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| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS*** ***VOL. 76, 2019*** | A publication ofaidiclogo_grande |
| The Italian Associationof Chemical EngineeringOnline at www.cetjournal.it |
| Guest Editors: Sauro Pierucci, Jiří Jaromír Klemeš, Laura PiazzaCopyright © 2019, AIDIC Servizi S.r.l.**ISBN** 978-88-95608-73-0; **ISSN** 2283-9216 |

Continuous MuItiple Tube Reactor in the Hydrogen Production Using Sucrose

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Biohydrogen (BioH2) is considered a promising fuel characterized by the higher energetic content per units of mass, easy electricity production by fuel cells (FCs) that generate water as the only by-product. The objective of this study was to evaluate the continuous multiple tube reactor (CMTR) performance, which has internal surfaces in the tubes adapted to rotating screw model, in the continuous production of hydrogen. The useful volume of the reactor comprised 1065.5 mL. The reactor was operated under anaerobic conditions, with continuous ascending flow and kept in an air-conditioned chamber at 25 ºC. Two assays were conducted: E1: COD of 2 g  L-1, hydraulic retention time (HRT) of 2 h and organic loading rate (OLR) of 24 g L-1d-1; E2: COD - 4 g  L-1, HRT – 2h, OLR - 48 g COD L-1d-1. The monitoring to evaluate the reactor performance included chemical oxygen demand (COD) removal efficiency (ERCOD, %); sucrose conversion efficiency (ECsuc, %); biogas flow rate (Qbiogas, in mL h-1); hydrogen molar flow (MHFR, in mmol H2 h-1), hydrogen yield (HY, in mol H2 mol-1 sucrose) and volumetric hydrogen production rate (VHPR, in mL H2 L-1 d-1). The CMTR presented stability in H2 production, allowing the production maintenance for long operation periods. The tubes internal suface adaptation (rotating screw) improved the system performance, which indicated that the biomass retention was sufficient to attend the system necessity

* 1. Introduction

It is possible to realize that exists a worldwide strong tendency on prioritize research and development focused on technologies that use renewable sources to contribute with environmental sustainability. Between the alternatives, hydrogen technologies were considered as the fossil fuels successors, due to the fact that is a fuel with significant energetic value. Compared with others conventional fuels, BioH2 just produce water as a by-product, which means less pollutants production.

The hydrogen (H2) production presents four basic processes by non-fossils energy primary sources: (i) water electrolysis; (ii) thermochemical; (iii) radiolytic and (iv) biological processes (SHAHLAN et al., 2018). The anaerobic fermentation has been highlighted between the biological processes of H2 obtainment, especially on account of the higher production of this gas when compared to other biological processes, and the possibility of use and treatment of different residual materials as substrate (HAFEZ et al., 2010; HASYIM et al., 2011; LEE et al. 2008; SREETHAWONG et al., 2010; WEI et al., 2010; DE SÁ et al., 2014).

Hydrogen production by fermentative processes is largely approached by literature on different reactors configurations, especially in continuous process and batch operation. But, many researches has reported about the maintenance difficulties on continuous production and high hydrogen rates by long operation times. It should be noted that exist many factors that can affect the continuous hydrogen production, such as pH, hydraulic retention time, temperature, carbon source, applied organic loading rate (OLR), metabolic paths involved and microbial diversity.

Some studies suggest that the instable hydrogen production can be related with the organic loading rate (OLR) or food-to-microorganism ratio (F/M), which can reach unfavourable values in the hydrogen production with the biomass increase in the reactor (ANZOLA-ROJAS et al., 2013; HAFEZ et al., 2010).

In this context, Gomes et. al. (2015) propose a continuous multiple tube reactor configuration (CMTR) as an alternative to overcome the limitations related to specific organic loading rate (SOLR), gaining a continuous biomass discard by using higher superficial flow velocity in the tubes. However, the biomass retention in the system was low, prejudice the H2 production. So, the authors suggested that the internal surface of the tubes were adapted to rotating screw model, to improve the biomass retention in the reactor.

Therefore, in this study was avaliated the hydrogen production in a CTMR which has the internal surface of the tubes adapted to rotating screw model, using sucrose as a carbon source, with mesophilic temperature conditions.

* 1. Material and Methods
		1. Continuous Multiple Tube Reactor and experimental conditions

The CTMR used in this study was constructed with three compartments, as proposed by Gomes et al. (2015): charge and discharge chambers (manufactured in acrylic) and intermediary region (composed by 12 PVC tubes with external diameter and length of 12 and 680 mm respectively). The tubes internal surface were adapted to rotating screw model to increase the biomass adhesion on the wall. The reactor useful volume comprehended 1065.5 mL: 80 mL (charge chamber) + 545.5 mL (tubes zone) + 440 mL (discharge chamber). The experimental apparatus used and the constructive characteristics of the reactor were presented in Figure 1.

**(a)**

**(b)**

*Figure 1 (a) Experimental apparatus and (b) Reactor constructive characteristics. Legend: 1-Synthetic wastewater reservoir; 2-Peristaltic pump; 3-Multiple tubes reactor; 4-Effluent outlet; 5-water seal; 6 -Gas meter*

*Fonte: Gomes et. al (2015)*

The reactor was operation upflow and continuous flow and maintained in an acclimatized chamber with temperature of 25° C. The CMTR was fed with synthetic substrate with sucrose as carbon source. Two essays were carried out in the CMTR: essay 1 (E1) – organic loading rate (OLR) of 24 g DQO L-1d-1 (DQO affluent of 2000 mg L-1; hydraulic retention time (HRT) of 2 h) and essay 2 (E2) – OLR of 48 g DQO L-1d-1 (DQO affluent of 4000 mg DQO L-1; HRT of 2 h). The essays period comprehended 96 days.

* + 1. Synthetic Wastewater and Inoculum

The synthetic wastewater was based on sucrose (1781.24 mg L-1) and urea as carbon and nitrogen sources and nutrients solution (Del Nery, 1987, according to Lima e Zaiat, 2012). The DQO of synthetic wastewater was 2000 mg L-1 and C/N rate of 140 (ANZOLA-ROJAS 2010). The pH was adjusted to 6.5 with addition of 0.25 mL L-1 of hydrochloric acid solution (HCL) 12 M. The inoculum was obtained by natural fermentation process in accordance with the method described by Leite et al. (2008). Fifteen liters of synthetic substrate were prepared and maintained in an open vessel to promote the autofermentation in an ambient temperature (~25° C). After this period, the substrate was recirculated in the reactor for five days to promote the biomass adhesion in the tube walls.

* + 1. Analytical methods

The determined monitoring system parameters were pH, chemical oxygen demand (COD), total carbohydrates (TC), total suspended solids (TSS), volatile suspended solids (VSS) and volatile fatty acids (VFA), in samples collected four times per week. The pH, COD, TSS and VSS parameters were realized according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2012). On the TC determination were used the method proposed by Dubois et al. (1956).

The acids analysis (acetic, propionic, butyric, formic and lactic) were realized by high performance liquid chromatography (HPLC) in a Shimadzu® system equipped with Aminex® column HP-87H (300 mm x 7.8 mm Bio-Rad), CTO-20ª oven in a temperature of 64° C, CBM-20A controller, UV detector with diodes arrangement SPD-20ª in a 208nm wave length and LC-20AT pump. The mobile phase was composed by ultrapure water Milli-Q (Millipore®) acidified with 0.005 M of H2SO4 in a 0.5 mL.min-1 flow and injection volume of 20µL (LAZARO et al., 2014; PENTEADO et al., 2013). The reactor discharge samples were filtered in cellulose acetate membrane with porosity of 0.2 µm and acidified with H2SO4 (2 M) solution.

The biogas flow was daily monitored by gasometer model MGC-1 V30 (*Ritter*®). For the biogas composition determination was used a needle with lock (model Sigma®) to collect 500 µL of gas in the reactor four times a week. The biogas constituent parts (hydrogen, carbon dioxide and methane) were determinated by gas chromatography in GC system 2010 (*Shimadzu*®), equipped with thermal conductivity detector (TCD), Supelco Carboxen® column 1010 Plot (30m x 0.53mm extern diameter, 0.30μm of thickness), using argonium as a carrier gas (make-up gas flow of 8 mL.min-1). The injector and detector temperature were 220° C and 230° C respectively. The column-heating ramp was 130° C to 135° C, in a rate of 46°C.min-1 during 6 minutes (PENTEADO et al., 2013).

The response variables calculated by the determined parameters were COD removal (ERCOD, in %), sucrose conversion (%), biogas flow rate (Qbiogas, in mL.h-1), volumetric hydrogen production rate (VHPR, in mLH2.h-1.d-1), molar hydrogen flow rate (MHFR, in mmolH2.h-1), hydrogen yields (YH2, in molH2.mol-1 sucrose) and acids ratio.

* 1. Results and Discussion

In Table 1 are shown the medium values of the CMTR performance in E1 and E2 related to the following parameters: sucrose conversion, COD removal, biogas flow and composition (H2 e CO2), MHFR, YH2, and VHPR. The essay 2 (4000mg COD L-1; HRT = 2 h) presented better results to most of the parameters. In the essays 1 and 2, the carbohydrates conversion efficiency reached values superiors to 65% during all the operating period, which corroborate with the values reached by Fontes Lima e Zaiat (2012) (67.2 to 79.2%).

Comparing the results obtained with the literature, Anzola-Rojas et al. (2013), operating a downflow structured bed reactor fed by synthetic effluent based in sucrose (OLR of 24 g COD L-1d-1) reached the Qbiogas, VHPR and YH2 of 131.7 mL h-1, 20.83 mL.L-1.h-1 e 0.59 mol H2 mol-1 sucrose respectively, which can be comparable to E2 (Table 1). The VPHR (48.3 mL H2 h-1 L-1) was similar to Penteado et al. (2013), (47.3 mLH2 L-1 h-1) operating an Anaerobic Packed-Bed Reactor (APBR) in the same conditions as this study. In Estevam et al. (2018) the MHFR was 2.2 mmol H2 h-1 using an anaerobic reactor with mechanical agitation (AnsBBR), fed by brewery effluent, and obtained results closed to those founded in this study.

Pachiega et al. (2018) observed higher performance that CMTR, operating a batch anaerobic reactor, with capacity of 2L, conducted by approximately 169h and fed with 2 g L-1 of sucrose. In this case, the YH2 values and carbohydrates consumption were respectively of 0.9 mol H2 mol-1 sucrose and 88.7%.

Table 1: *Continuous multiple tube reactor performance in the assays E1 and E2*

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| **Assay** | **ERCOD (%)** | **ECsucrose (%)** | **Qbiogás (mL.h-1)** | **H2 (%)** | **CO2 (%)** | **MHFR (mmol H2 h-1)** | **HY (mol H2 mol-1 suc)** | **VHPR (mL H2 L-1 h-1oi)** |
| **E1** | 19.1 | 77.7 | 89.9 | 61.5 | 38.5 | 0.99 | 0.48 | 48.3 |
| **E2** | 17.5 | 65.4 | 103.7 | 70.0 | 30.0 | 1.84 | 0.51 | 67.5 |

In Figure 2 are presented the time profiles of the parameters related to hydrogen production during the essays (biogas composition, MHFR, VHPR and HY). In E1 all the parameters presented high instability in the production during the operation period. On the other hand, E2 obtained higher gas production and stability than the first essay. On both essays, the production was maintained the entire experiment period, suggesting that the H2 production it is possible in this reactor configuration, especially with the grooves in the intern walls of the tubes.

The CMTR presented stability on the H2 production, allowing the production maintenance at long operation times. The H2 production period on the essays was superior to those observed in Anzola-Rojas et al. (2013), Fernandes et al (2013), Lima e Zaiat (2012) and Penteado et al. (2013).

The best CMTR performance in E2 was probably due to the biomass retention in the internal tube wall, which was sufficient to supply the biomass need in the system. As previously discussed, the biomass concentration in the reactor is a relevant factor to H2 production, been related on the literature the YH2 increasing with the biomass increasing as well (HAFEZ et al., 2010). In reactors with no fixed bed or support material can turn the biomass retention time on the system into critical values, under the minimum recommended (3h) to H2 production (HAFEZ et al., 2009).

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*Figure 2 Temporal profiles regarding the biogas and hydrogen production: (a) biogas flow rate, (b) volumetric hydrogen production rate, (c) molar hydrogen flow rate and (d) hydrogen yield. Legend: E1 (**) e E2 (**)*

Gomes et al. (2015), operating a CMTR in the same feed conditions (2 g L-1 e HRT 2h), observed that in their essays the hydrogen production decrease gradually in a few days of operation (10 to 24 days), ending by the 30th day. The authors supposing that the poor system performance could indicate that the intern surface of the PVC tubes probably did not supplied adequate conditions to the biomass fixation, and, as a result, the solids content was not sufficient to attend the biomass rates in the system.

Figure 3 presents the results of the parameters related to sucrose conversion and biomass production in the essays. During all the reactor operation period the sucrose conversion was higher on the E1 (Figure 3a), with similar values than Anzola-Rojas et al. (2016). The effluent pH values were stables, with inferior values on E2 (3.34 to 4.13) (Figure 3b). In the E1, the pH varied of 3.6 to 5.1.

The effluent VSS data in the reactor can indicate that the biomass liberation was stable on E2 and relatively instable on E1 (Figura 3c). These results corroborate to those presented in Table 1, which showed the best performance on E2, probably due to the high biomass retention in the system.

In E1 and E2 the main metabolites generated on the fermentation were acetic and butyric acid with small concentrations of formic and propionic acids. The acid and butyric fermentation occurred mostly in pH of 5.5 to 6.5; when the pH of 4 to 5.5 is typical to ethanol fermentation (MURI et al., 2018). The predominance of acid final products during the H2 production is renowned for occurred in the acidogenic phase, which decrease the pH of the medium.

It is known that the low pH value can prejudice the microorganism’s growth. Has been suggested that the acid absorption (normally followed by increase of the pH) that occurred during the production phase works as a detoxing process, initiated in response to the acid final products accumulation (ZHÃO et al., 2016). Maintinguer et al. (2008), Ramos & Silva (2018) e Ottaviano et al. (2017) also observed that the H2 production was dominated by butyric and acetic acids metabolic paths.

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*Figure 3 Reactor general performance in the essays: (a) sucrose convertion; (b) pH variation in the effluent; and (c) VSS concentration in the efluente. Legend: E1 (**) e E2 (**)*

* 1. Conclusions

The CMTR presented stability in H2 production, allowing the production maintenance for long operation periods. The grooves made in the internal walls of the tube improve the system performance, which indicate that the biomass retention was sufficient to attend the system necessity. This way, this study points the technical viability of continuous hydrogen production in a continuous multiple tube reactor under mesophilic conditions. The main metabolic products were acetic and butyric acids, followed by a reduced ethanol content.

Acknowledgments

The authors are grateful to the Coordination for the improvement of higher level personnel – Brazil (CAPES) and Araucária Foundation for supporting the development of this study.

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