Biotechnology Reports 5 (2015) 46–54

Contents lists available at ScienceDirect

Biotechnology Reports

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

The use of the carbon/nitrogen ratio and specific organic loading rate as tools for improving biohydrogen production in fixed-bed reactors☆

Mélida del Pilar Anzola-Rojas [a,*], Samantha Gonçalves da Fonseca [b], Cynthia Canedo da Silva [b], Valeria Maia de Oliveira [b], Marcelo Zaiat [a]

[a] Laboratory of Biological Processes, Center for Research, Development and Innovation in Environmental Engineering, São Carlos School of Engineering, University of São Paulo 1100, João Dagnone Ave., Santa Angelina, 13563-120 São Carlos, São Paulo, Brazil

[b] Microbial Resources Division, Research Center for Chemistry, Biology and Agriculture (CPQBA), Campinas University, CP 6171, CEP 13081-970 Campinas, São Paulo, Brazil

ARTICLE INFO

Article history:
Received 23 August 2014
Received in revised form 26 October 2014
Accepted 31 October 2014
Available online 4 November 2014

Keywords:
Fermentation process
Carbon/nitrogen ratio
Specific organic loading rate
Natural inoculum
Hydrogen-producing microbial consortium

ABSTRACT

This study assessed the effect of the carbon/nitrogen (C/N) ratio on the hydrogen production from sucrose-based synthetic wastewater in upflow fixed-bed anaerobic reactors. C/N ratios of 40, 90, 140, and 190 (g C/g N) were studied using sucrose and urea as the carbon and nitrogen sources, respectively. An optimum hydrogen yield of 3.5 mol H_2 mol^{-1} sucrose was obtained for a C/N ratio of 137 by means of mathematical adjustment. For all C/N ratios, the sucrose removal efficiency reached values greater than 80% and was stable after the transient stage. However, biogas production was not stable at all C/N ratios as a consequence of the continuous decreasing of the specific organic loading rate (SOLR) when the biomass accumulated in the fixed-bed, causing the proliferation of H_2-consuming microorganisms. It was found that the application of a constant SOLR of 6.0 g sucrose g^{-1} VSS d^{-1} stabilized the system.

1. Introduction

Over the last few decades, the fast population growth has increased the global energy demand, leading to the excessive use of fossil fuels. Hydrogen gas is a clean alternative energy carrier due to its high energy yield (122 kJ g^{-1}), and it can be converted into electricity using a fuel cell. In addition, the hydrogen combustion process is attractive because it generates only water vapor as waste [12,43,54]; thus, hydrogen is an environmentally friendly, combustible source of energy.

The lack of pure hydrogen (H_2) in the environment and its high production cost are the main barriers to using hydrogen as a fuel source [4]. Processes such as electrolysis and thermal decomposition of water are more expensive than the standard methods that are used for the production of fossil fuels [9]. Nonetheless, the Hydrogen National Program of the United States estimates that in 2025, hydrogen will account for 10% of the total global energy market [43].

Currently, hydrogen is produced primarily by reformation of natural gas, high-density fuels, and naphtha. However, it can also be produced biologically by phototrophic organisms or through a fermentation process [11]. Biological hydrogen production is sustainable when the energy source is obtained from organic compounds that are present in industrial or domestic wastewater or solid waste. These sources are widely available and inexpensive, and it is necessary to treat them to control environmental pollution [6]. Furthermore, fermentative hydrogen production can be performed under non-sterile conditions at ambient temperatures and pressures without light, and there are no oxygen limitation problems [2,8,25].

Through the fermentative process, complex organic compounds such as carbohydrates are broken into simpler compounds, e.g., organic acids and alcohols; this process is accompanied by hydrogen release from both facultative and strict anaerobic microorganisms [52]. In this process, several factors such as the type of reactor [6,22,46,57], hydraulic retention time (HRT) [5,13], organic loading rate (OLR) [21,26,50], inoculum and pre-treatments [45,47], degree of back-mixing [34], and pH can affect the hydrogen production [57]. Furthermore, the characteristics of the wastewater are extremely important because the hydrogen yield depends on the organic compounds and nutrients that are available to the microorganisms.

Macronutrients (N, P, and S) and micronutrients (K, Mg, Ca, Fe, Mn, Co, Cu, Mo, and Zn) are essential for biological metabolism

* Corresponding author. Tel.: +55 1633738360.
E-mail address: melida@sc.usp.br (M.d.P. Anzola-Rojas).

http://dx.doi.org/10.1016/j.btre.2014.10.010
2215-017X/

[41]. Often, the nutritional needs of a microorganism are defined by an analysis of the cell's chemical composition, and the substances necessary for the vital functions of a microorganism are called limiting factors [38]. Thus, excess or insufficient levels of nutrients in the medium might affect the biological activity and microbial diversity, causing variations in the predominant fermentation products [43].

According to Wang and Wan [57], nitrogen concentration plays an important role in fermentative hydrogen production because nitrogen is part of the proteins, nucleic acids, and enzymes that are responsible for the growth of hydrogen producers [57]. Excess nitrogen may inhibit hydrogen production because of changes in the microbial structure and the consequent shifting of the metabolic pathway [29,42]. In addition, low nitrogen concentrations might compromise cell growth [35].

Several papers have been published regarding adequate nitrogen concentrations for improving hydrogen production, but it is not possible to define an optimal carbon/nitrogen ratio due to the differences between the configuration and operation of the fermentative process. For example, Lin and Lay [35] showed that the best hydrogen yield of 4.8 mol H_2 mol^{-1} sucrose was reached with a C/N ratio of 47, and Cheong and Hansen [9] observed a maximum hydrogen rate of 25 mL H_2 h^{-1} g^{-1} at a C/N ratio of 30 [9,35]. The aforementioned authors concluded that a high concentration of nitrogen is necessary to improve the hydrogen production.

In contrast, Argun et al. [4] observed that an adequate nitrogen concentration depends on the phosphorus concentration in the medium. That is, systems with a low phosphorus concentration require a low nitrogen concentration and vice versa. However, in their research, the best hydrogen yield of 281 mL H_2 g^{-1} starch was obtained at a C/N ratio of 200 and a C/P ratio of 1000, namely, for lower concentrations of nutrients [4].

Peixoto et al. [44] showed a similar example when added urea (COD:N of 100:0.7) was used as the nitrogen source in one of their upflow fixed-bed reactors. Under that condition, the hydrogen production ceased completely after eight days of operation. In contrast, the reactor with a COD:N ratio of 100:0.3 produced hydrogen continuously for seventy days with an average hydrogen yield of 3.5 mol H_2 mol^{-1} substrate. These authors suggested that the excessive cell growth caused by the addition of nutrients affected the reactor hydrodynamic pattern, hindering the liquid–gas transfer mass of hydrogen. In addition, the decrease of the HRT increased the production of non-reduced compounds Peixoto et al. [44].

Wang and Wan [57] summarized six studies in which the optimal nitrogen concentration varied from 0.01 to 7 g N L^{-1} in batch reactors with various substrates, inocula, and nitrogen sources. However, the differences among the hydrogen measurement parameters were the main source of difficulty in comparing the performance of those systems. These authors suggested that additional research on the effects of the nitrogen concentration in continuous systems must be performed [57].

The published data in the literature indicate the importance of the C/N ratio in the fermentative process. However, on one hand, low nitrogen concentrations may lead to nutritional deficiency, affecting hydrogen yield and productivity. On the other hand, high nitrogen concentrations tend to improve the assimilative metabolism, thus resulting in a high cellular concentration, which in turn could lead to adverse changes in the microbial pathways. Furthermore, it is noted that the biomass accumulation, mainly in fixed-bed reactors, leads to low specific organic load rates (SOLRs), which could give rise to improved homoacetogenic activity, thus resulting in a decrease in the overall hydrogen production. In this context, the existence of an optimum C/N ratio for fixed-bed reactors is hypothesized in this study.

Based on both the literature data and the stated hypothesis, this paper aimed to identify the C/N ratio that maximizes the hydrogen production of a fermentative process in a continuous system. C/N ratios of 40, 90, 140, and 190 were used in a continuous upflow fixed-bed anaerobic reactor to produce hydrogen from synthetic wastewater. A second objective, namely, to estimate the SOLR as a function of time in upflow fixed-bed anaerobic reactors, was achieved through this research.

2. Materials and methods

2.1. Reactors

Experiments were carried out in upflow fixed-bed anaerobic reactors, as depicted in Fig. 1. Each reactor consisted of an acrylic tube with an internal diameter of 80 mm, an external diameter of 88 mm, and a length 750 mm, with a total volume of 3.8 L. Each tube had four compartments: feeding (100 mm), fixed-bed (500 mm), effluent outlet (100 mm) and headspace for gas collection (50 mm). The reactors were sealed to avoid gas leakage during the experiments.

2.2. Support for biomass attachment

Cylinder-shaped particles of recycled low-density polyethylene with diameters between 7.1 mm and 17.5 mm and a length of approximately 30 mm were used as support for biomass attachment. The material provided a surface area of 7.9 m^2 g^{-1} with no porosity. Each bed contained 374 g support L^{-1} bed with a uniformity coefficient of 1.20, resulting in a bed porosity of 60%.

2.3. Lab-made wastewater

The synthetic wastewater with a COD of 2 g L^{-1} was mainly composed of sucrose (1789.2 mg L^{-1}) and urea (40.6 mg L^{-1}, 17.9 mg L^{-1}, 11.5 mg L^{-1}, and 8.5 mg L^{-1} for C/N ratios of 40, 90, 140, and 190, respectively). The C/N ratios were calculated based on the percentages of carbon and nitrogen by mass in sucrose ($C_{12}H_{22}O_{11}$) and urea (CH_4N_2O). Micronutrients were added according to Peixoto et al. [44]: $NiSO_4{\cdot}6H_2O$ (0.5 mg L^{-1}),

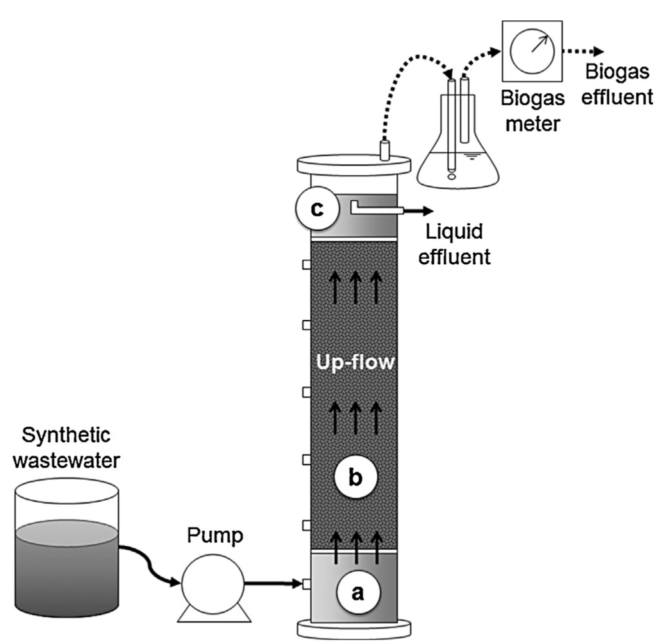

Fig. 1. Upflow fixed-bed anaerobic reactor for biological hydrogen production.