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PREPARATION AND APPLICATION OF IMMOBILIZED CELLS FROM THE FUNGUS *Mucor miehei*

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A laboratory bioreactor was developed for functionalizing immobilized cells of the mycelial fungus Mucor miehei. *The ability to use the cells to purify wastewaters from the oil-refining industry is demonstrated. With proper operation of the reactor, immobilized cells (1 g) can purify >100 l of wastewater containing 0.10-0.15% of lipid-like substances.*

Key words: *Mucor miehei*, immobilized cells, polymeric support, bioreactor, wastewaters, continuous processes.

Wastewaters from the oil-refining industry are purified of lipophilic contaminants by various means. Fats and fatty wastes are good substrates for the growth and development of many microorganisms. They are also indicators of the synthesis of lipolytic enzymes by microorganisms [1, 2]. The use of microorganisms, including cultures of *Mucor* species of fungi in the free state, for biological purification of fat-containing wastewaters has been reported [3, 4].

Immobilized cells bonded to supports have several advantages over free cells for use as biocatalysts. These include the ability to retain the enzyme activity over long periods, the increased stability to changes in the environment, and the capability to be stored in the bioreactor [5].

In particular, immobilized cells can be kept in an active state by periodic incubation in nutrient medium, which continuously renews the population on the sorbent [6-8]. Also, microscopic monitoring of the morphological and physiological changes during the biotransformation enables the kinetics of this process to be regulated [9].

Our goal was to prepare immobilized cells of the mycelial fungus *Mucor miehei*, strain UzLT-3, which actively produces extracellular lipases, in order to use them for microbiological purification of wastewaters from oil-refining plants.

The sorbent for immobilization of *M. miehei* cells was polyvinyl alcohol. The biological destruction of the oil was followed in a laboratory bioreactor. The fats are destroyed during passage of the fatty wastes through a column with biocatalyst that is constantly irrigated with mineral medium. The support surface drives the oil through a thin layer of adsorbent with subsequent utilization of the substrate. The constant irrigation of the biocatalyst with mineral medium facilitates the stable functioning of the adsorbent and the fungus itself. It should be noted that hydrolysis of the oil in a buffer without mineral replenishment also proceeds at a sufficiently high level for 4-5 days with full retention of the fungus morphology. However, subsequent use of the biocatalyst without mineral replenishment decreases the quantity of intact fungus cells. The process completely stops after seven days.

A different picture is observed for immobilized cells, the activity of which depends on the conditions under which the biocatalyst is used. In this instance, the population within the gel can be renewed by using mineral feed over three days at 40-42°C. This causes the immobilized cells to grow and form mycelium on the adsorbent surface. The whole process is related to formation of autolysis products by the immobilized fungus cells. This does not adversely affect the course of the reaction.

The kinetics of accumulation of fatty acids by free cells during repeated growth in a buffer are plotted in Fig. 1 (curve 1). The accumulation of free fatty acids persists at the maximum for five days, after which the activity declines irreversibly. By the eighth day the yield of fatty acids is only 3-5% of the maximum. On the other hand, immobilized cells in phosphate buffer (curve 2) function stably for seven days. Then the lipase activity decreases. By the eleventh day it reaches a minimum. However, the activity again increases after irrigation of the biocatalyst with mineral medium although the initial level is not reached. Immobilized cells of *M. miehei* remain functionally active for 30 and more days with constant mineral replenishment

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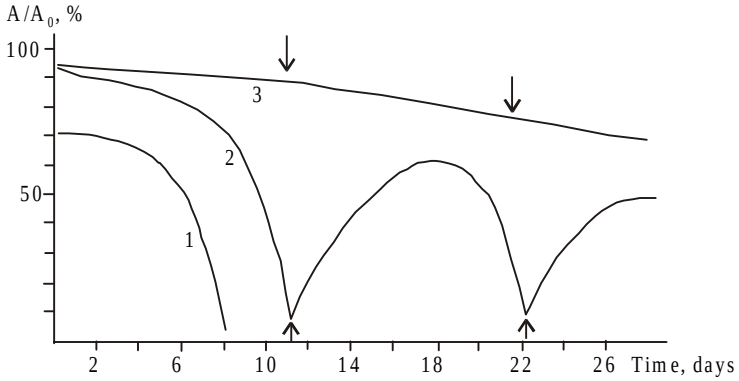

Fig. 1. Formation kinetics of free fatty acids by native and immobilized cells of the fungus *Mucor miehei*: native cells (1), immobilized with phosphate buffer (2), immobilized with mineral irrigation (3). The arrows indicate the times of mineral injection.

(curve 3). The results showed that immobilized fungus cells (1 g of biomass) can purify up to 110-120 l of wastewaters containing 0.1-0.15% of lipid-like substances.

Thus, the ability to use immobilized cells of the fungus *M. miehei* to purify wastewaters was demonstrated.

EXPERIMENTAL

A culture of *Mucor miehei* fungus cells from the Institute of Microbiology, Academy of Sciences of the Republic of Uzbekistan, strain UzLT-3, a thermophilic producer of extracellular lipases, was used [10]. The mineral feed was nutrient medium of the following composition (g/l): malt extract, 7.0; $(NH_4)_2SO_4$, 0.3; $CaCO_3$, 0.1; and cottonseed oil, 0.7. The culture was grown in 750-ml Ehrlenmeyer flasks on an orbital shaker (180 rpm) for four days at 40-42°C.

The cells were immobilized on polyvinyl alcohol by the procedure developed previously by us [8]. The quantity of fatty acids, hydrolysis products, was determined by titration [11].

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