CHEMICAL ENGINEERING TRANSACTIONS

VOL. 37, 2014

A publication of
AIDIC
The Italian Association
of Chemical Engineering
www.aidic.it/cet

Guest Editors: Eliseo Ranzi, Katharina Kohse- Höinghaus

ISBN 978-88-95608-28-0; **ISSN** 2283-9216

DOI: 10.3303/CET1437068

Study of Recovery of Fermentable Sugars with Subcritical Water and Carbon Dioxide from Palm Fiber and Grape Seed

Mauricio Ariel Rostagno*[a], Juliana M. Prado[b], Renata Vardanega[a], Tânia Forster-Carneiro[a], M. Angela A. Meireles[a]

[a]LASEFI/DEA/FEA (School of Food Eng.)/UNICAMP (University of Campinas) – R. Monteiro Lobato, 80; 13083-862, Campinas, SP, Brazil
[b]CTBE (Brazilian Bioethanol Science and Technology Laboratory) / Integrate Brazilian Center of Research in Energy and Materials (CNPEM), R. Giuseppe Máximo Scolfaro, 10.000; Campinas, SP, Brazil
rostagno@fea.unicamp.br

The biorefinery concept is anchored to many types of biomass, including the residues of food processing (seeds, bark, roots, etc.) and agricultural residues (straws, bagasse, etc.). Brazil is recognized as a world leader in exports of food. The industrial activities of the agri-food sector generate large amounts of organic waste. These residues can become a major renewable resource for the production of new chemicals, fuels and energy. The supercritical water technology allows the hydrolysis of organic waste to obtain the so-called precursors: carbohydrates, lignin, oils and proteins and carbon-5 and carbon-6. In this work, it was studied the supercritical water hydrolysis (SWH) of two food industry residues commonly found in Brazil: defatted grape seeds and pressed palm fibre. The hydrolytic process was conducted in semi-batch at 257°C, 20 MPa and constant water and carbon dioxide flow rates of 33 $mLmin^{-1}$ and 7g min^{-1}, respectively. The process was performed for 30 min. The results of the hydrolysis experiments with the addition of CO_2 as an acid catalyst were compared to data from experiments conducted with pure water. For pressed palm fibre there was no change in the content of reducing sugars in the hydrolysate (11.5%), but the pH varied, and total fermentation inhibitors increased. For the defatted grape seed the content of total reducing sugars increased 56%, the pH also varied, and the fermentation inhibitors also increased. The results indicated large differences between raw materials. Therefore, the addition of carbon dioxide improves the SWH process, depending on the raw material used.

1. Introduction

The current energy supply model of the world is largely based on the consumption and the distribution of fossil fuels, such as oil, coal and gas. One of the main problems of burning fossil fuels to produce energy is the emission of greenhouse effect gases. These products have been contributing to the global warming and to changes in the environment (Goldemberg, 2007). The increasing demand of energy associated with the dependence on fossil fuels is one of the greatest challenges (Inman et al., 2010). Additionally, making renewable fuels economically attractive is a difficult task. Although some innovative technologies are technically feasible, they remain more expensive than non-renewable sources (Serrano-Ruiz and Dumesic, 2011).

Brazil is recognized as a world leader in exports of food. The industrial activities of the agri-food sector generate large amounts of organic waste. Residues generated from biomass worldwide represent a great potential resource for energy achievement (Olivarez et al., 2012). These residues can become a major renewable resource for the production of new chemicals, fuels and energy (Berni et al., 2013). The combustible ethanol provides more power than gasoline, and it can take advantage of the installed infrastructure for distribution of liquid fuels. Because of that it has been largely used as fuel in Brazil, and

Please cite this article as: Rostagno M.A., Prado M J., Vardanega R., Forster-Carneiro T., Meireles M.A.A., 2014, Study of recovery of fermentable sugars with subcritical water and carbon dioxide from palm fiber and grape seed, Chemical Engineering Transactions, 37, 403-408 DOI: 10.3303/CET1437068

relatively simple modifications of the engines are needed so they can run with ethanol. Thus, it is a natural candidate as a short-term solution to the problem of increasing the share of our energy supply by renewable sources (Leite et al., 2009). Moreover, the knowledge generated about processes to produce other products from the bagasse is sufficient to indicate that this raw material can be better exploited (Mudhoo et al., 2011).

The lignocellulosic biomass is a particularly attractive feedstock because it is the cheapest, most abundant, and fastest growing form of terrestrial biomass. The sub/supercritical water hydrolysis (SWH) is a clean technology and fast hydrolysis method applicable to lignocellulosic biomass releasing the pre-treatment step and this is a great advantage. Others advantage of this technology are short reaction time, low corrosivity, low residue generation and no use of toxic solvents. The simple sugars that can be obtained from the cellulosic and hemicellulosic fractions of lignocellulosic materials can be used as substrate to produce second-generation bioethanol or other precursors of bio-products (Petchpradab et al., 2009).

In Brazil, agricultural residues are potential raw materials for other processes through of current technology capable of processing them into fermentable sugars (Prado et al., 2014).There are relatively few examples of applications of sub and/or supercritical water for the hydrolysis of agricultural and food industry residues. Most relevant examples include corn stalks and stover, sugarcane bagasse and rice bran. The sugarcane bagasse is an agricultural residue produced in large amounts in Brazil with a great potential to be used for the recovery of value added sub-products (Prado et al., 2014). Other potential raw materials that can be used to produce simple sugars using SWH include grape seeds. The grape seeds are a waste of winery industry that are mainly composed by lignin and hemicellulose. They also present a high content of oil, which can be extracted and used in several applications, such as cosmetics and foods. After the oil fraction is extracted, the grape seeds have no other immediate use. Other raw material with potential is the pressed palm fiber, this material is a residue from the pressing of palm fruits to produce oil, presenting a high content of hemicellulose. The reuse of these residues from agriculture or by the pre-processing of the food industry is a great opportunity because they are abundant waste and can produce the others high-value sub-products. However, considering the complexity of polymers interaction and the diversity of their composition, each raw material represents a technological challenge that should be studied separately. In the produce second-generation bioethanol, the main challenge is to achieve high yields of fermentable sugars and low concentrations of compounds that inhibit the fermentation microorganisms. It is expected that the feasibility of using palm fiber and grape seed in Brazil can to contribute the reuse of other wastes in the biorefinery, resulting in more effective action to obtain renewable energy from agro-industrial residues, including second-generation bioethanol, fuel gas, electricity, heat, and upgraded products (Forster-Carneiro et al., 2013).

The supercritical technology has become an attractive alternative to different process applications due to the use of environmental friendly solvents and small reaction time (Albarelli et al., 2011). A few studies on subcritical water hydrolysis have been conducted with palm fiber and grape seed and however, the process is far from being optimized. Furthermore, adding dioxide carbon (CO_2) as an acid catalyst could improve the supercritical process of differents raw materials (Nobre et al., 2008). Therefore, the objective of this work was to optimize process conditions for subcritical water hydrolysis of palm fiber and grape seed using CO_2 as catalyst.

2. Material and Methods

2.1 Raw materials origin

The pressed palm fiber was donated by Agropalma (Tailândia, PA, Brazil) after the palm had been used to manufacture palm oil. The raw material was stored at -18 °C and then it was commented in a knife mill (Marconi, model MA 340, Piracicaba, Brazil) equipped with a 1 mm. The other sample, object of this study, the grape seeds were provided by Villa Francioni winery (São Joaquim, SC, Brazil). The seeds were collected after wine fermentation, and were separated from stalks and peels by sieving and air blowing. The resulting sample was dried under the sun for 7 days. The seeds were ground frozen to preserve their vegetable oil and then their oil was extracted by supercritical CO_2 (Prado et al., 2014). The residue from the supercritical extraction process was stored at -18 °C and subsequently it was used in this study. Distilled water was used in all experiments.

2.2 Hydrolysis equipment

The semi-batch unit shown in Figure 1 was built to hydrolyze lignocellulosic biomasses using sub-supercritical water conditions (200-375 °C of temperature and 20-25 MPa of pressure). The equipment can operate up to 400 ºC of temperature and 40 MPa of pressure. The system is composed by a liquid high pressure pump that works up to 400 bar (LabAlliance, model Series III, State College, PA) for water pumping, an air driven liquid pump (Maximator GmbH, model M18, Thüringen, Germany) for carbon dioxide pumping, a thermostatic bath (Marconi, model MA-184, Piracicaba, Brazil) operated at -10 °C to assure that dioxide carbon is liquid before entering the pump, two static mixers (Autic, Campinas, Brazil), one stainless steel heating coil (Autic, 6 m × 1/8” i.d., Campinas, Brazil) for the reaction medium heating, one stainless steel 50 mL reactor (Autic, 2.34 cm i.d. × 11.7 cm, Campinas, Brazil) with metal-to-metal fit to allow using temperatures up to 400 °C, one micrometric needle valve (Autoclave Engineers, Erie, PA) for pressure control, one stainless steel refrigeration coil coupled to a thermostatic bath (Marconi, model MA-184, Piracicaba, SP, Brazil) operating at 40 °C to assure that the reaction is quickly quenched after the hydrolysate exits the reactor, and one glass gas-liquid separator to separate the liquid hydrolysate from the CO_2. The equipment also contains block valves, thermocouples and manometers.

The operating unit operates at subcritical hydrolysis conditions (200-250 °C of temperature and 20 MPa of pressure) and critical hydrolysis conditions of temperature of 375-400 °C and pressure of 25-40 MPa was as follows: the water was pumped and a static mixing was performed before the heating coil with addition of dioxide carbon (CO_2); the reaction medium entered the reactor containing the biomass, and the residence time was determined by the water flow rate; after the reaction there are rapid depressurization followed by cooling, and in the end the dioxide carbon was separated from the liquid hydrolysate; finally, the CO_2 was released to the atmosphere and the hydrolysate was collected in plastic flasks.

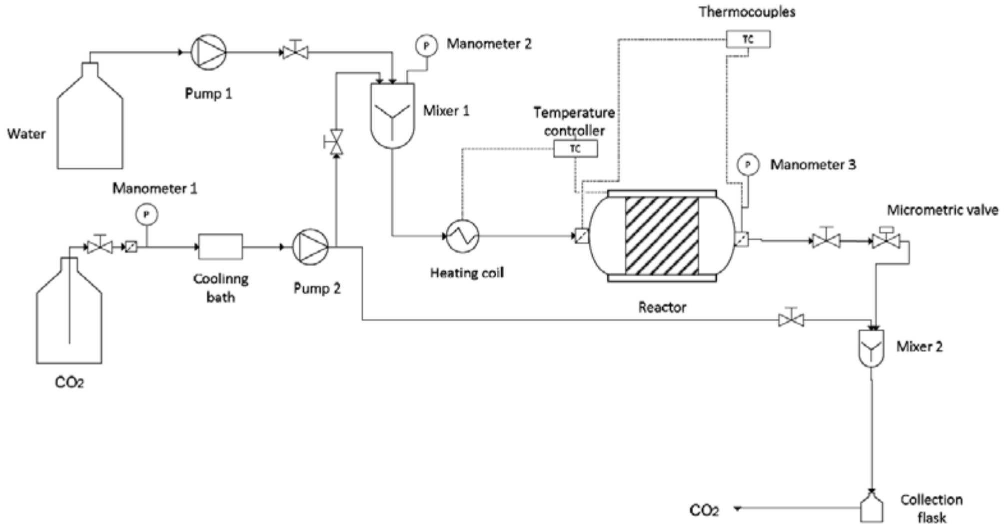

Figure 1. Hydrolysis equipment

2.3 Hydrolysis of raw materials

Experiments were carried out using 10 g of raw material, approximately. The sample was inserted in the reactor after this reactor and the electrical resistance were connected to the equipment. Distilled water was pumped to the system until reaching the specified pressure (20 MPa). After the pressurization the pump the process was stopped, the micrometric valve was closed and the heating of the coil and of the reactor was started. The electrical resistance was set at 250 °C of the temperature, while the reactor was pre-heated to 120 °C to assure that there was no hydrolysis of cellulose during the pre-heating time. After stabilizing the temperature throughout the system, the dynamic period of the process was started, the pumping water was set at 33 mL/min (ambient conditions) and the pumping of CO_2 was set at 7 g/min through the system for 30 min. Finally, the reactor temperature was set to 250 °C, causing a temperature profile until its stabilization. The experiments were performed in duplicate. The samples of hidrolysates were collected each 2 min.

2.4 Analysis of the hydrolysate

The colorimetric Somogyi-Nelson method allows calculating the percentage total reducing sugars, that is, sugars with open-chain form with an aldehyde group or a free hemiacetal group (Nelson, 1944; Miller, 1954). Acoording Nelson (1944) and Miller (1959) the total reducing sugars or the sugars with open-chain form with an aldehyde group or a free hemiacetal group can be analysed by the colorimetric Somogyi-

Nelson method. The hydrolysate should be subjected to acid hydrolysis to assure all the oligomers would be broken to monomers, and could be detected as reducing sugars (Miller, 1959). After the coloring reaction (Nelson, 1944) the absorbance was recorded at 730 nm by a spectrophotometer (Femto, model 800 XI, São Paulo, Brazil). The concentration of total reducing sugars was calculated using an external calibration curve of glucose (10-600 mg/L), and expressed as glucose equivalents. The pH of the hydrolysates was determined using a digital pHmeter (Digimed, model DM-22, Santo Amaro, Brazil).

The analyses were carried out using a Waters system (Waters Corp., Milford, Massachusetts), consisting of separation module (2695A) with integrated column heater and auto-sampler, and a photodiode array detector (2998). Some of the samples of the hydrolysates were diluted in deionized water and filtered through a 0.45 µm nylon membrane for degradation products of sugars and of the lignin analyses by HPLC (Rostagno et al., 2011). Separation of compounds present in the samples was carried out using a fused-core type column (XSelect HSS, C18, 2.5 µm, 100 Å, 100 × 4.6 mm, Waters Corp.) (Farías-Campomanes et al., 2013). The temperature of the column was maintained at 40 °C and the mobile phase consisted of water containing 0.1 % of acetic acid (solvent A) and acetonitrile containing 0.1 % of acetic acid (solvent B). Separation was achieved using the following gradient: 0 min: 10% B; 2.0 min: 10% B; 5.0 min: 20% B; 6.0 min: 20% B; 10 min: 90% B; 12 min: 90% B; 13 min: 10% B. Equilibration time between runs was 7 min; total time was 20 min per sample. The mobile phase flow rate was 0.8 mL/min and the injection volume was 10 µL. UV absorbance was monitored from 200 to 400 nm with a sampling rate of 10 points per second. The software for control of equipment and data acquisition was Empower 3. The identification of each compound was achieved by comparison of retention times and UV spectra of each compound. The standard curve (8 points) of each compound was prepared by plotting concentration (0.1-100 mg/L) against area. Regression equations and correlation coefficient (r2) were calculated using Microsoft Excel 2010 software and were higher than 0.99 for all compounds. The standard calibration curve was built with pure standards of furfural, 4-hydroxybenzoic acid (4-HBA), 5-HMF, and vanillin (Sigma-Aldrich, Milwaukee, WI).

3. Results and Discussion

The results of the hydrolysis experiments with the addition of carbon dioxide as an acid catalyst were compared to data from experiments conducted with pure water in both subcritical conditions of temperature and pressure (Table 1). For pressed palm fiber the results indicated that no change in the content of reducing sugars in the hydrolysate (11.5 %), or is both in processe using water or carbon dioxide no representative difference between them. The pH varied between experiments and observed significant difference between them due the increased total fermentation inhibitors. The pH of hydrolysates obtained with CO_2 addition was higher, which indicates lower amount of organic acids, which are products of sugars degradation, in the hydrolysates obtained with dioxide carbon. Furfural + 5-HMF, which are also products of sugars degradation, increased 230% with CO_2 addition, while phenolic compounds, which are products of lignin degradation, increased 45% with CO_2 addition.

Table 1- Experimental results obtained for subcritical water hydrolysis of palm fiber with and without CO_2

	Acid catalyst	
	Without CO_2	**With CO_2**
Total reducing sugars	11.9 %	11.2 %
pH	5.34-3.79	5.81-4.28
Furfural + HMF	47.2 mg/g	155.1 mg/g
Phenolic compounds	5.1 mg/g	7.4 mg/g

Therefore, the addition of CO_2 in supercritical water hydrolisis of pressed palm fiber was not effective. The SWH with water only, improved the depolymerisation of the lignocellulosic complex also the process was favored for the degradation of more sugars, leading to a constant sugars yield. Cardenas and collaborates (2014) studied the subcritical water hydrolysis without dioxide carbon with defatted pressed palm fiber in a semi-bath unit of laboratory scale and the results indicated that this raw materials is indicated too for the production of fermentable sugars, the biomass conversion (40 - 97%) increased with temperature indicating the formation of by-products and there is the highest selectivity for saccharide formation was found at 423 K (20 - 59 mol glucose/mol furfural equivalent).

In the supercritical water hydrolisis for the defatted grape seed the results were completely different compared with the experiment of pressed palm fiber (Table 2). The SWH with dioxide carbon addition increased the yield of total reducing sugars by 56%. The pH remained nearly constant. The results of analysis furfural + 5-HMF yield indicated an increased 42.5% with CO_2 addition, while that the results of yield of phenolic compounds showed an increase of the 47% due the CO_2 addition. The results indicating that the higher depolymerization of the lignocellulosic complex was also accompanied by higher sugars degradation. Even though, in this case total sugars recovered increased, therefore, for defatted grape seed the addition of CO_2 improved the process.

Table 2- Experimental results obtained for subcritical water hydrolysis of grape seed with and without CO_2

	Acid catalyst	
	Without CO_2	**Without CO_2**
Total reducing sugars	6.4 %	10.0 %
pH	4.25-4.27	4.36-4.19
Furfural + HMF	13.7 mg/g	72.0 mg/g
Phenolic compounds	0.68 mg/g	1.0 mg/g

In studies of subcritical water hydrolisis the results indicated the high glucose yield in a continuous batch system at 332 °C of temperature for 4.8 s of time and 355 °C of temperature for 15 s of time, respectively (Kumar and Gupta, 2008). In all these studies, furfural, 5-HMF and other degradation products were detected, therefore, there is still much to be improved in this technology (Sakaki et al., 2003).

In summary all analyses between different processes with and without acid catalyst or palm fiber or grape seed, probably associated with the different composition of the raw materials. The grape seed residue presented higher lignin content than pressed palm fiber. Lignin protects the lignocellulosic complex from degradation, therefore, less aggressive conditions (using water alone) are sufficient to depolymerize it when the lignin content is low. On the other hand, as lignin content increases, it is necessary to use more aggressive conditions (higher temperature or a catalyst) to achieve the same depolymerisation degree. Therefore, the addition of CO_2 can improve the SWH process, depending on the raw material used.

4. Conclusion

In the experiment of subcritical water hydrolisis with CO_2 or without CO_2 the results indicated no change in the content of reducing sugars in the hydrolysate (11.5%) for pressed palm fiber but the pH decreased, and total fermentation inhibitors increased indicating improved depolymerisation of the lignocellulosic complex associated with more sugars degradation. In the experiment of subcritical water hydrolisis of defatted grape seed the content of total reducing sugars increased 56.0%, the pH increased, and the fermentation inhibitors also increased, indicating a more pronounced effect of dioxide carbon addition on the lignocellulosic complex depolymerization than on the sugars degradation. The results indicated large differences between raw materials. Therefore, the addition of dioxide carbon can improve the subcritical water hydrolysis process, depending on the raw material.

Acknowledgments

The authors thank raw material donation by Agropalma, and Villa Francioni. The authors thank funding and support from São Paulo Research Foundation (FAPESP, projects 2010/08684-8, 2011/19817-1, 2013/10037-9, 2013/15049-5 and 2013/ 04304-4).

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