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Research paper

Ozonolysis of alkaline lignin and sugarcane bagasse: Structural changes and their effect on saccharification

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ABSTRACT

Alkaline lignin (AkL) and sugarcane bagasse (SCB) were pretreated by an ozonification process with the aim to study its effect on the solubilization of lignin. We study the ozonification of Alkaline lignin (AkL) and sugarcane bagasse (SCB) with the aim to understand its effect on lignin solubilization and its effect on further acid and enzymatic saccharification. Here, infrared spectroscopy permitted to elucidate the structural changes on the ozonated materials. Indeed, the spectra analysis showed that AkL suffered evident oxidation after 60 min and the corresponding soluble lignin from SCB was observed within 30 min of the ozonification reaction. The total (TS) and reducing sugars (RS) produced from ozonized SCB in acid hydrolysis were 30.83 and 27.17%; while enzymatic hydrolysis resulted in 18.41 and 13.43% after 120 min of ozonation. Furthermore, there is apparently no pH effect on the solubilization of lignin and biomass saccharification. Nevertheless, the highest values of AkL, TS and RS, 0.97, 24.0 and 20.5% were obtained at pH 7, respectively. The ozone is a potential oxidizing agent to remove lignin from lignocellulosic biomass.

1. Introduction

Lignocellulosic materials such as agricultural and urban residues are potential source to produce low cost energy and fuels and extensive research occurs to their conversion into ethanol [1]. Bioethanol production from lignocellulosic materials by a biological approach involves two processes, enzymatic hydrolysis of carbohydrate fraction into the feedstock to sugars and their fermentation to ethanol through yeast or bacteria [2].

Lignin is the most complex natural and amorphous polymer with a three-dimensional structure with phenyl propane units as the predominant building blocks. More specifically, p-coumaryl, coniferyl, syringyl, guaiacyl and sinapyl alcohols [3]. In many cases, lignin is a physical barrier to access to the cellulosic polymer, but they exist different alternatives to increment cellulose and hemicellulose access to depolymerization agents. Some involve the use of physical, chemical, physicochemical and/or biological methods, e.g. ammonia fiber explosion [4–6], steam explosion, hot water extraction, wet oxidation [1,4], and biological pretreatment that usually involves the use of microorganism to degrade the lignin and hemicellulose [4]. Also, there are common methods such as use of sulfuric acid, sodium hydroxide, hydrogen peroxide, peracetic acid and ozonolysis to remove lignin [7]. Some solvents like low molecular alcohols, dioxane, acetone, pyridine, and dimethyl sulfoxide; may remove lignin. Furthermore, thermal softening of lignin takes place at elevated temperatures, which allows depolymerization reactions of acidic or alkaline lignin [8].

However for industrial purposes, an effective pretreatment technologies should (1) result in efficient sugar generation at low energy consumption, (2) not cause significant loss or degradation of carbohydrates, (3) not form byproducts that inhibit subsequent hydrolysis and fermentation processes, and (4) be cost effective [9]. In this context we studied the ozonification process.

Ozone is a powerful oxidant, soluble in water and readily available. It is a nonlinear triatomic molecule containing an obtuse bond angle and two oxygen-oxygen bonds of equal length. The ozone attacks lignin selectively and mainly attacks the aromatic structures [10,11], preserving the side chains attached onto these

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aromatic rings [12]. Ozone disrupt the structure of many different lignocellulosic materials such as wheat straw, bagasse, pine, peanut, cotton straw and poplar sawdust [9]. The driving force of this process is the high reactivity of ozone molecule towards compounds with conjugated double bonds and functional groups with high electron densities. Therefore, the most likely fraction to be oxidized in ozonolysis of lignocellulosic materials is lignin due to its high content of C=C bonds. Ozone attacks lignin releasing soluble compounds of less molecular weight, mainly organic acids such as formic and acetic acid which can result in a drop in pH 6.5 to 2. The main advantages linked to this process are the lack of any degradation products, which might interfere with subsequent hydrolysis or fermentation and the reactions occurring at ambient temperature [13].

Hence, the aims of this work were to determine: (1) the changes in lignin structure of alkaline lignin (AkL) and sugar cane bagasse (SCB) caused by low concentration of ozone (<0.056%); (2) the influence of time and pH on the ozonolysis pretreatment of sugar cane bagasse (SCB) and its chemical changes along the process; and (3) the acid and enzymatic hydrolysis of ozonated SCB. The soluble lignin (SL), TS, and RS content as well as the infrared and UV–Vis spectra were obtained. We used the Alkaline lignin as a model material to analyze the changes in their chemical structure caused by ozone.

2. Materials and methods

2.1. Cane bagasse

Sugar cane bagasse was provided by a Mexican cane industry located in Veracruz State. It was washed, dried, and sieved into 40–60 mesh (0.420–0.250 mm). The initial characterization of sugar cane bagasse was done in accordance to NREL methodology, except total and reducing sugars, in % w/w: total sugar 57.26, reducing sugar 50.78, acid soluble lignin 4.46, acid insoluble lignin 22.53, total lignin 27.0, ash 4.4, and moisture 7.31 [14]. The holocellulose (67.4% w/w) and cellulose (35.56% w/w) contents were determined using the ASTM D 1104 and D 1103, respectively [15a–b]. The hemicellulose content of 31.85% w/w was determined by difference between holocellulose and cellulose contents. Alkaline lignin (AkL) was acquired from Aldrich Co. and was used as received on experiments.

2.2. Ozone

The ozone was produced from air, using a PEAK Ozonator model CM-1 O_3 SYSTEM. Ozone concentration in the gas phase was determined according to the iodometric method [16]. An ozone trap containing a potassium iodine solution 0.05 M was connected to the reactor to determine the ozone concentration in the outlet gas stream (See Supplementary information, SI).

2.3. Ozonolysis measurements of AkL

AkL solution (1.0% w/v) was bubbled with ozone during 3 h, room temperature and pressure. The ozone concentration was 0.0014–0.054% v/v (4.3×10^{-3}–7.8×10^{-2} g O_3/g of dry material), and the inlet gas flow was 200–400 mL/min, the samples were taken at 0, 10, 20, 30, 45, 60, 75, 105, 135 and 180 min. To evaluate the effect of this experiment, we have determinate the SL by UV–Vis (UV–Vis spectrophotometer Thermo Spectronic Helios, USA) and were obtained infrared spectra from the solids.

2.4. Ozonolysis measurements of sugar cane bagasse

On this case, 1 g of SCB with 1 mL of distilled water was gasified with ozone during 2 h, under room conditions, ozone concentration was 0.0014–0.056% v/v (4.3×10^{-3}–5.4×10^{-2} g O_3/g SCB) with an inlet gas flow 200–400 mL/min. Samples were taken at 0, 15, 30, 45, 60, 90 and 120 min. The ozonated bagasse was dried in an oven at 40–50° C during 24 h for a subsequent structural analysis by infrared spectroscopy.

Besides, 1.0 g of SCB with 1 mL of distilled water was gasified with ozone under room conditions with an ozone concentration of 0.051% v/v (8×10^{-2} g O_3/g SCB), and the inlet gas flow was 655 mL/min during 2 h.

In later cases, the remaining solid was washed with 15 mL of distilled water and SL was determined according to the literature procedure [14]. The ozonated bagasse was dried in an oven at 40–50 °C during 24 h for subsequent acid and enzymatic hydrolysis.

2.5. Effect of pH on the ozonolysis of SCB

Ozonolysis was performed using 1 g of SCB with 1.5 mL of phosphate buffer 1 M at different pH values (3, 7 and 11) during 2 h. The material was washed with 15 mL of distilled water and SL was determined as mentioned above [14]. The ozonated bagasse was dried in an oven at 40–50 °C during 24 for a subsequent acid hydrolysis.

2.6. Analysis of structure by infrared spectroscopy

The IR spectra of materials were obtained using a Nicolet Infrared Spectrophotometer NEXUS 40 FT-IR using a diffuse reflectance mode and KBr powder as dispersive phase. The solid materials were dried in an oven at 40–50 °C during 24 h. Then, about 3 mg mg were taken in order to obtain the infrared spectra using 285 mg of KBr. Each spectrum was analyzed taken a normalization of a baseline and using the area ratio of the significant functional groups.

2.7. Acid hydrolysis of ozonated SCB

Acid hydrolysis of ozonated SCB was performed using H_2SO_4 2.5% (v/v), 10% (w/v) dry matter at 121 °C during 1 h in an autoclave (YAMATO SM200 Autoclave). The hydrolyzed bagasse was separated by filtration and the filtrate was used to determined RS and TS by DNS [17] and phenol-sulfuric acid [18] methods respectively.

2.8. Enzymatic hydrolysis of ozonated SCB

Enzymatic hydrolysis of ozonated SCB was carried out with a commercial enzyme cocktail provided by ENMEX containing the following activities: cellulase activity 462.5 FPU/mL, endocellulase 13.65 IU/mL, exocellulase 0.158 μmol glucose/min, β-glucosidase 0.812 μmol p-NP/mLmin. The cellulase activity was determined according to the procedure of Adney and Baker [19]. The rest of the activities were performed using citrate buffer 50 mM, pH 4.8, at 50 °C, 180 rpm, 1 h using as substrates pNPG 2 mM (β-glucosidase), carboxymethylcellulose 0.5% (endocellulase), cellulose microcrystalline 1.25% (exocellulase). All other chemicals were obtained from local retails.

The hydrolysis was performed with 10% of solids (w/v), dosage of 0.9 mL of enzyme cocktail/g biomass and citrate buffer 50 mM, pH 4.8 (416.25 FPU/g biomass). The samples were incubated at 50 °C, 180 rpm for 1 h. After this time, the samples were placed on a boiling water bath to inactivate the enzyme. The samples were