

## INFLUENCE OF MEDIUM PH ON SURFACTANT SYNTHESIS BY *ACINETOBACTER CALCOACETICUS* IMV B-7241 ON HYDROPHILIC SUBSTRATE ETHANOL

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**Abstract.** The synthesis of exocellular metabolites with surface-active and emulsifying properties was investigated at the maintaining pH at 5.0–8.0 rate during the cultivation of *Acinetobacter calcoaceticus* IMV B-7241 on the medium with ethanol (2% by volume). It was stated that the maintaining pH at 7.0 by the KOH solution was accompanied by the increase of surfactant synthesis in 1.8 times comparing with the process without pH regulation. The replacement of KOH on NaOH for the maintaining pH at an optimal level led to the decrease of surfactant concentration in 1.2–1.5 times that was caused by the inhibitory effect of sodium cations on the activity of enzymes of surface-active amino- and glycolipids biosynthesis.

**Key words:** *Acinetobacter calcoaceticus* IMV B-7241, surfactant, pH regulation

**Introduction.** Microbial surface – active substances (surfactants) 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 mannosilerythritolipid 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.

**Materials and methods.** Bacteria were cultivated on the liquid medium with the following composition (g/L): NaCl – 1.0, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O – 0.6, (NH<sub>2</sub>)<sub>2</sub>CO – 0.35, KH<sub>2</sub>PO<sub>4</sub> – 0.14, MgSO<sub>4</sub>·7H<sub>2</sub>O – 0.1, distilled water – up to 1 L. Yeast autolysate 0.5 % (by volume) and

solution of microelements 0.1 % (by volume) were also added to the medium [3]. Ethanol concentration of 2 % (by volume) was used as a source of carbon and energy. Inoculum was cultivated in the media with the composition mentioned above with 0.5 % ethanol and was taken from the mid-exponential phase of growth (48 h). The quantity of taken inoculum was 5 % from the total sown medium ( $10^4$ – $10^5$  cells/ml).

1 N HCl and 1 N KOH were used to bring the value of pH medium up to 5.0, 6.0, 7.0 and 8.0. In the process of cultivation, starting from 20–24 h the culture fluid pH was maintained at a neutral level by adding of 1 N KOH (NaOH).

The cultivation was carried out in 750 ml flasks with 100 ml of medium on a shaker (320 rev/min) at 30 °C for 24–120 h.

The indexes of surfactant synthesis and bacteria growth-concentration of biomass, the surface tension ( $\sigma_s$ ) of cell-free cultural liquid, the conditional surfactant concentration (CSC\*, dimensionless), the emulsification index of cultural liquid ( $E_{24}$ , %) – were determined as it was described in our previous works [3, 6, 9].

The amount of the exocellular surfactant (g/L) was determined by the gravimetric method after the extraction from the supernatant of cultural liquid with mixture of methanol and chloroform [3]. The surfactant synthesizing ability was determined as the ratio of the concentration of the synthesized surfactant (g/L) to the concentration of the biomass and expressed in g of surfactant/g of biomass.

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) as previously described [3].

**Results and discussion.** Depending on the initial pH rate its value decreased to 4.7–5.2 on the second day. Further maintenance of pH at 5.0–8.0 rate was conducted by adding solutions of KOH and NaOH to the culture liquid. It is shown that the concentration of the synthesized biosurfactant and surfactant-synthesizing capacity increased to 3.0–3.1 g/L and 2.4–2.6 g surfactant/g biomass, respectively, while maintaining the pH at 6.0–7.0 rate by adding solution of KOH to the medium. These results were in 1.6–1.8 times higher than without pH regulation. At neutralization of the cultural liquid by NaOH the indicators of biosurfactant synthesis were 1.2–1.3 times lower comparing with KOH using.

Further experiments showed that sodium cations were inhibitors of the enzyme activity of the surface-active glyco- (PEP-synthetase) and aminolipids (NADP<sup>+</sup>-dependent glutamate dehydrogenase) biosynthesis by *A. calcoaceticus* IMV B-7241. Thus, the activity of PEP-synthetase and glutamate dehydrogenase decreased by 1.8 and 5 fold respectively in the presence of 50 mM of Na<sup>+</sup> in the reaction mixture. It was interesting to note that 2-fold reduction of PEP- carboxylase (the enzyme of the anaplerotic reaction filling the pool of C<sub>4</sub>-dicarboxylic acids) activity was observed in the presence of sodium cations in the medium with ethanol for strain IMV B-7241 cultivation.

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.

**Table**  
**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

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.

**Conclusion.** 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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