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T.P. Pirog, A.P. Sofilkanych, A.D. Konon, Kh.A. Pokora

National University of Food Technologies

INTENSIFICATION OF SURFACTANTS' SYNTHESIS ON FRIED OIL AND GLYCEROL CONTAINING MEDIUM

The possibility of waste use for biosurfactant production was determined. Presence of fumarate and citrate intensified biosurfactant synthesis by 2.5-fold. It was shown that C_4-dicarboxylic acids enhanced activity of surfactant biosynthesis enzymes. Cultivation on mixture of substrates resulted in increased surfactants quantity.

Searching for the ways to process waste has become very topical today. Biotechnology is one of the most environmentally attractive methods, which has the ability to solve the problem of waste utilization and to produce the valuable microbial products, for example biosurfactants. Due to their physico-chemical properties, the use of microbial surfactants have been proposed for various industrial applications, as additives in foods, cosmetics and detergent formulations [1, p. 427, 4, p. 478]. In the food industry, the most useful property is the ability to form stable emulsions, which improves the texture and creaminess of dairy products. Biosurfactants are also used to retard staling, solubilise flavour oils and improve organoleptic properties in bakery and ice cream formulations and as fat stabilisers during cooking of fats.

Despite the outstanding properties and advantages of biosurfactants their price is still too high compared to the synthetic ones. One of the ways to reduce the cost of biosynthesis is with the use of low-cost growth substrates, such as wastes of other industries [2, p. 660].

The oil-oxidizing bacteria were isolated from the oil-polluted samples of soil and identified as *Rhodococcus erythropolis* IMV Ac-5017, *Acinetobacter calcoaceticus* IMV B-7241 and *Nocardia vacinii* K-8. The ability of these strains to synthesize the metabolites with surface-active and emulsifying properties was determined during the cultivation in medium with hydrophobic (*n*-hexadecane, liquid paraffin) and hydrophilic (glucose, ethanol) substrates [3, p. 273].

The aim of present work – development of approaches for increasing biosurfactant synthesis by *R. erthropolis* IMV Ac-5017, *A. calcoaceticus* IMV B-7241 and *N. vacinii* K-8 on wastes.

Our experiments showed the possibility of cultivation of *R. erythropolis* IMV Ac-5017 on a medium with 1–2 vol. % of fried sunflower oil (after repeated use in public catering) and oil-containing wastes (soapstock), and indexes of biosurfactant synthesis were 1.2–2.6 times higher than in a medium containing *n*-hexadecane (traditional substrate). It was found that the addition of glucose (0.1 %) at the beginning of cultivation into the medium containing 2 vol. % of fried oil, accompanied by an increment of surfactant concentrations of 4-fold compared with the cultivation of *R. erythropolis* IMV Ac-5017 on a glucose-free medium.

The study of growth and the biosurfactant synthesis by *A. calcoaceticus* IMV B-7241, *R. erythropolis* IMV Ac-5017 and *N. vaccinii* K-8 in the medium containing glycerol (0.5–1.0 vol. %) showed that the strains were able to assimilate this substrate and synthesize metabolites with surface active and emulsifying properties. But conditional surfactant concentration (CSC*) was lower than in the case of *n*-hexadecane or ethanol as substrates. Due to the fact that the lowest rates of synthesis of surfactant have been observed in *N. vaccinii* K-8 (CSC* = 1.1), the aim of further experiments was to optimize the nutrient medium and cultivating conditions for the strain K-8. With single-factor experiments and mathematical methods of planning, the composition of nutrient medium for *N. vaccinii* K-8 was optimized (0.5 g/L of $NaNO_3$, 0.3 g/L of yeast autolysate and 1.5 vol. % of glycerol). It was established that after optimizing the composition of the medium, the amount of surfactant of *N. vaccinii* K-8 increased by 4 fold.

It was shown that the increase in the synthesis of *R. erythropolis* IMV Ac-5017 surfactant in the medium with ethanol or *n*-hexadecane observed in the case of simultaneous introduction of citrate and fumarate in concentrations of 0.1 and 0.2 % respectively in the early stationary phase of growth. Experiments have shown that the introduction of citrate and fumarate while cultivating *N. vaccinii* K-8 in the medium with glycerol was also accompanied by an increase of surfactant synthesis. The

highest indexes – increase of the conditional surfactant concentration of 35–40 % and emulsification index of 20 % compared with the cultivation of strain K-8 in a medium without organic acids – were achieved by the simultaneous addition of fumarate and citrate (0.1–0.2 % of each). Similar studies conducted with strain IMV B-7241, showed that the simultaneous introduction of fumarate and citrate (0.01–0.02 %) made the concentration of exocellular surfactant increase twofold compared to bacteria growing in the medium without organic acids. Importantly, under these conditions we did not observe an increase in biomass. An increase in surfactant concentration of IMV B-7241 strain was the result of the simultaneous functioning of two anaplerotic pathways, also resulting in a 3–5 fold increase in activity of biosynthesis enzymes.

Further studies were devoted to the surfactant synthesis by *A. calcoaceticus* IMV B-7241 and *R. erythropolis* IMV Ac-5017 on a mixture of *n*-hexadecane and glycerol. Cultivating on a mixture of glycerol and *n*-hexadecane (0.5–1.0 vol. %) led to a 1.5–3 fold increasing surfactant synthesis.

We showed that biosurfactant preparations of IMV B-7241 (0.15–0.22 mg/mL) and IMV Ac-5017 (0.61–2.1 mg/mL) strains were effective against *Escherichia coli* IEM-1 (67 % of cell loss), and vegetative (45–100 % of cell loss) and spore (75 % of spore loss) cells of *Bacillus subtilis* BT-2.

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