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The continuous bioconversion of glycerol to 1,3-propanediol using immobilized *Citrobacter freundii*

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Abstract

The purpose of the study was to compare the effectiveness of carriers used for Citrobacter freundiiimmobilization in 1,3-propanediol (1,3-PD) production over a continuous process in using different hydraulic retention time (HRT) values / dilution rates. The objective of this work was to investigate the effects of different HRT and immobilization material (keramsite or modified polyurethane foams - PUFs) on cell growth, glycerol utilization and 1,3-PD production using column systems.
*The results of the study revealed HRT as a significant operational parameter which directly affected the bioconversion of crude glycerol to 1,3-PD. The highest 1,3-PD concentrations for PUFs or keramsite were found to be 18.24 g/L and 15.93 g/L respectively. These data were obtained for the optimum value of HRT (4 h).The highest glycerol consumption percentage was found to be 99.6% with modified PUFs under 4 h HRT condition.Insufficient (e.g., 0.5 h) HRT duration resulted in lower 1,3-PD production in all cases (10.32 g/L for PUFs and 9.06 g/L for keramsite). The most effective productivity for PUFs (20.6 g/L*h) as carriers was higher than for keramsite (18.1 g/L*h). The results of these tests proved that the modified PUFs were more superior carriers for immobilization than keramsite.*

Keywords: bioimmobilization, PU foam, keramsite, bioconversion, *Citrobacter freundii*.

1. Introduction

One of the major limitations for industrial microbial production of 1,3-propanediol(1,3-PD) is the relatively high cost of the typical substrate, such as glucose. A economically attractive solution to this problem might be the use of a crude glycerol (devoid of prior purification) as a fermentative substrate (A. DROŻDŻYŃSKA& al. [1]). Crude glycerol is generated as a by-product, not only when biodiesel fuels are produced chemically, but also when they are manufactured enzymatically and during the production of bioethanol. The bioconversion of glycerol to 1,3-PD seems to be an attractive waste treatment practice in which both pollution control and energy recovery can be achieved (C. SANTIBANES& al. [2]). The majority of experiments on 1,3-PD production by *C. freundii* strain have been performed under freely suspended cell batch conditions. Improvements in process productivity have been obtained under fed-batch conditions. However, fed-batch conditions are not always advantageous. In order to obtain higher productivities, biomass content should be increased. This approach may reached more easily in continuous cultures (R. BOENIGK& al. [3], S. CASALI& al. [4]). One drawback of this particular process is culture instability and strain degeneration. Culture degeneration, characterized by a progressive loss of solvent production capability, has been known for a long time and has been observed in both continuous and batch cultures (J.C. ANDRADE& al. [5]). The solution of the problem could be the immobilization of bacterial cells using carriers stable in medium containing phosphates.

Immobilization not only imparts a special stability to the bacteria against the

negative effects of operational conditions, but additionally avoids the washing out of the cell populations upon the use of high dilution rates in continuous operation mode. While immobilized cell technology offers several important advantages such as a higher cell densities per unit bioreactor volume that result in very high fermentation rates, the re-use of the same biocatalysts for prolonged periods, the development of continuous process that may be operated beyond the nominal washing-out flow rate, and a smaller bioreactor volumes that may decrease capital costs in comparison to the traditional fermentation by suspended cell culture systems. In immobilized cell reactors, high production rates can be obtained with an effective retention of the bacteria (M. GUNGORMUSLER& al. [6]). In general, the immobilization of bacteria occurs in two main ways: adsorption by physical and chemical bonds, or physical entrapment of cells within the carriers (SILVA & al. [7]). Adsorption is a form of cellular adhesion. It is based on the ability of microorganisms to fix themselves to solid surfaces. This situation occurs in materials such as active carbon, ceramic rings,ceramic balls, pumice stones, polyurethane foams, polyHIPEs polymers and sintered glass (I. DE ORY& al. [8], M. GUNGORMUSLER& al. [6],[9]).

The work described here involved the immobilization of *C. freundii* by adsorption on two different immobilization materials: modified polyurethane foams (PUFs) and keramsite. These materials were chosen in consideration of the possibilities for the microorganisms to be adsorbed on the porous surface of the materials (J. HRENOVIC& al. [10], H.C. LEE& al. [11], M. KURANSKAK& al.[12], SHAN& al. [13]). Peanut shells were used to increase the PUFs structural ability for adsorption, transport properties and bacterial cells adhesion (D.S. DLAMINI& al. [9], E. GÓRECKA& al. [10], A.N. ABDELMAJEED& al. [12]).The effect of hydraulic retention time (HRT) on the production of 1,3-PD was also investigated. It was proved that HRT affects productivity of continuous process. As has been emphasized by M. GUNGORMUSLER& al. [9] HRT was an important factor for both immobilized and suspended systems and a HRT of 2 h was the most successful in terms of volumetric production rate. The purpose of the study was to compare the efficacy of carriers used for *Citrobacter freundii*immobilization in 1,3-PD production over continuous process while using different hydraulic retention time (HRT) values ⁄ dilution rates. The objective of this work was to investigate the effects of different HRT and immobilization material (keramsite or modified PU foams) on cell growth, glycerol utilization and 1,3-PD production using immobilized column systems.

2. Materials and methods

The *C. freundii* strain used in this study was obtained from the collection of the Department of Biotechnology and Food Microbiology of Poznan University of Life Sciences (Poland). The culture media: "M" that has been used for the studies consisted of 50 (g/L) (gram per liter) of waste glycerol (Trzebinia Rafinery, Poland), 2.4 g/L K_2HPO_4, 0.6 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 0.4 g/L $MgSO_4*7H_2O$, 0.1 g/L $CaCl_2*2H_2O$, 0.004 g/L $CoCl_2*H_2O$, 2 g/L yeast extract, 2.5 g/L bactopeptone, 1.5 g/L meat extract. All other reagents that have been used to compose the medium were from Merck and Chempur companies. The medium was prepared according to F. BARBIRATO& al. composition [17,18]. The amount of substrates was modified to obtain a high yield process. Keramsite, fraction 2-4 mm (Liapor, Germany) before using were sterilized in autoclave at 121ºC with 2.2 bar pressure for about 15 minutes. Peanut shells (Lidl, Poland) were used as an organic additive during PU foamspreparation. The mixture of polyols (PCC Prodex, Poland) (density =1.02 g/cm^3, viscosity = 2800 mPas)

was used as component A, and the mixture of polyisocyanates(density =1.21 g/cm^3, viscosity = 150 mPas) was used as a component B. Foams without additives were prepared according to PCC Prodex protocol.

For continuous productiontwo chromatographic columns (one for each carrier) with thermo jacket XK26/20 (GE Healthcare Life Sciences, USA), thermostat (Julabo, Poland), peristaltic pump (MRC, Israel) and magnetic stirrer with heating (DragonLab, China) were used. The foam composites were obtained from a conventional formulation for flexible polyurethane foams containing component A (polyethylene glycol, polypropylene glycol, triethylenediamine as the catalyst for the reaction and less than 1% of ethylene glycol as an extending-agent). The composition of component B, with respect to the polyols content, was 4.4-diisocyanate methylenediphenyl. The peanut shells as a modification were added into component B (0.56g of peanut into 2g of polyols). A conventional procedure for foam preparation was adopted. It consisted of vigorously mixing of 1.2g of the component A and 2.56g of component B for around 15s. The modified foams were prepared in a 1.2 cm diameter and 12 cm long falcon test-tube before expansion began to take place. Later during polymerization (5min) the polyurethane foams were left to rest for 24h at room temperature to complete PUF solidification. The prepared foams were cut into disks and tested.

Before and after immobilization, microscopic analysis was performed using a microscope Vega 3 LMU (Tescan) scanning electron microscope (SEM). The tests were necessary to examine the porous structure of the carriers and to confirm the adhesion of *C. freundii* cells to the surface of the carriers. Analysis was performed at room temperature with tungsten filament, and an accelerating voltage of 20 kV was used to capture SEM images for both of the pure carriers samples and immobilized carriers. All specimens were viewed from above.

In the first step of the experiments the *C. freundii* cells were pre-grown on Medium "M'' with agar for 24h at 30°C. After incubation, the biomass was suspended in a sterile 0.85% NaCl solution (1.2x10^7 CFU/mL). Then the suspended biomass was added to a sterile flask with the "M" broth (at a ratio of 1:10) and stirred using a magnetic stirrer (DragonLab, China) for 15 minutes. After stirring, the medium with the bacteria culture was added to a sterile flask with sterile keramsite or the modified PU foams and incubated at 30°C for 24h. After immobilization, 30mL of keramsite and 30mL of PUFs (with bacteria) were used in the bioconversion process. The immobilization effectiveness was evaluated by checking the bacterial cell adhesion using three samples of PU foams and three samples of keramsite. After 24h of incubation, the "M'' broth was taken off the flasks. The PUFs and keramsite were placed in a new flask and rinsed with a sterile NaCl solution. The samples were then suspended in the "M" sterile broth, and squeezed/homogenized with a sterile glass rod for around 1 minute and vortex about 4 minutes. From each suspension serial dilutions were made. The cell concentration was expressed as colony-forming units (CFU) per 1 mL and determined by making a serial decimal dilutions and plating on Medium "M'' with an agar. The test results were presented as the average of three samples. The adhesion of bacterial cells on the surface of the keramsite, as well as the surface of the foams were checked before their transfer into column and after the bioconversion process. After immobilization, the carriers were rinsed with a sterile 0.85% NaCl solution and transferred to a sterile column set (without being too closely packed), then filled with a sterile "M" broth. The temperature in the column was kept at 30°C using thermo jacket connected to thermostat (Julabo, Poland).The whole continuous process including columns with the immobilized foams and the keramsite under varying

hydraulic retention times (ranging between 0.5 and 4 h) lasted for 192 hours.

The total 1,3-PD and the glycerol contents were determined by HPLC (Knauer, Germany) using an Aminex HPX-87H organic acid and alcohol analysis column and RI detector. The injection volume of the sample was 10 µL. The column, maintained at 25°C, was eluted with 5 mM H_2SO_4 at a flow rate of 0.6 mL/min, samples ran for 30 minutes. The samples for chromatography analysis were taken every day after 24h. The production of 1,3-PD and the consumption of the glycerol were obtained by dividing the final concentration (g/L) by fermentation process time (h).

The samples from the effluent were taken and analysed for the viability of *C. freundii* cells. An 100 µL of each sample was put into 900 µL of the "M" medium and mixed using a vortex. Serial dilutions were made from each suspension. Cell concentration was expressed as CFU per 1 mL and determined by making a serial decimal dilutions and plating on the "M'' medium with agar (15 g per litre).

3. Results and Discussions

In order to achieve high productivities in glycerol fermentation to 1,3-propanediol, and to maintain a stable process, it was necessary to immobilize an active biomass of *C. freundii*. Therefore, a complete growth medium was supplied to the columns and temperature was maintained at an optimal range (30ºC). Polyurethane foam and keramsite were chosen as carriers during bacteria immobilization. The keramsite is preferable due to its porous and hydrophilic characteristics and the PUFs were commonly used as immobilization supports for a various biotechnological applications due to their open porous structure. Additionally PUFs were modified by the addition of peanut shells. The reason for the addition of organic waste was to increase the adhesion of bacterial cells to the foam surface. The PUFs and the keramsite were especially suitable for a microbial colonization and importantly, the columns filled with carriers were able to work in small reactor volumes.

A scanning electron micrograph revealed the porous structure of the modified PUFs and keramsite (Fig. 1). A network of bacteria rapidly colonized the carriers. After 24 h of immobilization the biofilm on the PUFs and the keramsite were macroscopically visible. M. GUNGORMUSLER& al. [9] immobilized *Clostridium beijerinckii* NRRL B-593 strain to convert crude glycerol to 1,3-PD. As immobilization carriers they used ceramic rings and pumice stones. The SEM images indicated that immobilization was very successful. M. GUNGORMUSLER& al. [6] also converted glycerol to 1,3-PD. The authors also used SEM to check the adhesion of *Klebsiella pneumoniae* to the surface of the two carriers. Scanning electron micrographs revealed an open porous structure to the ceramic rings and ceramic balls. A network of bacteria rapidly colonized the material surface. In this case SEM images also indicated that immobilization was successful. Similarly, the porous structure of the carrier was determined using SEM by U. PFLUGMACHER& al. [19]. The authors used PUR 90/16 to immobilize the *C. freundii* strain to convert glycerol to 1,3-PD.

In the supernatant from the columns, viable cells were detected throughout all experiments indicating that immobilization was complete. The results of the experiments showed that there were $1.27x10^7$ CFU/mL of *C. freundii* cells on the surface of the polyurethane foams (Fig. 2) and $1.29x10^7$ CFU/mL of these cells on the surface of the keramsite before the process began (Fig. 3). During the first hour of the bioconversion there were $6.17x10^5$ CFU/mL of bacterial cells in effluents in both columns. After 8 days, the process of glycerol conversion to 1,3-PD was finished, and a decrease in the amount of *C. freundii* in effluents was observed. There were

$3x10^7$CFU/mL of bacteria in the effluent from column containing the foam and $7.71x10^6$ CFU/mL in the effluent from the column containing keramsite. A decrease of a number of bacteria in the effluents could be explained by the fact that *C. freundii* produced 1,3-PD in the primer metabolism during logarithmic phase (M. GUNGORMUSLER & al. [9]). As explained by the authors, the log phase takes place during the 8–14th h of fermentation. Later on the microorganism goes into a stationary phase, in this phase 1,3-PD is produced slower than it is consumed. Only when the microorganism is kept in a log phase, does the product concentration reach its maximum value.

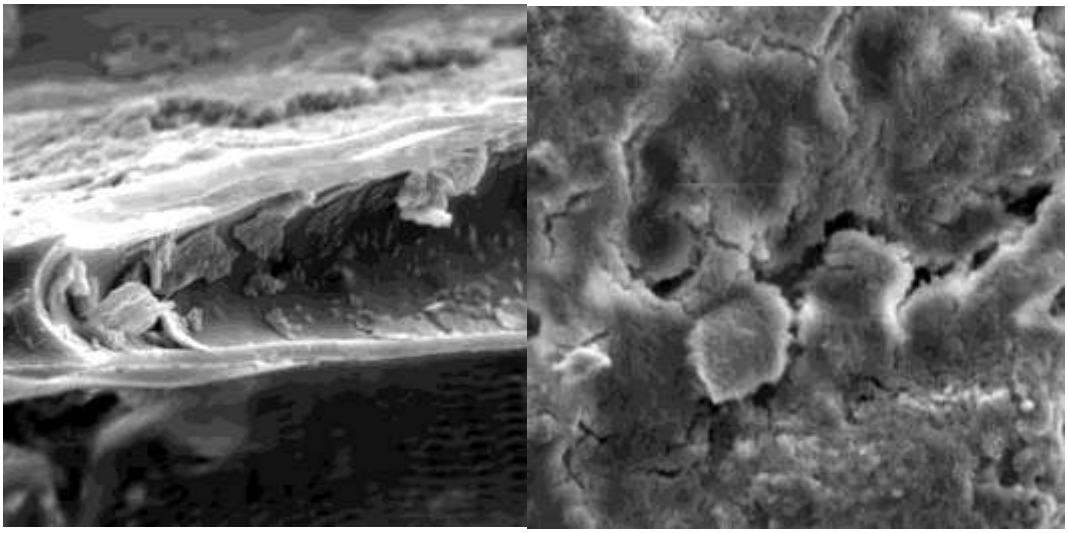

Fig. 1. Adhesion of *C. freundii* cells to the surface of modified PUFs (left) and keramsite (right).

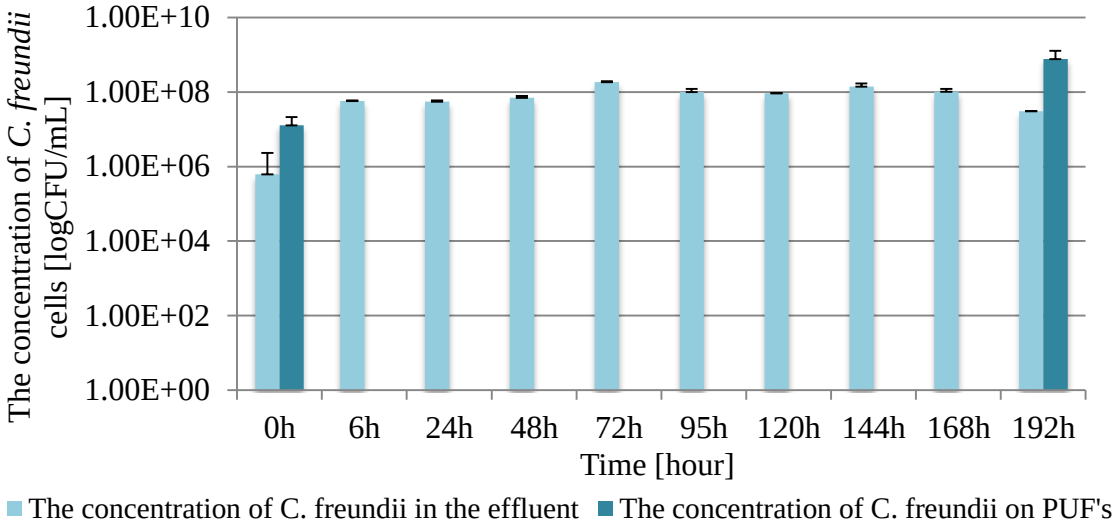

Fig. 2. The concentration of *C. freundii* cells (immobilized on PUFs) before, during and after bioconversion process (average values of triplicates).

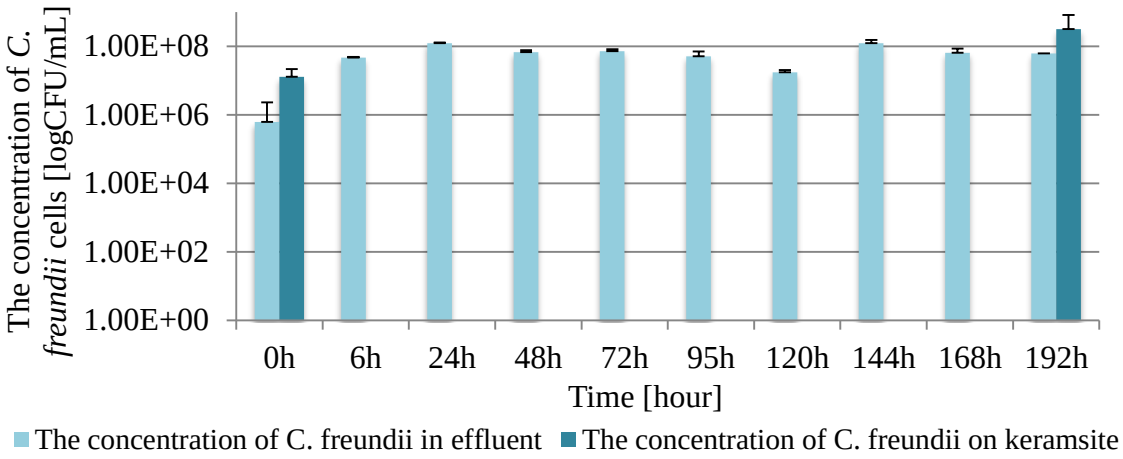

Fig. 3. The concentration of *C. freundii* cells (immobilized on keramsite) before, during and after bioconversion process (average values of triplicates).

It is important that in the case of modified polyurethane foams after 192 hours of bioconversion there were $7.57x10^8$ CFU/mL of bacterial cells on the surface (Fig. 2) and $3.17x10^8$ CFU/mL of microorganisms on the surface of keramsite (Fig. 3). These results as well as SEM images proved that the immobilization was complete. The modified PU foams proved to be a better carrier than keramsite due to the higher number of *C. freundii* cells on the surface of PUFs than on the keramsite surface. M. GUNGORMUSLER& al. [6] compared two materials ascarriersfor immobilization. They concluded that it was obvious that both of the materials that were analysed as a carriers were suitable for immobilization. It was also important that more biomass was attached on the ceramic balls than on ceramic rings.

The Table 1 shows the average 1,3-PD concentrations obtained from the each column as functions of HRT. As seen in the tables, there was an optimum value for HRT parameter that was 4 h for both of bioreactors. It was observed that a too short (e.g., 0.5 h) HRT resulted in lower 1,3-PD productions in all cases. The bioreactor containing bacteria immobilized by using PUFs performed much better in terms of 1,3-PD concentration in the product except where the bioreactor containing microorganisms immobilized by using keramsite, which performed relatively similar to PUFs bioreactor under 0.5 h HRT conditions. The highest 1,3-PD concentrations for the each bioreactor were found to be 18.24 g/L and 15.93 g 1,3-PD/L for PUFs bioreactor, and keramsite respectively. Similar results were obtained byM. GUNGORMUSLER& al. [9]. Their results showed an optimum value for HRT parameter that was 12 h for immobilization of *Clostridium beijerinckii* NRRL B-593 on ceramic and pumice stone. They observed that both too short (e.g., 2 h) and too long (e.g., 16 h) HRT resulted in lower 1,3-PD production in all cases. The highest 1,3-PD concentrations for the each bioreactor were found to be 31 g of 1,3-PD/L for both ceramic ring and pumice stone. It should be noted that differences between concentrations of 1,3-PD obtained in this work and by other authors could not only be caused by different carriers used for immobilization. The reason might also be bacterial strains that characterize different metabolisms. These considerations were confirmed by M. GUNGORMUSLER& al. [6]. The authors concluded that the differences in production rates were mostly dependent on the microorganism, and the second important parameter that affected the final product was immobilization material.

Table 1. The concentration of glycerol/1,3-PD at different HRTs (average values of triplicates).

HRT [h]	**1,3-PD [g/L]**		**Glycerol [g/L]**		**Glycerol consumption [g/L]**	
	PUFs	keramsite	PUFs	keramsite	PUFs	keramsite
4	18.24	15.93	0.20	0.80	49.58	48.98
3	16.23	13.54	1.17	0.49	48.61	49.29
2	15.79	13.26	6.64	7.67	43.14	42.11
1	13.12	10.97	11.12	13.62	36.48	33.98
0.5	10.32	9.06	19.14	17.55	29.60	31.45
Average glycerol consumption					41.48	41.16

Table 2. The yield and productivity of bioconversion process at different HRTs.

	PUFs		**Keramsite**	
HRT [h]	**The yield [g/L]**	**The productivity [g/(L*h)]**	**The yield [g/L]**	**The productivity [g/(L*h)]**
4	0.37	4.60	0.33	4.00
3	0.38	5.40	0.28	4.50
2	0.37	7.90	0.31	6.60
1	0.36	13.10	0.32	11.00
0.5	0.35	20.60	0.29	18.10

Table 2 shows the 1,3-PD productivity under various HRT conditions. The productivity at lower HRT conditions was found to be a much higher than at higher HRT. In the case of PUFs as carriers of the highest productivity was 20.6 g/L*h and was found under 0.5 h HRT condition. Due to this value of productivity, the modified polyurethane foams were found to be better carriers than PUR 90/16. In the case of PUR 90/16 the productivity ranged from 4.1 – 8.1 g/L*h (U. PFLUGMACHER& al. [2]). Similarly in the case of keramsite, the highest productivity (18.1 g/L*h) was determined under 0.5 h HRT conditions (Table 2). The lowest productivity was evaluated under 3 and 4 h HRT conditions for both of carriers. Similarly M. GUNGORMUSLER& al. [9] analysed productivity under various HRT conditions for bioreactors containing immobilized bacteria. They proved that even though lower HRT conditions did not result in higher 1,3-PD concentrations, the productivity at lower HRT conditions were found to be much higher, which could be considered favourable in terms of capital and operational costs. A concentration of 12.92 g/L of 1,3-PD measured inpacked bed bioreactors, was obtained by S. CASALI& al. [4] when *C. freundii*was immobilized on the PUF (at a HRT of 8 h). Similarly, the maximumproductivity obtained with *C. freundii*, was observed in a packed-bedreactor filled with VUK (Vukopor®S10) and fed at a HRT of 2 h. The results obtained in this study were superior at a HRT of 1 h (Table 1). The authors mentioned above used pure PUFs and VUK as carriers for microorganisms immobilization. The polyurethane foams that were used in this work were modified with the addition of peanut shells. It was necessary to increase cell adhesion and to increase the yield of conversion of the crude glycerol to 1,3-PD. The experiments of S. CASALI& al. [4] showed that a concentration of 1,3-PD obtained using immobilized bacterial cells was 8% higher thanthat with the same strain employed as a freely suspendedculture at the same HRT value. Thus, clear improvements in process productivity was attained by cell immobilizationthrough biofilm technology developed in their study.

Analysing the productivity of the bioconversion of the crude glycerol to 1,3-PD,

it can be concluded that PUFs were better carriers than keramsite. Table 2 also shows the yield of bioconversion process under various HRT conditions for each column. The amount of glycerol converted to 1,3-PD (grams glycerol per grams 1,3-PD) was described as the 1,3-PD yield. As seen in Table 2, 1,3-PD yields ranged between 0.23 and 0.79 (g 1,3-PD/g glycerol), which was compatible with theoretical expected ranges (X.CHEN& al. [13]). The results of the study also demonstrated that there were no significant differences between the values of the yields, and it was important that in the case of a yield, PUFs were better carriers than keramsite. Similarly, the results of the study by M. GUNGORMUSLER& al. [9] showed that values of yields ranged between 0.23 and 0.79, compatible with the theoretical expected ranges. They also proved hydraulic retention times that were both too short and too long resulted in lowest molar yields of 1,3-PD for all bioreactors studied. The authors noted that microorganisms were unable to consume glycerol when at short reaction times, and as a result production of the main product (1,3-PD) was lower and the yields decreased consequently. They also stated that the optimization of by-products (concentration reduction) as a cause of low yields should be studied with crude glycerol. Similarly it was necessary to minimize the concentrations of by-products for M. GUNGORMUSLER& al. [6]. This was because as reported, a crude glycerol broth may contain not only acetate but also some inorganic salts from the glycerol fermentation medium and these organic and inorganic substances may cause cell growth inhibition and subsequently a decrease in 1,3-PD production. A by-product that can be produced at higher amounts is 2,3-BD. However, an accumulation of the aforementioned by-product does not negatively affect 1,3-PD production. It should be also noted that researchers confirmed (P. ANAND& al.[7]) that *C. freundii* was not resistant to the various impurities in crude glycerol derived from biodiesel preparation. The pre-treatment of crude glycerol has proved to be an economical and easy method to remove the most potent growth inhibitors. In addition, it was observed that removing impurities can cause not only a growth inhibition decrease but also 1,3-propanediol production increase to a level which can be a comparable to pure glycerol.

Analysing the amount of 1,3-propanediol during 8 days of bioconversion we found that the highest concentration of this product was obtained after 72 h of the process. A decrease in the concentration of 1,3-PD was found after 192 h of bioconversion (Table 3). Table 1 summarizes the residual glycerol concentrations as a function of HRT in the effluent stream during the fermentation process. As seen in the Table, the average glycerol consumptions for all HRTs were found to be 41.48 g/L and 41.16 g/L for the culture immobilized by using PUFs and keramsite, respectively. Glycerol consumption increased with increasing HRT in PUFs and keramsite. The highest glycerol consumption percentage was found to be 99.6% with modified polyurethane foams under 4 h HRT condition. In contrast to the results obtained in this study, the experiments of M. GUNGORMUSLER& al. [6] demonstrated that the substrate with an initial concentration of around 40 g/L, was not consumed at lowest HRT values. However, at 4, 6 and 12 h of HRT, glycerol was utilized almost completely. Similarly, just as the results obtained in this study, their tests showed that the maximum utilization was observed at 4 h of HRT for the both bioreactors (39 and 40 g/L ofglycerol consumption for ceramic balls and ceramic rings, respectively). The authors also observed that the residual substrate concentration increased rapidly with incremented dilution rates. The tendency for substrate consumption in various HRT values was in a parallel for both of the immobilization materials. The glycerol utilization in the bioreactor filled with ceramic balls reached around 50% of the glycerol

consumption at an HRT of 1 h, likewise in a bioreactor filled with ceramic rings, glycerol utilization was again around 50% of the glycerol consumption. In comparison to results obtained by these authors the results of this study showed that glycerol utilization in the column containing PUFs reached around 76.6% of glycerol consumption at an HRT of 1 h, and in the column filled with keramsite utilization was 71.4% of glycerol consumption (Table 1).

Table 3. The concentration of glycerol and 1,3-PD during 192h of the bioconversion.

Time [h]	Glycerol/1,3-PD [g/L]			
	PUFs		Keramsite	
	Glycerol	1,3-PD	Glycerol	1,3-PD
1	36.10	0.37	31.70	0.83
2	36.50	0.88	36.31	2.70
3	50.10	2.44	39.25	3.30
4	36.42	4.90	31.76	5.00
5	33.50	5.40	33.80	5.48
24	2.10	19.74	8.12	14.54
48	1.62	19.79	10.62	14.41
72	0.08	19.90	0.37	18.28
96	0.01	17.96	0.65	17.76
120	0.01	18.68	1.20	14.64
144	8.08	13.00	7.67	13.26
168	0.27	17.58	0.07	15.63
192	13.67	12.51	5.49	13.51

To summarize, this study has shown that an efficient fermentation of glycerol to 1,3-PD can be carried out with *C. freundii* immobilized. In addition, immobilization with the use of modified polyurethane foams were a more efficient means of 1,3-PD production in comparison to culture immobilized on the surface of keramsite. The hydraulic retention time was found to be a highly significant operational parameter directly affecting the bioreactor performance. The immobilization was also important for the yield of the bioconversion process. It served a potential for reaching higher concentrations of the cells and creating a strong microenvironment that cannot easily be affected by the extreme changes in physical conditions. Where immobilized cells (biofilms attached to the materials) became more adapted to the environment, the higher yields of product were achieved. The immobilization system allowed the use of smaller reactor volumes while obtaining higher concentrations of the desired product (1,3-PD), in shorter fermentation periods. Based on the results presented above, it can be concluded that both materials were suitable candidates for immobilization purposes, but PUFs are better support materials than keramsite in terms of immobilization ratios. The results reported in this study may help to save the cost of 1,3-PD as higher production in smaller bioreactors could be achieved.

4. Conclusions

The hydraulic retention time was a significant operational parameter directly affecting the bioconversion of crude glycerol to 1,3-propanediol. The highest 1,3-PD concentrations for the PUFs and keramsite bioreactors were found to be 18.24 g/L and 15.93 g 1,3-PD/L, respectively. These data were obtained for the optimum value of HRT at 4 h.Insufficient (e.g., 0.5 h) HRT resulted in lower 1,3-PD productions in all

cases (10.32 g/L for PUFs and 9.06 g/L for keramsite). The best productivity for PUFs (20.6 g/L*h) as carriers was higher than for keramsite (18.1 g/L*h) as a carrier. This data proved that the modified PUFs were better carriers for immobilization than keramsite.

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References

1. A. DROŻDŻYŃSKA, K. LEJA, K. CZACZYK, Biotechnological production of 1,3-propanediol from crude oil. *J.Biotechnol.*, 92 (1), 92, 100(2011).
2. C. SANTIBANES, M.T. VARNERO, M. BUSTAMANTE,Residual glycerol from biodiesel manufacturing, waste or potential source of bioenergy: A Review, *Chil. J.Agr. Res.*,71(3), 469, 475(2011).
3. R. BOENIGK, S. BOWIEN, G. GOTTSCHALK,Fermentation of glycerol to 1,3-propanediol in continuous cultures of *Citrobacter freundii*. *Appl.Microbiol.Biotechnol.*, 38, 453, 457(1993).
4. S. CASALI, M. GUNGORMUSLER, L. BERTIN, F. FABIA, N. AZBAR, Development of a biofilm technology for the production of 1,3-propanediol (1,3-PDO) from crude glycerol. *Biochem. Eng. J.*, 64, 84, 90(2012).
5. J.C. ANDRADE, I. VASCONCELOS,Continuous cultures of *Clostridium acetobutylicum*: culture stability and low-grade glycerol utilization. *Biotechnol.Lett*., 25, 121, 125(2003).
6. M. GUNGORMUSLER, C. GONEN, N. AZBAR,Use of ceramic-based cell immobilization to produce 1,3-propanediol from biodiesel-derived waste glycerol with *Klebsiellapneumoniae*. *J. Appl.Microbiol*., 111, 1138, 1147 (2011).
7. M.F. SILVA, D. RIGO, V. MOSSI, R.M. DALLAGO, P. HENRICK, G.O. KUHN, C.D. ROSA, D. OLIVEIRA, J.V. OLIVEIRA, H. TREICHEL,Evaluation of enzymatic activity of commercial inulinase from Aspergillusniger immobilized in polyurethane foam. *Food Bioprod. Process*, 91 (1),54, 59(2013).
8. I. DE ORY, L.E. ROMERO, D. CANTERO,Optimization of immobilization conditions for vinegar production. Siran, wood chips and polyurethane foam as carriers for Acetobacteraceti. *Process.Biochem*., 39, 547, 555(2004).
9. M. GUNGORMUSLER, C. GONEN, N. AZBAR,Continuous production of 1,3-propanediol using raw glycerol with immobilized *Clostridium beijerinckii* NRRL B-593 in comparison to suspended culture. *Bioprocess Biosyst. Eng*., 34, 727, 723(2011A).
10. J. HRENOVIC, T. IVANKOVIC,D. TIBLJAS, The effect of mineral carrier composition on phosphate-accumulating bacteria immobilization. *J Hazard Mater* 166, 1377, 1382(2009).
11. H.C. LEE, Y.G. JEONG, B.G. MIN, W.S. LYOO, S.C. LEE,Preparation and Acid Dye Adsorption Behavior of Polyurethane/Chitosan Composite Foams. *Fiber Polym*., 10 (5), 636, 642(2009).
12. M. KURANSKA, A. PROCIAK, Porous polyurethane composites with natural fibres. *Comp. Sci. Technol*., 72, 299, 304(2012).
13. C.W. SHAN, M.I. IDRIS, M.I. GHAZALI,Study of Flexible Polyurethane Foams Reinforced with Coir Fibres and Tyre Particles. *Int. J. Appl. Phys. Math*.,2 (2), 123, 130(2012).
14. D.S. DLAMINI, A.K. MISHRA,B.B. MAMBA,Morphological, Transport, and Adsorption Properties of Ethylene Vinyl Acetate/Polyurethane/Bentonite Clay Composites. *J. Poly. Sc*.,35600, 4978, 4985(2011).
15. E. GÓRECKA, M. JASTRZĘBSKA, Immobilization techniques and biopolymer carriers. *Biotechnol. FoodSci*., 75 (1), 65, 86(2011).
16. A.N. ABDELMAJEED, O.A. KHELIL, E.N. DANIAL, Immobilization technology for enhancing bio-products industry. *AFR J.Biotechnol*.,11 (71), 13528, 13539(2012).

17. F. BARBIRATO, C. CAMARASA-CLARET, J.P. GRIVET, A. BORIES,Glycerol fermentation by a new 1,3-propanediol-producing microorganism: Enterobacteragglomerans. *Appl.Microbiol.Biotechnol*., 43, 786, 793 (1995).
18. F. BARBIRATO, E.H. HIMMI, T. CONTE, A. BORIES,1,3-propanediol production by fermentation: An interesting way to valorize glycerin from the ester and ethanol industries. *Ind. Crop. Prod*., 7, 281, 289(1998).
19. U. PFLUGMACHER, G. GOTTSCHALK,Development of an immobilized cell reactor for the production of 1,3-propanediol by *Citrobacter freundii*, *Appl.Microbiol.Biotechnol*., 41, 313, 316(1994).
20. X. CHEN, Z. XIU, J. WANG, D. ZHANG, P. XU, Stoichiometric analysis and experimental investigation of glycerol bioconversion to 1,3-propanediol by *Klebsiella pneumoniae* under microaerobic conditions. *Enzyme.Microb. Technol*., 33, 386, 394(2003).
21. P. ANAND,R.K. SAXENA,A comparative study of solvent-assisted pretreatment of biodiesel derived crude glycerol on growth and 1,3-propanediol production from *Citrobacter freundii*. *New.Biotechnol*., 29, 199, 205(2012).