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Support material dictates the attached biomass characteristics during the immobilization process in anaerobic continuous-flow packed-bed bioreactor

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ABSTRACT

Hydrogen is considered to be an ideal energy alternative to replace environmentally burdensome fossil fuels. For its long-term production the immobilized biofilm system is the most promising and to choose the right support material the most challenging. In this respect, the anaerobic up-flow bioreactors packed with four most used support materials (polyethylene, polyurethane, activated carbon and expanded clay) were tested to investigate the crucial bacteria sensitive period-the immobilization process. Seven-day-operation was necessary and sufficient to reach metabolic and microbial stability regardless of support material used. The support material had an influence on the microbial metabolic activity as well as on quantity and quality characteristics of the immobilized microbial community, being polyethylene and expanded clay more appropriate as supports among the materials evaluated; this could be attributed to pH alteration. The obtained results suggest that the support material dictates the outcome of the immobilization process in the anaerobic continuous-flow bioreactor.

1. Introduction

One of the most important challenges of this century is to develop new sources of renewable energy that might be able to replace fossil fuels. Hydrogen is a promising fuel because it is clean, renewable, and has a high energy density. Hydrogen can be produced by a number of processes (reviewed in Ref. [1]). Currently, it is commercially produced almost exclusively by electrolysis of water or thermochemical processes, both of which are highly energy intensive. Therefore, environmentally friendly technologies with little energy consumption are required. In nature hydrogen can be produced biologically (biohydrogen) by autotrophs as well as heterotrophs, and via two main pathways: photosynthesis and fermentation (reviewed in Ref. [2]). Anaerobic fermentation, also known as dark fermentation, is a light-independent process conducted by fermentative microorganisms, such as strict anaerobes (*Clostridium* sp. [3], thermophiles [4] and methanogens [5]), facultative anaerobes (*Enterobacter* sp. [6], *Escherichia coli* [7], *Bacillus* sp. [8], *Klebsiella* sp [9]. and *Citrobacter* sp [10]), or mixed cultures. All these bacteria are known to produce biohydrogen [11], formation of which is coupled to the production of volatile fatty acids and solvents. The most common fermentation products are ethanol, acetate, propionate, lactate and butyrate, and depend on the catabolic pathways involved in the fermentation process [12].

Bacteria mainly exist in one of two types of population: planktonic (freely existing) and sessile (attached to a surface or within the biofilm). Biofilm attached to the surface consists of many bacteria co-adhered by means of physical appendages and extracellular polymeric substances as well as various other components (water, proteins, nucleic acids, ions) [13]. Biofilm growth is governed by a number of physical, chemical and biological processes (reviewed in Ref. [14]).

Hydrogen producing microorganisms can be rapidly grown in bioreactors. Previous studies have shown that the co-cultures achieve better results of biohydrogen production than monocultures in the batch reactor system (reviewed in Ref. [11]), although the largest disadvantage of using mixed cultures is the coexistence of both hydrogen producers and consumers. Therefore, it is very important to select an appropriate pretreatment operation to enrich the hydrogen producing bacteria; this can be, for instance, achieved using heat treatment, acid/base treatment, methanogen inhibiting chemicals or others (reviewed in Refs. [15,16]). Furthermore, some of previous studies have also shown that biohydrogen

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production in immobilized biofilm system outcompete the suspended cell culture system [17,18]. Various natural and artificial materials to support cell growth have been evaluated in hydrogen production and under different operational conditions. The most used support materials are expanded clay [19–21], activated carbon [20,21], ceramic rings or balls [18,21], low-density polyethylene [20,21], polyurethane [22], polystyrene [19] and polypropylene [23]. It is known that surface characteristics dictate microbial adhesion capacity [24] and that the support material might select different microbial populations in biofilm [21]; however, the support material might even have no significant effect [20]. Consequently, there is a similar outcome also in the assessment of hydrogen production, with supports exhibiting either significant [21] or marginal influence on hydrogen production [20].

The great majority of studies of biohydrogen production in continuous-flow bioreactors evaluated the biomass attachment and microbial characteristics at the end of reactor operation, i.e. within the mature biofilm (e.g. Refs. [20,21,25]), but we are not aware of reports concerning the biomass characterization during the process of biomass attachment (immobilization process). Therefore, the aim of this study was to thoroughly investigate the biomass characteristics during the immobilization process and regarding to most used natural (activated carbon, expanded clay) and polymeric (polyethylene, polyurethane) support materials in an up-flow continuous-flow packed-bed bioreactor. The metabolic products as a reflection of microbial metabolic activity during each day of the immobilization process were evaluated. To check further whether the roughness of surface influences the biomass attachment, the surface of polyethylene support was smoothened due to the exposure to elevated temperatures reaching the melting point of the material.

2. Materials and methods

2.1. Inoculum and inoculum pretreatments

The seed sludge in this study used as inoculum was obtained from the anaerobic digester treating waste sludge from the municipal wastewater treatment plant (central waste water treatment plant of Ljubljana, Slovenia; Ljubljana CWWTP) operating in the mesophilic temperature conditions. The collected anaerobic sludge with mixed unknown cultures was filtered through sieves (Retsch, S/N 07039992, d_{mesh} = 2.8 mm and S/N 16022155, d_{mesh} = 0.09 mm) to remove larger solid particles that could clog the reactor tubing, and then stored at 4 °C. The characteristics of the employed inoculum are described elsewhere [26].

In order to enrich the concentration of hydrogen producing microorganisms in the inoculum, the following pretreatments were performed [26]. Inoculum was first stabilized at 37 ± 1 °C in the incubator (type SP-45C, Kambič, Slovenia) for 2–3 days to allow the mesophilic bacteria to multiply and spores to germinate. The pH value of inoculum was adjusted to 5.5 using 4 M HCl and maintained constant (using 1 M HCl) during the batch fermentation process for 24 h using a pH control unit, consisting of a titrator (model 718 STAT Titrino, Metrohm, Switzerland) and a pH electrode (model Easyferm food 325, Hamilton, Switzerland). The batch fermentation was carried out in a 500-ml glass reactor (model LF 100, Lenz, Germany) equipped with a heating jacket and connected to the heating circulator (Julabo, Germany) in order to maintain the reactor system at 37 ± 1 °C. Mixing was carried out using a magnetic stirrer (Rotamix 550 MMH, Tehtnica Železniki, Slovenia) rotating at minimum speed (approx. 60 rpm). Prior to use the batch reactor was flushed with N_2 (10 min, 500 ml/min) to ensure anaerobic conditions. During the performed batch fermentation the hydrogen producing microbial community was stabilized.

2.2. Biofilm support materials

To promote high and stable biomass growth in the continuous mode, two polymeric and two natural support materials were used as solid matrices for biomass immobilization: (i) polyethylene (Mutag BioChip™; Multi Umwelttechnologie AG, Germany; PE), (ii) polyurethane (Bio Contact-N; Nisshinbo Chemical, Japan; PU), (iii) activated carbon (Comelt S.p.A., Italy) and (iv) expanded clay, respectively. These support materials were chosen because they are the most used materials as biomass supports and have provided the best results in the production of biohydrogen (e.g. Refs. [21,27]). Their characteristics are presented in Table 1. The surface area and porosity analyzer (TriStar II 3020, Micromeritics, USA) was used to determine the BET specific surface area and pore size of support materials. The characteristics of polymeric materials investigated are according to manufacturers' specifications. For effective disinfection (reviewed in Ref. [28]) all support materials were, prior to use, rinsed thoroughly with ethanol (70%) followed by sterile double distilled water in order to remove the alcohol residues.

2.3. Experimental set-up and sampling

The glass reactor is a lab-scale custom made (at Jožef Stefan Institute, Ljubljana, Slovenia) up-flow anaerobic reactor packed with carrier materials, with an inner diameter of 3 cm and a total height of 40 cm. The total volume and working volume after packing are 230 and 100 ml, respectively. This glass reactor is equipped with a heating jacket and connected to the heating circulator (Julabo, Germany) to maintain the reactor temperature at 37 ± 1 °C. Prior to use the reactor packed with carriers was flushed with N_2 (10 min, 500 ml/min) to ensure anaerobic conditions. To maintain these conditions during the operation, the reactor system was continuously purged with Ar. The reactor was continuously operated at the hydraulic retention time (HRT) of 3.5 h and glucose (D-(+)-Glucose monohydrate, p.a.; Sigma-Aldrich®) as a substrate at the concentration of 2.5 g/l was used as a feed solution.

Four up-flow anaerobic reactors were packed with four different support materials described above and the immobilization process was carried out until the system reached the balance stage (±10% of differences between the values) regarding to the concentration of intermediate products. In order to monitor the metabolic status during the immobilization process, the liquid effluent samples

Table 1
Characteristics of the support materials used in the experiments.

Support material	Polyethylene	Polyurethane	Activated carbon	Expanded clay
Shape	Cylinder	Cylinder	Cylinder	Spherical
Length (mm)	1	4	4 ± 1	/
Diameter (mm)	4	4	4	4 ± 1
[a]Specific surface area (m^2/g)	<0.01	<0.01	767	0.33
Pore size (μm)	50–300	5–10	0.002–0.005	0.002–0.040

[a] BET method.