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HYDROGEN PRODUCTION AND PERFORMANCE OF ANAEROBIC FIXED-BED REACTORS USING THREE SUPPORT ARRANGEMENTS FROM CASSAVA STARCH WASTEWATER

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ABSTRACT: Fixed-bed reactors have been considered promise alternatives for hydrogen production due to their simple construction and increase in the biomass retention. The purpose of this study was to investigate the biological production of hydrogen in anaerobic fixed-bed reactors with cassava starch wastewater used as substrate. Different support materials and arrangements of fixed-bed were used to evaluate the biological production of hydrogen in anaerobic continuous fixed-bed reactors, with cassava starch wastewater as substrate - recycled low-density polyethylene scraps, in packed bed (R1), recycled low-density polyethylene cylinders, in ordained bed (R3) and bamboo stems, in vertical arrangement (R2 and R4). In R1 the initial pH was adjusted for 6.0, with hydraulic retention time (HRT) of 4 h and organic loading rate (OLR) of 9.5 $g.L^{-1}.d^{-1}$. In R2 the initial pH was maintained in 4.5, with HRT of 4 h and OLR of 9.5 $g.L^{-1}.d^{-1}$. R3 and R4 were operated with initial pH of 4.5, HRT of 4 h and OLR of 13.5 $g.L^{-1}.d^{-1}$. The volumetric hydrogen production (VHPR) was favored by lower OLR applied, evenin different pH ranges (6.0 and 4.5). VHPR values of 229 mL $H_2.L^{-1}.d^{-1}$and 248 mL $H_2.L^{-1}.d^{-1}$ were obtained in R1 and R2, respectively. Both in the bamboo stems bed as in the polyethylene cylinders bed, the increase of OLR and the reduction of the initial pH resulted in a diminishing of VHPR to 175 mL $H_2.d^{-1}.L^{-1}$ (R3) and 145 m LH_2 $.d^{-1}.L^{-1}$ (R4). Higher concentrations of butanol (821.32 and 1,529.22 $mg.L^{-1}$) and ethanol (915.41 and 924.41 $mg.L^{-1}$) were verified in the reactors with bamboo stems. In R4, the increase of OLR and the reduction of the initial pH contributed to the increase of butanol concentration in 1.8 times, diminishing the VHPR in 41.68%, yield in 63.95% and H_2 in 37.6%, and indicating that the effect of pH is more pronounced with the increase of OLR, leading to the solventogenesis.

KEYWORDS: bio-energy, fermentative processes, bamboo, polyethylene, agro-industrial effluent.

INTRODUCTION

The increase in global demand for energy, reduction in fossil fuel reserves and environmental impacts has motivated the search for alternative fuels. In this context, hydrogen is a promising substitute, a clean and efficient source, because it is its burning results in zero emissions of greenhouse gases and with energy potential three times higher than that of gasoline (141.8 $kJ.g^{-1}$) (SRIRANGAN et al., 2012; ARIMI et al., 2015). Considerable amounts of bio-hydrogen can be produced from renewable resources.

The use of solid wastes and wastewater generated from agriculture and agribusiness are extremely convenient due to their abundance, lowest price and biodegradability (URBANIEC & BAKKER, 2015). The biological production includes bio-photolysis, photo fermentation and electrochemical processes (AZWAR et al., 2014). The use of cheaper substrates and the application of technologies that require lower energy expenditure are some of the advantages of biological processes.

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To achieve the industrial scale and compete with the hydrogen produced by means of fossil fuels, different approaches have been extensively studied in the fermentative production of bio-hydrogen with different configurations of reactor, operational parameters and the choice of suitable substrates (ARIMI et al., 2015).

Fixed-bed reactors have been considered as a promising alternative for the production of hydrogen from wastewaters due to their simple construction and the enhancement in the cell retention time, which is essential for hydrogen production (REIS & SILVA, 2014). Anaerobic fixed-bed reactors have many advantages compared to other types of reactors that use suspended cells, including the possibility of achieving a higher cell concentration and a longer cell retention time (FERRAZ et al., 2014). The improvement in retention cellular increases the resistance of reactor to organic shock loads, inhibitory compounds and changes in substrate concentration (SIVAGURUNATHAN et al., 2016; KESKIN et al., 2012). In addition, the support operating as a separator mechanism of gas and solid, increases the contact between wastewater and biomass, leading to a uniform flow rate and improvement in the mass transfer phenomenon (KASSUWI et al., 2013).

Several reactors have been tested for biohydrogen production, being the fixed-bed bioreactor a promising technology due to its capacity to retain biomass in a wide spectrum of support material and bed porosity (LIMA et al., 2013). Low-density polyethylene has been used successfully as support material in fixed-bed reactors applied to the production of hydrogen from different substrates: vinasse (FERRAZ et al., 2014), saccharose (FERNANDES et al., 2013), soft-drink (PEIXOTO et al., 2011).

In reactors, bamboo has been applied in the immobilization of microorganisms for different uses as biogas production and organic matter removal (KUNZLER et al., 2013; WENPING et al., 2012); quinoline biodegradation (ZHUANG et al., 2015), nitrate removal (LIU et al., 2012) and biohydrogen production (ANDREANI et al., 2015).Among the advantages of these reactors are the availability, low cost, higher superficial area, adsorption capacity and physical-chemical stability (LIN et. al., 2010).

Wastes rich in organic matter have a high potential for boosting bio-hydrogen production by dark fermentation processes. The successful use of raw material, cheaper and abundantly available, is one of the main factors for an efficient production of bio-hydrogen (ARIMI et al., 2015). The cassava starch processing wastewater is a carbohydrate-rich waste and thus ischaracterized as a potential substrate for the fermentative production of hydrogen, ensuring the sustainability of the process.

This application presents the advantage of adding value to a highly pollutant wastewater converting it to aclean energy source (CAPPELLETTI et al., 2011). The wastewater of starch production contains carbohydrates as well as nitrogen and phosphorus, among other nutrients (LUCAS et al., 2015). According to URBANIEC & BAKKER (2015), the identification of substrates for future economic applications in industrial scale is a priority task at the present stage of the development of hydrogen fermentation technology.

Therefore, this paper sought to evaluate anaerobic fixed-bed reactors using three support arrangements in hydrogen production from cassava starch wastewater. Additionally, the effects of the organic loading rate increasing and pH values adjustment were tested.

MATERIALS AND METHODS

Reactors

This study employed upflow anaerobic fixed-bed reactors made of 5 mm of thickness transparent plexiglass, with 75 cm of height, 8 cm of inner diameter and 3.6 L of total volume (PEIXOTO et al., 2011). Each reactor was formed by three compartments with inlet and outlet chambers and reactional region completed with support material (fixed-bed). These compartments were separated by a stainless steel mesh.

Figure 1 shows the constructive characteristics of the reactor. Three support materials were evaluated in hydrogen production: i) recycled-low polyethylene scraps with particle size of ¾"; ii) recycled-low polyethylene cylinders with 3.0 cm of length and 2.5 cm of diameter, iii) bamboo stems with 40 cm of length, 1.5 cm of width and 79% of porosity. The reactors were kept in a heated chamber at temperature of 36 °C.

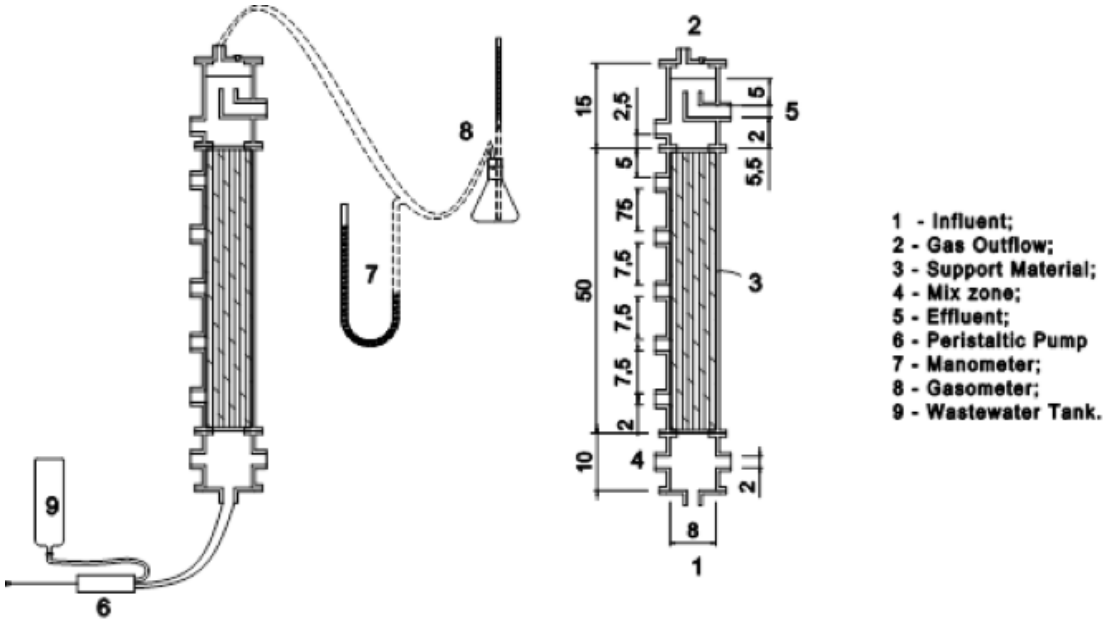

FIGURE 1. Scheme of the upflow anaerobic fixed bed reactor, with dimensions in cm.

Inoculum

The sludge used as inoculum was collected from bamboo pieces used as support material in a pilot scale anaerobic reactor, treating effluent from starch production. The sludge was pre-treated by boiling at 95 °C for 15 min (SREETHAWONG et al., 2010), to suppress the activities of hydrogen-consuming bacteria. The reactors were inoculated with 300 mL of anaerobic sludge with Total Volatile Solids (TVS) of 25 $g.L^{-1}$ and the working volume was completed with cassava starch wastewater. The sludge was then recirculated into the reactor in a closed circuit, providing a batch operation system for 96 h to activate the hydrogen-producing biomass (SIVAGURUNATHAN et al., 2016).

Wastewater

The cassava starch wastewater used in the reactor feeding, was collected in the municipality of Toledo, State of Paraná, Brazil. The effluent consists on the water resulting from the starch extraction and purification processes and the water used to the cassava roots washing. According to CAMPOS et al. (2006) the variability observed in the cassava starch wastewater is closely related to the origin of the cassava plant, age of the root, storage period and also to the type of process used. A summary of the results obtained in the characterization of cassava starch wastewater is shown in Table 1.

TABLE 1. Characterization of cassava starch wastewater.

Parameter	Average values	SD	Min.	Max.
COD ($mg.L^{-1}$)	8,152	3,659	3,471	15,662
Total Carbohydrates ($mg.L^{-1}$)	2,119	930	881	3,508
pH	4.34	0.30	3.88	4.76
TS ($mg.L^{-1}$)	2,252	2,457	527	6,497
VTS ($mg.L^{-1}$)	2,832	3,267	606	8,530
VTS/TS	0.8	0.1	0.7	0.9
N ($mg.L^{-1}$)	212	92	76	391
P ($mg.L^{-1}$)	31	14	12	51
C/N	51	37	9	106
Ca ($mg.L^{-1}$)	33	20.3	17.4	70.8
Fe ($mg.L^{-1}$)	10.9	3.2	6.7	15.7

Arithmetic average; SD – standard deviation; Min. – minimum value; Max. – maximum value; COD – Chemical Oxygen Demand; pH – Hydrogenionic Potential; TS – Total Solids; VTS – Volatile Total Solids; C/N – carbon/nitrogen ratio.Number of samples used to calculate the mean values and standard deviation = 12.

Reactor operation

Two series of experiments were carried out to investigate the effect of support materials in the hydrogen production. In the first series two reactors with beds of recycled low-density polyethylene scraps (R1) and bamboo stems (R2) were operated with hydraulic retention time (HRT) of 4 h, organic loading rate (OLR) of 9.5 $g.L^{-1}.d^{-1}$. Initial pH was adjusted to 6.0 in R1 and maintained in 4.5 in R2. In the second series two reactors were operated with beds of polyethylene cylinders (R3) and bamboo stems (R4), both conduced in HRT of 4 h, OLR of 13.5 $g.L^{-1}.d^{-1}$ and initial pH of 4.5.

The reactor was fed using a peristaltic dosing pump connected to a tank of 18 L that stored the substrate at temperature of 7 °C. At the top of the reactor, a gasometer and the effluent output were set up. Table 2 shows the operational conditions of the upflow anaerobic fixed-bed reactors.

TABLE 2. Operational conditions of the upflow anaerobic fixed-bed reactors.

Reactors*	Fixed-bed	Number of samples	Period of operation (d)	pH	HRT (h)	C/N	Influent Concentration ($mg.L^{-1}$)**	OLR ($g.L^{-1}.d^{-1}$)
R1	Polyethylene scraps	30	34	6.0	4	93	1,400	9.5
R2	Bamboo stems	30	34	4.5	4	241	1,400	9.5
R3	Polyethylene cylinders	33	59	4.5	4	25	2,200	13.5
R4	Bamboo stems	33	59	4.5	4	25	2,200	13.5

*Working volume R1 (2.9 L); R2 (2.9 L); R3 (2.9 L); R4 (3.0 L); **In terms of total carbohydrates; HRT – Hydraulic retention time; C/N – carbon (C) and nitrogen (N) ratio; OLR – organic loading rate.

Effluent samples of the reactor were characterized by: total carbohydrates with a colorimetric method (DUBOIS et al., 1956); Chemical Oxygen Demand (COD), pH and Volatile Suspended Solids (VSS) according to the Standards Methods for the Examination of Water and Wastewater

(APHA, 2005). Volatile organic acids concentrations were measured using a high performance liquid chromatograph (HPLC Shimadzu Prominence) equipped with an Aminex® HPX-87H column according to PENTEADO et al. (2013). The composition of the biogas (carbon dioxide, hydrogen and methane) was determined by gas chromatography (GC 2010 Shimadzu) with a Carboxen® 1010 Plot capillary column according to ANZOLA-ROJAS et al. (2015). The volume of biogas produced was quantified based on the methodology of CAPPELLETTI et al. (2011).

The response variables used to assess the performance of the reactors under different conditions included: total carbohydrates conversion efficiency (%); COD removal efficiency (%); biogas flow rate (Q_{biogas}, $mL.h^{-1}$); volumetric hydrogen production rate (VHPR, mL $H_2.d^{-1}$. L^{-1}), molar hydrogen flow rate (MHFR, in mmol $H_2.h^{-1}$) and hydrogen yield (HY, mol $H_2.mol^{-1}$ carbohydrates). Student's t-test was used to compare averages values of biogas flow rate, volumetric hydrogen production rate and hydrogen yield between R1, R2, R3 and R4 reactors operated at the same conditions ($p < 0.05$).

RESULTS AND DISCUSSION

The average results for the carbohydrate conversion, COD removal, percentage of hydrogen and carbon dioxide, volumetric hydrogen production rate, molar hydrogen flow rate and hydrogen yield verified to the reactors are provided in Table 3.

TABLE 3. Reactors performance in terms of carbohydrates conversion efficiency, COD removal efficiency, biogas composition, production rates and hydrogen yields.

Response-variable	Assay			
	R1	R2	R3	R4
EC_{TS} (%)	94.9±3.05(24)	91.59±10.23 (23)	92.76±10.5(35)	93.15±6.83(33)
ER_{COD} (%)	17.84±10.23(22)	14.82±12.11(23)	19.76±13.7(35)	18.65±15.13(33)
H_2 (%)	20.83±12.32(23)	22.77±11.17(23)	11.47±7.5(35)	14.21±5.02(33)
CO_2 (%)	60.62±17.4(23)	61.52±21.12(23)	75.7±15.46(35)	69.77±16.51(33)
VHPR (mL $H_2.d^{-1}.L^{-1}$)	228.97±169.4(23)	248.36±171.5(23)	175.6±142.6(35)	144.84±69.4(33)
MHFR (mmol $H_2.h^{-1}$)	1.03±0.76(23)	1.43±0.99(23)	0.68±0.65(35)	0.84±0.4(33)
HY (mol $H_2.mol^{-1}$ Carb)	0.20±0.17(22)	0.86±0.61(23)	0.15±0.2(35)	0.31±0.22(33)

Notes: EC_{TS} = carbohydrate conversion efficiency; ER_{COD} = COD removal efficiency; H_2= percentage of hydrogen in biogas; CO_2= percentage of carbon dioxide in biogas; VHPR = volumetric hydrogen production rate; MHFR = molar hydrogen flow rate; HY = hydrogen yield. Values between parentheses indicate the number of samples considered in the calculation of each response variable.

In all assays the carbohydrate conversion efficiency has reached values above 90%. This higher conversion observed is closely related to the biodegradability of cassava starch wastewater, explained by TVS/TS ratio of 0.82. Higher values of substrate conversion may be attributed to cellular immobilization, which guarantees longer periods of solids retention and greater resistance to hydraulic shock loads (SIVAGURUNATHAN et al., 2016; KESKIN et al., 2012).

According to ANTONOPOULOU et al. (2008), the process of fermentative hydrogen production does not significantly reduce the organic content of affluent. Usually, the chemical oxygen demand removal is below 20% during this process, as observed in this work. Glucose metabolism by acidogenic microorganism generates organic volatile acids, alcohols, hydrogen and dioxide carbon, therefore, the initial COD is converted into COD of the degradation products. Thus, these carbohydrates are consumed while low reductions in COD values are verified, once that the intermediate metabolites remained in the system (MADIGAN et al., 2010; REIS & SILVA, 2011; THANWISED et al., 2012).

The average values of molar hydrogen flow rate (MHFR) were 1.03±0.86; 1.43±0.99; 0.8±0.65 and 0.84±0.4 mmol $H_2.h^{-1}$ to the reactors R1, R2, R3 and R4, respectively. In R1 and R3, the maximum value of MHFR was similar (2.4 and 2.3 mmol $H_2.h^{-1}$), however, in different stages of operation, on the 21^{th} and 51^{th} day, respectively. In R2, the MHFR achieved 3.37 mmol $H_2.h^{-1}$ (22^{th} day), and after this peak a negligible value (0.04 mmol $H_2.h^{-1}$)of molar hydrogen flow rate was

noted on the 26th day. In R4 the maximum value of MHFR was 1.8 mmol $H_2.h^{-1}$ (44th day) and the minimum value was 0.12 mmol $H_2.h^{-1}$. PENTEADO et al. (2013) also observed high variability in the values of MHFR, with minimum value of 3.08, average value of 7.73 and maximum value of 28.18 mmol $H_2.h^{-1}$ in fixed-bed reactor using sucrose as substrate.

Yield of 0.86 mol $H_2.mol^{-1}$ofcarbohydrate was verified in R2, five times higher than the others assays, possibly justified by the higher nitrogen concentrations verified in the effluent used in R1, R3 and R4 (Table 2). No statistical difference was observed in the hydrogen yield among R1 and R3 reactors (p-value = 0.318). ANZOLA-ROJAS et al. (2015) obtained 1.7 and 3.5 mol $H_2.mol^{-1}$ of saccharose with the increase of C/N ratio from 40 to 140, an increase of 3.5 times in the yield. Yield decreasing can be possibly caused by changes in the metabolic pathway of the microorganisms due to the excess of nitrogen that leads to cellular growth rather than the biogas production, and collaborates to the production of reduced compounds, using hydrogen to the production of acids and solvents (PEIXOTO et al., 2011).

In starch-based synthetic wastewater, the reduced activity of α-amilase enzyme can compromise the yields in the hydrogen production. In Clostridium genera bacteria, the biosynthesis of the enzyme is inhibited due to the catabolic repression caused by the glucose concentration in the medium (CHOJECKI & BLASCHEK, 1986). In assays with mutant microorganisms able to do simultaneous saccharification and fermentation of cassava starch, JIANG et al. (2013) obtained yield of 3.2 mol $H_2.mol^{-1}$ of glucose, 1.65 times higher than the results verified in the strains of those microorganisms that did not hydrolyse starch.

Bed porosity obtained was 79% in packed-bed of polyethylene scraps (R1), ordered-bed with polyethylene cylinders (R3) and bamboo stems (R2 and R4) reactors. As it was verified biomass accumulation in the reactor with polyethylene scraps, the ordained bed with polyethylene cylinders could be an alternative to prevent this.In R1 and R2 reactors the average volumetric hydrogen production rates of 229 and 248 mL $H_2.d^{-1}.L^{-1}$ were similar (p-value = 0.702); however, the hydrogen yield in R2 was 4 times higher than in R1. Concentrations of VSS in the effluent of R2 and R4 (406 and 607 mg $VSS.L^{-1}$, respectively) were up to 5.5 times higher than those observed in the effluent of R1 and R3 (110 and 280 mg $VSS.L^{-1}$, respectively, p-value = 0.259). Although the same porosity of the beds, the higher concentration of VSS in the effluent of R2 and R4 can be attributed to the arrangement of the bamboo stems that favoured the biomass washout and the maintenance of the porosity in R2, justifying the yields verified in this reactor.

Reduction in the values of volumetric hydrogen production rate (VHPR) in R3 and R4 is related to the biomass accumulation in the system, once higher concentrations of nitrogen were noted in the affluent of these reactors, causing reduction of the bed porosity and loss of the working volume of the reactors. FERNANDES et al. (2013) verified diminishing of the working volume in reactors with bed porosity between 50 and 75% due to the greater biomass accumulation among the interstices of the support material. However, beds with higher porosity (91%) caused the washing out of the excess of biomass from the system, collaborating with the maintenance of the void spaces. Increasing the porosity from 75 to 91% increased the VHPR from 0.80 to 1.11 L $H_2.h^{-1}$ and resulted in the cyclic production of hydrogen during longer periods. The authors verified that the bed porosity affects more hydrogen production rather than the support material used in bed.

An increase in the concentrations of organic acids and alcohols occurred with the accumulation of biomass (Table 3) and decrease of the hydrogen production. According to JUNG et al. (2011) significant increases in the concentrations of the intermediates lead to inhibition of the metabolic pathways and the microbial activity. SREETHAWONG et al. (2010) observed an increase of the concentration of total organic acids from 5,450 to 15,570 $mg.L^{-1}$ with the decrease in the C/N ratio from 45 to 22, leading to a diminishing of 40% in the hydrogen production rate, from 990 to 590 $mL.h^{-1}$.

Figure 2 shows the average percentages of hydrogen, dioxide carbon and methane of the biogas in R1, R2, R3 and R4 reactors, corresponding to data presented in Table 3.

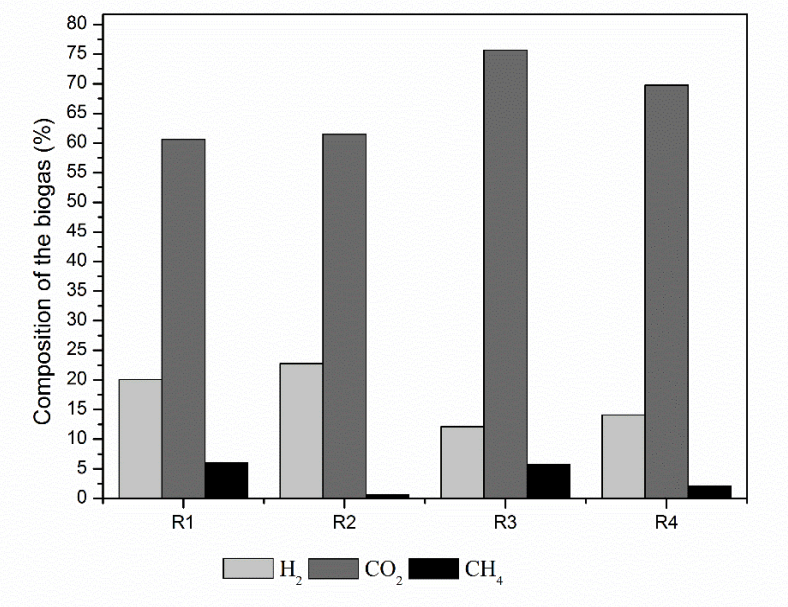

FIGURE 2. Composition of the biogas in R1, R2, R3 and R4.

The biogas generated in the assays consisted mainly of dioxide carbon and hydrogen, with lesser amounts of methane. Hydrogen percentage in the biogas were up to 44.5, 45.5, 32.4 and 22.4% in R1, R2, R3 and R4, respectively, indicating no statistical differences between R1 and R2 (p-value = 0.579) and R3 and R4 (p-value = 0.080).The proportion of hydrogen in the biogas was higher at the beginning of the operation and decreased during its conduction, probably due to the growth of the biomass that also contributed to the diminishing of the theoretical HRT of the reactor. The reduction in the percentages of hydrogen can be verified by the lower average values of 20.8±12.32; 22.77±11.2; 13.7±7.49 and 14.2±5.0% obtained for R1, R2, R3 and R4 reactors, respectively. According to CHANG et al. (2002), the increase in organic loads can decrease the hydrogen production rate and percentage of hydrogen into biogas in fixed-bed reactors. The aforementioned authors observed reduction of 85% in the hydrogen content when the HRT decreased from 3 to 1 h, diminishing also the production rate of this gas.

In the first phase of the experiment, the initial pH of the cassava wastewater was adjusted to 6.0 (R1) and maintained at 4.5 (R2) and in the second phase (R3 and R4), the pH was kept at 4.5, characteristic value of this residue. In R1 the pH of the effluent remained between 5.0 and 5.5, whereas in R2, R3 and R4 it was observed pH of the effluent around 4.5 and 5.0. Usually, the pH of the effluent in fermentative systems for hydrogen production from starch-based substrates varies in the range of 5.0 to 6.0 (THANWISED et al., 2012). Reductions to a pH below 4.5 can change the metabolic pathway, leading to the production of solvents (PHOWAN & DANVIRUTAI, 2014).

In anaerobic conditions the carbohydrate degradation can occur by 3 metabolic pathways: butyric acid pathway in which are generated butyric and acetic acids; ethanol pathway in which the metabolites are ethanol and acetic acid; and propionic pathway in which the fermentation products are propionic, acetic and valeric acids (REN et al., 1997; XIA et al., 2015). Average concentration of acetic, propionic, butyric and lactic acids; ethanol and butanol in R1, R2, R3 and R4 are presented in Table 4.

TABLE 4. Average concentrations of soluble metabolites.

Soluble Metabolites	Reactors			
($mg.L^{-1}$)	R1	R2	R3	R4
Acetic	209.85±79.66(30)	179.62±96.31(30)	388.53±195.67(33)	586.15±232.21(33)
Propionic	139.32±58.67(30)	138.72±66.78(30)	233.08±95.52(33)	360.01±139.81(33)
Butyric	252.81±114.84(30)	275.41±165.71(30)	467.72±184.66(33)	672.10±175.78(33)
Lactic	46.62±45.36(30)	83.64±178.50(30)	62.05±83.42(33)	51.65±34.77(33)
Ethanol	118.98±37.05(30)	915.41±578.04(30)	96.78±66.86(33)	924.41±467.51(33)
Butanol	134.33±43.49(30)	821.32±852.51(30)	146.56±43.66(33)	1,529.22±725.06(33)

Values between parentheses indicate the number of samples considered in the calculation of each response variable.

Butyric and acetic acids were the metabolites verified with higher concentrations in R1 and R3 reactors. According to LEE et al. (2008) in starch-based substrate is common to verify the development of the butyric pathway when pH of the medium is kept in the range of 5.0 to 6.0. The pattern of distribution of the soluble metabolites in R2 and R4 was marked by concentrations of ethanol and butanol higher than those to acetic, propionic, butyric and lactic during the entire experimental period. Except by ethanol in R1 and R3 and lactic acid in R2 and R4, the increase in the concentration of acids and alcohols was proportional to the increase of OLR from 9.5 to 13.5 $g.L^{-1}.d^{-1}$ (Table 3). Variations in the concentration of the metabolites suggest that the system presented changes among the fermentative pathways that were established in accordance with the operational conditions imposed to the reactor or even by the growth of different microbial communities (KOSKINEN et al., 2007; GUO et al., 2008).

Both in R2 and R4 the production of ethanol was favoured until the 15th day of operation; from this period the main metabolite produced was the butanol with average concentrations of 821.32 and 1,529.22 $mg.L^{-1}$, respectively. In accordance with MOREIRA et al. (1981) the presence of butanol in concentrations between 0.1 and 0.5 M can inhibit up to 50% the cellular growth and the sugar assimilation rate due to the limitations of the ATPase enzyme activity. With the increase of butanol concentration in R4, even in concentrations lower than those previously cited, decreases of 41.68% in the volumetric production, 63.95% in the yield and 37.6% in the content of H_2 in the biogas were observed in relation to R2.

According to ANZOLA-ROJAS et al. (2016) and ZHU et al. (2009), hydrogen and ethanol can be produced concomitantly without competition between the two metabolites (Equation 1), while acetic and butyric acids can be generated additionally, by other metabolic pathways (Equations 2 and 3).

$$C_6H_{12}0_6 \rightarrow C_2O_5OH + CH_3COOH + 2H_2 + 2\,CO_2 \quad (1)$$

$$C_6H_{12}0_6 \rightarrow 2H_2 + 2CO_2 + ½\,CH_3COOH + ¾\,CH_3(CH_2)_2COOH \quad (2)$$

$$C_6H_{12}0_6 \rightarrow {}^4/_3\,CH3CH_2COOH + {}^2/_3\,CH_3COOH + {}^2/_3\,CO_2 + 2H_2O \quad (3)$$

According to REN et al. (1997), the proportion of NADH/NAD^+ ratio and the accumulation of fermentative products are among the main factors responsible for the direction of the metabolic pathways. When the hydrogen is produced from the ethanol pathway, the balance between NADH/NAD^+ is maintained. Since ethanol is a neutral product, the production of acids is reduced, accelerating the fermentation in a quickly and efficient manner.

R1 and R2 achieved volumetric production of hydrogen of 228.97 and 248.36 mL $H_2.d^{-1}.L^{-1}$, respectively, similar to those obtained by distinct metabolic pathways, when submitted to the same OLR and HRT, but with different initial pH values. These results are due to the fact that the yields achieved in the ethanol pathway are the same observed when the fermentation product is the butyric acid (2 mol H_2/mol glucose). However, the pathway of butyrate production also can be directed to the butanol production with consumption of hydrogen (XIA et al., 2015). These alterations in the metabolic pathway can justify higher concentrations of butyric acid and butanol and lower volumetric production of 144.84 mL $H_2.d^{-1}.L^{-1}$ and yield of 0.31 mol $H_2.mol^{-1}$ carbohydrates verified in R4.

BARROS & SILVA (2012) evaluated the use of different support materials in the production of hydrogen and ethanol from glucose. Polyethylene terephthalate (PET) was the best support to the production of ethanol with concentrations of 1,359.24 $mg.L^{-1}$. Hydrogen production was favoured in the reactor with grounded tyre, achieving yield of 2.11 mol H_2 mol^{-1} glucose and 60% of H_2 in the biogas. These results are attributed to the porosity of the bed that favoured the accumulation of the biomass and the presence of H_2-producers microorganisms.

The fermentation of butyric/acetic acids has predominated in R1 and R3. This metabolic pathway is characteristic from systems operated in the pH range from 5.0 to 6.0 from starch-based substrates (LEE et al., 2008; SREETHAWONG et al., 2010; LUCAS et al., 2015). Even at pH values up to 4.5 a slight change in the metabolic pathway was verified for acetate/ethanol route. According to MADIGAN et al. (2010), few species of *Clostridium* shift the metabolic pathway to the production of neutral products, such as alcohols, in response to the decrease in pH. However, concomitant production of ethanol and hydrogen are reported in continuous systems in some studies (KIM et al., 2006; XIA et al., 2015). Propionic acid was observed throughout the experimental period at average concentrations of 139.3±58.7 and 233.0±95.5 $mg.L^{-1}$ in R1 and R3.

About the support material, the most promising results were verified in the reactor with immobilized biomass in bamboo stems (R2), followed by polyethylene scraps (R1) and polyethylene cylinders (R3). The bamboo stems and polyethylene cylinders showed higher porosities in bed, which improved the mass transfer phenomenon and enhanced the degree of mixing in the fixed-bed reactor.

CONCLUSIONS

The hydrogen yield from cassava wastewater was favored for bamboo stems arrangement (0.86 and 0.31 mmol H_2.mol Carb in R2 and R4, respectively), followed by polyethylene cylinders(0.2mmol H_2.mol Carb) and polyethylene scraps(0.15 mmol H_2.mol Carb) in the anaerobic fixed-bed reactor. Highest hydrogen yield was favored by OLR, lower C/N ratios and maintenance of bed porosity of bamboo stems in R2. The biomass accumulation in fixed-bed, due to the higher nitrogen concentrations in the wastewater, could lead to changes in the metabolic pathways decreasing both hydrogen yields and hydrogen molar flow rates in R1 and R3.

In the reactors with bamboo stems and fed with cassava wastewater, better results of hydrogen production were obtained with initial pH of 4.5; and also with initial pH of 4.5 and 6.0 when applying organic loading rate of 9.5 $g.L^{-1}.d^{-1}$. The increase of OLR in R4 (9.5 to 13.5 $g.L^{-1}.d^{-1}$) resulted in solventogenesis, leading to the production of higher concentrations of butanol (from 821.32 to 1,529.22 $mg.L^{-1}$) and to the decrease in the yield, molar flow rate and hydrogen production. Even with the ethanol as the main metabolite, there is the possibility of producing hydrogen and alcohol at the same time, allowing the use of these products as sources of energy.

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