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Research paper

Effect of sodium sulfite on acid pretreatment of wheat straw with respect to its final conversion to ethanol

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ABSTRACT

A pretreatment process that combines dilute acid and sodium sulfite has been applied to wheat straw to study the effect of temperature (120–180 °C) and sodium sulfite concentration (0–3%) on the yield of glucose in subsequent enzymatic hydrolysis and ethanol production by fermentation. The results were compared with both dilute acid pretreatment (without Na_2SO_3 addition) and hot water pretreatment. Formation of furfural and hydroxymethylfurural, which can inhibit ethanol-producing microorganisms, were measured and the ethanol yield in a subsequent fermentation was evaluated. The results indicate that a combination of 180 °C, 30 min, 1% H_2SO_4 and 2.4% Na_2SO_3 during pretreatment produced the highest ethanol yield; 17.3 g/100 g dry weight of initial biomass, which corresponds to 75% of the theoretical yield from glucose. 28 mg of furan inhibitors (sum of furfural and hydroxymethylfurfural) per gram dry weight of initial wheat straw were generated under this condition. Increasing sulfite loading up to 2.4% decreased inhibitor formation, leading to increased delignification and preservation of cellulose from dissolution. On the other hand, an elevated temperature in combination with low pH reduced the amount of solid phase after pretreatment, increased the level of inhibitors and reduced the concentration of ethanol produced by fermentation.

1. Introduction

The world production of wheat was about 730 million tonnes in 2014 and, according to FAO, is anticipated to reach 720 million tonnes in 2015 [1]. Wheat is the third most-produced cereal globally, after maize and rice and annual estimates of wheat straw, a by-product of wheat processing, that would be available for bioethanol production in Europe alone varies from 133 to 180 million tons [2,3]. Wheat straw thus represents a huge renewable resource with a lower lignin content and higher levels of cellulose and hemicellulose than corn stover, the by-product of corn production [4]. Therefore much research work has been carried out to develop technologies for utilizing constituent sugars of wheat straw for ethanol biofuel production. In most of these processes, it is necessary to decompose polymeric sugars before fermentation is carried out [5]. Establishing suitable conditions for biomass pretreatment is thus a crucial prerequisite for efficient fermentation of simple sugars that originate from enzymatic hydrolysis. Although there are several methods of biomass pretreatment [6], dilute acid pretreatment typically using sulfuric acid (H_2SO_4) at an elevated temperature is most commonly used.

During acid pretreatment, hemicellulose, a branched polymer of pentoses and hexoses and acetylated sugars is easily hydrolyzed into its respective monosaccharides, while lignin is condensed and precipitated. Cellulose fibers are released from their lignin casing, crystallinity is reduced and the surface area is increased so that the cellulose chains become more accessible to cellulolytic enzymes [7,8]. Although acid pretreatment is effective in breaking down lignocellulosic material, many toxic products are generated during decomposition, especially if low pH is combined with elevated temperatures and pressures. In addition to free aliphatic acids (e.g. acetic, formic, levulinic acids) or phenolic derivatives (4-hydroxybenzoic acid or vanillin) arising from lignin and hemicellulose decomposition, 2-furaldehyde (furfural, FF) and 5-hydroxymethyl-2-furaldehyde (hydroxymethylfurfural, HMF) are produced and are major inhibitors of enzymatic hydrolysis and fermentation [9]. Furfural is formed by dehydration of pentose sugars (xylose and arabinose) while HMF is a dehydration product of hexose sugars (glucose, mannose and galactose). Their inhibitory effects on the growth of yeast and their role in decreasing the yield of ethanol and reducing productivity have been reported elsewhere

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[10]. In addition to these toxic products, investments into special corrosion-resistant reactors [9], additional costs of neutralization of the acid prior to downstream processing, and the problem of commercial exploitation of highly condensed lignin, are drawbacks of this technology. Although some of these problems may be overcome using milder conditions, e.g. sulfur dioxide catalyzed pretreatment, insufficient lignin sulfonation and toxicity of gaseous SO_2 are important issues.

Some of these problems can be reduced in a SPORL process (sulfite pretreatment to overcome recalcitrance of lignocellulose), which combines H_2SO_4 and neutral sulfite pulping pretreatments [11,12]. Typically, SPORL is a two-step process, where biomass is firstly treated with sulfite or bisulfite salts under acidic conditions (pH 2–4) at 160–180 °C for a short time, then mechanical size reduction is carried out using a disc refiner. This pretreatment can be carried out with equipment (pulp digester and mechanical disk refiner) typically used in the pulp and paper industry. The pretreatment liquor can be prepared and recovered using existing techniques so costs associated with chemicals and cleaning waste streams can be reduced. Sulfites are known for their efficient depolymerization of carbohydrates and excellent delignification, even under acidic conditions, which further facilitate dissolution of hemicelluloses. The dissolved lignin can act as a surfactant to substantially enhance enzymatic saccharification [13] whereas the retained lignin on the solid substrate is sulfonated, resulting in a lower affinity for cellulases, substantially reducing nonproductive binding [14]. The SPORL process has been demonstrated to be an effective and robust pretreatment technology for woody biomass bioconversion to sugar and bioethanol [11–13,15,16]. In comparison with dilute acid pretreatment, it results in a lower level of inhibitors, such as hydroxymethylfurfural (HMF) and furfural, typical concentrations of which are 5 mg/g and of 1 mg/g dry weight (DW), respectively.

Previous studies on the SPORL process have focused mainly on woody biomass, including softwoods and hardwoods, and only a few studies on herbaceous crops have been carried out [11–13,15,16]. In the work reported here, a combination of dilute (1% w/v) H_2SO_4 and sodium sulfite (Na_2SO_3) was applied to wheat straw to study the effect of temperature and Na_2SO_3 concentration on glucose yield in subsequent enzymatic hydrolysis and ethanol production by fermentation. The temperature and Na_2SO_3concentration varied from 120 to 180 °C and 0–3.0% (w/v) respectively while the pretreatment time was kept constant (30 min). The conditions for pretreatment of wheat straw were selected based on previous literature [12,15,17,18] and was optimized for our equipment and materials.

Results obtained in these experiments were compared with both dilute acid and steam pretreatments. The amounts of furfural and hydroxymethylfurfural formed in all processes were measured and their influence on ethanol yield was examined.

2. Material and methods

2.1. Biomass

Raw wheat straw (prior to hydrolysis) contained 40.87% cellulose, 20.39% hemicellulose, 27.42% lignin and 5.54% ash on a dry weight basis (see 1st row in Table 1). Air dried wheat straw was milled using a knife mill Gindomix GM 200 (Retsch) at 6000 RPM for 2 min and then sieved to obtain particles smaller than 1 mm. The ground samples were stored in sealed bags at room temperature.

2.2. Determination of the chemical composition of wheat straw

Structural carbohydrates, lignin and ash content of raw and pretreated wheat straw were analyzed according to the NREL Laboratory Analytical Procedure [19].

2.3. Pretreatment of wheat straw

Pretreatment using the SPORL method was performed using a combination of dilute H_2SO_4 and Na_2SO_3. To see the effect of temperature, 15 g milled wheat straw mixed with 150 ml of 1% (w/v) H_2SO_4, and 1.8% (w/v) of Na_2SO_3 were placed in a customized reactor (inner height and diameter ratio $H_i/D_i = 2:1$) equipped with electrical heating and air cooling. The biomass pretreatments were carried out for 30 min at 120 °C, 140 °C, 160 °C and 180 °C. To investigate the effect of Na_2SO_3 concentration, pretreatments were carried out at 0%, 2.4%, 3.0% (w/v) of Na_2SO_3 while keeping the temperature at 180 °C; other factors (concentration of acid and time of pretreatment) were unchanged.

After pretreatment, the samples were filtered to separate solid and liquid phases and the composition of pretreated wheat straw was determined. The solid phase was washed with 60 ml of demineralized water and stored at 4 °C before using for enzymatic hydrolysis and ethanol fermentation. The liquid phase was assayed for inhibitor content.

2.4. Determination of concentration of furan inhibitors

A rapid spectrophotometric method [20] was used to determine total furan (furfural and hydroxymethylfurfural) concentrations. Prior to measurement, samples were diluted with distilled water (from 1:100 to 1:1000) to fit the calibration curve (sum of furans was 0.5–10 mg/l).

2.5. Media

2.5.1. Inoculation medium

Medium used for inoculation contained: 20 g/l glucose, 10 g/l yeast extract, 1 g/l NH_4Cl, 1 g/l KH_2PO_4, 0.5 g/l $MgSO_4.7\ H_2O$. It was sterilized at 121°C for 20 min and kept at 4 °C before use in enzymatic saccharification and fermentation.

2.5.2. Fermentation medium

Medium of the same composition as the inoculation medium was used for fermentation. Glucose was replaced by pretreated biomass, its amount corresponding to 20 g/l cellulose.

2.6. Microbial strain

Zymomonas mobilis CCM 2770 (Czech Collection of Microorganisms, Brno) was used for ethanol fermentation. It was stored at −70 °C in a solution of 30% glycerol.

2.7. Inoculum

A glycerol stock (1.5 ml) was thawed and used to inoculate an Erlenmeyer flask with 100 ml of inoculation medium. A shake culture was grown for 24 h at 30 °C and 100 rpm and then transferred to an Erlenmeyer flask with fermentation medium to achieve a 10% inoculation ratio.

2.8. Saccharification and fermentation

Enzymatic hydrolysis of pretreated samples was carried out in fermentation medium at 55 °C (orbital shaker, rotation frequency