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SHORT COMMUNICATIONS

Immobilization of Oligotrophic Bacteria by Absorption on Porous Carriers

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The approaches to immobilization of viable microbial cells on various carriers are currently well developed with respect to various groups of microorganisms. However, the particular working environment should be taken into account when the microbes actively degrading substances that pollute the indoor atmosphere are immobilized [1]. Because of the high resistance of oligotrophic bacteria to stressful factors, they survive and retain their activity for a long time and therefore can be used as immobilized microorganisms. Oligotrophic bacteria can be reactivated and they are resistant to sterilizing agents [2]. Hence, repeated use of immobilized oligotrophs is possible, which is also of great importance. Some oligotrophic bacteria immobilized by incorporation into gels and cryogels of polyacrylamide, silica gel, and cryosilica gel were successfully used to remove gaseous compounds [3, 4]. The microbial cells are mostly incorporated into the carrier matrix. However, immobilization by adsorption of microbial cells is a rather competitive method with respect to methods based on mechanical cell incorporation and chemical binding. The advantages of the absorption method are the following: cheapness, universality, absence of stressful cell treatment, and simplicity [5].

In this study, we made an attempt to immobilize oligotrophic bacteria capable of utilizing volatile substances. The bacteria were immobilized on porous carriers, and their activity with respect to some volatile substances was determined. In addition, two distinct methods of viable cell immobilization were compared: the absorption method and the method of biomass incorporation into the carrier structure.

The subjects of this study were three cultures of oligotrophic bacteria, *Renobacter vacuolatum, Blastobacter viscosus* strain 7d, and *Methylobacterium organophilum* NP-220, from the collection of the Laboratory of Classification and Storage of Unique Microorganisms (Institute on Microbiology, Russian Academy of Sciences). The biomass of the former two microorganisms was grown on PYG medium (peptone, yeast extract, glucose, 0.1% each), whereas that of *Methylobacterium organophilum* NP-220 was grown on a mineral medium containing 1% methanol [6]. The gas phase was analyzed for hydrogen and oxygen consumption and for carbon dioxide release on a model 3700 gas chromatograph as described previously [4]. Methanol concentration was measured on a Chrom-5 gas-liquid chromatograph (Czech Republic) by the conventional technique. Viable cells of oligotrophic bacteria were immobilized on both macroporous carriers (granite crumb, claydite) and microporous carriers (polypropylene (PP) and polyvinyl formal foam (PVFF)). We also tested some microporous carriers with different degree of hydrophily, which were prepared in the laboratory of LNPO Soyuz on the basis of propylene and polyamide. The polypropylene filter sheet has the following specification: density in dry condition, 0.3–0.5 g/cm^3; pore diameter, 16–25 μm; porosity of the material, 30–80%. Polyvinyl formal foam (PVFF) was manufactured at the Research and Industrial Center of Structural Materials (Vladimir). PVFF is a novel promising carrier with a high water-retaining capacity, which is used for immobilization of microorganisms. One unit of PVFF weight retains up to 8–10 units of weight of water suspensions; PVFF density in dry condition is 0.14 g/cm^3; its pores are completely open structures; pore volume in moist condition is 93%; and pore size is 200 μm. The porous carriers were used as microblocks (granules) sized $10 \times 10 \times 10$ mm. Viable cells of oligotrophic bacteria were immobilized by the absorption method with some modifications. The carrier granules were washed in distilled water, sterilized in an autoclave, and dried thoroughly. Afterwards, the granules were placed in a thick suspension of grown bacterial biomass (2–2.5 units of optical density, depending on the culture) and incubated for 1–1.5 h to reach saturation of the carrier with cells. The granules with absorbed cells were washed three times with sterile distilled water, drenched with fresh nutrient medium, and placed on a shaker at 28°C for one day. Afterwards, to remove cells from their surface, the microblocks with absorbed cells were washed three times with sterile water and again drenched with fresh nutrient medium. This procedure was repeated five to seven times to obtain granules with well-attached cells within pores. The activity of the immobilized cells was determined from their ability to oxidize methanol and utilize hydrogen as an energy source. For this purpose,

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Utilization of various substrates and respiration activity of oligotrophic bacteria immobilized in PP and PVFF

Carrier	Substrate	Residual substrate, %*			Content in the gas phase					
					CO_2 (%)**			O_2 (%)***		
Time, h		24	48	72	24	48	72	24	48	72
Renobacter vacuolatum										
PP	Methanol	40	0	0	3.0	5.2	6.0	90	75	60
	Hydrogen	50	10	0	1.5	2.0	2.5	85	72	50
PVFF	Methanol	30	0	0	2.8	5.5	6.8	85	70	50
	Hydrogen	40	0	0	2.0	2.5	3.0	87	75	60
Blastobacter viscosus 7d										
PP	Methanol	45	0	0	3.8	7.0	8.3	90	70	55
	Hydrogen	80	30	0	1.5	2.2	3.0	92	78	60
PVFF	Methanol	35	0	0	4.2	7.5	9.2	90	65	50
	Hydrogen	72	25	0	1.8	3.0	3.7	90	75	55
Methylobacterium organophylum Np-220										
PP	Methanol	20	0	0	3.5	8.0	9.5	85	62	50
	Hydrogen	80	65	20	1.0	2.0	3.5	90	75	40
PVFF	Methanol	30	0	0	2.5	7.5	8.8	90	70	65
	Hydrogen	85	70	40	1.0	1.8	2.5	92	75	65

Note: The weight of granules with immobilized cells was 3 g.

* The results are expressed in percent of the initial amount of substrate, taken as 100%: methanol concentration was 0.5 vol %; hydrogen concentration was 6 vol %.

** Increase in CO_2 (%) relative to the initial CO_2 concentration in atmospheric air (0.03%).

*** Initial O_2 concentration in atmospheric air (21%) was taken as 100%.

30 granules (about 3 g) with immobilized cells were placed in 100-ml glass flasks, which were closed with rubber stoppers with metallic clamps. According to a technique that we have previously developed to estimate hydrogen oxidation [3, 4], the flasks were turned upside down to prevent gas escape. The volume of medium in a flask with granules was 20 ml; methanol and hydrogen were the substrates of oxidation. Prior to reuse, the granules with immobilized cells were sterilized either with 0.5% H_2O_2 for 5 min or with 5% methanol for 24 h, depending on the aim of the experiment.

The granite crumb and claydite used as possible cheap carriers proved to be unfit because of poor cell absorption and attachment within the internal cavities. In addition, these carriers were partially disrupted during sterilization and shaking. The microporous carrier PVFF and the hard microporous carrier made of polypropylene (PP) were the most suitable for our tasks. With the method used, the maximum population of the internal carrier cavities with cells was reached. Visual analysis of the granule sections was possible because of cell biomass pigmentation. In addition, imprints of microblock sections were examined under a light microscope. Both approaches confirmed biomass attachment through the entire block thickness. As calculated, 1 cm^3 of the porous material contained 80–100 mg of wet biomass. All three bacterial cultures showed maximum activity in utilization of both substrates immediately after the beginning of incubation. The PVFF carrier was noticeably superior when used with *Renobacter vacuolatum* and *Blastobacter viscosus* (table). *Methylobacterium organophilum* was more active on the PP carrier. A release of carbon dioxide in variants with hydrogen was caused by utilization of the latter as the energy source during consumption of the stored cell reserves [7]. After freezing of the granules with immobilized cells and storage for three, six, and twelve months in the frozen state, the level of activity of the studied bacterial cultures remained unchanged [3, 4]. The immobilized cells proved to be resistant to sterilization with H_2O_2 and methanol, which makes it possible to use them repeatedly in further experiments [2]. The data obtained in this study are in agreement with those obtained previously with the same bacteria immobilized in PAAG and cryoPAAG, silica gel, and cryosilica gel, when these bacteria were incorporated into the matrix of the carrier [3, 4]. In experiments with cells immobilized by absorption, no adaptation period and no delay in the expression of the maximum enzymatic activity were observed, as distinct from the case of chemical binding of bacterial cells, which were affected by toxic monomers. Thus, with respect to the activity of substrate utilization, the cells of oligotrophic bacteria immobilized on porous carriers by absorption

are not the least bit inferior to the same microorganisms incorporated into the carrier structure. On the contrary, the method of microorganism absorption on carriers is undoubtedly more advantageous.

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