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PRODUCTION OF REDUCING SUGARS FROM LIGNOCELLULOSIC KIKUYU GRASS RESIDUES BY HYDROLYSIS USING SUBCRITICAL WATER IN BATCH AND SEMIBATCH REACTORS

PRODUCCIÓN DE AZÚCARES REDUCTORES A PARTIR DE RESIDUOS LIGNOCELULÓSICOS DE PASTO KIKUYU POR MEDIO DE HIDRÓLISIS CON AGUA SUBCRÍTICA EN REACTORES BATCH Y SEMICONTINUOS

PRODUÇÃO DE AÇÚCARES REDUTORES DE RESÍDUOS LIGNOCELULÓSICOS DE PASTO KIKUYU POR HIDRÓLISES UTILIZANDO ÁGUA SUBCRÍTICA EM REATORES BATCH E SEMI-BATCH

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ABSTRACT

A subcritical hydrolysis of Kikuyu grass lignocellulose residues was carried out in batch and semi batch mode operations. Experiments assessed the effect of temperature (250-300 °C), mass ratio (6:1-30:1), pressure (1 490-3 190 psi), and water flow rate (3-9 ml/min) in reducing sugars (RS) yield. Reducing sugar production was measured by means of the DNS method, and efficiency was calculated as the ratio between the mass of produced reducing sugars and the total mass of the residue fed to the reactor. A maximum RS of 9.7% was measured in batch hydrolysis experiments at 300 °C, 30:1 mass ratio and 3 190 psi, whereas 22% accumulated RS yield was obtained in semi batch experiments at 300 °C, 2 000 psi and 9 ml/min. The lower yield was attributed to the extended reaction time in batch experiments in comparison to semi batch experiments, in which the reaction time is not only shorter but also the hydrolysis products are continuously removed from the reactor. Analysis of Variance of data for batch experiments showed only the interaction between temperature and mass ratio to be significant, whereas the pressure had no significant effect. A notorious decrease in pH was measured with increasing reaction times due to the formation of acidic degradation products. The results showed the feasibility of producing reducing sugars from lignocellulosic residues available in large amounts and currently discarded without any utilization through subcritical hydrolysis.

Keywords: *Subcritical, Hydrolysis, Reducing sugars, Kikuyo grass, Lignocellulose biomass.*

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RESUMEN

Se estudió la hidrolisis subcrítica en modo de operación batch y semicontinuo de residuos lignocelulósicos de pasto Kikuyu. Los experimentos evaluaron el efecto de la temperatura (250-300 °C), relación másica (6:1-30:1), presión (1 490-3 190 psi), y flujo de agua (3-9 ml/min) en la productividad de azucares reductores (RS). La producción de azucares reductores se midió por medio del método DNS y la eficiencia se calculó como la relación entre la masa de azucares reductores producida y la masa total de residuo alimentado al reactor. Una productividad máxima de RS de 9.7% se obtuvo en los experimentos batch a 300 °C, relación masa de 30:1 y 3190 psi, mientras que una productividad acumulada de 22% se obtuvo en los experimentos semibatch a 300 °C, 2000 psi y 9 ml/min. La productividad más baja en los experimentos batch se atribuyó al elevado tiempo de reacción en comparación con los experimentos semibatch, en los cuales el tiempo de reacción no es solo más corto sino que también los productos de la hidrolisis se remueven continuamente del reactor. El análisis de varianza de los datos para los experimentos batch mostró como significativa solo la interacción entre la temperatura y la relación másica, mientras que la presión no tuvo efecto. Una notoria disminución de pH se midió a medida que incrementaba el tiempo de reacción debido a la formación de productos ácidos de degradación. Los resultados demuestran la factibilidad de producir azucares reductores a partir de residuos lignocelulósicos disponibles en grandes cantidades y actualmente desechadas sin ningún aprovechamiento por medio de hidrólisis subcrítica.

Palabras clave: *Hidrólisis, Subcrítica, Azucares reductores, Pasto Kikuyo, Biomasa lignocelulósica*

RESUMO

A hidrólise subcrítica de resíduos lignocelulósicos de vidro Kiyuku foi realizada em uma operação de lote e semi-lote. Os experimentos avaliaram o efeito da temperatura (250-300 °C), a proporção de massa (6:1-30:1), a pressão (1 490-3 190 psi), e o débito de água (3-9 ml/min) no rendimento de açucares redutores (RS). A produção de açúcar redutora foi estimada através do método DNS e sua eficácia foi calculada como sendo igual à proporção entre a massa de açucares redutores produzidos e a massa total do resíduo alimentado no reator. Um RS máximo de 9,7% foi calculado nos experimentos de hidrólise em lote a 300 °C, proporção de massa de 30:1 e 3 190 psi, comparado com um RS acumulado de 22% em experimentos semi-lote a 300 °C, 2 000 psi e 9 ml/min. O menor rendimento foi atribuído ao tempo de reação estendida nos experimentos de lote quando comparados com os experimentos de semi-lote, onde o tempo de reação não é só mais curto mas também os produtos da hidrólise são continuamente removidos do reator. A análise da variância estatística dos dados para os experimentos de lote mostrou apenas uma interação significativa entre temperatura e proporção de massa, enquanto a pressão não teve nenhum efeito significativo. Uma diminuição notória em pH foi verificada com o aumento dos tempos de reação produto da formação de produtos de degradação acídica. Os resultados mostraram a viabilidade da produção de açucares redutores de resíduos lignocelulósicos disponíveis e atualmente descartáveis e sem usos potenciais através da hidrólise subcrítica.

Palavras-chave: *Subcrítica, Hidrólises, Açúcares redutores, Pasto Kikuyo, Biomassa lignocelulósica.*

1. INTRODUCTION

Kikuyu (*Pennisetum clandestinum*) lignocellulos residues are produced in large amounts in big cities and are basically discarded to landfills without any potential utilization. For example, in Bogotá (Colombia), around 2 500 tons of Kikuyu grass clipping residues were produced during 2016 in maintenance operations of green zones and public spaces (UAESP, 2010). Kikuyu grass is a fast growing forage and must be cut around every 3 weeks. Due to its abundance and cellulose and hemicellulose composition, this lignocellulosic biomass could be employed for the production of reducing sugars, and fermented to produce second generation bioethanol. Nevertheless, studies on its utilization as a biomass for producing reducing sugars are scarce in literature. It is well known that cellulose and hemicellulose can be hydrolyzed to hexoses and siloxes using several processes that mainly involve a physical, chemical, physic-chemical or biological pretreatment (Haghighi *et al*.,2013), followed by the addition of acid, basic or enzymatic catalyst. However, industrial application of such processes has been limited due to several factors involving the complexity of the process, sugars degradation, corrosion, byproducts formation that prevent the fermentation or poor catalyst recyclability and economic feasibility (Prado *et al*.,2014). Yet, enzymatic hydrolysis has reached industrial scale production and commercialization. Dupont has a cellulosic ethanol plant in Nevada, which processes corn stover and produces 30 million gallons of fuel grade ethanol annually. It also offers cellulosic ethanol licenses by using a technology that implements enzymes and ethanologens. Also, Abengoa Bioenergy operates a 25 million gallons per year plant since 2014, which utilizes a proprietary enzymatic hydrolysis technology.

In this regard, sub and supercritical water hydrolysis has been reported for the production of reducing sugars from different lignocellulosic residues as a promising alternative to acid and enzymatic hydrolysis. The technology takes advantage of the well-known tunable properties of water in the vicinity or above the supercritical point of water (374 °C). For example, the ionic product of water (K_w) can be manipulated in order to favor or disfavor acid/basis catalysis (Cantero,Tapia, Dolores Bermejo, & Cocero, 2015), avoiding the use of corrosive acids such as sulfuric and hydrochloric acid commonly employed in the conventional acid catalyzed hydrolysis. As a matter of fact, subcritical water hydrolysis has been regarded as a clean and fast hydrolysis method with the advantages of no pretreatment required, shorter reaction time due to high temperature conditions, less corrosion and avoidance of catalyst residue generation, as well as lower formation of degradation products (Prado *et al*., 2015).

Several subcritical and supercritical water hydrolysis studies have been conducted through batch, semi batch and continuous mode operations with different lignocellulose residues, as recently reviewed in detail by Prado *et al* (2015). In batch mode, biomass and water are charged simultaneously to the reactor, heated up and left to react for a specific time. Since no product is removed during the reaction, produced sugars could be easily degraded. In a semi batch operation, a fixed amount of lignocellulosic biomass is charged to the reactor and water flows continuously, removing reaction products to avoid degradation. Data from semi batch reactors may be transferred to industrial scale reactors (Schacht, Zetzl, & Brunner, 2008). Batch and semi batch experiments are usually carried out in the temperature range of 180 °C to 230 °C. Continuous processes simultaneously pump up water and lignocellulosic biomass as slurry to the reactor. Since it is easier to adjust the residence time in the reactor, supercritical water conditions at very short reaction times have been preferred (Cantero, Dolores Bermejo, & Cocero, 2013). Corn stover, corn stalks, sugarcane bagasse and rice bran are the most widely used lignocellulosic residues in subcritical or supercritical hydrolysis experiments, which can be attributed to its relatively high content of cellulose and abundance. Yet, hydrolysis rates and yield will depend not only on cellulose and hemicellulose composition, but also will depend on cell wall composition, structure and lignin content. Therefore, each lignocellulosic material represents a technological challenge that needs to be address individually (Prado *et al*., 2015).

Accordingly, in this work we report on the production of reducing sugars by subcritical water treatment of Kikuyu grass clipping residues in batch and semi batch reactors. Although cellulose and hemicellulose content of this residue is inferior to other kinds of lignocellulosic biomasses, its abundance and accessibility make it an attractive raw material to produce high added-value products such as bioethanol.

2. EXPERIMENTAL DEVELOPMENT

Lignocellulose residues

Kikuyo grass clipping residues were collected from Universidad de La Salle campus in Bogotá, Colombia, during garden maintenance operations, comminuted in a knife mill (Cole-Parmer IKA analytical mill IL USA) and sieved below 400 µm, as shown in Figure 1. Comminuted grass samples were stored in a freezer (Cole-Parmer Marvel Scientific Refrigerator 24" IL USA) without any other pre-treatment. Reported residues humidity is 73% (UAESP, 2010). Lignocellulosic residue composition of kikuyu grass, corn stover and sugarcane bagasse, are shown in Table 1 for comparison purposes. While cellulose and hemicellulose composition in Kikuyu grass is inferior to other kinds or lignocellullosic residues, lignin content is considerably lower. Lignin is a specially problematic component of agricultural residues (Prado *et al*., 2014). Decomposition of lignin produces phenolic compounds, which strongly inhibit the action of yeast (Schacht *et al*., 2008).

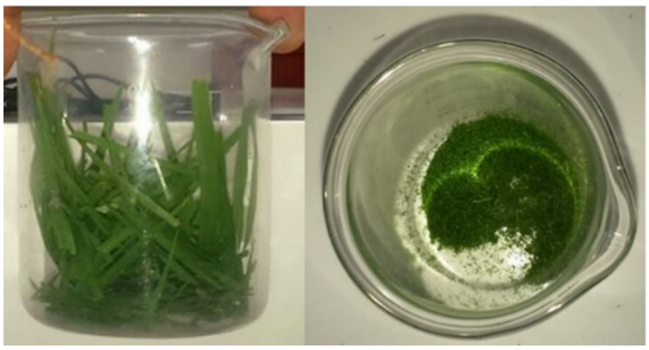

Figure 1. Kikuyu grass clipping residues

Experimental subcritical hydrolysis apparatus

Experimental runs were conducted in batch reactor equipment and a semi batch lab scale unit. The reactor was made of 316SS Swagelok tubing 0.5 in O.D (1.27 cm), 0.065 in (0.17 cm) wall thickness, length of 10 cm and volume of 6.94 cm^3. In batch experiments, a fixed amount of comminuted biomass and water, calculated according to the mass ratio for a specific run and the expected water pressure calculated at reaction conditions through steam tabs, were loaded to the reactor and sealed with screw-caps. Then the reactor was placed in an electrical insulated oven previously heated at the desired reaction temperature, as shown in Figure 2.

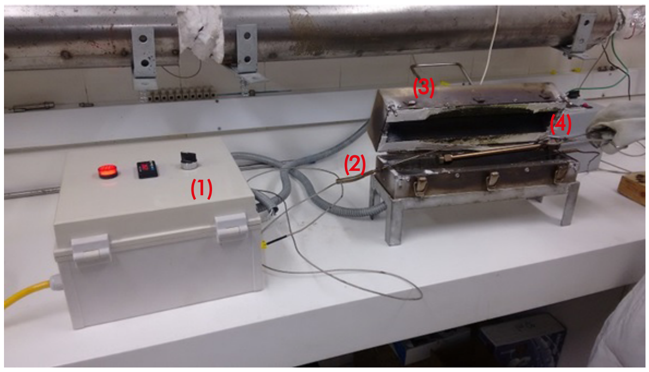

Figure 2. Batch experimental setup. (1) PID temperature controller, (2) K type Thermocouple, (3) Insulated high temperature oven, (4) ½" tubular reactor

The semi batch lab scale unit consisted of a deionized water tank, a Williams Milton Roy (PA, USA) pneumatic high pressure pump model CP250V225, a preheater made of 316SS Swagelok tubing 1/8 in O.D (3.175 mm), length of 3 m coiled tubing and electrical resistances, an insulated tubular reactor with a length of 10 cm, a concentric tube heat exchanger with water as cooling media, designed and constructed at Universidad de la Salle, a needle depressurization model SS-1RS4 (Swagelok, Barranquilla Colombia) valve and sample collection recipient, pressure gauges (Ashcroft CT, USA) and thermocouples, as shown in Figure 3

Table 1. Lignocellulosic residue composition of Kikuyu grass, corn stover and sugarcane bagasse

Lignocellulosic biomass	Cellulose wt %	Hemicellulose wt %	Lignin wt %	Source
Kikuyu grass	26.9	26,2	5,6	(Cardona & Rios, 2012)
Corn stover	38.3	25.8	17.4	(Haghighi Mood et al., 2013)
	34.5	27.7	17.8	(Kumar et al., 2011)
Sugarcane bagasse	43.1	31.1	11.4	(Cardona & Rios, 2012)
	35	35.8	16.1	(Sasaki et al., 2003)

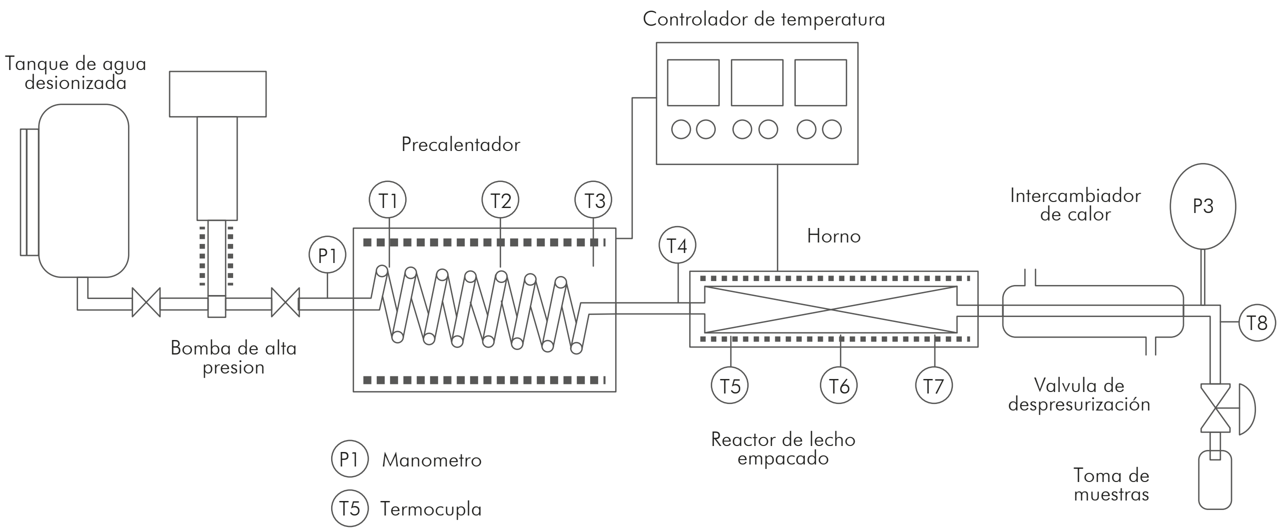

Figure 3. Semi batch lab scale unit

In a typical run, the reactor is removed from the system and packed with a fixed amount of 2 to 4 g comminuted lignocellulosic biomass. Glass beads are also added to the reactor to avoid biomass compaction and plugging. Once the reactor was put back in place, the system is pressurized with water at room temperature up to 2 000 psi to avoid water phase change during the heating up period at subcritical hydrolysis conditions. Once the temperature in the preheater is constant, pumping is started and pressure is adjusted through the needle valve. Samples are collected in sample tubes each 1 or 2 minutes. Pneumatic pump frequency and piston length was adjusted to have deionized water flowrates of 3 and 9 ml/min. The effect of reactor volume in result´s reproducibility was not assessed at this time of the research. Yet, it is expected to draw insights on the water flow rate and residence time from runs.

Analytical methods

Reducing sugars (RS) determination, which accounts for hexoses and pentoses produced from cellulose and hemicellulose fractions, was measured in hydrolysate samples by the dinitrosalicylic (DNS) colorimetric method using d-glucose as standard (Panreac, Barcelona Spain) (Miller, 1959). For batch experiments, the RS was measured at the end of the batch reaction time, whereas in semi batch, runs were measured in samples taken every 1 or 2 minutes. Briefly, for each ml of hydrolysate sample, 1 ml DNS reagent previously prepared was added, boiled for 15 min, and 1 ml deionized water added afterwards to keep for 10 min in cold water before reading the absorbance in a Macherey-Nagel Nanocolor 500 D photometer at 540 nm. The concentration of reducing sugars was calculated based on a standard curve obtained with glucose concentrations of up to 1 mg/ml. RS yield in batch experiments was calculated with the total water volume loaded to the reactor and the lignocellulosic biomass loaded to the reactor, whereas in semi batch experiments the volume of each sample and its respective RS concentration were measured to calculate the total mass. RS method does not distinguish hexoses from pentoses. To do so, HPLC analysis with standards for each sugar is required. Yet, this was not carried out at this stage of the research. The organic acids formation, which might potentially inhibit fermentation reactions, was also verified by means of the pH of hydrolysate samples using a digital WTW pH meter 315i.

Statistical analysis

Batch experimental runs were planned according to a 2^k full factorial design with factors temperature (A), pressure (B) and water to biomass mass ratio (C), using reducing sugars yield as a response variable. Low and high levels for each factor (-1,+1) were selected according to preliminary experiments. Thus, levels for temperature were 250 and 300 °C, pressures of 1450 and 3190 psi and 6:1 and 30:1 weight/weight, reaction time was kept constant at 30 min. The design is comprised by 8 experimental runs, made in a randomized order.

Statistical analysis of the results was made according to the analysis of variance (ANOVA), as well as the graphical analysis of the significant main effects and interactions plots, by using the statistical software package Minitab 16®.

3. RESULTS AND DISCUSSION

Subcritical batch hydrolysis experiments

Table 2 summarizes batch hydrolysis experimental conditions and the obtained reducing sugars yield (wet basis) . A maximum yield of 9.7 % was measured at 300 °C, 30:1 water to biomass ratio and 3190 psi. However, RS concentrations were generally higher when working with a 6:1 mass ratio due to the increased biomass amount loaded to the reactor. Not all of the experimental runs but some were replicated, as indicated in concentration and RS yield columns in Table 2. Yield in replicated runs was expressed as the mean value and the standard deviation. Although deviation was as high as 2 in some runs, the results showed that repeatability was generally good for a batch reactor. Yet, only the first data was used for the ANOVA analysis. Table 3 shows RS yield production from different raw materials by batch hydrolysis at similar subcritical conditions (wet basis). The 9.7% yield obtained through batch experiments is high when considered cellulose and hemicellulose content in Kikuyu grass. However, a direct comparison is not entirely appropriate because yield will depend not only on cellulose and hemicellulose biomass composition but also on different factors such as lignin content, biomass pretreatment, heating rate and heating time, reactants mass ratio and soluble sugars initially present in the raw material.

Table 2. Summary of batch reaction conditions

Temperature (°C)	Mass ratio	Pressure (psi)	Concentration (mg/ml)	RS yield %
250	6	1 450	15.0/13.5	8.6±0.6
250	6	3 190	10.8	6.5
250	30	1 450	1.3/2.2	5.2±2
250	30	3 190	1.5/2.4	5.8±2
300	6	1 450	7.1	4.3
300	6	3 190	6.1	3.7
300	30	1 450	2.7/1.8	6.7±2
300	30	3 190	3.2/2.5	8.5±1.7

Table 3. yield from different raw materials by batch hydrolysis

Raw material Cellulose/ hemicellulose	Reaction conditions	RS yield concentration	Reference
Kikuyo grass 26.9%/26.2%	300°C,3190psi	8.5%	This work
Wheat straw 21.7%/11.7%	280°C,NA	6.7%	(Zhao et al., 2009)
Corn stover 34.5%/27.7%	170°C,4350psi	4.91%	(King et al., 2012)
Rapeseed straw 49.48%/14.55%	255°C,1450psi	18%	(Pińkowska et al., 2013)

Figures 4 and 5 show the normal probability plot of the residuals and the normal plot of stardardized effects for data in Table 2. As can be observed in Fig. 4, the normal probability plot of the residuals resembles a straight line. Therefore, the error distribution is normal. Figure 5 shows only the interaction between temperature and the reactants mass ratio has a significant effect on the response, with a P-value of 0.017, whereas the reaction pressure had no significant effect, and a model regression coefficient R^2= 89.06% according to the performed Minitab ANOVA analysis.

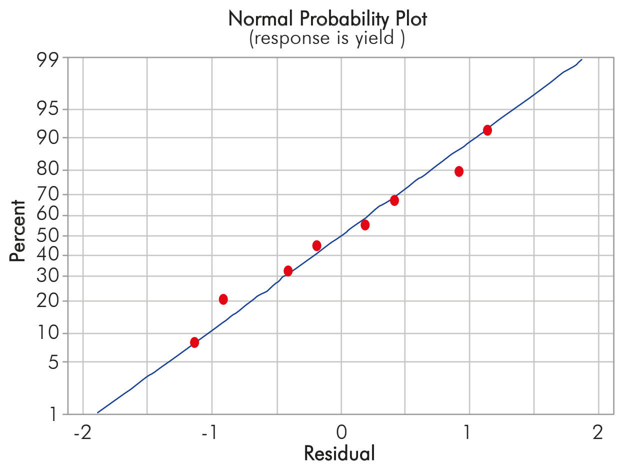

Figure 4. Normal probability plot of the residuals for data in Table 2

The interaction plot AC, temperature-mass ratio, is shown in Figure 6. It can be observed that the highest yields are obtained when working at 300 °C and 30:1 water to biomass ratio, which could be attributed to the higher reaction temperature speeding up the reaction when compared to the experiments with the same ratio at lower temperature. High yields are also obtained at 250 °C and a 6:1 mass ratio, which could be attributed to the higher biomass amount loaded to the reactor. Yet,

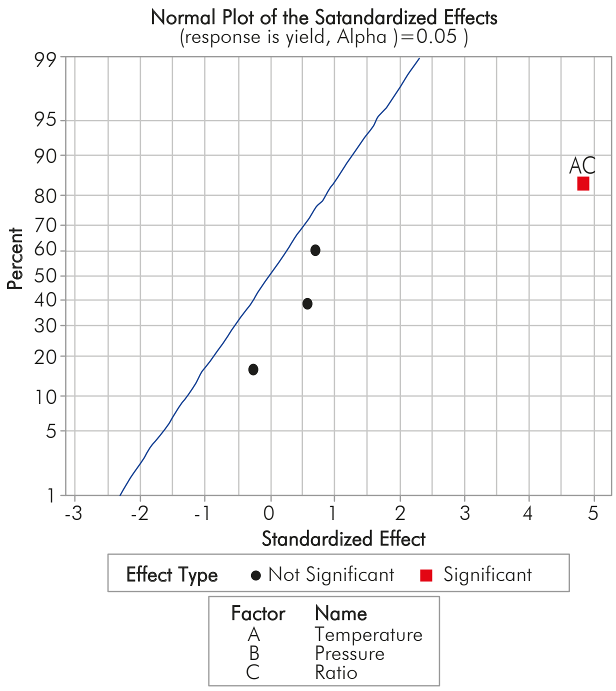

Figure 5. Normal plot of standardized effects for data in Table 2

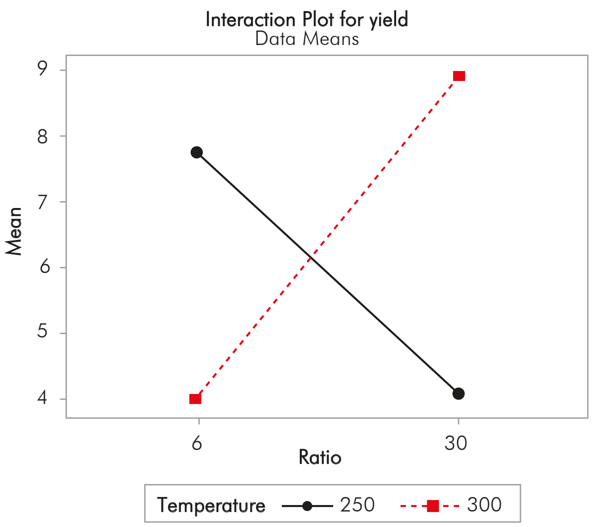

Figure 6. Interaction plot AC, temperature-mass ratio

it rapidly decreases upon increasing the temperature to 300 °C possibly explained by sugars degradation

Subcritical semi batch hydrolysis experiments

Semi batch subcritical hydrolysis experiments were carried out at temperatures between 240 and 300 °C, pressures up to 2 000 psi and water flowrates of 3 and 9 ml/min. A fixed amount of 2-4 g of kikuyu grass biomass together with glass beads were packed in the reactor for each run. Reactor effluent samples were taken every 2 minutes right after the pumping was started. Figure 7 shows the effluent color for the run conducted at 275 °C, 1 500 psi and 3 ml/min. At first, effluent samples are clear but soon after they developed a brownish color and pleasant sugary odor that could be directly related to reducing sugars concentration through the DNS method; the darker the brown color the higher the measured absorbance or RS concentration in the obtained hydrolysate at that specific reaction time. After some time has passed effluent samples turned clear again, which served as an indicative of the end of the run because of the depletion of lignocellulosic biomass in the reactor. After the pumping was stopped, the reactor was removed from the system to check the residue left. In some runs, there was practically no biomass left, only the glass beads, which means all cellulosic material was converted. RS concentration was measured in each sample taken, and sugar mass was calculated with the measured concentration and sample volume in order to approach the accumulated reducing sugar mass with time for each experimental run. Due to the large number of samples taken for RS analysis in each semi batch run, no replication was carried out. Reducing sugars accumulated per 100 g of biomass for each run are displayed in Figures 8 and 9.

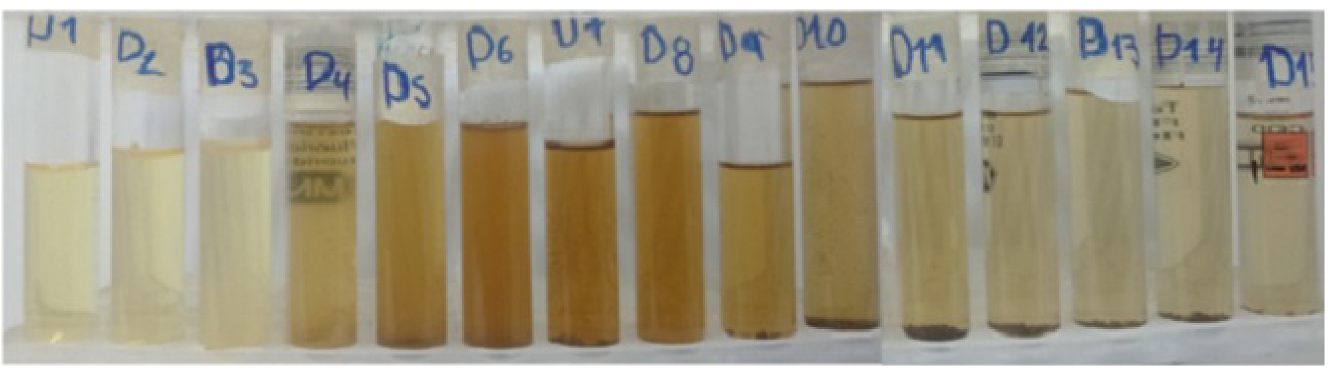

Figure 7. Aspect of effluent samples of semi batch run carried out (left to right) at 275 °C, 1 500 psi and 3 ml/min

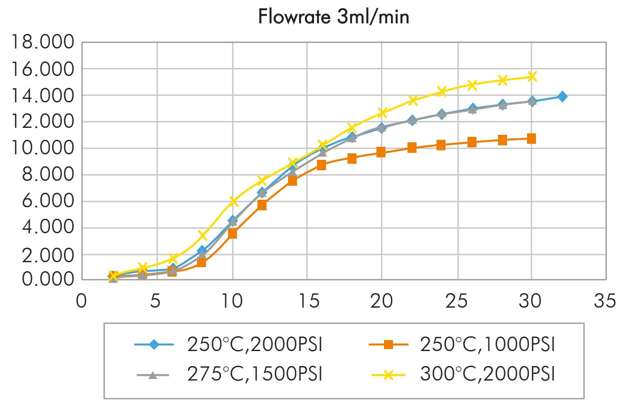

Figure 8. Reducing sugar accumulated with hydrolysis time in semi batch runs with 3 ml/min

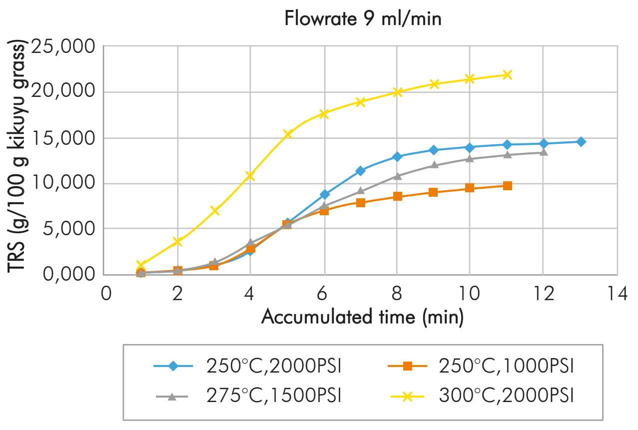

Figure 9. Reducing sugar accumulated with hydrolysis time in semi batch runs with 9 ml/min

Figures 8 and 9 shows that reaction times for runs with water flowrates of 3 ml/min and 9 ml/min were around 30 and 12 min whereas highest RS yields of 15% and 22%, were obtained at 300 °C and 2 000 psi, respectively. The maximum yield of 22% is considerably higher than the yield obtained by batch hydrolysis at 300 °C, which could be attributed to reducing sugar decomposition in comparison to the rapid removal and cooling of formed RS in the semi batch reactor setup. As also shown by Prado (2014), the higher the flowrate the higher the RS yield, probably due to the lower residence time in the reactor, which decreases the time for sugar degradation. Table 4 shows a comparison of reaction conditions and RS yields reported for semi batch subcritical hydrolysis experiments with different raw materials (wet basis). Obtained yield of 22% through semi batch experiments is similar to figures reported in other studies that utilize biomass sources with higher cellulose and hemicellulose fractions.

It is well known that produced reducing sugars, either in semi batch or batch reactor setups, are prone to degradation, raising concerns not only for productivity loss but also for the formation of by products such as furfural, hydroxymethyl furfural, acetic acid, acrylic acid, formic acid, glycolic acid, to name only a few, that could prevent the fermentation process as it is usually carried out (Mussatto & Roberto, 2004). Fermentability of produced sugars by subcritical water has been addressed in a few studies. For example, Abdelmoez *et al.* (2014) studied the subcritical hydrolysis of wheat straw, and under optimum hydrolysis conditions obtained 51.5 wt% RS, from which 3.2% were glucose, 7.6% xylose and the rest other polysaccharides. A hydrolysate sample was fermented by using *Saccharomices Cerevisiae* baker yeast, and ethanol production was higher than the theoretical expected from glucose only, which was attributed to the presence of glucose oligomers not accounted for, or the fermentation of some of the xylose.

Decomposition of lignin produces phenolic compounds which strongly inhibit the action of yeast (Schacht *et al.*, 2008). In order to assess the formation of acidic degradation byproducts, the pH of hydrolysates was taken every two minutes and was also measured, and it was found to decrease with the increase of the total time in semi batch runs as shown in Figure 10 for runs carried out with a water flowrate of 9 ml/min.

Table 4. RS yield from different lignocellulosic materials by semi batch subcritical hydrolysis

Raw material Cellulose/hemicellulose	Reaction conditions	RS yield concentration	Reference
Kikuyo grass (this work) 26.9%/26.2%	300 °C, 3 190 psi, 9ml/min	22%	This work
Sugar cane bagasse 35%, >30%	194-214°C, 2900 psi, 33 ml/min	23%	(Prado et al., 2014)
Coconut husk	259 °C, 2 900 psi, 33ml/min	11.7%	(Prado et al., 2014)
Palm fiber	250 °C, 2175 psi	23%	(Cardenas-Toro et al., 2014)

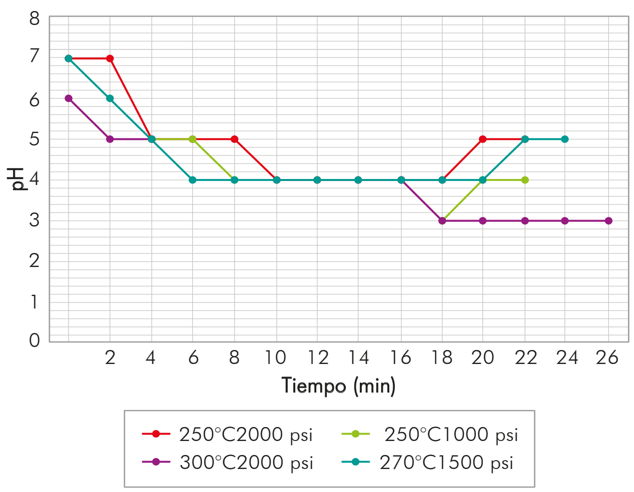

Figure 10. pH of hydrolysates by semi batch hydrolysis with flowrate of 9 ml/min

In Figure 10 can be observed that pH rapidly drops with the semi batch total reaction time, reaching a minimum of 3 in the run carried out at 300°C and 2 000 psi, which is also the run with the highest RS yield. Yet, degradation byproducts, not only from sugars degradation but also from lignin and compounds derived from the cellulosic structure, are not an issue exclusive to subcritical or supercritical hydrolysis experiments, but also to conventional acid catalyzed processes. In this regard, several biological, physical and chemical technologies have been proposed to detoxify or reduce the concentration of these compounds in lignocelllosic hydrolysates, as extensively reviewed in literature (Mussatto & Roberto, 2004).

4. CONCLUSIONS

- Hydrolysis using subcritical water in batch and semi batch configurations was applied in order to assess the effect of temperature, biomass to water mass ratio, pressure and water flowrate in the yield of reducing sugars obtained from cellulose and hemicellulose fractions in Kikuyo grass. A maximum yield of 9.7 % was measured at 300 °C, 30:1 water to biomass ratio and 3 190 psi in batch experiments, whereas a yield of 22 % was measured at 300°C, 2 000 psi and water flowrate of 9 ml/min in semi batch experiments. ANOVA analysis showed only the interaction between temperature and mass ratio to be significant, whereas the pressure had no significant effect. Semi batch experiments with water flow rates of 3 and 9 ml/min showed that the higher the flow rate the higher the RS yield, and a maximum of 22% was measured at 300 °C, 2 000 psi and 9 ml/min. Degradation acidic byproducts formation was verified by means of pH measurements, and it was found that pH rapidly decreases with time in semi batch experiments up to 3.

- The results indicate that semi batch subcritical hydrolysis of Kikuyu grass, an abundant lignocellulose residue currently discarded in sanitary landfills without any utilization, is an efficient technology to produce hydrolysates with a high concentration of reducing sugars. The issue still remains of the fermentation inhibitory effect of sugars and lignin decomposition products which needs to be further addressed.

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