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**INTENSIFICATION OF SURFACE-ACTIVE SUBSTANCES SYNTHESIS OF
RHODOCOCCUS ERYTHROPOLIS IMV Ac-5017 AND *ACINETOBACTER*
CALCOACETICUS IMV B-7241 ON GLYCEROL**

In recent years the interest to glycerol as a substrate for microbial synthesis is growing due to the expansion of biodiesel production in the world that caused the move of this alcohol from the category of "target" technology products into the category of wastes. Search for new, more advanced methods of utilizing this waste will not only help to get rid of the problems of its accumulation, but also increase the efficiency of biodiesel production. One of the possible ways of utilization of glycerol is to use it as a substrate in biotechnological processes for receiving of practically valuable products, including for surface-active substances (SAS) [1, p.32; 2, p. 213]. SAS of microbial origin are the subject of intensive theoretical and applied researches due to the possibility of their application in various industries and environmental biotechnology. The advantages of microbial surfactants in comparison with chemical analogues are biodegradability, reduced toxicity and stable activity in a wide range of pH, salinity and temperature [3, p. 262; 4, p. 2; 5, p. 427].

In previous studies it was shown the possibility of use of glycerol as a source of carbon and energy for the SAS synthesis by *Rhodococcus erythropolis* IMV Ac-

5017 and *Acinetobacter calcoaceticus* IMV B-7241 but the level of the SAS formed remained lower than on traditional substrates (hexadecane, ethanol) [6, p.472; 7, p. 274]. Thus, the aim of this work was to investigate the possibility of intensifying the SAS synthesis of *R. erythropolis* IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 during the growth on glycerol.

The efficiency of technology of microbial synthesis of practically valuable metabolites, including SAS, could be increased in different ways such as optimization of cultivation conditions of producers, adding of the exogenous precursors of biosynthesis, identifying of possible 'bottlenecks' sites of metabolism and development the ways of their elimination, using the mixture of growth and nongrowth substrates etc [8, p. 5].

Chemically SAS of *R. erythropolis* IMV Ac-5017 are the complex glyco-, phospho- and neutral lipids, and SAS of *A. calcoaceticus* IMV B-7241 - glico-, amino- and neutral lipids. Glicolipids of both strains are presented by trehalose mycolates [6, p. 474; 7, p. 275]. Based on the chemical nature of formed SAS, we suggested the possibility of increasing of their synthesis by adding into the medium citrate – a lipid synthesis regulator and fumarate - C₄-dicarboxylic acid, precursor of gluconeogenesis [8, p. 103] (table 1).

As seen from the data in the table, the simultaneous introduction of citrate and fumarate in concentrations 0,1 and 0,2% (m/m) respectively in the early stationary phase of growth in medium with glycerol was accompanied by increase of SAS synthesis indexes of *R. erythropolis* IMV Ac-5017 up to 32%, adding of precursors of biosynthesis at concentrations of 0,01% in medium of *A. calcoaceticus* IMV B-7241 resulted in the increase in the amount of extracellular SAS almost 2 fold as compared to the cultivation of strains on a medium without organic acids.

For *R. erythropolis* IMV Ac-5017 change of sodium citrate at equimolar carbon

Table 1

**Effect of presence of exogenous precursors of the SAS synthesis of
R. erythropolis IMV Ac-5017 and *A. calcoaceticus* IMV B-7241 on glycerol**

| Strain | Organic acids concentration, % | SAS (g/L), % to control |
|---------------------------------------|---|-------------------------|
| <i>A. calcoaceticus</i> IMV B-7241 | Fumarate, 0,01 + sodium citrate, 0,01 | 190,9±9,55 |
| <i>R. erythropolis</i> IMV Ac-5017 | Fumarate, 0,2 + sodium citrate, 0,1 | 132,2±6,61 |
| | Fumarate, 0,2 + citric acid, 0,08 | 146,5±7,33 |
| | Fumarate, 0,2 + pH adjust (to 8,0) by citric acid | 176,7±8,84 |

Remarks. The control (100%) – rates of SAS synthesis on medium with glycerol without organic acids. Sodium citrate and citric acid were used at equimolar carbon concentrations. Organic acids were added in the early stationary phase of growth.

concentrations of citric acid made possible the increase in amount of SAS synthesized by 10-15% as compared to sodium citrate. Even with periodic adjust pH to 8,0 with citric acid after adding of 0,2% of fumarate observed the increase of SAS concentration by 30%, 45% and 77% compared to the process without pH regulation, simultaneous adding of fumarate and sodium citrate and cultivation of bacteria on the medium without organic acids respectively.

One of the ways of improving the technology of microbial synthesis is the cultivation of producers on a mixture of growth substrates that allows to avoid unproductive wastes of carbon and energy that occur in the time of use of monosubstrates and increase the efficiency of transformation of carbon of substrates in secondary metabolites [8, p.145].

The established broad substrate specificity of N,N-dimethylnitrosamine (NDMA)-dependent alcohol dehydrogenases of *A.calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 [9, p.23] allowed us to suggest the possibility of improving of SAS synthesis, using a mixture of energy unequivalent growth substrates, particularly energy energy excess hexadecane and energy deficient glycerol.

Because the parameters of growth and synthesis of target product on mixed substrates depend on the inoculum quality [8, p. 148], firstly we investigated the influence of the nature of the carbon source in the medium for unoculum preparation. The maximum amount of SAS synthesized for both strains was obtained after using the inoculums, raised on monosubstrate hexadecane. Under such conditions of cultivation of *A. calcoaceticus* IMV B-7241 the rate of SAS surfactant concentration was 1,5 and 3,6 fold, and for *R. erythropolis* IMV Ac-5017 – 1,3 and 1,6 fold higher compared to the rates gained on monosubstrates hexadecane and glycerol respectively.

During the cultivation of microorganisms on mixed substrates to secure maximum conversion of carbon into the target product it is necessary to determine the optimal for its synthesis molar ratio of monosubstrates in the mixture, which in turn requires theoretical calculations of the energy needs for SAS and biomass synthesis. [8, p.145]. For making such calculations it is need to know ways of metabolism of corresponding monosubstrates. As a result of enzymatic analysis it was found that glycerol metabolism in *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 is carried out in two ways: by the glycerol-3-phosphate pathway - involving glycerolkinase and glycerol-3-phosphate dehydrogenase and by dihydroxyacetone pathway, where work NDMA-dependent alcohol dehydrogenase and pyrroloquinoline quinone (PQQ) – dependent glyceroldehydrogenase, performing oxidation of glycerol, and dihydroxyacetone kinase. Theoretically calculated for *A. calcoaceticus*

IMV B-7241 optimal molar ratio in the mixture of hexadecane and glycerol is 1:6,9, and for *R. erythropolis* IMV Ac-5017 – 1:7,7. Theoretically calculated ratios were confirmed experimentally: the maximum levels of SAS synthesis of *R. erythropolis* IMV Ac-5017 were in 1,5-2,2 fold and for *A. calcoaceticus* IMV B-7241 – in 1,2-4 fold higher than those gained on monosubstrates.

Thus, the proposed approaches such as use of glycerol as a cheap substrate, adding of exogenous precursors of biosynthesis, using a mixture of growth substrates may be the basis for the development of economically beneficial industrial technologies of producing of SAS of *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 and help to solve the problem of disposal of waste of biodiesel production.

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