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INFLUENCE OF MEDIUM pH ON SURFACTANT SYNTHESIS BY *ACINETOBACTER CALCOACETICUS* IMV B-7241 ON ETHANOL

Microbial surface – active substances (surfactant) can reduce surface and interfacial tension, absorb heavy metals, increase the efficiency of decomposition of oil pollution of ecosystems, show antimicrobial and antiadhesive effect on pathogens. Due to such multifunctional properties they can be widely applied in various industries (oil, chemical, pharmaceutical, food processing), agriculture, medicine and remediation of the environment from xenobiotics. In addition, surfactants of microbial origin are non-toxic and biodegradable [1].

In previous studies it was shown that *Acinetobacter calcoaceticus* IMV B-7241, isolated from oil-contaminated soil samples, synthesized surfactants either on hydrophilic (ethanol, glucose) or on hydrophobic (*n*-hexadecane) substrates [2]. pH of the medium was declined by the end of the cultivation up to 4.3–4.8 on the condition of IMV B-7241 strain growth on ethanol, in contrast to other sources of carbon.

According to the literature it is known that the majority of the microbial producers synthesize surfactant at pH close to neutral. Thus, the maximum indexes of emulsan synthesis by *A. calcoaceticus* BD4 on ethanol or glucose were observed at pH 6.4 [3], for emulsan of *Acinetobacter venetianus* RAG-1 – pH was 5.0–7.5 using ethanol as the main carbon and energy source [4]. While cultivating *Pseudomonas aeruginosa* UG2 both hydrophilic (glucose) and hydrophobic (corn

oil) substrates the optimal pH for synthesis of rhamnolipids was 6.25 [5], for *P. aeruginosa* ATSS 9027 – 7.4 at the bacteria growth on *n*-hexadecane [6]. The authors of the article [7] notice that the most rhamnolipids were synthesized while cultivating *P. aeruginosa* EBN-8 on various vegetable oils at neutral pH. For the yeasts of *Pseudozyma* genus the maximum indexes of mannosilerythritollipid synthesis were observed at pH 6.0 [8]. Besides, for some microorganisms the pH regulation can influence the biosynthesis of surfactant. Thus, for *Torulopsis apicola* the pH maintenance at a certain level can regulate the activity of biosynthesis enzymes of surfactant glycolipids [9]. During the cultivation of *Rhodococcus erythropolis* EK-1 on *n*-hexadecane the stabilization of pH at 7.2–7.4 was accompanied with the increase of the surfactant concentration by 1.5–1.7-fold [10].

The aim of present work was to investigate the pH influence on the synthesis and qualitative composition of *A. calcoaceticus* IMV B-7241 surfactant while the strain growth on ethanol.

The qualitative composition of *A. calcoaceticus* IMV B-7241 lipids was determined by the thin layer chromatography (TLC) on the plates DC-Alufolien Kieselgel 60 ("Merck", Germany).

At the first stage of research pH was maintained at 5–8 by periodic adding of 1 N KOH. At pH 6–7 the increase of surfactant concentrations was observed more than by 2-fold compared to the cultivation without pH regulation. The pH maintenance at 8 during the process of the strain IMV B-7241 cultivation was accompanied by the increase of metabolite synthesis with emulsifying properties, the evidence of which was the increase of the emulsification index by 10 %. The obtained results show the ability to change the direction of biosynthesis process of metabolites with surface-active and emulsifying properties of *A. calcoaceticus* IMV B-7241 by the pH regulation.

1 N NaOH was used at the second stage of research for the regulation of pH during the cultivation of the strain IMB B-7241. pH was maintained at the

optimum level (6–7) for the synthesis of surfactant *A.calcoaceticus* IMV B-7241. The replacement of the titration agent is connected with the fact that potassium and sodium cations can be both activators and inhibitors of enzymes of surfactant biosynthesis. It was determined that in the case of 1 N NaOH addition to the cultural liquid the decline in surfactant synthesis compared with KOH solution was observed. The obtained results allow to assume that Na⁺ cations can serve as inhibitors of enzymes in the surfactant biosynthesis of the strain IMB B-7241. The verification of this supposition will be the subject of our further researches.

At the next stage we determined the chemical composition of surfactant, that were synthesized at different pH (table). As seen from the data presented in the table, at pH maintained at level 8.5 by periodic titration with KOH the qualitative composition of the synthesized neutral and phospholipids and glycolipids by *A. calcoaceticus* IMV B-7241 was almost unchanged compared with the cultivation without pH regulation. In the case of using NaOH as titration agent the strain IMB B-7241 synthesized the smallest range of neutral lipids that also may indicate about the inhibition of enzymes of surfactant biosynthesis by the sodium cations.

The obtained results show that the qualitative composition of the neutral, phospholipids and glycolipids almost did not depend on the conditions of the cultivation of the *A. calcoaceticus* IMV B-7241, unlike the chemical composition of *R. erythropolis* EK-1 glycolipids, which were changed depending on the nutritive medium composition and the mass transfer rate [10]. Besides, the qualitative composition of aminolipids of IMV B-7241 strain wasn't identified yet. We consider that the increase of surfactant synthesis at pH 6.7 may be caused by the activation of surfactant synthesis of exactly aminolipids. Our future researches will be devoted to the study of this issue.

Chemical structure of surfactant synthesized at different pH rate

Given pH	Titration agent	Qualitative composition	
		neutral lipids	glyco- and phospholipids
5	KOH	3-keto-2-alkyl fatty acids, <i>n</i> -alkanes acids, mycolic acids	Trehalosediacelate, trehalosemonomycolate, diphosphatidylglycerol
	KOH	3-keto-2-alkyl fatty acids, <i>n</i> -alkanes acid palmitic acid	Trehalosediacelate, trehalosemonomycolates, phosphatidylethanolamin, diphosphatidylglycerol
6	NaOH	3-keto-2-alkyl fatty acids, <i>n</i> -alkanes acid	Trehalosemonomycolates, diphosphatidylglycerol
	KOH	3-keto-2-alkyl fatty acids, <i>n</i> -alkanes acids, mycolic acids	Trehalosediacelate, trehalosemonomycolates, diacylglycerides, diphosphatidylglycerol
7	NaOH	<i>n</i> -alkanes, mycolic acid	Trehalosemonomycolates, phosphatidylethanolamin, diphosphatidylglycerol
8	KOH	3-keto-2-alkyl fatty acids, mycolic acid, palmitic acid	Trehalosemonomycolates, phosphatidylcholine
Control (without pH regulation)	–	3-keto-2-alkyl fatty acids, mycolic acid, palmitic acid	Trehalosediacelate, trehalosemonomycolates, trehalosedimycolates, diacylglycerides, diphosphatidylglycerol

Thus, as a result of the work it was stated that at the cultivation of *A. calcoaceticus* IMV B-7241 in the medium with ethanol the pH maintenance at 6–7 by the addition of KOH was accompanied by the 2-fold increase of the concentration of synthesized metabolites with surface-active properties. Under such conditions of the cultivation the qualitative composition of the synthesized neutral, phospho- and glycolipids was almost unchanged compared with the cultivation without pH regulation.

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