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Production of exopolysaccharide ethapolan by *Acinetobacter sp*. IMV B–7005 on fried oil and oil–containing mixed substrates

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Abstract. Nowadays, the majority of microbial EPS are not produced on an industrial scale because of high cost and low yield of the target product. In this study, the cultivation conditions of *Acinetobacter* sp. IMV B–7005 for the efficient process of exopolysaccharide ethapolan synthesis on refined or cheaper waste oil and oil–containing mixed substrates were established. The production efficiency on the selected substrates was evaluated by the amount of synthesized ethapolan (g/l) and EPS–synthesizing ability (g EPS/g biomass). Regardless of the quality (sunflower, corn, olive, rapeseed) and type (after frying meat or potatoes) of waste oil in the biosynthesis medium, it was found that the polysaccharide synthesis and its rheological properties were at the level obtained using the refined substrate. Use of waste oil, especially mixed one, in mixture with molasses or acetate allows increasing the amount of synthesized EPS to 14–16 g/l. The obtained results show the possibility of developing a universal technology of ethapolan production on the oil–containing substrates and their mixtures, which is independent of the type and quality of the waste oil, as well as its supplier.
Keyword*: Acinetobacter sp. IMV B–7005, waste cooking oil, mixed substrates, microbial exopolysaccharide ethapolan.*

Introduction

Many microbial exopolysaccharides (EPS) with various physicochemical (gelling and emulsifying abilities, ability to retain a large amount of water and alter rheological properties of water systems, etc.) [BARCELOS *et al.*, 2020] and functional properties (anticancer, antioxidant, antimicrobial, antiviral, anti–inflammatory, and immunomodulatory activities, etc.) [YILDIZ and KARATAS, 2018; MOSCOVICI, 2015; SAADAT *et al.*, 2019; CHAISUWAN *et al.*, 2020] has been discovered and deeply studied up to date [MOSCOVICI, 2015; RÜHMANN *et al.*, 2015]. Nevertheless, only some of these exogenous high–molecular products of microbial metabolism became commercially successful and are being used in cosmetic, agricultural, food, pharmaceutical, petroleum industries, and wastewater treatment, etc. [K *et al.*, 2018; YILDIZ and KARATAS, 2018; SAADAT *et al.*, 2019; BARCELOS *et al.*, 2020]. Thus, for decades xanthan has been the most famous example of these biopolymers. Nowadays, it takes approximately 6% of the polysaccharide market [BARCELOS *et al.*, 2020]. Meanwhile, other EPS, such as dextran, gelan, alginate, levan, pululuan, welan, scleroglucan, emulsan, hyaluronic acid, and some other less–known polymers, are also widely used nowadays [YILDIZ and KARATAS, 2018; BARCELOS *et al.*, 2020].

Despite the fact, that new polymers can have unique properties [ROCA *et al.*, 2020] their industrial implementation is impracticable due to high cost and low yield. The main approaches to solve these problems are using cheap substrates, increasing the efficiency of their conversion into the target product and carrying out the general optimization of the cultivation conditions (optimization of process parameters) [FREITAS *et al.*, 2017; SENGUPTA *et al.*, 2018; BARCELOS *et al.*, 2020].

In case of replacing classical expensive carbohydrate substrates (glucose, sucrose, sugar syrups, starch), which may constitute a significant part of the final product cost, with cheaper

analogues – the particular attention is paid to the use of agro–industrial waste (molasses, whey, etc.) [SINGH *et al.*, 2019, SIDDEEG *et al.*, 2020]. Among them the most attractive is using harmful and toxic compounds (technical glycerol [RAGHUNANDAN *et al.*, 2018; RONČEVIĆ *et al.*, 2020], industrial wastewater [BAJIĆ *et al.*, 2017; KAZEMI *et al.*, 2019; RONČEVIĆ *et al.*, 2019; SIDDEEG *et al.*, 2020], etc.), which cannot be further used in the food (production of dietary supplements) or agricultural (animal feed) industries.

Our review [PIROG *et al.*, 2016] provides recent data on the synthesis of microbial EPS on various industrial waste. It is worth noting that after the publication of this review, only a few new works have been reported about waste using to obtain polysaccharides [ANTUNES *et al.*, 2017; BAJIĆ *et al.*, 2017; PEDROSO *et al.*, 2019; RONČEVIĆ *et al.*, 2019; SENGUPTA *et al.*, 2019; SINGH *et al.*, 2019; ASGHER *et al.*, 2020; RISHI *et al.*, 2020; SIDDEEG *et al.*, 2020], but so far there is no information on their synthesis on waste oil (WO), also known as used oil.

Note that such oil–containing waste is very toxic because during frying at high temperatures (180 °C or over) it may undergo diverse chemical reactions (thermal alterations, hydrolysis, oxidation, polymerization, and cyclization) [DOBARGANE and MARQUEZ–RUIZ 2015; PANADARE and RATHOD, 2015], resulting in increased content of polar compounds and formation of toxic (cyclic fatty acid monomers, oxysterols, monoaromatic hydrocarbons, aldehyde compounds, 2–thiobarbituric, etc.), carcinogenic substances (acrylamide, heterocyclic amines), and other degradation products (*trans* fatty acids, free radicals) [HOSSEINI *et al.*, 2016; LI *et al.*, 2019]. Due to the fact that more than 15–16.5 million tons of these waste vegetable oils generate annually in the world, from which 1–1.6 million tons accumulate in Europe [MANNU *et al.*, 2019; LOIZIDES *et al.*, 2019; MANNU *et al.*, 2020], and their emissions to the environment are strictly regulated only in highly developed countries (USA, Japan, European countries etc.), the effective disposal and processing of waste oil is an urgent problem.

Despite the fact that waste oils are collected and processed into biodiesel [GOH *et al.*, 2020] the most of it, especially oils used in households, are uncontrollably drained to the sewerage or disposed in landfill, resulting in a number of economic (drastically increase the purification cost of oil–contaminated water, cause blockage in the sinks and drains, and rapid wear of pipelines) and environmental (1 litre of oil may pollute up to 500 thousand litres of water) problems [PANADARE and RATHOD, 2015; LOIZIDES *et al.*, 2019].

At the same time, this waste after proper purification can be used for the livestock feeding (banned in some countries) or production of plasticizers, hydrogen–rich synthesis gas (syngas), biogas, binders, epoxides, surfactants, lubricants, soaps, greases, etc. [PANADARE and RATHOD, 2015; MANNU *et al.*, 2019; MAOTSELA *et al.*, 2019; MARCHETTI *et al.*, 2020; ORJUELA and CLARK, 2020]. Another promising method of waste oil processing is its biotreatment [GAO *et al.*, 2019] or use in biotechnology as a substrate for the microbial synthesis of practically valuable products (surfactants, polyhydroxyalkanoates, organic acids, enzymes, vitamins, etc.) [PANADARE and RATHOD, 2015; XIAOYAN *et al.*, 2017; NIU *et al.*, 2019; ORJUELA and CLARK, 2020; CRUZ *et al.*, 2019; ADLIN *et al.*, 2020].

In our previous work [PIDHORSKYY *et al.*, 2010] the possibility of synthesis of microbial polysaccharide ethapolan (produced by *Acinetobacter* sp. IMV B–7005) on a wide variety of different C_2–C_6–substrates (carbohydrates, hydrolyzed molasses, ethanol, acetate, organic acids) and their mixtures has been established.

We suggested that due to the emulsifying properties and structural features of the polysaccharide (containing palmitic, oleic, palmitoleic, lauric and stearic acids residues) [PIDHORSKYY *et al.*, 2010], it is possible to further expand the raw material base for its synthesis through the use of oil–containing materials.

Taking into account the above information, the purpose of this work was to establish the cultivation conditions of *Acinetobacter* sp. IMV B–7005 for the maximum indicators of the exopolysaccharide ethapolan synthesis on sunflower oil and oil–containing mixed substrates, as well as to study the

possibility of replacing the refined oil on a waste one.

Material and methods

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

Medium composition and cultivation conditions. The IMV B–7005 strain was grown in semi–defined medium of the following composition (g/l): *medium 1*: KH_2PO_4– 6.8; KOH – 0.9; $MgSO_4 \times 7\ H_2O$ – 0.4; $CaCl_2 \times 2\ H_2O$ – 0.1; NH_4NO_3 – 0.6; $FeSO_4 \times 7\ H_2O$ – 0.001; *medium 2* is similar to medium 1, but the concentration of NH_4NO_3 is 0.2 g/l; *medium 3* is similar to medium 1, but NH_4NO_3 is absent; *medium 4* is similar to medium 1 the concentration of KH_2PO_4 is halved, KOH is absent and NH_4NO_3 is replaced with 0.8 g/l NH_4Cl.

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 (in medium 2–4) and 0.00095% (in medium 1) (w/w by pantothenate).

The following types of substrates were used as a carbon and energy source: *monosubstrates*–refined sunflower, olive, corn, or rapeseed oil at concentration 5% (v/v); *mixed substrates* –the mixture of refined sunflower oil (0.25–1.5%, v/v) with molasses (1.5%, w/w by carbohydrates) or sodium acetate (0.5–3.0%, w/w).

In some variants refined oil was replaced with the waste ones: sunflower oil after frying meat or potatoes (from McDonald's restaurant network, Kyiv), after frying vegetables (obtained at home after three times frying of vegetables for 20 minutes), mixed (after frying meat, potatoes, onions, cheese, from "RockerPub", Kyiv), or after idle frying (obtained at home after three times frying of oil for 20 minutes); olive, corn, or rapeseed after frying meat or potatoes (obtained at home after three times frying of corresponding products for 20 minutes).

Since the producer of ethapolan does not assimilate sucrose, the molasses was pre–hydrolyzed: 100 g of molasses was diluted to a final volume of 200 mL, and afterwards, the solution was acidified to pH 4.0 with 1N H_2SO_4. The obtained solution was autoclaved at 112 °C for 30 min.

In one variant, the initial concentration of acetate in the medium was 0.5–1.5 and oil 0.25–0.75%, and during the cultivation process these substrates were fractionally applied (fed–batch) in portions of 0.5–1.5% (acetate) and 0.25–0.75% (oil). If before supplementation the pH of the culture fluid exceeded 8.0–8.5, acetic acid was added into the equimolar (by carbon) concentration (0.35%, v/v) instead of acetate.

The culture in exponential growth phase, grown in a medium with 0.5% of refined or waste oil, glucose, or molasses was used as inoculum. Concentration of inoculum was 10%.

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

Biomass and ethapolan estimation. Biomass concentration was determined by optical density of cell suspension with subsequent recalculation to dry biomass in accordance with the calibration curve.

The amount of EPS was determined gravimetrically. For this purpose, 1.5–2 volumes of isopropanol were added to a certain volume of the culture fluid (usually 10–15 mL). The EPS precipitate was washed with pure isopropanol and dried at room temperature for 24 hours.

EPS–synthesizing ability was calculated as the ratio of the EPS concentration to the biomass and expressed in g EPS/g biomass.

Rheological characterization of EPS. Rheological properties of 0,05% solution of culture fluid or dried EPS were performed by the changes of the viscosity degree in the presence of 0,1 M KCl and in the Cu^{2+}–glycine system, what is the individual property of ethapolan [PIDHORSKYY *et al.*, 2010].

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When determining the viscosity of ethapolan solutions in the presence of 0.1 M KCl in 0.05% EPS solution dry potassium chloride was added to a final concentration of 0.1 M. The solution was stirred until the salt was completely dissolved and held for one hour.

To study the behavior of ethapolan solutions in the Cu^{2+}–glycine system 0.003 M $CuSO_4 \times 5\ H_2O$, then 0.015 M glycine were added while stirring to the 0.05% EPS solution. The obtained solution was heated to 80 °C and held at this temperature for 5 minutes, cooled in air to 20 °C, and the viscosity of the resulting solution was measured.

The kinematic viscosity of ethapolan solutions was measured using an Oswald glass viscometer at 20 °C.

Statistical data processing. All experiments were conducted in three repetitions; the number of parallel definitions in the experiments was from three to five. Statistical processing of experimental data was carried out as described earlier [PIDHORSKYY *et al.*, 2010]. Differences in average indicators were considered reliable at the level of significance $p < 0.05$.

Results and discussion

In the first stage of the study, the possibility of using oil–containing substrates for ethapolan production was studied. Experiments showed that under cultivation of *Acinetobacter* sp. IMV B–7005 in the medium with 1% of sunflower oil approximately 6.5 g/l of EPS was obtained. Further increase in the oil content in the cultivation medium up to 5% with a simultaneous raising the concentration of ammonium nitrate (to 0.6 g/l) and pantothenate (0.00095%) was accompanied by an increasing EPS synthesis in 1.92 times (up to 12.5 g/l).

In subsequent experiments refined sunflower oil was replaced with various types of waste one (after frying meat or potatoes) (Table 1).

Table 1.

Ethapolan production on sunflower oil of different quality depending on the method of inoculum preparation

Quality of sunflower oil for EPS biosynthesis	Substrate for inoculum preparation	EPS concentration, g/l	EPS–synthesizing ability, g EPS/g biomass
Refined	Refined oil	13.1±0.66	7.5±0.38
	Glucose	9.0±0.45	7.2±0.36
	Molasses	7.9±0.39	8.6±0.43
	Waste oil after frying meat	9.7±0.49	5.9±0.29
Waste oil after frying meat	Refined oil	14.4±0.72	6.3±0.32
	Glucose	7.1±0.36	5.7±0.28
	Molasses	8.4±0.42	6.0±0.29
	Waste oil after frying potatoes	8.1±0.41	4.3±0.22
Waste oil after frying potatoes	Refined oil	4.2±0.21	3.3±0.17
	Glucose	2.5±0.13	1.6±0.08
	Molasses	5.7±0.29	4.1±0.21
Idle frying	Waste oil after idle frying	3.0±0.15	6.5±0.33

Notes: Oil concentration in the medium for biosynthesis was 5%. Cultivation was carried out in the medium 1.

It was found that under using oil after frying meat (5%), the concentration of EPS reached 14.4 g/l and EPS–synthesizing ability was 6.3 g EPS/g biomass. Meanwhile, the cultivation on the oil after frying potatoes, on the contrary, resulted in a significant inhibition of polysaccharide synthesis (EPS concentration was 4.2 g/l). We consider that it may be caused by the formation of toxic compounds, such as acrylamide, during frying of potatoes [HOSSEINI *et al.*, 2016]. At the same time, the growth of inoculum on this substrate may promoted the adaptation of the producer, which led to an increase of the EPS synthesis almost in 2 times. It should be noted that under producer cultivation on sunflower oil after idle frying the concentration of ethapolan was 3.0 g/l. For a more thorough understanding of the cultivation features of the strain IMV B–7005 on oil–containing substrates, in subsequent experiments ethapolan biosynthesis was

investigated on waste oils, characterized by different concentration of polyunsaturated (sunflower, corn) and monounsaturated (olive, rapeseed) fatty acids (Table 2).

It was found that replacing sunflower oil with corn one, accompanied by decrease in polysaccharide synthesis (up to 24%), but EPS–synthesizing ability remained unchanged and was 6.3 to 7.6 g EPS/g biomass. Thus, the patterns of the ethapolan synthesis under strain IMV B–7005 cultivation on the waste oils with the high content of polyunsaturated fatty acids were very similar. In the next stage, the possibility of ethapolan production on waste oil with a high content of monounsaturated fatty acids (olive, rapeseed) was studied.

Experiments showed that patterns of the EPS synthesis on olive and rapeseed oils, characterized by a high content of monounsaturated fatty acids, were practically the same.

Thus, regardless of the quality of oil (refined or waste) in the medium for inoculum preparation and the type of oil (olive or rapeseed) in the medium for biosynthesis, the maximum concentration of ethapolan (8–9.5 g/l) was slightly lower than that obtained on sunflower or corn oil (10–14.5 g/l). At the same time, EPS–synthesizing ability (10–15 g of EPS/g biomass) was in 1.6–2.4 times higher than on sunflower and corn oils. One approach to the intensification of microbial synthesis is the use of a mixture of growth substrates [PIDHORSKYY *et al.*, 2010].

Table 2.

Synthesis of EPS on oils with different content of poly– and monounsaturated fatty acids

Type of oil	Quality of oil in the medium for		EPS concentration, g/l	EPS–synthesizing ability, g EPS/g biomass
	inoculum preparation	EPS biosynthesis		
Corn	Refined oil	Refined oil	10.0±0.50	7.6±0.38
		Waste oil after frying meat	11.2±0.56	6.3±0.32
	Waste oil after frying meat		8.5±0.43	7.4±0.37
	Waste oil after frying potatoes		8.1±0.41	7.8±0.39
Olive	Refined oil	Refined oil	7.7±0.39	14.0±0.70
		Waste oil after frying meat	9.6±0.48	14.8±0.74
		Waste oil after frying potatoes	9.0±0.45	13.8±0.69
	Waste oil after frying meat		8.3±0.42	10.2±0.51
	Waste oil after frying potatoes		8.0±0.40	8.4±0.42
Rapeseed	Refined oil	Refined oil	8.1±0.41	9.3±0.47
		Waste oil after frying meat	8.9±0.45	10.2±0.51
		Waste oil after frying potatoes	8.6±0.43	10.3±0.52
	Waste oil after frying meat		7.8±0.39	9.6±0.48
	Waste oil after frying potatoes		6.1±0.31	6.8±0.34

Notes: Oil concentration in the medium for biosynthesis was 5%. Cultivation was carried out in the medium 1.

In this case, it becomes possible not only to utilize simultaneously several substrates [WU *et al.*, 2016], but also to increase overall the cultivation efficiency [PIDHORSKYY *et al.*, 2010; LIU *et al.*, 2020]. To date, there is practically no information in the literature about the use of mixed substrates for the biosynthesis of microbial EPS, although this technique has been successfully used to obtain primary (microbial oils [HASSANPOUR *et al.*, 2019], α, ω–dicarboxylic acids [CAO *et al.*, 2017], fumaric acid [KOWALCZYK *et al.*, 2018], secondary metabolites (polyhydroxyalkanoates [RAY *et al.*, 2018], natamycin [ZENG *et al.*, 2019], and fermentation products (n–butanol, 1,3–propanediol [SABRA *et al.*, 2016], lactic acid [HASSAN *et al.*, 2019], as well as other practically valuable microbial metabolites. In our previous work this approach was used to increase ethapolan production on the mixture of glucose (molasses) with ethanol, fumarate, or acetate [PIDHORSKYY *et al.*, 2010]. In that regard, the next stage of the research was devoted to study the possibility of EPS synthesis on the mixture of molasses and oil. Experiments showed that the highest synthesis rates of the target product (concentration of ethapolan was 10.0 g/l, EPS–synthesizing ability was 3.6 g EPS/g biomass) were observed at the monosubstrate

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concentrations of 1.5% and under using inoculum grown on refined oil (Table 3). Further increasing the concentrations of molasses and oil resulted in a reduction of ethapolan production.

It should be noted that under replacing refined oil with various types of waste one (after frying potatoes, meat or vegetables) in the mixture with molasses, regardless of the carbon source nature (molasses, various types of waste oil) in the medium for inoculum preparation, the concentration of ethapolan (10–13.5 g/l) was the same as that obtained with the use of the refined substrate (10–12.5 g/l).

Meanwhile, the results of EPS biosynthesis on mixed waste oil, which is now the most common, were of particular interest. Thus, when it was used for inoculum preparation and biosynthesis, EPS concentration reached 14 g/l and EPS–synthesizing ability was 3.5 g EPS/g biomass, which was higher compared to the use of other types of waste oils.

Table 3.

Influence of inoculum preparation method on ethapolan synthesis on the mixture of molasses and sunflower oil

Quality of oil in the mixture with molasses	Substrate for inoculum preparation	EPS concentration, g/l	EPS–synthesizing ability, g EPS/g biomass
Refined oil	Refined oil	10.09±0.50	3.60±0.18
	Molasses	12.25±0.61	3.35±0.17
Waste oil after frying potatoes	Waste oil after frying potatoes	11.06±0.55	2.61±0.13
	Molasses	13.52±0.68	3.21±0.16
Waste oil after frying meat	Waste oil after frying meat	12.41±0.62	3.42±0.17
	Molasses	11.33±0.57	2.87±0.14
Waste oil after frying vegetables	Waste oil after frying vegetables	9.94±0.50	2.95±0.15
	Molasses	10.71±0.54	3.15±0.16
Mixed waste oil	Mixed waste oil	13.92±0.70	3.49±0.17
	Molasses	12.90±0.65	3.28±0.16

Notes: Molasses and oil concentrations in the medium for biosynthesis were 1.5%. Inoculum preparation and biosynthesis were carried out in the medium 2 and 3 respectively.

It is worth noting that under cultivation bacteria in the mixture of molasses and oil, a significant decrease in EPS–synthesizing ability was observed compared to the use of oil monosubstrate (Table 1–2).

Table 4.

Synthesis of ethapolan in the mixture of sodium acetate and oil during fed–batch cultivation

Monosubstrate concentrations in the mixture, %	Fed–batch mode, %	pH	EPS concentration, g/l	EPS–synthesizing ability, g EPS/g biomass
Acetate, 0.5 + refined oil, 0.25	Without fractional application	7.7	2.30±0.17	2.69±0.13
Acetate, 1.0 + refined oil, 0.5	Without fractional application	7.8	4.70±0.24	2.00±0.10
Acetate, 1.5 + refined oil, 0.75	Without fractional application	8.7	4.02±0.20	1.07±0.05
	Three portions of 0.5% acetate and 0.25% oil	6.4*	5.67±0.28	2.00±0.10
Acetate, 3.0 + refined oil, 1.5	Without fractional application	9.5	2.84±0.14	0.68±0.03
	Three portions of 1.0% acetate and 0.5% oil	7.8*	13.82±0.69	4.53±0.23
	Two portions of 1.0% acetate and 0.5% oil, the third portion is 0.35% acetic acid and 0.5% oil	7.9*	17.27±0.86	6.47±0.32
Acetate, 3.0 + waste oil, 1.5	Without fractional application	9.6	2.31±0.12	0.55±0.03
	Two portions of 1.0% acetate and 0.5% oil, the third portion is 0.35% acetic acid and 0.5% oil	7.7*	16.36±0.82	7.34±0.37

Notes: Inoculum was grown on the corresponding oil. Cultivation was carried out in the medium 4.

We suggested that this problem can be solved by replacing the molasses in the mixture with oil with another substrate that does not contain nitrogen, in

particular acetate. Taking this into account, in the next stage of the study the possibility of ethapolan synthesis on the mixture of sunflower oil and sodium acetate was investigated. It was found that when the concentration of acetate in the mixture exceeded 1.0%, the pH of the medium was increased to a level not optimal for the production of ethapolan (optimum pH 7.0–8.0) (Table 4).

Such an increase in the pH of the culture fluid is due to the peculiarities of sodium acetate consumption, namely, its transportation into cells of the producer by symport with proton.

One approach to solving this problem is the fed–batch cultivation. The following experiments showed that reduction of the initial substrate concentrations in the mixture up to 1/3 of their total content, followed by fractional addition in portions during process to the final concentration of sodium acetate 1.5–3.0% and oil 0.75–1.5% allowed to stabilize the pH of the medium during cultivation at the level of 6.4–7.8 and increase rates of the ethapolan synthesis compared to a single addition of the corresponding substrate concentrations (Table 4). Note that after addition of the second portion of 1.0% acetate and 0.5% refined oil the pH of the culture fluid was increased to 8.0–8.2. In this case, for further stabilization of the pH, the third portion of acetate was replaced with an equimolar quantity (by carbon) of acetic acid. This allowed not only to maintain the pH of the medium at the optimal level, but also led to an increase in the production of EPS by 1.25 times (to 17.27 g/l).

Table 5.

Rheological properties of ethapolan solutions

Substrate concentrations for EPS biosynthesis, %	Relative increase of kinematic viscosity, % of control			
	0,05% solution of culture fluid		0,05% solution of ethapolan	
	0.1 M KCl	Cu^{2+}–glycine system	0.1 M KCl	Cu^{2+}–glycine system
Refined sunflower oil, 5.0*	100±5.00	100±5.00	100±5.00	100±5.00
Waste sunflower oil after frying meat, 5.0**	102±5.10	106±5.30	99±4.95	110±5.50
Waste sunflower oil after frying potatoes, 5.0	86±4.30	113±5.65	92±4.60	105±5.25
Refined olive oil, 5.0*	100±5.00	99±4.95	100±5.00	102±5.10
Waste olive oil after frying meat, 5.0**	99±4.95	105±5.25	95±4.75	100±5.00
Waste olive oil after frying potatoes, 5.0**	104±5.20	108±5.40	101±5.05	112±5.60
Molasses, 1.5 +mixed waste sunflower oil, 1.5	107±5.35	119±5.95	98±4.90	115±5.75
Acetate, 3.0 +mixed waste sunflower oil, 1.5***	110±5.50	122±6.10	103±5.15	107±5.35

Notes: Inoculum was grown on the corresponding oil. * Control. ** Inoculum was grown on refined oil. *** Fed–batch cultivation.

In further experiments, it was found that replacement of refined oil in the mixture with acetate on mixed waste oil leaded to a slight decrease in the concentration of EPS compared to the use of refined substrate (from 17.27 to 16.36 g/l). At the same time, an increase in EPS–synthesizing ability by 1.13 times was observed.

The practical value of microbial exopolysaccharides is determined primarily by their ability to significantly change the rheological properties of water systems at low concentrations. In view of this, in the next stage of the research, the influence of the type of used substrate (refined or waste oil, mixture of oil with molasses or acetate) on the quality of ethapolan solutions was investigated.

The study of rheological properties of ethapolan solutions prepared from EPS obtained by the cultivation of *Acinetobacter* IMV B–7005 on waste oil–containing monosubstrates and their mixtures showed that the viscosity increase in the presence of 0.1 M KCl and in the Cu^{2+}–glycine system did not exceed 15–30% compared with the use of EPS obtained on refined substrates.

Conclusions

It was established the possibility of polysaccharide ethapolan synthesis by *Acinetobacter* sp. IMB B–7005 on various types of refined oils (sunflower, corn, olive, rapeseed), as well as shown that under replacing them with waste oil of different quality (after frying potatoes or

meat), only a slight change in EPS production occurred.

Further intensification of EPS production by using oil in the mixture with molasses or acetate resulted in an increase of ethapolan synthesis to 14–16 g/l. It should be noted that under using waste oil–containing mono– and mixed substrates, the obtained EPS retained its valuable rheological properties.

Conflict of Interest: The authors declare that they have no conflict of interest.

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