Evaluation of different support materials used with a photo-fermentative consortium for hydrogen production

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ABSTRACT

Four different support materials were evaluated for immobilization of a consortium of purple non-sulfur bacteria (PNS) producing hydrogen gas. The inoculum was enriched from a microbial fuel cells. Tested materials were acrylic rods, silica gel, high-density polyethylene cylinders and luffa (dispersed fibers and cubes). The area/volume ratio, volatile solids per volume and the amount of hydrogen produced using volatile fatty acids as substrate were determined. The experiments were conducted in serological bottles of 120 mL, continuously illuminated at 7 klux, mixed at 100 rpm and 32 °C. It was found that the PNS bacteria generated 16.7 ± 2.6 mLH_2/gVS-h. The highest biomass quantity (as volatile solids, VS) was found in dispersed luffa fibers (8.0 ± 1.7 $mgVS/cm^3$) followed by luffa in cubes, high-density polyethylene cylinders and acrylic bars. Dispersed luffa allowed a better light distribution and contact with the media than luffa in cubes. Silica gel was not suitable for the immobilization and hydrogen production. Experiments with the colonized supports indicated that no significant differences regarding specific hydrogen production were observed among luffa, acrylic bars and polyethylene. In conclusion the luffa in the form of fibers is a suitable support for purple non-sulfur consortium because of this provide the highest area/volume ratio and the highest attached quantity of VS per volume. After several batches the biomass remained attached to the support.

Keywords: Hydrogen, purple non-sulfur bacteria, cell immobilization

1. Introduction

Hydrogen is the most promising alternative to fossil fuels since it combust cleanly producing only water, can be converted to electricity via fuel cell technology, can be transported for domestic/industrial consumption through conventional means and it is a renewable energy [1,2]. Most importantly, hydrogen has the highest energy content per unit weight (142 kJ/g) [1]. Hydrogen can be produced from renewable sources, even waste materials, by biological processes operated at ambient temperatures and pressures; therefore, less energy intensive than thermochemical and electrochemical ways generally used [3].

Purple non-sulfur (PNS) photosynthetic bacteria are found as the most promising microbial system for biohydrogen production, due to high substrate conversion, lack of oxygen-evolving activity, ability to use a wide wavelength of light and capability to use organic substrates (sugars or volatile fatty acids, derived from wastes) for hydrogen production [4]. Even more, gas produced by PNS bacteria consists of 80-95% (v/v) of H_2. The remaining part is mostly CO_2, which can be removed easily [5]. This biochemical process is catalyzed by nitrogenase in the absence of nitrogen. Sufficient source of ATP is required for efficient hydrogen production. Energy from light enables PNS bacteria to overcome the thermodynamic barrier in the conversion of organic acid into hydrogen [6].

Several studies have been conducted to evaluate the different factors affecting the hydrogen production in the photofermentation process as type of substrate and concentration [7, 8, 9], C/N ratio [10], pH [8], wavelength [11, 12], light intensity [11, 13, 14, 15] and illumination protocols [11, 16, 17]. However, the yields are still low. Hydrogen yields can be improved by maintaining a maximal activity of nitrogenase, a favorable molar C/N ratio and a uniform distribution of light through the culture [6].

Processes with immobilized cells have different advantages: the systems can be operated in exponential growth phase for a long period of time, less space and volume of growth medium are required, higher catalytic stability and resistance against toxins or enzymatic inhibitors, higher concentration of cells per unit volume of the bioreactor than suspended cells process and easier recovery of cells and reuse of these [6, 18].

These advantages have been tested entrapping cells of *R. rubrum* in agar or agarose [19], *R. sphaeroides* in chitosan, polyethyleneimine, trimethylammonium glycol chitosan iodide and poly-L-lysine [20], *R. faecalis* in agar [21] and *R. palustris* in polyvinyl alcohol, sodium alginate and carrageenan powder [12]. However, the surface attachment seems to be more proper for photofermentation because this technique offers less mass transfer resistance, higher operation stability and easier light penetration than entrapment [2, 22]. Different supports have been evaluated for biofilm formation, *R. sphaeroides* on porous glass [23], glass textile fiber [24] and filter porous glasses [18], *R. palustris* on activated carbon, silica gel and clay [25, 26], polymethyl methacrylate [27], glass slide [28], glass beads [2] and optical fiber [22], and *R. faecalis* on activated carbon fibers [29]. Nevertheless, all this studies were conducted with pure cultures. The use of mixed cultures can be beneficial for the treatment of complex substrates [30].

This work evaluates four different support materials for immobilization of a consortium of purple non-sulfur bacteria (PNS) producing hydrogen gas. The main objective was to identify the support that can allow the highest quantity of cells immobilized per volume and the highest production of hydrogen using a synthetic medium containing sodium acetate and sodium butyrate as carbon source. The performance of the hydrogen production using the colonized supports batch cultures was evaluated.

2. Experimental

2.1. Bacterial consortium and media

The hydrogen producing bacterial consortium was isolated from the anode of a bioelectrochemial system used in hydrogen production. Bacteria were grown on basal medium [31] consisting of (g/L) K_2HPO_4 750, $MgSO_4$ 200,

$FeSO_4 \cdot 7H_2O$ 11.78, H_3BO_3 2.80, $Na_2MoO_4 \cdot 2H_2O$ 0.75, $ZnSO_4 \cdot 7H_2O$ 0.24, $MnSO_4 \cdot 4H_2O$ 2.10, $CuCl_2.2H_2O$ 0.04, $CaCl_2.2H_2O$ 0.75, EDTA-Na 2.00, thiamine 3.78 and Biotin 3.57. The medium was supplemented with sodium acetate (2.46 g/L), sodium butyrate (3.30 g/L) and sodium glutamate (0.37 g/L). The C/N ratio was 80 [1]. The initial pH was adjusted to 6.8. Argon was used to obtain anaerobic conditions in the medium. The cultures were conducted to 32°C with continuous illumination to 5 klux in 1 L bottles mixed with a magnetic stirrer. The biomass was collected by centrifugation (Centrifuge Solbat C-40) to 3500 rpm for 15 min.

2.2. Support materials

Four different materials were selected as possible supports for the bacterial consortium. The material were acrylic rods (6 mm diameter and 7 mm length, in average), silica gel (Yamani, JIS Z 0701), high-density polyethylene (PE) cylinders (BCN 009 plus, 2H Germany) and luffa (dispersed fibers and 1 cm^3 cubes). Densities of the support materials was determining by weight in analytical balance (Ohaus Adventurer Pro AV114C) and displacement of water in Falcon tubes. The volume and area of the luffa fibers were determined measuring the diameter and length of the fibers through microscopy (Leica DM 500, Optical Len ICC50 HD). For that, the fibers were previously stained with methylene blue. Plastic materials (acrylic and polyethylene) were immersed in wastewater during three days to oxidize the surface and help to the biofilm formation.

2.3. Immobilization procedure

For the colonization, the supports were placed in 120 mL serum bottles with 75 mL of growth medium and were inoculated with 100 mg/L of inoculum. The bottles were kept, during 29 days, at 32±2 °C, with continuous illumination at 7 klux, and 100 rpm orbital shaking (Barnstead Lab-Line SHKE2000). The cultures were illuminated with yellow LED (590 nm) and tungsten lamps. Bottles inoculated in similar manner but without support material were used as control. During the immobilization period, biogas production was measured and samples of the biogas were taken off to measure the gas composition. At the end of the immobilization period, suspended biomass concentration and fixed biomass were determined. Biomass was separated of the medium by centrifugation and COD and pH of the medium were measured.

2.4. Hydrogen production by immobilized consortium

To evaluate the hydrogen production by the immobilized biomass, a sample of the colonized supports were taken off and washed with fresh medium to eliminate suspended cells. Then, they were transferred to fresh medium, but with the COD diluted ten times (sodium acetate 0.246 g/L, sodium butyrate 0.330 g/L and sodium glutamate 0.037 g/L), to maintain a similar COD/biomass ratio to that used in the colonization experiments. Culture conditions were the same than described in Section 2.3 and they were kept for four cycle cultures with a total time of 18 days. Volume and composition of produced biogas were determined during this time. At the end, pH, COD and biomass concentration were evaluated.

2.5. Analytical methods

Light intensity was measured on bottles surface with a luxmeter (Extech LT300) and pH value with a pH meter (OAKTON 510, probe Orion 9156BNWP). Suspended cell concentrations of the cultures were determined by optical density at 660 nm using a UV-VIS spectrometer (Perkin Elmer, Lambda 25). Calibration curves were previously elaborated to relate this measurements with Volatile Suspended Solids determined according the Standard Methods [32]. The amount of biomass attached to the supports was determined by estimating the protein concentration according to a modified Lowry method [33]. The supports were sonicated during 45 min in a bath (Branson 2510R-DTH) previous the protein determination. COD was measured by dichromate method [34]. A spectrophotometer (HACH DR/2010) was used.

The composition of biogas was measured by a gas chromatograph (SRI 8610C) equipped with a thermal conductivity detector (TCD) and a 1.82 m long silica gel column followed by a 1.82 m long (3.175 mm) molecular sieve column 13x. The initial column temperature was 40°C, which was held for 4 min and then gradually increased 20°C/min up to 110°C. The injector and detector temperatures were 90°C and 150°C, respectively. Nitrogen was used as the carrier gas at a flow rate of 20 mL/min.

Hydrogen production performance was fitted to modified Gompertz equation (1) [29, 35]:

$$H = H_{max} exp\left\{-exp\left[\frac{R_{max} e}{H_{max}}(\lambda - t) + 1\right]\right\} \quad (1)$$

where H (mL/g SVT) is the cumulative amount of hydrogen produced at culture time t(h), H_{max} (mL H_2/g SVT) is the maximum amount of hydrogen produced, R_{max} (mL H_2/g SVT/h) in the maximum hydrogen production rate; λ (h) is the lag phase time.

3. Results and discussion

3.1. Support characterization

Supports materials were selected by choosing a high A/V ratio and the facility to be suspended in the medium. In addition, a suitable light distribution was searched to be present with the supports. Table 1 shows the results for density and A/V ratio for supports evaluated. Silica gel and luffa presented the lowest density and highest A/V ratio, which can provide a high surface for the immobilization and facility for mixing.

Table 1 Characteristics of the support materials.

Support material	Density (g/cm^3)	A/V (cm^2/cm^3)
Luffa	0.82	244.0
Acrylic rods	1.25	9.6
PE cylinders	0.92	68.7
Silica gel	0.70	5000000

3.2. Hydrogen evolution during colonization

The hydrogen production per gram of total volatile solids, VS, (suspended and attachment cells) was fitted to the Gompertz model as shown in Figure 1. Parameters obtained with the model are presented in Table 2. The maximum hydrogen production rate was reached with acrylic rods; however, the maximum specific hydrogen production was similar for luffa fibers, luffa cubes, polyethylene cylinders and control. Cultures with luffa cubes had a lower rate and hydrogen production than luffa fibers. The difference among the two configurations is explained because of the different light distribution between them. Luffa cubes is a more compact structure that can block the light pass through the support. In the case of silica gel, minimal biogas quantity was detected in the first 200 h, after that, production stopped. Although silica gel has been reported like a support for PNS bacteria [25], this has been previously sterilized. In contrast, in this study minimal treatment was used (only washed with deionized water). For all the cultures, a pH increment of 0.3±1 units was observed, which can be associated to the ammonia production. Nevertheless, in the case of silica gel, the pH decreased to 5.6 ± 0 1, which may indicate that other microorganisms non-hydrogen producers (fermentation bacteria) were developed. COD removal percentages (average of 72.2 ± 4.2 %) were similar for luffa, acrylic rods, polyethylene cylinder and control.

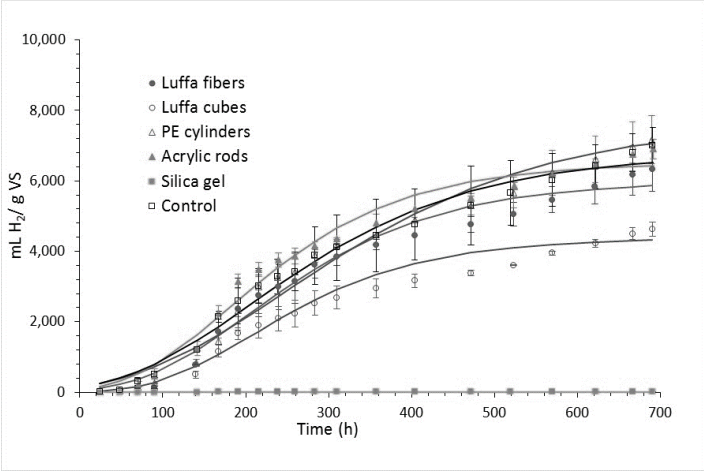

Figure 1. Cumulative hydrogen production and Gompertz fit as a function of time.

Table 2. Gompertz parameters, COD removal and final pH during colonization experiments

Support material	Gompertz parameters			ΔCOD %	Final pH
	$H_{máx}$ (L H_2/g SV)	$R_{máx}$ (mL H_2/g SV/h)	λ (h)		
Luffa fiber	6.0	17.0	73.7	71.6	7.1
Luffa cubes	4.4	14.0	92.0	69.4	7.2
Acrylic rods	6.5	20.2	61.5	70.9	7.2
PE cylinders	7.7	15.7	66.0	79.6	7.2
Control	6.8	16.4	54.1	69.4	7.1

3.3. Immobilized biomass

Figure 2 shows the results for the biomass quantification for the tested supports. After immobilization, a maximum amount of biomass per volume of support was found for the dispersed luffa fibers (8.0 ± 1.7 mg VS/cm^3). The highest amount of attached biomass correlated with the highest A/V ratio. When the supports were transferred to fresh medium to evaluate the hydrogen production by immobilized biomass, the biofilm increased between 2.5 and 4.5 times on the supports. At the end of the experiment, the biomass attached to the luffa fibers was 19.6 ± 1.5 mg SV/cm^3, 1.8 times more than the observed for the luffa cubes, 9.8 times more than PE cylinders and 16.2 times than acrylic rods. This increase in the immobilized biomass had been observed through several cycles of semicontinuous operation for *R. palustris* on filter porous glasses [18], reaching a final concentration of 19 mg/cm^3, similar to founded in the luffa fibers tested in this study. A lower biomass concentration has been found with *R. sphaeroides* in porous glass (11.2 mg/cm^3) [23]. In contrast with this study, porous glass required chemical modification to ensure biofilm formation. Silica gel was not appropriate for immobilizing the hydrogen producer consortium.

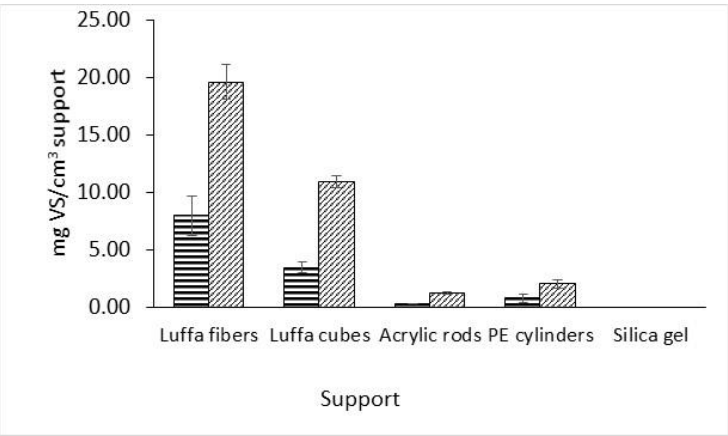

Figure 2. Immobilized biomass quantification. ▤ Immobilization, ▨ Evaluation of supports in hydrogen production.

3.4 Hydrogen production by immobilized consortium

Figure 3 presents the results for the evaluation of hydrogen production using the colonized supports. During the first batch cycle, the cumulative hydrogen production was lower than in subsequent cycles, which can be explained by an adaptation period of the cells. Zagrodnik et al. [18] explained this behavior because of the low bacteria concentration in the biofilm. The maximum specific hydrogen production for the subsequent batch cultures were similar in the different supports. In contrast with colonization experiments, here the luffa fibers and luffa cubes had similar productivities, because the suspended cells were lower than in the colonization experiments, and then the light presented a better distribution. Luffa productivities were slightly higher than with plastic materials, which may indicate a higher affinity of cells by natural materials. Luffa is mainly constituted by cellulose, lignocellulose and lignin. Patel et al. [36] observed the production of H_2 in dark fermentation with cells immobilized in ligno-cellulosic materials and PVC, and they observed that some ligno-cellulosic materials improved the hydrogen productivity in comparison with immobilization with PVC.

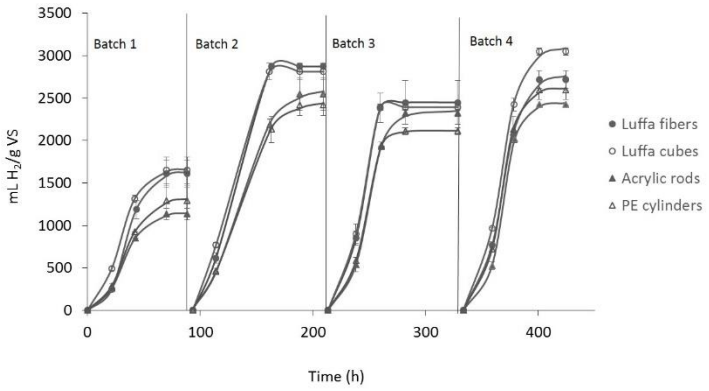

Figure 3. Hydrogen production with immobilized cells during four batch cycles (425 h)

COD removal obtained for all the tests were similar to colonization experiments (76±6.1 %). The results of this study were compared with other studies using immobilized cells in biofilms (Table 3). The obtained maximum hydrogen production rates are in accordance with that reported for other supports materials [18, 25-26, 29]. However, a highest hydrogen production was observed in the present study with the luffa present the additional advantage that sterilization or pre-treatments were no required.

Table 3. Comparison of hydrogen production using different support materials.

Bacteria	Support	R_{H2} (L/L_{cult}-h)	$H_{máx}$ (L/L_{cult})	Ref
R. sphaeroides	Filter porous glasses	0.007-0.059	-	18
R. palustris	Clay	0.038	1.54	25
	Silica gel	0.035	1.79	
	Active carbon	0.029	1.37	
R. palustris	Clay	0.039	3.19	26
R. faecalis	Active carbón fibers	0.033	3.45	29
Bacterial consortium	Luffa fibers	0.014	4.88	This study

4. Conclusions

Maximum hydrogen production rates of 16.7 ± 2.6 mLH_2/gVS-h were obtained with the evaluated supports. Maximal hydrogen production is mainly influenced by the concentration of the cells in the support materials. It was found that luffa fibers is the most suitable support for the hydrogen producer consortium because allowed the highest

amount of attached biomass (19.6 ± 1.5 mg SV/cm^3) and allowed a better light distribution and the highest cell concentration. Percentage of COD removal was 76±6.1 %.

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