



Research paper

Pretreatment of rye straw with aqueous ammonia for conversion to fermentable sugars as a potential substrates in biotechnological processes



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ABSTRACT

Alkaline pretreatment causes the disruptions in the lignin structure and breaks the linkage between lignin and the other carbohydrate fractions in lignocellulosic biomass. The aim of the current study was to investigate the possibility of rye straw hydrolysis with 2% aqueous ammonia for 0–8 h, at 60 °C and 90 °C, under atmospheric pressure. The study investigated how rye straw hydrolysis with aqueous ammonia at low concentration and relatively low temperatures affect such parameters as total and volatile solids (TS & VS), chemical oxygen demand (COD) and pH at low concentration. The experimental results indicated that the concentration of reducing sugars and volatile fatty acids (VFA) in rye straw after 8 h hydrolysis with ammonia were about 1.3 and 5.2 times higher at 60 °C, respectively and 1.8 and 5.2 times higher at 90 °C, respectively compared to samples in which water hydrolysis of rye straw. The percentage content of glucose in the hydrolysates ranged between 17.98% and 50.98% in water and 21.62%–41.75% in 2% aqueous ammonia. SEM and FTIR analysis of rye straw after 8 h hydrolysis confirmed the positive effect of 2% aqueous ammonia on changes in the material structure.

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1. Introduction

Lignocellulosic materials are natural, abundant, the most economic and renewable resources in the world, essential to the functioning of industrial societies and critical to the development of a sustainable global economy [1,2]. Production of plant biomass on earth reaches 200 billion tons per year, of which 90% are lignocellulosic wastes [3]. In contrast to petrochemicals, which are the subject of economic and environmental concern, lignocellulosic materials are relied upon as feedstock for the production of chemicals, fuels and biocompatible materials. According to statistical data of the European Union, in 2013 about 10 million tons of rye from 2.5 million hectares have been collected. Forecasts for 2014 predict seeding of 2.3 million hectares and gathering of 9.25 million tons of rye. It is estimated that from 1 ton of rye 0.5–2 tons of straw can be obtained (depending on the soil type or variety).

This gives from 5 to 20 million tons of rye straw, of which 40% is potentially used for non-agricultural purposes [4].

These materials contain sugars polymerized to cellulose and hemicellulose that can be liberated by hydrolysis and subsequently fermented by microorganisms to form different chemicals [2,5]. Therefore polymers from the plant biomass are considered as useful resources convertible to not only pulp and foodstuff but also energy resources such as alcohol, methane and chemical raw materials like furfural and organic acids. Effective and economical methods for such uses, however, remain underdeveloped. Since cellulose and hemicellulose in the cell wall undergo lignification by the lignin, an efficient pretreatment for separating cellulose and hemicellulose from plant biomass with ease and at a low cost is a very important goal [2,6].

Because of the chemical barriers such as lignin, hemicellulose and acetyl group that inhibit the accessibility of enzyme to the cellulose substrate, pretreatment of lignocellulosic materials for the purpose of efficient enzymatic hydrolysis is the key element in the bioconversion of this biomass [2,7,8]. The pretreatment must meet

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the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by hydrolysis, (2) avoid the degradation or loss of carbohydrate, (3) avoid the formation of byproducts that are inhibitory to the subsequent hydrolysis and fermentation processes, and (4) be cost-effective [9]. Without the pretreatment the degree of polysaccharides hydrolysis reaches values lower than 20%, while the use of pretreatment increases it to 90% or higher [3]. There are different methods of pretreatment of lignocellulosic material which can be divided into: biological, physical, chemical, and physicochemical. Chemical pretreatment methods include, among others processes, using dilute-acid [2,10,11], ammonia fiber explosion (AFEX) [2,12], soaking in aqueous ammonia (SAA) [2,13], ammonia recycle percolation (ARP) [14] and alkaline pretreatments [2,11,15].

Alkaline pretreatment causes the disruptions in the lignin structure and breaks the linkage between lignin and the other carbohydrate fractions in lignocellulosic biomass, which makes the carbohydrates in the hetero-matrix more accessible. The reactivity of remaining polysaccharides increases as the lignin is removed. This method also allows for removing of acetyl and other uronic acid substitutions on hemicellulose, which lessen the accessibility of enzymes to cellulose surface [16].

The use of ammonia for the pretreatment of lignocellulosic materials results in biomass swelling and increased porosity of biomass, reduction in the degree of polymerization and the crystallinity of cellulose and an effective delignification [3,17]. Ammonia is thought to act selectively with lignin bonds, especially C–O–C bonds, as well as ester and ether bonds [13,18], which selectively reduces lignin in the lignocellulose structure. Aqueous ammonia as a chemical has several desirable traits that make it a good candidate for an industrial platform. Additionally, ammonia is relatively inexpensive, and minimal harmful byproducts that could inhibit enzymatic hydrolysis are created from the interaction of aqueous ammonia with lignocellulosic biomass [17,18]. However most of the ammonium pretreatments require relatively high temperatures (over 100 °C) and high pressure.

An effective pretreatment method should be economical, therefore low temperature may be more appropriate because they would lead to low pressure requirement for reaction vessels and would not require excessive heating [19]. In addition, low temperature pretreatment could maximally recover carbohydrate with minimal formation of fermentation inhibitors. Several low temperatures, long residence time pretreatments have been proposed [13,19–21].

In the present study we have focused on using 2% aqueous ammonium as an active agent for rye straw hydrolysis performed in 60 °C and 90 °C for short time (maximum 8 h). In this paper we show potential possibility of rye straw pretreatment with 2% aqueous ammonium for obtaining substrates (sugars, volatile fatty acids etc.) for biotechnological processes (e.g. anaerobic digestion, biofuels production).

2. Materials and methods

2.1. Material

Rye straw was collected from a local farm in Lodz, Poland, and then stored in a sheltered area before using. The moisture content of the straw was 5.52%. For aqua ammonium pretreatment, the rye straw was milled into pieces measuring between 2 and 4 mm. The milled material was stored in a room temperature (25 °C) for further use. This was defined as untreated rice straw throughout this study. The initial compositions of the rye straw are presented in Table 1.

Table 1
Chemical compositions of untreated rye straw.

Parameter	Value
TS ^a [g/kg]	942 ± 1.35
VS ^a [g/kg]	921 ± 0.45
TS ^b [g/kg]	121.7 ± 4.7
VS ^b [g/kg]	119.0 ± 3.8
TS ^c [g/kg]	116.4 ± 2.7
VS ^c [g/kg]	114.1 ± 3.1
COD ^d [g O ₂ /kg]	1611 ± 78
COD ^e [g O ₂ /kg]	1303 ± 46
VFA ^d [mg C ₂ H ₅ COOH/dm ³]	186 ± 5
VFA ^e [mg C ₂ H ₅ COOH/dm ³]	159 ± 9
Reducing sugars ^d [g C ₆ H ₁₂ O ₆ /dm ³]	0.48 ± 0.02
Reducing sugars ^e [g C ₆ H ₁₂ O ₆ /dm ³]	0.11 ± 0.01
Sodium [mg/kg]	242.5 ± 2
Calcium [mg/kg]	2275.5 ± 12
Magnesium [mg/kg]	80 ± 3
Potassium [mg/kg]	3775 ± 41
Copper [mg/kg]	6 ± 0.2
Iron [mg/kg]	487.5 ± 4
Manganese [mg/kg]	108.5 ± 0.6
Zinc [mg/kg]	0.1 ± 0.0
Cadmium [mg/kg]	ND
Lead [mg/kg]	ND
Chrome [mg/kg]	ND
Nickel [mg/kg]	ND
Cobalt [mg/kg]	ND

Result = mean ± standard deviation (SD).

^a Raw rye straw.

^b Raw rye straw suffused with water.

^c Raw rye straw suffused with 2% aqueous ammonia.

^d Liquor after suffusion of ryes straw with water.

^e Liquor after suffusion of ryes straw with 2% aqueous ammonia.

2.2. Aqueous ammonia pretreatment of rye straw

The milled rye straw was pretreated with 2% (w/v) aqueous ammonia solution in 250 mL volume flasks under the conditions of 60 °C and 90 °C and a S:L (solid: liquid) ratio of 1:20 (5 g rye straw in 100 mL of 2% (w/v) aqueous ammonia solution), for 2, 4, 6, and 8 h, respectively. No stirring occurred during the pretreatment. A control containing untreated rye straw stored in a room temperature suffused with aqueous ammonia in 60 °C and in 90 °C for 1 min was performed. Also water pretreatment of rye straw in higher temperatures (60 °C and 90 °C) has been investigated. The water hydrolysis conditions were the same as in the case of 2% aqueous ammonia pretreatment. After a certain period of time (2, 4, 6, 8 h), the process of chemical and physical hydrolysis was intermittent. The slurry was separated by centrifugation (18,000 rpm, 4 °C, 10 min) and the supernatant was collected and stored at –18 °C for reducing sugars, volatile fatty acids (VFA), chemical oxygen demand (COD) and pH analysis. The solid was recovered using a vacuum filtration on a Buchner funnel and washed with distilled water. After that the solid was air-dried and stored at –18 °C.

2.3. Characterization of untreated and pretreated rye straw

2.3.1. Scanning electron micrograph (SEM)

Scanning electron microscopy JEOL JSM-6610LV was used for the microstructural analysis of the untreated and 2% aqueous ammonia and water pretreated rye straw. All images were taken at an accelerating voltage of 30 kV. Representative images were chosen to illustrate the physical changes caused by the aqueous ammonia and water pretreatment.

2.3.2. Fourier transform infrared spectroscopy (FTIR) analysis

In order to determine the changes in functional groups that may

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