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Economic process to produce biohydrogen and volatile fatty acids by a mixed culture using vinasse from sugarcane ethanol industry as nutrient source

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HIGHLIGHTS

- Industrial wastewater was utilized to produce bioenergy and volatile fatty acids.
- Complete metabolic analysis of 2 different anaerobic consortia were carried out.
- Very high biohydrogen production, yields and purity were achieved.
- The developed technology has a high potential to be transferred to the industry.

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ABSTRACT

This work evaluates the potential of vinasse (a waste obtained at the bottom of sugarcane ethanol distillation columns) as nutrient source for biohydrogen and volatile fatty acids production by means of anaerobic consortia. Two different media were proposed, using sugarcane juice or molasses as carbon source. The consortium LPBAH1 was selected for fermentation of vinasse supplemented with sugarcane juice, resulting in a higher H_2 yield of 7.14 molH_2 molsucrose^{-1} and hydrogen content in biogas of approx. 31%, while consortium LPBAH2 resulted in 3.66 molH_2/molsucrose and 32.7% hydrogen content in biogas. The proposed process showed a rational and economical use for vinasse, a mandatory byproduct of the renewable Brazilian energy matrix.

1. Introduction

Almost 100% of the (increasing) energetic demand is supplied by carbon-containing fossil sources such as oil, coal and natural gas. The environmental concerns involving the use of such sources of energy are related to the increase in atmospheric carbon concentration, which is the main cause of global warming and climate change.

The gradual introduction of fuels with increasingly lower carbon content (wood, coal, oil, natural gas) results in continuous decarbonization of the global fuel mix, which ends up as hydrogen. Hydrogen has a higher gravimetric energy density than any other known fuel and is compatible with electrochemical and combustion processes for energy conversion without producing the carbon-based emissions that contribute to environmental pollution and climate change (Cuetos et al., 2007).

Hydrogen can be produced through chemical, physical and biological processes. Among the biological ones, the most studied are photo-fermentation and dark fermentation. The advantages of dark fermentation over other biological processes are: (i) better process economy for lower energy requirements, (ii) process simplicity, (iii) higher rates of hydrogen production, and (iv) utilization of low-value waste as raw materials (Kim et al., 2008).

Among the wide range of by-products of diverse microbial metabolism, the two pathways producing hydrogen from

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carbohydrates are associated with acetate and butyrate, resulting respectively in 4 and 2 molH_2 molglucose^{-1}. The formation of relatively reduced organic molecules (e.g. ethanol, lactate, propionate) under non ideal conditions can inhibit H_2 production if allowed to accumulate (Redwood et al., 2009) and their production might be avoided.

The proper choice of microorganism(s) and substrate is crucial in the development of a feasible biohydrogen production technology. Although many studies have been carried out using pure cultures, when complex substrates are used, mixed cultures present some advantages. Besides being less susceptible to oxygen or contamination by other microorganisms, the presence of different microorganisms generally improves substrate degradation and consequently hydrogen production due to development of a food web where direct inhibition of microbial activity by metabolic intermediates is greatly reduced (Angenent and Wrenn, 2008).

Currently, the cost of H_2 generated from biological processes is very high. Several novel approaches have been proposed and studied to surpass the economical drawbacks (Hallenbeck, 2009) of H_2 production, such as the use of (agro) industrial waste. As media composition significantly affects the production of organic acids and hydrogen by dark fermentation, the composition of complex media plays an important role. Complex carbon sources, such as molasses (Ren et al., 2006), food wastes (Agrawal et al., 2007), dairy wastewater (Mohan et al., 2007), mushroom waste (Lay et al., 2012), rice slurry (Fang et al., 2006), cheese whey (Ferchichi et al., 2005), lignocellulosic materials, glycerol waste (Ito et al., 2005), vegetable waste (Mohan et al., 2009) and others proved to be susceptible for dark fermentation.

In Brazil, the industrial wastewater produced in larger amount is vinasse. Vinasse or stillage is a liquid residue removed from ethanol distillation columns at an impressive rate of 12–15 liters per liter of alcohol. Since Brazil's ethanol production in 2012/2013 is estimated in 25 billion liters, approx. 370 billion liters of vinasse will have to be disposed off. Vinasse is generally used as fertilizer, presenting some advantages in terms of sugarcane growth and productivity on optimal use. Due to high Chemical Oxygen Demand (COD) of approx. 15,000–27,000 mg/L, low pH (approx. 4.5) (Monteiro, 1975) and high potassium content, its use as fertilizer is limited. However, in practice, it has been used indiscriminately to a point that ground water contamination is being observed in some areas (Hassuda et al., 1989). Da Silva et al. (2007) stated that vinasse can promote changes of soil physical properties in two different ways by: (i) raising the capacity of infiltration water in the soil thus causing ions leaching and contamination of the groundwater; and (ii) reducing the rate of infiltration and increasing the runoff, resulting in possible contamination of surface water.

The long-term use of vinasse in productive lands can cause desertification and land salinization, causing productivity decrease, late maturing and decrease in sucrose content (Pinto et al., 1994). In this context, it is of great importance to give a more rational destination to vinasse.

In this work, the potential of vinasse as a medium for biohydrogen production by means of anaerobic consortia was evaluated. The main goal was to develop an economic and simple to handle process. Hence, only pH and the carbon/nitrogen ratio were modified to optimize H_2 production.

2. Methods

2.1. Microorganisms

Two consortia were evaluated as potential biohydrogen producers. One originated from a sample of fruit bat feces (LPB AH1) and the other from a lake of a dairy farm (LPB AH2). The choice of these 2 consortia was based on screening (data not shown) of 8 different consortia (besides LPBAH1 and LPBH2, the samples were taken from soil used for sugarcane cultivation; domestic sewage; swine feaces; mangrove from Matinhos/PR; cow feces; and puddle in a cave at São Paulo).

2.2. Medium composition and culture conditions

The procedure for promoting an anaerobic culture was based on the Balch technique (Balch et al., 1979). The removal of oxygen was achieved by boiling the medium under an anoxic ambient (CO_2 atmosphere). Bicarbonate was added at the temperature of 85 °C and cysteine–HCl at 65 °C as reducing agents to lower the redox potential of medium.

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

Vinasse was a courtesy of Usina São Manoel (São Manoel, São Paulo, Brazil) and was collected from the first storage tank situated after the distillation unit. Vinasse composition was determined by BioAgri Laboratories (Paulínia, São Paulo, Brazil). Anaerobic media containing vinasse and different carbon sources were used: (i) sucrose + vinasse, (ii) sugarcane molasses + vinasse and (iii) sugarcane juice + vinasse. Molasses was also a courtesy of Usina São Manoel while the sugarcane juice was collected at Curitiba (Paraná, Brazil).

2.3. Optimization and data analysis

Optimization was carried using the statistical tool "Essential Experimental Design", version 2.213. An inscribed central composite design with 2 factors at 3 levels and 3 repetitions at the center point was used for each strain. The response used for optimization was total biogas produced (in L gas/L medium) since a direct relationship was observed between biogas production and hydrogen content. Table 1 presents the values assigned to each level of the statistical plan.

2.4. Bioreactor cultures

The optimized condition was scaled up to an Inceltech LH-SGi set 2 M bioreactor (Inceltech, S.A., France). About 1.5 L cultures were carried out at controlled temperature (37 °C) with no agitation. pH was monitored by a pH sensor but was not controlled.

2.5. Biogas production and composition analysis

Biogas production in Hungate tube cultures was periodically measured using 60 mL plastic syringes. Gas quantification was carried daily in cultures considered free of H_2 pressure or twice a week (more precisely in the 4th and 7th day of culture) on those cultures subjected to H_2 pressure. Purification was carried out by an adaptation of a widely used technique that involves the

Table 1
Values of pH and carbon source assigned to each level of the optimization plan.

Carbon source (g/L)					
Level	−1.414	−1	0	1	1.414
Value	7.93	10	15	20	22.07
pH					
Level	−1.414	−1	0	1	1.414
Value	4.88	5.5	7	8.5	9.12