

## ISOLATION OF SURFACTANTS SYNTHESIZED BY THE *PSEUDOMONAS* BACTERIA AND STUDY OF THEIR PROPERTIES

T.Ya. Pokynbroda<sup>1\*</sup>, I.V. Karpenko<sup>1</sup>, H.H. Midyana<sup>1</sup>, O.Ya. Karpenko<sup>2</sup>

<sup>1</sup>Department of Physical Chemistry of Fossil Fuels InPOCC, NAS of Ukraine, Lviv, Ukraine

<sup>2</sup>Lviv Polytechnic National University, Lviv, Ukraine

\*Corresponding author: pokynbroda@ukr.net

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**Background.** The important problem of biosurfactants production is biosynthesis optimization. But lack of effective isolation methods elaboration with simultaneous new costeffective products is the greatest weaknesses of existing technologies.

**Objective.** The aim of the study is rational technology elaboration for isolation of biosurfactants obtained from strains *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573. Investigation of the influence of different acids and temperatures on the efficiency of isolation of the surfactant products, study the properties of the obtained products. Determination of the possible directions of the use of supernatant obtained after precipitation of the biocomplexes (SPL) as a new inexpensive product.

**Methods.** Rhamnolipid surfactant concentrate was precipitated from culture liquid supernatant (CLS) by acidification to pH 3–4 with acid solutions (HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH), kept at 100 °C for 25 min, cooled to room temperature, centrifuged before the phase separation, the supernatant was decanted. The surface tension of the SPL was determined by du Nouüy method (with a platinum ring). The emulsification index of biosurfactants was determined regarding mineral oil and sunflower oil. The RLs were isolated from the SPL by extraction with a mixture of ethyl acetate and isopropanol, their composition was determined by thin layer chromatography. Influence on plants was assessed by their morphometric parameters after presowing seed treatment.

**Results.** The rational technology for surfactants isolation from strains *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 was developed. It was shown that the suitable method of the isolation of the biosurfactants of *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 is acidic precipitation from CLS with heating. As a result, the product yield was increased by 20%, and the duration of the process was reduced. The physico-chemical properties of the SPLs after the isolation of biosurfactants from the CLS were studied. SPLs have been shown to be effective oil emulsifiers, foaming and wetting agents for various surfaces. It was shown that SPLs (at dilutions 1:10) do not exhibit phytotoxic effects and stimulate the growth of watercress.

**Conclusions.** The new wasteless technology for *Pseudomonas* strains biosurfactants isolation has been proposed, which provides for the elimination of the extraction stage with solvents, as a result, the yield of the target products has been increased. Thus, the technology has economic and environmental advantages. It was shown that SPLs, being inexpensive and effective products, can be used in environmentally friendly technologies: in agriculture (for stimulation of plant growth), for remediation of contaminated soils, production of detergent compositions.

**Keywords:** rhamnolipid surfactants; physicochemical properties; acid precipitation; plant growth stimulators.

### Introduction

Nowadays environmental technologies have become a priority, so biogenic surfactants are considered as an alternative to synthetic [1]. Among the promising producers, the bacteria of the genus *Pseudomonas* deserve the attention, since they synthesize the rhamnolipids (RLs) with high surface activity. According to their chemical structure, RLs are the ethers of rhamnose and fatty acids. Due to their diphylic structure, the biosurfactants are characterized by the same properties as synthetic ones: surface and interfacial tension, critical mi-

celle concentration, hydrophilic-lipophilic balance, stabilization of hydrocarbon emulsions, foaming ability, etc. Rhamnolipids are able to reduce the surface tension of solutions up to 29 mN/m, emulsify a number of hydrocarbons, vegetable oils, mineral oils, petroleum, etc. In aqueous solutions, biosurfactants are adsorbed on solid surfaces, changing their adhesion and wetting. RLs specifically influence the microorganisms and viruses: they provide antimicrobial effects in higher concentrations while increasing the permeability of cell membranes in lower concentrations. These properties of biosurfactants determine their ability to enhance the ac-

tion of other substances while using them. They can be used in agriculture (complex plant protection and nutrition), in the food, pharmaceutical and cosmetic industries. Thus, due to physico-chemical properties combined with the biocompatibility the rhamnolipid surfactants can replace synthetic products in environmentally priority technologies.

Nowadays the economic accessibility of biosurfactants is limited by the cost of their synthesis, and even more by the costs of isolation and purification of the products. In addition, the knowledge of physico-chemical and biological properties of biosurfactants, which are necessary for assessing their practical potential, is not always complete [2]. The most problematic stage in the production of RLs is their isolation from the culture liquid. Even with optimized biosynthesis, the production efficiency depends heavily on rational methods of isolation of the target products. In this connection, there is a growing need for the optimization of the processes of isolation of biosurfactants. For instance, the cost of raw materials is up to 20–40% of the total cost of production [3]. To replace traditional substrates (sugar, alcohol) it is proposed to use economically viable carbon sources (industrial wastes, renewable substrates, etc.) [4, 5]. Previously, soybean, sunflower oils, technical glycerol – a by-product of biodiesel production were used for the rhamnolipid biosynthesis [6–8], the mathematical methods were applied for the synthesis optimization [9–11]. However, it is known, that the processes of biosurfactants allocation are up to 50–80% of the total cost of their production [2]. Currently, solvents are often used for extraction of pure biosurfactants, but they do not fully extract products, and also are toxic [12]. The economic availability of rhamnolipids technology can be increased via obtaining their complexes: culture liquid supernatant (CLS), rhamnolipid biocomplex, etc. The maximum use of components of the post-fermentative culture liquid is also a rational way to the production efficiency.

**The aim** of the present study is the rational technology elaboration for isolation of biosurfactants obtained from strains *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573. The tasks of the study: to investigate the influence of different acids and temperature conditions on the efficiency of isolation of the surfactant products of *Pseudomonas* strains and to study the properties of the obtained products. To determine the possible directions of the use of supernatant obtained after precipitation of the biosurfactants as a new inexpensive product.

## Materials and methods

The following strains were used for the study: *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 – producers of extracellular biosurfactants from the collection Department of Physico-Chemistry of Fossil Fuels of L.N. Litvinenko InPOCC, NAS of Ukraine. Cultivation was carried out on a rotary shaker, 220 rpm, (WL-2000, JV Electronic, Poland), 30 °C, 5 days [6]. Rhamnolipid biocomplex was precipitated from CLS [13] by acidification to pH 3–4 with acid solutions (HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH), kept at 100 °C 25 min, cooled to room temperature, centrifuged (8000 rpm, 20 min) before the phase separation, the supernatant obtained after precipitation of the biosurfactants (SPL 1) was decanted. For comparison, biosurfactant was isolated from the acidified CLS upon cooling (4 °C) – SPL 2. The surface tension of the SPL was determined by du Noüy ring method with a platinum ring on the Tensiometer KRÜSS K6 ("KRÜSS" GmbH, Germany) [14], the relative concentration of biosurfactants was determined by the critical micellar dilution (CMD) [15]. The emulsification index of SPL (E<sub>24</sub>) was determined with respect to vaseline oil and sunflower oil. The RLs were isolated from the SPL by extraction with a mixture of ethyl acetate – isopropanol 2:1, their composition was determined by thin layer chromatography (Merck Silica gel 60, Germany), chloroform-methanol-water 65:2:4, visualization – by 5% phosphomolybdic acid [16]. Presowing treatment of watercress seeds was carried out by soaking in SPL, sprouting, germination was determined according to State Standard of Ukraine DSTU 4138-2002 [17]. The contact angles of wetting of the SPL were determined on the cathetometer KM-8, foaming formation – according to State Standard of Ukraine DSTU 3789-98 [18]. The experiments were repeated three times. The results were statistically processed using Microsoft Excel software by the method of average error.

## Results

The new technology for surfactants isolation of the strains *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 was developed.

The CLS was acidified (to a pH of 2.5–4.0), then heated (Method 1) or cooled (Method 2), centrifuged to obtain SPL 1 and SPL 2 and a concentrate of rhamnolipid surfactant (Table 1).

It was shown that when the acidified CLS of both strains was heated, the yield of the rhamnolipid surfactant concentrate increased by 7–28%, depending on the acid nature. The maximum yield was 6.6 g/L for *Pseudomonas* sp. PS-17 (with HCl), for *P. fluorescens* 8573 – 6.2 g/L (with H<sub>2</sub>SO<sub>4</sub>). The properties of the obtained SPL were studied to assess their practical potential. It was found that 3–5 g/L of the lipids have remained in the SPL (depending on the method used) (Tables 1, 2). SPLs, obtained by acidification and followed CLS cooling (method 2), are capable of emulsifying hydrophobic substances (E<sub>24</sub> – 45–59% – for sunflower oil, 40–50% – for vaseline oil), so they can be used as emulsifying agents of various oils, fats, hydrocarbons, etc.

SPLs promotes the wetting of surfaces (Table 2). The contact angle of wetting the geranium leaves

by solutions of SPLs, obtained by the method 1 was 75°, and by the method 2 – 15–30° (for water – 110°). A similar effect was obtained for a hydrophobic surface of polytetrafluorethene (PTF). Thus, SPLs can be used in the composition of agricultural agents and other means for wetting enhancement.

For practical application of SPLs of *Pseudomonas* sp. PS-17 strain, their phytotoxicity was assessed with the test plant – *Lepidium sativum* (Table 3).

SPLs (1:10) do not exhibit phytotoxic effect; they even stimulate the growth of *Lepidium sativum*: its morphometric indices were increased. The length of the shoots was on 5–18% higher than control (4 days), the length of the roots – on 6–17%, similar effects were observed after 9 days and 16 days. Therefore, SPL is a low-toxic product.

**Table 1:** Effect of various acids and temperatures on the yield of *Pseudomonas* biosurfactants

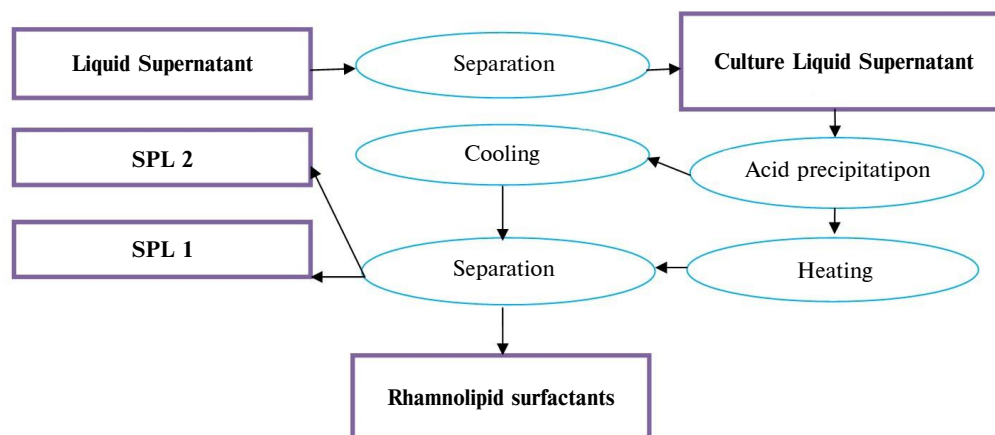
Acid	<i>Pseudomonas</i> sp. PS-17				<i>P. fluorescens</i> 8573			
	4 °C, 24 h		100 °C, 25 min		4 °C, 24 h		100 °C, 25 min	
	Rhamnolipid concentrate, g/L	RL in SPL, g/L	Rhamnolipid concentrate, g/L	RL in SPL, g/L	Rhamnolipid concentrate, g/L	RL in SPL, g/L	Rhamnolipid concentrate, g/L	RL in SPL, g/L
CH <sub>3</sub> -COOH	4.57 ± 0.16	3.9 ± 0.3	5.88 ± 0.12	3.5 ± 0.2	4.39 ± 0.12	4.2 ± 0.3	5.71 ± 0.13	3.4 ± 0.3
H <sub>3</sub> PO <sub>4</sub>	5.39 ± 0.12	4.6 ± 0.3	6.20 ± 0.13	3.9 ± 0.3	5.68 ± 0.15	4.8 ± 0.2	5.79 ± 0.21	3.7 ± 0.2
HCl	5.44 ± 0.15	4.6 ± 0.4	6.59 ± 0.15	3.3 ± 0.3	5.21 ± 0.16	4.4 ± 0.2	6.13 ± 0.26	3.5 ± 0.3
H <sub>2</sub> SO <sub>4</sub>	5.41 ± 0.21	4.5 ± 0.5	5.86 ± 0.15	3.7 ± 0.3	5.17 ± 0.13	4.2 ± 0.3	6.20 ± 0.19	3.6 ± 0.3
HNO <sub>3</sub>	5.32 ± 0.31	4.8 ± 0.5	6.39 ± 0.18	3.1 ± 0.2	5.54 ± 0.14	4.6 ± 0.2	5.63 ± 0.18	3.9 ± 0.2

**Table 2:** Physico-chemical properties of the SPLs of *Pseudomonas* sp. PS-17 strain obtained by various methods (pH 7)

Product	Acid for precipitation	Foam stability, %	E <sub>24</sub> , %		Contact angles θ, deg.		Surfactant content, g/L
			Sunflower oil	Vaseline oil	PTF	Leave of geranium	
SPL 1	HCl	50.3 ± 0.7	20.5 ± 0.5	1.5 ± 0.5	40.3 ± 0.7	75.3 ± 0.7	3.3 ± 0.4
	H <sub>2</sub> SO <sub>4</sub>	80.5 ± 0.5	5.0 ± 0.5	1.2 ± 0.8	75.6 ± 0.4	75.4 ± 0.5	3.7 ± 0.3
SPL 2	HCl	92.2 ± 0.8	59.2 ± 0.8	50.2 ± 0.8	40.3 ± 0.7	15.5 ± 0.5	4.6 ± 0.3
	H <sub>2</sub> SO <sub>4</sub>	93.5 ± 0.5	45.7 ± 0.3	40.3 ± 0.7	42.5 ± 0.5	30.4 ± 0.6	4.5 ± 0.3

**Table 3:** Morphometric indices of *Lepidium sativum* after seed treatment with SPL solutions of *Pseudomonas* sp. PS-17 strain

Dilution	Germination, %	4 days		9 days		16 days	
		Shoot length, cm	Root length, cm	Shoot length, cm	Root length, cm	Shoot length, cm	Root length, cm
Control (H <sub>2</sub> O)	98	2.64 ± 0.15	4.31 ± 0.14	4.16 ± 0.13	7.72 ± 0.16	6.11 ± 0.14	9.10 ± 0.14
1:150	98	2.78 ± 0.13	5.08 ± 0.14	4.18 ± 0.15	7.36 ± 0.16	6.13 ± 0.18	9.12 ± 0.18
1:100	98	2.76 ± 0.13	5.06 ± 0.13	4.66 ± 0.16	7.38 ± 0.18	6.64 ± 0.19	9.55 ± 0.18
1:50	98	2.79 ± 0.14	4.38 ± 0.15	5.02 ± 0.18	7.62 ± 0.19	6.12 ± 0.18	11.09 ± 0.17
1:25	98	3.12 ± 0.15	3.84 ± 0.13	5.42 ± 0.16	6.84 ± 0.17	6.21 ± 0.19	10.08 ± 0.17
1:10	97	2.94 ± 0.15	3.14 ± 0.14	5.46 ± 0.18	6.54 ± 0.15	6.31 ± 0.17	11.52 ± 0.19
1:5	84	1.58 ± 0.13	0.86 ± 0.11	3.16 ± 0.17	2.78 ± 0.18	5.02 ± 0.18	5.31 ± 0.18
1:1	76	0	0	0	0	0	0



**Figure:** The scheme of *Pseudomonas* strains biosurfactants isolation

Thus, a new wasteless technology for *Pseudomonas* strains biosurfactants isolation has been elaborated (the Figure). The technology includes the solvent extraction stage.

The identified physico-chemical properties (emulsification, wetting, foaming) of the obtained products, as well as their low toxicity, are a real basis for their use in agricultural technologies (e.g. plant growth stimulation), the remediation of contaminated soils, in detergent compositions as it was shown in our previous works [19, 20].

## Discussion

Rhamnolipids are effective surface-active products synthesized by the bacteria of the genus *Pseudomonas*. It is known, that the processes of biosurfactants isolation make up to 50–80% of the total cost of their production [2]. In addition, physico-chemical and biological properties of biosurfactants, which are necessary for the assessment of practical potential in specific industries, are still not sufficiently characterized.

Currently, solvents are often used for extraction and purification of biosurfactants, but they do not extract products completely, and also are toxic. The economic feasibility of rhamnolipids can be increased via obtaining their complexes: culture liquid supernatant, rhamnolipid biocomplex, etc. The maximum use of components of the post-fermentative culture liquid can also be a rational method to the increase in production efficiency.

In this work, the methods of rhamnolipid surfactant isolation from the CLS of *Pseudomonas* strains using different acids and different temperature regimes were improved. The rational parameters for the deposition of biosurfactants of *Pseudo-*

*monas* strains were developed. The advisability of surfactants isolation from the CLS of *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 by acid precipitation with obtaining of biosurfactant precipitate and supernatant was established. The optimum mode of acid precipitation of surfactants was determined – heating of the CLS to 100 °C for 25 min. As a result, the yield of the rhamnolipid surfactant concentrate was increased up to 20%. Thus, the advantages of the developed method are the time saving, energy costs reduction due to the absence of the cooling stage of the acidified CLS as well as an increase in the yield of the product.

The physico-chemical properties of SPL (after the precipitation of biosurfactants from CLS of the *Pseudomonas* strains) for the evaluation of their practical potential were investigated. SPLs, obtained by acidification and followed cooling, are capable of emulsifying hydrophobic substances (various oils, fats, hydrocarbons).

The differences in emulsifying and foaming properties of SPL, obtained by various methods, are explained by a lower concentration of surfactants in SPL, obtained with heating, due to their more complete precipitation. Obviously, SPL obtained by this method are promising for composite detergents (with other surfactants), in cosmetics – for emulsifying oils and fats, etc. SPL obtained with cooling can also be used for the remediation of oil-contaminated soils (due to emulsifying properties). The wetting properties of SPL are important for the removal of oily stains, soot, and metallic dust from hydrophobic surfaces. Moisturizing properties of SPL also can be used in the pharmaceutical, cosmetic means. It is also shown that SPL (in dilutions of 1:10 and more) do not show a phytotoxic effect, but even stimulate the growth of watercress.

**Table 4:** Comparative characteristics of biosurfactants of *Pseudomonas* strains with commercial preparations

Product characteristics	<i>Pseudomonas</i> biosurfactants	Sodium dodecyl-sulphate	Triton X-100	AGAE Technologies	Urumqi Unite Biotechnology
Critical micelle concentration, g/l	0.05–0.14	0.26	0.65	0.02–0.15	0.05–0.10
Interfacial tension (water-kerosene), mN/m	0.009–0.2	0.1	0.4	0.08	0.09
Foaming capacity	7	8	6	9	6
Price, USD/kg	70–180	90	70	320	400–1600

SPLs can be a promising agent for crop production, a basis for detergents (with other surfactants), as components of anticorrosive compositions, etc. [21, 22].

Thus, a new wasteless technology for *Pseudomonas* strains biosurfactants isolation has been proposed. This technology has economic and environmental advantages.

Thanks to the strategic planning technique used to identify strengths, weaknesses, opportunities, and threats related to the obtained biosurfactant products (SWOT-analysis) [23] it has been shown that their advantages over synthetic ones are high efficiency, heat resistance, non-toxicity, biocompatibility and biodegradability (Table 4). Renewable raw materials including production waste can be used for the biosynthesis. It is shown that their predicted cost is 70–180 USD/kg depending on the concentration, whereas for commercial biosurfactant (Urumqi Unite Bio-Technology, AGAE Technologies) – 320–1600 USD/kg [24]. Thus, the unique physicochemical and biological properties of the obtained substances, their low toxicity, and the mild effect on the cells of microorganisms and plants are a powerful argument for their use as environmentally safe products in modern industrial and agricultural technologies. So, thanks to poly-functional properties and environmental safety, the

obtained surfactants of *Pseudomonas* strains will be competitive in the modern market as independent products and components of complex products for modern industrial and agricultural technologies.

### Conclusions

The rational technology for *Pseudomonas* strains biosurfactants isolation has been proposed. It was shown that an effective way to isolate biosurfactants *Pseudomonas* sp. PS-17 and *P. fluorescens* 8573 is acidic precipitation from CLS with heating. As a result, the yield of the biosurfactant concentrate is increased by 20%, and the duration of the process is reduced.

The physico-chemical properties of SPL after isolating the rhamnolipid concentrate from the culture liquid supernatant were studied. SPLs have been shown to be effective oil emulsifiers, foaming and wetting agents for various surfaces.

It was found that SPL (at 1:10 dilutions) do not show phytotoxic effects and stimulate the growth of watercress.

SPL as a cheap effective product can be used in environmentally friendly technologies: for agriculture, remediation of contaminated soils, detergents, etc.

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Т.Я. Покинъброда, І.В. Карпенко, Г.Г. Мідяна, О.Я. Карпенко

#### ВИДІЛЕННЯ СУРФАКТАНТІВ БАКТЕРІЙ *PSEUDOMONAS* І ДОСЛІДЖЕННЯ ЇХ ВЛАСТИВОСТЕЙ

**Проблематика.** Важливою проблемою виробництва біосурфактантів є оптимізація біосинтезу, проте найбільші труднощі існуючих технологій – відсутність ефективних методів їх виділення для отримання нових економічно вигідних продуктів.

**Мета.** Розробка раціональної технології виділення біосурфактантів штамів *Pseudomonas* sp. PS-17 і *P. fluorescens* 8573. Вивчення впливу різних кислот і температурних умов на ефективність виділення сурфактантів штамів *Pseudomonas* і дослідження властивостей отриманих продуктів. Визначення потенційних напрямів використання одержаного продукту – надосадової рідини, отриманої після осадження біокомплексів.

**Методика реалізації.** Рамноліпідний біокомплекс осаджували із супернатанту культуральної рідини підкисленням розчинами кислот (HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH), витримували при 100° С 25 хв або охолоджували, центрифугували, надосадову рідину декантували. Поверхневий натяг біогенних поверхнево-активних речовин визначали методом дю Нуї з платиновим кільцем. Індекс емульгування біосурфактантів визначали щодо вазелінової та соняшникової олій. Ліпіди виділяли з надосадової рідини екстракцією етилацетатом з ізопропанолом, склад визначали за допомогою тонкошарової хроматографії. Вплив на рослини оцінювали за морфометричними показниками після передпосівної обробки насіння.

**Результати.** Розроблено раціональну технологію виділення поверхнево-активних речовин штамів *Pseudomonas* sp. PS-17 і *P. fluorescens* 8573. Показано, що найбільш прийнятним методом виділення рамноліпідних біосурфактантів є їх кислотне осадження із супернатанту культуральної рідини з нагріванням. У результаті вихід продукту збільшився на 20 %, а тривалість процесу скоротилася. Вивчено фізико-хімічні властивості надосадової рідини після виділення біокомплексів. Показано, що вона є ефективним емульгатором, піноутворювачем і змочувальним агентом для різних поверхонь. Надосадова рідина в розведенні 1:10 не проявляє фітотоксичного ефекту й стимулює ріст крес-салату.

**Висновки.** Запропоновано нову безвідходну технологію виділення біосурфактантів штамів роду *Pseudomonas*, яка передбачає виключення стадії екстракції розчинниками, у результаті підвищується вихід цільових продуктів. Показано, що надосадова рідина як дешевий продукт може використовуватися в екологічно чистих технологіях: для сільського господарства (стимулювання росту рослин), рекультиватії забруднених ґрунтів, у складі мийних композицій.

**Ключові слова:** рамноліпідні сурфактанти; фізико-хімічні властивості; кислотне осадження; стимулятори росту рослин.

Т.Я. Покинсьброта, И.В. Карпенко, Г.Г. Мидяна, А.Я. Карпенко

#### ВЫДЕЛЕНИЕ БИОСУРФАКТАНТОВ БАКТЕРИЙ *PSEUDOMONAS* И ИЗУЧЕНИЕ ИХ СВОЙСТВ

**Проблематика.** Важной проблемой производства биосурфактантов является оптимизация биосинтеза, но наибольшие трудности существующих технологий – отсутствие эффективных методов их выделения для получения новых экономически выгодных продуктов.

**Цель.** Разработка рациональной технологии выделения биосурфактантов штаммов *Pseudomonas* sp. PS-17 и *P. fluorescens* 8573. Изучение влияния различных кислот и температурных условий на эффективность выделения сурфактантов штаммов *Pseudomonas* и исследование свойств полученных продуктов. Определение потенциальных направлений использования полученного продукта – надосадовой жидкости, полученной после осаждения биокомплексом.

**Методика реализации.** Рамнолипидный биокомплекс осаждали из супернатанта культуральной жидкости подкислением растворами кислот (HCl, H<sub>2</sub>SO<sub>4</sub>, H<sub>3</sub>PO<sub>4</sub>, HNO<sub>3</sub>, CH<sub>3</sub>COOH), выдерживали при 100° С 25 мин или охлаждали, центрифугировали, надосадовую жидкость декантировали. Поверхностное натяжение биогенных поверхностно-активных веществ определяли методом дю Нуи с платиновым кольцом. Индекс эмульгирования биосурфактантов определяли относительно вазелинового и подсолнечного масел. Липиды выделяли из надосадовой жидкости экстракцией этилацетатом с изопропанолом, состав определяли тонкослойной хроматографией. Влияние на растения оценивали по морфометрическим параметрам после предпосевной обработки семян.

**Результаты.** Разработана рациональная технология выделения поверхностно-активных веществ штаммов *Pseudomonas* sp. PS-17 и *P. fluorescens* 8573. Показано, что наиболее приемлемым методом выделения рамнолипидных биосурфактантов является их кислотное осаждение из супернатанта культуральной жидкости с нагреванием. В результате выход продукта был увеличен на 20 %, а продолжительность процесса уменьшилась. Изучены физико-химические свойства надосадовой жидкости после выделения биокомплексом. Показано, что надосадовая жидкость является эффективным эмульгатором, пенообразователем и смачивающим агентом различных поверхностей. Надосадовая жидкость в разведениях 1:10 не проявляет фитотоксического эффекта и стимулирует рост крес-салата.

**Выводы.** Предложена новая безотходная технология выделения биосурфактантов штаммов рода *Pseudomonas*, которая предусматривает исключение стадии экстракции растворителями, в результате повышается выход целевых продуктов. Таким образом, эта технология имеет экономические и экологические преимущества. Было показано, что надосадовые жидкости как дешевый продукт могут использоваться в экологически чистых технологиях: для сельского хозяйства (стимулирование роста растений), рекультивации загрязненных почв, в составе моющих композиций.

**Ключевые слова:** рамнолипидные сурфактанти; физико-химические свойства; кислотное осаждение; стимуляторы роста растений.