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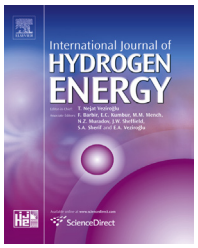

Long-term effect of acid and heat pretreatment of sludge from a sugarcane vinasse treatment plant on the microbial community and on thermophilic biohydrogen production

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ARTICLE INFO

Article history:
Received 29 May 2015
Received in revised form
12 August 2015
Accepted 22 August 2015
Available online 26 September 2015

Keywords:
Vinasse sludge pretreatment
Lactic acid
Clostridium
Thermoanaerobacterium

ABSTRACT

This work investigated how heat and acid pretreatment affected thermophilic biohydrogen production by a mixed culture present in the sludge of a sugarcane vinasse treatment plant. Heat- (80 °C for 15 min) or acid- (pH 3 for 24 h) treated sludge samples were employed to seed batch bioreactors along 36 days. The heat- and acid-treated sludge samples afforded H_2 production rates (HPRs) of 8.4 and 9.5 mmol/L.d, respectively. The lactic acid concentration increased in the bioreactor seeded with heat-treated sludge, whilst the lactic acid concentration diminished in the bioreactor inoculated with the acid-treated sludge. Identification of the microbial community revealed that Clostridiaceae predominated in all the sludge samples. The most abundant Clostridiaceae in the heat- and acid-treated sludge samples were *Clostridium* and *Thermoanaerobacterium*, respectively. Acid pretreatment modified the microbial community and enhanced consumption of lactic acid, a metabolite that is normally associated with decreased H_2 production.

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http://dx.doi.org/10.1016/j.ijhydene.2015.08.096

Introduction

Hydrogen is a clean energy source. However, the methods that are currently available for H_2 production, such as oil or coal processing, consume fossil fuels and a large amount of energy [1]. In recent years, researchers have considered the use of fermentation to produce H_2: this process occurs at ambient temperature and pressure and employs carbohydrate-rich wastes and wastewater as raw material [2,3].

Some studies on fermentative H_2 production have used pure cultures under sterile conditions [4]. An interesting alternative has been to employ mixed cultures instead of pure cultures, which dismisses the need for sterilization. Other advantages of mixed cultures include the ability to adapt the microbial diversity and handle different substrates and the possibility of conducting continuous processes [5–7].

Mixed cultures from bioreactors employed in the anaerobic treatment of wastewater constitute the preferred seed inoculum for fermentative H_2 production. Examples of such inoculum are the sludge from the Upflow Anaerobic Sludge Blanket (UASB) reactor used to treat the wastewater from brewery production processes [8], the anaerobic sludge from palm oil mill effluent (POME) treatment plants [9], and the anaerobic sludge from municipal treatment plants [10].

Brazil is the world's largest producer of bioethanol. This activity generates a huge amount of vinasse—ca. 12 L per liter of produced ethanol. This wastewater is rich in nitrogen and sulfur compounds. Part of the vinasse is used as fertilizer; it is also biologically treated in anaerobic bioreactors for methane production [11]. Therefore, the anaerobic sludge obtained from sugarcane vinasse treatment is a widely available regional inoculum source. Some works have employed vinasse as substrate for biohydrogen production [12–14], but application of sludge from vinasse anaerobic treatment systems as inocula and the effects of different pretreatments on such vinasse sludge for biohydrogen production remain unexplored.

Successful H_2 production by a mixed bacterial culture, like anaerobic sludge, requires inhibition of the bacteria involved in the latest step of anaerobic digestion; i.e., methanogenesis. Another strategy is to inhibit H_2 consumption mediated by microorganisms that comprise the anaerobic sludge [6]. Several sludge pretreatments can avoid the presence of H_2 consumers and enrich a mixed culture with H_2-producing bacteria [5]. Most of the pretreatment methods involve heat [15,16] or acid pretreatment [17–19], or a combination of both [20–23]. However, the efficacy of each pretreatment method depends on the inoculum source, on the substrate, and on the bioreactor operation conditions [5,24]. In other words, each mixed culture deserves to be investigated for the effect of inoculum pretreatment on biohydrogen production [5].

There is a literature consensus that it is necessary to pretreat mixed cultures for H_2 production. Nevertheless, the majority of studies on the pretreatment of mixed cultures have relied on batch tests accomplished for a short time, which may not reflect the effect of pretreatment throughout long operation periods [24].

Here, we have explored the use of a mixed culture obtained from a thermophilic anaerobic sugarcane vinasse treatment plant for biohydrogen production in long-term assays carried out in Anaerobic Sequential Batch Reactors—ASBRs, which are simple to operate. More specifically, we have evaluated the effects of acid (pH 3/24 h) and heat (80 °C/15 min) pretreatment on hydrogen production, soluble metabolites, and microbial community of the sludge from a vinasse treatment plant.

Materials and methods

Inoculum

The inoculum consisted of a sludge collected from a UASB reactor operated at 50 °C, used to treat the effluent from a sugar and ethanol mill (vinasse) situated in the Region of Ribeirão Preto, state of São Paulo, Brazil. During the experiments, the sludge was maintained by feeding with glucose (Diprolab, Brasil) at 5.0 g/L (27.8 mmol/L) and with a nutrient solution containing $NiSO_4.6H_2O$ (0.50 mg/L), $FeSO_4.7H_2O$ (2.50 mg/L), $FeCl_3.6H_2O$ (0.25 mg/L), $CoCl_2.2H_2O$ (0.04 mg/L), $CaCl_2.6H_2O$ (2.06 mg/L), SeO_2 (0.042 mg/L), KH_2PO_4 (5.36 mg/L), K_2HPO_4 (1.30 mg/L), Na_2HPO_4 (2.76 mg/L), and urea (20.0 mg/L). All the chemicals were analytical grade.

Mixed culture pretreatments

At the beginning of the pretreatments, the volatile solids (VS) concentration in the sludge was analyzed according to the *Standard Methods of Water and Wastewater* [25] and adjusted to 2.5 g/L. This guaranteed the same VS concentration throughout the pretreatments.

Preliminary sludge pretreatment tests with different temperatures, pH, and times were conducted. The effect of these pretreatments on H_2 production was assayed in short-term batch tests (results not shown) and analysed by kinetic models [26]. The conditions previously tested for the heat pretreatments were 100 °C for 15 min and 60 min, and 80 °C for 15 min and 60 min, as suggested in the literature [20–24]. For the acid pretreatments, the following conditions were assayed: pH 3 for 12 and 24 h. The combined pretreatment was tested at pH 3 for 24 h, which was followed by heat treatment at 80 °C for 15 min [23]. These preliminary assays revealed that the following conditions enhanced H_2 production in short-term tests: 80 °C for 15 min followed by cooling to room temperature and maintenance of pH 3 for 24 h by addition of HCl at 5.0 mol/L. Thereafter, the pH was adjusted to 6.0 with NaOH at 5.0 mol/L, and the pretreated sludge was used to seed bioreactors.

Disaggregating the sludge before the pretreatments was not necessary because the granules had soft consistency. Disaggregation occurred after the pretreatments.

Long-term hydrogen production in anaerobic sequential batch Reactor—ASBR

Three 450-mL bioreactors were operated as ASBRs for each inoculum (sludge without pretreatment (control), acid-treated sludge, and heat-treated sludge). For the experiments, 150 mL of sludge sample was added to the bioreactors at the beginning of the operation.