

**USAGE OF MIXED WASTE OIL IN THE MIXTURE WITH ACETATE
FOR MICROBIAL EXOPOLYSACCHARIDE ETHAPOLAN
BIOSYNTHESIS**

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One of the important indicators of the effectiveness of technologies for obtaining practically valuable microbial metabolites that directly affects the possibility of their industrial implementation is the final cost of the target product [1].

In our previous work [2] the possibility of synthesis of microbial exopolysaccharide (EPS) ethapolan (produced by *Acinetobacter* sp. IMV B-7005) on mixture of sodium acetate and sunflower oil has been shown. The disadvantage of this technology was the need to use expensive refined sunflower oil in the mixed substrate. It should be noted that substrate costs can be up to 50% of the final cost of the target product [1].

In connection with the above, the aim of this work was to study the possibility of replacing the refined oil in the mixture with a waste one during polysaccharide ethapolan synthesis on the mixture of sodium acetate and sunflower oil.

For this purpose we chose mixed sunflower oil after frying various foods (meat, potatoes, onions, and cheese), which is the most common type of waste oil, because different overcooked oil are usually mixed before being sent for utilization.

Experiments have shown that after the replacement of refined oil in the mixture with acetate on mixed oil after frying various foods the amount of final product reached 16.36 g/l, which was only slightly lower than that obtained using a refined substrate (17.27 g/l) (Table). Meanwhile, EPS-synthesizing capacity increased up to 7.34 g EPS/g biomass, that was 2.2-3.7 times higher than the indicators obtained on the mixture of mixed waste oil after frying various products and other C₂-C₆-substrates (ethanol and molasses) [3].

It should be noted that during refined oil replacement on mixed waste oil after frying various foods additional influence of the concentration of Mg²⁺ cations in the culture medium was studied.

These cations are one of the activators of acetyl-CoA synthetase – key enzyme C₂-metabolism in *Acinetobacter* sp. IMV B-7005. Low activity of acetyl-CoA synthetase in the IMV B-7005 strain is one of the reasons of acetate metabolism limitation.

It was found that when the content of Mg²⁺ increased to 5 mM a moderate increase in the ethapolan synthesis on the mixture of acetate (3.0%) and mixed waste oil after frying various foods (1.5%) was observed. Under such cultivation conditions concentration of EPS was 18.21 g/l, EPS-synthesizing capacity was 7.82 g EPS/g biomass.

At the same time, the additional introduction of Mg²⁺ in the medium with acetate and refined oil led to a slight decrease in the synthesis of EPS. We suggest that this may be due to the different effects of these cations on the enzymatic systems

responsible for the catabolism of fatty acids and other related components that are part of sunflower oil or formed during frying.

For instance, Li et al. [4] found that in lactic acid bacteria *Lactococcus lactis* ssp. *lactis* concentration of Mg^{2+} cations, at which the maximum enzymatic activity is observed, differs for various enzymes of the β -oxidation system.

Table – Biosynthesis of ethapolan on the mixture of sunflower oil (1.5%) of different quality and sodium acetate (3.0%)

Concentration of Mg^{2+} in the medium, mM	Type of oil in the mixture with acetate	pH _{end}	EPS, g/l	EPS-synthesizing ability, g EPS/ g biomass
1.6	Refined (control)	7.9	17.27±0.86	6.47±0.32
	Mixed waste oil after frying various foods	7.7	16.36±0.82	7.34±0.37
5.0	Refined (control)	7.8	16.95±0.85	6.21±0.31
	Mixed waste oil after frying various foods	7.7	18.21±0.91	7.82±0.39

Notes: The inoculum was grown on the refined or mixed waste oil after frying various foods. Fractional application of substrates was carried out three times every 24 hours: two portions of 1.0% acetate and 0.5% of oil, and one portion of 0.35% acetic acid and 0.5% of oil.

Thus, as a result of this work it was found that increase of the Mg^{2+} cations (acetyl-CoA-synthetase activator) concentration to 5.0 mM allowed not only to replace refined oil in the mixture with acetate on a mixed one, but also to increase the maximum synthesis of the final product.

Obtained data are the basis for the development of a universal technology for the ethapolan synthesis on mixed substrates, independent of the supplier and the quality of waste sunflower oil.

References

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