Production of bioethanol from rice husk pretreated with alkalis and hydrolyzed with acid cellulase at pilot scale

IOP Conf. Series: Journal of Physics: Conf. Series **1126** (2018) 012034 doi:10.1088/1742-6596/1126/1/012034

Production of bioethanol from rice husk pretreated with alkalis and hydrolyzed with acid cellulase at pilot scale

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Abstract. Rice husk (RH) is the main biomass byproduct of the rice industry, so much that it corresponds to 20% of the total cereal production and is generally considered a waste. Due to its content of cellulose and hemicellulose, the RH has a great potential for its alternative use in the production of bioethanol, since it has a great advantage with respect to the conventional raw materials used (sugar cane, corn, beet and sweet sorghum, among others), given that this lignocellulosic material is abundant and does not compete with the food industry. The objective of this work was to obtain bioethanol from rice husk at a pilot scale, taking advantage of its cellulose and hemicellulose content. For this, a sample of 450g of RH was taken, which was pretreated with a 2.00% w/v NaOH solution and subsequently hydrolyzed with acid cellulase (CFB3S). The alcoholic fermentation of total reducing sugars (TRS) obtained in the enzymatic hydrolysis was carried out with *Saccharomyces cerevisiae*, finally, the fractional distillation of the must was carried out, by means of which four bioethanol samples were obtained with concentrations of 71.3% v/v, 30.0% v/v, 10.3% v/v, and 5.3% v/v of alcohol, which when mixed produced a sample of 95mL of solution with a concentration of 27% v/v alcohol (4.70g/100g of RH).

1. Introduction

The treatment of solid waste that produces environmental pollution is one of the most important issues today, since we should look for suitable forms for its handle and final disposal, in such a way that do not affect the environment. At present, organic materials with a high content of lignin and cellulose are no longer considered waste byproducts, but have become the raw material of multiple industrial processes such as the production of fuel alcohol, although many complications occur when obtaining ethanol from lignocellulosic tissues that complicate the degradation of biomass [1]. The incorporation of lignocellulosic materials in the elaboration of new products is a trend that gains more strength, since products similar to those coming from commercial raw materials are obtained, and which are also friendly to the environment [2]. The lignocellulosic residues generated in the processing of rice, such as husk and chaff, which is the waste left in the collection site, are considered materials of little value and in some cases are a waste.

It is important to highlight the economic and social influence of rice cultivation in Colombia, since more than 28000 farmers and their families directly depend on it. Rice cultivation is one of the few short-cycle crops that has remained stable despite the variability of Colombian agriculture [3]. The annual production of rice is more than two million tons, generating as a byproduct around 400000 tons of husk. In Colombia, the burning of this waste is usually a quick strategy for its elimination, however,

the negative environmental impact that is generated must not be ignored [4], due to the fact that, during the combustion process of the rice husk (RH), high levels of greenhouse gases are emitted [5].

RH has a high content of polysaccharides such as cellulose and hemicellulose, so this is considered a valuable resource in the production of sugars that can be fermented [4]. Because the national production of rice is considerable, large quantities of husk are obtained that currently have little use, therefore, it is important to find other applications, in addition to their use as fuel for the ovens, among them, their use as raw material for the production of sugars and its subsequent fermentation and distillation to obtain bioethanol.

The literature reports obtaining bioethanol from RH using *Kluyveromyces marcianus* CK8 [5]. With respect to the use of the waste generated during the production of rice, it is found the use of these residues to obtain sugars [6,7], obtaining a carbonated alcohol beverage [4] and obtaining lactic acid [8], among others. Kinetic analysis of the thermal decomposition of four lignocellulosic biomasses including rice husk [9] and a literary review of the synthetic silica production pathways, focusing on the possibility of using rice husk ash as a possible raw material for reinforcement of polymeric materials [10] are also reported.

In San José de Cúcuta (Norte de Santander) there are several companies that process and market rice, among them Coagronorte Ltda, Arrocera Agua Blanca S.A., and Arrocera Gelvez SAS, among others, which generate as a byproduct a large amount of husk. These companies generally use the RH as fuel for the ovens, generating problems for the environment due to the emission of polluting smoke. With this, it is inferred that these companies have no established plans to use or adequately dispose of the large quantities of husk that are produced. For this reason, the proposal was made to produce bioethanol from this agroindustrial waste, taking advantage of its content of cellulose and hemicellulose, which are broken down into sugars through enzymatic hydrolysis with acid cellulase (CFB3S) and subsequent fermentation of sugars obtained for the production of alcohol. Initially, bioethanol was obtained on a laboratory scale in order to determine the appropriate conditions, to then produce it on a pilot scale.

2. Materials and methods

2.1. Obtaining bioethanol from RH at laboratory scale

2.1.1. Pretreatment of the sample. This stage was carried out in a Bioflo®/celliGen®115 Fermenter & Bioreactor with a maximum capacity of 5.0L. For pre-treatment of the RH, three batches of 300.0g of husk were weighed, each of which was mixed with 4.0L of NaOH (Merck) solution at 2.00% w/v for two hours, the mixture was made at 70°C and 400rpm, the method was modified in terms of temperature (85°C) compared to what was reported by [6] and [7], because the maximum temperature reached by the Bioflo®/celliGen®115 is 70°C.

2.1.2. Enzymatic hydrolysis. To carry out the enzymatic hydrolysis process, exact quantities around 10.00g of pretreated RH were measured, these were placed in 250mL Erlenmeyer flasks with cotton stoppers, to which were added sodium citrate buffer solution (pH 5.00), in a ratio of 10g of RH pretreated by 100mL of buffer [11]. The Erlenmeyer flasks were placed in a Multipurpose Digital Shaker OrbitTM 1000, with 120 rpm shaking, this assembly was taken to a drying oven at 55°C and when the temperature was reached, 2.5mL volumes of acid cellulase enzyme (CFB3S) were added, the enzymatic treatment was carried out in triplicate. During the hydrolysis process, the concentration of total reducing sugars (TRS) was monitored at time intervals of 0.5, 24, 48 and 72 hours, in order to establish the adequate time for enzymatic hydrolysis.

2.1.3. Analysis of total reducing sugars (TRS) by the DNS method. For the analysis of the TRS in the samples obtained during the process of enzymatic hydrolysis on a laboratory scale, 1.00mL of the supernatant of each Erlenmeyer was collected at times of 0.5, 5, 24, 48 and 72 hours, which were transferred to plastic vials of 1.5mL and allowed to decant for 10 minutes for further analysis by the

DNS method (3,5-dinitrosalicylic acid). The analysis of the samples was carried out in the Dinko 2300 II spectrophotometer at a wavelength of 540nm. The conditions for the enzymatic hydrolysis were set according to the technical sheet of the acid cellulase enzyme (CFB3S), supplied by Sunson Industry Group Co. Ltd., where they establish the optimum pH range of 4.50-5.50 and the optimal temperature between 45 and 65°C, so the average value of the pH and temperature ranges, respectively, was used.

2.1.4. Fermentation of glucosed syrups. The fermentation of the glucosed syrups obtained with the enzymatic hydrolysis was carried out in two stages, first the reproduction of the yeast Saccharomyces cerevisiae, in order to obtain the greatest amount of biomass possible, this stage goes until the obtaining of acetaldehyde and the carbon dioxide liberation; and then alcoholic fermentation, in which acetaldehyde is reduced by the action of dehydrogenase to ethyl alcohol [12].

The reproduction stage of the biomass is very important because if the fermentation starts too early, the population will not be large enough to obtain a good conversion rate to ethanol [13]. For this reason, oxygen was supplied to the syrups obtained in the enzymatic hydrolysis, by agitation of 200rpm, and the cell count was performed by Neubauer chamber using a Leica DM500 microscope, to plot a growth curve of Saccharomyces cerevisiae, in order to establish the approximate time in which biomass production ends. On the other hand, to determine the approximate time of the alcoholic fermentation, the syrups were left to rest without agitation; during this period, a daily measurement of the Brix degrees was made to the samples using an automatic digital refractometer ATAGO RX-007 α, and at the time they remained stable, the time of the alcoholic fermentation was determined.

2.1.5. Preparation of glucosed syrups to obtain alcohol. Once the adequate conditions were established, both to obtain the highest concentration of TRS during the enzymatic hydrolysis and the time required to carry out the alcoholic fermentation, the syrups were again prepared in triplicate starting from three samples of 30.00g of RH pretreated with NaOH 2.00% w/v, mixed with 300mL of sodium citrate buffer solution pH 5.00 and 7.5mL of acid cellulase enzyme (CFB3S). After the time previously determined for enzymatic hydrolysis, the glucosed syrups were filtered. Subsequently, each syrup was added 0.750g of urea and 0.750g of ammonium hydrogen phosphate, to ensure the presence of sufficient nitrogen and phosphorus for the metabolism of the yeast [14].

Finally, the syrups were sterilized in an autoclave at 120°C and 15psi for 20 minutes, in this way the syrups free of microorganisms that could alter the fermentation process were obtained. Three inocula of yeast (*Saccharomyces cerevisiae*) active commercial Levapan mark were prepared, mixing in each 0.300g in 10.00mL of distilled water (40-50°C) previously sterilized, since the concentration of yeast in each syrup should be 1g/L [14]. The cell concentration of the inocula was determined by means of the cell count by Neubauer chamber, later each inoculum of yeast was added to the respective 300mL of syrup. After the yeast reproduction stage, the syrups were left to rest for several days in a tray with water at 28°C±2, making a daily measurement of the Brix degrees, when these stabilized the alcoholic fermentation was finished. The syrups were shaken in a shaker at 200rpm to start the yeast reproduction stage and perform the cell count by Neubauer chamber. A curve of growth of *Saccharomyces cerevisiae* was plotted, in order to establish the time of reproduction of the yeast, for these measurements were made every hour during 12 hours and some at 24 hours, taking samples of 1.00mL of the syrups which were diluted to 10^{-1}, to perform the count under the microscope on the 40X objective. After the stage of reproduction of *Saccharomyces cerevisiae*, the syrups were left at rest for several days in a tray with water at 28°C±2, the Brix degrees were measured daily using an automatic digital refractometer ATAGO RX-007 α, and when these remained established the alcoholic fermentation was finished.

2.1.6. Distillation of alcohol. After the alcoholic fermentation, the alcohol was obtained by simple distillation. The alcohol content expressed in %v/v was measured with the METER TOLEDO Densito30PX densitometer.

2.2. Obtaining bioethanol from RH on a pilot scale

The methodology for obtaining bioethanol from the RH at pilot scale was the same as that of the laboratory scale. To carry out the enzymatic hydrolysis process, exact quantities were measured around 450.00g of pre-treated RH, to which sodium citrate buffer solution (pH 5.00) was added, in a ratio of 1:10 of buffer [11]. It was carried out at a temperature of 55°C, residence time of 24 hours and agitation of 350 rpm (according to the Bioflo® conditions). When the temperature of 55°C was reached, volumes of 2.5mL of acid cellulase enzyme (CFB3S) were added per 10g of pretreated RH, the enzymatic treatment was carried out in triplicate.

At the end of the enzymatic hydrolysis time, the nutrients were added to the glucose syrup for a concentration of 0.25% w/v each (11.250g of urea and 11.250g of ammonium hydrogen phosphate), sterilized in autoclave at 120°C, 15psi, for 20 minutes and allowed to cool to room temperature. A yeast inoculum Saccharomyces cerevisiae was prepared, mixing 4.500g in 150.0mL of distilled water (temperature 40-50°C) to have a yeast concentration of 1g/L [14] and then added to the glucose syrup content in the Fermenter & Bioreactor. The fermentation process was carried out taking into account the conditions established at the laboratory scale.

After the time required for the fermentation, the distillation of the must was carried out in a fractional distillation equipment with interchangeable columns PS-DA-490/EL, which uses a packed column of 100cm height, in order to obtain a better separation of alcohol and therefore better performance. To the distillate obtained, the alcohol content expressed as %v/v was measured, using the METER TOLEDO Densito30PX densitometer.

3. Results

3.1. Obtaining bioethanol from RH at laboratory scale

3.1.1. Pretreatment of the sample. For the chemical pretreatment, an RH sample of 300.00g was taken, after the pretreatment process, drying to the environment, grinding and sieving, a sample quantity of 129.71g was obtained. According to these results, the performance of the process was 43.24%.

3.1.2. Analysis of total reducing sugars (TRS) by the DNS method. The monitoring of TRS concentration (g/L) by the DNS method during enzymatic hydrolysis was performed at times of 0.5, 5, 24, 48 and 72 hours. The results obtained can be seen in Table 1. Figure 1 shows a curve of TRS concentration as a function of time.

Table 1. Average concentration of TRS obtained with respect to time during enzymatic hydrolysis.

Time (Hours)	TRS (g/L)	Std Dev
0.5	3.671	0.011
5	5.521	0.002
24	7.778	0.001
48	7.693	0.001
72	7.746	0.001

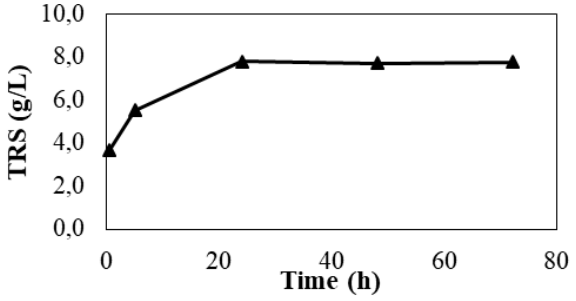

Figure 1. Concentration of TRS respect to time during enzymatic hydrolysis.

According to the results shown in Table 1 and Figure 1, the highest concentration of TRS in g/L in the samples hydrolyzed enzymatically was obtained in a time of 24 hours of hydrolysis (7.778g TRS/L), and from this time the concentration tended to remain stable.

3.1.3. Fermentation of glucosed syrups. The fermentation stage was developed with the glucosed syrups obtained by the treatment of 2.5mL enzyme per 10.00g of RH. For this, three yeast inocula (Saccharomyces cerevisiae) were prepared so that their concentration in each syrup was 1g/L. The cell concentration of each inoculum was determined by Neubauer chamber using the Leica DM500 microscope, with an average count of $2.62x10^9$cells/mL. This concentration of yeast was added to each of the glucose syrups, a cell count was performed every hour for 12 hours and at 24 hours, in order to estimate the time in which the yeast stopped reproducing, to establish the time of yeast reproduction. The growth graph of the yeast was elaborated with respect to the time observed in Figure 2.

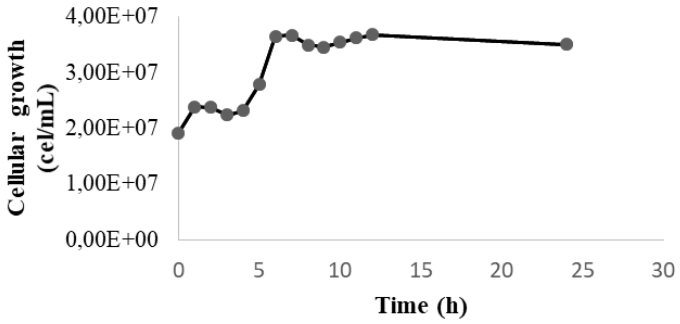

Figure 2. Growth of *Saccharomyces cerevisiae* in the fermentation stage.

According to Figure 2, the concentration of *Saccharomyces cerevisiae* in glucosed syrups, initially (zero hours) is $1.92x10^7$cells/mL, it is observed that after one hour it increases to $2.39x10^7$cells/mL, and at six hours reached its maximum growth, with a cell concentration of $3.65x10^7$cells/mL, so it was established that the stage of reproduction of the yeast, should be performed for six hours, therefore, the syrups should be in shaking at 200rpm during this time. After the aerobic stage, the glucose syrups were left to rest without agitation and the Brix degrees were measured daily for 7 days, in order to observe when they stabilized, in order to establish the time of the resting stage in the fermentation, to finally proceed to the alcohol distillation stage. According to the results shown in Table 2, the Brix degrees tend to remain stable after six days of rest, so this time, which is necessary to carry out the alcoholic fermentation stage, was established.

After the alcoholic fermentation, the musts were filtered and by simple distillation volumes of 50mL of distillate were obtained, to which the alcohol concentration (%v/v) was measured with the METER TOLEDO Densito 30PX densitometer, with an average concentration 3.7% v/v alcohol.

3.2. Obtaining bioethanol from RH on a pilot scale

The enzymatic hydrolysis was performed with a sample of 450.00g of RH pretreated with NaOH solution 2.00% w/v, mixed with 4.5L of sodium citrate buffer solution pH 5.00 and 112.5mL of acid cellulase enzyme (CFB3S), to maintain the proportions of 2.5mL of enzyme per 10g of husk. Once the enzymatic hydrolysis process was finished and the nutrients [$(NH_4)_2HPO_4$ and $(NH_2)_2CO$] were added, the glucosed syrup was sterilized in an autoclave and the inoculum was added (cell suspension of 4.500g of dry active yeast commercial Levapan brand in 150mL of distilled sterilized water). In this way, the aerobic stage of fermentation was started by agitation at 200rpm. After six hours of aerobic stage and six days in rest state, the obtained must was filtered and the bioethanol was separated by fractional distillation, obtaining four samples of distillate, the first of 30mL with a concentration of 71.3% v/v of alcohol. Table 2 shows the volumes obtained from each distillate sample and their respective concentrations.

Table 2. Data of the samples obtained in the fractional distillation process.

Sample number	Volume (mL)	Concentration of alcohol (% v/v)
1	30	71.3
2	20	33.0
3	20	10.3
4	25	5.30

Finally, the four distillate samples were mixed, and the concentration was 27.0% v/v alcohol. According to [11] the theoretical yield is 30g ethanol/100g of RH, based on the calculation of the 95mL of mixture of the samples obtained in the fractional distillation, whose concentration was 27.0% v/v of alcohol, the percentage conversion of RH to bioethanol (Equation (1)) and the yield of the bioethanol production process (Equation (2)) were calculated. The calculations are shown below (Equations (1) to (4)):

$$\%\ Conversion = \frac{g\ of\ bioethanol}{g\ rice\ husk} * 100 \tag{1}$$

$$R = \frac{real\ g\ of\ bioethanol}{theoretical\ perfomance} * 100 \tag{2}$$

$$\%\ Conversion = \frac{20.2379 g\ of\ bioethanol}{430.47 g\ rice\ husk} * 100 = 4.70\% \tag{3}$$

$$R = \frac{4.70 g\ of\ bioethanol}{30 g\ of\ bioethanol} * 100 \tag{4}$$

According to the percentage of conversion obtained, for every 100g of RH, 4.70g of bioethanol are produced; when comparing this with the theoretical value (30g of bioethanol/100g of RH), it was found that the efficiency of the process was 15.67%, which indicates that the process of obtaining bioethanol made in this work was 6.10% more efficient, than that made by [11], whose yield was 9.57% when obtaining bioethanol from RH pretreated with 6.25% w/v NaClO by simultaneous hydrolysis and fermentation.

In accordance with the result obtained (for each 450.0g of RH, 95mL of bioethanol at 27.00% v/v are produced) it can be inferred that when processing a ton of RH, could be produced 211.11L of ethanol at 27.00% v/v, therefore, 59.37L of 96% v/v ethanol would be obtained, and when comparing this result with the yield of ethanol from sugar cane (70.0L/ton) [15], producing ethanol from RH on a large scale, could be considered as an alternative of interest.

4. Conclusions

During the process of enzymatic hydrolysis of the pretreated RH, using the acid cellulase enzyme (CFB3S), it was found that the highest concentration of TRS in the samples was 7.788g TRS/L, obtained in a time of 24 hours of hydrolysis. After the alcoholic fermentation of the glucosed syrups obtained, at a laboratory scale, a distillate with a concentration of 3.7% v/v was achieved by simple distillation.

For the pilot-scale process, 450.00g of RH pretreated with NaOH solution 2.00% w/v (430.47g dry base) were treated, with the subsequent enzymatic hydrolysis with acid cellulase (CFB3S), the alcoholic fermentation of the sugars reducers obtained in the hydrolysis, and fractional distillation of the must, it was possible to obtain four samples of bioethanol with concentrations of 71.3, 30.0, 10.3, and 5.3%v/v of alcohol, which when mixed produced a sample of 95mL of solution with a concentration of 27%v/v of alcohol (4.70g/100g of RH), therefore, taking as a reference the theoretical value of 30g ethanol/100g of RH, a yield of 15.67% was obtained.

References

[1] Riaño A, Morales A, Hernández J and Barrero C 2010 Producción de bioetanol a partir de subproductos agroindustriales lignocelulósicos *Tumbaga*, **1** 61-91
[2] Vargas J, Alvarado P, Vega-Baudrit J and Porras M 2013 Caracterización del subproducto cascarillas de arroz en búsqueda de posibles aplicaciones como materia prima en procesos *Revista Científica* **23** 1-15
[3] Fuentes C, Segundo A, Granados J and Piedrahita W 2006 *Flora arvense asociada con el cultivo del arroz en el departamento del Tolima-Colombia* (Bogotá: Universidad Nacional de Colombia)
[4] Proaños J and Castro Y 2014 Evaluación de la producción de ácido láctico a partir de cascarilla de arroz por Lactobacillus del brueckii *Revista Mutis* **4** 7-18
[5] Nachaiwieng W, Lumyong S, Yoshioka K, Watanabe T and Khanongnuch C 2015 Bioethanol production from rice husk under elevated temperature simultaneous saccharification and fermentation using *Kluyveromyces marxianus* CK8 *Biocatalysis and Agricultural Biotechnology* **4** 543-549
[6] Zhang Q and Cai W 2008 Enzymatic hydrolysis of alkali-pretreated rice straw by Trichoderma reesei ZM4-F3 *Biomass and Bioenergy* **32** 1130-1135
[7] Zhang Q, He G, Wang J, Cai W and Xu Y 2009 Two-stage co-hydrolysis of rice straw by Trichoderma reesei ZM4-F3 and Pseudomonas aeruginosa BSZ-07 *Biomass and Bioenergy* **33** 1464-1468
[8] Collaguazo Simbaña P E 2011 *Estudio de la tecnología para la obtención de una bebida alcohólica carbonatada a partir de la degradación de la cascarilla de arroz (oryza sativa) tratada enzimáticamente con hemicelulasa* (Ambato: Universidad Técnica de Ambato Ecuador)
[9] Ordóñez Y, Tannous K 2017 Análisis cinético de la descomposición térmica de biomasas aplicando un esquema de reacciones paralelas independientes *Revista UIS Ingenierías* **16** 119-28
[10] Uribe N, Echeverry C, Betancur M, Zapata L, Martínez J 2018 Possibilities of rice husk ash to be used as reinforcing filler in polymer sector-a review *Revista UIS Ingenierías* **17** 127-42
[11] Piñeros Y and Otálvaro A 2010 Evaluación de la producción de etanol a partir de cascarilla de arroz pre-tratada con NaClO, mediante hidrólisis y fermentación simultaneas *VIII Congreso Nacional de Biotecnología y Bioingeniería, VII Simposio Internacional de Producción de Alcoholes y levaduras* (México: Sociedad Mexicana de Biotecnología y Bioingeniería)
[12] Karlson P 2014 *Introduction to Modern Biochemistry* (California: Elsevier Science)
[13] Montenegro Vega Y N and Peñaranda Fuentes A 2015 *Obtención de etanol a partir de residuos de madera utilizando sistema biológico (Aspergillus niger - Saccharomyces cerevisiae) con pretratamiento con ultrasonido* (Cucuta: Universidad Francisco de Paula Santander)
[14] Benítez L, Tovar C, Ortiz Á, Dunoyer A, Alvear M and Castillo C 2010 Producción de bioetanol a partir de la fermentación alcohólica de jarabes glucosados derivados de cáscaras de naranja y piña *Revista. Educación en Ingeniería* **5** 120-125
[15] Argote F E, Cuervo R A, Osorio E, Delgado J and Villada H S 2015 Evaluación de la Producción de Etanol a partir de melaza con cepas nativas saccharomyces cerevisiae *Revista Biotecnología en el Sector Agropecuario y Agroindustrial* **13** 40-48