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2G Ethanol Production From Palm Lignocellulosic Biomass

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ABSTRACT

Brazil presents the world's largest potential for the production of palm oil due to nearly 75 million hectares of land suitable for palm culture and advantageous soil and climate. The biomass generated in the production of palm oil (palm pressed fiber, PPF) is mainly composed of lignocellulosic material that can be hydrolyzed into fermentable sugars for further conversion to ethanol. This work evaluated alkaline pretreatment of this palm oil residue and subsequent Simultaneous Saccharification and Fermentation (SSF), achieving a conversion of glucose to ethanol higher than 90% and a concentration equivalent to 22.40 g/L of the alcohol.

Indexing terms/Keywords

Ethanol; lignocellulosic residues; SSF; Palm pressed fiber

Academic Discipline And Sub-Disciplines

Science, Biotechnology

SUBJECT CLASSIFICATION

Biotechnology, Bioprocess Engineering

TYPE (METHOD/APPROACH)

Saccharification, Fermentation, Bioproduction, Bioprocess Engineering

INTRODUCTION

Indiscriminate use of hydrocarbons for industrial development has led to a decrease in oil reserves and generated high levels of environmental contamination. These factors have stimulated mankind to seek new energy and industrial alternatives less dependent on fossil and non-renewable feedstocks, therefore reducing damages to the environment. Shifting from an oil-dependent economy to a new, more clean, one, however, is not an easy task, since oil industry has been long established and provides more than 36% of energy needs. Besides, oil plays a fundamental role as a raw material for the petrochemical industry [1].

Expand the energy supply, while promoting development with reduced environmental impact and preserving energy sources, is the biggest challenge of the scientific community today. The concept of alternative energy, intensively investigated in the last century, is, today, synonymous of renewable energy [2].

Brazil presents many advantages over other countries regarding the use of renewable energy and biofuels production from a wide range of oil-rich types of plants. Located predominantly in tropical and subtropical regions, it receives intense solar radiation throughout the year, providing a high energy density per unit area, and its biodiversity is amongst the richest in the world. Also, Brazil shows the largest potential for oil palm production due to its almost 75 million hectares of land suitable for cultivation without competing against food agriculture [3].

Oil Palm industry generates large quantities of residues. According to the principles of Biorefinery, palm bagasse, a lignocellulosic material, could be explored for further ethanol production. Composed mainly of cellulose, hemicellulose and lignin, these feedstocks cannot be readily fermented into valuable products. However, once pretreated and saccharified, they provide sugars that serve as substrates for the production of a wide array of products, biofuels amongst them. Also, palm is one of the few plant species that are able to supply large amounts of both vegetable oil (including biodiesel) and ethanol [4,5].

In order to obtain biodiesel, though, not only pretreatment and fermentative stages are required: the Saccharification step, concerning enzymatic hydrolysis of cellulose, which releases glucose, must be carried out.The use of cellulolytic enzymes for the hydrolysis of complex lignocellulosic biomass to obtain sugar liquors is of great interest. The lignocellulosic raw material, usually rich in cellulose, can be converted to glucose - a monosaccharide assimilated by most microorganisms - through enzyme action and, further, submitted to different processes, resulting in a vast range of products, from fuels to polymers [6].

Seeking a more integrated bioprocess, different strategies have been conceived and Simultaneous Saccharification and Fermentation (SSF) stands as a prominent one. In this process, cellulose hydrolysis and fermentation occur both in only one stage, and once glucose is released in the cultivation media, it is promptly consumed, overcoming cellulose inhibition

due to high levels of glucose. Hemicellulose fractioning and hydrolysis, with its subsequent fermentation, though, are held separately (Figure 1) [4,5].

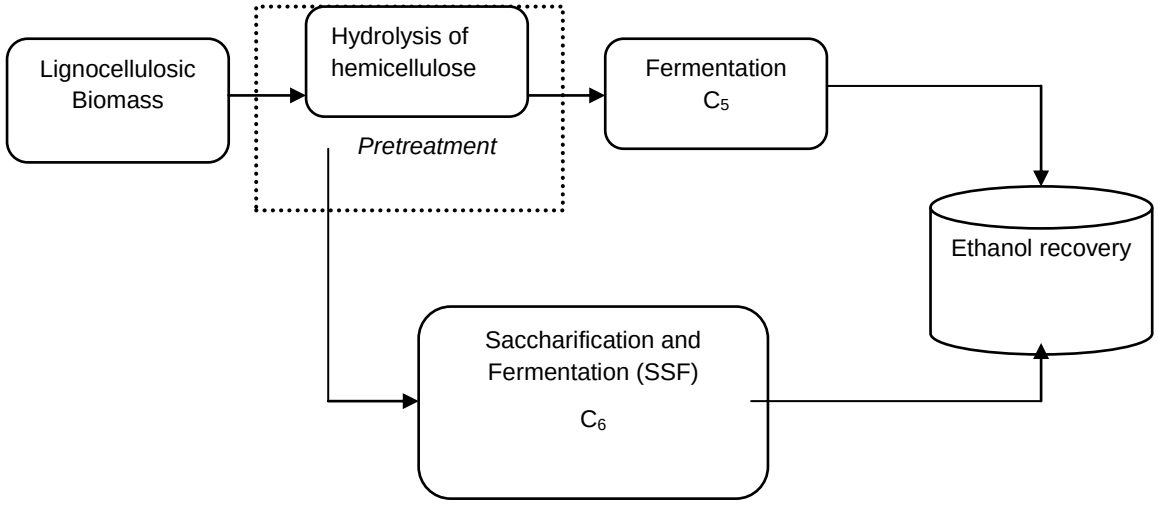

Fig 1: SSF schematic diagram [4].

This work studies palm bagasse potential to produce ethanol through a SSF process using pretreated lignocellulosic material and commercial enzyme and yeast.

MATERIALS AND METHODS

Feedstock

Raw palm bagasse was provided by Agropalma Company, located in Belém/ PA, Brazil.

Before serving for scientific purposes, this feedstock undergoes a series of steps. Once oil palm fresh fruit bunches (FFB) are harvested in the field, they are transferred to a sterilizer, where they are cooked under pressure of 2 kg/cm^2 to 3 kg/cm^2 for approximately 60 minutes at 135 ºC [7]. After cooking, FFB are tilted through thresher and fruits and bunches are separated (empty fruit bunches, EFB) with the former being transferred, through conveyor, to a digester. The carrier transports empty clusters to the storage area, while the mixed fruit is sent to a screw press where crude oil is separated from the pie, which corresponds to palm pressed fiber (PPF). PPF, processed in the carrier, is, then, dried. This is the material used in this work.

Alkaline pretreatment

For further saccharification steps, palm bagasse previously acid pretreated was washed until pH reached 5.5. Later, it was dried at 50ºC for 24 hours and, finally, submitted to alkaline pretreatment in order to remove lignin, resulting in partially delignified cellulignin (PDCL). This pretreatment was carried out at 1 atm and 121 ºC with a solid-liquid ratio of 1:20 (g of cellulignin/ mL of alkali) for 30 minutes. Three different concentrations of NaOH (% w/v) were investigated and are exhibited in Table 1:

Table 1. NaOH concentrations for alkaline pretreatments

Assay	NaOH concentration (% w/v)
A	1%
B	2%
C	4%

Microorganism, enzyme and fermentation media

Enzymatic hydrolysis was performed using commercial enzyme Cellic®CTec2 (Novozymes) in a citrate buffer solution (pH = 5.0), while fermentation assays were carried out with commercial yeast strain *Saccharomyces cerevisiae* (Fleischmann), used in the baking industry.

Fermentation media consisted of 10.0 g/L of yeast extract , 1.25 g/L of urea, 1.1 g/L of KH_2PO_4, 40.0 mL/L of mineral salt solution and partially delignified cellulignin (PDCL), obtained from acid and alkaline pretreatment, as substrate [8].

Morphological analysis of palm bagasse

The micrographs of the samples were obtained through scanning electron microscope (SEM) Hitachi TM-1000 model. Procedure for preparing material for analysis consisted in depositing a portion of the solid on a tape fixed carbon in the sample port. Scale of micrographs ranged from 1 mm to 300 µm.

Enzymatic hydrolysis and enzyme activity

Enzymatic hydrolysis for saccharification was carried out for palm bagasse previously submitted to both acid (solid : liquid ratio equivalent to 1:3.25, H_2SO_4 0.85 %v/v) and alkaline pretreatment (NaOH 1% (A), NaOH 2% (B) and NaOH 4% (C)).

Assays were performed in triplicate, and glucose concentration (g/L) was chosen as response variable for the process.

Enzyme activity of commercial enzyme Cellic®CTec2 was determined as proposed by [9].

Simultaneous saccharification and fermentation (SSF)

Before starting SSF process for ethanol production, an enzymatic pre-hydrolysis step was carried out at 47ºC – 50ºC at 200 rpm in a rotary shaker (New Brunswick™ Innova® 44) for 15 hours with sodium citrate buffer solution (pH = 5,0), an enzyme load of 26 FPU/ g of PDCL and a solid: liquid ratio equivalent to 1: 5 (g of PDCL : mL of citrate buffer) [10].

Once SSF started, process temperature shifted to 37 °C, which is the highest temperature that allows development of the yeast. Initial cell concentration was about 8 g/L and the process lasted for 48 hours at 200 rpm in 500 ml (nominal volume) conical shake flasks containing 150 mL of medium. These flasks were closed to avoid air entrance, enabling only CO_2 exit through a slightly acidified solution with hydrochloric acid.

Along the process, at adequate time intervals, ethanol concentration was estimated by monitoring weight loss of each flask, due to CO_2 release (ethanol equivalent, EE). Fermentation efficiency (FE) was calculated relative to stoichiometry of the reaction of alcoholic fermentation:

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2$$

Samples were collected at the beginning and at the end of the SSF process and centrifuged at 10000 rpm for 10 minutes. Also, all the assays were held in triplicate.

Analytical methods

Supernatants from samples collected at the beginning and at the end of SSF process were used to evaluate ART concentration through DNS method [11]. Glucose was quantified by glucose-oxidase method (GOD) using analytical kit from Interteck Katal (Belo Horizonte, MG, Brazil).

Also, sugars such as glucose, xylose and arabinose, besides ethanol, in order to confirm previous GOD, DNS and EE results, were analyzed individually by high-performance liquid chromatography (HPLC) using a Shimadzu cromatograph coupled to a Hi-Plex H column at 60 °C with H_2SO_4 (5 mM) as mobile phase at 0.6 mL/min flow rate.

RESULTS AND DISCUSSION

Morphological analysis of palm bagasse

Table 2 shows *in natura* palm bagasse composition, according to the methodology described:

Table 2. In natura palm bagasse composition

Components	(%) w/w
Cellulose	42.28
Hemicellulose	24.34
Lignin	28.99
Ash	2.25
Others	2.14

It can be observed that cellulose is the most abundant component of the material (42,28% w/w), followed by lignin (28.99 % w/w) and hemicellulose (24,34% w/w). These results corroborate previous studies reported in the literature [13]. High content of both lignin and hemicellulose indicate that material must be pretreated prior to saccharification and fermentation in order to achive efficient hydrolysis and ethanol conversion.

In order to visualize and better understand possible physical changes in lignocellulosic fibers after each pretreatment to which palm bagasse was submitted, scanning electron microscopies (SEM) were performed and its results are shown in Figure 2:

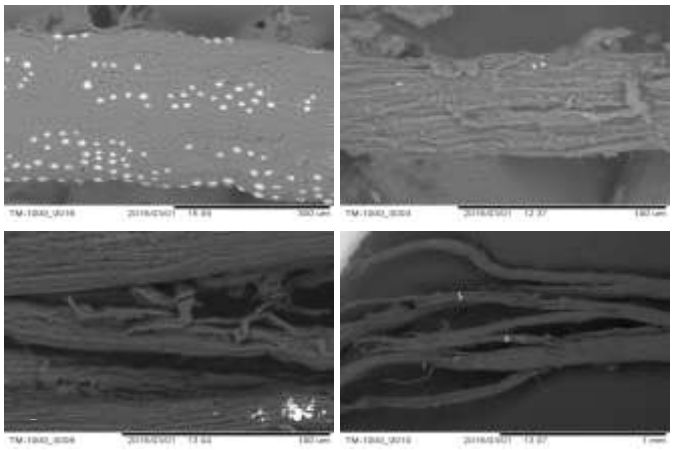

Fig 2: Scanning electron microscopy (SEM) of palm bagasse. (a) *in natura* palm bagasse, (b) material after acid pretreatment (cellulignin), (c) and (d) material after acid and alkaline pretreatments (partially delignified cellulignin)

It can be seen, from micrograph of Figure 2a, that *in natura* palm bagasse fibers are compact and homogeneous, while cellulignin fibers, presented on the micrograph of Figure 2b, seem more disorganized.

Micrographs of Figures 2c and 2d illustrate, respectively, disruption of fibers after acid pretreatment only and both acid and alkaline pretreatment. Material submitted to the latter exhibits greater disruption, since, besides removing hemicellulosic fraction (acid pretreatment), alkaline pretreatment partially removes lignin, resulting in more exposed cellulose fibers, which are more accessible to subsequent enzymatic action.

Enzymatic hydrolysis

From the micrographs, it is clear the importance of both acid and alkaline pretreatment for further enzymatic access to cellulose. Hence, previously acid and alkaline pretreated palm bagasse (A, B and C, as described in Materials and Methods) were submitted to enzymatic hydrolysis, using Cellic®CTec2 (FPase activity = 281.61 ±1.79 FPU/ml, Filter Paper Units per millimeter of enzyme cocktail) and the results concerning released glucose are exhibited in Figure 3.

Glucose levels for enzymatic hydrolysis of material previously pretreated (PDCL) with the lowest NaOH concentration - 1% (A) - are the lowest, reaching, at its highest, after 15 hours, 19 g/L. However, for those whose previous alkaline pretreatment used higher alkali concentrations - 2% (B) and 4% (C) -, approximately 45.9 g/L and 46.5 g/L of glucose were released, respectively, after, 15 hours, confirming that these pretreatments, as suggested by the micrographs, enhance enzyme access to cellulose, indeed, being, therefore, much more efficient. These results corroborate those reported by Menezes et al. (2002) and Sewalt et al. (1997) [14,15].

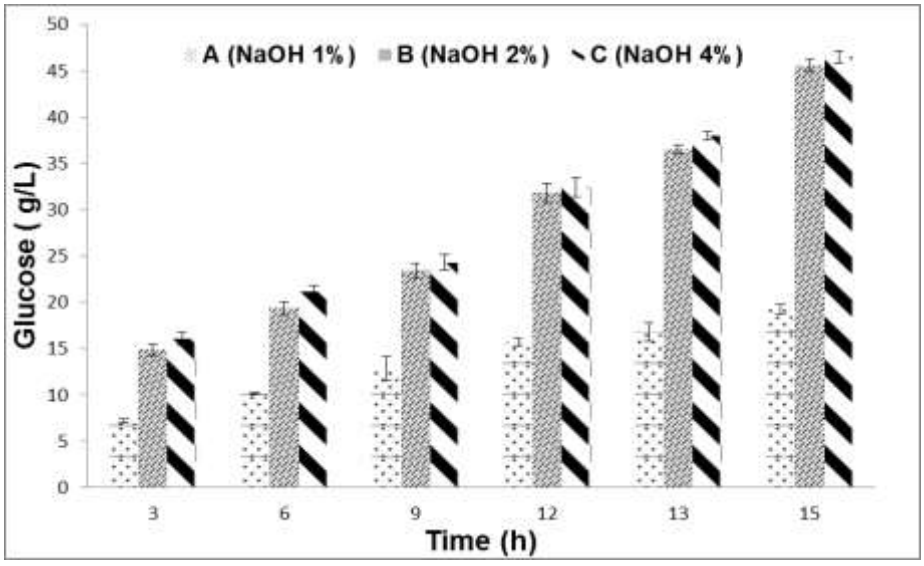

Fig 3: Results for enzymatic hydrolysis using as substrate partially delignified celluligin (PDCL) subjected to three different alkaline pretreatments: A (NaOH 1%), B (NaOH 2%) and C (NaOH 4%). Conditions: 200 rpm, 47ºC - 50ºC, pH=5.0 (sodium citrate buffer) and solid: liquid ratio=1:5 (g of PDCL : mL of citrate buffer).

Since results for released glucose are similar for material submitted to NaOH 2% (B) and NaOH 4% (C), seeking a cost-efficient process, NaOH 2% (B) seems to be the best alternative. In order to better evaluate these two conditions, fermentation studies were carried out.

Simultaneous saccharification and fermentation (SSF)

SSF process for ethanol production, comprising a pre-hydrolysis step for initial conversion of cellulose to glucose, prior to Saccharomyces cerevisiae inoculation, as described in Materials and Methods, was performed and lasted for 48 hours (Figure 4)

PDCL previously pretreated with NaOH 2% (B), once subjected to SSF, produced 22.4 g/L of ethanol, with $Y_{P/S}$ factor (ethanol yield) equivalent to 0.50 g/g, corresponding to a fermentation efficiency (FE) of 98.5 % and a volumetric productivity (QP) of 0.47 g/L.h, denoting that high conversions of cellulose to glucose and, further, to ethanol, were achieved. Meanwhile, PDCL pretreated with NaOH 4% (C) produced 18.21 g/L of ethanol, with $Y_{P/S}$=0.50 g/g, FE = 86.4 % and QP=0.39 g/L.h. Hence, higher concentrations of NaOH, besides elevating costs, seem unnecessary.

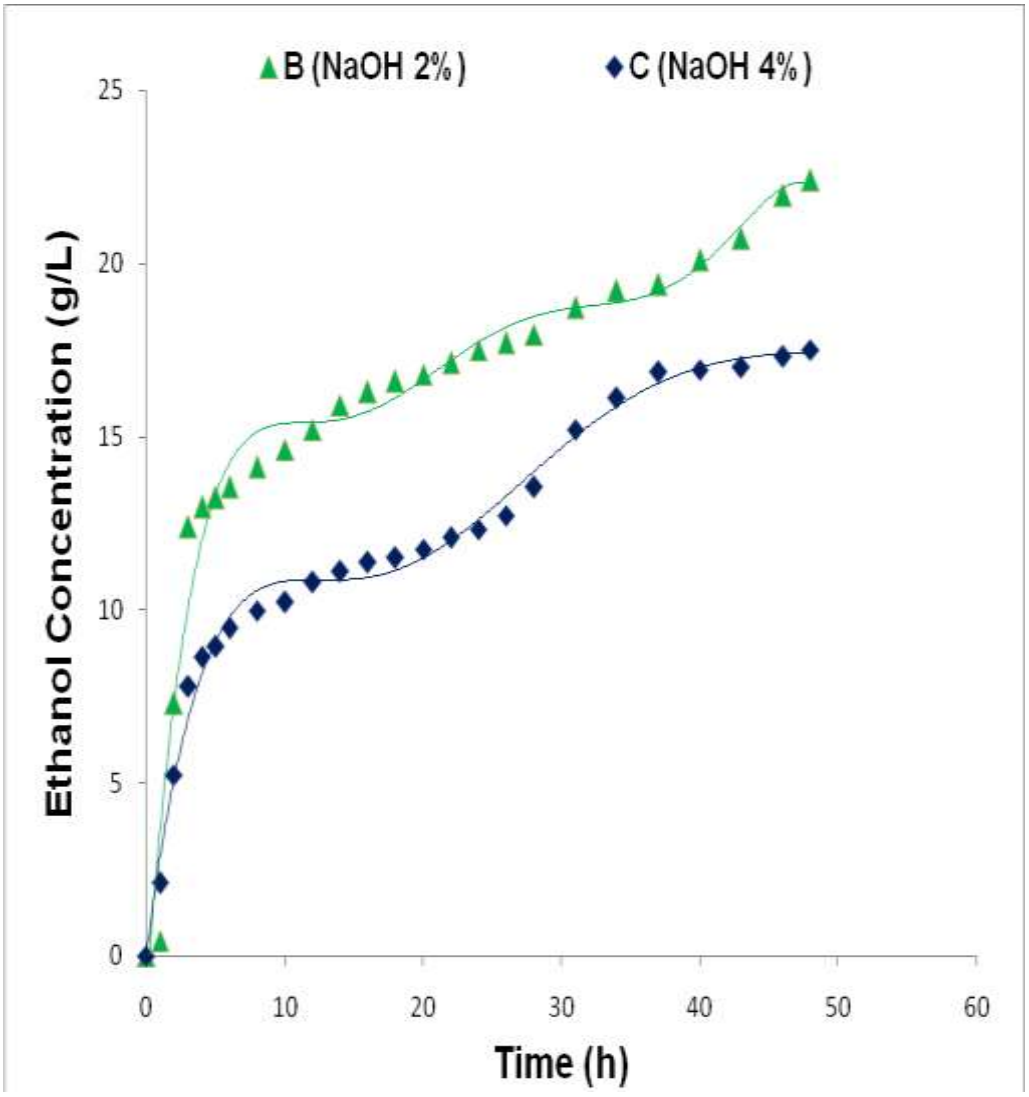

Fig 4: Ethanol production through SSF process for PDCL subjected previously to two different alkaline pretreatments: B (NaOH 2%) and C (NaOH, 4%). Conditions: 200 rpm, 37ºC and solid: liquid ratio=1:5 (g of PDCL : mL of citrate buffer).

Also, assays regarding SSF of *in natura* palm bagasse, alkali pretreated only (without acid) and PDCL (pretreated with both acid and NaOH 2%) were held (Figure 5). This test was performed due to the fact that the feedstock, as mentioned before, is subjected to a baking thermal treatment, which may weaken the rigid structure of the lignocellulosic material, therefore, increasing enzymatic access to cellulose and subsequent conversion to glucose. The results, however, show that this treatment alone is not enough, since ethanol production for *in natura* bagasse was lower than for material alkali pretreated only and for PDCL.

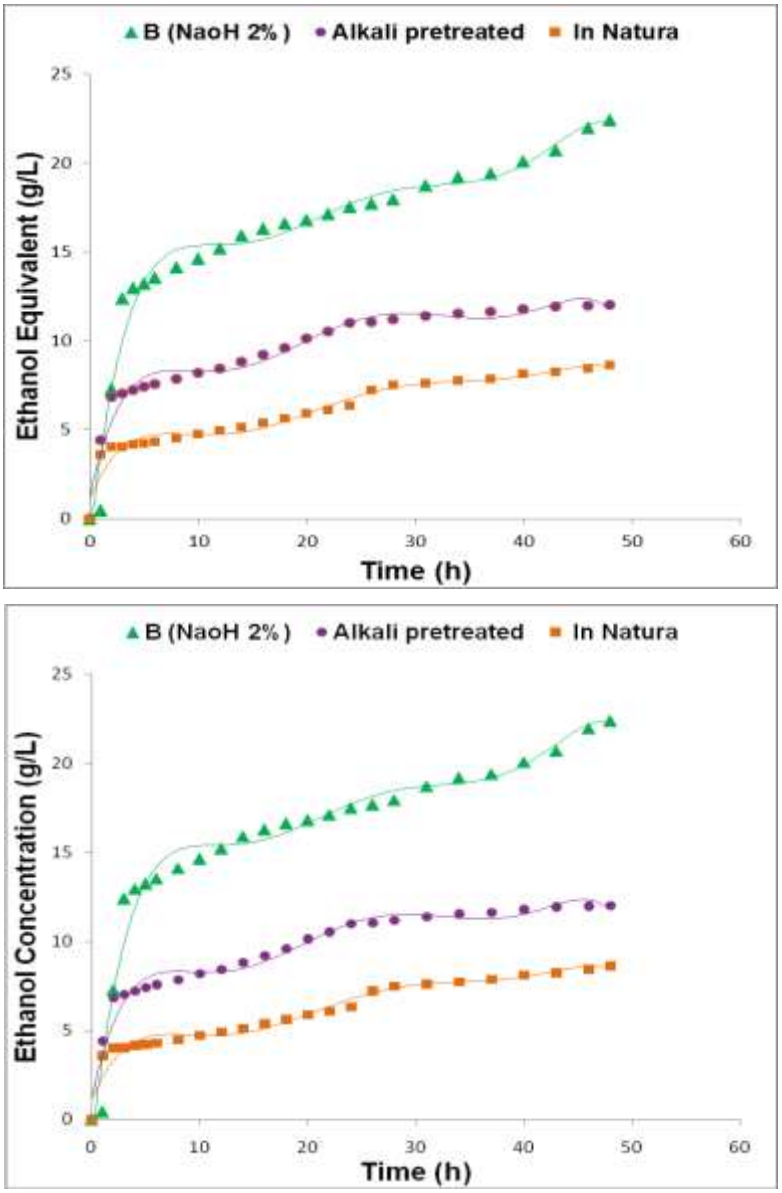

Fig 5: Ethanol production through SSF process for *in natura* palm bagasse, alkali pretreated only palm bagasse and both acid and alkali (NaOH 2%, B) pretreated palm bagasse. Conditions: 200 rpm, 37ºC and solid: liquid ratio=1:5 (g of PDCL : mL of citrate buffer).

Parameters presented in Table 3 confirm the relevance of alkaline pretreatment to promote higher enzymatic digestibility of lignocellulosic complex. Ethanol volumetric productivity for PDCL (B) is 5 and 2.5 times higher than those for *in natura* and alkali pretreated palm bagasse, respectively. Hence, although palm fibers are previously cooked, this process does not promote a disruption or disorganization of these fibers that allows for an efficient enzymatic access to substrate for further fermentation.

Table 3. Parameters for SSF process for acid and alkali pretreated (B), alkali pretreated and *in natura* palm bagasse

Parameter	B (PDCL)	Alkali pretreated	In Natura
$Y_{P/S}$ (g/g)	0.50	0.50	0.42
FE (%)	98.5	97.8	82.3
Q_P (g/L.h)	0.47	0.19	0.086

Where: $Y_{P/S}$ = ethanol yield factor, FE=Fermentation efficiency and Q_P= Volumetric productivity

CONCLUSIONS

This work shows that palm lignocellulosic biomass presents potential as feedstock for bioproduction of ethanol. The relevance of performing both acid and alkali pretreatment on palm oil bagasse, in order to partially delignify the material and, hence, enhance enzymatic access to cellulose, was demonstrated. High values of ethanol concentration and fermentation efficiency during SSF process (22.4 g/L and 98.5%, respectively) of material previously pretreated with acid and NaOH 2% (w/v) were achieved. Results for material pretreated with NaOH 4% indicate that is unnecessary to perform pretreatment under more severe conditions. These encouraging results denote that it is possible for palm bagasse to play an important role within the context of biofuels and Biorefinery.

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