INTERNATIONAL JOURNAL OF HYDROGEN ENERGY 39 (2014) 6402–6406

Available online at www.sciencedirect.com

ScienceDirect

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

On the independence of hydrogen production from methanogenic suppressor in olive mill wastewater

M.R. Gonçalves, J.C. Costa, M.A. Pereira, A.A. Abreu*, M.M. Alves

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

ARTICLE INFO

Article history:
Received 20 August 2013
Received in revised form
18 November 2013
Accepted 9 February 2014
Available online 11 March 2014

Keywords:
Biohydrogen
Biomethane
Homoacetogenesis
Olive oil mill effluent
Methanogenic suppressor
Anaerobic digestion

ABSTRACT

Anaerobic degradation of olive mill wastewater (OMW) at concentrations ranging from 2 to 100 g/L of chemical oxygen demand (COD) was assessed in batch assays. Methane was the main final product obtained for the lower concentrations tested. For 25 g COD/L, H_2 was temporarily produced, albeit H_2 depletion occurred, likely due to homoacetogenesis, since acetate was formed concomitantly. Hydrogen was produced and accumulated permanently in the assays containing 50 g COD/L of OMW. Methanogenesis and homoacetogenesis were naturally inhibited, suggesting that hydrogen recovery from OMW can be performed without the addition of methanogenic suppressors such as 2-bromoethanosulfonate. This fact opens new perspectives for the utilization of high OMW concentrations in a two-stage valorisation process combining biohydrogen and biomethane production.

1. Introduction

Olive mill wastewater (OMW) is a complex effluent obtained from the traditional press and the continuous three-phase mills of olive oil production. Large amounts of OMW are generated every year and yet there are no feasible solutions to its treatment [1]. The production of biofuels (methane or hydrogen) from OMW is a promising solution for the treatment and valorisation of this pollutant [2]. However, there are still some problems associated with both processes.

Anaerobic digestion of raw OMW has been reported as a difficult process mainly due to their intrinsic characteristics, such as acid pH, high organic loads and the presence of complex and toxic compounds (lipids and phenolic compounds) [1]. Anaerobic batch experiments have shown that high concentrations of OMW, such as 50 g/L chemical oxygen demand (COD), may lead to the inhibition of the microbial consortium [3]. The high concentration of raw OMW (130 g COD/L) has led researchers to use highly diluted streams (5 g COD/L) during the start-up of continuous anaerobic reactors, whereas 45–50 g COD/L of OMW was only used after one year of operation [4].

Hydrogen production from OMW has been performed by dark and photofermentation [5–8]. One of the main issues concerning hydrogen production through anaerobic processes is to assure that hydrogen-consuming microorganisms' are inhibited, and the activity of hydrogen-producing microorganisms is preserved and stimulated. Under anaerobic conditions, hydrogen is used mainly by hydrogenotrophic

* *Corresponding author*. Tel.: +351 253604400; fax: +351 253604429.
E-mail addresses: angela_abreu@deb.uminho.pt, abreu.angela@gmail.com (A.A. Abreu).
http://dx.doi.org/10.1016/j.ijhydene.2014.02.056

methanogens to produce methane and by homoacetogenic bacteria to produce acetate [9]. Sludge pre-treatment with heat [10,11] and the addition of chemicals such as 2-bromoethanesulfonate (BES) [12,13] and chloroform [14] have been used to inhibit H_2 utilizers during the anaerobic degradation of wastewaters such as OMW and palm oil mill effluent. Alternatively, pure cultures have been used to produce hydrogen from these types of effluents [15]. Nevertheless, these strategies increase the overall cost of the process. In addition, chemical and heat treatments have usually a short time effect on methanogeneses and are not effective to prevent homoacetogenesis [16,17]. So far, there are no studies correlating OMW concentration with hydrogen production without applying strategies to inhibit H_2 utilizers.

Preliminary studies carried out in our research group (not published) suggested that hydrogen is selectively produced at high OMW concentration, in detriment of methane, without the need of applying strategies to inhibit H_2 utilizers. In this vein, the main objective of this work is to get more insights on the influence of OMW concentration on biohydrogen production and on the requirement of a methanogenic inhibitor.

2. Material and methods

Anaerobic batch experiments were carried out at different initial OMW concentrations, ranging from 2 to 100 g chemical oxygen demand per litter (COD/L), in the presence and absence of a methanogenic suppressor 2-bromoethane sulfonate (BES) – an analogue of coenzyme M in methanogens and inhibitor of methane-producing *Archaea*. These experiments were performed to evaluate the influence of the substrate concentration on H_2 and CH_4 production and to assess the need of a methanogenic inhibitor to promote H_2 production.

2.1. Inoculum and substrate

The anaerobic suspended sludge used in the batch experiments was obtained from a domestic wastewater treatment plant. The specific methanogenic activity of the sludge was <0.05 and 0.26 ± 0.01 g COD-$CH_{4(STP)}$ gVS^{-1} d^{-1} for acetate and H_2/CO_2 (80/20 v/v), respectively. The OMW was obtained from a three-phase continuous olive oil extraction process (Amarante, Portugal) and stored at −20 °C for further utilization. OMW was characterized and the values obtained are summarized in Table 1.

2.2. Experiment set-up

Batch assays were performed in closed vials with volumes of 70 and 160 mL. The working volume was 20 mL. The sludge was added to the vials at a final concentration of around 3 g volatile suspended solids per litter (VSS/L). The basal medium used in all batch experiments was made up with demineralised water and sodium bicarbonate (3 g/L) and the pH was adjusted to 7.0. The OMW, previously adjusted to pH 7.0, was diluted at different final concentrations of 2, 10, 25, 50, and 100 g COD/L. The vials were flushed with N_2/CO_2 (80:20 v/v) and finally the medium was reduced with $Na_2S.9H_2O$ at final concentration of 1 mM. The batch experiments were performed in the presence (15 mM) and absence of BES. The vials were placed on a rotary shaker (100 rpm) and incubated at 37 °C. The batch experiments performed with OMW concentrations of 2, 10 and 25 g COD/L were done in duplicate. pH, methane and hydrogen were determined along the experiment time. For the batch assay containing 25 g COD/L, volatile fatty acids (VFAs) were also analysed. Batch experiments with 50 and 100 g COD/L of OMW were carried out in quadruplicate, since the results variability is high for these substrate concentrations. In this case, VFAs and pH were only measured at the end of the experiment. Methane and hydrogen accumulated in the vials headspace were measured along the experiments. The measured values of each gas were corrected to standard temperature and pressure (STP) conditions. The amount of methane produced was converted to equivalent COD (mg COD-CH_4), considering the theoretical biochemical methane potential (350 L CH_4 kg^{-1} COD).

Table 1 – Olive mill wastewater (OMW) characterization.

Parameter	OMW[a]
pH	4.7 ± 0.1
Total COD (g/L)	130.1 ± 7.4
Total Solids (g/L)	75.5 ± 3.1
Total Nitrogen (mg/L)	460.0 ± 53.2
Total Phenols (Gallic acid, g/L)	4.3 ± 0.4
Oil and Grease (g/L)	13.6 ± 1.5
Total free-long chain fatty acids (g COD/L)	6.2 ± 3.8
% C18:1	78.1 ± 10.9

[a] Data expressed as an average ± error (95% confidence).

2.3. Analytical methods

Total chemical oxygen demand (COD), total solids (TS), total phenols and biogas were determined as described in previous studies [3,4]. VFAs analysis has been described previously [18].

3. Results and discussion

The initial production of hydrogen and methane from OMW at concentrations ranging from 2 to 100 g COD/L, in the presence and absence of a methanogenic inhibitor (BES), is represented in Fig. 1.

In BES-free vials, the highest methane production (49 mg COD-CH_4) was achieved with 2 g COD/L of OMW (Fig. 1(a)) in 19 days, representing a biodegradability of 81%. Lower methanisation was obtained for OMW concentrations of 10 and 25 g COD/L, in a similar time range, and no methane production was observed in batch experiments with 50 and 100 g COD/L. A lag-phase of 7 days was observed in the batch experiment performed with 25 g COD/L.

Regarding hydrogen production (Fig. 1(b)), the accumulation of H_2 was only verified in batch experiments with 50 g COD/L. A production of 0.53 mmol H_2 was attained after 3 days and it was practically stable until the end of experiment (32 days). After day 1, 0.3 mmol of hydrogen was produced, with