The influence of PU foams modification on the efficiency of *Citrobacter freundii* cells immobilization

Małgorzata Mizielińska*, Urszula Kowalska, Łukasz Łopusiewicz

Center of Bioimmobilisation and Innovative Packaging Materials, Faculty of Food Sciences and Fisheries, West Pomeranian University of Technology 35 Janickiego Str., Szczecin, 71-270 Szczecin, Poland

*E-mail address: Malgorzata.Mizielinska@zut.edu.pl

ABSTRACT

The purpose of the study was to modify PU (polyurethane) foams to increase the efficiency of *Citrobacter freundii* cells adhesion. The immobilization can contribute to better productivity during the bioconversion of glycerol to 1,3-propanediol (1,3-PD). The results of the study showed that *C. freundii* immobilized on foams with organic additives could decompose more glycerol and produce more 1,3-PD than cells immobilized on pure foams. The immobilization made production in long-term operations or repeated runs possible. The results of the experiments have also proved that the foams with additives, especially with wood chips and peanut shells, would be the most suitable carriers because they allow the immobilization of a higher number of bacterial cells. It was demonstrated that the initial number of *C. freundii* cells in all samples (before incubation) was $1.2 \times 10^6$ CFU/mL. The results showed that the accumulation of bacterial cells in the control sample with pure PUF (polyurethane foams) was $1.5 \times 10^7$ CFU/mL after 24 hours of incubation (the number of bacterial cells attached to surface of foams). It was determined that the accumulation of bacterial cells in samples with 15% of additives at 30 °C increased to $2.17 \times 10^7 / 3.37 \times 10^7 / 1.25 \times 10^7 / 1.65 \times 10^7 / 5.65 \times 10^7$ CFU (PUFbtb, PUFab, PUFwch, PUFps, PUFrc) CFU/mL after 24 hours of incubation.

*Keywords:* bioimmobilization, polyurethane foam, bacterial adhesion, *Citrobacter freundii*
1. INTRODUCTION

The immobilization of bacterial cells and enzymes takes place in two main ways: adsorption by physical or chemical bonds, or physical entrapment of enzymes or cells within the carriers (De Ory et al., 2004, Silva et al., 2013). The most widely studied methods for the immobilization of bacteria have been adsorption to surfaces and encapsulation within gels or porous materials.

Polyurethane (PU) foam has been widely used as a carrier for the immobilization of various microorganisms because of its high mechanical strength and resistance to organic solvents and microbial attack and also because of its biochemically inert characteristics. PU makes open cell foam as a result of the condensation of polycyanates and polyls. Upon polymerization, carbon dioxide escapes from the matrix, leaving pore spaces behind. Typically, porous matrices of PUF not only increase the surface areas but also minimize the diffusion limitation for the substrate and product. A diffusion-limited environment is a common disadvantage of polymers currently used for encapsulation such as acrylamide, alginate, and carrageenan (Silva et al., 2013, Gu et al., 2013, Riviera-Armenta et al., 2004).

It has been reported that polyurethane foams can provide high surface area and open porous structures and thus are adopted as matrix materials for immobilizing processes (Lee et al., 2009, Shan et al., 2012, Kuranska and Prociak 2012). There are compounds that may be used to modify the PUFs properties and structure. Several research studies using starch as a modifier have been carried out. Other natural materials like coir, pine wood fibres, hemp, chitosan, saccharides, cashew nut shell liquid, soybean oil and soy flour have been also used in PU foam preparation. There are also a variety of cellulose derivatives having different properties such as solubility and thermal behavior. The research work focused on the use of carboxymethyl cellulose (CMC), cellulose sulphate (CS), cellulose acetate (CA) and trimethylsilyl cellulose (TMSC) to modify PUF’s properties (Riviera-Armenta et al., 2004, Lee et al., 2009, Shan et al., 2012, Kuranska and Prociak 2012) for better adsorption, transport properties and bacterial cells adhesion (Dlamini et al., 2011, Gorecka and Jastrzebska 2011, Abdelmajeed et al., 2012).

The shortage of resources of crude oil has induced an increase in biofuels production (Drozdzynska et al., 2011). The microbial production of 1,3-propanediol is an exciting method of valorizing waste glycerol from biodiesel (Kaur et al., 2012). The productivity of 1,3-PD can be improved through the application of metabolic and genetic engineering procedures (Kaur et al., 2012). It is also possible to improve the efficiency of the bioconversion process by using immobilized microorganisms.

The purpose of the study was to modify PU foams to increase the efficiency of Citrobacter freundii immobilization which can contribute to better productivity during the bioconversion of glycerol to 1,3-propanediol.

2. MATERIAL AND METHODS

2.1. Material

- C. freundii strain used in this study was obtained from the collection of Poznań University of Life Sciences (Poland).
- TSB, TSA and MacConkey agar (Merck, Germany) were used to check adhesion
properties of bacteria cells. All mediums were prepared according to the Merck protocols.

- The culture media: “M” that has been used for the studies consisted of (g/L) 50 waste glycerol (Trzebinia Rafinery), 2.4 K2HPO4, 0.6 KH2PO4, 2 (NH4)2SO4, 0.4 MgSO4·7H2O, 0.1 CaCl2·2H2O, 0.004 CoCl2·H2O, 2 yeast extract, 2.5 bactopeptone, 1.5 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 Barbitarro et al. composition (1995, 1998).
- Birch-tree bark, alder bark, wood chips (ANPER, Gryfice), rapeseed cake (REM S.A., Nowogard) and peanut shell (Lidl) were used as organic additives during the PU foams preparation.
- A mixture of polyols (PCC Prodex) (density = 1.02 g/cm³, viscosity = 2800 mPas) was used as component A, and a mixture of polyisocyanates (density = 1.21 g/cm³, viscosity = 150 mPas) was used as component B. The foams without additives were prepared according to the PCC Prodex protocol.

2.2. Methods

2.2.1. The foams synthesis

PUF 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 the extending-agent). The composition of component B, with respect to the polyols content, were 4,4-diisocyanate methylenediphenyl. The conventional procedure for foams preparation was adopted. It consisted of vigorously mixing component A and component B (in ratio 100:60) about 15 s. The foams were prepared in a falcon test-tube 1.2 cm in diameter and 12 cm in length before expansion started to take place. Later during the polymerization stage (5min) the polyurethane foams were left to rest for 24 h at room temperature complete solidification of PUF. The prepared foams were cut into disks and tested.

The foam composites were prepared by the addition of either ground birch-tree bark (btb) or alder bark (ad) or wood chips (wch) or rapeseed cake (rc) or peanut shells (ps) (separately) in the first stage to the polyol and other components. The additives were ground before foams synthesis. In the first stage, the same maximum concentrations (0%, 15%, 20%) of additives were used in the experiments in order to obtain comparable results. In the second stage concentrations of chosen additives used were: 0, 5, 10, 15, and 20% w/w, with respect to the total mass. The composites obtained were designated as PUF0 (matrix foam), PUFbtb, PUFab, PUFwch, PUFrc, and PUFps, according to their respective organic component content.

SEM: 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 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 the top.
2.2.2. Immobilization

In the first step of the experiments the bacteria cells of *C. freundii* were pre-grown on a MacConkey agar for 24h at 30 °C. After incubation the biomass was suspended in sterile 0.85% NaCl solution (1.2×10⁷ CFU/mL). Then the suspended biomass was added to a sterile flask with the broth “M” (in 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 sterile flasks with sterile PU foams or PU foams with 15% of each additives and incubated at 30°C for 24h. Three samples of each of the PU foams were used for immobilization. In the second step after the analysis as the results all experiments were repeated for PU foams with 5% and 10% of wood chips or rapeseed cake or peanut shell.

2.2.3. Adhesion

After 24h of incubation, broth “M” was taken off the flasks. Three samples of each of the PUFs placed in these flasks were rinsed with a sterile NaCl solution, suspended in the sterile broth “M”, squeezed/homogenised with a sterile glass rod for about 1 minute and via vortex for about 4 minutes. Serial dilutions were made from each suspension. Cell concentration was expressed as colony-forming units (CFU) per mL and determined by making serial decimal dilutions and plating on a MacConkey agar. Results are presented as the average of the three samples with standard deviation.

2.2.4. Bioconversion process

After selection of the best foams with additives, *C. freundii* (1.5×10⁸ CFU/mL) was immobilized on them for 48 hours. After immobilization PUFs were transferred to 100 mL flasks. The flasks with 10 cm³ of each carrier were filled with sterile medium “M” and incubated in a shaker with 250 rpm (IKA® KS 4000) for 48 hours. The temperature in the shaker were kept at 30 °C. The efficiency of the crude glycerol and production of 1,3-propanediol was the final concentration (g/L) by fermentation process time (h). The second cycle began with the removal from each flask of a calculated number of foams in such way that, after their introduction into the new sterile medium “M”, a decrease of 1,3-propanediol was registered. This process represents the start of a new semi-continuous fermentation cycle taking place. After the second bioconversion process the foams were taken off the flasks and transferred to new 100 mL flasks. The next flasks were filled with the sterile medium “M” and put into a shaker to start a new bioconversion process (in the same conditions). The immobilized cells of *C. freundii* were used to produce 1,3-propanediol three times. The whole immobilization process involved successive semi-continuous cycles, with 1,3-propanediol concentration values in the range of 17.08-20.73 g/L.

Chromatography analysis: the total 1,3-PD and glycerol content was determined by HPLC (Knauer, Germany) using an Aminex HPX-87H organic acid 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 H₂SO₄ at a flow rate of 0.6 mL/min, samples ran for 30 minutes. Samples for chromatography analysis were taken every day after 24 h. The production of 1,3-PD and consumption of glycerol were obtained by dividing the final concentration (g/L) by the fermentation process time (h).
3. RESULTS AND DISCUSSION

The present work has focused on modification of polyurethane foams by addition of natural carriers to improve adhesion of bacterial strains onto surface of these foams. As has been shown (Figure 1) the modified foams have been prepared.

![Fig. 1. The foam containing a) alder bark; b) rapeseed cake; c) wood chips; d) peanut shells.](image)

The results of the study showed that all foams with 5%, 10% and 15% of the organic additives were as flexible as pure PU foams were. This testifies to the fact that additives did not change the foams significantly. On the other hand all of the PUFs with 20% of the additives were rigid and these were not used during the experiments. Wang et al. (2012) proved that PU foams could be used to immobilize bacterial cells. The authors mixed prepolymer A, prepolymer B and bacterial suspension (10⁹ CFU/mL). About 15 minutes after mixing of three components, PU foams formed and bacterial cells were embedded inside the foams. The authors showed that prepolymer B was active against bacterial cells. It is why
this was assumed that neither polyurethane foams (polymer) nor organic additives (natural substances) were active against living cells. The study proved that all carriers used for immobilization do not interact negatively on the \textit{C. freundii} strain. The initial number of \textit{C. freundii} cells in all samples (before incubation) was $1.2 \times 10^6$ CFU/mL. The results showed that the accumulation of bacterial cells in the control sample with pure PUF was $1.5 \times 10^7$ CFU/mL after 24 hours of incubation (Table 1) (the number of bacterial cells attached to surface of foams). De Ory et al. (2004) explained that the internal structure of polyurethane foam could allow immobilization of bacteria on their surface. The authors proved that after the initial stage, a sudden increase in adhered biomass was observed and, within a few hours the maximum colonization of PUF carrier was reached. From that point on, further adsorption was not registered.

These authors explain that this situation could be directly related to the hydrodynamic behavior of PU foams submerged in the liquid phase. During the first hours the particles of a carrier remain dry, but as the process continues they gradually become completely wet through capillary action. After this point the cellular colonization of the carrier begins at a high rate. According to these authors (De Ory et al. 2004) the highly porous structure of polyurethane foams facilitates the total exposure of the surface and precludes problems associated with accessibility for the cell after soaking. As a consequence, a high homogeneity for the bacterial adhesion was observed with this material. Based on the results of the experiments of Ribeiro et al. (2005) with different substrates, the following conclusion was drawn: ”the nature of the carbon source influenced adhesion dynamics on polyurethane foam”. These authors also showed that carbon source influences the efficiency of the conversion process using immobilized microorganisms cells. In the case of these experiments only the medium “M” containing glycerol was used as a carbon source. Additionally, the comparison of the adsorption capacity of pure foams and natural polymer (cotton fiber) were done by Kilonzo and Bergougnou (2012).

Their experiments showed that the adsorbed bacterial cells were only about 50% of the amount on the cotton fiber. It seemed plausible to attribute the worse adsorption efficiency of polyurethane foams to the inferior roughness. The only way to increase the adhesion capacity of foams was to introduce chosen, porous additives into PU foams during their synthesis. Based on this assumption experiments were done. It was determined that the accumulation of bacterial cells in samples with 15\% of additives at 30 °C increased to $2.17 \times 10^7 / 3.37 \times 10^7 / 1.25 \times 10^8 / 1.65 \times 10^8$ CFU/ $5.65 \times 10^7$ (PUFbtb, PUFab, PUFwch, PUFps, PUFrc) CFU/mL after 24 hours of incubation (Table 1). Peanut shells proved to be the best carriers because of the biggest adhesion of \textit{C. freundii} to surface of PU foams containing these additives. Slightly worse additives were wooden chips. Unfortunately the accumulation of bacterial cells on foams containing birch-tree bark and alder bark was the worst in comparison to PUFps, PUFwch and PUFrc.

It is also very important that the adhesion of bacterial cells to the surface of foams with additives was better than in the case of pure foams. The higher adhesion of bacterial cells was obtained for PUFps and PUFwch in comparison to pure PU foams. Even SEM images showed bigger clusters of cells on the surface of PUF with additives than on the surface of pure foams. The experiments indicate the improvement of adhesion properties of PUF by adding organic additives (Table 1, Fig. 1, 3).
Table 1. The concentration of bacterial cells on PUF surface depending on the amount of additives

<table>
<thead>
<tr>
<th>The amount of additive [%]</th>
<th>Type of additive in foam</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PUFbtb</td>
</tr>
<tr>
<td></td>
<td>C* [cfu/mL]</td>
</tr>
<tr>
<td>0</td>
<td>1.50E+07</td>
</tr>
<tr>
<td>5</td>
<td>2.47E+07</td>
</tr>
<tr>
<td>10</td>
<td>2.37E+07</td>
</tr>
<tr>
<td>15</td>
<td>2.17E+07</td>
</tr>
<tr>
<td>20</td>
<td>1.98E+07</td>
</tr>
</tbody>
</table>

C* - concentration  
SD** - standard deviation

The adsorption capacity and strength of binding are the two major factors that affect the selection of a suitable support material. Similarly to pure foams, PUFs with additives provide adequate supporting surfaces for cell adsorption, due to their high specific surface area, mechanical properties, non-toxicity, maximum loading, durability and high availability. However, the adhesion capacity of PUFs with wood chips confirmed that foams containing 15% of this additive can attach even $1.25 \times 10^8$ CFU/mL in comparison to pure PUF which can accumulate $1.8 \times 10^7$ CFU/mL. The PUFwch with content of 10% and 5% adsorbed $1.16 \times 10^8$ CFU/mL and $1.05 \times 10^8$ CFU/mL (Fig. 4).
Fig. 2. Adhesion of *C. freundii* cells to the surface of PUFwch.

Figure 3. Adhesion of *C. freundii* cells to the surface of pure PUF.
These results showed that 15% of the wood chips as additives can allow to obtain the best adhesion properties and it is why the 15% PUFwch were used in the bioconversion process. The analysis of adhesion properties of PUFs with peanut shells as additives also indicated that foams containing 15% of additives can attach the highest number of *C. freundii* cells. These foams adsorbed $1.16 \times 10^8$ CFU/mL of bacterial cells while 10% and 5% PUFps adsorbed only $1.6 \times 10^7$ CFU/mL and $2.2 \times 10^7$ CFU/mL (Fig. 5). The results demonstrated that 15% peanut shells foams can be used in the 1,3-propanediol production process.

Unfortunately the comparison of adhesion properties of PUFs with rapeseed cake depending on the content of additives demonstrated the opposite results. In this case the foams containing only 5% of the additives accumulated $1.96 \times 10^8$ CFU/mL. 10% and 5% PUFrc adsorbed only $4.9 \times 10^7$ CFU/mL and $5.65 \times 10^7$ CFU/mL. It is why they were not used during the bioconversion process (Fig. 6).

![Fig. 4. The comparison of adhesion properties depending on the kind and on the amount of additive.](image)

Microorganisms retained on a carrier can be used in many production processes, allowing for significant cost decrease. The attachment of bacterial cells creates a protective barrier around the immobilized microbes, ensuring their prolonged viability during processing. Adsorption is the elementary and probably the simplest method of reversible immobilization. Adsorption is based on weak forces, however still enabling an efficient binding process (Gorecka and Jastrzebska 2011, Abdelmajeed et al. 2012). Immobilization of *C. freundii* by adsorption was very important because of the possibility of re-use of the PU foams in the bioconversion process which decreases costs. It was also assumed that the organic additives that were used during PUF synthesis would increase the adhesion of cells to the surface of foams which has influence the growth of efficiency of the bioconversion...
process. Pure PU foams and PU foams with 15% peanut shells, 15% wood chips and only 5% rapeseed cake were used as carriers in bioimmobilization process. Analyzing the results obtained from the first run it can be determined that *C. freundii* immobilized on PUFwch produces the highest number of grams (20.17 g/L) of 1,3-propanediol. Bacterial cells accumulated on PU Frc synthesizes 20.01 g/L while cells immobilized on PUFps 19.82 g/L. The lowest number of grams (18.5 g/L) produces bacteria adsorbed by pure PU foams (Fig. 6). It can be associated with the greatest adhesion of microorganisms to the surface of PUFwch (1.25×10^8 CF/mL) in comparison to the lowest adhesions on PU Frc/PUFs/PUF (1.96×10^8 CF/mL / 1.16×10^8 CF/mL / 1.5×10^7 CF/mL) (Table 1, Fig. 3, 4, 5).

These results indicated that the number of grams of 1,3-propanediol increases with the increasing adhesion of cells to a surface of foams. They also showed that organic additives influence the increase of adhesion properties of *C. freundii* to the surface of foams. In this section the results showed that it is possible to obtain a complete set of reproducible fermentation cycles for each of the foams studied especially for PUFwch. Similarly the activity of biomass immobilized to the surface of PUF were observed by De Ory et. al. (2004).

Their results demonstrated that the activity of bacterial cells remained steady with constant fermentation rates. The authors compared three carriers: wood chips, saran and PU foams. The experiments showed that foams stood out from the other two assayed supports in that it led to the maximum fermentation process. In the second passage PUFwch were the best carriers. Microorganisms immobilized to its surfaces produced 20.73 g/L of 1,3-propanediol. In this case the worst carrier with additives was PU Frc. In comparison to our experiments the worst results were obtained by Casali et al. (2012) who also prepared a medium with glycerol to produce 1,3-PD using free *C. freundii* cells. The initial concentrations of glycerol used by these authors were 30 g/L and 60 g/L. The amount of 1,3-PD was very low 4.9 g/L and 5.1 g/L. The authors concluded that the productivities observed in their study were lower than those obtained so far by using immobilized bacteria on crude glycerol. Rossi et al. (2012) compared the abilities of a consortium of bacteria to grow in either raw or pure glycerol. Pure glycerol was exhausted with a production of 22.8 g/L of 1,3-PD. For raw glycerol consumption, was approximately the same amounts of 1,3-PD being produced (19.9 g/L).

The lower amounts of 1,3-PD were obtained by these authors using a selected single *Klebsiella pneumoniae* strain. The experiments under aerobic conditions showed a lower production of 1,3-PD when compared to the anaerobic fermentation. The highest amount of 1,3-propanediol which was obtained during process was 9.4 g/L under anaerobic conditions and 6.2 g/L for aerobic cultivation. The results obtained by these authors were obtained using free bacterial cells. It should be noted that our results obtained by using immobilized bacteria were better. In comparison to our study a very high amount of 1,3-PD was obtained by Mu et al. (2008) who investigated a novel integrated bioprocess with microbial production of 1,3-PD by *K. pneumoniae* using a hollow fiber membrane. The final amount of 1,3-PD obtained by these authors was 50.7 g/L. The results of Himmi et al. (1999) also showed that it is possible to obtain a higher amount of 1,3-propanediol than in our experiments. These authors even obtained 26 g/L of 1,3-PD from 50 g/L of glycerol but they used an anaerobic *Clostridium butyricum* strain.

The results of our study showed that in comparison to the first and second run, opposite numbers of grams of 1,3-propanediol were obtained during the third run. In this case PU Frc was the best carrier (Fig. 5).
It is important to say that in the case of the second and third run an increase of adhesion properties of foams with additives did not influence the amount of 1,3-propanediol. Nevertheless it is important to note the overall decrease in the bioconversion yield as the passages progressed, especially in the third run. The decrease in the fermentation yield as the cycles progressed was observed by the other authors (De Ory et al. 2004). They found that a decrease was due to evaporation losses.

![Comparison of the number of grams of 1,3-propanediol produced by immobilized C. freundii cells depending on PUF with additives.](image)

**Fig. 5.** Comparison of the number of grams of 1,3-propanediol produced by immobilized *C. freundii* cells depending on PUF with additives.

Through analyzing biotechnological processes it is known that bacterial cells need a carbon source to produce 1,3-propanediol. It is also known that bacterial mediums used during these processes are expensive. One of the major factors governing the economic viability of any bioprocess is the cost of the starting material. It is why it is expected that conversion processes were optimized to use less carbon source. In contrast to those situations when bacterial strains should degrade some wastes and use them as a carbon source in the bioconversion processes it is very important to use the wastes as much as possible. The biotechnological process of bioconversion of glycerol to 1,3-propanediol would give us a high number of grams of product and complete degradation of the waste substratum at the same time. The results of the study showed that *C. freundii* immobilized on foams with organic additives could decompose more glycerol than cells immobilized on pure foams (Fig. 6). The differences between the degradability of foams with different additives were noticed. There were also differences between glycerol consumption depending on the number of run. Results obtained in the first run showed that *C. freundii* immobilized to the surface of PUFps degraded more glycerol than those cells immobilized on PUFwch or PUFrc. The same results were obtained in the second run. PUFps were the best carrier also in the third run.
In summary it was important to obtain a high productivity of 1,3-PD after the bioconversion process using the immobilized \textit{C. freundii} strain. The waste glycerol created as the byproduct of the biodiesel production usually contains dissolved acid or base or other impurities. Previous studies indicated that most bacterial strains with the ability to produce 1,3-PD can not survive in a medium with crude glycerol (Wong et al. 2011). Very often the efficiency of the bioconversion of the crude glycerol to 1,3-PD is lower than the production of 1,3-PD using pure glycerol (Himini et al. 1999).

The summarizing, the results obtained in the study demonstrated that the choice of carrier for the immobilization of \textit{C. freundii} cells is very important because it can increase the productivity of 1,3-PD and make production in long-term operations possible, the immobilized bacteria were used to carry out three repeated runs. The glycerol to 1,3-PD yield decreased from 50.25 g/L to an even 0.1 g/L in the first run, from 50.78 g/L to 1.29 g/L in the second run and from 50.5 g/L to 1.2 g/L in the third run (Table 2). The results obtained by other authors (Wong et al. 2011) indicated that the immobilized cells could be repeatedly used at least 6 times with a nearly constant 1,3-PD yield. Our experiments and the results obtained by the authors (Wong et al. 2011) proved that the immobilized-cell system appeared to be effective, promoting the operational stability and reusability of cells. If only the immobilization capacity is considered, it is clear that the foams with additives, especially with wood chips and peanut shells, would be the most suitable carriers because they allow the immobilization of a higher number of \textit{C. freundii} cells. However, the pure foams are also more than acceptable. De Ory (2004) also confirmed that PU foams are more than acceptable for immobilization. As this author said this is very important to take into account the time

**Fig. 6.** Comparison of the number of grams of glycerol decomposed by immobilized \textit{C. freundii} cells depending on PUF with additives.

![Glycerol consumption by Citrobacter freundii (g/mL)](Image)
required to reach the maximum immobilization capacity. In this sense the porous structure of polyurethane foams is highly uniform, a fact that facilitates rapid cellular adhesion.

Table 2. The amount of 1,3-PD and of glycerol depending on the type of foam.

<table>
<thead>
<tr>
<th>Type of foam</th>
<th>Initial glycerol concentration (\text{g/L})</th>
<th>glycerol concentration (\text{g/L})</th>
<th>1,3-PD concentration (\text{g/L})</th>
<th>1,3-PD productivity (\text{g/L/h})</th>
<th>1,3-PD yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUF</td>
<td>50.25</td>
<td>1.36</td>
<td>18.5</td>
<td>0.385</td>
<td>37.8</td>
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<tr>
<td>PUFrc</td>
<td>0.1</td>
<td>20.01</td>
<td>0.417</td>
<td>39.9</td>
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<tr>
<td>PUFwch</td>
<td>0.11</td>
<td>20.17</td>
<td>0.420</td>
<td>40.2</td>
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<tr>
<td>PUFps</td>
<td>0.08</td>
<td>19.82</td>
<td>0.413</td>
<td>39.5</td>
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<tr>
<td>PUF</td>
<td>50.78</td>
<td>3.27</td>
<td>17.08</td>
<td>0.356</td>
<td>36</td>
</tr>
<tr>
<td>PUFrc</td>
<td>1.29</td>
<td>19.78</td>
<td>0.412</td>
<td>40</td>
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<tr>
<td>PUFwch</td>
<td>2.25</td>
<td>20.73</td>
<td>0.432</td>
<td>42.7</td>
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<tr>
<td>PUFps</td>
<td>1.41</td>
<td>20.19</td>
<td>0.421</td>
<td>40.9</td>
<td></td>
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<tr>
<td>PUF</td>
<td>50.5</td>
<td>3.63</td>
<td>18</td>
<td>0.375</td>
<td>38.4</td>
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<td>PUFrc</td>
<td>2.22</td>
<td>19.61</td>
<td>0.409</td>
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<td>18.54</td>
<td>0.386</td>
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<td>PUFps</td>
<td>1.2</td>
<td>18.14</td>
<td>0.378</td>
<td>36.8</td>
<td></td>
</tr>
</tbody>
</table>

4. CONCLUSIONS

1. The foams with additives, as wood chips and peanut shells were the most suitable carriers for immobilization of a higher number of bacterial cells.
2. The immobilized-cell system appeared to be effective for 1,3-PD production in long-term operations or repeat runs.
3. The \textit{C. freundii} cells immobilized on foams with organic additives decomposed more glycerol and produced more 1,3-PD than cells immobilized on pure foams did.

References


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