Bioresource Technology 202 (2016) 42–49

Contents lists available at ScienceDirect

Bioresource Technology

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

Improvement of hydrogen production via ethanol-type fermentation in an anaerobic down-flow structured bed reactor

Mélida del Pilar Anzola-Rojas [a,b,*], Marcelo Zaiat [b], Heleen De Wever [a]

[a] Flemish Institute for Technological Research (VITO), Boeretang 200, 2400 Mol, Belgium
[b] Laboratory of Biological Processes, Center for Research, Development and Innovation in Environmental Engineering, São Carlos School of Engineering, University of São Paulo, João Dagnone Ave. 1100, Santa Angelina, 13563-120 São Carlos, São Paulo, Brazil

HIGHLIGHTS

- Anaerobic down-flow structured bed reactor improves the H_2-producing capacity.
- The organic loading rate and specific organic load drive the fermentation-type.
- Hydrogen is mainly produced via ethanol-type fermentation.
- The total energy conversion rate maximum is 23.40 kJ h^{-1} L^{-1}.

ARTICLE INFO

Article history:
Received 3 September 2015
Received in revised form 27 November 2015
Accepted 28 November 2015
Available online 8 December 2015

Keywords:
Biohydrogen
Fixed-bed anaerobic reactor
Specific organic load
Soluble fermentation products
Substrate concentration

ABSTRACT

Although a novel anaerobic down-flow structured bed reactor has shown feasibility and stable performance for a long-term compared to other anaerobic fixed bed systems for continuous hydrogen production, the volumetric rates and yields have so far been too low. In order to improve the performance, an operation strategy was applied by organic loading rate (OLR) variation (12–96 g COD L^{-1} d^{-1}). Different volumetric hydrogen rates, and yields at the same OLR indicated that the system was mainly driven by the specific organic load (SOL). When SOL was kept between 3.8 and 6.2 g sucrose g^{-1} VSS d^{-1}, the volumetric rates raised from 0.1 to 8.9 L H_2 L^{-1} d^{-1}, and the yields were stable around 2.0 mol H_2 mol^{-1} converted sucrose. Furthermore, hydrogen was produced mainly via ethanol-type fermentation, reaching a total energy conversion rate of 23.40 kJ h^{-1} L^{-1} based on both hydrogen and ethanol production.

1. Introduction

Nowadays, the growing demand for energy associated with the high fossil fuel-dependence is aggravating troubles caused by the greenhouse gas emission. In order to reduce that impact, efforts have been targeted to research focused on cleaner energy alternatives such as production and utilization of biofuels. Hydrogen and ethanol produced via anaerobic digestion have a significant interest, commercially and environmentally (Reis and Silva, 2014), since both can be produced simultaneously from wastes rich in organic matter. Hydrogen is considered an ideal and clean energy carrier, that generates only water as a product when combusted. Furthermore, hydrogen can be easily converted to electric energy through fuel cells (Ren et al., 2007). Alternatively, ethanol can be used as a fuel for transportation and as a substrate for biodiesel production (Han et al., 2012).

In anaerobic processes, the evolution of hydrogen can occur via acetic acid, butyric acid and ethanol-type fermentation. Via the first 2 ones, 4 or 2 mol of hydrogen per mole of glucose can be produced when the final products are solely acetate or butyrate, respectively. However, during the acetic acid-type pathway, the increase of hydrogen and carbon dioxide availability in the medium can induce changes in the trophic characteristics of the microorganisms, i.e. from heterotrophic to autotrophic growth (Noori, 2013). Thus, hydrogen and carbon dioxide can be consumed

---

Abbreviations: ADSBR, anaerobic down-flow structured bed reactor; APBR, anaerobic packed-bed reactor; HRT, hydraulic retention time; HY, hydrogen yield; OLR, organic loading rate; SCE, sucrose conversion efficiency; SOL, specific organic load; SFP, soluble fermentation products; VHP, volumetric hydrogen production.

* Corresponding author at: Laboratory of Biological Processes, Center for Research, Development and Innovation in Environmental Engineering, São Carlos School of Engineering, University of São Paulo, João Dagnone Ave. 1100, Santa Angelina, 13563-120 São Carlos, São Paulo, Brazil

E-mail addresses: pilaranzola@gmail.com, melida@sc.usp.br (M.P. Anzola-Rojas).

http://dx.doi.org/10.1016/j.biortech.2015.11.084
0960-8524/© 2015 Elsevier Ltd. All rights reserved.

for producing more acetate via homoacetogenesis. Likewise, the hydrogen obtained via the butyric acid-type of pathway can be used for butanol production (Hwang et al., 2004).

Therefore, the production of hydrogen via ethanol-type fermentation can be an attractive pathway, forming products like ethanol, acetate, H_2 and CO_2. Similar to the butyric acid-type of fermentation, 2 mol of hydrogen can be produced per mole of ethanol. Moreover, at a pH around 4.5, the ethanol-type of fermentation is neutral, preserving the balance between NAD and $NADH^+$ (Ren et al., 1997). Additionally, propionic acid production can be minimized, improving the stability of the anaerobic process (Ren et al., 1997). The potential of the ethanol-type fermentation from glucose has been assessed, reaching 100–200 mL H_2 g^{-1} glucose (Hwang et al., 2004) and 0.45 L H_2 g^{-1} COD removed (Ren et al., 2007). Likewise, Guo et al. (2008) achieved 3.47 mol H_2 mol^{-1} converted sucrose from molasses.

Among the fermentative systems, the anaerobic packed-bed reactor (APBR) can provide good mixing conditions, as well as a larger surface area available for microorganism deposition, and thus a high retention of biomass (Reis and Silva, 2014). The APBR has been widely used for hydrogen production, assessing different parameters (summarized by Barca et al. (2015)). However, in many cases, the long-term APBR performance and stability was not assessed. In other cases, the H_2 and CO_2 production occurred unstably even when the fermentation in the liquid medium was stable (Anzola-Rojas and Zaiat, 2015). That trend has been related with the development of H_2-consuming microorganisms such as homoacetogens, aided by aged and excessive accumulation of biomass on the bed, which in turn increased the substrate competition (Fernandes et al., 2013; Penteado et al., 2013; Anzola-Rojas et al., 2015; Ren et al., 2010).

Notwithstanding, in order to face those barriers, a new version of this reactor type was presented (Anzola-Rojas and Zaiat, 2015), as an anaerobic down-flow structured bed reactor (ADSBR), where hydrogen was produced for a long-term, steadily and continuously. The main features of this reactor are the structuration of the bed and flow inversion, aiding in the natural removal of biomass with the effluent and diminishing the interstices where H_2-consuming microorganisms are likely to settle. Additionally, the countercurrent gas and liquid flows favor biogas release to the gaseous phase, primarily at the top of the reactor, thus minimizing the contact of H_2 with possible H_2-consuming microorganisms throughout the bed.

To optimize a fermentative system directed to certain products, a range of Organic Loading Rates (OLR) has to be applied that the system can handle effectively, or an optimal OLR defined for maximum yields (Hafez et al., 2010). In acidogenic systems, where hydrogen is produced along with acids and solvents, the effect of OLR has been assessed by changes in the HRT (Amorim et al., 2009; Keskin et al., 2011) or substrate concentration (Hafez et al., 2010; Perna et al., 2013; Han et al., 2012; Ferraz-Júnior et al., 2015), indicating in both cases shifts in the microbial culture and the overall performance. For instance, the decrease of the HRT, with consequent OLR increase, led to suggested changes in the microorganism metabolism, passing from the use of substrate mainly for bacterial growth or cell maintenance to producing soluble fermentation products (SFP) (Amorim et al., 2009). On the other hand, changes of the OLR by incrementing the substrate concentration suggested a maximum limit, above which the system is inhibited owing to either the substrate or excess of product (Kim et al., 2006).

In that context, in order to improve hydrogen production in an ADSBR, the OLR range was varied between 12 and 96 g COD L^{-1} d^{-1}, by changing the HRT between 2 and 4 h, and the substrate concentration between 1 and 16 g COD L^{-1}. Moreover, the distribution of the soluble fermentative products was analyzed for determining the predominant fermentation type in the reactor, and the total energy efficiency of the ADSBR was estimated.

2. Methods

2.1. Anaerobic down-flow structured-bed reactor (ADSBR)

Hydrogen and SFP production were assessed in an ADSBR, as recently proposed by Anzola-Rojas and Zaiat (2015). The ADSBR of 4.35 L was built with an acrylic tube of 80 mm diameter and 800 mm length. The bed was structured by means of small cylinders of 20 mm in diameter and 25 mm in length, which were organized in alternated columns, and cantilevered in stainless steel rods. The material used for biomass attachment was recycled low-density polyethylene, as presented in Fig. 1. The physical features were: 0.96 g mL^{-1} density, 4.56 m^2 superficial area, and 7.9 m^2 g^{-1} specific superficial area. To control the temperature at around 25 °C, hot water was circulated over the reactor body.

2.2. Synthetic wastewater

The synthetic wastewater was based on sucrose and urea as carbon and nitrogen sources, respectively, maintaining a carbon/nitrogen ratio of 140 g/g. For each 1 g COD L^{-1} were added $C_{12}H_{22}O_{11}$ (892.85 mg L^{-1}), CH_4N_2O (5.75 mg L^{-1}) and the following micronutrients: $NiSO_4{\cdot}6H_2O$ (0.25 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (1.25 mg L^{-1}), $FeCl_3{\cdot}6H_2O$ (0.13 mg L^{-1}), $CoCl_2{\cdot}2H_2O$ (0.02 mg L^{-1}), $CaCl_2{\cdot}6H_2O$ (1.03 mg L^{-1}), SeO_2 (0.018 mg L^{-1}), KH_2PO_4 (2.68 mg L^{-1}), K_2HPO_4 (0.65 mg L^{-1}), $Na_2HPO_4H_2O$ (1.35 mg L^{-1}). Sodium bicarbonate

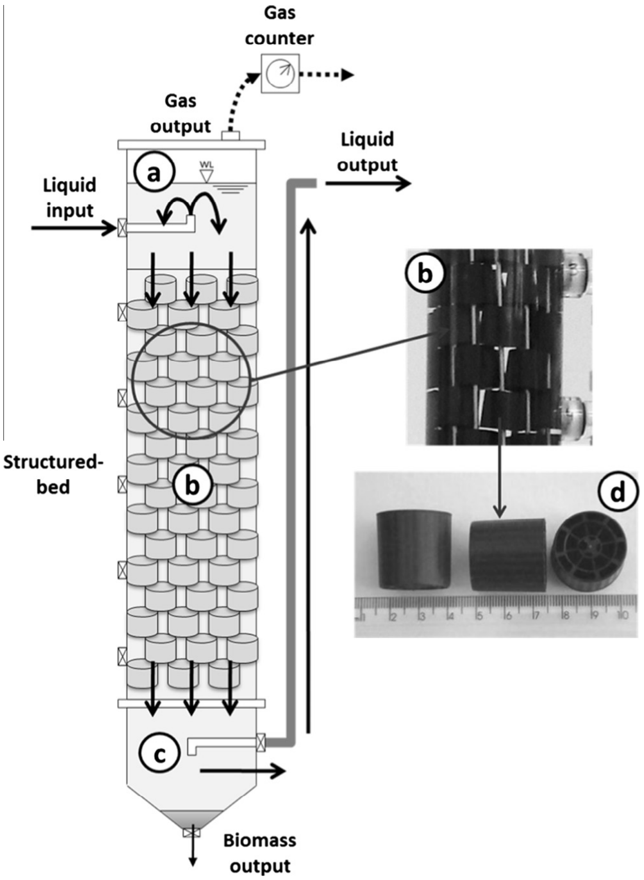

Fig. 1. Anaerobic down-flow structured-bed reactor (ADSBR) (a) influent input and headspace (b) structured bed, (c) effluent output and bottom (d) cylinders of recycled low-density polyethylene (Anzola-Rojas and Zaiat, 2015).