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Application of nanofiltration for production of 1,3-propanediol in membrane bioreactor

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ABSTRACT

Biodiesel production from rapeseed oil generates various by-products, for example crude glycerol. Glycerol can be utilized by microbial conversion to 1,3-propanediol (1,3-PD), which can be used as a raw material for the synthesis of polyesters and polyurethanes. The results of studies on the synthesis of 1,3-PD from glycerol by use of *Citrobacter freundii* bacteria were presented. A biosynthesis cost is generally determined based on the costs of nutrients used for culture medium preparation and the costs of broth separation by integrated downstream processes. In this work nanofiltration (NF) was used as a pre-treatment stage for 1,3-PD separation from the broth. Additionally, the NF retentate was reused for the preparation of culture medium, resulting in a change of the initial composition of broth. The catalytic activity of enzymes is strongly affected by broth composition. However, the efficiency of batch fermentations based on the commercial media and NF retentate was similar. Thus, the application of NF process enables a cheaper production of 1,3-PD by biosynthesis. The influence of some broth ingredients, such as carboxylic acids, on variations of biocatalytic properties of used bacteria was discussed.

1. Introduction

1,3-Propanediol (1,3-PD) finds applications in the production of polymers and various materials, such as: cosmetics, lubricants and medicines. The production of 1,3-PD was traditionally performed through the chemical synthesis based on hydroformylation of ethylene oxide (Shell route) or hydration of acrolein (Degussa-DuPont route) [1–3]. In the recent years, the application of crude glycerol, derived directly from biodiesel production, as a substrate for 1,3-PD synthesis has been considered in several papers [1–5].

Biodiesel is one of the alternative fuels produces from vegetable oils such as rapeseed oil, sunflower seed oil and spent frying oil or animal oil. The data indicated a sharp increase in biofuels production both within Europe and around the world [6]. Nevertheless, the cost of biodiesel production is still a relatively high. The main reason for such cost is a large amount of glycerol, which is generated as a by-product. Per every gallon of biodiesel simultaneously is produced approximately 1.05 pound of glycerol (10 weigh percent of biodiesel). Glycerol imposes environmental problems, since generated huge amounts cannot be disposed or converted only to soaps, pharmaceuticals and cosmetics.

A catalytic hydrogenolysis of glycerol to 1,3-PD is considered as a promising approach for the production of monomer for polyester fibers [2,4,5]. It is known that the secondary hydroxyl group of glycerol is less accessible to the active sites than the primary one due the steric hindrance effect. To improve the selectivity of 1,3-PD synthesis (up to 50%) a series of SiO_2 modified $Pt/WOx/ZrO_2$ catalysts with various SiO_2 content were applied [4]. In other work a series of WOx promoted Pt/Al_2O_3 catalysts were used for production 1,3-PD and yield about 42.4% was obtained [5]. A higher selectivity was obtained when 2-Tosyloxy-1,3-propanediol (TPD) was used as a precursor for the synthesis of 1,3-PD [2]. TPD was prepared from glycerol in a three-step sequence (acetalisation with benzaldehyde-tosylation-deacetalisation). Using Raney Ni catalyst the hydrogenolysis of TPD was performed under the conditions of 413 K and 1 MPa of H_2, and the conversion with 81% selectivity for 1,3-PD was obtained.

It is well established that crude glycerol from biodiesel production contains water and hydrogenolysis of glycerol leads to an unavoidable accumulation of by-product water. Moreover, beside 1,3-PD and water possible hydrogenolysis products are 1,2-propanediol, 1-propanol, 2-propanol, and some other degradation products (ethylene glycerol, ethanol, methanol and methane) [2]. This composition indicates, that besides using the selective catalysis, the separation of post-reaction mixture is difficult. Thus

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other alternative eco-friendly methods for 1,3-PD synthesis, such as based on the biocatalysis, are investigated [6–12].

Most biological reactions are catalysed by catalytic proteins (enzymes); therefore, they are generally carried out in the temperature range 298–353 K, the pH close to neutral and at atmospheric pressure [12]. The enzymatic activity is strongly affected by environmental conditions, such as substrate nature and its concentration, and by the presence other substances (activators or inhibitors) [10–15]. In technological processes, the performed transformation requires several different enzymes for catalysis. In this case is better to use whole microorganism cells rather than to separate individual enzymes from them [3,12].

The biotechnological methods that have been developed to obtain 1,3-PD by conversion of glycerol use bacteria of the genera *Clostridium* ssp., *Klebsiella* ssp., *Citrobacter* ssp., *Hafnia* ssp. and *Lactobacillus* ssp. [10,11]. Under consideration can be only organism resistance to osmotic, toxic and mechanical stresses or maintenance of growth capability under the conditions of industrial-scale synthesis [10,13]. Other criteria for the choice of a production strain are the low pathogenicity (risk class), aerobic metabolism and robustness, e.g. process stability after several bioconversions [14].

The biosynthesis of 1,3-PD from glycerol is performed under anaerobi conditions using glycerol as the sole carbon source. Under these conditions glycerol dissimilation involves two parallel pathways: reductive and oxidative [3,16,17]. The reductive pathway is carried out in two sequential reaction catalysed by Vitamin B12-dependent glycerol dehydratase and 1,3-PD oxidoreductase enzymes. The first enzyme removes a water molecule from glycerol to form 3-hydroxypropionaldehyde (3-HPA), which is then reduced to 1,3-propanediol by second enzyme ($NADH_2$). In the case of some microorganism the production of 1,3-propanediol is not a vitamin B12-dependent process, which created an economical advantage for an industrial application [3]. In the oxidative pathway glycerol is dehydrogenated by a nicotinamide adenine dinucleotide (NAD+) to dihydroxyacetone (DHA), which is transformed to glyceraldehyde-3-phosphate and further to pyruvate that in turn produces various byproducts (acetate, lactate, butyrate, ethanol, H_2, and CO_2) and $NADH_2$. The acetic acid is the unique by-products, because its synthesis does not consume the $NADH_2$ formed in glycolysis, which increasing the $NADH_2$ pool for 1,3-PD production [18]. Therefore, if only acetic acid is produced in the oxidative pathway, the glycerol fermentation results in maximum yield of 1,3-PD (Eq. (1)).

$$3CH_3OH{-}CHOH{-}CH_2OH \rightarrow CH_3COOH + CO_2 + H_2 + 2CH_2OH{-}CH_2{-}CH_2OH \quad (1)$$

On the other hand, during glycerol fermentation by *Clostridium butyricum* bacteria it was observed, that an increase of acetate concentration resulted in increased production of biomass and butyrate, together with a reduction in the production of 1,3-PD [13].

Various methods used for the production of 1,3-PD from glycerol via bacterial fermentation are based on repeated batch cultivation. In this case, a portion of the culture broth containing microbial biomass are removed at specified intervals from the bioreactor and replaced with fresh fermentation media so that the volume of the bioreactor contents remains constant [12]. A common problem in fed batch fermentation processes are incomplete substrate fermentation and the accumulation of metabolites in the fermentation medium [9–12,15]. The separation of such mixtures is difficult to perform and is costly [19]. Various unit operations are required for the purification, concentration and separation of organic compounds from the fermentation broth [9,19–21]. The efficiency of 1,3-PD production can be improved through the application of membrane processes such as nanofiltration (NF) [22–24].

NF process can be considered as a separation or concentration step. The fermentation generates a broth containing the dissociated forms of organic products and residual mineral salts. Surface charged membranes are used in NF process, and due to the charge interaction the ions are rejected more effectively. For that reason, the divalent ions usually exhibit a higher rejection than the monovalent ones. On the other hand, the rejection of solutes without electric charge relies primarily on both sieving and diffusion mechanisms. Thus, the NF membranes can reject ions, which are smaller than the membrane pore size (<1 nm); meanwhile, uncharged solutes are rejected only due to steric reasons [25,26].

Table 1
Composition of M media and price of media components (laboratory 1–2 kg packages). Chemicals from Polish producers: BTL and Chempur.

Substrate	Concentration [g/dm^3]	Price [€] for 1m^3 of broth
K_2HPO_4	3.4	52.86
KH_2PO_4	1.3	14.46
$(NH_4)_2SO_4$	2.0	11.43
$CuSO_4$ $7H_2O$	0.4	2.61
$CaCl_2$	0.01	0.11
Yeast extract	2.0	108.98
Peptone K	2.5	327.81
Meat extract	1.5	190.10
$CoCl_2$	0.004	0.70
glycerol	20.0	95.71
$\sum$	33.114	804.76

Considering the development of continuous fermentation it is advantageous to separate mainly the products from the broth, whereas the retentate is recycled to the bioreactor [9,12,23]. In these systems a fraction of broth is usually collected from bioreactor using either micro- or ultrafiltration, simultaneously dosing a new portion of nutrient into the bioreactor, in the amount equivalent to a volume of obtained filtrate. This operation ensures an increase of cells density in the broth and an appropriate dilution rate is achieved, what reduces the activity of glycerol dehydrogenase and other enzymes [16].

In the bioreactors connected with NF process, a higher productivity can be achieved in a comparison to the batch fermentation, due to higher biomass content and as a consequence of the removal of inhibitory components [27,28,29,30]. However, the NF membranes also selectively retain a fraction of broth components, and as a result, their concentration is enhanced. This may lead to a change of activity of further biocatalytic transformations, e.g. a slight increase of concentration $(NH_4)_2SO_4$ and $FeSO_4$ caused a dramatic decrease in 1,3-PD formation [31].

The primary objective of the present study was to evaluate wheather a composition of fermentation broth prepared from NF retentate did not affect substantially the biocatalytic activity of microorganisms used for 1,3-PD synthesis by fermentation of glycerol solutions.

2. Experimental

The influence of NF process on the biocatalystic activity of bacteria was studied through a comparison of the experimental results of glycerol fermentation carried out with a new culture medium as well as a culture medium prepared on the basis of raw materials recovered in the NF process. All experiments were conducted with *Citrobacter freundii* bacteria (from Department of Biotechnology and Food Microbiology Poznań University of Life Science, Poland). The fermentations (30 °C, pH = 7.0–NaOH addition) were conducted in a LiFlusGX bioreactor (Biotron Inc., South Korea). Before experimental use bacteria were pre-cultured in the M media (Table 1) and incubated at 30 °C for 24 h. The M media, supplemented in 2 mL of pre-cultured bacterial suspension and glycerol (about 20 g/dm^3) were used for the first series of fermentations.