Bioresource Technology 221 (2016) 598–606

Contents lists available at ScienceDirect

Bioresource Technology

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

The impact of furfural concentrations and substrate-to-biomass ratios on biological hydrogen production from synthetic lignocellulosic hydrolysate using mesophilic anaerobic digester sludge

Chinaza Akobi [a], Hisham Hafez [b,c], George Nakhla [a,b,*]

[a] Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario N6A 5B9, Canada
[b] Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario N6A 5B9, Canada
[c] GreenField Specialty Alcohols Inc., Chatham, Ontario N7M 5J4, Canada

HIGHLIGHTS

- Enhancements in biohydrogen production at furfural concentrations of up to1 g/L.
- Furfural threshold concentration of $\leqslant$1 g/L was established.
- A food- to microorganisms ratio of 4 gCOD/gVSS was optimal.
- Hydrogen yields correlated linearly with both furfural:sugar and furfural:biomass.
- Furfural:sugars exerted a greater effect on H_2 inhibition than furfural:biomass.

ARTICLE INFO

Article history:
Received 21 July 2016
Received in revised form 13 September 2016
Accepted 15 September 2016
Available online 24 September 2016

Keywords:
Furfural
Biohydrogen
Mesophilic anaerobic digester sludge
Lignocellulosic hydrolysate
Batch

ABSTRACT

This study evaluated the impact of furfural (a furan derivative) on hydrogen production rates and yields at initial substrate-to-microorganism ratios (S°/X°) of 4, 2, 1, and 0.5 gCOD/gVSS and furfural concentrations of 4, 2, 1, and 0.5 g/L. Fermentation studies were carried out in batches using synthetic lignocellulosic hydrolysate as substrate and mesophilic anaerobic digester sludge as seed. Contrary to other literature studies where furfural was inhibitory, this study showed that furfural concentrations of up to 1 g/L enhanced hydrogen production with yields as high as 19% from the control (batch without furfural). Plots of hydrogen yields against g furfural/g sugars and hydrogen yields versus g furfural/g biomass showed negative linear correlation indicating that these parameters influence biohydrogen production. Regression analysis indicated that g furfural/g $sugars_{initial}$ exerted a greater effect on the degree of inhibition of hydrogen production than g furfural/g VSS_{final}.

1. Introduction

Current global technologies for energy production and supply rely mainly on fossil fuels causing rapid depletion of these resources and increasing carbon dioxide emissions as energy consumption continues to increase. The biological production of hydrogen, which is a renewable energy carrier, can mitigate this trend and lessen the concerns linked with fossil fuel use. Biohydrogen production methods are more sustainable, environmentally-friendly, and less energy intensive compared with current methods of energy production (Gomez-flores et al., 2015; Kapdan and Kargi, 2005; Lay, 2001).

The main light-independent process for biohydrogen production is dark fermentation. Biomasses that are rich in carbohydrates are the most-suitable feedstocks for biohydrogen production using fermentative anaerobic bacteria (Chen et al., 2006; Ntaikou et al., 2010). The use of low-cost feedstock is necessary in creating a cost-effective technology. Lignocellulosic materials such as agricultural residues (e.g. corn stalks, corn cobs, sugar cane bagasse, rice straw), hardwood (e.g. poplar wood, aspen wood) and softwood (e.g. red cedar, red oak) are generally found in abundance as agricultural or industrial by-products with little or no commercial value (Cantarella et al., 2004; Costa Lopes et al., 2013; Du et al., 2010; Fenske et al., 1998; Lynd et al., 1996; Polman, 1994). In agriculture, most of these wastes are left unused on the fields after

* Corresponding author at: Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario N6A 5B9, Canada.
E-mail address: gnakhla@uwo.ca (G. Nakhla).

http://dx.doi.org/10.1016/j.biortech.2016.09.067
0960-8524/

harvest, thus creating environmental problems and a waste of potential renewable resource (Pan et al., 2010).

Carbohydrates in lignocellulosic biomass are usually complex and not just simple hexose or pentose sugars (Galbe and Zacchi, 2012). It is relatively difficult for microorganisms to degrade the cellulosic and hemicellulosic polymers of lignocellulosic materials to yield sugars. These complex compounds need to be broken down into simpler forms for easy conversion to hydrogen gas (Cao et al., 2010). In order to enhance the yield and rate of biohydrogen production, lignocellulosic biomass must therefore undergo pre-treatment.

Several pre-treatment methods using steam explosion, ammonia fiber explosion (AFEX), acid, alkali, liquid hot water and many others have been employed (Cantarella et al., 2004; Du et al., 2010; Siqueira and Reginatto, 2015). Acids, particularly dilute acid pre-treatment, have been widely used with respect to biohydrogen production as it is considered the easiest, most efficient, and cost-effective method that produces high sugar yields and suitably changes the structure of the substrate to facilitate fermentation (Cao et al., 2010; Chang et al., 2011; Cui et al., 2009; Mosier et al., 2005; Panagiotopoulos et al., 2009; Pattra et al., 2008). However, this process generates fermentation inhibitors such as furan derivatives (aldehydes including furfural and hydroxymethylfurfural), ketones, phenols (such as vanillin, syringaldeyde) and organic acids (such as acetic acid) (Allen et al., 2010; Klinke et al., 2004). The hemicellulosic fraction of lignocellulose undergoes hydrolysis at high temperatures and pressures; and in the presence of dilute acids, yield monomeric sugars (mainly pentoses) in a reaction known as the Maillard reaction (Cantarella et al., 2004; Navarro, 1994). Under these conditions, the inhibitor furfural, is released as a by-product when the pentose sugars undergo dehydration. This inhibitor has been shown to have toxic effects on cells causing damage by inhibiting enzymes produced by microorganisms during hydrolysis and fermentation of sugars resulting in low biohydrogen production rates and yields (Allen et al., 2010; Cantarella et al., 2004). Furfural also alters the growth of microorganisms by impeding enzymes responsible for fermentation, thus affecting their membrane integrity (Mills et al., 2009; Quéméneur et al., 2012). Due to these inhibitory effects, furfural is considered to be a limiting factor in the biological conversion of lignocellulosic materials.

Microorganisms have the ability to minimize the effects of furfural as an inhibitor by metabolic switch between pathways where furfural is converted to less toxic compounds such as furfuryl alcohol or furoic acid, if its concentration does not exceed levels that the microorganisms can tolerate (Boopathy et al., 1993; Boyer et al., 1992; Liu et al., 2004, 2005). Hydrolysates can also be detoxified using charcoal, diethyl ether, ion exchange resin, activated carbon, $Ca(OH)_2$ (overliming) or with yeasts in order to increase hydrogen yields (Mateo et al., 2013; Zhao et al., 2011).

It is, however, important to determine the inhibition threshold levels of furfural prior to fermentation so as to maximize biohydrogen production rates and yields as well as reduce toxicity to tolerable levels in order to preserve microbial activity. The inhibitory effects of furfural on fermentative hydrogen production using mixed cultures have not been thoroughly studied. Literature on this subject are few in number with little or no data regarding furfural's inhibitory concentrations (Siqueira and Reginatto, 2015). Some studies have been done with furfural using pure substrates such as glucose (Siqueira and Reginatto, 2015) and xylose (Quéméneur et al., 2012); real hydrolysates (Cantarella et al., 2004; Cao et al., 2010); and pure cultures (Cao et al., 2010; Monlau et al., 2013); and other studies have been carried out to ascertain the effects of pH, substrate concentration and other intermediate products like acetate, butyrate etc., (Ginkel et al., 2001; Khanal et al., 2004). The present study employs the use of a mix of pure sugars and volatile fatty acids as substrate, simulating the composition of a typical real waste hydrolysate at different S°/X° ratios.

Therefore, the main objectives of this study were to assess the impact of furfural on lignocellulosic hydrolysate using mixed cultures to evaluate hydrogen production potential and ascertain the threshold furfural concentrations that resulted in the maximum hydrogen production rates and yields.

2. Materials and methods

2.1. Seed sludge and substrate

Mesophilic anaerobic digester sludge was collected from St. Marys Wastewater Treatment Plant, Ontario, Canada and pre-heated at 70 °C for 30 min prior to use so as to inactivate non-hydrogen producers (Hafez et al., 2010; Kumar et al., 2014). The pH, total suspended solids (TSS) and volatile suspended solids (VSS) concentration of the sludge were 6.97, 16.2 g/L and 12.2 g/L respectively. Synthetic hydrolysate was prepared in the laboratory using substrate characteristics simulating the composition of a typical pretreated lignocellulosic hydrolysate. The substrate comprised mainly sugars and volatile fatty acids (VFAs) and included (per liter of distilled water): arabinose, 5.9 g; xylose, 50 g; mannose, 0.3 g; galactose, 2.5 g; glucose, 6.7 g; formate, 1.2 g and acetate, 1.8 g.

2.2. Batch setup

Experiments were conducted in batches using 250 mL Wheaton glass serum bottles with working volumes of 200 mL under anaerobic conditions. 40 mL of seed was added per bottle and the volume of substrate ($V_{substrate}$) added to each bottle was calculated using the substrate-to-biomass ratio equation as described by Nasr et al. (2014).

$$S^{\circ}/X^{\circ}\,(gCOD/gVSS) = \frac{V_{substrate} * TCOD_{substrate}}{V_{seed} * VSS_{seed}} \quad (1)$$

where $V_{substrate}$ and V_{seed} are the volumes of substrate and seed respectively in L, $TCOD_{substrate}$ is the total chemical oxygen demand of the substrate in g/L and VSS_{seed} is the volatile suspended solids content of the seed also in g/L. Four furfural concentrations (4, 2, 1, and 0.5 g/L) were tested at S°/X° of 4, 2, 1, and 0.5 $gCOD_{substrate}/gVSS_{seed}$ in triplicates. Batch controls consisted of substrate and seed sludge without furfural while blanks were run with seed sludge only. The composition of nutrient media added to each bottle in mg/L included: $CaCl_2$, 140; $MgCl_2{\cdot}6H_2O$, 160; $MgSO_4{\cdot}7H_2O$, 160; Urea, 1500; Na_2CO_3, 200; $KHCO_3$, 200; K_2HPO_4, 15; H_3PO_4, 500; trace metal solution (TMS), 500 (Hafez et al., 2010). The initial pH of the mixture was adjusted to 5.5 ± 0.04 using HCl but was not controlled during the experiment. Buffering capacity was however provided by adding 5 g/L $NaHCO_3$ to each bottle. Ten-mL samples were collected from each bottle for initial analysis before the bottles were purged with nitrogen gas to create an anaerobic condition. The batch was operated at a temperature of 37 °C in a swirling shaker (MaxQ 4000 Thermo Scientific CA benchtop shaker) at a speed of 180 rpm. At the end of the batch, final samples were taken for analysis.

2.3. Analytical methods

Glass syringes in the range 5–100 mL were used at regular intervals to release the gas in the bottles in order to equilibrate with ambient pressure (plunger displacement method) (Chen et al., 2006; Gupta et al., 2015). Hydrogen was analyzed using a