IMPROVEMENT OF BIOFILM FORMATION IN TRICKLE BED REACTORS BY SURFACE MODIFICATION OF DIFFERENT PACKING MATERIALS

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ABSTRACT

The microorganism *Cupriavidus necator* has been chosen as a platform to biologically transform CO_2 from gas streams into 3-hydroxypropionic acid (3-HP) inside trickle bed reactors. Usually, packing materials are used as a support for microorganisms' adhesion and growth. To optimize the production of 3-HP this study aims to develop advanced packing materials with improved surface and sorption properties. Hardwood, polyurethane and polyisocyanurate-based foams, polypropylene pellets and polyester textiles have been studied as innovative packing materials, and compared with Raschig rings, as standard packing materials. An evaluation of contact angle, surface morphology, surface area, porosity and biofilm formation has been performed.

Key Words: PLASMA TECHNOLOGY, TRICKLE BED REACTOR, PACKING MATERIALS, CUPRIAVIDUS NECATOR, 3-HYDROXYPROPIONIC ACID

1. INTRODUCTION

Transforming CO_2 into added-value chemicals and plastics, *via* biological processes, is highly promising and valuable. CO_2 is widely abundant and its use as feedstock avoids overexploitation of natural resources and reduces GHG (Greenhouse Gases) emissions. In this frame, CO_2 becomes a secondary raw material and a valuable commodity rather than a pollutant. In this project, a TBR is being developed for the production of 3-hydroxypropionic acid (3-HP), a building block for the production of many plastic materials and plasticizers. This technology is presented as an alternative to the currently used petroleum-based materials. The production of 3-HP is carried out using an engineered *Cupriavidus necator*, which uses CO_2 as carbon source and metabolically transforms it into the target product.

Packing materials are commonly used in TBRs as a support for adhesion and growth of these microorganisms. The strategy followed in this study to achieve higher production of 3-HP is the development of advanced packing materials with improved surface and sorption properties. A rational research has been conducted on the effects of surface properties of packing materials on attachment, growth and biofilm formation. The main parameters and characteristics influencing the whole process of biofilm formation include electrostatic interaction between support and bacteria, surface area and surface roughness of the support, size and shape of the bacteria, hydrophobic or hydrophilic nature of the support and bacteria, temperature, nutrients and shear forces in the bioreactor, among others [1,2].

Different packing materials have been used in TBRs and bioreactors. Woodchips, and more specifically hardwood chips, have been used extensively. Beech wood shavings have been widely used with different types of bacteria for the production of a variety of chemicals, such as acetone and butanol [3], among others. Plastic based materials have been also used and found to provide good adhesion and biofilm growth properties. The most interesting, in terms of productivity, were found to be polypropylene (PP), combined with *Zymomonas mobilis* in a biofilm reactor for ethanol production [4], and reticular foam plastics, such as polyurethane foams combined with *Saccharomyces cerevisiae* for ethanol production [5]. Additionally, for lactic acid production have also been utilised sophisticated configurations made of fibrous beds of cotton affixed to the surface of a perforated stainless steel cylinder mounted on the agitation shaft as packing material with *Rhizopus oryzae* [6].

The aim of this research is to study innovative, efficient, environmentally friendly and low-cost packing materials, by analyzing their characteristics, bio-adhesion properties and growth of bacteria. The results of this research will provide a more suitable selection of materials, which will be then surface modified with low pressure air plasma. Plasma treatments can produce different effects on the material's surface, and in the frame of this project, two complementary effects are considered: (i) surface activation by introducing polar groups on packing material's surface, to promote wetting and to enhance adhesion and biofilm formation, and therefore achieving higher 3-HP production efficiency, and (ii) increase of surface roughness, and consequently, increase of the surface area. Finally, optimum packing materials with adapted surface properties, increased accumulated attached biomass and bioreactor performance will be obtained.

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2. MATERIALS AND METHODS

Based on the literature review conducted on current packing materials and the parameters influencing attachment and growth of bacteria, the following materials have been selected:

Table 1 Alternative packing materials selection

Category	Packing material	Relevant characteristics
Standard materials	Raschig rings	Materials commonly used in bioreactors
Hardwood	Beech wood chips	High surface energy, roughness and porosity
	Eucalyptus wood chips	
Foams	Soft polyether-polyurethane foam	High surface area and macro and micro-porosity.
	Hard polyether-polyurethane foam	
	Polyisocyanurate (PIR) based foam	
Polypropylene	Polypropylene pellets	Have been proved to have adhesion properties with bacteria
Polyester textiles	Polyester woven textile	High surface area
	Polyester nonwoven	
	Polyester 3D textile	

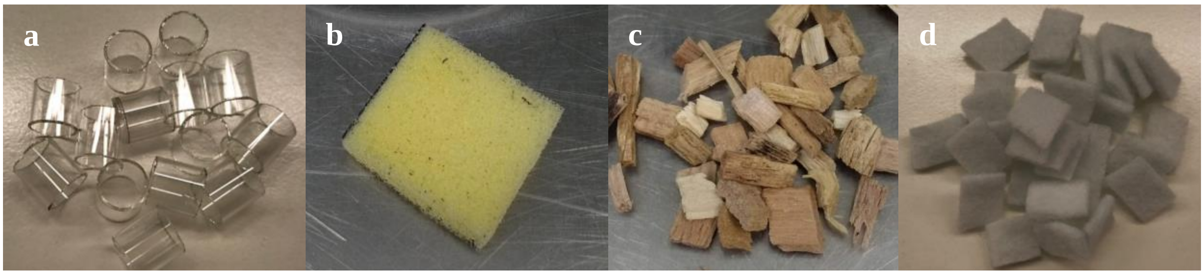

Figure 1 Images of packing materials: Raschig rings (a), PU foam (b), beech wood (c) and PES nonwoven (d)

The selected packing materials have been characterized using different techniques. Wettability properties have been analyzed by performing water contact angle (WCA) measurements with a Krüss K100 MK2 Tensiometer. Wilhelmy method has been applied and an average value of contact angle has been calculated by measuring three replicates of each sample. Surface morphology has been examined with a JEOL JSM-6010LV Scanning Electron Microscope (SEM). Surface area and porosity have been evaluated with a Quantachrome Nova 2200e surface area analyzer, using BJH (Barrett, Joyner and Halenda) method.

Simultaneously, the ability of *C. necator* to form biofilm on each of the different packing materials has been tested. This has been performed by incubating the bacteria, at a known cell density of 1-3x10^5 (colony formation units) cfu·mL^{-1}, with each material in triplicate at 30°C with rotational shaking. After 24h, five consecutive washes in phosphate buffer solution (PBS) have been performed to remove the cells that did not adhere to the surface. 1 minute of vigorously shaking with glass beads has been used to recover the cells forming the biofilm, and finally, different cell dilutions have been plated to quantify the cfu·mL^{-1} for each sample. In order to compare the different materials, the apparent volume (cm^3) has been used to normalize the amount of microorganisms adhering to the materials.

The materials that present better microorganism adhesion results, as well as better surface properties will be selected and surface-treated in a low pressure plasma TETRA 30 PC LF laboratory system (from Diener Electronic GmbH). The effectiveness of the different treatments will be evaluated using the same characterization techniques presented above.

3. EXPERIMENTAL RESULTS AND DISCUSSION

3.1 Contact angle

WCA on packing materials can provide information of their hydrophilicity, which is related to the wettability of the materials. An angle below 90º is considered to be hydrophilic. Therefore, as it can be seen in Table 2, all the packing materials present a hydrophilic surface. Raschig rings, as standard packing materials, present the lowest WCA and thus the best wettability. This material is followed by beech wood and polyester textiles, which are the alternative packing materials with the highest wettability. Finally, eucalyptus wood and polyurethane foams have a WCA close to 90º, meaning that they could be considered almost hydrophobic.

Table 2 Contact angle of untreated packing materials

Category	Contact angle
Raschig rings	58.2º
Beech wood chips	64.1º
Eucalyptus wood chips	88.2º
Soft polyether-polyurethane foam	88.8º
Hard polyether-polyurethane foam	88.8º
Polyester woven textile	66.6º
Polyester nonwoven	66.6º
Polyester 3D textile	65.8º

Plasma treatment of the alternative packing materials is expected to introduce polar functional groups on their surface, which will increase their hydrophilicity to similar or higher levels than Raschig rings.

3.2 Surface morphology

Surface morphology of packing materials has been observed by SEM in order to analyze their structure, roughness and pore size. In Figure 2 the surface morphology of the studied packing materials is presented.

The interior and exterior sides of Raschig ring show a completely flatten surface, without scratches. Their cross-section shows a burr that increases the surface roughness on the edges of the Raschig ring. Beech wood chips present vessels on their surface and the cross-section images show pores of about 40-50 μm. Eucalyptus wood chips have a more irregular surface and lower pore sizes of about 2-10 μm. Soft and hard polyurethane foams show an open cell structure with cell size of approximately 0.4 and 0.5 mm, and pore size of about 0.15 and 0.21 mm, respectively. Polyisocyanurate-based foams present a closed cell structure of about 50-200 μm. Polypropylene pellets show a flat surface with higher roughness on the material edges. Regarding the polyester textiles, SEM images show the different fiber distribution: the woven textile shows the typical structure with warp and weft, the nonwoven fibers are randomly distributed and 3D textiles have different structures on the same material.

During low pressure plasma treatments, the plasma species will interact with the material and, due to their high energy, the surface roughness will be modified.

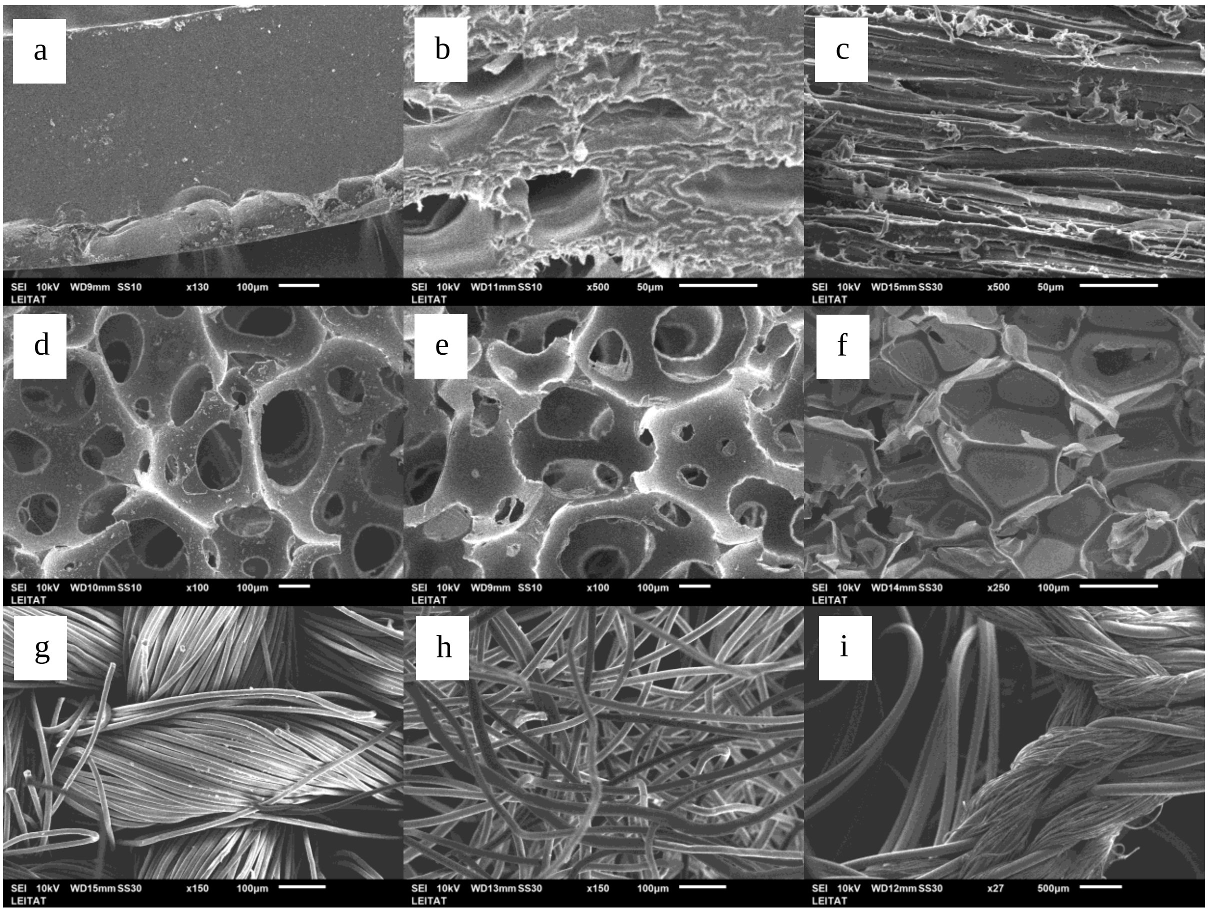

Figure 2 SEM images of surface morphology of Raschig ring corss-section (a), beech wood chip cross-section (b), eucalyptus wood chip (c), hard (d) and soft (e) PU foam, polyisocyanurate-based foam (f) and polyester woven (g), nonwoven (h) and 3D (i) textile.

3.3 Surface area and porosity

Surface area is also a relevant property, since it is the area available for microorganisms to adhere and grow on the packing materials. Analysis for determining surface are and pore volume are being currently carried out, following BJH method.

Preliminary results show that polyisocyanurate-based foams and polyester nonwoven present the highest surface area, whereas the total pore volume is higher in polyurethane and polyisocyanurate-based foams.

The surface modification of the packing materials by low pressure plasma is expected to increase the surface roughness and, therefore, the surface area and total pore volume.

3.4 Bacterial adhesion

The microorganism *C. necator* displayed ability to adhere to all the selected materials. The results have been normalized by using the apparent volume (cm^3) of packing materials and are shown in Figure 3. According to these results, the best supports for biofilm formation are PES nonwoven textiles with $2.3 \cdot 10^9$ $cfu \cdot cm^{-3}$ followed by PES woven textile with $1.6 \cdot 10^9$ $cfu \cdot cm^{-3}$. PES 3D textiles and hard wood chips also showed a high biofilm formation. In the case of Raschig rings, the normalized results are $8.8 \cdot 10^6$ $cfu \cdot cm^{-3}$, being this material worse than textiles and hard wood chips, in terms of biofilm formation. Finally, polyisocyanurate foams, polyurethane foams and PP pellets were the materials with the lowest microorganism adhesion.

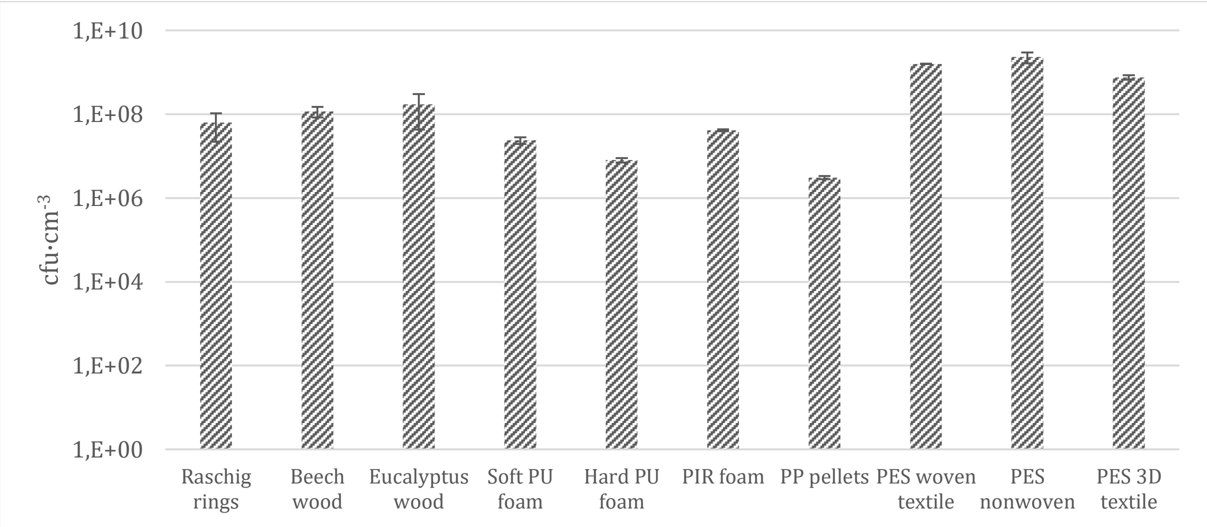

Figure 3 Biofilm formation of *C. Necator* ($cfu \cdot cm^{-3}$) in each packing material..

Further analysis will be performed on the untreated materials in order to determine biofilm distribution, susceptibility of the packing material to degradation, accessibility of nutrients and biofilm survival over time. The plasma-treated materials, which are meant to achieve a higher biofilm formation, will be tested with the same procedures.

4. CONCLUSION

BIOCON-CO2 project is still ongoing and currently only results from untreated materials characterization are available. These results show that the alternative packing materials studied present lower wettability, compared to the standard Raschig ring. SEM images show different surface morphologies, taking into account roughness, structure and pore size and distribution. Regarding the biofilm formation, polyester textiles and hard wood chips showed the highest biofilm formation.

A final selection of the pre-selected materials will be made taking into account the following aspects: adhesion of bacteria *C. necator*, adaptability of the packing materials inside the reactor, degradation suffered by the packing materials over time, accessibility to nutrients by the bacteria, biofilm survival over time and cost of packing materials. The results obtained so far show that polyester textiles and hard wood chips seem to be the most suitable alternative packing materials which could be potentially used in the TBR to produce 3-HP.

The next step of the project is the low pressure plasma treatment of the alternative packing materials, aiming to promote wetting and adhesion of bacteria, as well as to increase surface area. The plasma-treated packing materials are meant to enhance adhesion and biofilm formation, and therefore achieve a higher 3-HP production efficiency.

5. AUTHOR'S BIOGRAPHY

Ruth Garcia-Campà is Researcher at LEITAT Technological Center, in the Surface Technologies Unit. Her main research lines are focused on surface modification and functionalization of fibers, yarns and fabrics, as well as on the reduction of the environmental and human health impact caused by the conventional textile dyeing and finishing processes. She has also experience in plasma treatments of different textile substrates, for surface activation and plasma-enhanced chemical vapor deposition processes.

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