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Integration of first and second generation biofuels: Fermentative hydrogen production from wheat grain and straw

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HIGHLIGHTS

- The feasibility of integrating lignocellulose- and starch-rich biomass-based hydrogen production was investigated with the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus*.
- Wheat grain hydrolysate showed limited fermentability whereas wheat straw hydrolysate showed relatively good fermentability.
- The mixed hydrolysate showed good fermentability at the highest tested sugar concentration.

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ABSTRACT

Integrating of lignocellulose-based and starch-rich biomass-based hydrogen production was investigated by mixing wheat straw hydrolysate with a wheat grain hydrolysate for improved fermentation. Enzymatic pretreatment and hydrolysis of wheat grains led to a hydrolysate with a sugar concentration of 93.4 g/L, while dilute-acid pretreatment and enzymatic hydrolysis of wheat straw led to a hydrolysate with sugar concentration 23.0 g/L. Wheat grain hydrolysate was not suitable for hydrogen production by the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus* at glucose concentrations of 10 g/L or higher, and wheat straw hydrolysate showed good fermentability at total sugar concentrations of up to 10 g/L. The mixed hydrolysates showed good fermentability at the highest tested sugar concentration of 20 g/L, with a hydrogen production of 82–97% of that of the control with pure sugars. Mixing wheat grain hydrolysate with wheat straw hydrolysate would be beneficial for fermentative hydrogen production in a biorefinery.

1. Introduction

One of the major challenges in the utilization of hydrogen gas as a biofuel is the need for inexpensive production methods from renewable resources. A significant amount of research work has already been done on fermentative hydrogen production from various types of biomass. Sugary biomass such as sugar beet (Hussy et al., 2005); Panagiotopoulos et al., 2010a) and sweet sorghum (Claassen et al., 2004; Panagiotopoulos and I.A., 2008; Antonopoulou et al., 2010) and starch-containing biomass such as sweet potato residues (Yokoi et al., 2002), potato industry coproducts (Mars et al., 2010), wheat flour industry coproducts (Hawkes et al., 2008) and corn and barley grains (Panagiotopoulos et al., 2009) have been investigated. However, the utilization of lignocellulosic biomass would be preferable as it is cheap, abundant and does not directly compete with food production. Therefore, wheat and barley straw (Eriksen et al., 2011; Panagiotopoulos et al., 2011a), *Miscanthus* (de Vrije et al., 2009), corn stover (Datar et al., 2007; Panagiotopoulos et al., 2011a), sweet sorghum bagasse (Panagiotopoulos et al., 2010b), olive pulp (Koutrouli et al., 2009) and carrot pulp (de Vrije et al., 2010) have been used for biological hydrogen production. The strategy of the present work was the integration of lignocellulose- and starch-rich biomass-based hydrogen production in a biorefinery, which potentially could facilitate the introduction of the technology of lignocellulose-based hydrogen into the market.

In the current work, the extreme thermophilic bacterium *Caldicellulosiruptor saccharolyticus* (Rainey et al., 1994; Jones, 2008; Blumer-Schuette et al., 2008) was used. *C. saccharolyticus* is a cellulolytic bacterium of the order Clostridiales and grows at an optimum temperature of 70 °C. It is advantageous over other microorganisms because it leads to high hydrogen yields (van Niel

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et al., 2002; de Vrije et al., 2007, 2009; Panagiotopoulos et al., 2010a, 2011b) and simultaneously utilizes hexoses and pentoses (de Vrije et al., 2009; Kengen et al., 2009; Zeidan and van Niel, 2009; Panagiotopoulos et al., 2010b).

As an example of a lignocellulose-containing raw material, wheat straw was studied as substrate for fermentation. Although extensive work has already been reported on bioethanol production from pretreated wheat straw (Saha et al., 2005; Linde et al., 2008; Talebnia et al., 2010), very little research effort has focused on the pretreatment of wheat straw for fermentative hydrogen production. In Europe wheat straw is the most abundant agricultural waste and its production equals about 32% of the worldwide production (Kim and Dale, 2004). As an example of a starch-containing raw material, wheat grains were studied because of their high content of fermentable sugars and their high availability in Europe.

The focus of this study was on the effect of mixing a wheat grain hydrolysate with a wheat straw hydrolysate on the fermentability for hydrogen production. A fermentability test (Panagiotopoulos et al., 2009) with the extreme thermophilic bacterium *C. saccharolyticus* was used to evaluate the fermentation characteristics of the hydrolysates.

2. Methods

2.1. Raw materials

Wheat grains obtained in the spring of 2005 from a research farm in the province of Acheleia, Cyprus were ground in a laboratory hammer mill. The samples were stored, protected from the weather at room temperature. The moisture content of the wheat grains was 10.0%. Wheat grains had a starch content of 62.5% and a total polysaccharide content of 79.4% (Table 1).

Wheat straw was obtained in the spring of 2006 from a research farm in Acheleia, Cyprus. The wheat straw was air dried (93.2% w/w dry matter), chopped to a length of 1–2 cm and ground through a 2-mm screen. The samples were stored and protected from the weather at room temperature. The polysaccharide content of the straw was 60% of the total dry matter, with cellulose being the most abundant component of the polysaccharides, followed by hemicellulose (Table 1).

2.2. Pretreatment and enzymatic hydrolysis

2.2.1. Wheat grains

The starch of the wheat grains was liquefied and subsequently hydrolysed according to Panagiotopoulos et al. (2009). Starch liquefaction was performed with a commercial alpha-amylase (Termamyl 120, Novo Nordisk) at 90 °C for 1 h. Enzymatic hydrolysis was subsequently performed with a commercial glucoamylase (AMG 300, Novo Nordisk) at 60 °C for 16 h. Prior to enzymatic hydrolysis, the pH of the samples was adjusted to 5.0 with 25% acetic acid (v/v). The alpha-amylase and the glucoamylase were used at a concentration of 1.2% and 2.4% (w/w dry matter), respectively. After completion of the hydrolysis, the hydrolysates were centrifuged at 10,000*g* for 15 min and the supernatant samples were analyzed for glucose, fructose, sucrose, acetic acid, HMF and furfural.

Table 1
Chemical composition of wheat grains and wheat straw expressed as (%) percentage of dry matter.

Component	Wheat straw[a]	Wheat grains
Cellulose	35.4	n.d.[b]
Hemicellulose	24.6	n.d.
Starch	n.d.	62.5
Non-starch polysaccharides[c]	n.d.	16.9
Acid-insoluble lignin	17.7	1.1
Protein	2.4	17.6
Ash	6.9	1.0

Note: Minor components are not included in this list, so the numbers do not sum to 100%.

[a] Data obtained from Panagiotopoulos et al. (2011a).

[b] Not determined.

[c] The content of non-starch polysaccharides is presented as the sum of the cellulose content and the hemicellulose content of wheat grains.

2.2.2. Wheat straw

Dilute-acid pretreatment of wheat straw was conducted with sulfuric acid. Wheat straw (8.0 g; 7.46 g dry matter) was mixed with 27.6 mL of sulfuric acid solution (50 mM) and 47.1 mL of water at a resulting acid loading of 1.8% w/w dry matter and a solid:liquid ratio of 1:10, and placed in a 100-mL reactor. Heating of the material was performed with an oilbath (Fisons Haake N3) at 170 °C for 30 min. Enzymatic hydrolysis was carried out with the samples of pretreated barley straw. Hydrolysis was performed in an orbital shaker (Infors AG, CH-4103 Bottmingen) with commercial cellulase GC 220 (Genencor, Rochester, USA) with an activity of 116 FPU (filter paper unit)/mL (Kabel et al., 2006). Prior to enzymatic hydrolysis, the pH of the samples was adjusted to 5.0 with sodium hydroxide.

Standard conditions of the enzymatic hydrolysis of pretreated wheat straw were: cellulase 30 FPU/g dry biomass; solid:liquid ratio 1:10; 50 °C; incubation time 24 h; stirring speed 170 rpm. After completion of the enzymatic hydrolysis, the hydrolysates were centrifuged at 10,000*g* for 15 min and the supernatant samples were analyzed for glucose, xylose, arabinose, acetic acid, HMF and furfural.

2.3. Fermentation

2.3.1. Methodology

Growth of the extreme thermophilic bacterium *C. saccharolyticus* on substrates derived from biomass was compared to growth on corresponding sugars of analytical grade (control media). The total sugar concentration of the fermentation media was 20 g/L coming from the pure glucose/pure sugar mixture, the hydrolysate or a combination of both.

2.3.2. Microorganism, medium, cultivation

C. saccharolyticus DSM 8903 was obtained from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ). The culture medium consisted of (per liter) KH_2PO_4 0.3 g, K_2HPO_4 0.3 g, $MgCl_2{\cdot}6H_2O$ 0.4 g, NH_4Cl 0.9 g, yeast extract 1.0 g, cysteine-HCl 0.75 g, $FeCl_3{\cdot}6H_2O$ 2.5 mg, SL-10 trace elements 1 mL, and resazurine 0.5 mg. 4-Morpholine propanesulfonic acid (MOPS, 10.5 g/L) was used as buffer. The pH was adjusted to 7.0 at room temperature. Inoculation was done by adding 10% (v/v) of a preculture that was grown overnight on glucose. The medium was made anaerobic by flushing with nitrogen. Experiments were carried out under non-sterile conditions at 72 °C. The fermentability was tested using closed flasks of 118 mL with 20 mL of culture medium under a nitrogen atmosphere and without pH control. The detailed procedure of the fermentability experiments has been described by Panagiotopoulos et al. (2009).

2.4. Analytical methods

The chemical composition of wheat grains and wheat straw was determined with methods described by the Technical Association of the Pulp and Paper Industry (Tappi). The detailed procedure of the analysis of wheat straw is described by Panagiotopoulos et al. (2011c). The starch content of wheat grains was determined