Renewable Energy 93 (2016) 302–311

Contents lists available at ScienceDirect

Renewable Energy

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

Impact of furfural on biohydrogen production from glucose and xylose in continuous-flow systems

Basem Mikhaeil Haroun [a, b], George Nakhla [a, c], Hisham Hafez [c, d, *], Fayza Aly Nasr [b]

[a] Department of Chemical and Biochemical Engineering, University of Western Ontario, London, Ontario, N6A 5B9, Canada
[b] Department of Water Pollution Research, Environmental Research Division, National Research Center, 33 El Bohoth St. (former El Tahrir St.), P.O.12622, Dokki, Giza, Egypt
[c] Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario, N6A 5B9, Canada
[d] GreenField Ethanol Inc., Chatham, Ontario, N7M 5J4, Canada

ARTICLE INFO

Article history:
Received 25 September 2015
Received in revised form
21 February 2016
Accepted 23 February 2016
Available online 8 March 2016

Keywords:
Acclimatization
Furfural
Hydrogen
Continuous-flow system
Glucose
Xylose

ABSTRACT

Continuous biohydrogen production by acclimatized anaerobic sludge was investigated using glucose and xylose individually at a concentration of 10 g/L and furfural concentrations of (0, 0.25, 0.5, 1, 2, and 4 g/L). The glucose-fed reactor showed that the initial hydrogen yield of 2.27 mol H_2/mol glucose increased by 17% and 6% at furfural concentrations of 0.25 and 0.5 g/L, respectively, and decreased by 21%, 29% and 62% at furfural concentrations of 1, 2, and 4 g/L, respectively. The inhibition threshold for furfural was in the range of 2–4 g/L. The revivability of the inhibited sludge was confirmed by eliminating furfural addition, which resulted in a hydrogen yield of 1.64 mol H_2/mol glucose comparable to the 2.27 mol H_2/mol glucose observed initially without furfural. A similar trend was observed in the xylose-fed reactor, in which the hydrogen yield decreased by 63% at the 4 g/L furfural to 0.57 mol H_2/mol xylose.

1. Introduction

Biological hydrogen production via dark fermentation of lignocellulosic biomass is feasible because lignocellulosic materials such as agricultural residues [24], grass, forestry waste, and municipal solid waste contain polymerised sugars such as cellulose and hemicellulose [11] that can be liberated by hydrolysis [28]. Lignocellulosic biomasses hydrolysis is necessary for efficient saccharification for ethanol production [32] by yeast.

During hydrolysis of lignocellulosic materials, wide ranges of by-products, which are inhibitory to anaerobic microorganisms, are generated, primarily weak acids, furan derivatives, and phenolic compounds [28,30]. Among the aforementioned groups, furan derivatives strongly inhibit hydrogen production compared to weak acids or phenolic compounds [10,30]. The main toxic furan derivatives are furfural and 5-hydroxymethyl furfural (HMF) [10] with furfural significantly more potent than HMF [3,9,10,20,32,33]. Furfural exhibits negative influence on microbial fermentation by reducing the cells growth rate, lowering cell membrane permeability, inducing reactive oxygen species [1,17,20,28] that interfere with glycotic and/or fermentative enzymes [30], breaking down DNA and inhibiting protein and RNA synthesis [10,19]. Furfural inhibition was determined to be dose-dependent at concentrations from 10 to 120 mM for bioethanol production [19].

Most studies focused on the influence of furan derivatives on ethanol fermentation [2,6,33] and to a lesser extent on methane production [7] with biohydrogen production recently receiving attention [9,15,22,29,30]. Specifically Liu et al. [20], studied the effect of furan derivatives on mesophilic anaerobic digested sludge (ADS) in batches at a pH of 6.5 using steam-exploded cornstalk at a concentration of 8% TS (73% VS) and observed that hydrogen productivity decreased by 50% at 0.5 g/L furfural but increased by 40% at 0.5 g/L HMF. Quemeneur et al. [30] who investigated the effect of different inhibitors such as furan derivatives, phenolic compounds, and lignin on mesophilic biohydrogen production using ADS at a pH of 5.5 using xylose at a concentration of 5 g/L in batches and reported that furan derivatives were most toxic with a 70% drop in hydrogen yield to 0.51 mol H_2/mol xylose at 1 g/L furfural. Monlau et al. [22] studied mesophilic biohydrogen production from glucose

* Corresponding author. Department of Civil and Environmental Engineering, University of Western Ontario, London, Ontario, N6A 5B9, Canada.
E-mail address: hisham.hafez@GFSA.com (H. Hafez).

http://dx.doi.org/10.1016/j.renene.2016.02.072
0960-1481/

as a carbon source at a concentration of 5 g/L in presence of increasing volumes (0%, 3.75%, 7.5%, 15% and 35% (v/v)) of dilute acid hydrolysate generated from sunflower stalks pre-treatment using ADS as seed, pH of 5.5. A 78% reduction in biohydrogen yield to 0.45 mol H_2/mol hexose was observed at a furfural concentration of 86 mg/L with a complete inhibition at 172 mg/L. Furthermore, furfural induced a microbial shift as evidenced by a change of end products from VFAs to lactic acid and ethanol. Fangkum & Reungsang [10], studied batch mesophilic biohydrogen production from sugarcane bagasse hydrolysate at a concentration of 10 g/L using elephant dung as inoculum at a pH of 6.5 and achieved a maximum hydrogen yield of 0.84 mol H_2/mol sugar consumed with 86% substrate degradation. Substrate degradation was observed to decrease with the increase in sugarcane bagasse hydrolysate concentrations since the hydrolysate that was produced during dilute acid pretreatment contained furfural, the main furan derivatives in the hydrolysate, at a concentration of 220 mg/L. [9] studied the effect of furfural generated by dilute acid pretreatment of corn stover on thermophilic batch hydrogen production using a pure culture of *Thermoanaerobacterium thermosaccharolyticum W16* and observed complete inhibition at 2 g/L furfural. On the contrary Lin et al. [18], studied the effect of furan derivatives (i.e. furfural and 5-hydroxymethyl furfural (HMF)) and phenolic compounds (i.e. vanillin and syringaldehyde) individually at a concentration of 15 mM on mesophilic batch hydrogen fermentation using mixed hydrogen producing bacteria isolated from anaerobic digester sludge [(ADS) – with *clostridium butyricum* as the predominant species] and glucose at a concentration of 10 g/L at a pH of 6. The aforementioned authors reported hydrogen production yield of 248 ml H_2/g glucose for control (without inhibitor) compared to 242 ml H_2/g glucose at furfural concentration of 15 mM (1.44 g/L) with complete furfural degradation after 48 h.

Microorganisms generally are able to reduce furfural to its corresponding alcohol, furfuryl alcohol, which is less inhibitory [19,25,33,34]. On the other hand Boopathy and Daniels [8], also found that furfural was converted to acetic acid by anaerobic fermentation using a sulfate reducing bacterium, *Desulfovibrio* sp. [20]. Additionally Almeida et al. [2], reported that furfural could be converted to furoic acid by *Saccharomyces cerevisiae* in an aerobic reactor. The two possible microbiological solutions to minimize the furfural inhibition are gradual acclimatization and genetic engineering of new strains, with the latter having limited practical application with real feedstocks that contain a variety of microorganisms that can out-grow genetically-modified microorganisms [2].

Since furfural is a primary breakdown product from pentoses and therefore likely to be present in hydrolysates, the effect of furfural on yeast fermentation has been the subject of several investigations, while only few papers addressed the effect of furfural on bio-hydrogen fermentation testing ADS in batches [9,20,22,30]. It is apparent that while the short-term inhibitory impact of furfural on biohydrogen production in batches have just recently been assessed, the performance of furfural in hydrogen fermentation from glucose and xylose by acclimatized anaerobic microbial consortium in long-term continuous-flow studies was never reported. Thus the main objectives of the current study are: (1) Assess the long-term impact of furfural and potential acclimatization of ADS; and (2) Assess the revivability of the inhibited biomass.

2. Materials and methods

2.1. Seed sludge

Anaerobic digester sludge (ADS) was collected from St. Mary's wastewater treatment plant (St. Mary's, Ontario, Canada) and preheated at 70 °C for 30 min [23] to be used as seed sludge in the continuous integrated bioreactor clarifier system (IBRCS). The total suspended solids (TSS) and volatile suspended solids (VSS) concentrations of the ADS were 10.1 and 6.9 g/L, respectively.

2.2. Systems set up and operation

Two patented IBRCSs [14] (Fig. 1), R1 and R2, were operated for biological hydrogen production at 37 °C for 143 days, at an organic loading rates (OLR) of 32.01 gCOD/L/d, excluding furfural, and hydraulic retention time (HRT) of 8 h. The IBRCSs comprised a continuously stirred tank reactor (CSTR) for biological hydrogen production (7 L working volume), followed by an uncovered gravity settler (volume 8 L) for the decoupling of solids retention time (SRTs) from the HRT [12]. Water was recirculated through a water jacket to maintain a constant temperature of 37 ± 1 °C. Nitrogen gas was initially purged in the headspace in order to maintain anaerobic conditions. The pH was controlled (5.5 ± 0.1) by chemical feed pumps (Romania, BL1.5, HANNA, Blackstone, 1.5 L/h, 13 BAR) with 2 N NaOH and HCl solutions. In order to enrich the hydrogen-producing bacteria, anaerobically digested sludge was treated at 70 °C for 30 min [13]. The testing program included seven phases denoted henceforth as phases 1 to 7 lasting 16, 20, 15, 17, 45, 15, and 15 days respectively. Glucose was the feed in R1 at a concentration of 10 g/L while xylose was in R2 at a concentration of 10 g/L throughout the experiment together with increasing furfural concentrations (0, 0.25, 0.5, 1, 2, 4 g/L) in both reactors. Thus, the microbial cultures were acclimatized by long-term exposure to increasing furfural concentrations. Finally, R1 was tested for revivability by reverting to glucose at a concentration of 10 g/L with no furfural added (phase 7). The feed contained sufficient inorganics as described by Hafez et al. [13]. The systems were monitored for total chemical oxygen demand (TCOD), soluble chemical oxygen demand (SCOD), volatile fatty acids (VFAs), glucose, xylose, furfural, ethanol, lactate, total suspended solids (TSS), volatile suspended solids (VSS), and biogas composition including hydrogen and methane. The quantity of produced biogas was recorded daily using a wet-tip gas meter (Rebel wet-tip gas meter company, Nashville, TN, USA).

2.3. Analytical methods

The biogas produced from the IBRCS was measured using wet-

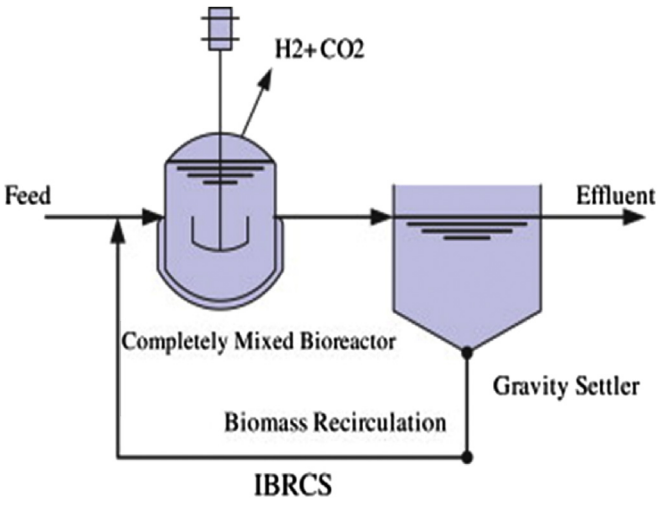

Fig. 1. Integrated Biohydrogen Reactor Clarifier system.