Bioepoxidation of Propylene by Non-Growing Cells of *Rhodococcus* sp.

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Abstract

The biotechnological potential of *Rhodococcus* sp. bacteria for direct selective bioepoxidation of propylene to propylene oxide has been investigated. The kinetics of accumulation of propylene oxide in suspensions of the non-growing bacterial cells has been studied. The effect of propylene oxide produced on biocatalytic activity of *Rhodococcus* cells has been investigated. The study of bacteria adsorption on various carbon-containing inorganic supports has been started to develop efficient adsorbents for *Rhodococcus* immobilization.

INTRODUCTION

Due to the progress in biotechnology and applied industrial microbiology, the biocatalytic processes appear to be quite competitive with traditional chemistry. Such processes suggest alternative approaches for solving the chemical problems of selective direct oxidation of hydrocarbons because of high selectivity (close to 100 %) and stereospecificity of the biocatalysis. Also the biotech processes are ecologically friendly and safe, and their waste is not toxic. If to compare biocatalysis with chemical catalysis for direct selective oxidation of low alkanes and alkenes, one can see that the basic parameter of biocatalysts (activity and yield of the desired product) are comparable to those of the best heterogeneous catalysts [1].

Direct selective oxidation of light gaseous alkanes and alkenes into valuable oxy-products (alcohols, methylketons, epoxides) is the key process of chemical processing of hydrocarbon feedstock as gas and oil. A number of large chemicals companies perform the purposeful and rather expensive investigations of the alternative biotech processes [1]. For example, NIPPON MINING Co. (Japan) has brilliantly implemented an alternative pilot-scale production of chiral epoxides of unsaturated hydrocarbons (31 epoxides were described) using a suspension of microorganisms of *Nocardia (Rhodococcus)* strains. The yield of R-isomers of 1,2-epoxides was 80 %; the optical purity was 80–90 % [1].

Recently the authors have investigated the reaction of methane hydroxylation and propylene epoxidation by the non-growing bacterial cells of methane-assimilating microorganisms *Methylococcus capsulatus* IMV 3021 at homogeneous and heterogeneous conditions [2, 3]. Also the research and development of efficient adsorbents for immobilization of various microorganisms have been carried out [3–7].

In the present study the authors investigated the biotechnological potential of propane-assimilating microorganisms of *Rhodococcus* sp. strains to run the reaction of direct selective bioepoxidation of propylene. The kinetics of generation of "biotechnological" propylene oxide at homogeneous conditions was investigated. The effect of product of bioepoxidation (propylene oxide) on monooxygenase activity of *Rhodococcus* cells was studied. The research and development of the efficient adsorbents for immobilization of the non-growing *Rhodococcus* sp. cells was initiated.

EXPERIMENTAL

The strains of *Rhodococcus* sp. 1r и *Rhodococcus* sp. 276 were obtained from the microorganisms' collection of Zabolotny Institute of Microbiology and Virusology (Kiev, Ukraine). The strains of *Rhodococcus ruber* AC 225 и *Rhodococcus ruber* AC 333 were donated by Institute of Ecology and Genetics of Microorganisms (Perm', Ural region, Russia) from Regional Specialized Collection of Alkanotrophic Microorganisms [8].

Various inorganic supports, in particular ones described in refs. [3–7] were studied for adsorption of the *Rhodococcus* sp. The adsorption was estimated by the difference between the amount of bacteria before and after adsorption and expressed in milligrams of dry cells per 1 g of support.

Monooxygenase (MO) activity of the non-growing *Rhodococcus* cells was determined as the rate of extracellular accumulation of propylene oxide in cell suspensions at the following conditions: 35 °C; 0.02 M phosphate buffer pH 7.0; intensive stirring; saturation of cells suspensions for 5–7 min by gaseous mixture of 20 % vol. propylene, 20 % vol. oxygen, the balance being helium. The gas-to-liquid volume ratio was 10 : 1, the concentration of the dissolved propylene and oxygen maintained approximately constant during the experiment run (1–4 h). Periodically (every 10–15 min) a 10 μl sample of the liquid-phase reaction medium was immediately injected to a gas chromatograph supplied with a flame ionization detector. The 3-m column was packed with Porapak Q (80/100), carrier gas helium was passed through column with flow rate 3.6 l/h. The temperature for oven with the column was set at 150 and 250 °C for injection block. MO activity of the non-growing *Rhodococcus* cells was expressed in nanomoles of accumulated propylene oxide per 1 min per 1 mg of dry cells. The experimental error did not exceed 10–15 %.

RESULTS AND DISCUSSION

Biocatalytic activity of non-growing Rhodococcus cells (homogeneous conditions)

A comparison of the biotechnological potential of various stains of *Rhodococcus* sp. to perform the propylene bioepoxidation demonstrated that *Rhodococcus rube*r AC 333 and AC 225 exhibited the highest biocatalytic activity (Table 1). Also a comparative analysis showed

TABLE 1

Biocatalytic monooxygenase (MO) activity of *Rhodococcus* sp. and relative microorganisms *Nocardia* sp. in the reaction of propylene bioepoxidation

Strains of microorganism	MO initial activity, nmol propylene oxide/min/mg of dry cells	Ref.
Rhodococcus sp. 1r	6.4*	This work
Rhodococcus sp. 276	0.4*	»
Rhodococcus ruber AC 333	9.8*	»
Rhodococcus ruber AC 225	9.8*	»
Nocardia sp. 55	2.9	9
Nocardia sp. 57	6.3	9
Nocardia sp. 64	6.3	9
Rhodococcus erythropolis BPSd1	6.6	10
Rhodococcus rhodochrous RNKb1	8.1	10
Rhodococcus erythropolis 3/89	10.4	11
Rhodococcus erythropolis	2.4	12
Nocardia corallina B-276	0.5	13
*Nocardia corallina***	11.7	14

*Activity was observed in cells suspensions at bacteria concentration of ~1 mg of dry cells/ml.

**Activity of cell-free extracts (not of non-growing whole cells) was expressed in nanomoles propylene oxide / min/mg of protein, when NADH was added into reaction medium.

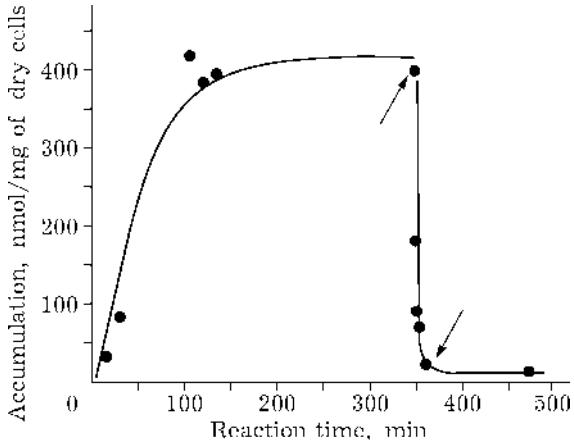

Fig 1. Kinetics of accumulation of propylene oxide in suspension of non-growing bacterial cells of *Rhodococcus ruber* AC 333. *Upper arrow* indicated the removing of propylene oxide from reaction medium by intensive air stream; *lower arrow* indicated the feeding of propylene/oxygen substrates into suspension.

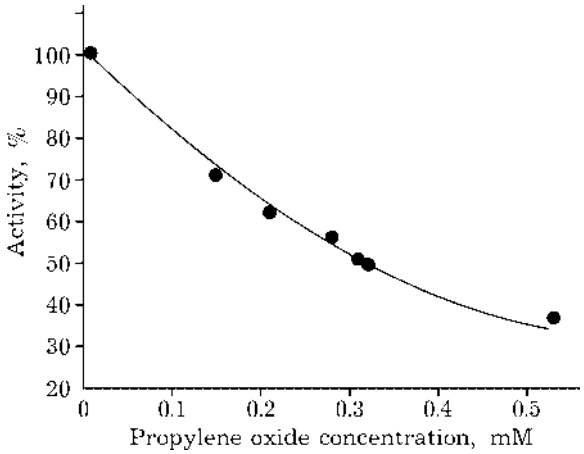

Fig. 2. Dependence of biocatalytic activity of *Rhodococcus ruber* AC 333 on propylene oxide concentration added in reaction medium at initial moment.

that the activity of the microorganisms in study was comparable to the best reference data (see Table 1).

A typical kinetic curve of extracellular propylene oxide accumulation during propylene bioepoxidation by the all strains of studied *Rhodococcus* sp. was represented in Fig. 1. As the concentration of propylene oxide in the reaction medium increased, the reaction rate decreased to zero during 100–120 min and the process of propylene bioepoxidation stopped. After removal of propylene oxide with an intensive air stream and re-feeding of the propylene/oxygen substrates into the reaction medium, the oxidative activity of *Rhodococcus* cells did not restore (see Fig. 1). One of the reasons for the kinetics represented in Fig. 1 was inhibition (reversible or irreversible) of the bacteria monooxygenase by the reaction product (propylene oxide). The effect of epoxide on MO activity was studied when propylene oxide was added into the reaction medium at initial moment before starting of bioepoxidation. As evident from Fig. 2, the presence of propylene oxide in the reaction medium resulted in decreasing the initial *Rhodococcus* activity. If to describe the decay of MO activity by exponential equation, excellent agreement between experimental and calculation results were observed: the reaction rate decreased respectively by a factor of 2 and 100 when the concentrations of propylene oxide were 0.22 and 1.4 mM. Such exp-equation described the irreversible inhibition of enzymes when the inhibitor concentration far exceeded the enzyme concentration. The inhibition rate constant for *Rhodococcus* cells was estimated to be ~10^3 min/mol, *i. e* the inhibition of MO activity by propylene oxide occurred very rapidly. Actually, the incubation of the suspended non-growing cells with propylene oxide at a low concentration (<0.4 mM) for 10 min before starting bioepoxidation resulted in complete deactivation of the cell monooxygenase. The obtained results suggested that propylene oxide was irreversible inhibitor of the *Rhodococcus* monooxygenase. Also, epoxides of ethylene, propylene and butylene inhibited irreversibly the monooxygenase activity of *Mycobacterium* bacteria (relative to *Rhodococcus* microorganisms) [15]. As a conclusion, in order to enhance the productivity of bioepoxidation process the efficient removal of propylene oxide from reaction zone was required. To solve this problem, a heterogeneous biocatalyst by immobilizing bacterial cells on the solid supports should be developed and the process of propylene bioepoxidation should be performed at the flow mode.

Adsorption of Rhodococcus on the inorganic supports

The first key stage for the obtaining a heterogeneous biocatalyst was the development an efficient adsorbent for immobilization of bac-

TABLE 2

Some parameters of inorganic supports in study and its adsorptive properties for non-growing bacterial cells *Rhodococcus ruber*

Adsorbent	Carbon content, % mass	$S_{acces.}$, m^2/g*	Strenghth for crushing, kg /cm^2	Adsorption, mg of dry cells/g**	Adsorption, mg of dry cells/m^2 of accessible surface**
		Granulated supports			
Minerals (Al_2O_3, SiO_2, TiO_2)	0	0.08	50	1.4–2.0	25
Carbonized alumina (SUMS-1), carbonized Al/Si	7–20	0.02	70	2.5	125
Sibunit	100	0.03	3.3	5.1–9.4	170
CFC bulk	100	0.07–0.12	0.3	2.4–4.2	210
		Macrostructured supports			
Foam-like ceramics	0	0.1	>70	0	0
Foam-like carbon	100	4.2	<<0.02	53	12

*Accessible surface area was estimated by comparative method described in [7].

**Adsorption of *Rhodococcus* sp. was determined at initial cells concentration in suspension of ~1 mg of dry cells/ml.

terial cells. A number of supports (Table 2) with different texture and macrostructure (granules, foams, honeycomb monoliths) as well as chemical surface properties and morphology of the carbon layer were tested as adsorbents [3–7]. Unlike mineral supports, carbon-mineral and carbon supports were found to be the most efficient adsorbents for bacterial non-growing cells (see Table 2), the catalytic filamentous carbon (CFC) exhibiting the best absorption capability. Adsorption properties of the CFC-based supports depended strongly on the surface morphology, which was determined by the carbon yield (Y) expressed in grams of synthesized filamentous carbon per 1 g of catalyst. The "looser" was the CFC surface (Y < 40); the higher adsorption ability was exhibited by a support with respect to various microorganisms [7], including *Rhodococcus* cells (Fig. 3).

Based on the comparative analysis of the experimental results (see Table 2), the requirements for the development of optimal adsorbent for immobilization of microorganisms, including *Rhodococcus,* were stated. The adsorbent should be characterized by the following properties: (i) carbon-coated surface, (ii) morphology of the carbon layer similar to that of catalytic filamentous carbon with low carbon yield, *i. e.* rough and loose, (iii) well-developed macrostructure, *e. g.* foam-like structure, (iiii) high mechanical strength comparable to that of mineral materials. Such adsorbents were prepared using macro-structured ceramics coated by a CFC layer, and the adsorption of *Rhodococcus* was carried out (Fig. 4). For the non-carbonized ceramics (cordierite), massive bulk CFC and CFC-coated cordierite the value of adsorption of *Rhodococcus ruber* was respectively 0, 2.5, and 7.1 mg of dry cells per 1 g of support. Obviously, CFC-coated macrostruc-

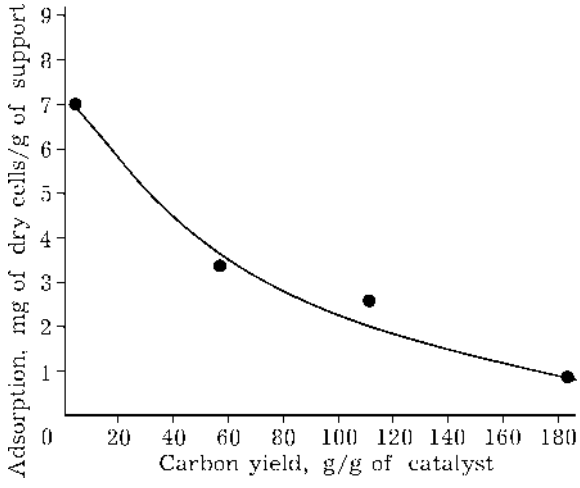

Fig. 3. Influence of the carbon yield of bulk CFC-supports on adsorption of *Rhodococcus ruber* AC 333.

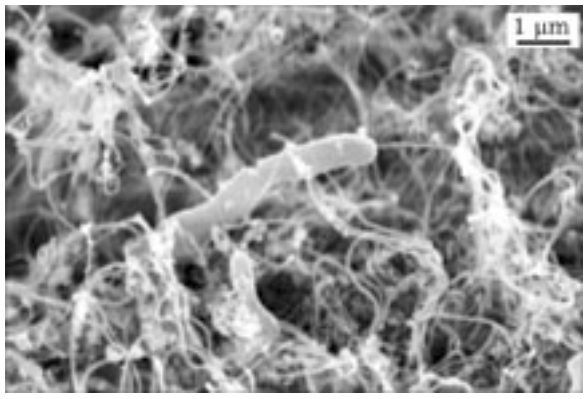

Fig. 4. Electron microscopic image of *Rhodococcus ruber* AC 333 adsorbed on macrostructured foam-like ceramics coated by catalytic filamentous carbon (CFC). The bar on micrograph corresponds to the distance in micrometers.

tured ceramics were found to be the efficient adsorbents for the non-growing cells of *Rhodococcus sp*.

CONCLUSIONS

The non-growing bacterial cells of *Rhodococcus* strains were shown to exhibit the biocatalytic activity in the process of direct selective epoxidation of propylene. Suspended *Rhodococcus ruber* AC 333 and AC 225 exhibited the comparatively high monooxygenase activity, the initial rate of propylene oxide generation running up to *ca.* 10 nmol/min/mg of dry cells. Propylene oxide was found to inhibit *Rhodococcus* monooxygenase irreversibly and completely. To enhance the productivity of the propylene bioepoxidation by the non-growing bacterial cells of *Rhodococcus* sp. the approach to develop the heterogeneous biocatalyst based on immobilized *Rhodococcus* cells was proposed. Foam-like ceramics coated by catalytic filamentous carbon layer was found to satisfy the requirements for the most efficient adsorbents for immobilization of bacterial cells. The study of immobilization of *Rhodococcus* sp. bacteria was started.

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