Ecology & Safety Journal of **International Scientific Publications**
ISSN 1314-7234, Volume 11, 2017 www.scientific-publications.net

EFFECT OF ALKALINE PRETREATMENT ON CORN LEAVES

Panagiotis Kandylis, Maria Kanellaki

Department of Chemistry, University of Patras, 26500-Patras, Greece

Abstract

Lignocellulose is the most abundant renewable biomass, however pretreatment is needed prior its utilization. In the present study the effect of dilute alkaline pretreatment on corn leaves structure was investigated. Different concentrations of NaOH solution and different pretreatment times were used for the treatment of corn leaves. Porosimetry was performed to investigate the effect of dilute alkaline pretreatment on specific surface area, pore volume and pore diameter. The results showed that low NaOH loading (5 and 10 g L^{-1}, either for 1 or 3 h) gave the highest surface area and pore volume. These results are important and very promising for potential enzymatic hydrolysis of the biomass, improving the accessibility of enzymes to cellulose.

Key words: *corn, alkaline pretreatment, porosimetry, hydrolysis, surface area*

1. INTRODUCTION

Lignocellulosic biomass includes forest residues (e.g. oak, poplar, pine, fir, etc., softwood and hardwood), agricultural wastes such as cereal harvesting residues (e.g. stalks, leaves, straw, and husk from wheat, corn, rice, oats, etc.), sugarcane bagasse, and grasses (Chandra et al. 2007; Gomez, Steele-King & McQueen-Mason 2008). Lignocellulose is the most abundant renewable biomass, with an annual worldwide production of about 220 billion tonnes on dry weight basis (Chandra, Takeuchi & Hasegawa 2012; Chandra et al. 2007). Therefore, the use of renewable resources such as lignocellulosic biomass for the production of biofuel and other chemicals can reduce the world’s dependence on oil and also reduce the CO_2 emissions, since the carbon contained in such biofuels derives from natural atmospheric CO_2 sequestration by photosynthesis (carbon neutral energy sources) (de Souza et al. 2014; Sanna 2014). Generally, it is a global industrial aim to turn to green, sustainable technologies, replacing synthetic, fossil-fuel based industrial chemicals to similar or even better materials that will be produced from renewable resources (Sheldon 2014; Yabushita, Kobayashi & Fukuoka 2014). In addition, the large scale application of such technologies will result to new employment opportunities, which is particularly important due to the current economic crisis.

Biofuel and other chemicals production using lignocellulosics have many advantages, however economical and technical problems still prevent their establishment (Sánchez & Cardona 2008). The early research targeted to the production of glucose by cellulose degradation and its use as feedstock in fermentation processes, however nowadays research is targeting to exploitation of all biomass-derived sugars (hexoses and pentoses) to increase yields and improve the final process profits. This trend has affected the whole process design strategies for biofuel and other chemicals production from lignocellulosics, especially the biomass pretreatment and conversion steps, introducing new microbial species and enzymes for efficient biomass saccharification and fermentation (Gawand et al. 2013; Liu et al. 2013).

Lignocellulose is the primary building block of plant cell walls and mainly consists of cellulose (38-50%), hemicellulose (23-32%) and lignin (15-25%), as well as, in lower concentrations, pectin, protein, extractives and inorganic minerals (Jorgensen et al., 2007). Cellulose, a β-1-4-glucan, consists of cellobiose (a disaccharide consisting of two glucose units in beta (1-4) glycosidic linkage) units, forming a linear polysaccharide (Delmer & Amor 1995). These chains are linked by hydrogen bonding which form the cellulose chains into microfibrils. Between these microfibrils of cellulose is enough space and there are located hemicellulose, lignin and pectin (Beguin & Aubert 1994) making the final structure of cellulose extremely complicated and thus resistant to biological or chemical treatments (Eriksson & Bermek, 2009).

Pretreatment of lignocellulosic materials is a crucial procedure which aims to the improvement of material's structure for the following microbial and enzymatic processes. This improvement is facilitated through breakdown of lignin and hemicellulose, disruption of the crystalline structure of cellulose and increase of the material's porosity. This procedure makes cellulose and hemicellulose more easily accessible to enzymes and chemical hydrolysis to simple sugars. Various pretreatment techniques (physical, physico-chemical, chemical, and biological) have been used for lignocellulosic materials (Kandylis et al. 2016). Among these methods alkaline pretreatment is considered as one of the most effective leading to products with lower lignin content, less furan derivatives, requiring less energy and resulting to less sugar degradation (Behera et al. 2014; Whitfield, Chinn & Veal 2012).

Nowadays the residues of corn production have been proven ideal raw material for the production of several fermentation products. The lignocellulosic biomass of corn is composed of cellulose (35-45%), hemicelluloses (25-45%) and lignin (10-17%), as well as smaller amounts of other components. After appropriate treatment, the corn stalk residues have been used, among others, for the production of lactic acid (Ma et al. 2016), ethanol (Li et al. 2016; Cai et al. 2016a), biogas (Li et al. 2015), butanol (Ding et al. 2016) and microbial fat (Cai et al. 2016a). However, in most processes using lignocellulosic biomass, including that of corn, biomass was treated as integrity. However, each part of the plant has different composition and characteristics, and it is reasonable its behavior to be different in each treatment (Jin et al. 2013; Li et al. 2016). Therefore, in order to achieve more efficient use of biomass, it is necessary to study the biomass from each corn part separately. Therefore, recently, several researchers began to study each of the different parts of the raw materials separately (Cai et al. 2016b; Mei et al. 2016; Li et al. 2016), however, the need for more focused studies still exists.

In this study, corn leaves were pretreated by NaOH solutions. The effects of NaOH loading, temperature and pretreatment time on biomass characteristics were evaluated. The changes of compositions morphologies of the corn leaves biomass prior and after alkaline pretreatment were investigated by porosimetry.

2. MATERIALS AND METHODS

2.1. Corn stalk

Corn stalks were provided by a local farm in Mornos river valley near the city of Nafpaktos, Central Greece. After corn harvesting, corn stalks were collected, separated and classified into flower, leaf, cob, husk and stem. In the present study only the corn leaves were used.

2.2. Pretreatment

Corn leaves were pretreated using NaOH in different concentrations (5 g L^{-1}, 10 g L^{-1}, 15 g L^{-1} and 20 g L^{-1}) for 1 hour. The temperature of the solution was maintained at 80-90ºC. In addition a three hours pretreatment of corn leaves with 10 g L^{-1} was also performed. After pretreatment the solid part washed with deionized water until pH≈8 and filtered (average pore diameter of filter 1 mm). The bagasse was collected and then dried in oven at 105ºC until constant weight. The dried bagasse was milled to powder (less than 1 mm).

2.3. Analytical methods

The recovery rate of the solid fraction of corn leaves was calculated using the following formula (Li et al. 2016):

$$\%\ \text{recovery} = \frac{W_{pre}}{W_{raw}}\ x\ 100\%$$

where W_{pre} and W_{raw} were the weight of the pretreated and raw corn leaves, respectively.

The average pore diameter and cumulative surface area of pores were measured using a Micromeritics TriStar 3000 porosimeter using Brunauer-Emmett-Teller (BET) surface area analysis and Barrett-Joyner-Halenda (BJH) pore size and volume analysis.

3. RESULTS AND DISCUSSION

Pretreatment of lignocellulosic biomass is a crucial step for its exploitation as raw material for various value added products. More specifically is important for the step of enzymatic hydrolysis of cellulose to fermentable sugars. An effective and economical pretreatment should meet the following requirements (Taherzadeh & Karimi 2008; Sun & Cheng 2002): (1) produce a reactive cellulosic fiber for enzymatic attack, (2) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis, (3) avoid the destruction of hemicelluloses and cellulose, (4) avoid the degradation or loss of carbohydrate, (5) avoid the formation of byproducts which will be possible inhibitors for hydrolytic enzymes and fermenting microorganisms, (6) be cost effective by (6a) minimizing the energy demand, (6b) reducing the cost of size reduction for feedstocks, (6c) reducing the cost of material for construction of pretreatment reactors, (7) produce less residues, (8) consume little or no chemical and (9) use a cheap chemical.

3.1. Alkaline pretreatment of corn leaves

In the present study alkaline pretreatment with sodium hydroxide solution (NaOH), which satisfy many of the previously mentioned requirements, was used on corn leaves, and the effect of this process on several characteristics of biomass was evaluated. NaOH, has been extensively studied in pretreatment of several lignocellulosic materials. The main result and advantage of sodium hydroxide pretreatment is the disruption of the lignin structure and thus improvement of the accessibility of enzymes to cellulose and hemicelluloses (Park & Kim 2012). This is made through degradation of lignin mainly, by the cleavage of ether bonds, the swelling of cellulose, as well as the partial degradation of cellulose and hemicelluloses. In addition acetyl and other uronic acid substitutions on hemicelluloses are also removed, which also increase the accessibility of enzymes to cellulose surface (Zhang et al. 2014).

Alkaline pretreatment with NaOH is considered a mild pretreatment method for low lignin content materials like corn stalk. The effect of NaOH pretreatment on solid recovery of corn leaves are shown in Figure 1. In can be concluded that the solid recovery was significantly affected by the NaOH concentration and pretreatment time. More specifically 5 g L^{-1} NaOH led to 47.5% recovery and as the NaOH concentration increased the recovery rate decreased to 25.6% for 20 g L^{-1} NaOH. The same observed with the increase in pretreatment time from 1 h with 10gL^{-1} NaOH (recovery 40.6%) to 3 h with 10 g L^{-1} NaOH (recovery 30.0%).

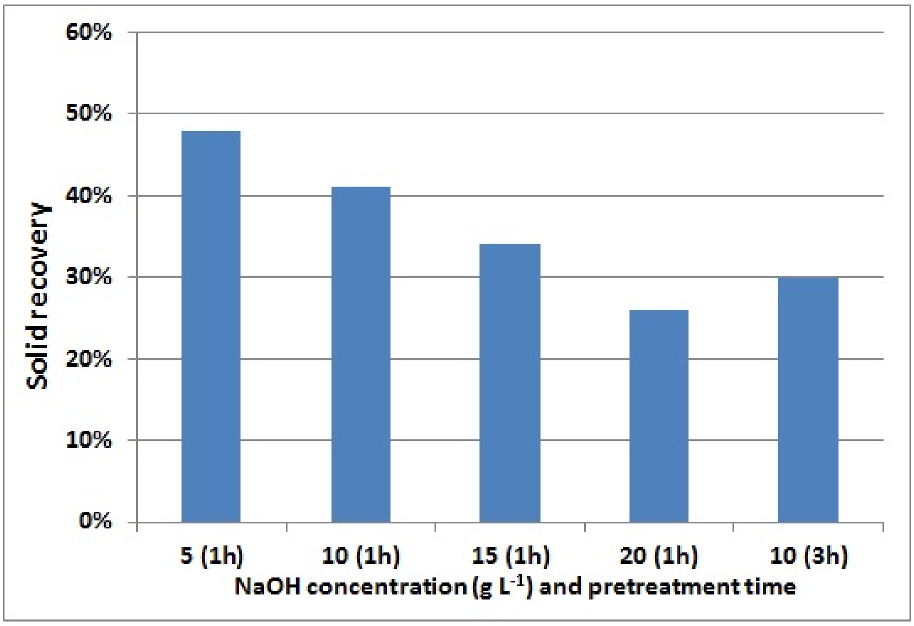

Figure 1. Solid recovery of alkaline pretreated corn leaves.

3.2. Effect of pretreatment on surface area and porosity of corn leaves

The process of cellulose hydrolysis greatly depends upon the adsorption of enzyme on to the substrate. This is determined by the accessible surface area making it a limiting factor for efficient enzymatic digestion (Arantes & Saddler 2011). The accessible surface area is in turn dependent upon particle size, porosity and pore volume (Ravindran & Jaiswal 2016). Alkaline pretreatment improves enzymatic hydrolysis of lignocellulosic materials by removing lignin and this is related to the cellulose accessible surface area. The surface area of lignocellulosic materials can be categorized into external and internal surface area. The external surface area is related to the size and shape of the particles, while, the internal surface area depends on the capillary structure of cellulosic fibers (Behera et al. 2014).

The particle size and pore volume (or porosity) show great influence on the accessible specific surface area of lignocellulosic materials. As the particle size is reduced, a relatively high enzymatic hydrolysis rate and glucose yield can be achieved (Yeh, Huang & Chen 2010). In addition the reduction of particle size lead to more cellulose exposure to the enzymes, resulting in almost complete digestion of cellulose within a short time (Martin-Sampedro et al. 2012).

Surface area of substrate is an important parameter affecting the maximum adsorption capability of enzyme and thus the hydrolysis of cellulose (Ye & Berson 2014). Several studies have correlated the increased enzymatic hydrolysis rate with corresponding increase in available surface area using different cellulose and lignocellulosic substrates (Sinitsyn, Gusakov & Vlasenko 1991; Ye & Berson 2014), as well as substrates following pretreatments (Rollin et al. 2011). Therefore, effective pretreatments to increase the surface binding area of substrate are believed good practice for improvement of the cellulose hydrolysis rate (Kim et al. 2011). Particularly, the surface area accessible to a molecule the size of the enzyme (51 Å) plays an important role in the hydrolysis of cellulosic substrate (Grethlein, Allen & Converse 1984). The surface area of pores (BJH adsorption surface area of pores) accessible to a molecule greater than the size of an enzyme (51 Å) for corn leaves prior pretreatment was 0.80 m^2 g^{-1}. In the case of different pretreated corn leaves were found to increase in the order of 20 g L^{-1} NaOH (0.92 m^2 g^{-1}), 15 g L^{-1} NaOH (1.02 m^2 g^{-1}), 5 g L^{-1} NaOH (1.17 m^2 g^{-1}) and 10 g L^{-1} NaOH (1.41 m^2 g^{-1}). Further increase of pretreatment time from 1 to 3 hours had no effect on the surface area (1.36 m^2 g^{-1}). The total surface area of pores of corn leaves biomass, after 1 hour pretreatment with 10 g L^{-1} NaOH found, almost, three times higher than that reported in previous study with alkaline pretreated softwood sawdust (Koutinas et al. 2012).

Figure 2 presents the BJH adsorption and desorption cumulative volume of pores between 17 Å and 3000 Å width for non-treated and alkaline pretreated corn leaves. Similar values of adsorption and desorption are indicators in the reliability of experimental data obtained in this study (Lee et al. 2007). The results indicate that the average pore volume increased with the alkaline pretreatment. More specifically the highest pore volume obtained with lower NaOH loading (5 g L^{-1} and 10 g L^{-1} NaOH solution). Further increase of NaOH concentration led to lower pore volume but higher than the untreated corn leaves. Different pretreatment time had no effect on pore volume.

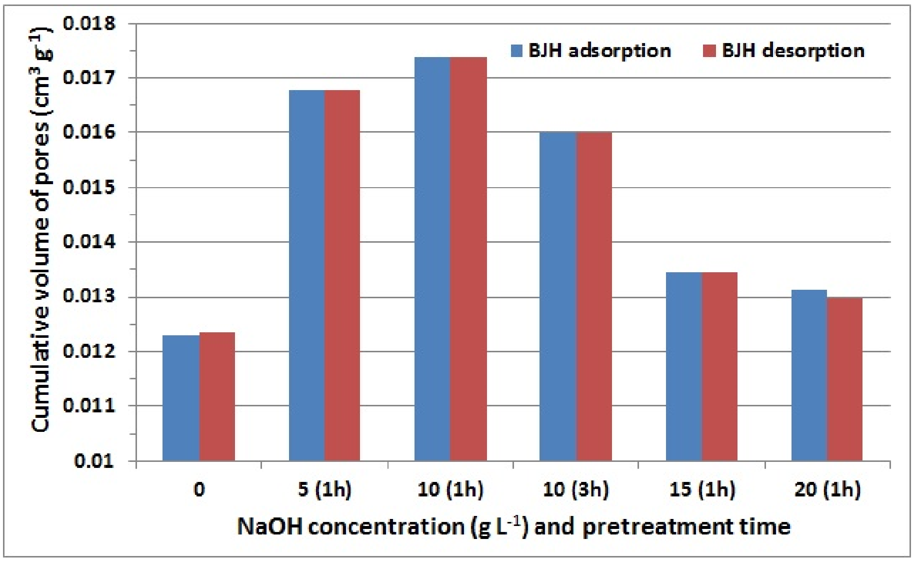

Figure 2. BJH adsorption and desorption cumulative volume of pores between 17 Å and 3000 Å width for non-treated and alkaline pretreated corn leaves.

Previous studies also described a good correlation between the pore volume of lignocellulosic materials and their enzymatic digestibility and hydrolysis rate (Grethlein 1985). They concluded that the pore size of the substrate plays a limiting factor in the enzymatic hydrolysis of biomass (Yang et al. 2011). Removal of lignin and hemicellulose increases the mean pore size of the substrate, and therefore, increases the probability of the cellulose to get hydrolyzed (Hendriks & Zeeman 2009). The digestibility of lignocellulose can be significantly enhanced when the pores of the substrate are large enough to accommodate both large and small enzyme components to maintain the synergistic action of the cellulase enzyme system (Sun et al. 2016). The pore size distribution for non-treated and alkaline pretreated corn leaves is shown in Figure 3.

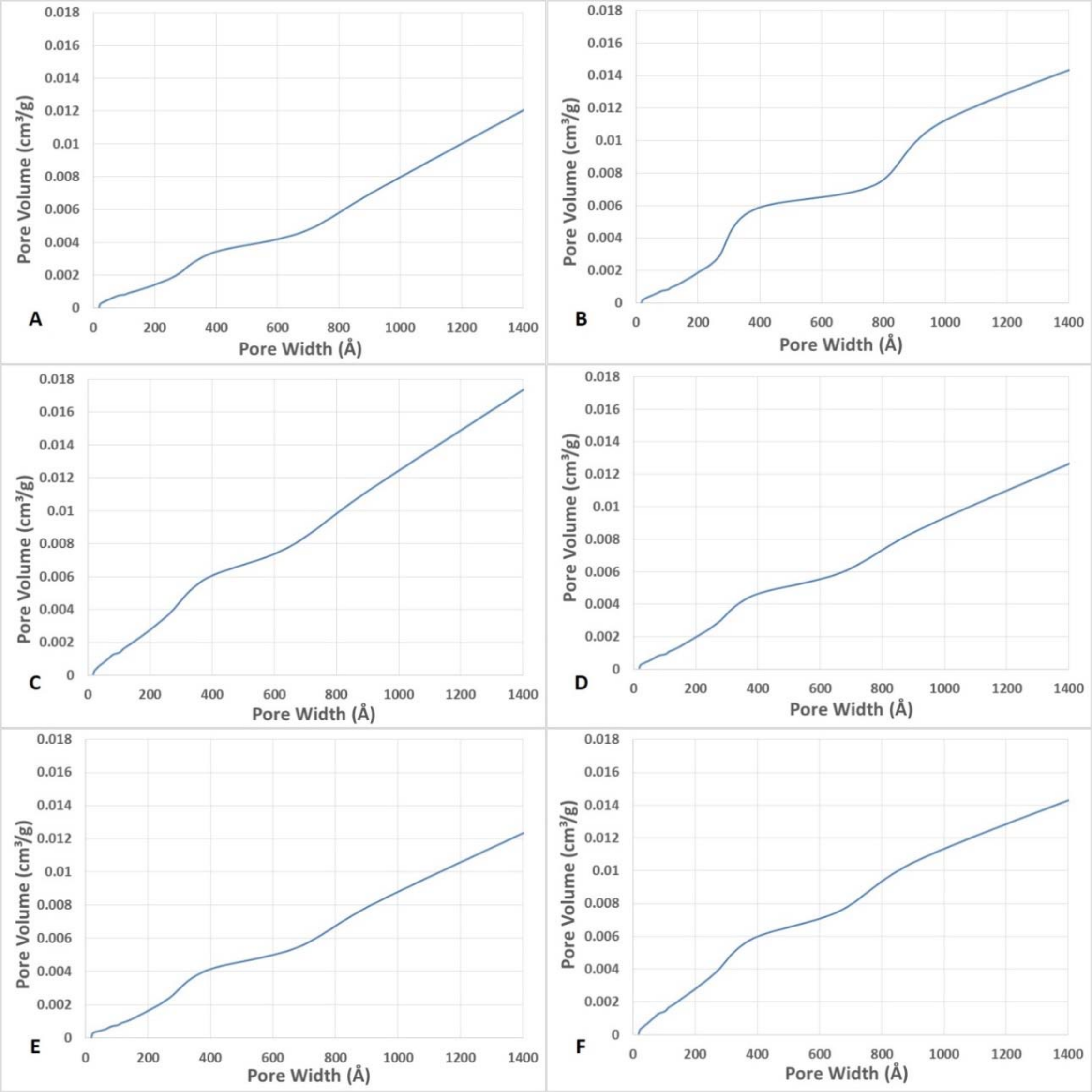

Figure 3. Pore size distribution for non-treated and alkaline pretreated corn leaves. (A) non-treated corn leaves, (B) pretreated corn leaves with 5 g L^{-1} NaOH for 1 h, (C) pretreated corn leaves with 10 g L^{-1} NaOH for 1 h, (D) pretreated corn leaves with 15 g L^{-1} NaOH for 1 h, (E) pretreated corn leaves with 20 g L^{-1} NaOH for 1 h, (F) pretreated corn leaves with 10 g L^{-1} NaOH for 3 h.

4. CONCLUSIONS

In order to make enzymatic hydrolysis more feasible method and to increase its application, it is important to reduce its operational costs. Therefore the economics of such methods may be improved by increasing the hydrolysis rate and reducing the enzyme production costs. In order to increase the hydrolysis rate, a more careful and specific research in the pretreatment of lignocellulosic materials should apply. The present study focused on corn leaves and their alkaline pretreatment was studied. Also the effect of several different methods on available surface area for the enzymes and porosity of the pretreated biomass was studied. The results showed that low NaOH loading (5 g L^{-1} and 10 g L^{-1}, either for 1 or 3 h) gave the highest surface area and pore volume. These results are very promising for the improvement of accessibility of enzymes to cellulose. However further research is needed to study pretreatment in other lignocellulosic materials but also to study the hydrolysis rate of the pretreated corn leaves.

ACKNOWLEDGMENT

This scientific publication was implemented within the framework of the action "Supporting Postdoctoral Researchers" of the Operational Program "Human Resources Development, Education and Lifelong Learning", 2014-2020, which implemented by I.K.Y. and co-financed by the European Social Fund and the Greek State.

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