Biocatalytic membranes for ultrafiltration treatment of wastewater containing dyes

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Abstract  A possibility to prepare the biofunctional membranes showing the biocatalytic properties and use those in post-treatment of wastewater containing synthetic dyes have been established. Selected Pseudomonas mendocina and Bacillus subtilis cultures were used as biocatalysts for dye destruction. It has been established that cells in spore form are able to survive in N-methylpyrrolidone that allow to use method of polymer solution casting for membrane preparation. The optimal conditions for entrapping of whole cells of microorganisms into the polymer matrix have been determined. Membrane biocatalytic activity has been studied depending on method of casting solution preparation, biocatalyst loading and operating parameters. Dye destruction occurs both in membrane pores and on membrane surface. Membrane obtained provide discoloring of treated solutions (permeate). The dye concentration in retentate depends on the trans-membrane fluxes. The concentration in retentate need not be observed at relatively low fluxes (up to 20 l/m² h).

1 Introduction
Treatment of the wastewater containing dyes is one of the most important ecological problems. Effective purification of such wastewater demands using of integrated technologies based on different physico-chemical methods [1]. Selection of microorganism cultures that are able to destroy synthetic dyes have allowed to develop biological methods for wastewater neutralization [1–3]. For instance, some strains of genus Pseudomonas and Bacillus destroy effectively dyes at their concentrations up to 100 mg/l [4].

At present the membrane methods of separation are widespread in practice of wastewater treatment. Nano- and ultrafiltration are the most promising methods in purification of waste water containing dyes. Nano- and ultrafiltration membranes are characterised by low retention of inorganic electrolytes, that allow to increase degree of wastewater concentration with respect to dyes, the most harmful components of waste water. In spite of high retention of synthetic dyes by nano- and ultrafiltration membranes, in most of cases, especially at high levels of concentration, their residual contents in treated water are higher than the maximal acceptable levels [5, 6]. Integration of membrane and biological processes have been took as a principle of new technical systems called membrane bioreactors [7, 8]. In such bioreactors, membranes are used for re-circulation of biocatalyst or as its carrier.

The purpose of this paper is to develop the biocatalytic membranes containing immobilized whole cells that are able to destroy synthetic dyes and to study the process of biocatalytic destruction of synthetic dyes in the trans-membrane flux. The use of such membranes in the wastewater treatment process provides the means to enhance performances of nano- and ultrafiltration. For instance, it is possible to exclude membrane fouling by dyes as a result of dye destruction on the membrane surface. Moreover the safe levels of dyes in treated water could be achieved because of their destruction in permeate flux.

2 Materials and methods

2.1 Microorganisms and cultural medium
In our study, Pseudomonas mendocina and Bacillus subtilis were used as destructors of synthetic dyes [3]. Cells were enriched on the cultural medium containing 0.1 g/l of KH₂PO₄, 0.15 g/l of Na₂HPO₄, 0.2 g/l of NH₄Cl, 0.1 g/l of MgSO₄, 0.4 g/l of KCl and 2% w/w of agar–agar.

The vofolan dye at its concentration of 10 mg/l was used as a carbon source.

A bacterial culture has been centrifuged in the physiological solution at 8000 rpm for 15 min. The cell mass after their rinsing has been introduced into the organic solvent which has been used as a solvent of the membrane-forming polymer. Then it was thoroughly stirred to reach a homogeneous suspension.

The died cells used as a control were obtained in the following way. The suspension with a density of 10⁸–10⁹ cell/ml in a layer of thickness about 1 cm was irradiated under ultraviolet light at 250–260 nm. A control seeding of irradiated cells on the nutrient medium demonstrated the death of all the cells.
2.2 Membrane formation
Biocatalytic membranes based on sulfonated aromatic polysulfone (SPSF) were obtained by the wet and dry-wet methods from polymer solution containing suspended cells [9].

Three schemes were used to get the casting solutions containing whole cells:

1. The culture of microorganisms was introduced into the polymer solution (method A).
2. Dissolution of membrane-forming polymer in the bacterial suspension in organic solvent (method B).

Before the formation process, all the solutions prepared according to any of the above methods were degased for 1 h under normal pressure.

The polymer solution was spread on a calibrated glass plate as a layer of thickness of 200 μm. The polymer solution formed as a film was immediately precipitated (wet method of membrane formation); or solvent was allowed to evaporate in air at 25 °C for 15 min with subsequent precipitation (dry/wet method of membrane formation). Distilled water or 8% w/w aqueous solution of KCl were used as precipitation bath.

The concentration of SPSF and amount of biomass varied between 15 to 20% w/w and 0.07 to 0.25 g/ml, respectively.

2.3 Determination of survival of microorganisms in the organic solvent medium
To estimate the possibility of using organic solvents for preparation of casting solutions containing bacterial cells, the survival of microorganisms were determined in pure organic solvents. Ethanol, acetone and N-methylpyrrolidine (NMP) were used as solvents. The cells were incubated in the organic solvents for 15, 30 and 60 min at room temperature. The amounts of introduced and survived cells were determined by the utmost dilution method [10].

2.4 Characterization of transport properties and biocatalytic activity of the membranes
The membrane transport properties and biocatalytic dye destruction were studied using the thermostated dead-end cell with stirring at the rate about 500 rpm. The cell volume and its membrane area were 150 ml and 26 cm². The operating pressure was varied in a range of 20–500 kPa. Membrane cell was thermostated at 30 °C.

The “natural red” dye was used as substrate-dye. It contains one Cr-atom that fits on the one end of the dye molecule. The commercial term is vofolan. Its solutions at a concentration of 20 mg/l were ultrafiltrated at different trans-membrane fluxes up to permeate recovery about 80%. Biocatalytic effectiveness was determined in terms of the degree of biocatalytic destruction of dye, $\alpha$,

$$\alpha = \frac{W_d}{W_{\text{feed}}}$$

where $W_d$ – total amount of destructed dye calculated as a sum of dye contents in total permeate volume and in retentate, $W_{\text{feed}}$ – total amount of dye in the feed solution.

2.5 Analytical methods
The dye concentration was determined by calorimetry at a wavelength of 560 nm. The macroscopic structure of biocatalytic membranes was established by optical microscope POLYVAR (Austria) at a magnification of 100, 400 and 1000 times.

3 Results and discussion

3.1 An effect of organic solvent on the physiological state of microorganisms
As a rule the immobilisation of microorganisms needs in the soft conditions and it is carried out from aqueous suspension of cultures. This predetermined the use of water soluble polymers in order to introduce microorganisms in the polymeric materials such as films and fibres. These materials and hydrogels. They possess weak mechanical strength and cannot be used under the conditions of mechanical action, for example pressure, forced flow.

There is a little information about entrapping of whole cells in membranes based on synthetic water insoluble polymers by the wet formation method [11, 12]. Such polymers are more mechanically resistant than hydrogels. Their use would give the means to obtain the semi-permeable flat membranes or hollow fibres for performing of biocatalytic processes in the conditions of convective membrane transfer. The problem is the necessity to use organic solvents which are as a rule incompatible with living organisms.

The effect of the organic solvents on the physiological activity of bacteria – destructors was studied using NMP, acetone and ethanol as solvents. A control seeding of microorganisms on nutrient medium after their exposition in pure solvents showed that cells remain viable in NMP only. The examination of cells by optical microscope showed that the NMP does not cause cell lysis as well as albumin denaturation. Survival of P. mendocina cells (vegetative) is as low as 0.01%. Survival of B. subtilis cells is greater (Table 1) and increases with growth of biomass content. It should be noted that survival of B. subtilis in spore form is practically full. Thus, NMP can be used as a solvent of the membrane-forming polymer suitable to form the biocatalytic membranes with entrapped whole cells of B. subtilis. Maintenance of cell viability for an hour allows to degas the casting solutions. This operation is necessary and important stage to obtain defect free membranes.

3.2 Morphology of biocatalytic membrane
The study of the membrane morphology with entrapped bacteria by the optical microscope has shown that method