ACIDOGENIC FERMENTATION OF ALKALINE PRETREATED CORN STEM BIOMASS

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Abstract

Worldwide corn production is continuously increased in order to satisfy the growing demand for more food. However this leads to the production of a large amount of solid wastes that either left in the field or at best used as animal feed of low quality. In the present study a more sustainable exploitation of corn wastes (especially corn stem) was studied. Firstly corn stem biomass was effectively pretreated using NaOH solution, in order to improve its structure for the following acidogenic fermentation. The effect of alkaline pretreatment on biomass structure (using scanning electron microscopy and porosimetry) and on biomass lignin content was also evaluated. The anaerobic acidogenic fermentation experiments of the pretreated corn stem biomass were carried out using an up-flow anaerobic sludge blanket reactor (UASB) culture, either as free cells or immobilized on corn cob and kissiris. In all cases the immobilized cells increased the organic acids yields during the acidogenic fermentation. The main organic acids produced were lactic, acetic and butyric acid. The organic acids could be subsequently used either as chemicals or as substrate for the production of ester-based biofuels, as a cost-effective and environmentally friendly alternative for second generation biofuels production.

Keywords: *corn stem, lignocellulose, organic acids, acidogenesis, alkaline pretreatment, BET, SEM, lignin*

1. INTRODUCTION

Green chemistry deals with the efficient use of (preferably renewable) sources in combination with the minimization of waste and avoidance of the use of toxic and/or dangerous reagents and solvents in the preparation and application of chemicals [1]. Although attention continues to focus on those, at present there is a growing emphasis on the replacement of non-renewable mineral resources - crude oil, coal and natural gas - from renewable biomass as a viable raw material for the production of basic chemicals and liquid fuels [2]. Nowadays, more than ever, it is a global industrial goal to shift to green, sustainable technologies. Thus, it is a global trend and desire to replace fossil-based industrial chemical compounds with similar or even better materials produced from renewable sources [2-3]. The ideal scenario, in terms of a truly circular economy, involves the utilization of agricultural biomass waste, for example, sugar cane residues, corn residues, wheat straw, rice husks and fruit peels by applying appropriate processes [4]. Following that trend, the present study focuses on the exploitation of corn residues (especially corn stem), for the production of added value products such as organic acids. It is estimated that for every 1 kg of corn produced, 1 kg of lignocellulosic biomass was also produced [5]. In Greece, the production of corn, and therefore waste, is estimated to 2,169,900 tons [6]. The management of these wastes is limited and either left in the field or at best used as low quality feed [5].

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 stems were used.

2.2. Pretreatment

Corn stems 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. In addition pretreatment with 10 g L^{-1} NaOH was carried out for 3 h. The temperature

of the solution was maintained at 80-90^{o}C. After pretreatment the solid part washed with deionized water until pH≈8 and filtered (average pore diameter of filter 1 mm). The corn stem biomass was collected and then dried in a freeze drying system for 24 h. The dried biomass was milled to powder (less than 1 mm).

2.3. Anaerobic acidogenic fermentations of pretreated corn stem biomass

2.3.1. Culture and growth media

The acidogenic fermentations were carried out using a mixed culture from an up-flow anaerobic sludge blanket reactor (UASB) at the Department of Chemistry, University of Patras, Greece [7]. The culture was grown at 37°C in a medium with cellobiose 50 g L^{-1}, $NaHCO_3$ 4 g L^{-1}, yeast extract 4 g L^{-1} and aqueous NH_3 with 50% H_3PO_4 solution at a COD:N:P ratio of 100:5:1 [8].

2.3.2. Cell immobilization and acidogenic fermentations

Cell immobilization was carried out using the same methodology described by Lappa et al. [7] with a modification of the growth medium (cellobiose was used instead of sucrose). In brief, a 250 mL bioreactor was filled with 100 g either kissiris or non-treated corn cob and equal volumes of 20 g L^{-1} cellobiose medium and anaerobic culture suspension. The system was left to ferment for two days at 37^{o}C without feeding in order to achieve cell immobilization. Subsequently, the immobilized culture was used to ferment the medium that contained the pretreated corn stem biomass as the only carbon source. The broth also contained: $NaHCO_3$ 4 g L^{-1}, yeast extract 4 g L^{-1}, and aqueous NH_3 with 50% H_3PO_4 solution. The bioreactor was sealed to maintain anaerobic conditions and fermentations took place at 37°C. Anaerobic fermentations were also carried out with free cells. All experiments were carried out in triplicate and the data are presented as mean values.

2.4. Analytical methods

2.4.1. Recovery rate

The recovery rate of the solid fraction of corn stems was calculated using the following formula [9]:

% recovery = (W_{pre}/W_{raw}) x 100%, where W_{pre} and W_{raw} were the weight of the pretreated and raw corn stem, respectively.

2.4.2. Average diameter and cumulative surface area of pores

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. To determine the specific surface area and pore volume of the samples, a small amount of sample was placed in the cells (accurately weighed) and heated at 90^{o}C for 1.5 h under N_2 flow to clean the surface of the sample from any adsorbed gas. After degassing, the sample cells were attached to the specific sites in the system chamber and the amount of N_2 absorbed at liquid nitrogen temperature in different N_2 - He mixtures was determined. The BET equations of the instrument software were used to calculate the specific surface area while the BJH method was used to calculate the pore size distribution.

2.4.3. Lignin content

The acid soluble lignin content was analyzed according to the standard of National Renewable Energy Laboratory [10].

2.4.4. Organic acids determination

The organic acids produced during the anaerobic acidogenic fermentations were analyzed on a HPLC system (Jasco Inc., Japan) LC-2000 Series equipped with a size-exclusion organic acids analysis column (Aminex HPX-87H, 300×7.8mm i.d., 9 μm particle size, Bio-rad, France) fitted in a CO-2060 PLUS column oven. A PU-2089 pump, an AS 2050 PLUS autosampler and a MD-2018 Photodiode array detector (set at 210 nm) were employed. Isocratic separation at 50 °C, with a flow rate of 0.6 mL/min and 0.008 N H_2SO_4 as mobile phase, was performed. The detector signals were recorded and analyzed

by ChromNav software. Amounts of 20 μL of the samples were filtered through a 0.2 μm filter. Standard solutions of acids (Sigma-Aldrich Ltd) were prepared at various concentrations for quantification.

2.4.5. Scanning electron microscopy

Dried samples were sputter-coated with gold in BALTEC MED020 sputter-coater and examined in a scanning electron microscope (JSM5600LV, JEOL, Japan), operating at an accelerating voltage of 25kV.

3. RESULTS

In the present study a more sustainable exploitation of corn stem through acidogenic fermentation was studied. The process employed are presented in Figure 1. More specifically, corn stem was pretreated using dilute alkaline solution (NaOH) at 80-90^{o}C for lignin removal. After pretreatment the residual solid biomass of corn stem was dried and milled. The pretreated corn stem biomass was subsequently used for anaerobic acidogenic fermentation with a mixed UASB anaerobic culture for organic acids production. The produced organic acids may be used either as chemicals or as substrate for a new biofuel production. The first step of the proposed exploitation of corn stem is the mild alkaline pretreatment and especially with NaOH solution. The effect of NaOH pretreatment on the surface characteristics of corn stem biomass was highlighted using SEM and the micrographs are presented in Figure 2. The effect of NaOH pretreatment on solid recovery of corn stem is shown in Figure 3. After pretreatment, the solid recovery rate of corn stem was 65.4 % for 5 g L^{-1} NaOH and then decreased, with the increase of NaOH content, to 37.8 % for 20 g L^{-1} NaOH. In addition the pretreatment time affected the solid recovery rate of corn stem biomass and more specifically the use of 10 g L^{-1} NaOH for 1 h resulted to 43.4 % while after 3 h to 29.7 %. The removal of acid soluble lignin in the corn stem of the present study was up to 26.8 % after alkaline treatment using 10 g L^{-1} NaOH for 1 h. The results from the porosimetry analysis (surface area, pore volume and size), indicated that as the NaOH content increases during delignification, an increase of the specific surface area of the corn stem biomass was also observed. Finally the pretreated corn stem biomass was subsequently used for anaerobic acidogenic fermentation with a mixed UASB anaerobic culture. The UASB culture was used either as free cells or immobilized on kissiris or non-treated corn cob. The immobilization of the UASB cells was confirmed by the fermentation ability of the immobilized cells and by scanning electron microscopy (Figure 4). The results indicated that the use of immobilized cells presented a promoting activity on the yield of the acidogenic fermentation of corn stem biomass. In the present study in all cases the main organic acids produced were lactic, acetic and butyric acid.

4. DISCUSSION

Worldwide corn production is continuously increased in order to satisfy the growing demand for more food. However this leads to the production of a large amount of solid wastes that either left in the field or at best used as animal feed of low quality. Nowadays the solid residues of corn production have been proven ideal raw material for the production of several fermentation products such as volatile fatty acids [11], bioethanol [12], methane [13] and hydrogen [14]. However, in most cases biomass is treated as integrity, which leads to low productivities since is well known that each part of the plant has different composition and characteristics, and it is reasonable its behavior to be different in each treatment [15]. Therefore, in order to achieve more efficient use of biomass, it is necessary to study the biomass from each corn part separately. In the present study corn stem has been studied during dilute alkaline pretreatment and direct acidogenic fermentation, in order to develop a sustainable process from waste corn stem biomass to value products, such as organic acids (Figure 1).

4.1. Alkaline pretreatment of corn stem

In the present study alkaline pretreatment with sodium hydroxide solution (NaOH) was used on corn stem biomass, and the effect of this process on several characteristics of biomass was evaluated.

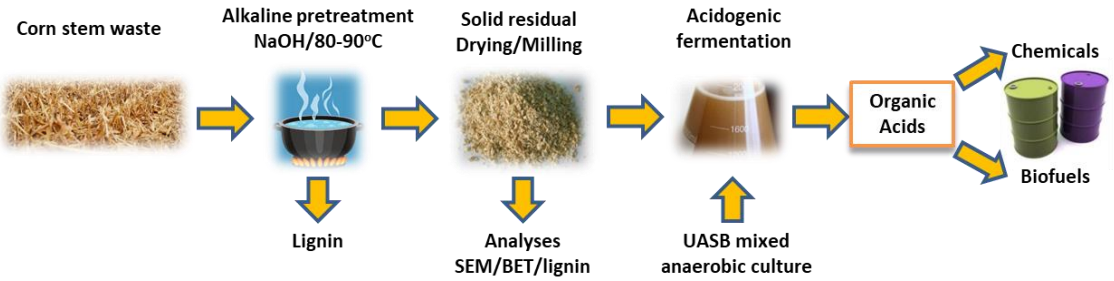

Figure 1. Sustainable exploitation of corn stem wastes through acidogenic fermentation

4.1.1. Scanning Electron Microscopy

The effect of pretreatment with NaOH on the morphology of corn stem surface was studied using SEM (Figure 2). The photographs clearly showed that the pretreatment significantly affected the corn stem surface. More specifically after pretreatment the surface is not the same as before pretreatment since the cell walls were swollen and fibers, with possible cellulose and hemicellulose origin, were exposed. This indicated that the porosity and the external surface area of the pretreated corn stem biomass had increased [16]. This image may be attributed to the lignin removal and to the exposure of cellulose and hemicellulose structures. These results are very important for possible use of the pretreated biomass for enzymatic hydrolysis, since cellulose is now more easily accessible.

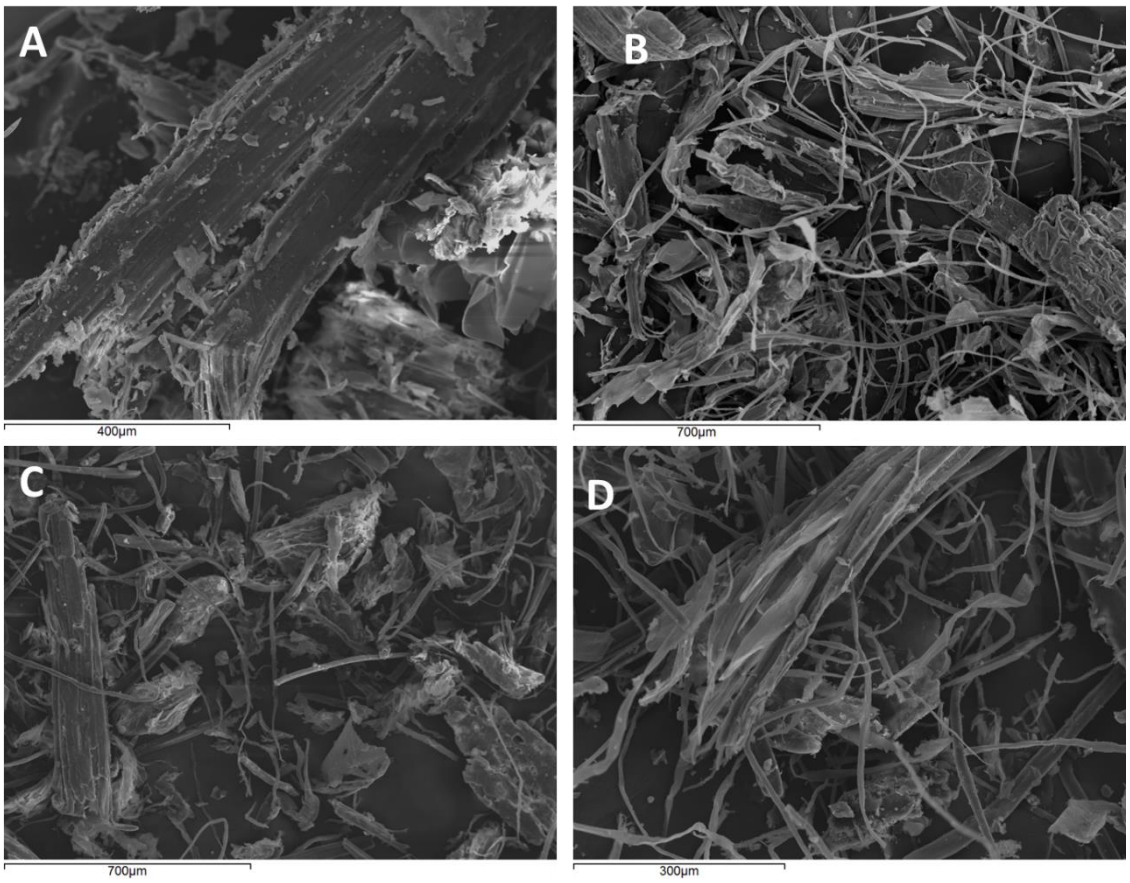

Figure 2. Scanning electron micrographs of corn stem biomass before and after pretreatment with NaOH solution for 1 h (A: before pretreatment; after pretreatment with different NaOH concentrations B: NaOH 10 g L^{-1}, C: NaOH 15 g L^{-1}, D: NaOH 20 g L^{-1}).

4.1.2. Solid recovery rate

Alkaline pretreatment and especially with NaOH solution, has been extensively studied in pretreatment of several lignocellulosic materials. Alkaline pretreatment with NaOH is considered a mild pretreatment method for low lignin content materials like corn stem. In the present study the solid recovery was significantly affected by the NaOH concentration (Figure 3). The solid recovery rate of corn stem decreased, with the increase of NaOH content, which was also observed in the case of wheat straw [17] and corn leaves with lower recovery rates [18]. Since lignin is easily to be degraded under alkaline conditions [19], the reduction of the solid recoveries was likely caused by lignin degradation and removal.

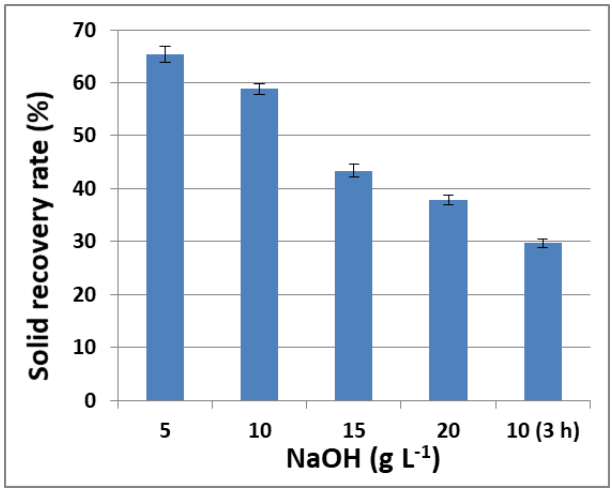

Figure 3. Solid recovery of alkaline pretreated corn stem.

4.1.3. Lignin removal

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 hemicellulose [20]. The lignin content in corn stem is 20.5 % w/w and is not relatively high compared to other agricultural residues and wastes [21]. Compared to other parts of corn stalk, corn stem presents the higher lignin content along with corn flower [22]. In the present study as the NaOH content increased, an increase also observed in acid lignin removal. The same effect of NaOH concentration was also observed in the case of wheat straw [17]. These results are important and very promising for potential enzymatic hydrolysis of the biomass, improving the accessibility of enzymes to the remaining cellulose of corn stem. In fact several studies have demonstrated strong positive correlations between lignin removal and sugar released by enzymatic hydrolysis [17, 23, 24]. In addition is well known that lignin directly acts as a physical barrier, restricting cellulase access to cellulose, and reduces the enzyme's activity through non-productive binding [25].

4.1.4. BET analysis

The results from the BET analysis (surface area, pore volume and size), indicated that as the NaOH content increases during delignification, an increase in specific surface area of corn stem biomass was observed. Similar observations reported in the case of alkaline pretreated corn leaves [18], while in the contrary alkaline pretreated wheat straw biomass underwent a collapse of the smaller pores, and therefore its surface was minimized [8]. The total surface area of pores of corn stem biomass, after 1 h pretreatment with 10 g L^{-1} NaOH found, almost, two times higher than that reported in previous study with alkaline pretreated softwood sawdust [26]. These results are very promising since the surface area of substrate is an important parameter affecting the maximum adsorption capability of enzyme and thus the hydrolysis of cellulose [27].

4.2. Anaerobic acidogenic fermentation of alkaline pretreated corn stem biomass

The pretreated corn stem biomass was subsequently used for anaerobic acidogenic fermentation with a mixed UASB anaerobic culture. The UASB culture was used either as free cells or immobilized on kissiris or non-treated corn cob (Figure 4). The results indicated that the use of immobilized cells presented a promoting activity on the yield of the acidogenic fermentation of corn stem biomass. Several studies have confirmed the promoting effect of kissiris on acidogenic fermentation of several sugars such as sucrose, raffinose, lactose, glucose and agricultural and food wastes and side streams like vinasse, whey and wheat straw [7, 28, 29]. In the present study in all cases the main organic acids produced were lactic, acetic and butyric acid.

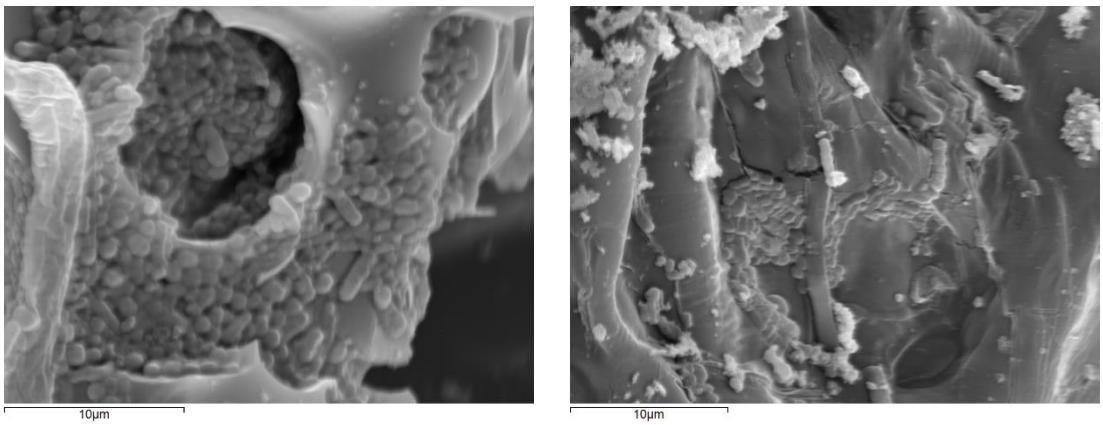

Figure 4. SEM photographs showing the immobilized cells of UASB culture on kissiris (A) and non-treated corn cob (B)

5. CONCLUSIONS

Acidogenic fermentations were performed on dilute alkaline pretreated corn stem biomass. Alkaline pretreatment led to the removal of significant amount of lignin and increase of surface area (BET analysis). The subsequent acidogenic fermentation of the pretreated corn stem biomass revealed the promoting effect of immobilization (either on kissiris or non-treated corn cob). Significant amounts of organic acids were produced and mainly lactic, acetic and butyric acid. The proposed exploitation of corn stem wastes is very promising since the produced organic acids could be subsequently used either as chemicals or as substrate for the production of ester-based biofuels, as a cost-effective and environmentally friendly alternative for second generation biofuels production [30].

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REFERENCES

1. Sheldon, RA, Arends, I and Hanefeld, U 2007, "Green chemistry and catalysis", John Wiley & Sons.
2. Sheldon, RA 2014, "Green and sustainable manufacture of chemicals from biomass: state of the art", *Green Chemistry*, vol. 16, no. 3, pp. 950-963.
3. Yabushita, M, Kobayashi, H and Fukuoka, A 2014, "Catalytic transformation of cellulose into platform chemicals", *Applied Catalysis B: Environmental*, vol. 145, pp. 1-9.
4. Tuck, CO, Pérez, E, Horváth, IT, Sheldon, RA and Poliakoff, M 2012, "Valorization of biomass: deriving more value from waste", *Science*, vol. 337, no. 6095, pp. 695-699.
5. Kadam, KL and McMillan, JD 2003, "Availability of corn stover as a sustainable feedstock for bioethanol production", *Bioresource Technology*, vol. 88, no. 1, pp. 17-25.
6. FAOSTAT 2013, "Food and Agriculture Organization of the United Nations", <http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E>.
7. Lappa, K, Kandylis, P, Bekatorou, A, Bastas, N, Klaoudatos, S, Athanasopoulos, N, Kanellaki, M and Koutinas, AA 2015, "Continuous acidogenesis of sucrose, raffinose and vinasse using mineral kissiris as promoter", *Bioresource Technology*, vol. 188, pp. 43-48.
8. Tsafrakidou, P, Bekatorou, A, Koutinas, AA, Kordulis, C, Banat, IM, Petsi, T and Sotiriou, M 2018, "Acidogenic fermentation of wheat straw after chemical and microbial pretreatment for biofuel applications", *Energy Conversion and Management*, vol. 160, pp. 509-517.
9. Li, P, Cai, D, Luo, Z, Qin, P, Chen, C, Wang, Y, Zhang, C, Wang, Z and Tan, T 2016, "Effect of acid pretreatment on different parts of corn stalk for second generation ethanol production", *Bioresource Technology*, vol. 206, pp. 86-92.
10. Sluiter, A, Hames, B, Ruiz, R, Scarlata, C, Sluiter, J, Templeton, D and Crocker, D 2012, "Determination of structural carbohydrates and lignin in biomass", *Laboratory Analytical Procedure*, NREL, Report No.TP-510-42618.
11. Murali, N, Fernandez, S and Ahring, BK 2017, "Fermentation of wet-exploded corn stover for the production of volatile fatty acids", *Bioresource Technology*, vol. 227, pp. 197-204.
12. Chen, S, Xu, Z, Li, X, Yu, J, Cai, M and Jin, M 2018, "Integrated bioethanol production from mixtures of corn and corn stover", *Bioresource Technology*, vol. 258, pp. 18-25.
13. Liu, CM, Wachemo, AC, Yuan, HR, Zou, DX, Liu, YP, Zhang, L, Pang, YZ and Li, XJ 2018, "Evaluation of methane yield using acidogenic effluent of NaOH pretreated corn stover in anaerobic digestion", *Renewable Energy*, vol. 116, pp. 224-233.
14. Wang, S, Ma, Z and Su, H 2018, "Two-step continuous hydrogen production by immobilized mixed culture on corn stalk", *Renewable Energy*, vol. 121, pp. 230-235.
15. Li, P, Cai, D, Luo, Z, Qin, P, Chen, C, Wang, Y, Zhang, C, Wang, Z and Tan, T 2016, "Effect of acid pretreatment on different parts of corn stalk for second generation ethanol production", *Bioresource Technology*, vol. 206, pp. 86-92.
16. Hong, E, Kim, D, Kim, J, Kim, J, Yoon, S, Rhie, S, Ha, S and Ryu, Y 2015, "Optimization of alkaline pretreatment on corn stover for enhanced production of 1.3-propanediol and 2, 3-butanediol by *Klebsiella pneumoniae* AJ4", *Biomass and Bioenergy*, vol. 77, pp. 177-185.
17. McIntosh, S and Vancov, T 2011, "Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw", *Biomass and Bioenergy*, vol. 35 no. 7, pp. 3094-3103.
18. Kandylis, P and Kanellaki, M 2017, "Effect of alkaline pretreatment on corn leaves", *Ecology and Safety*, vol. 11, pp. 246-254.

19. Jin, Y, Huang, T, Geng, W and Yang, L 2013, "Comparison of sodium carbonate pretreatment for enzymatic hydrolysis of wheat straw stem and leaf to produce fermentable sugars", *Bioresource Technology*, vol. 137, pp. 294-301.

20. Park, YC and Kim, JS 2012, "Comparison of various alkaline pretreatment methods of lignocellulosic biomass", *Energy*, vol. 47, no. 1, pp. 31-35.

21. Sun, Y and Cheng, J 2002, "Hydrolysis of lignocellulosic materials for ethanol production: a review", *Bioresource Technology*, vol. 83, no. 1, pp. 1-11.

22. Cai, D, Li, P, Luo, Z, Qin, P, Chen, C, Wang, Y, Wang, Z and Tan, T 2016, "Effect of dilute alkaline pretreatment on the conversion of different parts of corn stalk to fermentable sugars and its application in acetone–butanol–ethanol fermentation", *Bioresource Technology*, vol. 211, pp. 117-124.

23. Chen, F and Dixon, RA 2007, "Lignin modification improves fermentable sugar yields for biofuel production", *Nature Biotechnology*, vol. 25, no. 7, p.759.

24. Guo, GL, Hsu, DC, Chen, WH, Chen, WH and Hwang, WS 2009, "Characterization of enzymatic saccharification for acid-pretreated lignocellulosic materials with different lignin composition", *Enzyme and Microbial Technology*, vol. 45, no. 2, pp. 80-87.

25. Jeoh, T, Ishizawa, CI, Davis, MF, Himmel, ME, Adney, WS and Johnson, DK 2007, "Cellulase digestibility of pretreated biomass is limited by cellulose accessibility", *Biotechnology and Bioengineering*, vol. 98, no. 1, pp. 112-122.

26. Koutinas, AA, Sypsas, V, Kandylis, P, Michelis, A, Bekatorou, A, Kourkoutas, Y, Kordulis, C, Lycourghiotis, A, Banat, IM, Nigam, P, Marchant, R, Giannouli, M and Yianoulis, P 2012, "Nanotubular cellulose for bioprocess technology development", *PloS One*, vol. 7, no. 4, pp. e34350.

27. Ye, Z and Berson, RE 2014, "Factors affecting cellulose hydrolysis based on inactivation of adsorbed enzymes", *Bioresource Technology*, vol. 167, pp. 582-586.

28. Koutinas, A, Kanellaki, M, Bekatorou, A, Kandylis, P, Pissaridi, K, Dima, A, Boura, K, Lappa, K, Tsafrakidou, P, Stergiou, PY and Foukis, A 2016, "Economic evaluation of technology for a new generation biofuel production using wastes", *Bioresource Technology*, vol. 200, pp. 178-185.

29. Dima, A, Boura, K, Bekatorou, A, Stergiou, PY, Foukis, A, Gkini, OA, Kandylis, P, Pissaridi, K, Kanellaki, M, Papamichael, EM and Koutinas, AA 2017, "Scale-up for esters production from straw whiskers for biofuel applications", *Bioresource Technology*, vol. 242, pp. 109-112.

30. Kandylis, P, Bekatorou, A, Pissaridi, K, Lappa, K, Dima, A, Kanellaki, M and Koutinas, AA 2016, "Acidogenesis of cellulosic hydrolysates for new generation biofuels", *Biomass and Bioenergy*, vol. 91, pp. 210-216.