Chemical Engineering Journal 230 (2013) 158–165

Contents lists available at SciVerse ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

# Pretreatment strategies to improve anaerobic biodegradability and methane production potential of the palm oil mesocarp fibre

Adriana Guimarães Costa [a,b], Gleycielle Cavalcante Pinheiro [b], Francisca Gleyciara Cavalcante Pinheiro [c], André Bezerra Dos Santos [a], Sandra Tédde Santaella [d], Renato Carrhá Leitão [e,*]

[a] Department of Hydraulic and Environmental Engineering, Federal University of Ceará, Brazil
[b] Department of Chemistry and Environment, Federal Institute of Education, Science and Technology of Ceará, Brazil
[c] Department of Organic and Inorganic Chemistry, Federal University of Ceará, Brazil
[d] Sea Science Institute, Federal University of Ceará, Brazil
[e] Brazilian Agricultural Research Corporation, Tropical Agroindustry National Centre, Brazil

## HIGHLIGHTS

- Effect of physicochemical pretreatments on the anaerobic biodegradability of the oil palm mesocarp fibre.
- Delignification and sugar solubilisation of the lignocellulosic biomass.
- Methane production from the oil palm mesocarp fibre.

## ARTICLE INFO

*Article history:*
Received 28 March 2013
Received in revised form 27 May 2013
Accepted 16 June 2013
Available online 27 June 2013

*Keywords:*
Anaerobic biodegradability
Hydrolysis
Lignocellulosic waste
Pretreatment

## ABSTRACT

The use of lignocellulosic waste as a power source typically requires pretreatment of the material, either for sugars solubilisation or lignin removal. We evaluated the acid, alkaline and hydrothermal pretreatments on palm oil mesocarp fibre in order to increase the anaerobic biodegradability and methane production potential (MPP). The results show that the best MPP (199 L $CH_4$/kg substrate) was achieved by using acid pretreatment with a reaction time of 34 min, temperature of 103 °C and [HCl] of 1.97 M. However, the energy generated from methane is lower than merely burning the bagasse, and probably the lignin with high added-value has to be recovered for improving sustainability and profitability. In this case, the alkaline pretreatment with a reaction time of 47 min, temperature of 183 °C and [NaOH] of 1.8 M extracted 91% of the lignin present in the fibre, and the hydrolysate could generate 180 L $CH_4$/kg substrate.



## 1. Introduction

Over the last years, renewable energies are increasing their importance in the energy matrix of several countries. Biofuels, such as ethanol, biodiesel and biogas can reduce the use of fossil fuels and thereby minimize environmental impacts. However, despite the environmental and economic advantages, the waste from biofuel production chains requires proper disposal.

Among the oilseeds species that are used for biodiesel production, the palm oil is the most productive. The palm fruit comprises an outer fleshy pulp (mesocarp), which covers a nut with an inner seed. The palm fruits provides the following products and by-products: crude palm oil (20%), palm kernel oil (1.5%), palm kernel cake (3.5%), stem (22%), mesocarp fibres (12%), shells (5%), and a large quantity of liquid waste called Palm Oil Mill Effluent (POME) [1]. The palm oil plantation area in world was estimated to be approximately $12 \times 10^6$ ha in 2012 [2], with a yield of approximately 28 t/ha/year of bunches, from which approximately $40 \times 10^6$ t/year of mesocarp fibre are generated.

At first, the organic wastes from biofuel production chain are anaerobically biodegradable and can be used for energy production after its conversion to biogas. However, most of this by-product is lignocellulosic material, and the anaerobic digestion is limited by hydrolysis due to the physical barrier provided by lignin and hemicellulose, and the crystalline portion of cellulose [3]. Pretreatments are generally necessary to accelerate the hydrolysis and the material biodegradability [4].

Several pretreatments can be used to increase the biodegradability of lignocellulosic materials: (i) physical pretreatment such as milling which promotes a reduction of the cellulose crystallinity by breaking the intermolecular hydrogen bridges [5], and improves

* Corresponding author. Address: Brazilian Agricultural Research Corporation, Tropical Agroindustry National Centre, Rua Dra. Sara Mesquita, 2270, 60511-110 Fortaleza, CE, Brazil. Tel.: +55 (85) 3391 7343.
*E-mail address:* renato.leitao@embrapa.br (R.C. Leitão).

1385-8947/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.cej.2013.06.070

the accessibility by changing the size and shape of the particles, which promote a decrease in mass transfer resistance [6]; (ii) hydrothermal pretreatment that uses only water at high temperature and pressure to promote the release of acetic acid, which acts as a catalyst for hemicellulose hydrolysis, and consequently improves cellulose accessibility [7]; (iii) acid pretreatment, with concentrated or diluted acid, to provide the solubilisation of fermentable sugars such as glucose from the cellulose, as well as xylose, mannose, galactose, glucose and arabinose from hemicellulose [4]; (iv) alkaline pretreatment that causes disruption in the ester bonds between lignin and hemicellulose, resulting in a swelling of the material, with consequent increase of the accessible surface for the exoenzymes [8].

Each pretreatment must be optimised based on reaction time, temperature, pressure, mass/volume ratio and catalyst concentration, in order to maximise sugar production or lignin removal, and minimize toxic or recalcitrant compounds [9,10]. Depending on the pretreatment severity, sugars can be degraded into furfural and 5-hydroxymethylfurfural (HMF), and these may be further degraded to levulinic acid and formic acid [9,11].

This study aims to evaluate several pretreatments applied to palm oil mesocarp fibre in order to maximise the anaerobic biodegradability and hence the methane potential production (MPP). Prior to the anaerobic digestion assays, the fibre was subjected to hydrothermal pretreatment, acid hydrolysis with dilute hydrochloric acid, or alkaline hydrolysis with sodium hydroxide solution. Each pretreatment was optimised based on temperature, reaction time and catalyst concentration by applying the multivariate factorial design and response surface methodology.

## 2. Materials and methods

### 2.1. The palm oil mesocarp fibre

The palm oil mesocarp fibre (fruit fibre) was obtained in a biodiesel plant, after cooking and pressing the fruit for extracting the palm oil. The fibre was then milled in a knife mill, sieved to a size of 18 mesh (1 mm), homogenised and stored at room temperature. Prior to the acid, alkaline and hydrothermal pretreatments, the fibre was characterised in terms of cellulose, hemicellulose and lignin content (21.41% cellulose, 21.77% hemicellulose, 30.33% lignin, 7.38% moisture, 8.63% ashes and 9.99% of other extractives). After hydrolysis, the liquid fraction was characterised in terms of sugars (TRG), total and dissolved Chemical Oxygen Demand (COD), acetic acid, furfural and HMF concentrations (for the hydrothermal and acid pretreatments) and lignin concentration (for the alkaline pretreatment). The solid fraction of the hydrolysate of the best results from the different pretreatments was characterised in terms of total solids, total COD, and lignin content (for the alkaline hydrolysis).

Total solids, moisture and Chemical Oxygen Demand (COD) were determined according to the Standard Methods for Examination of Water and Wastewater [12]. Ash, extractives, holocellulose (cellulose and hemicellulose) and lignin were determined according to a modified method based on the standards TAPPI T211 om-02, T412 om-02, T204 cm-97, T222 om-02, T203 cm-09 [13,14]. The soluble lignin content evaluated after alkaline hydrolysis was determined using the spectrophotometric method [15]. The method TAPPI 222 om-02 was used for more accurate determination of lignin concentration, necessary to calculate the lignin removal efficiency of the best result of the alkaline pretreatment.

The total sugars, in terms of total reducing groups (TRG) were determined using the method of DNS (3.5-dinitrosalicylic acid) [16]. Furfural and HMF concentrations were determined by High Performance Liquid Chromatography (HPLC) under the following conditions: Column Agilent Zorbax SB C-18 kept at 25 °C; visible ultraviolet detector at 276 nm, acetonitrile/water (2:8) with 1% acetic acid as eluent at flow rate of 0.7 mL/min. The injected sample volume was 20 μL. Samples were pre-filtered on cellulose acetate membrane ME25 with a porosity of 0.45 μm and diameter of 13 mm.

### 2.2. Hydrolysis assays

The hydrolysis assays were conducted in 500 mL high pressure reactors (Berghof model BR-300). The hydrothermal pretreatment was evaluated based on sugars solubilisation ($P_{TRG}$) according to different sets of temperature ($T$ between 180 and 200 °C), reaction time ($t$ between 5 and 15 min), and fibre mass to catalyst solution volume ratio ($m/v$ between 5% and 15%). The acid hydrolysis with diluted HCl was also evaluated based on the $P_{TRG}$, and the independent variables were temperature ($T$) between 103 and 137 °C, acid concentration ([HCl]) between 0.63 and 1.97 M and reaction time ($t$) between 6.4 and 73.6 min. Alkaline hydrolysis with NaOH was evaluated based on lignin ($P_{Lig}$), with different sets of temperature ($T$ from 116 to 184 °C), alkali concentration ([NaOH] from 0.80 to 1.80 M) and reaction time ($t$ from 13 to 47 min).

All assays were performed using $2^3$ multivariate experimental design (two levels and three independent variables), with the central point in triplicate (level 0) and six star-points (when necessary) as shown in Table 1. Concentrations of acid or base, reaction time, reaction temperature and, in the case of hydrothermal pretreatment, fibre mass to catalyst solution volume ratio were the independent variables. Specific TRG production ($P_{TRG}$) and specific lignin production ($P_{Lig}$) were the dependent variables. Statgraphics® Centurion XV (StatPoint, USA) was used for statistical analysis and response surface modelling.

### 2.3. Hydrolysis calculation

Hydrolysis efficiency calculation in terms of TRG production was performed based on the percentage of cellulose and hemicellulose contained in the crude fibre (before hydrolysis), considering that all holocellulose content will be converted into TRG. The Eqs. (1) and (2) were used to calculate the mass of TRG in the hydrolysate and maximum achievable TRG, assuming that all holocellulose was hydrolysed [17].

$$M_{TRG} = [TRG] \times V_{Hid} \quad (1)$$

$$M_{TRG\_Max} = \left(\frac{\%Cell}{FCC \times FPC \times 100} + \frac{\%Hem}{FCH \times FPH \times 100}\right) \times m_{dry} \quad (2)$$

where $M_{TRG}$ is the mass of TRG in the hydrolysate (g); [TRG] is the concentration of TRG in the hydrolysate (g/L); $V_{Hid}$ is the

**Table 1**
Factors and levels of the several pre-treatments of the palm oil mesocarp fibre.

| Factors | Levels | | | | |
|---|---|---|---|---|---|
| | −1.682 | −1 | 0 | +1 | +1.682 |
| *Hydrothermal hydrolysis* | | | | | |
| Reaction time (min) | | 5 | 10 | 15 | |
| Temperature (°C) | | 150 | 175 | 200 | |
| $m/v$ ratio (%) | | 5 | 10 | 15 | |
| *Acid hydrolysis* | | | | | |
| [HCl] (M) | 0.63 | 0.9 | 1.3 | 1.7 | 1.97 |
| Reaction time (min) | 6.4 | 20 | 40 | 60 | 73.6 |
| Temperature (°C) | 103.2 | 110 | 120 | 130 | 136.8 |
| *Alkaline hydrolysis* | | | | | |
| [NaOH] (M) | 0.8 | 1.0 | 1.3 | 1.6 | 1.8 |
| Reaction time (min) | 13 | 20 | 30 | 40 | 47 |
| Temperature (°C) | 116 | 130 | 150 | 170 | 184 |

hydrolysate volume after the experiment (L); $M_{TRG\_Max}$ is the total mass of sugars in the crude fibre based on TRG (g); %Cell is the cellulose percentage in the crude fibre; %Hem is the hemicellulose percentage in the crude fibre; FCC is the conversion factor for cellulose (0.9); FPC is the factor for estimating cellulose loss during hydrolysis (1.055); FCH is the conversion factor for hemicellulose (0.88); FPH FPC is the factor for estimating hemicellulose loss during hydrolysis (1.155); $m_{dry}$ is the crude fibre mass used in the hydrolysis assays, based on dry matter (g).

Hydrolysis efficiency in terms of TRG was calculated using Eq. (3). The specific TRG production, based on dry matter, was calculated according to Eq. (4).

$$\eta_{TRG} = \frac{M_{TRG}}{M_{TRG\_Max}} \quad (3)$$

$$P_{TRG} = \frac{M_{TRG}}{m_{dry}} \quad (4)$$

where $\eta_{TRG}$ is the holocellulose conversion efficiency (cellulose + hemicelulose) fraction of the crude fibre into TRG (%); $P_{TRG}$ is the specific TRG production, based on dry matter (g TRG/g dry fibre).

The calculation of the hydrolysis efficiency in terms of lignin extraction was performed based on the lignin percentage contained in the crude fibre (before hydrolysis), which was determined according to the modified method based on the standards TAPPI [13,14], as previously mentioned. Eqs. (5) and (6) were used to calculate the mass of lignin in the hydrolysate and maximum achievable lignin assuming a complete solubilisation.

$$M_{Lig\_Hid} = [Lig] \times V_{Hid} \quad (5)$$

$$M_{Lig\_Sample} = \%Lig \times m_{dry} \quad (6)$$

where $M_{Lig\_Hid}$ is the mass of lignin in the hydrolysate (g); [Lig] is the lignin concentration in the hydrolysate (g/L), which was determined by the spectrophotometric method described previously; $V_{Hid}$ is the hydrolysate volume at the end of the experiment (L); $M_{Lig\_Sample}$ is the total mass of lignin in the crude fibre (g); %Lig is the percentage of lignin contained in the crude fibre (before hydrolysis), $m_{dry}$ is the crude fibre mass used in the hydrolysis assays, based on dry matter (g).

When the spectrophotometric method was used for lignin determination, the solubilisation efficiency was calculated through Eq. (7). The specific lignin production was calculated using Eq. (8).

$$\eta_{Lig} = \frac{M_{Lig\_Hid}}{M_{Lig\_Sample}} \quad (7)$$

$$P_{Lig} = \frac{M_{Lig\_Hid}}{m_{dry}} \quad (8)$$

where $\eta_{Lig}$ is the lignin solubilisation efficiency (%); $P_{Lig}$ is the specific lignin production, based on dry matter (g Lig/g dry fibre).

The lignin removal efficiency of the best result of the alkali pretreatment was calculated using Eqs. (9) and (10).

$$\%Lig_{Hid\_CF} = \frac{\%Lig_{Hid} \times \%Hol_{CF}}{(1 - \%Lig_{Hid})} \quad (9)$$

$$\eta_{Lig} = \frac{\%Lig_{CF} - \%Lig_{Hid\_CF}}{\%Lig_{CF}} \quad (10)$$

where $\%Lig_{Hid\_CF}$ is the lignin percentage in the hydrolysate based on the lignin mass in the crude fibre (%); $\%Lig_{Hid}$ is the lignin percentage in the hydrolysate based on the mass of hydrolysed fibre; $\%Hol_{CF}$ is the percentage of holocellulose in the crude fibre (%); $\%Lig_{CF}$ is the percentage of lignin in the crude fibre (%).

The hydrolysis yield ($\%y_{Hid}$) represents the percentage of the fibre that was recovered after hydrolysis. $\%y_{Hid}$ was calculated based on the ratio of the mass of the crude fibre and the mass of the hydrolysed fibre, both considering dry matter, according to the following equation:

$$\%y_{Hid} = \frac{m_{dry}}{m_{Hid\_dry}} \quad (11)$$

where $m_{Hid\_dry}$ is the hydrolysed fibre mass after hydrolysis assays, based on dry matter (g).

### 2.4. Anaerobic biodegradability and methane production potential assays

The hydrolysates from the best results of the pretreatments were evaluated in terms of biodegradability and methane production potential. After the acid and the hydrothermal pretreatments, the hydrolysates were neutralized with a solution of NaOH 2 M. The hydrolysates of the alkaline pretreatment were washed with 200 mL of NaOH at the same concentration used in the pretreatment, for removing the loose lignin, and then washed with distilled water until neutral pH. Only the solid fraction was used in the biodegradability assays.

The methodology for assessing the anaerobic biodegradability was based on specific methanogenic activity (SMA) assays [18]. The tests were carried out in 0.3 L serum bottles (0.2 L working volume), which were filled with inoculum (approximately 1.4 g of volatile solids (VS)/L), substrate (1.5 g COD/L), distilled water, and pH buffer (1.0 g/L $NaHCO_3$). Nutrients and trace elements were added to prevent deficiencies during the test: $NH_4Cl$ (0.28), $K_2HPO_4$ (0.25), $MgSO_4{\cdot}7H_2O$ (0.10), $CaCl_2{\cdot}2H_2O$ (0.01), and $CaCO_3$ (0.60); solution of trace elements (1 mL/L) containing the following substances (mg/L): $FeCl_2{\cdot}4H_2O$ (2000), $H_3BO_3$ (50), $ZnCl_2$ (50), $CuCl_2{\cdot}2H_2O$ (38), $MnCl_2{\cdot}4H_2O$ (500), $(NH_4)6Mo_7O_{24}{\cdot}4H_2O$ (50), $AlCl_3{\cdot}6H_2O$ (90), and $CoCl_2{\cdot}6H_2O$ (2000). The tests were performed over 30 days at 35 ± 1 °C, under shaking conditions of approximately 120 rpm.

Control flasks (without substrate) were used for monitoring the biogas produced by endogenous respiration. The inoculum consisted of a mixture in equal proportions of rumen liquid from goats and sludges withdrawn from three anaerobic sludge blanket reactors treating (i) wastewater, (ii) brewery effluent, (iii) glycerol with nutrients. The assays were performed in an anaerobic respirometer Micro-Oximax (Columbus Instruments, USA), where the biogas production was monitored automatically via pressure sensors, and methane analysed by an infrared sensor. All physical–chemical determinations followed the methods described in Standard Methods for the Examination of Water and Wastewater [12]. All experiments were performed in triplicate.

The maximum biodegradability (%), which is the maximum percentage of substrate COD that is converted to methane, was calculated according to Eq. (12). The MPP was calculated based on the cumulative methane production at the end of the biodegradability test and the mass of substrate used in the experiment, according to Eq. (13).

$$Bio(\%) = \frac{\left(COD^{30}_{CH_4-substrate} - COD^{30}_{CH_4-control}\right)}{DQO^{0}_{substrate}} * 100 \quad (12)$$

$$MPP = \frac{\left(V^{30}_{CH_4-substrate} - V^{30}_{CH_4-control}\right)}{M^{0}_{substrate}} \quad (13)$$

where Bio(%) is the sample biodegradability (%); $COD^{30}_{CH4-substrate}$, the total methane volume produced in the flask containing the substrate in terms of COD (g), considering 0.395 L $CH_4$/g COD at 35 °C and 1 atm; $COD^{30}_{CH4-control}$, the total volume of methane produced by the control flask, in terms of COD (g); $COD^{0}_{substrate}$, the initial quantity of substrate in terms of COD (g) added to each reactor; MPP, the methane production potential (L $CH_4$/kg substrate); $V^{30}_{CH4-substrate}$, the cumulative methane volume produced after 30 days in the flask containing the substrate (L $CH_4$); $V^{30}_{CH4-control}$, the methane volume produced by the control flask after 30 days (L $CH_4$); and $M^{0}_{substrate}$, the initial mass of substrate in the bottle (kg substrate).

The hydrolysis efficiency of the anaerobic digestion was calculated based on the production of methane and dissolved organic compounds. The last was determined in terms of dissolved COD. The hydrolysis efficiency was calculated as shown in Eq. (14) [19].

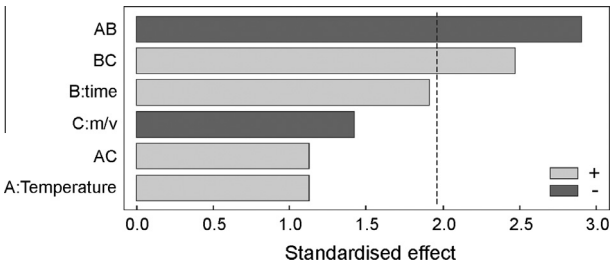


**Fig. 1.** Pareto diagram of the hydrothermal pretreatment, with the effects of $t$ and $T$ on $P_{TRG}$.

$$H(\%) = \frac{\left[\left(COD^{30}_{CH4-substrate} - COD^{30}_{CH4-control}\right) + \left(COD^{30}_{Diss-substrate} - COD^{30}_{Diss-control}\right) - COD^{0}_{Diss-substrate}\right]}{COD_{Total0}} * 100 \quad (14)$$

where $H(\%)$ is the anaerobic hydrolysis efficiency (%); $COD^{30}_{Diss-substrate}$ is the mass of dissolved organic compounds in the flask containing the substrate after 30 days in terms of COD (g); $COD^{30}_{Diss-control}$ is the mass of dissolved organic compounds in the control flask after 30 days in terms of COD (g); $COD^{30}_{Diss-control}$ $COD^{0}$Diss-substrate is the initial mass of substrate in terms of dissolved (g).

## 3. Results and discussion

### 3.1. Hydrothermal pretreatment

The results of hydrothermal hydrolysis were verified based on the specific TRG production ($P_{TRG}$). The values of dependent ($P_{TRG}$) and independent ($T$, $t$ and $m/v$) variables, and the results of [TRG], $\eta_{TRG}$, HMF and furfural are shown in Table 2.

The best results of $\eta_{TRG}$ (26.7%) and $P_{TRG}$ (0.125 gTRG/g substrate) were obtained with the shortest reaction time (5 min) and mass to volume ratio (5%), but with the highest temperature studied (200 °C). The coefficient of determination ($R^2$ = 0.33) obtained by regression analysis was very low, indicating that the response surface model is not suitable for determining the optimal point.

Fig. 1 shows the Pareto diagram, which represents the estimated effects according to their order of significance. Under the studied conditions, the independent variables ($t$, $T$ and $m/v$ ratio) had no significant effect ($p$ = 0.05), and the production of sugars is statistically similar in all experiments ($p$ > 0.05 in all cases). However, the interaction of the variables time and temperature presented linear and negative effect in $P_{TRG}$, which is an indication that an increase in the reaction time is related to a decrease in temperature or vice versa.

This is because at high pretreatment severity (high temperature and long reaction time), an increase of acetic acid concentration, which originates from the acetyl group, causes a catalytic degradation of the produced sugars, forming furfural and HMF, with consequent reduction of the $P_{TRG}$ [7]. The interaction effects on $P_{TRG}$ of the variables "$t$" and "$m/v$ ratio" was positive, indicating that an increase in reaction time should be accompanied with an increase in the fibre mass to catalyst solution volume ratio in order to maximise sugars solubilisation, since the larger the mass of substrate, the larger should be the reaction time for hydrolysis of lignocellulosic material.

Since the effects of the independent variables ($t$, $T$ and $m/v$) had no significant effect, the values of such parameters for hydrothermal pretreatment were estimated using the Pareto chart and the ranges used by other researchers [20–22]. Therefore, the conditions adopted to maximise solubilisation of sugars and subsequent anaerobic biodegradability improvement were: temperature of 200 °C, reaction time of 5 min and $m/v$ of 5%, following the hypothesis that the highest temperature is related to the lower reaction time (negative effect), and shorter reaction time is related to lower mass to volume (positive effect) ratio.

Under high temperature, it might occur sugars as well as the degradation to furfural and HMF, which are potential inhibitors to biological processes [7]. The methanogenic archaea, for instance *Methanococcus sp.*, was inhibited by furfural only in concentrations higher than 25 mM [23]. Hydrogen producing bacteria, for instance *Thermoanaerobacterium thermosaccharolyticum* W16, was not affected even when 0.5 g/L of furfural and 0.5 g/L of HMF were tested

**Table 2**
Results of the hydrothermal hydrolysis of the palm oil mesocarp fibre.

| Assay | $t$ (min) | $T$ (°C) | $m/v$ (%) | [TRG] (g/L) | $P_{TRG}$ (gTRG/g substrate) | $\eta_{TRG}$ (%) | HMF (g/L) | Furfural (g/L) |
|---|---|---|---|---|---|---|---|---|
| 1 | 5 | 180 | 5 | 3.95 | 0.058 | 12.6 | 0.01 | 0.01 |
| 2 | 15 | 180 | 5 | 7.65 | 0.121 | 25.9 | 0.26 | 0.38 |
| 3 | 5 | 200 | 5 | 8.30 | 0.125 | 26.8 | 0.13 | 0.12 |
| 4 | 15 | 200 | 5 | 3.69 | 0.054 | 11.4 | 0.02 | 0.04 |
| 5 | 5 | 180 | 15 | 10.99 | 0.069 | 14.7 | 0.11 | 0.11 |
| 6 | 15 | 180 | 15 | 11.67 | 0.074 | 15.8 | 0.11 | 0.13 |
| 7 | 5 | 200 | 15 | 9.83 | 0.059 | 12.6 | 0.38 | 0.82 |
| 8 | 15 | 200 | 15 | 20.87 | 0.116 | 24.8 | 0.40 | 0.78 |
| 9 | 10 | 190 | 10 | 12.69 | 0.098 | 21.0 | 0.15 | 0.17 |
| 10 | 10 | 190 | 10 | 9.38 | 0.087 | 18.6 | 0.12 | 0.26 |
| 11 | 10 | 190 | 10 | 11.85 | 0.093 | 19.9 | 0.15 | 0.20 |
| $OC_A$ | 5 | 200 | 5 | 8.30 | 0.125 | 26.8 | 0.13 | 0.12 |

$OC_A$ – Optimal condition (adopted).

[24]. According to Table 2, the concentrations of furfural and HMF produced in all the hydrolysis tests were below the inhibitory concentrations reported in literature.

### 3.2. Acid hydrolysis

The values of dependent ($P_{TRG}$) and independent ($T$, $t$ and [HCl]) variables, and the results of [TRG] $\eta_{TRG}$, HMF and furfural are shown in Table 3. Fig. 2 shows the Pareto chart, where all variables resulted in significant and negative effect on $P_{TRG}$. This is an indication that the smaller the values of the independent variables, the greater the production of sugars. However, all interactions also showed negative effect, which indicates that to maximise $P_{TRG}$, one variable should be reduced while increasing the other. Thus, under the studied conditions, increasing temperature causes negative effect on the final concentration of sugars when associated with an increase of [HCl] and vice versa.

In fact, increasing any variable leads to higher sugars degradation to furfural and HMF. However, none of the experiments resulted in concentrations of furfural and HMF that cause methanogens inhibition [23]. Bustos and co-authors [25] investigated the acid hydrolysis of sugarcane bagasse using HCl, and found that increasing temperature from 100 to 128 °C, and reaction time from 0 to 300 min, the furfural production increased from 0.56 to 12 g/L, which are in agreement with our findings. In other investigations, Herrera and co-authors [26,27] assessed the acid hydrolysis of sorghum straw with HCl at concentrations between 0.65 and 1.95 M, reaction times between 0 and 300 min and temperature of 100 °C and 122 °C. From these later works, it can be observed the same phenomenon, i.e. an increase in the temperature caused a negative effect on solubilisation of sugars, and promoted further degradation to furfural, which also corroborate with our results.

The coefficient of determination ($R^2 = 0.89$) obtained in the regression analysis was relatively high, and the optimal condition for the acid hydrolysis can be calculated based on the derivative of Eq. (15), which represents the statistical model of a surface response, with $P_{TRG}$ as a function of "$t$", "$T$" and "[HCl]". The result of this analysis was: reaction time of 34 min, temperature of 103 °C and [HCl] of 1.97 M. Under these conditions, the model result for $P_{TRG}$ was 0.293 g/g fibre. A further experiment using such conditions was carried out in order to compare the experimental and calculated results.

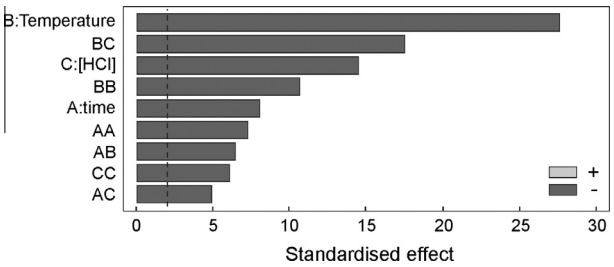


**Fig. 2.** Pareto diagram of the acid hydrolysis assays, with the effects of $t$, $T$ and [HCl] on $P_{TRG}$.

$$P_{TRG} = -3.1844 + 0.0098 \times t + 0.0468 \times T + 1067 \times [HCl] - 0.000027 \times t^2 - 0.000057 \times t \times T - 0.0011 \times t \times [HCl] - 0.00016 \times T^2 - 0.0077 \times T \times [HCl] - 0.056 \times [HCl]^2 \quad (15)$$

Fig. 3 shows the response surface plotted using Eq. (15), with $P_{TRG}$ as function of $T$ and [HCl], considering $t$ = 34 min. The experimental value found for $P_{TRG}$ was 0.263 g TRG/g fibre, as shown in Table 3, which is quite close to that predicted by the model at a confidence interval of 95%. The hydrolysis efficiency of the holocellulosic fraction was approximately 56.16%. The results of HMF and furfural concentrations obtained in all experiments of acid hydrolysis showed lower values than those reported in the literature that cause inhibition on methanogens [23,24].

### 3.3. Alkaline hydrolysis

The values of dependent ($P_{Lig}$) and independent ($T$, $t$ and [NaOH]) variables, and the results of [Lig] and $\eta_{Lig}$ are shown in Table 4. Fig. 4 shows the Pareto chart, where it is possible to observe that the variables "$t$" and "$T$" showed positive linear effect, as well as negative quadratic effect. This shows that the higher the temperature and the longer the reaction time, the higher the lignin extraction, but with a tendency to decrease from a certain value, generating a maximum $P_{Lig}$. The decrease on lignin content when applying high values of "$t$" and "$T$" (high severity factor) occurs due to its degradation to phenolic compounds such as: 4-hydroxybenzoic acid, originated in the rupture of ester bonds that links the

**Table 3**
Results of the acid hydrolysis of the palm oil mesocarp fibre.

| Assay | $t$ (min) | $T$ (°C) | [HCl] (M) | [TRG] (g/L) | $P_{TRG}$ (gTRG/g substrate) | $\eta_{TRG}$ (%) | HMF (g/L) | Furfural (g/L) |
|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 110 | 0.9 | 19.92 | 0.212 | 45.4 | 0.04 | 0.4 |
| 2 | 60 | 110 | 0.9 | 23.77 | 0.259 | 55.4 | 0.07 | 0.7 |
| 3 | 20 | 130 | 0.9 | 24.49 | 0.264 | 56.4 | 0.13 | 0.8 |
| 4 | 60 | 130 | 0.9 | 20.51 | 0.226 | 48.4 | 0.18 | 1.1 |
| 5 | 20 | 110 | 1.7 | 24.19 | 0.265 | 56.7 | 0.06 | 0.7 |
| 6 | 60 | 110 | 1.7 | 21.30 | 0.238 | 51.0 | 0.08 | 0.9 |
| 7 | 20 | 130 | 1.7 | 14.12 | 0.154 | 32.9 | 0.16 | 1.2 |
| 8 | 60 | 130 | 1.7 | 10.86 | 0.121 | 25.9 | 0.22 | 1.2 |
| 9 | 40 | 120 | 1.3 | 22.12 | 0.242 | 51.7 | 0.17 | 1.3 |
| 10 | 40 | 120 | 1.3 | 22.32 | 0.247 | 52.8 | 0.09 | 0.9 |
| 11 | 40 | 120 | 1.3 | 22.75 | 0.252 | 53.9 | 0.13 | 1.1 |
| 12 | 6,4 | 120 | 1.3 | 21.43 | 0.234 | 50.1 | 0.06 | 0.7 |
| 13 | 73,6 | 120 | 1.3 | 15.31 | 0.176 | 37.5 | 0.16 | 1.2 |
| 14 | 40 | 103 | 1.3 | 26.00 | 0.280 | 59.8 | 0.06 | 0.5 |
| 15 | 40 | 138 | 1.3 | 9.32 | 0.101 | 50.2 | 0.22 | 1.4 |
| 16 | 40 | 120 | 0.61 | 22.58 | 0.235 | 21.6 | 0.10 | 0.8 |
| 17 | 40 | 120 | 1.97 | 16.89 | 0.185 | 39.6 | 0.13 | 1.2 |
| $OC_M$ | 34 | 103 | 1.97 | | 0.293 | | | |
| $OC_E$ | 34 | 103 | 1.97 | 23.77 | 0.263 | 56.26 | 0.05 | 1.47 |

$OC_M$ – Optimal condition (model); $OC_E$ – Optimal condition (experimental).

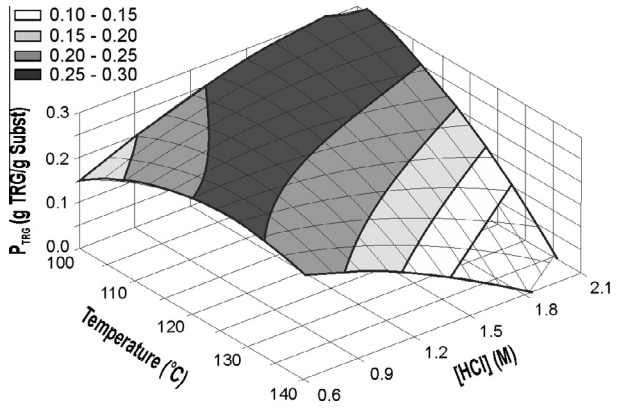


**Fig. 3.** Response surface plotted using Eq. (15), which describes the specific production of sugars as function of temperature and HCl concentration ($t$ = 34 min).

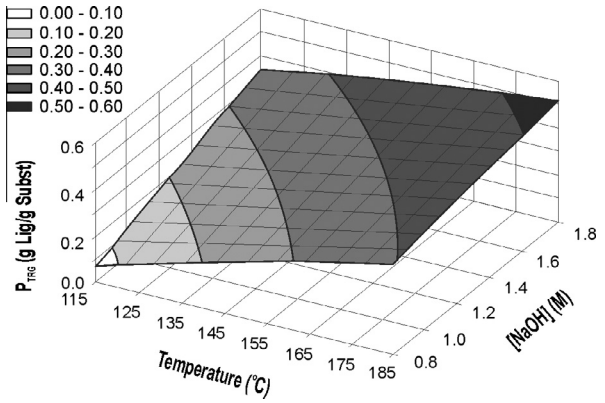


**Fig. 5.** Response surface plotted using Eq. (16), which describes the specific production of sugars as function of temperature and NaOH concentration ($t$ = 47 min).

**Table 4**
Results of the alkaline hydrolysis of the palm oil mesocarp fibre.

| Assay | t (min) | $T$ (°C) | [NaOH] (M) | [Lig] (g/L) | $P_{Lig}$ (g Lig/g substrate) |
|---|---|---|---|---|---|
| 1 | 20 | 130 | 1.00 | 23.24 | 0.27 |
| 2 | 20 | 170 | 1.00 | 30.24 | 0.33 |
| 3 | 40 | 130 | 1.00 | 21.91 | 0.24 |
| 4 | 40 | 170 | 1.00 | 32.75 | 0.37 |
| 5 | 20 | 130 | 1.60 | 25.72 | 0.29 |
| 6 | 20 | 170 | 1.60 | 27.10 | 0.30 |
| 7 | 40 | 130 | 1.60 | 31.70 | 0.36 |
| 8 | 40 | 170 | 1.60 | 38.57 | 0.43 |
| 9 | 30 | 150 | 1.30 | 30.45 | 0.34 |
| 10 | 30 | 150 | 1.30 | 31.79 | 0.35 |
| 11 | 30 | 150 | 1.30 | 30.87 | 0.34 |
| 12 | 30 | 116 | 1.30 | 22.03 | 0.24 |
| 13 | 30 | 184 | 1.30 | 38.53 | 0.43 |
| 14 | 13 | 150 | 1.30 | 25.92 | 0.29 |
| 15 | 47 | 150 | 1.30 | 31.20 | 0.35 |
| 16 | 30 | 150 | 0.80 | 28.02 | 0.31 |
| 17 | 30 | 150 | 1.80 | 34.55 | 0.38 |
| $OC_M$ | 47 | 183 | 1.80 | | 0.52 |
| $OC_E$ | 47 | 183 | 1.80 | 37.71 | 0.41 |

$OC_M$ – Optimal condition (model); $OC_E$ – Optimal condition (experimental).

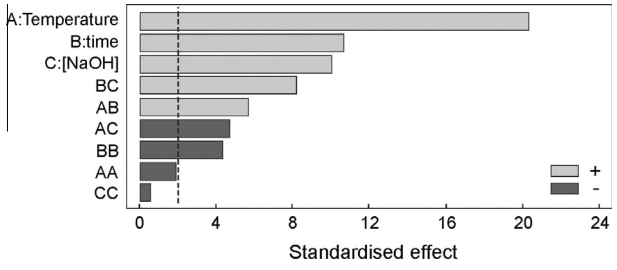


**Fig. 4.** Pareto diagram of the alkaline hydrolysis assays, with the effects of $t$, $T$ and [NaOH] on $P_{TRG}$.

hydroxyl groups of the cinnamic alcohol; and the syringaldehyde and syringic acid that are originated from the degradation of the siringilpropane units [28]. The variable [NaOH] caused a significant and positive linear effect on $P_{Lig}$ ($p$ = 0.05), thus the higher the concentration, the greater the removal of lignin.

The coefficient of determination ($R^2$) obtained in the regression analysis was 0.96, which shows that Eq. (16) can be used as a model of the surface response, with $P_{Lig}$ as a function of "$t$", "$T$" and "[NaOH]" (Fig. 5). The optimal condition for the alkaline hydrolysis was calculated based on the derivative of Eq. (16), and the result was: reaction time of 47 min, temperature of 184 °C and [NaOH] of 1.80 M. Under these conditions, the model result for $P_{Lig}$ was 0.52 g Lig/g fibre. The experimental value found for $P_{Lig}$ was 0.41 g Lig/g fibre, which is 90.9% of the total lignin in the fibre, according to Eq. (10). The difference between modelled and experimental results was probably due to the method for lignin determination. Another explanation is that the model was built based on a method which overestimates the extraction of lignin [14].

$$P_{Lig} = -0.273 + 0.0059 \times T - 0.0137 \times t + 0.2011 \times [NaOH] - 0.00001 \times T^2 + 0.00008 \times T \times t - 0.0022 \times T \times NaOH - 0.0001 \times t^2 + 0.0077 \times T \times [NaOH] - 0.0133 \times [NaOH]^2 \quad (16)$$

It is possible to get similar results to those found in this study but with lower temperatures, provided that some sort of pretreatment is applied to the fibre prior to the step of delignification [29]. They applied acid hydrolysis on the barley husks ($H_2SO_4$ at 0.52 M, temperature of 130 °C and reaction time of 15 min) before alkaline hydrolysis (NaOH at 2.5 M, temperature of 130 °C and reaction time of 34 min) and achieved 92% lignin extraction, according to Eq. (10).

### 3.4. Anaerobic biodegradability and methane production potential

The results of the biodegradability and MPP assays of the hydrolysates produced by the various pretreatments (hydrothermal, acid and alkaline) are presented in Table 5. The highest anaerobic biodegradability was achieved with alkaline pretreatment, which extracted approximately 91% of the lignin content of the fibre. This is because lignin acts as a mechanical barrier, being responsible for the integrity, structural rigidity, impermeability, and adhesion of cellulose and hemicellulose, increasing its resistance to microbial attack.

Therefore, the process of delignification improves the rate and the extent of enzymatic hydrolysis and consequently the hydrolysis step of the anaerobic digestion [4]. However, the alkaline pretreatment also hydrolyses part of the holocellulosic fraction. The sugars that were solubilised remain dissolved in the liquid phase of the hydrolysate and are lost during the process of fibre washing to remove the lignin, thus reducing the methane production potential.

The results show that the lignin is a hindrance to hydrolysis, since the anaerobic hydrolysis ($H$%) of the crude fibre reached only 7.8%. The alkaline pretreatment produced a fibre with lower lignin content and with increased accessible surface for the exoenzymes [8], which promoted the hydrolysis step of the anaerobic digestion

**Table 5**
Results of the anaerobic biodegradability and MPP assays.

| Pre-treatment | $\%y_{Hid}$ (%) | H (%) | Bio $(\%)_{Hid}$[a] | Bio $(\%)_{Total}$[b] | $MPP_{Hid}$[a] ($LCH_4$/kg Subst) | $MPP_{Total}$[b] ($LCH_4$/kg Subst) |
|---|---|---|---|---|---|---|
| Control[c] | 100.0 | 7.8 | 8.7 | 8.7 | 77.8 | 77.8 |
| $NaOH_{Fibre}$ | 25.5 | 22.8 | 25.3 | 6.4 | 180.0 | 45.8 |
| HCl | 84.0 | 4.4 | 22.4 | 18.8 | 236.9 | 198.9 |
| Hydrothermal | 95.9 | 1.8 | 21.5 | 20.7 | 180.5 | 173.2 |

[a] Anaerobic biodegradability and MPP based on dry fibre after hydrolysis.
[b] Anaerobic biodegradability and MPP based on dry fibre before hydrolysis.
[c] Dry milled fibre was used in the assays, without pre-treatment, as control.

(*H*% = 22.8%), the biodegradability (Bio(%) = 25.3%) and the MPP (180.0 L $CH_4$/kg substrate). However, only part of lignocellulosic material ($\%y_{Hid}$ of 25.5%) was used for anaerobic digestion, due to losses during the washing process. Considering all the material used for hydrolysis, the recalculation of biodegradability and MPP resulted in lower values for biodegradability (6.4%) and MPP (45.8 L $CH_4$/kg substrate).

The highest values for anaerobic biodegradability and MPP were obtained with hydrothermal and acid hydrolysis. The MPP of the palm oil mesocarp fibre can reach up to 198.9 L $CH_4$/kg substrate, which is comparable with the MPP of other by-products of the biofuel production chain. For example, glycerol derived from biodiesel production has MPP of 220 L $CH_4$/kg substrate [30]. Us and Perendeci [31] evaluated a mixture of different greenhouses waste (roots, stalks, leaves, tomato, pepper, cucumber, eggplant and courgette) after acid pretreatment, using diluted $H_2SO_4$ (0–5% $H_2SO_4$; 1–3 h; 60–100 °C). The results show that the MPP increased 18.5% (from 210 to 249 $LCH_4$/kg dry sample) when a mild pretreatment condition (0% $H_2SO_4$; 1 h; 78 °C) was applied. The best results was achieved using 0% of sulphuric acid probably because the addition of sulphur may have caused inhibition of the methanogenic consortia, or competition with the sulphate reducing bacteria. In this work, HCl was used as catalyser at a much higher severity, and the pretreatment of the palm oil mesocarp fibre increased 60% of the MPP.

Fernandes et al. [32] evaluated the effect of thermochemical pretreatment on the anaerobic biodegradability of three different lignocellulosic biomass (hay, straw and bracken). The authors found that the higher lignin content, the lower the biodegradability, e.g. bracken with 17.3% lignin presented 160 L CH4/kg fibre after pretreatment, whereas hay with 2.3% lignin presented 300 L CH4/kg fibre without any pretreatment. This is in agreement with the findings of this work, because when the lignin was extracted from the palm oil mesocarp fibre the MPP increased from 78 to 180 L CH4/kg fibre.

Considering that methane has a lower heating value (LHV) of 34,450 kJ/$m^3$ [33], the best power yield that can be obtained by anaerobic digestion is 6.9 MJ/kg dry fibre. On the other hand, the mesocarp fresh fibre of the palm oil, with moisture of approximately 40%, has LHV of 9.6 MJ/kg [1], indicating that if the only goal for palm oil mesocarp fibre is the energy production, the inclusion of pretreatments is not economically feasible.

An alternative is to use the extracted alkali lignin in the chemical industry [34]. The lignin can be used in the manufacture of pesticides, additives for paints and resins, production of vanillin for food industry, production of lignosulfonates for the soap and glue industries, additive to improve the viscosity of the drilling mud of oil wells, conditioner for improving soil, among other applications [35]. In this case, besides the value added by the use of lignin, the hydrolysed fibre can generate 180 L $CH_4$/kg substrate, and will enable the energy production of 6.2 MJ / kg substrate.

The anaerobic hydrolysis of the hydrolysate generated upon the application of acid and hydrothermal pretreatments (Table 5) are very low, 4.4% and 1.8% respectively. This occurred largely because the holocellulosic fraction was previously solubilised during the pre-treatment processes, and only the recalcitrant material remains as suspended COD. This may be also associated with remaining lignin, which was not extracted and prevent the enzyme access to the holocellulosic fraction, and/or the high crystallinity of the cellulosic fraction.

The wastes of palm oil refinery (empty fruit bunch, palm press fibre or mesocarp fibre, palm kernel cake, palm kernel shell and palm oil mill effluent) can be used as biomass source for energy production, using different conversion technologies (pyrolysis, gasification, direct combustion, pelletising and anaerobic digestion) to produce syngas, bio-oil, methane, briquette, and acetone–butanol–ethanol [36]. MPP of palm oil mesocarp fibre using gasification and pyrolysis can reach values of up to 203 and 50 L $CH_4$/kg fibre, respectively, which is similar to the values found in this work. However, despite the high investment costs of pyrolysis of syngas reactors, hydrogen is also generated and can improve the energy recovery.

## 4. Conclusions

Based on the initial mass of fibre, the acid pretreatment improved the solubilisation of the sugars (56%), which maximised the MPP (199 L $CH_4$/kg substrate) of the palm oil mesocarp fibre. However, the methane produced by the anaerobic digestion of this hydrolysate generates less energy than the direct burning of the crude fibre. Thus, the economic feasibility of this process depends on the use of other by-products besides biogas. The alkaline hydrolysis is a promising alternative, as this ensures extraction of up to 91% of the lignin present in the fibre as well as producing 180 L $CH_4$/kg hydrolysed fibre.

## Acknowledgements

This work was carried out with the support of the Brazilian Agricultural Research Corporation (Embrapa), through funds of Call 01/2009 PAC-Embrapa-Macroprograma 2; of the Ceará State's Foundation for the Scientific and Technological Development (FUNCAP) and of the Federal Institute of the Ceará State (IFCE) for the scholarships.

## References

[1] J. Furlan Jr. Dendê: manejo e uso dos subprodutos e dos resíduos. Embrapa Amazônia Oriental (Oil palm: management and use of by-products and Waste). Documentos 246, Embrapa Amazônia Oriental, Belém, Brazil, 2007.
[2] M. Barrientos, C. Soria. Palm Oil Production by Country – Index Mundi. <http://www.indexmundi.com/agriculture/?commodity=palm-oil&graph=production> (accessed 27.03.12).
[3] I. Angelidaki, M. Alves, D. Bolzonella, L. Borzacconi, J.L. Campos, A.J. Guwy, S. Kalyuzhnyi, P. Jenicek, van J.B. Lier, Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays, Water Sci. Technol. 59 (5) (2009) 927–934.
[4] M.J. Taherzadeh, K. Karimi, Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: a review, Int. J. Mol. Sci. 9 (9) (2008) 1621–1651.

[5] V.S. Chang, M.T. Holtzapple, Fundamental factors affecting biomass enzymatic reactivity, Appl. Biochem. Biotechnol. 84–86 (2000) 5–37.
[6] Y.H.P. Zhang, L.R. Lynd, Toward an aggregated understanding of enzymatic hydrolysis of cellulose: noncomplexed cellulase systems, Biotechnol. Bioeng. 88 (7) (2004) 797–824.
[7] N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple, M. Ladisch, Features of promising technologies for pretreatment of lignocellulosic biomass, Bioresour. Technol. 96 (2005) 673–686.
[8] X.F. Sun, F. Xu, R.C. Sun, Y.X. Wang, P. Fowler, M.S. Baird, Characteristics of degraded lignins obtained from steam exploded wheat straw, Polym. Degrad. Stabil. 86 (2004) 245–256.
[9] M. Pedersen, A.S. Meyer, Lignocellulose pretreatment severity-relating pH to biomatrix opening: review, New Biotechnol. 27 (6) (2010) 739–750.
[10] M. Vázquez, M. Oliva, S.J. Téllez-Luis, J.A. Ramírez, Hydrolysis of sorghum straw using phosphoric acid: evaluation of furfural production, Bioresour. Technol. 98 (2007) 3053–3060.
[11] E. Palmqvist, B. Hahn-Hägerdal, Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition, Bioresour. Technol. 74 (2000) 25–33.
[12] APHA, Standard Methods for Examination of Water and Wastewater, 21th ed, American Public Health Association, 2005.
[13] J.P.S. Morais, M. de F. Rosa, J.M. Marconcini, Procedimentos para análise lignocelulósica. Documentos 236. Embrapa Algodão. Campina Grande, Brazil, 2010.
[14] T. Yokoyama, J.F. Kadla, H.M. Chang, Microanalytical method for the characterization of fibre components and morphology of woody plants, J. Agric. Food Chem. 50 (5) (2002) 1040–1044.
[15] G.J.M. Rocha. Deslignificação de bagaço de cana de açúcar assistida por oxigênio. São Carlos, SP, Brazil, Institute of São Carlos, University of São Paulo-USP, Doctor thesis, 2000.
[16] G.L. Miller, Use of dinitrosalicylic acid reagent for determination of reducing sugars, Anal. Chem. 31 (1995) 426–428.
[17] T.J. Irick, K. West, H.H. Brownell, W. Schwald, J.N. Saddler, Comparison of colorimetric and HPLC techniques for quantitating the carbohydrate components of steam-treated wood. Scientific note, Appl. Biochem. Biotechnol. 17 (1988) 137–149.
[18] M. Soto, R. Méndez, J.M. Lema, Methanogenic and non-methanogenic activity tests. Theoretical basis and experimental setup, Water Res. 27 (8) (1993) 1361–1376.
[19] G. Zeeman, Mesophilic and Psychrophilic Digestion of Liquid Manure. PhD. Thesis, Department of Environmental Technology, Agricultural University, Wageningen, 1991.
[20] M. Laser, D. Schulman, S.G. Allen, J. Lichwa, M.J. Antal Jr., L.R. Lynd, A comparison of liquid hot water and steam pretreatments of sugar cane bagasse for bioconversion to ethanol, Bioresour. Technol. 81 (2002) 33–44.
[21] M.O. Petersen, J. Larsen, M.H. Thomsen, Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals, Biomass Bioenergy 33 (5) (2009) 834–840.
[22] M.J. Díaz, C. Cara, E. Ruiz, I. Romero, M. Moya, E. Castro, Hydrothermal pretreatment of rapeseed straw, Bioresour. Technol. 101 (2010) 2428–2435.
[23] R. Boopathy, Anaerobic biotransformation of furfural to furfuryl alcohol by a methanogenic archaebacterium, Int. Biodeter. Biodegr. 63 (2009) 1070–1072.
[24] G.-L. Cao, N.-Q. Ren, A.-J. Wang, W.-Q. Guo, J.-F. Xu, B.-F. Liu, Effect of lignocellulose-derived inhibitors on growth and hydrogen production by Thermoanaerobacterium thermosaccharolyticum W16, Int. J. Hydrogen Energy 35 (2010) 13475–13480.
[25] G. Bustos, J.A. Ramírez, G. Garrote, M. Vázquez, Modeling of the hydrolysis of sugar cane bagasse with hydrochloric acid, Appl. Biochem. Biotechnol. 104 (2003) 51–68.
[26] A. Herrera, S.J. Téllez-Luis, J.A. Ramírez, M. Vázquez, Production of xylose from sorghum straw using hydrochloric acid, J. Cereal Sci. 37 (2003) 267–274.
[27] A. Herrera, S.J. Téllez-Luis, J.J. González-Cabriales, J.A. Ramírez, M. Vázquez, Effect of the hydrochloric acid concentration on the hydrolysis of sorghum straw at atmospheric pressure, J. Food Eng. 63 (2004) 103–109.
[28] L.J. Jönsson, E. Palmqvist, N.O. Nilvebrant, B. Hahn-Hägerdal, Detoxification of wood hydrolysates with laccase and peroxidase from white-rot fungus *Trametes versicolor*, Appl. Microbiol. Biotechnol. 49 (1998) 691–697.
[29] A.B. Moldes, J.M. Cruz, J.M. Domínguez, J.C. Parajó, Production of a cellulosic substrate susceptible to enzymatic hydrolysis from pre hydrolyzed barley husks, Agric. Food Sci. Finland 11 (2002) 51–58.
[30] M.B. Viana, A.V. Freitas, R.C. Leitão, S.T. Santaella, Biodegradability and methane production potential of glycerol generated by biodiesel industry, Water Sci. Technol. 66 (10) (2012) 2217–2222.
[31] E. Us, N.A. Perendeci, Improvement of methane production from greenhouse residues: optimization of thermal and $H_2SO_4$ pretreatment process by experimental design, Chem. Eng. J. 181–182 (2012) 120–131.
[32] T.V. Fernandes, G.J.K. Bos, G. Zeeman, J.P.M. Sanders, J.B. Van Lier, Effects of thermo-chemical pre-treatment on anaerobic biodegradability and hydrolysis of lignocellulosic biomass, Bioresour. Technol. 100 (2009) 2575–2579.
[33] K.R. Salomon, E.E.S. Lora, Estimativa do potencial de geração de energia elétrica para diferentes fontes de biogás no Brasil, Biomass Energy 2 (1) (2005) 57–67.
[34] C. Vila, V. Santos, J.C. Parajó, Recovery of lignin and furfural from acetic acid–water–HCl pulping liquors, Bioresour. Technol. 90 (2003) 339–344.
[35] J.M. Harkin, Lignin and its uses. Research note. US Department of Agriculture, Forest Service Forest Products Laboratory, Madison, Wis., EUA, 1969.
[36] H. Kasivisvanathan, R.T.L. Ng, D.H.S. Tay, D.K.S. Ng, Fuzzy optimisation for retrofitting a palm oil mill into a sustainable palm oil-based integrated biorefinery, Chem. Eng. J. 200–202 (2012) 694–709.