Process Biochemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Process Biochemistry

journal homepage: www.elsevier.com/locate/procbio

Screening and bioprospecting of anaerobic consortia for biohydrogen and volatile fatty acid production in a vinasse based medium through dark fermentation

Eduardo Bittencourt Sydney[a,*], Alessandra Cristine Novak[a], Drielly Rosa[b], Adriane Bianchi Pedroni Medeiros[b], Satinder Kaur Brar[c], Christian Larroche[d], Carlos Ricardo Soccol[b]

[a] Federal University of Technology – Paraná, Department of Bioprocess Engineering and Biotecnology, 84016-210, Ponta Grossa, Paraná, Brazil
[b] Federal University of Paraná, Department of Bioprocess Engineering and Biotechnology, Centro Politécnico, 81531-990, Curitiba, Paraná, Brazil
[c] Institut National de la Recherche Scientifique, Quebec, Canada
[d] Clermont Université, Université Blaise Pascal, Labex IMobS3, Institut Pascal, Polytech Clermont-Ferrand, 24 avenue des Landais, BP 20206, 63174 Aubière Cedex, France

ARTICLE INFO

Keywords:
Anaerobic fermentation
Mixed culture
Biohydrogen
Vinasse
Volatile fatty acids

ABSTRACT

The use of microbial consortia and industrial effluents for the production of biohydrogen by dark fermentation is seen as a key strategy in an attempt to overcome the economic and technical drawbacks of this potential technology. Three mesophilic microbial consortia were sampled and identified. Fermentation was carried out in a vinasse-based medium supplemented with pure or complex carbon sources under different conditions of H_2 partial pressure. Consortia LPBAH1 and LPBAH2 were predominantly composed of *Oxalobacteraceae* and *Lactobacillaceae*, while LPBAH3 was rich in sporulating *Lactobacillaceae* (> 96%). Each consortium presented specificities related to biohydrogen and VFAs production: (i) the highest biohydrogen yield was achieved with LPBAH1 (> 50% *Oxalobacteraceae*) in a vinasse medium supplemented with sugarcane juice ($1.59 \pm 0.21\ mol_{H2}/mol_{glucose}$); (ii) The lower H_2 yields were achieved with LPBAH3, which otherwise produced the highest amount of butyric acid (up to $10\ g\ L^{-1}$); (iii) LPBAH2 presented great stability in H_2 production in different conditions of H_2 partial pressure.

1. Introduction

The gradual introduction of fuels with increasingly lower carbon content per unit of energy (wood – coal – oil – natural gas) results in a continuous decarbonization of the global fuel mix. This chain of lower carbon content fuel ends in Hydrogen (H_2).

Currently, the cost of H_2 generated from biological processes is very high, especially due to medium cost and process sensitivity. In some bioprocesses for biohydrogen production (such as dark fermentation of organic matter) volatile fatty acids, which are platform molecules that can be used as raw material for green chemistry, are produced and accumulated in the liquid phase. The production and commercialization of these platform molecules will have a great impact on the economics of biohydrogen production technology [1], and thus deserve attention.

In the development of biohydrogen processes, two points are of great interest: (i) the use of agroindustry liquid and solid wastes as feedstock, promoting economic (and environmental) advantages; and (ii) the use of mixed cultures or consortia. The use of consortia in industrial fermentations presents some challenges, especially related to process stability, but offers less susceptibility to contamination by H_2-consuming bacteria and oxygen [2]. Moreover, a diverse microbial community is more adaptable to substrate variation (an intrinsic characteristic of (agro)industrial wastewater) due to the presence of alternative metabolic pathways capable of developing a food web where specific groups of organisms maintain a low concentration of critical intermediate products and promote the flux of carbon and electrons from the feedstock material to the desired end product [3].

With respect to the range of potential substrates which can be utilized by the broad range of hydrogen-producing bacteria, it can be stated that, at present, it is a vast and open field for further exploration. One of the major bottlenecks in developing large-scale biohydrogen technologies using waste is its availability and volume of production. Since use of bioH_2 is mostly aimed at the production of energy, it can be

* Corresponding author.
E-mail address: eduardosydney@utfpr.edu.br (E.B. Sydney).

https://doi.org/10.1016/j.procbio.2018.01.012
Received 3 November 2017; Received in revised form 17 January 2018; Accepted 18 January 2018

considered a fair value-added product, and thus has to be produced in very large quantities.

Since the last decade, complex carbon sources, such as molasses [4], food waste [5], dairy wastewater [6], mushroom waste [7], rice slurry [8], cheese whey [9], lignocellulosic materials, glycerol waste [10], vegetable waste [11] and many others have been studied and proved to be viable for the production of biohydrogen by dark fermentation.

One of the largest industries in the world is the bioethanol industry. In tropical countries, bioethanol is produced through a classic fermentation process, in which yeasts transform sugarcane juice, molasses, or a molasses-juice mixture into ethanol. At the end of fermentation, almost 100% of the sugar (sucrose) present in the culture medium is consumed by the yeast (usually a *Saccharomyces*), resulting in a liquid called wine with a yield of approx. 50%. The wine has a concentration of ethanol (% by volume) between 6 and 10° GL, which is recovered by distillation at the top of distillation columns, where the volatile substances are separated based on their different boiling points [12]. From the base of the column, the fermented broth free of ethanol, called vinasse, is removed. Vinasse contains some organic solids in suspension as well as minerals, residual sugar, and some volatile compounds.

Considering the ethanol concentration in the wine, vinasse is generated in an average proportion of 12–15 L for each liter of alcohol produced. Because of its production rate and its chemical characteristics (see [13]), vinasse constitutes a large pollution source. It is used indiscriminately as fertilizer, representing a greater risk, especially to soil fertility and underground water [14]. In this perspective, it is of great importance to give a more rational destination to vinasse.

In this work, the bioprospecting, screening and identification of microbial consortia capable of producing biohydrogen in the vinasse-based medium are presented. Biohydrogen productivity, VFAs production, the effect of partial H_2 pressure on the metabolism of the microbial communities and their production stability through time were evaluated.

2. Material and methods

2.1. Consortia sampling

Three samples from Brazilian environments with proper conditions for the development of anaerobic microorganisms (known presence of strict/facultative anaerobes and/or absence of oxygen) were collected. The samples were transferred to screw cap glass bottles with the aid of 10 mL sterile syringes (in the case of liquid samples). Their transport was conducted in closed-cell extruded polystyrene foam with dry ice. The name of the strains and the sampling sites are described in Table 1.

2.2. Molecular identification

The microbial identification of consortia LPB AH1, LPB AH2 and LPB AH3 was carried out at the WEMSeq Biotechnology Laboratory (Curitiba/PR – Brazil). For the sequencing and bioinformatics analyses, approximately 5 ml of each consortium sample was taken. The extraction of the total genomic DNA from the samples was performed with phenol/chloroform, followed by the PCR analysis for the V4 region of the 16S rDNA gene with 10 ng of DNA, primers 515F and 806R, in the KlenTaq system (Sigma) according to the methodology of [15]. In thermocycling, 96 °C were applied for 3 min, 18 cycles of 96 °C for 20 s, 50 °C for 45 s and 68 °C for 1 min. The resulting amplicons were analyzed by electrophoresis in 1.5% agarose gel and quantified with a Qubit kit (Invitrogen). The amplicons were diluted to 16 pM and sequenced on the Illumina MiSeq pallet with the 500V2 set, which generated 250 bp readings. The sequences generated were analyzed using the Qiime program, using a cut line of 16000 readings/sample with the Silva database with 97% identity.

Table 1
Origin of the samples collected from the Brazilian environment with potential for biohydrogen and VFAs production.

Name	Origin	Location
LPB AH1	Feces from fruit bat (unknown species)	24°35′42.0“S 48°36′17.7“W
LPB AH2	Liquid waste from a dairy farm	25°51′32.9“S 49°29′45.4“W
LPB AH3	Soil used for sugarcane cultivation	21°17′13.9“S 47°44′56.1“W

Table 2
The composition of the vinasse used during the experiments [12].

Parameter	mg/l	Parameter	mg/L
Iron	41.8	Potassium	2386
Manganese	3.7	Magnesium	203
Lead	< 0.1	Sulfate	1700
Cadmium	< 0.1	Total Phosphorus	104.9
Arsenic	< 0.1	DBO	8358
pH	4.52	DQO	29600
Nitrate	< 10	Sodium	20.1
Total Nitrogen (Kjeldahl)	2.15	Calcium	791

2.3. Medium composition, culture conditions and consortia maintenance

Vinasse composition and characterization are described in Table 2. Sucrose (VS), sugarcane juice (VSJ) or molasses (VM) were added in a vinasse medium as a carbon source at an equivalent concentration of 10 g/L of sucrose. Total carbohydrates in molasses were estimated using a refractometer, and in the sugarcane juice by using the Dubois et al. method [16].

The procedure for promoting an anaerobic culture was based on the Balch technique [17]. The removal of oxygen was achieved by boiling the medium under anoxic conditions (CO_2 atmosphere). Bicarbonate (1 g/L) was added at 85 °C and Cysteine-HCl at 65 °C as reducing agents to lower the redox potential of the medium. To assure oxygen removal, Resazurin was used as an indicator (0.5 mg/L).

The experiments were carried out in 15 mL Hungate tubes, with a working volume of 6 ml, sealed with autoclavable Bakelite screw caps and rubber stoppers, and incubated at 37 °C. The medium pH was adjusted with 1N KOH to 7.0. The cultures were maintained under these conditions for one week and then inoculated in a new medium.

Consortia maintenance was carried out by transferring the cell passages to a new fresh medium every two weeks. Monthly production of volatile fatty acids and hydrogen was carried out to evaluate consortia stability.

2.4. Biohydrogen production and composition analysis

Biohydrogen production in Hungate tube cultures was periodically measured using 60 ml plastic syringes. Daily gas quantification was carried out in cultures considered free of H_2 partial pressure or twice a week (more precisely on the 4th and 7th day of culture) in the cultures where H_2 partial pressure was allowed. Gas was collected by inserting a graduated syringe through the flange-type butyl rubber septum and purified for hydrogen content estimation, as discussed later. Purification was carried out by employing CO_2 absorption in an NaOH solution [18,19]. The column consisted of a graduated cylinder filled at 50% of its volume with 2 mm glass beads to increase gas contact time with the basic solution. Gas was injected at approximately 3 mL/s through a porous stone.

2.5. Analysis of organic components

The organic components of the culture medium were determined by using High Performance Liquid Chromatography (HPLC). Before injection, the samples (2 mL) were centrifuged and filtered (Millipore