Research, Society and Development, v. 10, n. 1, e4921019902, 2021
(CC BY 4.0) | ISSN 2525-3409 | DOI: http://dx.doi.org/10.33448/rsd-v10i1.19902

Comparative study of the efficiency of pretreatment with alkaline hydrogen peroxide in pineapple bagasse in different granulometries submitted to acid and enzymatic saccharification

Estudo comparativo da eficiência do pré-tratamento com peróxido de hidrogênio alcalino no bagaço de abacaxi em diferentes granulometrias submetidas à sacarificação ácida e enzimática

Estudio comparativo de la eficacia del pretratamiento com peróxido de hidrógeno alcalino em bagazo de piña en diferentes granulometrías sometidas a sacarificación ácida y enzimática

Received: 11/03/2020 | Reviewed: 11/12/2020 | Accept: 01/21/2021 | Published: 01/25/2021

Danielle Pires Nogueira
ORCID: https://orcid.org/0000-0002-2097-2646
Public University of Navarra, Spain
E-mail: nogueira.dp@gmail.com
Lorena Costa Vasconcelos
ORCID: https://orcid.org/0000-0001-6242-9564
Federal University of Goiás, Brazil
E-mail: lorenacvmacedo@gmail.com
Gabriel Luis Castiglioni
ORCID: https://orcid.org/0000-0001-6941-148X
Federal University of Goiás, Brazil
E-mail: gabrielcastigli@gmail.com
Fernanda Ferreira Freitas
ORCID: https://orcid.org/0000-0002-1670-5094
Federal University of Goiás, Brazil
E-mail: ferferreirafreitas@gmail.com
Araceli Aparecida Seolatto
ORCID: https://orcid.org/0000-0002-6653-6620
Federal University of Goiás, Brazil
E-mail: araceliseolatto@ufg.br

Abstract

This work had the purpose of evaluating the efficiency of the pretreatment with alkaline hydrogen peroxide of pineapple bagasse in order to obtain fermentable sugars by applying acid and enzymatic hydrolysis to said residue. Four experimental designs were applied to study the best conditions for the pre-treatment. Total reducing sugars (TRS) concentration was the response and hydrogen peroxide concentration, time and temperature were the independent variables. The studies were conducted using pineapple bagasse with particle sizes of 20 mesh and 48 mesh. Acid saccharification, with 2.9% sulfuric acid (v/v), following the pre-treatment, yielded TRS concentrations that reached 0.094 g of TRS/g of raw bagasse for 20 mesh and 0.101 g of TRS/g of raw bagasse for 48 mesh. The enzymatic saccharification, with 9 FPU/g cellulase and 2% (m/v) of bagasse, reached 0.063 g of TRS/g of raw bagasse for both particle sizes. The peroxide concentration showed a significant influence, the use of high concentrations reduced the TRS output in both hydrolysis. With the results found in this work, it is possible to infer the feasibility of applying pineapple bagasse as a lignocellulosic raw material.

Keywords: *Ananás comosus*; Hydrolysis; Lignocellulosic-materials; Industrial residues.

Resumo

Este trabalho teve como objetivo avaliar a eficiência do pré-tratamento com peróxido de hidrogênio alcalino de bagaço de abacaxi para a obtenção de açúcares fermentáveis por meio da aplicação de hidrólise ácida e enzimática sobre o referido resíduo. Quatro projetos experimentais foram aplicados para estudar as melhores condições para o pré-tratamento. A concentração de açúcares redutores totais (TRS) foi a resposta e a concentração de peróxido de hidrogênio, o tempo e a temperatura foram as variáveis independentes. Os estudos foram conduzidos com bagaço de abacaxi com granulometria de 20 mesh e 48 mesh. A sacarificação ácida, com 2,9% de ácido sulfúrico (v / v), após o pré-tratamento, rendeu concentrações de TRS que alcançaram 0,094 g de ATR / g de bagaço bruto para 20 mesh e 0,101 g de ATR / g de bagaço bruto para 48 mesh . A sacarificação enzimática, com 9 FPU / g de celulase e 2% (m / v) de bagaço, atingiu 0,063 g de TRS / g de bagaço bruto para ambos os tamanhos de partícula. A concentração de

peróxido apresentou influência significativa, o uso de altas concentrações reduziu a produção de TRS em ambas as hidrólises. Com os resultados encontrados neste trabalho, é possível inferir a viabilidade da aplicação do bagaço de abacaxi como matéria-prima lignocelulósica.
Palavras-chave: Ananás comosus; Hidrólise; Materiais lignocelulósicos; Resíduos industriais.

Resumen
Este trabajo tuvo como objetivo evaluar la eficacia del pretratamiento con peróxido de hidrógeno alcalino del bagazo de piña para obtener azúcares fermentables mediante la aplicación de hidrólisis ácida y enzimática a dicho residuo. Se aplicaron cuatro diseños experimentales para estudiar las mejores condiciones para el pretratamiento. La concentración de azúcares reductores totales (TRS) fue la respuesta y la concentración de peróxido de hidrógeno, el tiempo y la temperatura fueron las variables independientes. Los estudios se realizaron utilizando bagazo de piña con tamaños de partículas de malla 20 y malla 48. La sacarificación ácida, con 2.9% de ácido sulfúrico (v / v), luego del pretratamiento, arrojó concentraciones de TRS que alcanzaron 0.094 g de TRS / g de bagazo crudo para malla 20 y 0.101 g de TRS / g de bagazo crudo para malla 48. La sacarificación enzimática, con 9 FPU / g de celulasa y 2% (m / v) de bagazo, alcanzó 0.063 g de TRS / g de bagazo crudo para ambos tamaños de partícula. La concentración de peróxido mostró una influencia significativa, el uso de altas concentraciones redujo la salida de TRS en ambas hidrólisis. Con los resultados encontrados en este trabajo, es posible inferir la factibilidad de aplicar bagazo de piña como materia prima lignocelulósica.
Palabras clave: Ananás comosus; Hidrolisis; Materiales lignocelulósicos; Residuos industriales.

1. Introduction

There is a correlation between energy and prosperity, making energy indispensable for modern societies (Nakanishi et al., 2018). Renewable energy sources are an upcoming trend, and lignocellulosic biomass is recognized as a prominent raw material for the production of biofuels and other value-added products, thus it can be of value to the global energy supply (Chaturved & Verma, 2013). The conversion of biomass into various value-added biochemical products, including biofuel, is generally termed as biomass valorization (Ho et al., 2019). This phrase has been widely used in recent research publications, indicating the shift of global research focal point to the alternative sources for producing chemicals and bioenergy, which could be derived from the waste. Biofuels are already being used to compose part of the fuels in many countries. Although, their raw materials are, in most of the cases, also food sources. Second and third generation fuels come to give a noble end to lignocellulosic and lipid residues that, otherwise, would be increasing pollution of land and water sources. Climate crisis is something we can no longer ignore, we must find environmental friendly ways to produce energy right now (Aranda-Martinez et al., 2017; Silva et al., 2018; Santo et al., 2018).

Second generation ethanol presents an enormous growth potential. It does not depend on the increase of agricultural lands for its production and, it has the added advantage of not competing with the production of food, since it repurposes residues (Candido et al., 2019). Lignocellulosic biomass (LB) represents the main potential raw material for lignocellulosic feedstock biorefinery, due to its high polysaccharide content, high availability, and low cost (Travanini, 2016). These residues have cellulose and hemicellulose in its composition. These structures can be broken down in sugars, such as glucose and xylose, by means of saccharification (Boussarsar et al., 2009). However, the biomass conversion into biofuels is only economically viable if 100% of its carbon is efficiently utilized in the industrial process. Including the cellulose, constituted of glucose units, and hemicellulose, mostly pentose units, fractions (Bettiga et al., 2008).

According to Oliveira (2012), the main effect of exposing the lignocellulosic matrix to pretreatment agents for extended periods is cellulose loss, resulting in low output in the process. Although, this step is necessary to reduce cellulose's degree of crystallinity; dissociate the lignin-cellulose complex; increase biomass surface area; preserve pentoses maximizing the sugars output; and minimizing the generation of process inhibitors, in both saccharification and fermentation steps (Shen et al., 2011, Santos et al., 2018). This step is a major bottleneck in second-generation ethanol production. There is a plethora of methods to choose from, with specific effects on biomass components. So the method and conditions should be chosen according to the raw material and subsequent process steps (Maurya et al., 2015). The excellent delignification using alkaline

peroxide is not the only reason why researchers are paying more attention in biomass valorization for the past decades. Unlike acid or alkaline pretreatment, alkaline peroxide pretreatment can be performed at relatively milder conditions (concentration, temperature) and at atmospheric pressure, while effectively removing lignin from various agricultural residues. Furthermore, alkaline peroxide is a relatively "green" reagent with low environmental impact as it can be easily decomposed to yield water and oxygen as end products. (Ho et al., 2019).

Alkaline peroxide pretreatment is more efficient than alkaline pretreatment to improve the lignin solubilization, and digestibility of the raw material (Karagöz et al., 2012).

Acid saccharification can occur with either concentrated or diluted acids. Sulfuric acid is the most commonly used one. However, other acids can be used, there are studies involving hydrochloric, nitric and phosphoric acids (Menon and Rao, 2012). Acid saccharification requires extreme temperatures and pressures. Opposed to that, enzymatic saccharification can occur at atmospheric pressures and temperatures around 50°C. It happens with the synergistic action among cellulolytic enzymes, and saves energy, besides being an expensive ingredient (Zhang et al., 2006). Enzymatic hydrolyses has the added advantage of not generating products that degrade the monomers, which will reduce the sugars output. Yet, these cellulolytic enzymes are unable to catalyze a complete and quick cellulose conversion. It means the conversion rates depend on the type of residues used, saccharification times, and enzymes concentration applied (Candido et al., 2019).

According to Dahunsi (2019), there is therefore need to evaluate the use of this alkaline pretreatment on other lignocelluloses as well to assess the optimal condition for the process. In 2017 Brazil was the third largest producer of pineapples in the world, behind Costa Rica and the Philippines (Shahbandeh, 2019). The consumption *in natura* represents 97% of all the production in Brazil. From the processed part, around 40% of the fruit is waste. The residues are solid and high in sucrose and fermenting sugars (Gil and Maupoey, 2018). They are also high in lignocellulosic material, with around 40% hemicellulose and 30% cellulose (Lousada Jr et al., 2006; Brito et al., 2020).

Based on the above, the objective of the present work was to study the performance of alkaline hydrogen peroxide pretreatment of the pineapple bagasse in two different granulometries. In order to do that the pretreatment was followed by diluted acid and enzymatic saccharification. The response analyzed was the total reducing sugars (TRS) output, to evaluate the application in producing second-generation (2G) ethanol.

2. Methodology

The present work was a quantitative experimental research. The authors Nogueira and Vasconcelos conducted the experimental work supervised by Castiglioni, Freitas, and Seolatto.

Biomass

Pineapple bagasse, composed of pulp and peel, was donated by the industry Doce Vida (Sweet Life), located in Anápolis – GO, Brazil. The material was dried in an air circulation oven at 60°C for 72 h. After drying, the material was milled and sieved. The fractions of 20 and 48 mesh were separated, and stored frozen (-6°C) in polyethylene containers.

Biomass Characterization

The pineapple bagasse, in its 20 and 48 mesh fractions, was characterized. The ash and moisture contents were tested according to the *Instituto* Adolfo Lutz (Adolfo Lutz Institute) (1976) methodology. The soluble and insoluble lignin contents were quantified using a methodology preconized by IUPAC as described by Oliveira (2012). The cellulose and hemicellulose contents were determined by methodology described by Browning (1967). The carbohydrate content was assessed by High Performance Liquid Chromatography (HPLC). The bagasse underwent an acid hydrolyses and the hydrolyzed portion was

tested in a LC-20A Prominence Shimadzu chromatographer, with a SUPERCOGEL Ca column, coupled to a refraction detector. The carrier solvent was deionized water with a pump flow of 0.5 mL/min. The oven temperature was 80°C and the injection volume 20µL (OLIVEIRA, 2012). All the analysis were done in triplicates.

Pretreatment with alkaline hydrogen peroxide.

The pretreatment with alkaline hydrogen peroxide was conducted according to methodology described by Krishna (2000). The factorial design chosen to be applied in this work was a 2^3 central composite, which indicates a factorial experiment with three control factors (variables studied), each tested in two levels (encoded by -1 and 1). The experimental design matrix was generated using the software Statistica™. The selected factors were time (h), temperature (T), and concentration of hydrogen peroxide. The response variable was the total reducing sugars (TRS). To make the study more precise and analyze the repeatability it was added of 6 central points and 2 axial points, totalizing 17 assays. The variables with their encoded and actual values are presented on Table 1. The experimental design was run four times. The same conditions were studied for acid and enzymatic saccharifications for each granulometry. The assays were done in triplicates.

Table 1. Coded and uncoded values of the independent variables.

Variable	-α	-1	0	+1	+α
Time (h)	2.55	8	16	24	29.45
Temperature (°C)	9.77	20	35	50	60.23
[H_2O_2] alkaline	0.64	2	4	6	7.36

Source: Authors.

Thus, 4 g of the homogenized dried biomass was put into 100 mL of hydrogen peroxide solution at different concentrations for the experimental design (Table 1). The pH was adjusted to 11.5 with sodium hydroxide (20 mol/L), since it is the pH that provides more precise results (Karagöz et al., 2012). The pretreatment reactions were conducted in 250 mL Erlenmyer flasks. Each flask was sealed hermetically before being put in an orbital shaker at 150 rpm. At the end of each reactional time, the samples were filtered and washed with distilled water until reaching neutral pH. After pre-treatment the material was subjected to acid and enzymatic hydrolysis to release reducing sugars which were quantified.

Acid and enzymatic saccharification

After filtration he solid part left from the pretreatment was dried at 40°C until constant weigh. In order to determine the best pretreatment conditions the acid and enzymatic saccharifications were conducted. At the end of each saccharification, the TRS were determined according to methodology described by Miller (1959) and the results were presented in g of TRS/g of raw bagasse. The results were analyzed using the software for windows Statistica 7.0™. The significance level considered for all the analyses was 10% (p-value<0.1).

For the acid saccharification, the material was transferred to 125 mL Erlenmeyer flasks. Then 25 mL of sulfuric acid (H_2SO_4) 2.9% m/v was added, and put in the autoclave for 30 minutes at 121°C (Moutta et al., 2011). After the autoclaving process the flasks were left to cool at room temperature. When they were cool, the solid portion was separated by vacuum filtration. The liquid fraction was neutralized with a sodium hydroxide solution (20 mol/L) before quantifying TRS.

The enzymatic saccharification assays applied Cellulase from *Trichoderma reesei*, obtained from Sigma Aldrich. The optimum pH and temperature were previously determined according to methodology described by Rabelo (2010). The dried pretreated bagasse was transferred to Erlenmeyer flasks. A buffer solution of sodium acetate pH 4.8 was added to each flask in

enough volume to make a 2% (m/v) concentration of solids, considering the moisture content of the samples. The amount of cellulase was fixed at 9 FPU/g of dried biomass. The saccharification occurred at 50°C and 150 rpm in an orbital shaker. To determine the best saccharification time, aliquots were taken at times of 0, 3, 6, 12, 24, 48 and 60 h. The best reaction time was 48 h, the one used to calculate the results. The test also included a blank, not to overestimate the TRS results.

3. Results and Discussion

Biomass Characterization

The cellulose and hemicellulose polymers require cleaving into their monomers, which is done by hydrolysis (Kučerová and Výbohová, 2017). As we can see on Table 2, the pineapple bagasse presented reducing and non-reducing carbohydrates.

Table 2. Determination of carbohydrates, cellulose, hemicellulose, and total lignin of pineapple bagasse in its 20 and 48 mesh fractions.

Components	20 mesh	48 mesh
Sucrose (%)	40.75 ± 0.69	44.75 ± 0.61
Glucose (%)	34.57 ± 1.60	29.28 ± 2.06
Xylose (%)	20.67 ± 0.62	21.77 ± 0.71
Cellobiose (%)	nd*	0.01 ± 0.00
Arabinose (%)	4.01 ± 0.22	4.18 ± 0.90
Total lignin (%)	23.03 ± 0.43	22.57 ± 0.86
Cellulose (%)	38.5 ± 0.82	36.7 ± 0.95
Hemicellulose (%)	35.9±0.39	33.4±0.44

*nd = not detected. Source: Authors.

Sucrose (non-reducing sugar) was the highest concentration monomer, for both fractions. Since sucrose, in certain conditions, like in acid hydrolyses, can breakdown into glucose and fructose, this can be an indication that the hydrolysis was not very drastic. Aziz et al., (2011), when characterizing pineapple juice found sucrose levels similar to the ones in this work (42.2 g/l).

Cassellis et al (2014), studied the structural, physical-chemical and functional properties of industrial pineapple residues to obtain information on the recommendation of its use as dietary fiber. Pineapple residues showed a high concentration of cellulose and low concentrations of lignin, which is an advantage when it is to be subjected to chemical or enzymatic hydrolysis.

The low amount of total of lignin, 23.03% and 22.57%, respectively to 20 and 48 mesh biomasses, permits the extraction of monomeric sugars from both the cellulosic and hemicellulosic portions (Kreangkrai Maneeintr et al., 2018). Sugarcane, an attractive biomass for 2G ethanol for its lignin content, has similar lignin levels (20.28%) to the pineapple bagasse in this study (Da Silva et al., 2020). It is an indication that pineapple bagasse has potential to be efficient in this segment.

The results from both granulometries were statistically compared using Student's t-test, with a 90% confidence level. The concentrations of glucose, xylose, arabinose, cellulose and hemicellulose in pineapple bagasse between the two granulometries were equal statistically. This shows that the damage degree does not affect the global composition of the raw material.

Silva (2011), obtained, in their study with pineapple bagasse, 31.69% for the cellulose fraction and 38.18% for hemicellulose applying the same methodology used in the present work. Maneeintr et al., (2018), had cellulose levels ranging from 25.23% to 27.58% in their work. The results in this work were higher, something that could be explained by the fact that the vegetable cells' composition vary depending on where they grow, rain levels, and other factors (Banerjee et al., 2018).

Evaluation of pretreatment for acid saccharification

For a profitable use of a potential source of sugars, cost effective pretreatment and hydrolysis have to be applied. Usually the pretreatment is the most expensive step in the whole process (Bhattacharyya and Gundupalli, 2019). The results yielded by the design matrix applied for the pretreatment with alkaline hydrogen peroxide followed by acid hydrolysis are shown in Table 3.

As we can see, when keeping time and concentration in their lowest levels and the temperature at 50ºC the TRS output is practically unaltered for both granulometries. The best TRS response for the 20 mesh fraction occurred on assay 5, which had the lowest levels of temperature and concentration of H_2O_2 ([H_2O_2]). When comparing assay 5 with assay 1, shortest and longest time levels, the result is practically unchanged. This could suggest that reaction time, in the tested conditions, is not relevant for the process. Assays 9 and 10 also corroborate said indications. Assay 13, the one with lowest [H_2O_2] -and intermediate time and temperature, had a result close to assays 5 and 1 as well. The lowest outputs of TRS were encountered in assays 4, 8, and 14, which had the highest temperatures and [H_2O_2]. Such results demonstrate that these conditions significantly disfavor the formation of reducing sugars.

The 48 mesh pineapple bagasse had its higher response in assay 13. It could indicate the lowest temperatures are more favorable for the pretreatment. Assays 4, 6, and 8, with high hydrogen peroxide concentrations, had the lowest responses in the experiment.

Between the two granulometries assay 8 had the lowest TRS output for both granulometries. This assay has the highest levels for all the factors, showing these conditions impair the formation of TRS. The highest amount of TRS happened in assay 13 for both granulometries. This experiment, contrary to assay 8, had the lowest [H_2O_2], and central points of temperature and time. It is possible to infer, then, that the use of high reaction times, high temperatures and high concentrations of peroxide is not advantageous. There is an indication that the cellulose suffered degradation in these levels of the studied variables.

This is an indication that high concentrations of peroxide can be removing part of the cellulose and hemicellulose along with the lignin. Under normal circumstances, hydrogen peroxide can only react with the aliphatic part of lignin, while no changes and degradation of phenolic compounds are observed. However, hydrogen peroxide is able to attack the phenolic compounds when it is used under alkaline conditions and heated to a relatively high temperature, which will expose the phenolic ring and cause carboxylic groups to be added to the macromolecular structure. Such alteration of biomass structure by AHP pretreatment has led to effective lignin and xylan removals, while decreasing cellulose crystallinity as well as swelling of biomass due to the insertion of polar groups into the molecule (Ho et al., 2019).

Table 3. Matrix of the factorial experimental design 2^3 with coded and actual values and TRS results of the acid saccharification of 20 and 48 mesh pineapple bagasse.

Assay	Time (h)	Temperature (°C)	$[H_2O_2]$ (%)	TRS Average Mass (g/g) 20 mesh	TRS Average Mass (g/g) 48 mesh
1	8 (-1)	20 (-1)	2 (-1)	0.092±0.0005	0.079±0.0007
2	8 (-1)	20 (-1)	6 (1)	0.051±0.0013	0.049±0.0023
3	8 (-1)	50 (1)	2 (-1)	0.077±0.0016	0.071±0.0008
4	8 (-1)	50 (1)	6 (1)	0.045±0.0000	0.038±0.0013
5	24 (1)	20 (-1)	2 (-1)	0.094±0.0008	0.072±1.3040
6	24 (1)	20 (-1)	6 (1)	0.048±0.0002	0.037±0.0008
7	24 (1)	50 (1)	2 (-1)	0.070±0.0030	0.075±0.0005
8	24 (1)	50 (1)	6 (1)	0.039±0.0005	0.035±0.0008
9	2.55 (-1.68)	35 (0)	4 (0)	0.051±0.0008	0.052±0.0013
10	29.45 (1.68)	35 (0)	4 (0)	0.054±0.0020	0.044±0.0002
11	16 (0)	9.77 (-1.68)	4 (0)	0.080±0.0004	0.048±0.0016
12	16 (0)	60.23 (1.68)	4 (0)	0.070±0.0047	0.041±0.0024
13	16 (0)	35 (0)	0.64 (-1.68)	0.091±0.0006	0.101±0.000
14	16 (0)	35 (0)	7.36 (1.68)	0.044±0.0017	0.043±0.0008
15	16 (0)	35 (0)	4 (0)	0.069±0.0000	0.043±0.0024
16	16 (0)	35 (0)	4 (0)	0.067±0.0000	0.047±0.0003
17	16 (0)	35 (0)	4 (0)	0.068±0.0005	0.045±0.0024

Source: Authors.

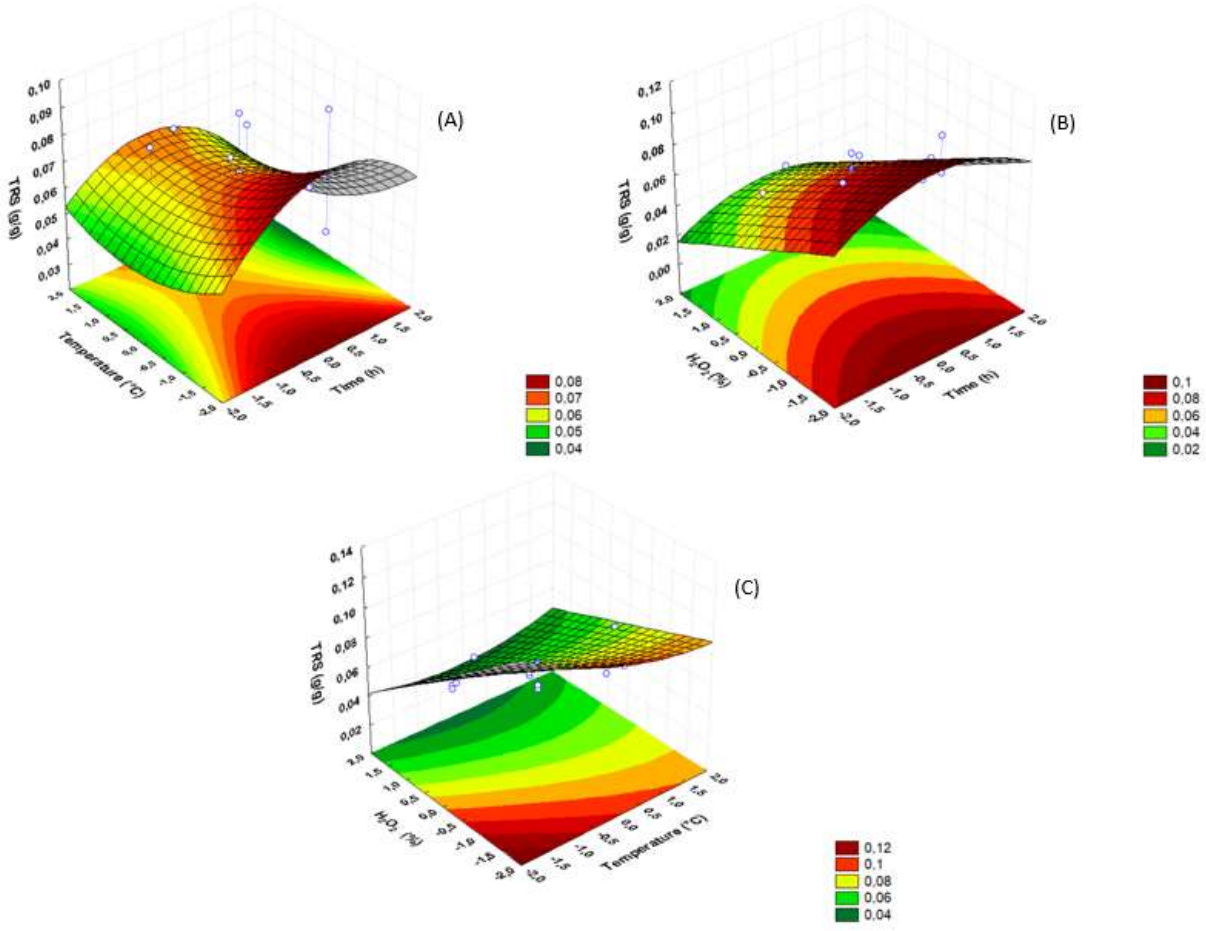
Evaluation of the pretreatment of 20 mesh pineapple bagasse for acid saccharification.

The results were analyzed by multiple linear regression. The experimental results had a good data adjustment to the model (R^2=97%) and the obtained model is presented in equation 1. Figure 1 presents the response surfaces generated with the proposed model.

$$[ART] = 0{,}067 - 0{,}0055t^2 - 0{,}0051T + 0{,}0024T^2 - 0{,}017C + 0{,}003C*T \quad [1]$$

The surfaces show a higher TRS concentration in temperatures around 20ºC. This indicates that running the pretreatment close to room temperature would not jeopardize the reaction's output (assays 1 and 5). When analyzing the effect of time (Figure 1 A and B), we can see it did not have a significant interference in the TRS response. There are good results in a broad range of times (assays 1, 5 and 13). However, the hydrogen peroxide had the best results at the lowest concentrations (Figure 1 B and C). Neto et al., (2013) compared pretreatments with alkaline hydrogen peroxide and phosphoric acid followed by delignification with sodium hydroxide for sugarcane bagasse. The results were significantly higher for alkaline hydrogen peroxide.

Figure 1. TRS amount response surface as a function of temperature and time (A); concentration and time (B); and temperature and concentration (C) of the acid saccharification of 20 mesh pineapple bagasse.



Source: Authors.

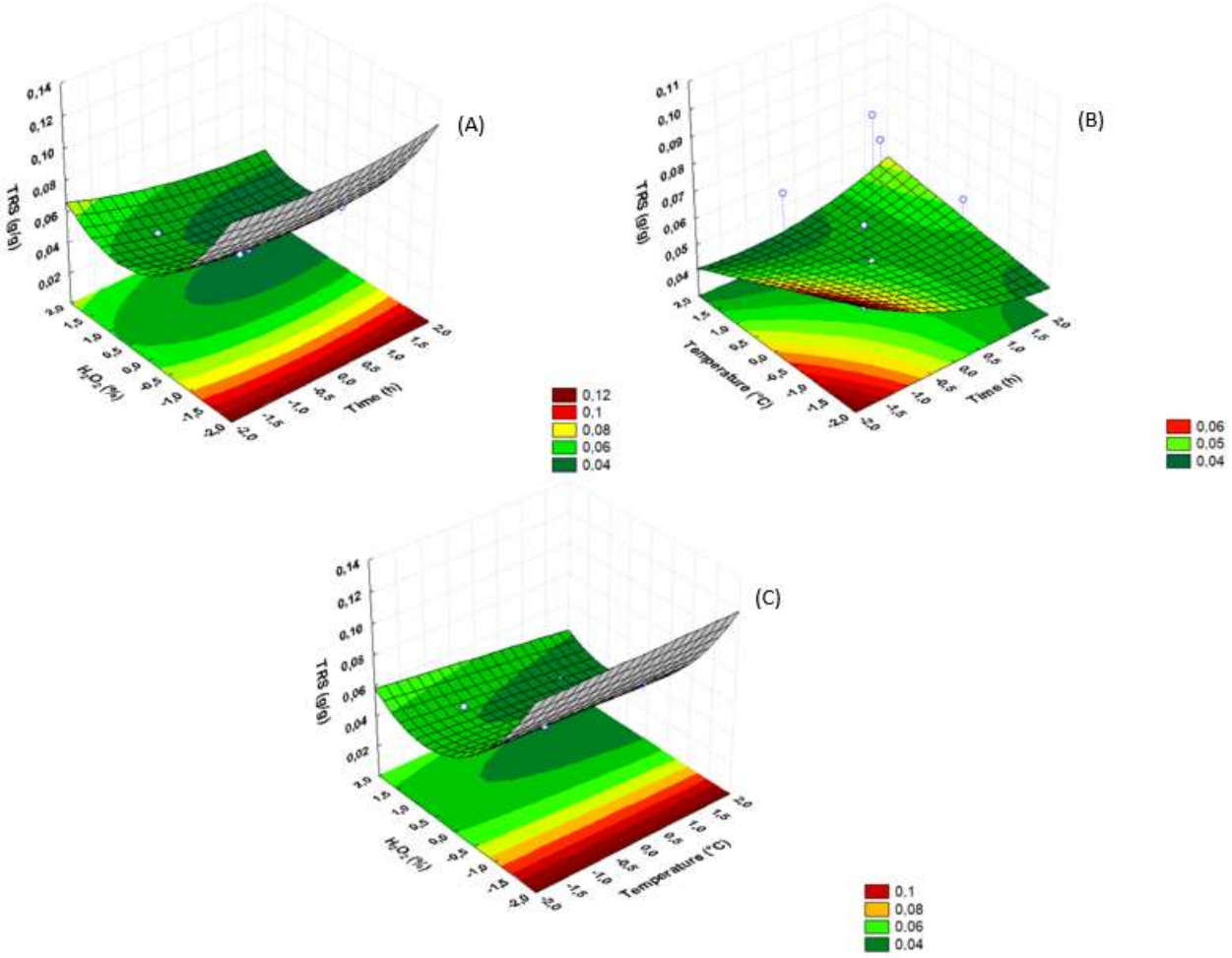
Evaluation of the pretreatment of 48 mesh pineapple bagasse for acid saccharification.

The multiple linear regression was also applied to this set of data and, after eliminating the non-significant parameters, the correlation coefficient (R^2) was 99% and the obtained model is presented in equation 2.

$$[ART] = 0{,}045118 - 0{,}002303t + 0{,}001358t^2 - 0{,}002180T - 0{,}017247C + 0{,}009844C^2 + 0{,}002500t*T - 0{,}001500T*C - 0{,}001T*C \quad [2]$$

In Figure 2 (A) it is possible to see the influence of time and temperature, in its lowest levels, was positive for the output of TRS. When comparing assays 1 and 7, both with level -1 for [H_2O_2] and other variables varying from -1 to +1, TRS only increased 5%. This results could suggest economy in the process, since long times and high [H_2O_2] are unnecessary to improve the results. Considering the influence of reaction time and [H_2O_2] (Figure 2 B and C), the best responses occurred in lowest [H_2O_2], independently of time.

Figure 2. TRS amount response surface as a function of time and temperature (A); time and concentration (B); and temperature and concentration (C) of the acid saccharification of 48 mesh pineapple bagasse.



Source: Authors.

Both analyzed granulometries of pineapple bagasse had similar behaviors in TRS output. Statistical analysis (Studend t-test) was used to compare the results. The TRS results of both granulometries were statistically the same (90% confidence) just for assays 2, 9 and 14. For the other tests, a significant difference was verified. This could indicate that there are no compositional differences between the 20 and 48 mesh bagasses. Da costa et al., (2015) pretreated cashew apple bagasse with alkaline hydrogen peroxide and quantified 10.0 g/l of glucose and 4.5 g/l xylose in their best hydrolysis condition. In this work, the best concentrations of TRS (assay 1, with all variables at its lower levels, hydrolyzed in 2.9% sulfuric acid (w/v), for 30 minutes at 121°C and 1 atm) were 16.84 g/l and 14.42 g/l (20 and 48 mesh, respectively). These results may be an indication that fruit bagasse is an interesting raw material for 2g ethanol.

Evaluation of pretreatment for enzymatic saccharification

The enzymatic saccharification process used cellulases for extracting sugar from the biomass. Cellulose and Hemicellulose in the biomass will be digested by this enzyme and hydrolyzed in to monosaccharides (Maneeintr et al., 2018). The results obtained using the design matrix applied to perform the pretreatment with alkaline hydrogen peroxide followed by enzymatic saccharification with cellulases in both granulometries are shown in Table 4.

Table 4. Matrix of the factorial experimental design 2^3 with coded and actual values and TRS results of the enzymatic saccharification of 20 and 48 mesh pineapple bagasse.

Assay	Time (h)	Temperature (°C)	$[H_2O_2]$ (%)	TRS Average Mass (g/g) 20 mesh	TRS Average Mass (g/g) 48 mesh
1	8 (-1)	20 (-1)	2 (-1)	0.063±0.0008	0.058±0.0012
2	8 (-1)	20 (-1)	6 (1)	0.044±0.0016	0.037±0.0003
3	8 (-1)	50 (1)	2 (-1)	0.057±0.0014	0.056±0.0020
4	8 (-1)	50 (1)	6 (1)	0.043±0.0008	0.034±0.0027
5	24 (1)	20 (-1)	2 (-1)	0.054±0.0003	0.049±0.0002
6	24 (1)	20 (-1)	6 (1)	0.042±0.0003	0.030±0.0008
7	24 (1)	50 (1)	2 (-1)	0.050±0.0001	0.049±0,0003
8	24 (1)	50 (1)	6 (1)	0.039±0.0010	0.032±0.0024
9	2.55 (-1.68)	35 (0)	4 (0)	0.052±0.0009	0.047±0.0047
10	29.45 (1.68)	35 (0)	4 (0)	0.044±0.0016	0.035±0.0048
11	16 (0)	9.77 (-1.68)	4 (0)	0.048±0.0003	0.044±0.0017
12	16 (0)	60.23 (1.68)	4 (0)	0.043±0.0001	0.043±0.0013
13	16 (0)	35 (0)	0.64 (-1.68)	0.059±0.0007	0.063±0.0034
14	16 (0)	35 (0)	7.36 (1.68)	0.036±0.0004	0.028±0.0017
15	16 (0)	35 (0)	4 (0)	0.041±0.0005	0.039±0.0004
16	16 (0)	35 (0)	4 (0)	0.040±0.0001	0.039±0.0027
17	16 (0)	35 (0)	4 (0)	0.040±0.0016	0.038±0.0030

Source: Authors.

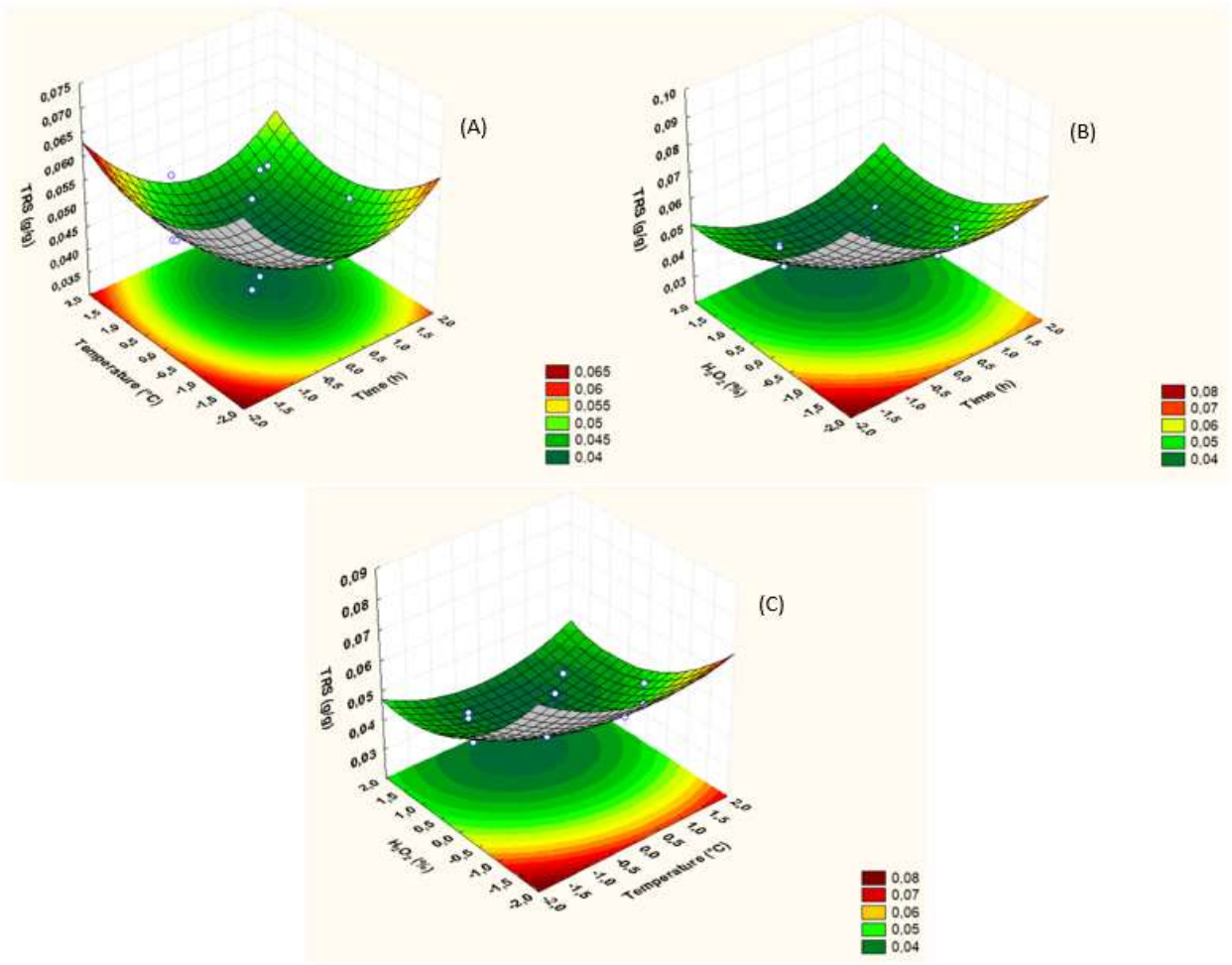
Ho et al., (2019) cites several other biomasses being pretreated with alkaline hydrogen peroxide followed by enzymatic hydrolisis, the same methodology used in this work. Analyzing the results in Table 4, we can see the highest output of TRS, for 20 mesh fraction, was in assay 1, and for 48 mesh in assay 13. Similar to the acid hydrolyses the best results were at the lowest conditions studied. Likewise, the lowest results occurred at the highest conditions of temperature (50°C), $[H_2O_2]$ (6%), and time (24h).

Evaluation of the pretreatment of 20 mesh pineapple bagasse for enzymatic saccharification.

Many factors can influence the performance of enzymatic hydrolysis, namely the type of biomass, enzymatic load, the cocktail of enzymes, and hydrolysis time, but the severity of the pretreatment seems to be of the greatest importance (Pábon et al., 2020). In this work, the results were treated statistically and the non-significant parameters were eliminated from the model. After the adjustments the determination coefficient (R^2) was 98% and the obtained model is presented in equation 3. Figure 3 presents the surfaces acquired from said model.

$$[ART] = 0{,}040646 - 0{,}002641t + 0{,}003227t^2 - 0{,}001670T + 0{,}002188T^2 - 0{,}007055C + 0{,}002814C^2 + 0{,}001163t*C \quad [3]$$

Figure 3. TRS amount response surface as a function of time and temperature (A), concentration and time (B), and temperature and concentration (C) of the enzymatic saccharification of 20 mesh pineapple bagasse.



Source: Authors.

The evaluation of the graphs shows that the highest outputs came from the mildest studied conditions, in consonance with the data presented this far. Tests with identical conditions for time and H_2O_2, and temperature varying from level -1 to +1 (For example, between assays 1 and 3 or 2 and 4), presented similar results. It implicates a low interaction effect between these variable and temperature, as can be checked in equation 3.

When analyzing Figure 3, it appears that the best responses come from lower temperature uses. Observe, for example, assays 1 and 3, where all experiments were set to peroxide concentration 2%. Assay 1 (8h time and 20°C temperature) obtained 0.063 g TRS/g dry bagasse, while assay 3 (8 h time and 50°C temperature) had an output of 0.057 g TRS/g of dry bagasse, showing that the temperature had a greater negative effect, reducing the response value. This indicates that, for this pretreatment, lower temperature values are more favorable. It was also clear that the highest TRS outputs were obtained when the lowest levels of the variables were combined. This is corroborated by the results yielded by experiments 1, 2 and 5.

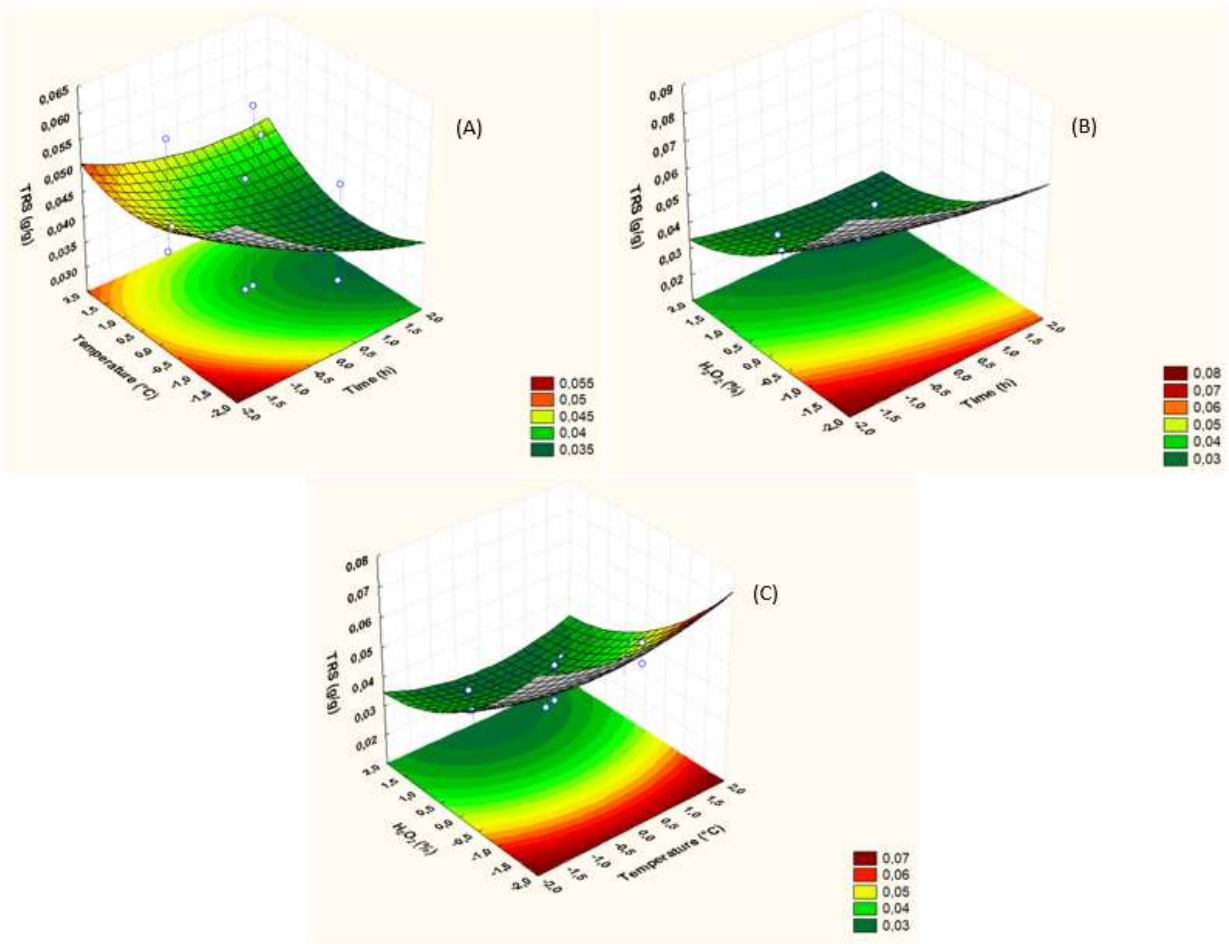
De Araújo et al., (2017), investigated the influence of chemical (Triton X-100) and biological surfactant preparation (rhamnolipids) in coconut husk hydrolysis that was subjected to pretreatment with alkaline hydrogen peroxide. They used as pre-treatment an alkaline hydrogen peroxide concentration close to the highest level used in this work (7.5%) followed by enzymatic hydrolysis and obtained 40% lignin content.

Evaluation of the pretreatment of 48 mesh pineapple bagasse for enzymatic saccharification.

From the results presented in Table 4, it was possible to analyze statistically the response behavior of each variable. After the adjustments the determination coefficient (R^2) was 97 % and the obtained model is presented in equation 4. Figure 4 presents the surfaces acquired from said model.

$$[ART] = 0{,}039 - 0{,}0033t + 0{,}0007t2 + 0{,}0016T^2 - 0{,}010C + 0{,}0023C^2 + 0{,}0009t*T + 0{,}0008t*C \quad [4]$$

Figure 4. TRS amount response surface as a function of time and temperature (A), concentration and time (B), and temperature and concentration (C) of the enzymatic saccharification of 48 mesh pineapple bagasse.



Source: Authors.

Figure 4 A shows that the highest TRS outputs were obtained at the lowest times and temperatures (assays 1). Figure 4 B presents that there was not much interference of time, but the lowest concentrations brought the highest TRS concentrations. Fact reinforced in Figure 4 C, where the highest TRS is also in the lowest concentrations of hydrogen peroxide. The temperature also does not present a significant influence in the output of TRS.

Enzymatic saccharification had similar results for both fractions analyzed. There was a constant positive effect on the application of low concentrations of hydrogen peroxide. It has also been observed in acid saccharification. Student's t test showed that most results were statistically equal, between fractions of 20 and 48 mesh. This indicates that there are no significant differences between the different particle sizes.

Acid saccharification, for both particle sizes, showed superior results when comparing Tables 2 and 3. Possibly, this is due to the use of only one enzyme, cellulase, in enzymatic saccharification. It is usual to suplement the hydrolysis with β-glucosidase (Söderström et al., 2003), that has a high cost. According to Martin et al., (2007), there are some advantagens in applying acid hydrolyses in detriments of the enzymatic process. The chemical reagents cost less; there is a wide range of acids that can be applied; and its reactions are better understood, because there are more studies. Nevertheless, we have disadvantages as well, as the coproducts that can reduce the sugars output, and inhibit the fermentation. Some disadvantages of enzymatic saccharification are the need for large amounts of enzymes or long times, to increase the formation of glucose (Martins et al., 2015).

Cao et al., (2016) evaluated the saccharification of sweet sorghum bagasse pretreated with hydrogen peroxide, applying Celullases and β Glucosidase and achieved a production of 40.26 g/L of TRS. This difference are likely due to the different biomasses or the combination of enzymes. Chen et al., (2009), state that alkaline pretreatment is a more efficient chemical process. When compared to acid pretreatments, it degrades sugars in a smaller degree. The authors compared alkaline hydrogen peroxide and sodium hydroxide. They observed that alkaline hydrogen peroxide is more effective in solubilizing lignin when compared to sodium hydroxide pretreatment. The lignin reduced from 23.3% to 5.6% with alkaline hydrogen peroxide and from 23.2% to 14.6% with sodium hydroxide. In the present work preliminary tests with sulfuric acid 2% for 24h at 20°C had a TRS yield of 0.032 g/g. It shows this method did not have satisfactory results when compared to the tests with alkaline hydrogen peroxide (0.094 g/g TRS, in assay 5).

According to Oliveira (2012), the main collateral effect from the exposure of lignocellulosic materials to hydrogen peroxide is cellulose loss. This will result in low process output. The results in this work show that alkaline hydrogen peroxide pretreatment is very effective. Although the best results occur at the lowest concentrations and shortest times. These is evidence that peroxide pretreatments may remove cellulose, as well as lignin. Therefore, further research is necessary to optimize the pretreatment conditions and maximize the sugars output.

4. Final Considerations

The pineapple bagasse characterization presents similar results to previous works on lignocellulosic residues. The total lignin 22.03% (20 mesh) and 22.57% (48 mesh) allows the extraction of monomeric sugars. Both types of saccharification had better responses at the lowest levels of alkaline hydrogen peroxide concentration, and time and temperatures did not have significant impacts. The fact that between the granulometries some of the experiments had statistically equal and for others statistically different outputs of reducing sugars impedes a conclusive analysis. It can indicate that the variations are more based on different portions of the biomass, than in the granulometry itself. Based on this data, the pineapple bagasse could be applied, not only as raw material for secondary processes, but as an alternative in producing 2G ethanol. Future studies on this subject should focus on locating the optimal contitions of pretreatment and hydrolysis to maximaze the glucose output.

Acknowledgments

The authors acknowledge CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) and Capes (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the financial support.

References

Aranda-Martinez, A., Ortiz, M. A. N., García, I. S. A., Zavala-Gonzalez, E. A., & Lopez-Llorca, L. V. (2017). Ethanol production from chitosan by the nematophagous fungus *Pochonia chlamydosporia* and the entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana*, *Microbiological Research, 204*, 30-39.

Aziz, M. G., Michlmayr, H., Kulbe, K. D., & Del Hierro, A. M. (2011). Biotransformation of pineapple juice sugars into dietetic derivatives by using a cell free oxidoreductase from Zymomonas mobilis together with commercial invertase, *Enzyme Microbiology and Technology, 48*(1), 85-91.

Banerjee, S., Ranganathan, V., Patti, A., & Arora, A. (2018). Valorisation of pineapple wastes for food and therapeutic applications, *Trends in Food Science & Technology, 82*, 60-70.

Bhattacharyya, D., & Gundupalli, M.P. (2019). Ethanol Production from Acid Pretreated Food Waste Hydrolysate using *Saccharomyces cerevisae* 74D694 and Optimizing the Process using Response Surface Methodology, *Waste Biomass Valor, 10*, 701-708.

Bettiga, M.; Hahn-Hägerdal, B.; & Gorwa-Grauslund, M. F. (2008). Comparing the xylose reductase/xylitol dehydrogenase and xylose isomerase pathways in arabinose and xylose fermenting Saccharomyces cerevisiae, *BioMedCentral, 16*(1), 107-115.

Boussarsar, H., Rogé, B., & Mathlouthi, M. (2009). Optimization of sugarcane bagasse conversion by hydrothermal, *Bioresource Technology, 100*(24), 6537-6542.

Brito, T. B. N., Pereira, A. P. A., Pastore, G. M., Moreira, R. F. A., Ferreira, M. S. L., & Fai, A. E. C. (2020). Chemical composition and physicochemical characterization for cabbage and pineapple by-products flour valorization, *Food Science and technology, 124*, 109028.

Browning, B. L. (1967). Methods of wood chemistry. New York/ London/ Sydney: *Interscience Publishers*, V. II.

Cao, W.; Sun, C.; Qiu, J.; Li, X.; Liu, R.; & Zhang, L. (2016). Pretreatment of sweet sorghum bagasse by alkaline hydrogen peroxide for enhancing ethanol production. Korean Journal of *Chemical Engineering, 33*(3), 873-879.

Candido, R. G., Mori, N. R., & Gonçalves, A. R. (2019). Sugarcane straw as feedstock for 2G ethanol: Evaluation of pretreatments and enzymatic hydrolysis, *Industrial Crops and Products, 142*, 111845.

Cassellis, M. E. R., Sánchez-Pardo, M. E., & Mora-Escobedo, R. (2014). Structural, physicochemical and functional properties of industrial residues of pineapple (Ananas comosus), Cellulose Chemistry and Technology, 48(7-8), 633-641.

Chaturved, V., & Verma, P. (2013). An overview of pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products, *3 Biotech, 3*(5), 415-431.

Chen, M., Zhao, J.; & Xia, L. (2009). Comparison of four different chemicals pretreatment of corn stover for enhancing enzymatic digestibility, *Biomass and energy, 33*, 1381-1385.

De Araújo, C. K. C., Campos, A. O., Padilha, C. E. A., Sousa Júnior, F. C., Nascimento, R. J. A., Macedo, G. R., & Santos, E. S. (2017). Enhancing enzymatic hydrolysis of coconut husk through Pseudomonas aeruginosa AP 029/GLVIIA rhamnolipid preparation. Bioresource Technology, 237, 20-26.

Da Costa, J. A.; Marques JR., J. E.; Gonçalves, L. R. B.; & Rocha, M. V. P. (2015). Enhanced enzymatic hydrolysis and ethanol production from cashew apple bagasse pretreated with alkaline hydrogen peroxide, *Bioresource Technology, 179*, 249-259.

Dahunsi, S. O. (2019). Liquefaction of pineapple peel: Pretreatment and process optimization, *Energy, 185*, 1017-1031.

Gil, L. S.; & Maupoey, P. F. (2018). An integrated approach for pineapple waste valorisation. Bioethanol production and bromelain extraction from pineapple residues, *Journal of Cleaner Production, 172*, 1224-1231.

Ho, M. C., Ong, V. Z., & Wu, T. Y. (2019). Potential use of alkaline hydrogen peroxide in biomass pretreatment and valorization – a review, *Renewable and Sustainable Energy Reviews, 112*, 75-86.

Karagöz, P.; Rocha, I. V.; Özkan, M.; & Angelidaki, I. (2012). Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by Same Vessel Saccharification and Co-Fermentation, *Bioresource Technology, 104*(87), 348-357.

Krishna, S. H., Rao, K. C. S., Babu, J. S., & Reddy, D. S. (2000). Studies on the production and application of cellulose from *Trichoderma reesei* QM-9414, *Bioprocess Engineering, 22*(98), 467-470.

Karagöz, P., Rocha, I. V., Özkan, M., & Angelidaki, I. (2012). Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by Same Vessel Saccharification and Co-Fermentation. *Bioresource Technology, 104*(87), 348-357

Kucerová, V., & Vybohová, E. (2018). Release of saccharides during hot-water pretreatment of Willow Wood, *Cellulose Chemistry and Technology, 52*(5-6), 381-386.

Maneeintr, K., Leewisuttikul, T., Kerdsuk, S., & Charinpanitkul, T. (2018). Hydrotermal and enzymatic treatments of pineapple waste for energy production, *Energy Procedia, 152*, 1260-1265.

Martin, C. et al. (2007). Dilute sulfuric acid pretreatment of agricultural and agro-industrial residues for ethanol production. In: J. R. Mielenz, K. T. Klasson, W. S. Adney, & J. D. McMillan (Ed.) *Applied Biochemistry and Biotechnology* (pp. 339-352). Nashville: The Twenty-Eighth Symposium Proceedings of the Twenty-Eight Symposium on Biotechnology for Fuels and Chemicals.

Maurya, D. P.; Singla, A.; & Negi, S. (2015). An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech, 5*(5), 597-609.

Menon, V.; & Rao, M. (2012). Trends in bioconversion of lignocelluloses: Biofuels, platform chemicals & biorefinery concept, *Progress in Energy Combustion and Science, 38*(4), 522-550.

Miller, G. L. (1959). Use of Dinitrosalicylic Acid Reagent for Determination of Reducing Sugar, Analytical Chemistry, Natick, *Mass, 31*(3), 426-428.

Moutta, R. O., Chandel, A. K., Rodrigues, R. C. L. B., Silva, M. B.; Rocha, G. J. M., & Silva, S. S. (2011). Statistical optimization of sugarcane leaves hydrolysis into simple sugars by dilute sulfuric acid catalyzed process, Sugar technology, *14*(1). 53-60.

Nakanish, S. C., Nascimento, V. M., Rabelo, S. C., Samapaio, I. L. M., Junqueira, T. L., & Rocha, G. J. M. (2018). Comparative material balances and preliminar technical analysis of the pilot scale sugarcane bagasse alkaline pretreatment to 2G ethanol production, *Industrial Crops & Products*, *120*, 187-197.

Neto, J. M.; Garcia, D. R.; Rueda, S. M. G.; & Da Costa, A. C. (2013). Study of kinetic parameters in a mechanistic model for enzymatic hydrolysis of sugarcane bagasse subjected to different pretreatments, *Bioprocess Biosystems and Engineering*, *36*, 1579-1590.

Oliveira, C. C. dos S. Otimização do pré-tratamento com peróxido de hidrogênio a alta concentração de sólidos para a hidrólise enzimática de bagaço de cana-de açúcar. 81f. Dissertação (Mestrado em Engenharia Química) - Curso de Pós-Graduação em Engenharia Química. Universidade Estadual de Campinas. São Paulo (2012).

Pabón, A. M. A., Felissia, F., Mendieta, C., Chamorro, E., & Area, M. C. (2020). Improvement of Bioethanol Production from Rice Husks, *Cellulose Chemistry and Technology*, *54* (7-8), 689-698.

Santo, M. E., Rezende, C. A., Bernardinelli, O. D., Pereira Jr., N., Curvelo, A. A. S., De Azevedo, E. R., Guimarães, F. E. G., & Polikarpovi, I. (2018). Structural and compositional changes in sugarcane bagasse subjected to hydrothermal and organosolv pretreatments and their impacts on enzymatic hydrolysis, *Industrial Crops & Products. 113*, 64-74.

Santos, L. C., Adarme, O. F. H., Baeta, B. E. L., Gurgel, L. V. A., & Aquino, S. F. (2018). Production of biogas (methane and hydrogen) from anaerobic digestion of hemicellulosic hydrolysate generated in the oxidative pretreatment of coffee husks, *Bioresource Technology*, *263*, 601 – 612.

Söderström, J.; Pilcher, L.; Galbe, M.; & Zacchi, G. (2003). Two-step steam pretreatment of softwood by dilute H_2SO_4 impregnation for ethanol production, *Biomass and Bioenergy*, *24*, 475-486.

Shahbandeh, M. (2019). Leading countries in pineapple production worldwide in 2017 (in 1,000 metric tons). *Statista*. Retrieved August 30, 2019, from: https://www.statista.com/statistics/298517/global-pineapple-production-by-leading-countries/

Shen, F., Saddler, J. N., Liu, R.; Lin, L.; Deng, S.; Zhang, Y.; Yang, G.; Xiao, H.; & Li, Y. (2011). Evaluation of steam pretreatment on sweet sorghum bagasse for enzymatic hydrolysis and bioethanol production, *Carbohydrate Polymers*, *86*(4), 1542-1548.

Silva, O. de O. Aproveitamento do bagaço de abacaxi (Ananas comosus L. Merril) para produção biotecnológica de xilitol. 142f. (Doctor Scientiae). Departamento de Ciência e Tecnologia de Alimentos Universidade Federal de Viçosa, MG (2011).

Silva, V., Ratti, R. P., Sakamoto, I. K., Andrade, M. V. F., & Vareshe, M. B. A. (2018). Biotechnological products in batch reactors obtained from cellulose, glucose and xylose, using thermophilic anaerobic consortium, *Renewable Energy*, *125*, 537-545.

Rabelo, S. C. Avaliação do pré-tratamento com peróxido de hidrogênio alcalino para a hidrolise enzimática de bagaço de cana-de-açúcar. 180f. Dissertação (Doutorado em Engenharia Química) - Curso de Pós-Graduação em Engenharia Química. Universidade Estadual de Campinas (2010).

Travaini, R., Martín-Juarez, J., Lorenzo-Hernando, A., & Bolado-Rodríguez, S. (2016). Ozonolysis: an advantageous pretreatment for lignocellulosic biomass revisited, *Bioresource Technology*, *199*, 2-12.

Zhang, H.P.Y., Himmel, M. E., & Mielenz, J. R. (2006). Outlook for cellulase improvement: Screening and selection strategies, *Biotechnology Advances*, *24*(5), 452-481.