BIOFUEL PRODUCTION FROM LIGNOCELLULOSIC BIOMASS

I.A. Panagiotopoulos [a, *], R.R. Bakker [b], T. de Vrije [b], P.A.M. Claassen [b], E.G. Koukios [a]
[a] Bioresource Technology Unit, School of Chemical Engineering, National Technical University of Athens, Zografou Campus, Athens, GR-15700, Greece
Tel.: 00306946712529, e-mail: iapan@chemeng.ntua.gr
[b] Wageningen UR Agrotechnology and Food Innovations, P.O. Box 17, 6700 AA Wageningen, The Netherlands

ABSTRACT: The main objective of this study was to investigate the feasibility of applying various dilute acids in the pretreatment of barley straw for biological hydrogen production. The conversion to fermentable substrates and the fermentability of barley straw pretreated with different acid catalysts and enzymatically hydrolyzed, was compared. At a fixed acid loading of 1% (w/w dry matter) approximately 28-32% of barley straw was converted to soluble monomeric sugars. The extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus* showed good hydrogen production on hydrolysates of straw pretreated with H_3PO_4 and H_2SO_4, and to a lesser extent, HNO_3. The fermentability of the hydrolysate of straw pretreated with HCl was relatively low.
Keywords: barley straw, conversion, biological hydrogen production, fermentability test, *Caldicellulosiruptor saccharolyticus*

1 INTRODUCTION

Conversion of biomass to fermentable sugars and finally hydrogen must be performed at low cost and with high technical efficiency, if it is to compete with other fuel conversion processes. Lignocellulosic biomass represents a cheap and abundant source for hydrogen production. Previous studies have focused on the identification of lignocellulosic feedstocks with high technical suitability for hydrogen production [1,2,3]. Barley straw, which is an important residue from the grain industry worldwide, is a promising feedstock for hydrogen production.

A key aspect of biological conversion of barley straw to hydrogen is the processing of the biomass [4]. The utilization of cellulose and hemicellulose present in barley straw is essential for the sustainable production of hydrogen. The cellulose and hemicellulose content of barley straw can be hydrolyzed either chemically or enzymatically or by a combination of both. In this study, pretreatment of barley straw includes dilute acid pretreatment and subsequent enzymatic hydrolysis. The mixed multiple sugars that are produced from the pretreatment of barley straw can be fermented to hydrogen by the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus*, which has recently attracted considerable research attention [5]. Another important aspect of processing barley straw for hydrogen production is the limitation of the release of fermentation inhibitors naturally present in barley straw.

Various acid pretreatments have been used over the years to hydrolyze straw for the production of fermentable substrates or chemicals. Concentrated acids have been used in the past, but they are corrosive and hazardous. Nowadays the most widely used approach is based on dilute sulfuric acid, because it is generally inexpensive, convenient and effective for a broad spectrum of lignocellulosic biomass, but dilute nitric and hydrochloric acids have also been used. The goal of this study was to investigate and compare the technical feasibility of employing dilute sulfuric, hydrochloric, nitric and phosphoric acid in the pretreatment of barley straw for biohydrogen production. Our investigation includes the conversion of barley straw to fermentable substrates and the fermentability of the barley straw hydrolysates to hydrogen. The experimental work performed was a part of a broader technical and economic feasibility study on the development of 2-stage biological hydrogen production from low cost biomass [6,7].

2 MATERIALS AND METHODS

2.1 Feedstock

Barley straw was obtained from a research farm in the province of Acheleia, Cyprus. It was ground in a knife mill with a 2 mm screen. The moisture content of barley straw was 4.8%.

2.2 Pretreatment

Hereafter all necessary experimental steps to produce fermentable substrates from barley straw are referred to in a single term as pretreatment. Thus, pretreatment means dilute-acid pretreatment and subsequent enzymatic hydrolysis.

Dilute-acid pretreatment experiments were conducted with sulfuric, hydrochloric, nitric or phosphoric acid. Barley straw was mixed with the dilute corresponding acid (acid loading 1%, w/w) at a solid:liquid ratio of 1:10, then placed in a stainless steel cylindrical reactor and heated in an oilbath (Fisons Haake N3) at 160^0C for 30 min.

Enzymatic hydrolysis was carried out with the solids collected after completion of experiments with dilute acid. All hydrolysis experiments were performed in an orbital shaker (Infors AG, CH-4103 Bottmingen) with commercial cellulase (GC 220, Genencor International). Prior to enzymatic hydrolysis, the pH of the samples was adjusted to 5 with sodium hydroxide. Standard conditions of the enzymatic hydrolysis of straw were: cellulase (45 FPU/g dry biomass), solid:liquid ratio 1:10, temperature 50^0C, incubation time 24 h, stirring speed 170 rpm. Details of the experimental procedure can be found elsewhere [8].

2.3 Fermentation

2.3.1 Methodology

Growth of the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus* DSM 8903 on substrates derived from barley straw was compared to growth of the bacterium on corresponding sugars of analytical grade. The total sugar concentration was 10, 20 or 30 g/L coming from the pure sugar mixture or the hydrolysate.

2.3.2 Microorganism, media, cultivation

Caldicellulosiruptor saccharolyticus DSM 8903 was obtained from the Deutsche Sammlung von Microorganismen und Zellculturen (Braunschweig, Germany). Its optimal growth temperature is 70^0C. A modified DSM 8903 medium consisted of (per liter): NH_4Cl 0.9 g, K_2HPO_4 0.3 g, KH_2PO_4 0.3 g, $MgCl_2 \cdot 6H_2O$ 0.4 g, 4-morpholine propanesulfonic acid (MOPS) 10.5 g, yeast extract 1 g, cysteine-HCl $\cdot$ H_2O 0.75 g, $FeCl_3 \cdot 6H_2O$ 2.5 mg, resazurine 0.5 mg, trace elements 20.0 mL. A mixture of glucose and xylose was used as carbon and energy source. The trace elements were (per liter): $FeCl_2 \cdot 4H_2O$ 1.5 g, $ZnCl_2$ 70 mg, $MnCl_2 \cdot 4H_2O$ 100 mg, H_3BO_4 6 mg, $CoCl_2 \cdot 6H_2O$ 190 mg, $CuCl_2 \cdot 2H_2O$ 2 mg, $NiCl_2 \cdot 6H_2O$ 24 mg, $Na_2MoO_4 \cdot 2H_2O$ 36 mg. The basal medium was made anaerobic by flushing with nitrogen (100%) and sterilized by autoclaving. The pH was adjusted to 7.0 with 5 M HCl or 5 M KOH. Anaerobic, non-sterile hydrolysates from barley straw were used for the experiments.

C. saccharolyticus was cultivated in anaerobic serum bottles (118 mL) in culture volumes of 20 mL at 70^0C. The flasks were inoculated with 1 mL of a culture in the exponential growth phase. Samples from the headspace and culture medium were regularly withdrawn for analysis of acetate and lactate, and measurement of the growth of *C. saccharolyticus*. Experiments were performed in duplicate.

2.4 Analytical methods

Analysis of monomeric sugars was performed with High-performance anion-exchange (HPAEC) at pH 12. HPAEC was performed on a Thermo Separation Products system equipped with a Borate trap (Dionex), a Dionex CarboPac PA-20 column (3 mm ID x 150 mm) in combination with a Dionex CarboPac PA-20 guard column (3 mm x 25 mm) and PAD-detection (Dionex, Sunnyvale, CA). Organic acids were analyzed by HPLC with a Waters Shodex ionpak KC811 column and detected by differential refractometry; the mobile phase was 0.003 M H_2SO_4 at a flow rate of 1 mL/min; the column temperature was kept at 80°C. Optical density was measured on a Pharmacia spectrophotometer at 580 nm. All data reported are means of two replicates.

3 RESULTS

3.1 Sugar yields

Four acids were compared in the pretreatment of barley straw. The dosage of the acids was based on the results of an earlier reported work [8], and was kept at a low level to limit the chemical requirements of the pretreatment process [7]. Sugar production was greatest with HCl and lowest with HNO_3 (Table I). Approximately 60-70% of the total carbohydrates of barley straw were converted to soluble monomeric sugars. The main sugars which were produced after dilute-acid pretreatment and enzymatic hydrolysis were mostly glucose, xylose and arabinose. All acid pretreatments resulted in significantly higher sugar yields compared to the control pretreatment (with water). The pH at the start of the pretreatment with H_2SO_4, HCl, HNO_3 and H_3PO_4 was 3.7, 3.6, 4.1 and 4.7, respectively. This suggests the relatively lower severity of the dilute-acid pretreatment in case of H_3PO_4, and to a lesser extent, HNO_3 when the dilute-acid pretreatment was set on a fixed acid loading (w/w dry biomass). When no acid catalyst was used, the initial pH was 6.7. The application of a longer incubation time of enzymatic hydrolysis may increase the yields of monomeric sugars; the products of the dilute-acid pretreatment included approximately 20% monomeric sugars and 80% di- and/or oligomeric sugars (data not shown), which probably means that some di- and/or oligomeric sugars are still present in the final hydrolysate.

Table I: Production of fermentable monomeric sugars by dilute acid pretreatment and enzymatic hydrolysis of barley straw with different acid catalysts

Dilute acid pretreatment	Sugar concentration (g/L)			Sugar yield
1% w/w	Glucose	Xylose	Arabinose	(g/100 g of dry straw)
Water	11.5	4.7	0.8	20
H_2SO_4	16.7	6.8	1.2	30
HCl	16.9	7	1.2	32
HNO_3	15.6	5.7	1	28
H_3PO_4	16.1	6.1	1.1	29

3.2 Fermentability

The fermentability of the substrates was determined by measuring the growth of *C. saccharolyticus*, the production of acetic acid and lactic acid, and the production of hydrogen. Under the applied experimental conditions, the sum of acetic acid production and lactic acid production reflected the fermentability of the substrate and its potential for hydrogen production.

3.2.1 Fermentability at sugar concentration 10 g/L

At fermentations with 10 g sugars/L the growth of *C. saccharolyticus* was generally similar in all hydrolysates of barley straw treated with the acids tested (Fig. 1A). In the hydrolysates with HCl- and HNO_3-treated straw the growth of the microorganism was slightly lower than the growth with H_2SO_4 and H_3PO_4. In contrast to the control fermentation, the growth of *C. saccharolyticus* was (almost) completed after 16 h of fermentation in the experiments with the acids tested. The production of organic acids was the highest in fermentations with hydrolysates of barley straw treated with H_2SO_4 and H_3PO_4. Fermentation end time results (Fig. 1B) showed that organic acid production from hydrolysates of barley straw treated with HCl and HNO_3 was 20% decreased, as compared to the control culture, at sugar concentration 10 g/L. Hydrogen production results at the end of fermentation (Fig. 1C) were generally similar to results of organic acid production and in hydrolysates of barley straw treated with acid, 95-120% hydrogen production, as compared to the control culture, was observed.

3.2.2 Fermentability at sugar concentration 20 g/L

At fermentations with 20 g sugars/L the growth of *C. saccharolyticus* was the highest in the hydrolysate of H_3PO_4-treated straw (Fig. 2A). In this medium the increase of optical density after 16 and 40 h of fermentation was, respectively, 50-100 and 40-90% higher than in the media with hydrolysates of straw treated with H_2SO_4, HCl and HNO_3. Similarly, the highest production of organic acids was observed with the hydrolysate of H_3PO_4-treated straw (Fig. 2B), possibly due to the residual phosphoric acid in the hydrolysate. The aforementioned hydrolysate also showed the highest hydrogen production, to a final level that was 50% higher than the control (Fig. 2C). The organic acid production in fermentations with hydrolysates of barley straw treated with H_2SO_4 and HNO_3 was about 30% higher than in the control after 16 h of fermentation (data not shown) and equal to the control after 40 h of fermentation. The fermentability of the hydrolysate of HCl-treated straw was good and the 10% inhibition in the production of organic acids after 40 h of fermentation might be of little importance.

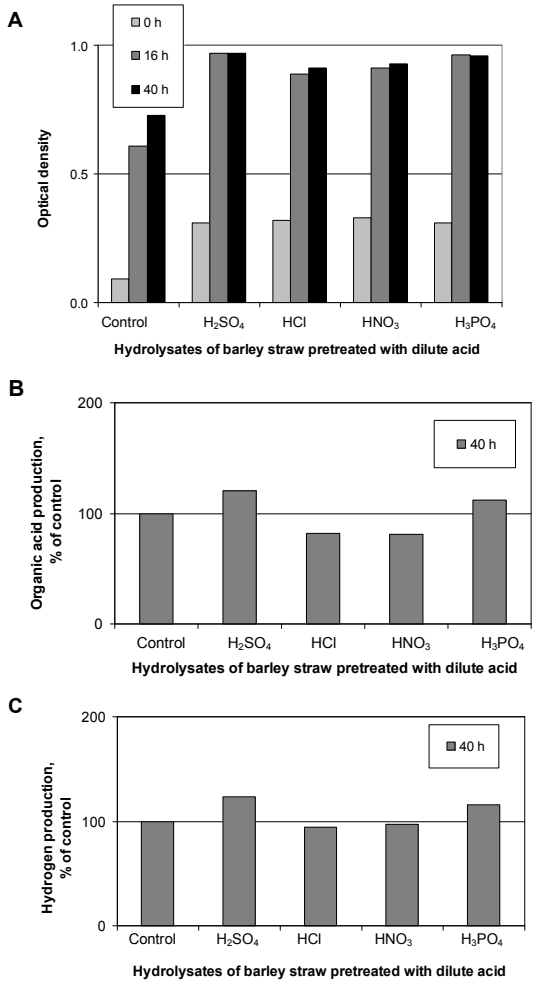

Figure 1: Growth of *C. saccharolyticus* (A), organic acid (acetate and lactate) production (B) and hydrogen production (C) on control medium with pure sugars (glucose:xylose=2.5:1), and on media with barley straw sugars after dilute acid pretreatment of barley straw with different acid catalysts and subsequent enzymatic hydrolysis. Total sugar concentration was 10 g/L.

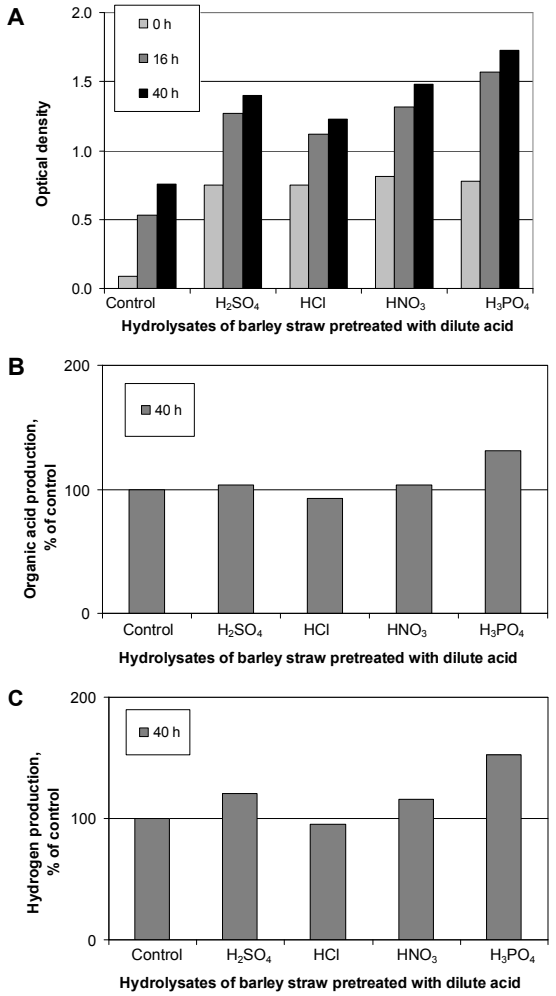

Figure 2: Growth of *C. saccharolyticus* (A), organic acid (acetate and lactate) production (B) and hydrogen production (C) on control medium with pure sugars (glucose:xylose=2.5:1), and on media with barley straw sugars after dilute acid pretreatment of barley straw with different acid catalysts and subsequent enzymatic hydrolysis. Total sugar concentration was 20 g/L.

3.2.3 Fermentability at sugar concentration 30 g/L

At fermentations with 30 g sugars/L the fermentability of all hydrolysates of barley straw treated with the acids tested was significantly inhibited. Production of organic acids from the hydrolysate of straw treated with HCl, and to a lesser extent, the hydrolysates of straw treated with the other tested acids, was poor as inhibition of organic acid production occurred already after 16 h of fermentation. By the end of fermentation, 50-60% inhibition in organic acid production, as compared to the control culture, was observed in all media (Fig. 3A). The concentration of Cl^- might have led to the relatively lower production of organic acids in media with barley straw sugars. Similarly, none of the hydrolysates of barley straw treated with dilute-acid showed sufficient hydrogen production (Fig. 3B). At experiments with 30 g sugars/L the growth of the microorganism could not be determined through the optical density because the formation of barley straw particulates took place by an early stage of fermentation (<16 h) probably due to high substrate concentration in the medium.

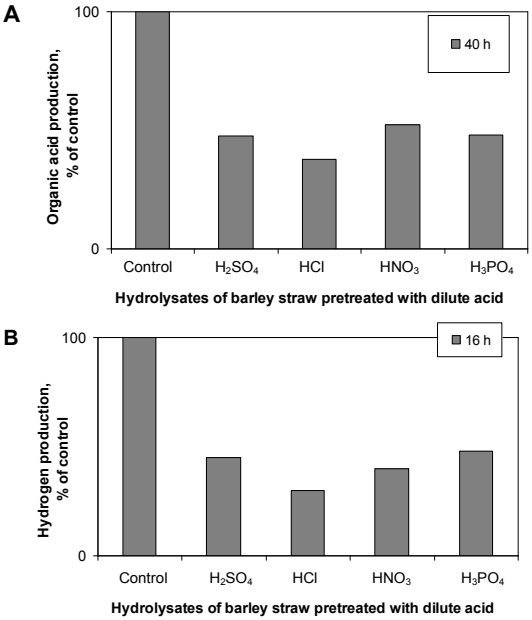

Figure 3: Organic acid (acetate and lactate) production (A) and hydrogen production (B) on control medium with pure sugars (glucose:xylose=2.5:1), and on media with barley straw sugars after dilute acid pretreatment of barley straw with different acid catalysts and subsequent enzymatic hydrolysis. Total sugar concentration was 30 g/L.

4 CONCLUSIONS

This study can serve as an initial step for the understanding of the effect of an acid catalyst, used in the pretreatment of barley straw, on the fermentability of the hydrolysate. The selection of the most advantageous acid will be based on simultaneous fulfilment of the criteria of low environmental pollution, low energy requirement, low cost, high sugar yields and good fermentability to hydrogen. In terms of sugar yield, at a fixed acid loading of 1% (w/w dry matter) approximately 28-32% of barley straw was converted to soluble monomeric sugars. This corresponds to approximately 60-70% conversion of the total carbohydrates of barley straw to soluble monomeric sugars. In terms of fermentability to hydrogen, the beneficial effect of the dilute acids tested in this work was ranked in the following order: H_3PO_4 > H_2SO_4 > HNO_3 > HCl. The residual phosphoric acid not only does not have inhibitory effects on the subsequent enzymatic hydrolysis of barley straw and fermentation, but it possibly ameliorates the fermentation conditions because phosphate is an obligatory nutrient in the fermentation medium. Moreover, combination of H_2SO_4 and H_3PO_4 could be used advantageously in the pretreatment of barley straw for the production of fermentable substrates and, subsequently, hydrogen, because H_2SO_4 produces sufficiently a hydrolysate with high level of sugar at relatively low cost, and H_3PO_4 produces a highly fermentable substrate. Further investigation on the fermentability of barley straw sugars after dilute-acid pretreatments of variable severity is required.

5 ACKNOWLEDGEMENTS

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