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Development of a biofilm technology for the production of 1,3-propanediol (1,3-PDO) from crude glycerol

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ABSTRACT

Glycerol is the main by-product of transesterification of fats in the biodiesel production. 1,3-Propanediol (1,3-PDO) is a valuable chemical that can be obtained from glycerol by microbial conversion. A number of *Enterobacteriaceae* species are able to produce 1,3-PDO from glycerol in stirred tank freely suspended cell bioreactors. Little is known about the use of crude glycerol in the production of 1,3-PDO and about the opportunity to intensify the process via strain immobilization in packed bed bioreactors.

In this work, *Citrobacter freundii*, strain DSM 15979, and *Pantoea agglomerans*, strain DSM 30077, were tested for their ability to produce 1,3-PDO from crude glycerol in shaken flask batch conditions and in packed bed biofilm reactors operating under continuous conditions. Three different hydraulic retention times (HRT) were comparatively tested (8, 4 and 2 h) in order to understand its effects on 1,3-PDO production under immobilized cell conditions. The study revealed that HRT significantly influenced the process performances. The best productivities were observed when a HRT of 2 h was applied. However, both strains were found to be good candidates for 1,3-PDO production in biofilm reactors, even though *P. agglomerans* displayed quite higher productivities (3.6 g/(L h)) than the other strain.

1. Introduction

Crude glycerol is the main by-product of transesterification of fats with short chain alcohols in the biodiesel producing industry which grew considerably during the past decade. Glycerol is therefore a cheap feedstock for the production of chemicals and also an interesting substrate for tailored biotechnological productions [1–3].

1,3-Propanediol (1,3-PDO) is one of the products that can be obtained from glycerol by microbial conversion [4]. 1,3-PDO has a large range of potential utilizations: it is a versatile intermediate for the synthesis of heterocycles and, given its chemical structure displaying two hydroxylic groups at 1 and 3 positions, it is a monomer for the production of polyesters, like polytrimethylene terephtalate (PTT), which have better properties compared to conventional polyesters like polyethylene terephtalate. The market demand for 1,3-PDO recently increased as a result of the increased production of PTT [5]. 1,3-PDO can also be used for the synthesis of polyurethanes, as a chain extender, lubricant, solvent and precursors for the chemical and pharmaceutical industries [6]. 1,3-PDO may also be used as biocides, e.g. PCT 3015. Besides, 2-bromo-2-nitro-1,3-propanediol is used as an industrial biocide for the prevention of bio fouling in cooling towers and evaporation condensers, air conditioners and humidifier systems [7].

Numerous species of *Enterobacteriaceae* are able to convert glycerol into 1,3-PDO. The most promising ones are *Klebsiella pneumoniae* [7] and *Citrobacter freundii* [8]. Little has been done with *Enterobacter agglomerans* [2]. *Lactobacillus brevis*, *L. buchneri* [9]; *L. reuteri* [10] have been reported to use glycerol as an external hydrogen acceptor during lactic acid fermentation. In all these cases, 1,3-PDO was produced under anaerobic conditions in the presence of pure glycerol as the unique carbon source [11]. 1,3-PDO production from glycerol with strains of the *Clostridium* genus has also been studied during the last years [12]. The best known producer within this group was *C. butyricum* followed by the acetone/butanol producers *C. acetobutylicum*, *C. pasteurianum*, [13,14] and *C. beijerinckii*. Apart from wild types, *C. butyricum* and *C. acetobutylicum* were also modified by genetic engineering in order to get more efficient yields over the microorganism [15]. The basic steps involved combining the two genes (B12-independent glycerol dehydratase and the 1,3-PDO dehydrogenase) in order to obtain both a vitamin B12-free metabolism and lower by-product formation. The tailored microorganism were reported as stable and reached high productivities [15].

Strains of the genera *Citrobacter*, *Klebsiella* and *Clostridium* normally produce 1,3-PDO from glycerol via dihydroxyacetone,

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dihydroxyacetone phosphate and pyruvate; the reducing equivalents formed are consumed by conversion of a part of the glycerol into 1,3-PDO via β-hydroxypropionaldehyde [14–17]. Different strategies to produce 1,3-PDO was investigated using over mentioned wild-types. The investigation on the inhibitory effects of industrial glycerol obtained from rapeseed oil biodiesel process on *Clostridium* species from culture collections were reported by Petitdemange et al. [18]. In order to obtain acclimated microorganisms to possible inhibitory effects, isolation of *Clostridium* species was carried out and isolated species successfully produced 1,3-PDO [18]. The existence of excess glycerol channeled the pathway for the production of 1,3-PDO in addition to lower concentrations of butanol when *C. pasteurianum* was used [19].

Most of the experiments on the 1,3 PDO production by *K. pneumoniae*, *C. freundii*, *Clostridia* and *Lactobacilli* have been performed with pure glycerol and under freely suspended cells batch conditions [20,21]. Improvements in process productivity have been obtained under fed-batch conditions [20]. However, fed-batch conditions were not advantageous when *C. pasteurianum* was used as the biocatalyst [19]. In order to obtain higher productivities, biomass content should be increased, this approach may easily be reached in continuous or immobilized cultures [17]. Interestingly, although there are some reports on continuous suspended cultures for the production [22,23], little is known about the use of crude glycerol in the 1,3-PDO production and about the opportunity to intensify the process via strain immobilization in packed bed reactors.

The purpose of this work was to investigate and compare the 1,3-PDO producing potential of *C. freundii*, DSM 15979 strain, and *Pantoea agglomerans*, DSM 30077 strain, in the presence of crude glycerol and under freely suspended cells conditions and biofilms in column bioreactors packed with Vukopor® S10 or polyurethane foam (PUF) operated under continuous mode of cultivation.

2. Materials and methods

2.1. Microbial cultures, crude glycerol and cultural media

P. agglomerans, strain DSM 30077, and *C. freundii,* strain DSM 15979, were obtained from DSMZ culture collection (Braunschweig, Germany) as lyophilized cultures. Nutrient broth (NB) was used to activate the cultures. Raw glycerol was obtained from a factory in Torbali, Izmir, Turkey. It was placed in 10 L plastic containers stored at 4 °C until used. The glycerol content in biodiesel waste was found to be 54.35% (w/v). It also contained 34.81% of water, 6.52% of soap, 3.53% of NaOH, 0.64% of NaCl and trace amount of methanol. The culture medium (CM) used to grow the strains was composed by (in 1 L of distilled water) 40 g of crude glycerol, 5.72 g K_2HPO_4, 1.5 g KH_2PO_4, 2.0 g $(NH_4)_2SO_4$, 0.24 g $MgSO_4{\cdot}7H_2O$, 1.0 g yeast extract (Merck), 0.5 mL $FeSO_4{\cdot}7H_2O$ (0.05 g/L) and 1.0 mL trace element solution. The last one, prepared in distilled water, contained $ZnCl_2$ (70 mg/L), $MnCl_2{\cdot}4H_2O$ (0.1 mg/L), H_3BO_3 (60 mg/L), $CoCl_2{\cdot}2H_2O$ (0.2 g/L), $CuCl_2{\cdot}2H_2O$ (20 mg/L), $NiCl_2{\cdot}6H_2O$ (25 mg/L), $Na_2MoO_4{\cdot}2H_2O$ (35 mg/L) and 0.9 mL/L HCl (37%, v/v). The final concentration of glycerol in the CM was 40 g/L, which means we dissolved 80 g/L of waste glycerol was dissolved in the fermentation media in order to obtain the desired concentration. CM pH was adjusted at 7.0 before autoclaving.

2.2. 1,3-PDO production under different fermentation conditions

The selected strains of *P. agglomerans* and *C. freundii* were preliminary tested for 1,3-PDO production (Fig. 1 and Table 1) in 200 mL CM in 0.5 L closed shaken flasks incubated at 30 °C and 120 rpm. In the first batch test 20 g/L of crude glycerol were used as the substrate and 1,3-PDO profiles versus growth were investigated as a result of hourly sampling during 25 h (Fig. 1). Following, crude glycerol in the range from 5 to 140 g/L were tested for 12 h (Table 1). The microorganisms used in this study are facultative anaerobe, therefore no strict anaerobic conditions were provided. Thus, the glass flasks were set up by covering them with polypropylene caps after immobilization was completed without purging nitrogen through the culture media. As a consequence, no oxygen was allowed to enter into the flask after they were closed. Continuous conditions in dedicated conventional fermentors and packed bed reactors were investigated, in order to compare the 1,3-PDO productivity among freely suspended and immobilized cell systems. Two suspended cultures of *P. agglomerans* and *C. freundii* were grown in Biostat-A+ and B+ fermenters (Sartorius, Germany), respectively. The fermentors had a working volume of 1 L, under a maintained pH value of 7.0, and operated with an agitation rate of 180 rpm. They were fed with a CM with a glycerol concentration of 40 g/L, under varying HRTs (ranging between 2 and 8 h). At the same time, four identically configured packed biofilm reactors were developed and employed under continuous mode of operation. The height and internal diameter of the column reactors were of 30 cm and 4.5 cm, respectively, whereas their total empty volume was 280 mL. Two of the columns were packed with Vukopor® S10 (VUK) (Lanik, Boskovice, CZ), while the other 2 with polyurethane foam (PUF) (Arslan Sunger, Turkey). VUK particles had dimensions and porosity of 25 mm × 25 mm × 18 mm and 10 ppi, respectively; PUF cubes had comparable dimension and a porosity of 20 ppi. These

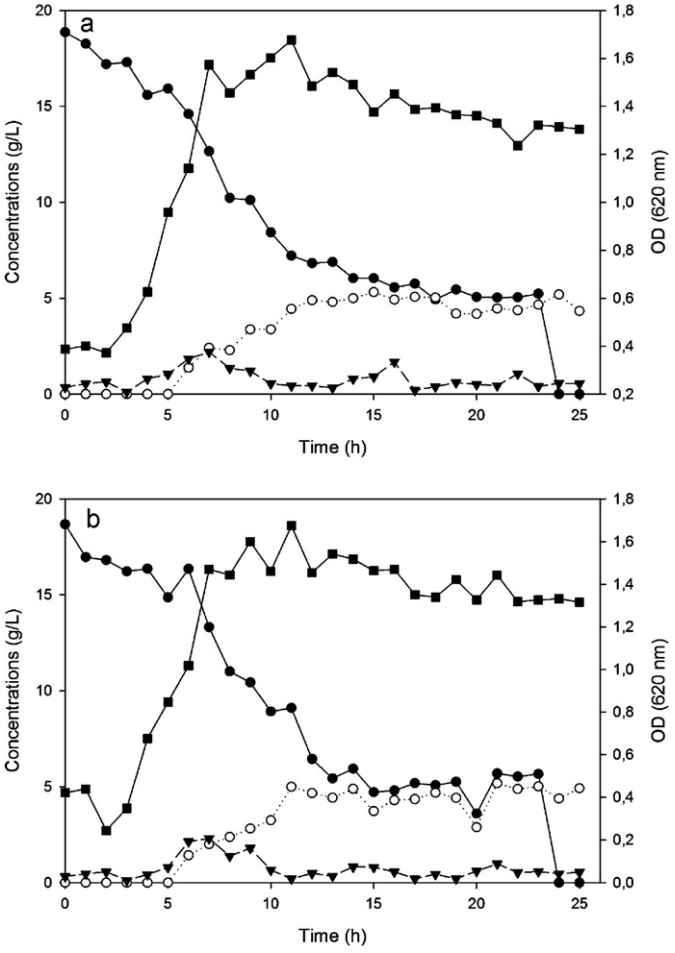

Fig. 1. 1,3-PDO (●), residual glycerol concentrations (○) and biomass (▼) in comparison to turbidity (OD) (■) in (a) *Citrobacter freundii* DSM 15979 and (b) *Pantoea agglomerans* DSM 30077.