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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

injection of the biogas into a column containing a 10% NaOH solution. The column consisted of a graduated cylinder filled 50% of its volume with 2 mm sized glass beads in order to increase gas contact time with the basic solution. Gas was injected at approximately 3 mL/s through a porous stone.

In the bioreactor, gas measurement was carried out using an inverted beaker connected by a rubber hose to the bioreactor gas exit. Gas production was considered as the related volume of displaced water. At the end of the fermentation, the accumulated gas was sampled and analyzed. Gas analysis was carried out at the Institute for Technology Development (Instituto de Tecnologia para o Desenvolvimento – LACTEC) in a Thermo Gas Chromatograph equipped with Petrocol DH150 (50 m × 0.25 mm), DC 200 (1.8 m) and Porapak-N (2.0 m × 1/8″), which were placed in bypass series flow path of gas chromatograph system. The columns were connected to a TCD detector (block temperature: 120 °C, transducer temperature: 120 °C, filament temperature: 190 °C). This system allowed the measurement of oxygen (O_2), nitrogen (N_2), carbon dioxide (CO_2) and methane (CH_4). The amounts of hydrogen (H_2), hydrogen sulphide (H_2S) and water vapour (H_2O) could not be determined. However, based on the samples of similar fermented broths (media based on vinasse) measured in a PLOT U (8 m × 0.32 mm) column operated at 70 °C, at injector temperature of 70 °C, using hydrogen as carrier gas at 15ψ, the amounts of H_2S and H_2O were considered equal to 0.30% and 1.20%, respectively. Hydrogen (H_2) content was then considered as the amount to reach 100%.

2.6. Analysis of organic components

The organic components of the culture medium were determined through High Performance Liquid Chromatography (HPLC). The HPLC equipment was a Shimadzu Liquid Chromatography equipped with an Aminex® HPX-87H 300 × 7.8 mm (Bio-Rad) column and a refractive index detector (RID-10A). The column was kept at 60 °C and 5 mM and H_2SO_4 at 0.6 ml/min was used as mobile phase. The chemical species quantified by this method are glucose, fructose, succinate, lactate, formate, acetate, propionate and butyrate. All the reagents used were of analytical grade.

As the retention time of butyrate and ethanol were very similar, it was impossible to differentiate them by HPLC. The method used for determining ethanol content was based on the oxidation of ethanol to acetic acid by reaction with potassium dichromate in an acidic medium before reading at 600 nm using a spectrophotometer.

3. Results and discussion

3.1. Vinasse composition

The vinasse composition is presented in Table 2. The presence of ions important for the production of biohydrogen, such as manganese, magnesium and phosphorus were observed. Iron is also an important nutrient for biohydrogen production and is present in sufficient amount (10 mg L^{-1} iron was determined to be the optimum in batch hydrogen production by *Clostridium pasteurianum* (Liu and Shen, 2004)).

Moreover, the low content of nitrogen indicated that microbial growth would be limited, which was considered interesting as higher hydrogen yields would most probably be achieved by limiting cell growth, thereby enhancing catabolic processes.

Table 2
Composition of the vinasse used during the experiments.

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	29,600
Nitrate	<10	Sodium	20.1
Total nitrogen (Kjeldahl)	2.15	Calcium	791

3.2. Preliminary studies

Initial experiments were carried out in order to confirm the possibility of using vinasse as medium for biohydrogen production. As very poor biogas production was achieved in pure vinasse, different carbon sources were added at 10 g L^{-1}: sucrose, molasses or sugarcane juice. The results for biogas and volatile fatty acids production are presented in Table 3.

Data in Table 3 demonstrated that both consortia presented a high capacity to produce biohydrogen in vinasse medium. In vinasse medium supplemented with sugarcane juice, consortium LPB AH1 presented low inhibition of H_2 production by the effect of H_2 partial pressure. This is very interesting from industrial application viewpoint because it could facilitate process handling in industrial scale. On the other hand, the profile of VFAs with and without H_2 removal was very different, resulting in higher propionic acid accumulation.

Metabolic analysis of the consortium LPB AH2 showed higher potential for biohydrogen production in molasses (2.17 LH_2/L medium) and sugarcane juice (2.16 LH_2/L medium) supplemented media (Table 3), which corresponds to 1.53 $molH_2 \cdot molglucose^{-1}$ and 1.52 $molH_2 \cdot molglucose^{-1}$, respectively. The use of molasses together with the maintenance of a low H_2 partial pressure environment resulted in the exclusive production of butyrate (6.1 g L^{-1}).

In sugarcane juice supplemented medium, the consortium LPB AH1 showed the best results: hydrogen production reached 2.25 L H_2/L medium (which corresponds to 1.59 $molH_2 \cdot molglucose^{-1}$), accompanied by considerable amounts of acetate and butyrate production (3.5 and 7.6 g L^{-1}, respectively), which is interesting for coupling to methane or solvent production.

Based on these observations, the consortium LPB AH2 was chosen for biohydrogen and VFAs process in vinasse medium supplemented with molasses and LPB AH1 in vinasse medium with sugarcane juice. Since higher hydrogen production was closely related to higher biogas production, biogas was considered as a response factor for optimization.

3.3. Optimization of biohydrogen production of the consortia grown in vinasse-based medium

Both consortia culture conditions were optimized prior to scaling up. Results are presented in Table 4. The effect of pH and substrate concentration on biogas production could be seen from the 3-D plot presented in Fig. 1a and b, respectively.

A maximum production of H_2-rich biogas of 8.29 Lgas L^{-1} medium occurred at pH 7.0 and 12 g L^{-1} substrate was achieved by LPB AH1 in sugarcane supplemented vinasse medium. For the consortium LPB AH2, the best result (7.67 L gas/L medium) was achieved at pH 7.0 and 15 g L^{-1} of carbon source (molasses).

The reported pH and substrate range for the maximum hydrogen yield varied among the studies reported in the literature and it should be determined for each specific case. Fang and Liu (2002) described an optimal pH of 5.5 for the fermentation of glucose (7 g L^{-1}) by mixed culture, while Liu and Shen (2004) described an optimum pH of 7.0–8.0 for their experiments using starch at concentration of 15 g L^{-1}.

Table 3
Metabolic products of the cultivation of the consortia in vinasse medium containing different carbon sources; results are net production against blank (not fermented medium) and represent the average of 5 analyses (VFAs concentration is shown in g L^{-1}).

(1) with H_2 accumulation					(2) with H_2 removal				
Carbon source	Succinic	Lactic	Formic	Acetic	Propionic	Butyric	Ethanol	Hydrogen (L/L)	Biogas (L/L)
LPH AH1									
Juice	(1) 0	(1) 0	(1) 0	(1) 0.599	(1) 1.321	(1) 6.793	(1) 0	(1) 2.03 ± 0.31	(1) 6.8 ± 0.5
	(2) 0	(2) 0	(2) 0.185	(2) 3.525	(2) 0.408	(2) 7.642	(2) 0	(2) 2.25 ± 0.29	(2) 7.3 ± 0.4
Molasses	(1) 0	(1) 0	(1) 0.067	(1) 1.157	(1) 0.118	(1) 4.322	(1) 0.2	(1) 1.15 ± 0.24	(1) 5.2 ± 0.5
	(2) 0.05	(2) 0	(2) 0	(2) 2.085	(2) −0.4025	(2) 4.421	(2) 0	(2) 1.97 ± 0.26	(2) 5.8 ± 0.5
Sucrose	(1) 0	(1) 0	(1) 0.163	(1) −0.581	(1) −0.2	(1) 6.995	(1) 0	(1) 2.08 ± 0.19	(1) 6.2 ± 0.2
	(2) 0	(2) 0	(2) 1.393	(2) 1.049	(2) −0.451	(2) 4.824	(2) 0	(2) 2.94 ± 0.31	(2) 7.5 ± 0.2
LPB AH2									
Juice	(1) 0	(1) 4.197	(1) 0	(1) 2.956	(1) 0.311	(1) 6.313	(1) 0	(1) 1.74 ± 0.42	(1) 5.3 ± 0.4
	(2) 0	(2) 0	(2) 0	(2) −2.62	(2) 0.478	(2) 8.000	(2) 0	(2) 2.16 ± 0.35	(2) 5.8 ± 0.2
Molasses	(1) 0	(1) 0	(1) 0	(1) 3.783	(1) 0.383	(1) 6.396	(1) 0	(1) 1.45 ± 0.29	(1) 4.4 ± 0.3
	(2) 0	(2) 0	(2) 0	(2) −1.793	(2) −0.1355	(2) 6.067	(2) 0	(2) 2.17 ± 0.25	(2) 4.7 ± 0.3
Sucrose	(1) 1.17	(1) 0	(1) 0.876	(1) 1.333	(1) −0.398	(1) 7.044	(1) 0	(1) 2.29 ± 0.42	(1) 5.9 ± 0.4
	(2) 2.749	(2) 0	(2) 0.518	(2) 4.709	(2) −0.720	(2) 6.809	(2) 0	(2) 2.37 ± 0.20	(2) 6.4 ± 0.2

Table 4
Volatile fatty acids and gas production of the consortium LPB AH1 and LPB AH2 under conditions according to the statistical model used for optimization. The concentration of the carbon source, lactic, formic, acetic, propionic and butyric acids are showed in g L^{-1} and represent the net production against non-fermented medium.

pH	Carbon Source	Lactic	Formic	Acetic	Propionic	Butyric	Gas (L/L_{medium})
Consortium LPB AH1 – vinasse medium supplemented with sugarcane juice							
4.88	15.00	7.994	3.175	0.969	−0.576	1.331	2.88
5.5	10.00	0.000	0.000	2.430	0.046	6.123	7.38
5.5	20.00	8.323	3.218	0.772	−0.371	2.321	2.04
7	7.93	0.000	0.000	1.995	−0.187	5.150	2.88
7	15.00	0.000	0.000	2.280	0.146	7.638	8.27
7	22.07	9.257	3.564	0.952	−0.009	2.589	2.25
8.5	20.00	12.998	4.946	1.050	−0.456	1.916	3.00
8.5	10.00	0.615	0.000	0.000	−0.218	6.728	7.46
9.12	15.00	10.737	4.146	1.343	−0.150	2.874	2.89
Consortium LPB AH2 – vinasse medium supplemented with molasses							
4.88	15	2.017	0.000	−0.493	1.390	6.794	1.83
5.5	10.00	0.000	0.000	−0.450	−0.877	1.850	5.88
5.5	20	0.000	0.000	−0.643	−0.755	3.675	3.88
7	7.93	0.364	0.000	0.092	−0.756	1.538	5.67
7	15.00	0.000	0.000	−0.846	−0.293	6.065	7.61
7	22.07	5.817	0.000	−0.655	−0.628	4.296	6.44
8.5	20	8.095	0.000	−0.948	−0.696	3.403	6.33
8.5	10.00	0.368	0.000	0.659	0.514	6.289	5.92
9.12	15.00	7.936	0.000	−0.012	−0.558	0.836	3.72

It is interesting to observe from Table 4 the effects of pH and carbon source on the global system. Medium pH usually affects hydrogen production yield, biogas content, type of the organic acids produced and the specific hydrogen production rate (Kapdan and Kargi, 2006) because of its effect on the microbiology chain. It could be demonstrated (Table 4) that for consortium LPB AH1 low amounts of biogas was produced simultaneously for higher production of lactic acid (high pH and/or high carbon source concentration). This was also observed by Kapdan and Kargi (2006). Fermentation carried out by this consortium in pH 7.0 and 7.93 g L^{-1} carbon source resulted in low gas production probably due to the low content of fermentable carbon.

On the other hand, biogas production by the consortium LPB AH2 did not present a close relation to lactic acid production, even though the best result was achieved in the absence of lactic acid production. It can be observed that in extreme conditions of pH and carbon source large amounts of lactate was observed. The production of lactic acid during molasses fermentation for biohydrogen production was also observed by Kapdan and Kargi (2006).

The best mathematical model that satisfactorily fitted the results achieved for both consortia was a full quadratic model (Table 5), with R^2 higher than 0.90 for both consortia. Low coefficients of variation (20% for LPB AH1 and 14% for LPB AH2) and low standard error (1.095 for LPB AH1 and 0.809 for LPB AH2) were observed, which was impressive as higher variation was expected because of the complex composition of the medium. VIF values indicated the absence of multicollinearity among the regressors in both cases (Table 5). Low first order autocorrelation values of 0.358 (LPB AH1) and 0.347 (LPB AH2) were observed and indicated a weak positive autocorrelation between residuals.

The ANOVA analysis showed a low percentage of residuals (8% LPBAH1 and 10% LPBAH2), indicating that predicted responses were close to the obtained ones. The F test confirmed that the model was valid in a confidence interval of 99% (Fsignif < confidence interval) for LPB AH1 and 98% for LPB AH2.

3.4. Biohydrogen and VFAs production in bioreactor scale by the consortium LPB AH1

Biohydrogen and VFAs fermentation process using consortium LPB AH1 was carried out in vinasse medium supplemented with sugarcane juice. VFAs and biomass production profile during the fermentation are shown in Fig. 2(a), while gas production, carbohydrate consumption and pH variation is shown in Fig. 2(b).

Initial sugar content was equal to 11.48 g L^{-1} and it was completely exhausted by the end of fermentation. Biomass production was equal to 0.25 g L^{-1}. A higher production of VFAs, mainly

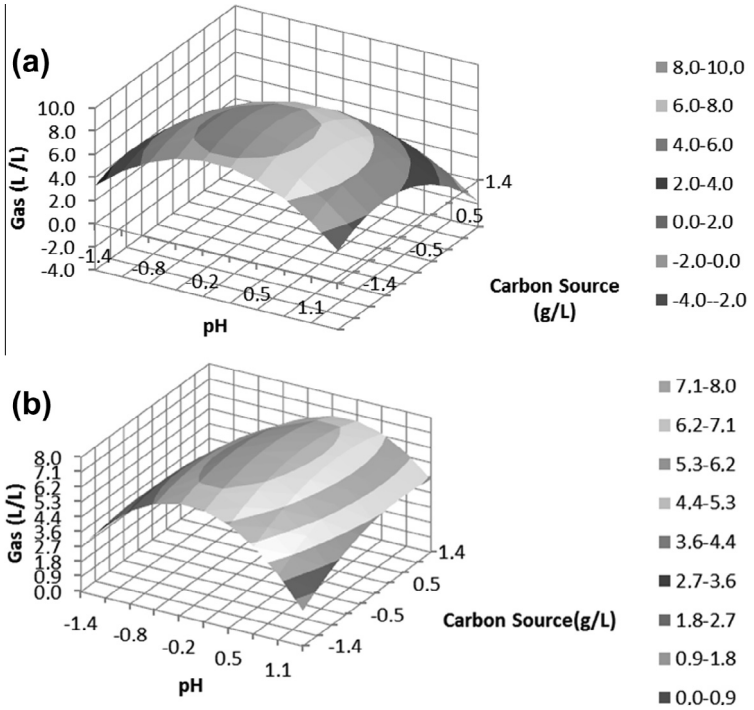

Fig. 1. Graphical 3-D display of the results for optimization of gas production by (a) LPB AH1 consortium cultivated in vinasse medium supplemented with sugarcane juice and (b) LPB AH2 consortium cultivated in vinasse medium supplemented with sugarcane molasses.

Table 5
The equations of the full quadratic model that fit best to the results achieved in the optimization of culture conditions of both consortia are presented. Coefficient values, standard errors, 95% interval of confidence and T student are also shown.

Gas (ml) = b0 + b1∗pH^2 + b2∗pH + b3∗substrate (g/L)^2 + b4∗substrate (g/L)∗pH + b5∗substrate (g/L)

		P value	Std error	−95%	95%	*t* Stat	VIF
Consortium LPB AH1							
b0	8.270	4.65041E-05	0.632	6.645	9.894	13.09	
b1	−1.946	0.00400	0.387	−2.941	−0.952	−5.029	1.000
b2	−2.347	0.00379	0.461	−3.531	−1.163	−5.095	1.095
b3	−1.644	0.01606	0.461	−2.829	−0.460	−3.569	1.095
b4	0.220	0.704	0.547	−1.187	1.627	0.402	1.000
b5	0.132	0.747	0.387	−0.863	1.127	0.341	1.000
Consortium LPB AH2							
b0	7.615	1.58788E-05	0.467	6.413	8.816	16.29	
b1	−2.148	0.00148	0.341	−3.024	−1.273	−6.306	1.095
b2	0.645	0.07381	0.286	−0.09032	1.381	2.255	1.000
b3	−0.508	0.196	0.341	−1.384	0.368	−1.491	1.095
b4	0.603	0.197	0.405	−0.438	1.643	1.489	1.000
b5	−0.06266	0.835	0.286	−0.798	0.673	−0.219	1.000

butyrate and lactate, in the first 24 h of fermentation was noted, which was accompanied by a high rate of sugar and propionic acid consumption, pH decrease and biogas production. More than 60% of the gas that has been produced until the end of fermentation was produced in the first 24 h.

On the second day of fermentation, it was noticed that the consumption of the carbon source and some of the VFAs produced in the first day (lactic, acetic and formic acids) resulted in propionic acid production. This might be due to a shift of the microbial population from lactic acid to propionic bacteria.

As no considerable differences in VFAs profile was noticed after the second day of fermentation, it could be stated that their consumption was directed towards hydrogen and biomass production (which is confirmed by comparing Fig. 3).

The biogas at the end of fermentation was composed of 2.13% N_2, 66.2% CO_2, 0.62% O_2, traces of CH_4, 1.2% of H_2O, 0.3% of H_2S and 29.55% H_2.

A yield of 5.95 molH_2·molsucrose^{-1} (corresponding to a yield of 2.97 molH_2·molglucose^{-1}) was achieved after the first day of fermentation, corresponding to 74.35% of the theoretical maximal yield. This was high and similar or even higher yields were achieved more frequently using thermophiles (de Vrije et al., 2009). Since biogas composition was analyzed only by the end of the fermentation, it is not possible to determine maximum productivity. Global production was approx. 2.4 LH_2/L medium.

Considering the hydrogen content in the biogas, the inferior and superior calorific powers (ICP and SCP) were calculated and estimated as 8613 kcal kg^{-1} and 10,212 kcal kg^{-1}, respectively. In comparison to a methane-rich biogas (65% CH_4 – ICP = 7735 kcal kg^{-1} and 8612 kcal kg^{-1}), the calorific power presented by the hydrogen rich biogas was superior (at least 11% higher).

According to the results presented in Fig. 2(a), a μmax of 0.06 g L^{-1} d^{-1} was achieved. The substrate consumption rate in the first 48 h was equal to 4.07 g L^{-1} d^{-1}. Approximately 2.2% of

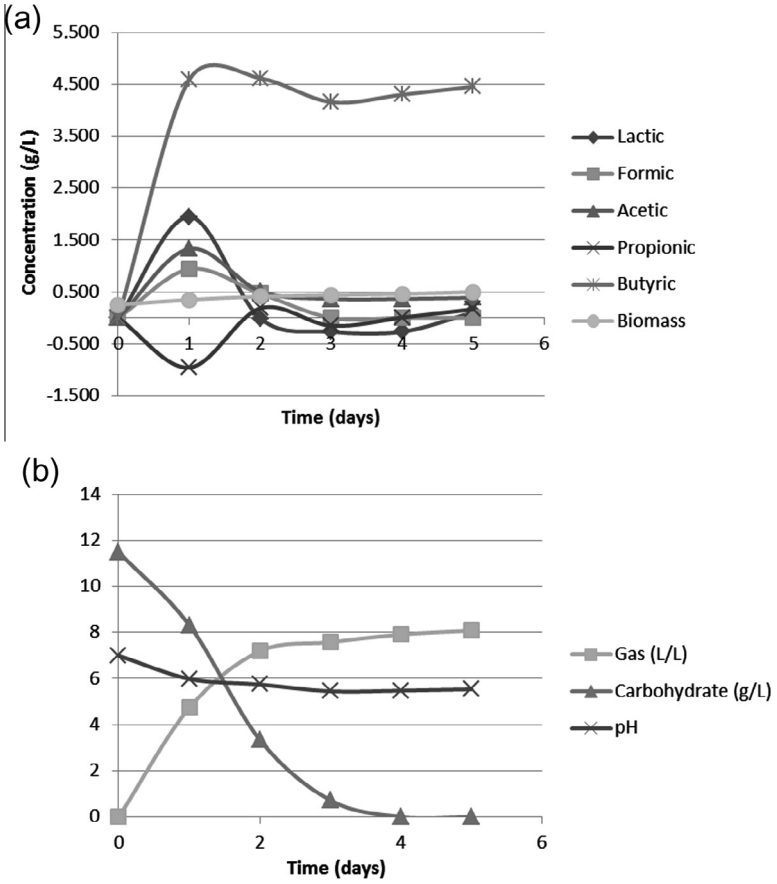

Fig. 2. Metabolic analysis of the consortium LPB AH1. (a) Curves of biomass and net VFAs concentration during the cultivation of the consortium LPH AH1 in vinasse medium supplemented with sugarcane juice. *Results are net production against blank (not fermented medium), therefore, negative values indicate that the corresponding VFA presented higher concentration in the blank than in the fermented broth and positive values mean the opposite. (b) Biogas production, substrate consumption and pH variation during fermentation of vinasse supplemented with sugarcane juice by the consortium LPB AH1.

the substrate was used in biomass production (YX/S), while almost 39% was used for butyrate production and 44.6% considering every acid produced (YVFAs/S). This meant that 53.2% of the consumed substrate was probably used for CO_2 production (YCO_2/S) and cell maintenance (Ym/S).

3.5. Biohydrogen and VFAs production in bioreactor scale by the consortium LPB AH2

Biohydrogen and VFAs fermentation by consortium LPB AH2 was carried using vinasse and sugarcane juice as medium, according to the results previously achieved. Carbohydrate concentration at the beginning of the fermentation was quantified as 13.42 g L^{-1} and the initial pH was 7.0.

Almost 50% of the carbon source was consumed in the first 24 h but was only exhausted in the last day of fermentation, biomass production achieved 0.65 g L^{-1} and final pH was 5.15 (Fig. 3a).

The HPLC analysis showed a complex metabolism of VFAs production (Fig. 3b). In the first 24 h butyric, acetic and lactic acids were produced in large amounts, while low quantities of formic and propionic acid were identified. This was accompanied by assimilation of most of the carbohydrates (6.21 g L^{-1}), high biogas production (58% of the gas that would be produced by the end of the fermentation) and a pH drop. In the second day of fermentation, the VFAs were consumed and gas production was lowered. In the 3rd day, little change was noticed but in the 4th day, butyric, acetic and low amounts of propionic acids were observed together with carbohydrate consumption. Biomass production was greatly increased between the 4th and 6th day of fermentation and gas production rate was kept relatively constant (Fig. 3b). The maximum H_2 yield was achieved considering 2 days of fermentation (2.83 molH_2·molsucrose^{-1}, corresponding to 1.41 molH_2·molsucrose^{-1}). Global biohydrogen productivity was 2.0 LH_2/L medium.

Only 6.41 Lgas L^{-1} medium was produced at the end of the fermentation, which is low in comparison to the predicted gas production during optimization (7.76 Lgas L^{-1} medium). This difference illustrates the expected variation of biogas (and consequently biohydrogen) production caused by the use of complex medium and a consortium of microorganisms. The biogas at the end of fermentation was composed of 3.93% N_2, 62.4% CO_2, 0.97% O_2, traces of CH_4, 0.3% H_2S, 1.2% H_2O and 31.2% H_2.

Considering the hydrogen content in the biogas, the lower and higher calorific powers (ICP and SCP) were calculated and estimated as 9048 kcal kg^{-1} and 10732 kcal kg^{-1}, respectively. In comparison to a methane rich biogas (65% CH_4 – ICP = 7735 kcal kg^{-1} and 8612 kcal kg^{-1}), the calorific power presented by the hydrogen rich biogas is higher (at least 17% higher).

According to the results presented in Fig. 3a, a μmax of 0.225 g L^{-1} d^{-1} was achieved. The maximum substrate consumption rate (first 24 h) was equal to 6.21 g L^{-1} d^{-1} (YX/S) (Fig. 3b). About 4.9% of the substrate was used in biomass production (YX/S), while almost 41.5% was used for VFAs production (YVFAs/S). This meant that 53.6% of the consumed substrate was probably used in CO_2 production (YCO_2/S) and cellular maintenance (Ym/S).

In comparison with other authors, the yield presented by consortia LPBAH1 on vinasses + sugarcane juice (2.97 molH_2·molglucose^{-1}) was similar to the yield obtaned by Liu and Shen (2004) (3.08 molH_2·molglucose^{-1}) where sucrose was fermented

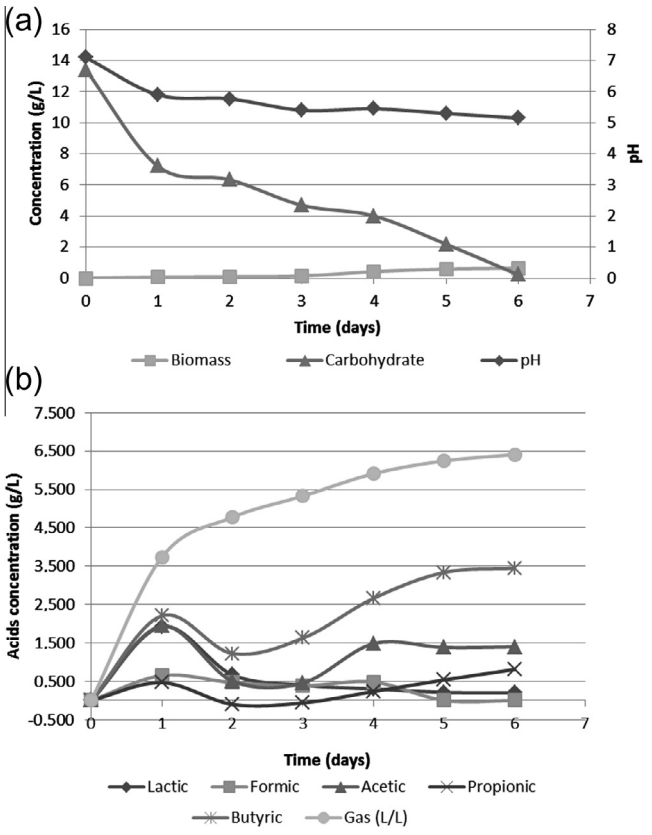

Fig. 3. Metabolic analysis of the consortium LPB AH2. (a) Biogas production, substrate consumption and pH variation during fermentation of vinasse supplemented with sugarcane molasses by the consortium LPB AH2. (b) Curves of biomass and VFAs production during the cultivation of the consortium LPB AH2 in vinasse medium supplemented with sugarcane molasses. *Results are net production against blank (not fermented medium), therefore, negative values indicate that the corresponding VFA presented higher concentration in the blank than in the fermented broth and positive values mean the opposite.

by a consortia originating from a digested wastewater sludge. Yield was higher than other reported studies, such as 2.58 $molH_2 \cdot molglucose^{-1}$ (obtained by Mu et al. (2006), where sugarbeet pulp was fermented by an anaerobic mixed culture), 2.40 $molH_2 \cdot molglucose^{-1}$ (obtained by Ren et al. (2006), where corn stover hydrolisate was fermented by *Thermoanaerobacterium thermosaccharolyticum*), 2.18 $molH_2 \cdot molglucose^{-1}$ (obtained by Doi et al. (2009), where glucose was fermented by a consortia from an anaerobic digester), 1.47 $molH_2 \cdot molglucose^{-1}$ (obtained by Levin et al. (2004), where delignified wood fiber was fermented by *C. thermocellum*) and 0.76 $molH_2$ $molglucose^{-1}$ (obtained by Lo et al. (2008), where rice straw hydrolysate was fermented by *C. butyricum*), for example.

4. Conclusion

In conclusion, vinasse was successfully used as source of nutrients for biohydrogen and VFAs production when supplemented with carbon sources. The achieved yields of hydrogen production ($molH_2$/mol substrate) were similar, to those achieved by thermophiles and higher than the majority achieved by Clostridia and Enterobacteria (Rittmann and Herwig, 2012).

The results indicated that the choice of microorganisms was a central point in biohydrogen processes development.

The proposed process is of importance for giving a more rational destination to vinasse and in turn expanding the Brazilian energy matrix, while reducing the dependence on fossil fuels.

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