Bioresource Technology 175 (2015) 152–159

Contents lists available at ScienceDirect

Bioresource Technology

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

Effects of furan derivatives on biohydrogen fermentation from wet steam-exploded cornstalk and its microbial community

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HIGHLIGHTS

- Understanding furan derivatives is important for gas biofuels from lignocellulose.
- HMF and FUR had various effects on hydrogen fermentation and microbial community.
- Hydrogen productivity was increased by up to 40% with the addition of HMF.
- Furan derivatives were almost degraded at an initial concentration below 1 g/L.

ARTICLE INFO

Article history:
Received 25 August 2014
Received in revised form 12 October 2014
Accepted 13 October 2014
Available online 18 October 2014

Keywords:
Anaerobic fermentation
Biohydrogen
Furan derivatives
Steam-exploded cornstalk

ABSTRACT

Understanding the role of furan derivatives, furfural (FUR) and 5-hydroxymethyl furfural (HMF), is important for biofuel production from lignocellulosic biomass. In this study, the effects of furan derivatives on hydrogen fermentation from wet steam-exploded cornstalk were investigated. The control experiments with only seed sludge indicated that HMF addition of up to 1 g/L stimulated hydrogen production. Similar results were obtained using steam-exploded cornstalk as the feedstock. Hydrogen productivity was increased by up to 40% with the addition of HMF. In addition, over 90% of furan derivatives with an initial concentration below 1 g/L were degraded. Pyosequencing showed that the addition of HMF and FUR resulted in different microbial communities. HMF led to a higher proportion of the genera *Clostridium* and *Ruminococcaceae*, supporting the increased hydrogen production. This study suggested that hydrogen fermentation could be a detoxifying step for steam-exploded cornstalk, and HMF and FUR exhibited different functions for hydrogen fermentation.

1. Introduction

Waste, otherwise known as low-grade biomass has become a worldwide problem in terms of environment and natural resources. China is one country that produces large amounts of low-grade biomass from agriculture each year. Low-grade biomass mainly consists of plant residues and livestock manure, which has caused serious environmental pollution and social problems, such as the emission of greenhouse gases, non-point source pollution, water eutrophication and pathogen contamination (Chen et al., 2009; Zhang et al., 2010). Waste biomass can be used to generate biohythane i.e. the mixture of biohydrogen and biomethane by two-stage anaerobic fermentation, which offers more environmental and social benefits than fossil-based hythane (Liu et al., 2013; Lu et al., 2009). Compared to biomethane fermentation, two-stage biohythane fermentation maximizes the total energy recovery from biomass and shortens the fermentation time (Liu et al., 2013). Efficient biohydrogen fermentation is therefore the first important step toward harvesting biohythane from low-grade biomass.

Steam explosion has already been recognized as an efficient method to destroy the structure of lignocelluloses, for instance cornstalk, using a high-pressure steam (Hendriks and Zeeman, 2009). However, furan derivatives are released during steam explosion, which are regarded as a notorious fermentation inhibitor (Li and Chen, 2008; Wang et al., 2013). The furan derivatives mainly include furfural (FUR) and 5-hydroxymethyl furfural (HMF), which exert negative influence on microbial fermentation

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http://dx.doi.org/10.1016/j.biortech.2014.10.067
0960-8524/

through reducing cell growth rate, lowering cell membrane permeability, and inducing reactive oxygen species (Allen et al., 2010; Eva and Barbel, 2000; Koopman et al., 2010). Most studies focused on the influence of furan derivatives on ethanol fermentation (Almeida et al., 2009; Ask et al., 2013; Taherzadeh et al., 1999). Recently, the inhibitory effects of furan derivatives on biohydrogen production have received increasing attention (Cao et al., 2010; Kongjan et al., 2010; Park et al., 2011; Quéméneur et al., 2012; Veeravalli et al., 2013). Specifically, Cao et al. investigated the effect of fermentation inhibitors, including furan derivatives, on thermophilic hydrogen production from acid pretreated cornstalk by using a pure culture, *Thermoanaerobacterium thermosaccharolyticum* W16 (Cao et al., 2010). Veeravalli et al. (2013) studied the effects of HMF/FUR (0–1 g/L) on hydrogen production from a glucose-containing medium and found that furans affected the hydrogen production and microbial diversity. Another study (Monlau et al., 2013) reported the inhibition of sunflower stalk hydrolyzates on hydrogen production from a glucose-containing medium. The hydrolyzates contained soluble sugars, and fermentation inhibitors, including furan derivatives and phenolic compounds. The hydrogen productivity was decreased largely to zero with the increase of the hydrolyzates concentration. The dominant population was shifted from *Clostridium* sp. to *Sporolactobacillus* sp. It has been suggested that the concentration of the inhibitors should be largely reduced to avoid their negative influence on the microbial metabolism.

As furan derivatives are always regarded as fermentation inhibitors, many studies have been devoted to detoxifying furan derivatives or screening microorganisms tolerant to furan derivatives (Li and Chen, 2008; Taherzadeh et al., 1999; Wang et al., 2013; Zhang et al., 2013). Detoxification through biochemical conversion has been employed since the 1960s (Kakinuma and Yamatodani, 1964; Wierckx et al., 2011). The commonly isolated furan-degrading microorganisms are aerobic gram-negative bacteria such as *Pseudomonas* sp. (Koenig and Andreesen, 1989; Lopez et al., 2004), and *Stenotrophomonas* sp. (Lopez et al., 2004). Koopman recently isolated a strain, *Cupriavidus basilensis* HMF14, capable of selectively degrading HMF and FUR, while leaving sugars intact (Koopman et al., 2010; Wierckx et al., 2010). However, anaerobic degradation of furan derivatives has seldom been reported. *Desulfovibrio* sp. is the only isolated anaerobic microorganism capable of converting FUR into acetic acid (Boopathy and Daniels, 1991; Brune et al., 1983).

Simultaneous saccharification and hydrogen generation from solid lignocellulosic biomass, such as cornstalk can be realized through anaerobic fermentation (Cheng and Liu, 2011; Lu et al., 2009; Xu et al., 2010). Steam explosion is useful for biomass pretreatment in order to improve the fermentation efficiency of cornstalk for hydrogen production, which always results in the formation of HMF and FUR as discussed above. Nevertheless, the performance of HMF and FUR in hydrogen fermentation from steam-exploded cornstalk by anaerobic microbial consortium was never reported, which will be presumably totally different from those reported for the fermentation from a glucose-containing medium. This is mainly because hydrogen fermentation from cornstalk is much different from other easily degradable substrates, such as organic wastewater, starch or kitchen wastes.

The purposes of the current study were (1) to compare the effects of HMF and FUR on hydrogen fermentation from wet steam-exploded cornstalk using anaerobic sludge as inocula; (2) to analyze whether furan derivatives can be detoxified through anaerobic hydrogen fermentation; and (3) to elucidate the different impacts of HMF and FUR on microbial communities during hydrogen fermentation from steam-exploded cornstalk using the 454 pyrosequencing technology.

2. Methods

2.1. Inoculum, feedstock and medium

Seed sludge was collected from an anaerobic digester at the Xiaohongmen Wastewater Treatment Plant (Beijing, China). Heat treatment was accomplished by boiling the samples using 100 °C hot water for 30 min. 7% (w/v) of the boiled seed sludge was inoculated for hydrogen fermentation. Steam explosion of the cornstalks was carried out with high-pressure steam at 1.5 MPa, 200 °C for 5 min in a steam-explosion reactor (Laihe Company, China). The wet steam-exploded cornstalk had a 28–30% total solid content (TS) and 73% VS (volatile solid) of TS. The VS consisted of 70% of cellulose, 6% of hemi-cellulose and 24% of lignin. The raw wet steam-exploded cornstalk contained HMF and FUR at the concentrations of 150–200 mg/L and 160–220 mg/L, respectively. The medium included the following (per L): yeast extract, 2.0 g; $(NH_4)_2SO_4$, 1.3 g; KH_2PO_4, 1.5 g; $K_2HPO_4{\cdot}3H_2O$, 2.9 g; $CaCl_2$, 0.075 g; $MgCl_2{\cdot}6H_2O$, 0.2 g; and $FeSO_4{\cdot}7H_2O$, 1.25 mg. The wet SEC was diluted with a prepared medium to reach a final TS concentration of 8% and then it was used as the substrate for hydrogen fermentation without any other treatment. The initial pH of the substrate was adjusted to 6.5 for hydrogen fermentation.

2.2. Experimental setup and procedure

The experiment device was a normal-pressure batch bioreactor system, consisting of a 250-mL glass flask (150-mL working volume), gas-tight plastic tubes, sampling valve, and gas balloon. The glass flask served as the anaerobic bioreactor, the produced gas was measured through the gas sampling valve and collected by using the gas balloon, and the fermented broth was evaluated by the sampling port embedded in the flask.

HMF and FUR were employed as model furan derivatives of the cornstalk hydrolysate through steam-explosion. The effects of furan derivatives on hydrogen fermentation from steam-exploded cornstalk were conducted in the following procedure: A control experiment was designed by investigating hydrogen production fed with inoculated seed sludge and externally added furan derivatives in different concentrations (0, 10, 100, 500 and 1000 mg/L) without any cornstalk. The purpose of this experiment was to obtain the baseline of hydrogen production from seed sludge and to study the effects of furan derivatives on the activities of seed sludge. Furan derivatives in five concentrations (0, 100, 500, 1000 and 2000 mg/L) were then applied to hydrogen fermentation from steam-exploded cornstalk using the same seed sludge to study how furan derivatives affected hydrogen fermentation. The microbial communities formed in the hydrogen fermentation with the additions of HMF and FUR were finally analyzed by pyrosequencing. Hydrogen fermentation was performed using closed flasks in a 100 rpm biochemical shaker at 37 °C.

In the verification experiment of hydrogen fermentation capable of utilizing HMF and FUR contained in SEC, a 5-L stirred batch reactor fed with steam-exploded cornstalk (TS, 8%) was used. The inocula were the same sludge as that used in the flask batch culture. Hydrogen fermentation in the 5-L batch reactor was carried out with an agitating speed of 100 rpm, a temperature of 37 °C and pH controlled at 6.5.

2.3. Analytical methods

Gas composition was detected as previously described (Liu et al., 2012) using a GC equipped with a thermal conductivity detector and a stainless-steel column packed with TDX-01