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INTENSIFICATION OF SURFACTANTS SYNTHESIS UNDER *Nocardia vaccinii* IMV B-7405 CULTIVATION ON A MIXTURE OF GLUCOSE AND GLYCEROL

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The purpose of this study was to establish optimal molar glycerol to glucose ratio for enhanced biosurfactant synthesis by the cultivation of *Nocardia vaccinii* IMV B-7405 on a mixture of energetically nonequivalent substrates (glucose and glycerol).

Based on theoretical calculations of the energy requirements for biomass production and the synthesis of surface-active trehalose monomycolates on the energy-deficient substrate (glycerol), the concentration of the energy-excessive substrate (glucose), which increased the efficiency of the substrate carbon conversion to biosurfactant, was determined. It was found that the synthesis of extracellular biosurfactant on a mixture of glucose and glycerol at molar ratio of 1.0:2.5 increased 2.0–2.3-fold compared to that on corresponding monosubstrates.

The increased level of biosurfactant on the mixed substrate is determined by intensification of surface-active trehalose monomycolate synthesis, confirmed by 1.2–5.7-fold increase in activity of the enzymes involved in their biosynthesis (trehalose phosphate synthase, phosphoenolpyruvate-carboxykinase, phosphoenolpyruvate-synthetase) compared to cultivation on monosubstrates glucose and glycerol. The results indicate the feasibility of using a mixture of energetically nonequivalent substrates for enhancing the synthesis of secondary metabolites, as well as possibility of achieving the high efficiency of these mixed substrates if they are properly chosen and the molar ratio of their concentrations are correctly determined.

Key words: *Nocardia vaccinii* IMB B-7405, biosurfactant, energetically nonequivalent substrates.

Literature data and results of own studies indicate the prospects of the use of a mixture of growth and non-growth substrate for the intensification of the microorganism growth and the synthesis of metabolites valuable for practical use. It has been shown by the example of microbial exopolysaccharide ethapolan and biosurfactants (BS) such as *Rhodococcus erythropolis* IMV Ac-5017 and *Acinetobacter calcoaceticus* IMV B-7241 [1–4]. This approach allowed avoiding inefficient loss of carbon and energy that occur when using monosubstrates, as well as improving the efficiency of substrate carbon conversion to products of microbial synthesis.

It was shown the possibility of using mixed growth substrates (*n*-hexadecane, glycerol, ethanol, glucose) to intensify the synthesis of surfactants by *Nocardia vaccinii* IMV B-7405, at that, the highest values were observed for a mixture of glucose and glycerol [5]. However, to attain the maximum efficiency of carbon conversion to the final product, the determining the optimal for its synthesis molar ratio of monosubstrate concentrations in the mixture is necessary, which in turn requires the theoretical calculations of the energy requirements for the biomass production and BS synthesis on the energy-deficient substrate followed by determining the concentration of energy-excessive substrate which compensate for the energy expenditure [1–4]. To implement these theoretical calculations, understanding of the metabolic pathways of relevant monosubstrate synthesis is important.

It was revealed in our previous work [6] that glucose in *N. vaccinii* IMV B-7405 was assimilated in the pentose phosphate cycle, and the catabolism of glycerol to dihydroxyacetone phosphate (glycolysis intermediate) could

proceed via both the glycerol-3-phosphate and the dihydroxyacetone pathway.

The aim of this work was to determine the optimal molar ratio of glucose to glycerol for the intensification of BS synthesis by *N. vaccinii* IMV B-7405 on a mixture of these substrates.

Materials and Methods

Nocardia vaccinii K-8 strain registered in the Depository of microorganisms of Institute of Microbiology and Virology of the National Academy of Sciences of Ukraine under the number IMV B-7405 was used in the study. *N. vaccinii* IMV B-7405 strain was grown in liquid mineral medium composed of (g/l): $NaNO_3$ — 0.5, $MgSO_4 \cdot 7H_2O$ — 0.1, $CaCl_2 \cdot 2H_2O$ — 0.1, KH_2PO_4 — 0.1, $FeSO_4 \cdot 7H_2O$ — 0.001, yeast autolysate — 0.5 **(vol/vol)**. Glucose 0.75%, glycerol 0.6% (vol/vol) as well as mixtures of 0.5% glucose and glycerol (0.2; 0.4; 0.5; 0.6 and 0.8% vol/vol) in molar ratios of 1:1; 1:2; 1:2.5; 1:3 and 1:4 respectively were used as the carbon and energy sources.

The mid-exponential-phase culture grown in the medium of the indicated above composition was used as the inoculum. Glucose (0.5%), glycerol (0.5% vol/vol) and a mixture of glucose (0.25%) and glycerol (0.25% vol/vol) were used as the carbon and energy sources for the inoculum preparation.

The amount of inoculum (10^4–10^5 cell/ml) was 5% of the medium volume. Cultivation was performed in 750 ml flasks with 100 ml of medium on a shaker (220 rpm) at 28–30 °C for 24–120 h.

Amount of the extracellular BS was determined after their extraction from the cultural liquid supernatant by chloroform/methanol (2:1) mixture using the modified method [7]. To obtain the supernatant, the culture liquid was centrifuged at 5000 g for 20 min.

Considering that *N. vaccinii* IMV B-7405 strain synthesizes complex of polar and non-polar lipids [6] and a reported method of BS extraction [7] allows to extract mainly non-polar lipids, we modified the classic Folch mixture by introducing into it 1N HCl (chloroform : methanol : water = 4:3:2). This mixture allows most efficient extraction of both polar and non-polar lipids.

The supernatant (25 ml) was placed into cylindrical separatory funnel (100 ml), 1N HCl was added to achieve pH 4.0–4.5 (about 5 ml), the funnel was closed with a ground stopper and stirred for 3 min, and then the chloroform-methanol (2:1) mixture (15 ml) was added and again stirred for 5 min (lipid extraction). The resulting mixture was left in separatory funnel for phase separation, thereafter the bottom fraction was poured off (the organic extract 1) and the aqueous phase was re-extracted. At the re-extraction, 1N HCl to achieve pH 4.0–4.5 (about 5 ml), chloroform-methanol (2:1) mixture (15 ml) were added to the aqueous phase and lipids were extracted for 5 min. After phase separation, the bottom fraction was poured off (the organic extract 2). At the third stage, chloroform-methanol (2:1) mixture (25ml) was added to the aqueous phase and extraction was performed as described above (the organic extract 3). The extracts 1–3 were combined and evaporated on a rotary evaporator IR-1M2 at 50 °C and absolute pressure 0.4 atm up to constant mass.

To determine the emulsification index (E_{24}, %), sunflower oil (5 ml) (emulsified substrate) was added to culture liquid (5 ml) and the mixture was stirred for 2 min. Thereafter the measurement of the emulsification index was determined after 24 h as of the ratio of the emulsion layer height to the total height of the liquid in the tube and it was expressed in percent.

Preparation of cell-free extracts and determination of the activity of enzymes involved in glucose and glycerol catabolism, anaplerotic pathways and BS biosynthesis were performed as described in [6]. The activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49) was measured by detecting $NADP^+$ reduction at 340 nm using glucose-6-phosphate as an electron donor. Dihydroxyacetone kinase activity (EC 2.7.1.29) was determined on dihydroxyacetonephosphate formation which was assayed by spectrophotometry measuring the oxidation of $NADH^+$ in the coupled reaction with glycerol-3-phosphate dehydrogenase. The activity of isocitrate lyase (EC 4.1.3.1) was estimated by measuring the rate of phenylhydrazone glyoxylate formation at 324 nm, the activity of phosphoenolpyruvate (PEP) synthetase (EC 2.7.9.2) — by measuring the rate of pyruvate formation which was detected through NADH oxidation at 340 nm in the coupled reaction with lactate dehydrogenase, the activity of PEP-carboxykinase (EC 4.1.1.49) — by assessing the PEP and pyruvate formation during NADH oxidation, and the activity of glutamate dehydrogenase (EC 1.4.1.4) — by measuring the glutamate formation at NADPH oxidation at 340 nm, the activity of PEP carboxylase (EC 4.1.1.31) — by measuring

NADH oxidation at 340 nm, the activity of trehalose-phosphate synthase (EC 2.4.1.15) — by measuring uridine diphosphate formation which was determined by the NADH oxidation at 340 nm in coupled reactions with pyruvate kinase and lactate dehydrogenase.

NAD^+, $NADP^+$, uridine-5'-diphosphate-glucose (Fluka, Switzerland), lactate dehydrogenase, isocitrate (Serva, Germany) were used for enzyme assays. Others reagents were Sigma, USA.

Enzyme activity was expressed in nmol of product formed per 1 min and calculated per 1 mg of protein. Protein concentration in cell-free extracts was determined by Bradford assay [8]. Enzyme activity assessment was performed at 28–30 °C (optimal temperature for the *N. vaccinii* IMV B-7405 growth).

All experiments were performed in 3 replicates, the number of parallel determination in the experiment was 3-5. Statistical processing of the obtained data was performed as previously described [3]. The differences between average means were considered significant at $P < 0.05$.

Results and Discussion

It was previously shown [1–4] that the auxiliary substrate concept, proposed by Babel [9] to increase the biomass yield, can serve as theoretical basis for studies on the enhanced synthesis of secondary metabolites on mixture of several substrates. According to "energy-based classification" (on which concept is based), all substrates are divided into energy-excessive and energy-deficient [10]. According to this classification, phosphoglyceric acid (PGA) is a central carbon precursor (key intermediate) for the synthesis of all cellular components. The energy needed for the synthesis of cellular components from this precursor, is a constant value and found to be 1 mol of ATP per 10.5 g of dry biomass [10]. Substrates are classified as energy-excessive if the amount of ATP as well as reducing equivalents, generated on the pathway from the substrate to PGA, is sufficient for the biomass synthesis. Substrates, which must be partly oxidized to CO_2 to produce the energy required for the synthesis of cellular components, are classified as energy-deficient. Knowledge of the metabolic pathways of carbon substrate conversion into key intermediate (PGA) of biomass synthesis enables to determine the substrate "energy value".

According to the "energy-based classification" by Babel [10], glycerol is always energy-deficient substrate, whilst glucose may be, depending on catabolism pathway, either energy-deficient (glycolysis, the Entner-Doudoroff pathway) or energy-excessive (pentose phosphate pathway) substrate.

Previously it was found that *N. vaccinii* IMV B-7405 synthesizes complex of neutral, glyco- and aminolipids, at that, glycolipids were presented as trehalose mycolates [11]. Enzymatic studies [9] confirmed the ability of *N. vaccinii* IMV B-7405 to synthesize surface active glyco- and aminolipids, as evidenced by the high activity of enzymes of gluconeogenesis (PEP-carboxykinase and PEP-synthetase) and $NADP^+$-dependent glutamate dehydrogenase as well as trehalose-phosphate synthase — a key enzyme of the trehalose mycolates biosynthesis.

Calculation of the glucose/glycerol concentrations ratio for N. vaccinii IMV B-7405 cultivation on their mixture. The calculation of the optimal glucose/glycerol ratio was based on the following assumptions: 1) trehalose mycolates are the main BS components; 2) catabolism of glucose is performed via the pentose phosphate pathway; 3) glycerol is involved in metabolism through dihydroxyacetone with the participation of pyrroloquinoline quinine- and nitroso-N,N-dimethylaniline-dependent alcohol dehydrogenases and dihydroxyacetone kinase; 4) glucose is used primarily as an energy source, and for the synthesis of biomass and trehalose mycolates carbon of glycerol is used; 5) the mycolic acid in the trehalose mycolates is 3-hydroxy-2-dodecanoyl-docosanoic acid containing 34 carbon atoms (similar to *Rhodococcus erytropolis* trehalose lipids [12]); 6) P/O ratio is 2.

A possible scheme for the synthesis of trehalose monomycolate from glycerol is shown in Fig. 1. This scheme is based on our following studies of glycerol metabolism in the IMV B-7405 strain [6]:

1. *Assimilation of this substrate through dihydroxyacetone*. It was found [6] that glycerol catabolism to dihydroxyacetonephosphate (glycolysis intermediate) may be performed in two ways: through glycerol-3-phosphate and through dihydroxyacetone. Since the dihydroxyacetone kinase activity was higher than glycerol kinase activity (732 and 244 nmol/min^{-1}·mg^{-1} protein, respectively), exactly this pathway of glycerol catabolism is indicated in Fig. 1.

2. *Peculiarities of the central metabolism* (enzyme activities of tricarboxylic acid cycle, including the succinate formation in the alternative 2-oxoglutarate synthase reaction, gluconeogenesis reaction).

It should be noted, according to [6], that both gluconeogenesis enzymes (PEP-carboxykinase and PEP-synthetase) function in the *N. vaccinii* IMV B-7405 cells. Since the PEP-synthetase activity was higher than PEP-carboxykinase activity (1071 and 820 nmol/min^{-1}·mg^{-1} protein, respectively), exactly this reaction is shown in Fig. 1.

3. *Biosynthesis of surface-active trehalose mycolates* (formation of trehalose-6-phosphate in the trehalose-phosphate synthase reaction).

Based on data [12], the formation of mycolic acid via 3-hydroxy-2-dodecanoyl docosanoic acid and the synthesis of trehalose-6-mycolate from trehalose-6-phosphate and mycolic acid are shown in Fig. 1.

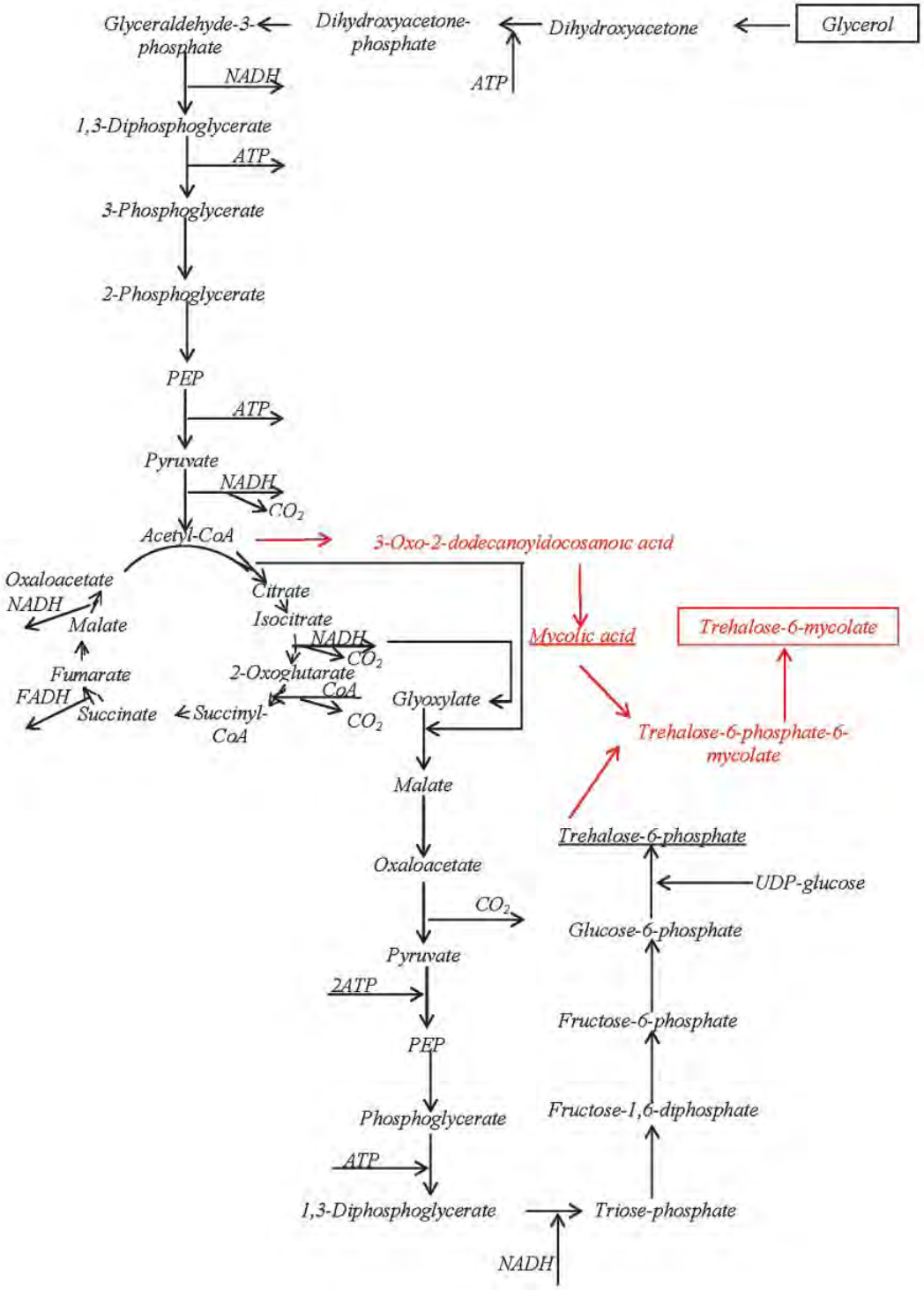

Fig. 1. **A proposed scheme for the biosynthesis of trehalose monomycolates from glycerol in *N. vaccinii* IMV B-7405.** The data from [12] are marked by red.

Energy consumption during the trehalose phosphate synthesis. As can be seen from the scheme, 8 mol of glycerol are required for the synthesis of one molecule of trehalose phosphate (4 mol to produce glyoxylate and 4 mol for the synthesis of acetyl-CoA, which reacts with glyoxylate to form malate). Thus, 8 mol of ATP are consumed during the formation of glycerol-3-phosphate from glycerol, 8 mol of ATP — in the formation of PEP from pyruvate, 4 mol of ATP — in the synthesis of 1,3-diphosphoglycerate from phosphoglyceric acid (PGA) and 8 (4 NADH) — during conversion of 1,3-diphosphoglycerate into triose phosphate. Hence, energy consumption is 28 mol of ATP. In addition, one mol of ATP is consumed during the formation of nucleoside-diphosphate-saccharide (glucose-6-phosphate → UDP-glucose) necessary for the synthesis of trehalose-6-phosphate. Hence, the energy consumption during synthesis of trehalose-6-phosphate from glycerol is 30 mol of ATP.

Energy consumption during the synthesis of mycolic acid. Considering the pathway of biosynthesis of fatty acids from acetyl-CoA described in [1], it can be calculated that for the producing of 3-hydroxy-2-dodecanoyl docosanoic acid containing 34 carbon atoms, 17 mol of acetyl-CoA are required, for the synthesis of which from glycerol, 17 mol of ATP is used. Given the number of cycles (16) in the synthesis of mycolic acids from acetyl-CoA, the energy consumption is 16 + 17 = 33 mol of ATP.

ATP generation during the synthesis of trehalose monomycolate from glycerol.

Energy is generated at the formation of acetyl-CoA:

$$\text{Glycerol} \rightarrow \text{Acetyl CoA} + \text{ATP} + 2\text{NADH}. \quad (1)$$

Since 17 mol of acetyl-CoA for mycolic acid synthesis and 8 mol of acetyl-CoA are required for trehalose phosphate synthesis, the equation (1) can be represented as:

$$25\ \text{Glycerol} \rightarrow 25\ \text{Acetyl-CoA} + 25\ \text{ATP} + 50\ \text{NADH}, \quad (2)$$

From equation (2), given P/O=2, it follows that during the formation of trehalose monomycolate from glycerol 25 + 100 = 125 mol of ATP are generated, or 5 mol of ATP per mole of glycerol. The total energy consumption during the synthesis of trehalose phosphate and mycolic acid from glycerol is 30 + 33 = 63 mol of ATP, or 2.52 mol of ATP per mol of utilized glycerol.

Thus, the energy generated during the trehalose monomycolate formation is 5–2.52 = 2.48 mol ATP / mol of utilized glycerol.

The energy consumption during the biomass synthesis. Synthesis of biomass from phosphoglyceric acid, a key intermediate of synthesis of cellular components, can be represented as follows [10]:

$$4\ \text{PGA} + NH_3 + 29\ \text{ATP} + 5.5\ \text{NAD(P)H} \rightarrow (C_4H_8O_2N)_3 \quad (3),$$

where $(C_4H_8O_2N)_3$ is a formula of mole of the biomass.

Total conversion reactions of glycerol and glucose in PGA are [8]:

$$\text{Glycerol} \rightarrow \text{PGA} + \text{NAD(P)H}; \quad (4)$$

$$\text{Glucose} \rightarrow \text{PGA} + 7\ \text{NAD(P)H} + 3CO_2. \quad (5)$$

For P/O = 2 equations (4) and (5) can be presented as:

$$\text{Glycerol} \rightarrow \text{PGA} + 2\ \text{ATP}; \quad (6)$$

$$\text{Glucose} \rightarrow \text{PGA} + 14\ \text{ATP}. \quad 7)$$

Considering the equation of biomass synthesis from PGA (equation (3) and the glycerol catabolism to PGA equation (equation (6), it can be calculated that the ATP amount required for biomass synthesis (per mol of glycerol) is 8 mol of ATP. In our opinion, this energy can be obtained from glucose. Given that 2.48 mol of ATP / mol of glycerol is generated during the synthesis of trehalosemonomycolate from glycerol, 8 — 2.48 = 5.52 mol ATP should be obtained at the expense of glucose. The equation (7) implies that 0.394 mol of glucose is needed for this amount of energy. Consequently, the glucose/glycerol molar ratio in the medium should be 0.394:1 or 1:2.5.

Effect of molar ratio of glucose/glycerol concentrations on BS synthesis. In the next step, BS synthesis by *N. vaccinii* IMV B-7405 at various molar ratios of glucose and glycerol concentrations in a mixture was studied (Fig. 2). As it can be seen (Fig. 2), the highest rates of BS synthesis were observed at molar ratio of monosubstrates 1:2.5–1,4, i.e. closest to the theoretical one.

Further experiments showed that in the cells of *N. vaccinii* IMV B-7405 strain grown on a mixture of glucose and glycerol (1:2.5), the enzymes of both substrates catabolism functioned simultaneously. Thus, the activity of $NADP^+$-dependent glucose-6-phosphate

Table 1. **Characteristics of BS synthesis under cultivation of *N. vaccinii* IMV B-7405 on glucose, glycerol and their mixture**

Substrate concentration,%	BS, g/l	E24,%
Glucose, 0.5+ Glycerol, 0.5	3.0±0.15*	55±2.8**
Glucose, 0.75	1.3±0.06	51±2.5
Glycerol, 0.6	1.5±0.07	53±2.6

Notes. The concentration of glycerol is given in% (vol/vol). The molar ratio of glucose/glycerol concentrations is 1:2.5 (Table 1 and 2). Mixed and monosubstrates are equimolar for carbon. Under cultivation on glucose and glycerol, the inoculum was grown on respective monosubstrates (0.5%), for the mixed substrate — on mixture of glucose (0.25%) and glycerol (0.25%). The concentration of biomass in all cases was the same 0.8–0.9 g/l.

* — $P < 0.05$ relative to control (BS concentration on the monosubstrates).

** — $P < 0.05$ relative to control (emulsification index on the monosubstrates).

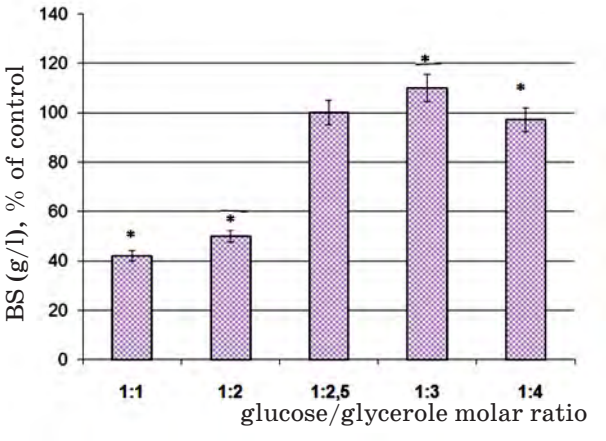

Fig. 2. **Effect of molar ratio of glycerol/glucose concentrations in the cultivation medium of *N. vaccinii* IMV B-7405 on BS synthesis:** *Hereinafter: *— $P < 0.05$* relative to the control (BS concentration at the theoretically calculated glucose/glycerol molar ratio 1:2.5). The inoculum was grown on a mixture of glucose (0.25%) and glycerol (0.25%, vol/vol)

dehydrogenase (one of enzymes of the pentose phosphate pathway) and dihydroxyacetone kinase (the key enzyme of the dihydroxyacetone pathway of glycerol assimilation) was was 400–450 nmol/min^{-1}·mg^{-1} protein). These data indicated the mixotrophic nature of the *N. vaccinii* IMV B-7405 growth on a mixture of glucose and glycerol.

Characteristics of BS synthesis by *N. vaccinii* IMV B-7405 on glucose and glycerol mixture (molar ratio 1:2.5), as well as on monosubstrates equimolar for carbon concentrations shown in Table 1. The concentration of extracellular biosurfactants obtained on the mixture of glucose and glycerol was 2-2.3 times higher than ones synthesized on monosubstrates and emulsification index of the culture liquid did not substantially change (51–55%).

These data may indicate that cultivation of IMV B-7405 strain on the mixed substrate is accompanied by enhanced synthesis of metabolites with surfactant properties. To test this assumption the activity of enzymes of the surfactants biosynthesis was determined (Table 2).

The data presented in the Table 2 have shown that under cultivation IMV B-7405 on the glucose and glycerol mixture, the activity of PEP-carboxylase (enzyme of anaplerotic reaction that replenishes the pool of C_4-dicarboxylic acids — precursors of gluconeogenesis) was 1.3-1.4 fold higher, and the activity of key enzymes of gluconeogenesis (PEP-carboxykinase and PEP-synthetase) was 1.2-5.7-fold higher than that under cultivation on monosubstrates. These data, as well as the higher activity of trehalose-phosphate synthase on mixed substrate (69 against 38–39 nmol/min^{-1}·mg^{-1} protein on glucose and glycerol) indicated an increase in the surface-active trehalosemycolate synthesis under such conditions. Enhanced synthesis of exactly glycolipids on a mixture of glucose and glycerol was also evidenced by almost the same (394–427 nmol/ min^{-1}·mg^{-1} protein) activity of $NADP^+$-dependent glutamate dehydrogenase, the enzyme of surface-active aminolipid biosynthesis, on both mixed and monosubstrates. It should be noted that under cultivation of IMV B-7405 strain on either glycerol or mixture of glucose and glycerol, activity of isocitrate lyase (one of the key enzymes of the glyoxylate cycle functioning in microorganisms growing on non-carbohydrate substrates) was not detected.

Thus, both, the data presented in Table 2 and the results of growth experiments (Table 1) have confirmed the enhanced synthesis of surface-active metabolites under cultivation of *N. vaccinii* IMV B-7405 on a mixed substrate.

Table 2. **The activity of enzymes of BS biosynthesis and anaplerotic pathways under *N. vaccinii* IMV V-7405 cultivation on the mono- and mixed substrates**

Enzyme	Activity (nmol/min·mg protein) in the cell-free extracts, obtained from cells grown on:		
	glycerol	glucose	mixture of glycerol and glucose
PEP-carboxylase	714±35	218±10	1250±62*
PEP-synthetase	1042±52	623±31	1290±64*
$NADP^+$-dependent glutamate dehydrogenase	394±19	427±21	417±20*
PEP-carboxylase	714±35	803±40	1036±51*
Isocitrate lyase	0	0	0
Trehalose-phosphate synthase	39±2	38±2	69±3*

Notes. Enzyme activity was determined in the cell-free extracts obtained from the cells in early exponential growth phase. * — $P < 0.05$ relative to control (enzyme activity in the cells grown on the monosubstrates).

We reviewed in [4] the literature data (mainly 2005–2010) on the application of the mixture of substrates for intensification of technologies of microbial synthesis of practically valuable products of fermentation (ethanol, lactic acid, butanediol), the primary (amino acid, *n*-hydroxybenzoate, triglycerides) and secondary (lovastatin, surfactants) metabolites as well as biodegradation of xenobiotics of aromatic nature (benzene, cresols, phenols, toluene) and pesticides (dimethoate).

Following this review, a little new information on the microbial synthesis of industrial products, including microbial surfactant mixtures on growth substrates was published. For example, in [13] the possibility of enhancing the synthesis of 1,3-propanediol by *Klebsiella pneumoniae* ME-303 on the mixture of xylose and glycerol was shown. It was found that under cultivation of ME-303 strain in a fermenter on a medium containing 30 g/l glycerol and 8 g/l xylose, the concentration of 1,3-propanediol was 13.2 g/l and was only 9.4% higher than under cultivation on monosubstrate glycerol.

Study of biomass production and polyols (mannitol and arabitol) synthesis by *Yarrowia lipolytica* on glycerol, glucose and mixture of these subsrtates (10 g/l) showed that cultivation of yeast on mixed substrate was accompanied by an increase in growth rate, levels of biomass, but not the amount of alcohol [14].

Formation of sophorolipids by strain *Starmerella* (*Candida*) *bombicola* ATCC 22214 on a mixture of glycerol (15%) and sunflower oil (10%), and a mixture in which the purified glycerol was replaced with glycerol-containing waste products of commercial fats lyposis was studied in [15]. It was found that regardless of the glycerol source in mixture, concentration of synthesized surfactant was almost the same (6.36–6.61 g/l).

Yeast *Cyberlindnera samutprakarnensis* $JP52^T$ when grown on a mixture of glucose (2%) and palm oil (2%, vol/vol) synthesized 1.89 g/l BS [16]. Interestingly, BS synthesis was not observed under cultivation of JP52T strain on palm oil, on monosubstrate glucose concentration of surfactant was only 0.03 g/l.

In [17], synthesis of mannosylerythritol lipid BS by *Pseudozyma hubeiensis* Y10BS025 on mixtures of several substrates was studied. It was established that cultivation of Y10BS025 strain on a mixture of glucose and glycerol at ratio 75:25 with addition of soybean oil (8%, vol/vol), accompanied by synthesis of 115 g/l BS, that higher than if olive oil was added (65 g/l).

In studies [15–17] (and other aforementioned publications), monosubstrates and their concentrations in the mixture were established empirically. It is noteworthy that the substrates were used in extremely high (sometimes 100 g/l) concentrations. In this case, the enhancement of the BS synthesis on the mixture of growth substrates compared to the cultivation on monosubstrates was not indicative, since the main criterion of efficiency of mixed substrates was the maximal carbon conversion into final product.

In previous researches [1–4] and in this study, to provide the highest carbon conversion

of the two energetically unequal substrates into microbial products (surfactants), we performed a theoretical calculation of optimal molar ratio of monosubstrate concentrations in the mixture that allowed increasing the concentration of the final product by several times in comparison with that on monosubstrates.

The theoretical calculation of the energy required for the synthesis of trehalose mycolates *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 on a mixture of energy-excessive hexadecane and energy-deficient glycerol showed that the optimum molar ratio of substrates for BS synthesis found to be 1:7 [3, 4]. Under such cultivation conditions the BS synthesis was 2.6–3.5-fold higher compared to synthesis on hexadecane or glycerol. In present work it was found that amount of BS synthesized by *N. vaccinii* IMV B-7405 at molar ratio of glucose and glycerol concentration 1:2.5–1:4 as close to the theoretically calculated 1:2.5 was 2.0–2.3-fold higher than those obtained on monosubstartes.

Thus, the obtained results confirmed own previous results [1–4] that the use of the mixture of energetically unequal growth substrates is rational for the increase of the synthesis of secondary metabolites and indicated that the high efficiency of these mixed substrates can be achieved by both the correct choice of substrates and the correct determination of the molar ratio of their concentrations.

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ІНТЕНСИФІКАЦІЯ СИНТЕЗУ ПОВЕРХНЕВО-АКТИВНИХ РЕЧОВИН ЗА КУЛЬТИВУВАННЯ *Nocardia vaccinii* IMB B-7405 НА СУМІШІ ГЛЮКОЗИ ТА ГЛІЦЕРОЛУ

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Метою роботи було визначення оптимального молярного співвідношення глюкози та гліцеролу для інтенсифікації синтезу поверхнево-активних речовин *N. vaccinii* IMB B-7405 на суміші енергетично нерівноцінних субстратів (глюкози і гліцеролу).

На основі теоретичних розрахунків енергетичних потреб синтезу поверхнево-активних трегалозомономіколатів та біомаси на енергетично дефіцитному субстраті (гліцерол) встановлено концентрацію енергетично надлишкової глюкози, що дає змогу підвищити ефективність конверсії вуглецю використовуваних субстратів у поверхнево-активні речовини. За молярного співвідношення концентрацій глюкози та гліцеролу 1,0:2,5 кількість синтезованих позаклітинних поверхнево-активних речовин була у 2,0–2,3 раза більша, ніж на відповідних моносубстратах.

Підвищення концентрації поверхнево-активних речовин на змішаному субстраті зумовлено збільшенням синтезу поверхнево-активних трегалозоміколатів, про що свідчило зростання в 1,2–5,7 раза активності ензимів їх біосинтезу (трегалозофосфатсинтази, фосфоенолпіруваткарбоксикінази, фосфоенолпіруватсинтетази) порівняно з культивуванням на моносубстратах глюкозі та гліцеролі. Отримані результати свідчать про доцільність використання суміші енергетично нерівноцінних ростових субстратів для підвищення синтезу вторинних метаболітів, а також про те, що високої ефективності таких змішаних субстратів може бути досягнено як за правильного вибору субстратів, так і коректного визначення молярного співвідношення їх концентрацій.

Ключові слова: *Nocardia vaccinii* IMB B-7405, поверхнево-активні речовини, енергетично нерівноцінні субстрати.

ИНТЕНСИФИКАЦИЯ СИНТЕЗА ПОВЕРХНОСТНО-АКТИВНЫХ ВЕЩЕСТВ ПРИ КУЛЬТИВИРОВАНИИ *Nocardia vaccinii* IMB B-7405 НА СМЕСИ ГЛЮКОЗЫ И ГЛИЦЕРОЛА

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Целью работы было определение оптимального молярного соотношения глюкозы и глицерола для интенсификации синтеза поверхностно-активных веществ *N. vaccinii* IMB B-7405 на смеси энергетически неравноценных субстратов (глюкозы и глицерола).

На основе теоретических расчетов энергетических потребностей синтеза поверхностно-активных трегалозомономиколатов и биомассы на энергетически дефицитном субстрате (глицерол) установлена концентрация энергетически избыточной глюкозы, позволяет повысить эффективность конверсии углерода используемых субстратов в поверхностно-активные вещества. При молярном соотношении концентраций глюкозы и глицерола 1,0:2,5 количество синтезируемых внеклеточных поверхностно-активных веществ было в 2,0–2,3 раза больше, чем на соответствующих моносубстратах.

Повышение концентрации поверхностно-активных веществ на смешанном субстрате обусловлено увеличением синтеза поверхностно-активных трегалозомиколатов, о чем свидетельствовало возрастание в 1,2–5,7 раза активности энзимов их биосинтеза (трегалозофосфатсинтазы, фосфоенолпируваткарбоксикиназы, фосфоенолпируватсинтетазы) по сравнению с культивированием на моносубстратах глюкозе и глицероле. Полученные результаты свидетельствуют о целесообразности использования смеси энергетически неравноценных ростовых субстратов для повышения синтеза вторичных метаболитов, а также о том, что высокая эффективность таких смешанных субстратов может быть достигнута как при правильном выборе субстратов, так и корректном определении молярного соотношения их концентраций.

Ключевые слова: *Nocardia vaccinii* IMB B-7405, поверхностно-активные вещества, энергетически неравноценные субстраты.