 1,0:0,9. Эксперименты показали, что наиболее высокие показатели синтеза микробного экзополисахарида наблюдались при молярном соотношении моносубстратов в смеси 1,0:1,1, максимально приближенном к теоретически рассчитанному. Повышение концентрации мелассы и рафинированного масла в смеси с 1,0 до 1,5% сопровождалось увеличением количества синтезированного микробного экзополисахарида и его синтезирующей способности в 1,2 и 1,3 раза соответственно. Установлена возможность замены рафинированного масла в смеси с мелассой на различные типы отработанного (после жарки картофеля, мяса, овощей и смешанного). Максимальные показатели синтеза микробного экзополисахарида (концентрация 14 г/л, синтезирующая способность 3,5 г микробного экзополисахарида/г биомассы) наблюдались при использовании смешанного отработанного масла как для получения посевного материала, так и биосинтеза микробного экзополисахарида. Полученные результаты свидетельствуют о возможности создания универсальной технологии получения микробного экзополисахарида этаполана на смеси отходов (мелассы и отработанного масла), не зависящей от типа и поставщика отработанного масла.

Ключевые слова: микробные экзополисахариды, интенсификация синтеза, смесь субстратов.