Journal of Multidisciplinary Cases
ISSN: 2799-0990
Vol: 02, No. 04, June- July 2022
http://journal.hmjournals.com/index.php/JMC
DOI: https://doi.org/10.55529/jmc.24.1.7

Bioethanol Production from Rice Husk through SHCF and SSCF Processing Strategies

Onuguh I.C[1*], Ikhuoria E.U[2], Obibuzo J.U[3]

[1]Department of Chemistry, Igbinedion University Okada, Edo State Nigeria.*
[2]Department of Chemistry, University of Benin, Benin City Nigeria.
[3]Department of Biochemistry, Nigerian Institute for Oil Palm Research, Edo State Nigeria.

Corresponding Email: [1]acoibenzinno@gmail.com*

Received: 06 March 2022 **Accepted:** 20 June 2022 **Published:** 29 July 2022

Abstract: ***In many developing countries, there is great quest for achieving sustainable energy from the conversion of the huge biomass of organic wastes into useful biofuels such as bioethanol. Bioethanol is a renewable clean-liquid biofuel produced by fermentation of sugars or converted starch or cellulose from plant based feedstocks. It is conventionally produced from sugar and starch containing feedstocks. However, these feedstocks are unable to meet the global demand of bioethanol production due to their primary food value and legal pursuits against the legitimacy of their schemes. This study investigated and improved on the feasibility of producing bioethanol from rice husk agro-waste generated from rice production. It was first subjected to different physico-chemical pretreatments in order to optimize the hydrolysate sugar yield and identify the most effective process. It was further hydrolyzed by cellulase enzymes from Trichoderma ressei micro-organism isolated from the soil. Separate hydrolysis and co-fermentation (SHCF) and simultaneous saccharification and co-fermentation (SSCF) strategies/methods were adopted using both hydrometer and Pycnometer measurements. The fermentation results revealed that the maximum bioethanol yield through SHCF and SSCF strategies were 4.64 and 6.45 (% w/v dry biomass) respectively. SSCF strategy was more effective as it gave better bioethanol yield and was less time consuming. This study also shows that rice husk agro-waste of no or little commercial value can be utilized in the production of good quality bioethanol with implications for improved waste management, income and efficient energy generation.***

Keywords: ***Bioethanol, Lignocellulosic Materials, Co-Fermentation, SHCF, SSCF, Hydrolysate Sugars, Physico-Chemical Pretreatment, Rice Husk Agro-Waste.***

1. INTRODUCTION

Energy consumption has steadily increased over the years with increase in population and industrialization. A liquid transportation biofuel bioethanol now represents a key contributor to the energy profile of most developed countries. Its demand has increased as a result of

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diminishing reserves of crude oil and environmental problems related to burning fossil fuels (Riungu et al., 2014).

Bioethanol is a fermentation derived alcohol that is obtained from carbohydrate materials as opposed to synthetically produced alcohol from petroleum sources (Graeme, 2010). It has the characteristics of being colourless, volatile and flammable making it a good biofuel that can be blended with petrol in any percentage. It is the world's leading transportation biofuel and is mostly produced from starch (US) and sugar (Brazil) feedstocks. However, the future lies with more sustainable substrates like agricultural/food wastes and woody biomass that do not compete with human food chain (Graeme, 2010). The main advantage of bioethanol over petrol is that it is renewable and it is not a net contributor to greenhouse gas emissions. This is due to the fact that the biomass cultivated for bioethanol is able to re-fix by photosynthesis the carbon dioxide produced during bioethanol production and combustion (Sun and Cheng, 2002).

In Nigeria, agricultural and food wastes are most often wrongly disposed in open dumps or drainage systems causing unpleasant odour, flooding and providing a breeding ground for diseases-carrying organisms. Solid wastes from food and fruit peels are largely generated from the daily activities of hotels, restaurants, juice processing houses etc. The piles of rice husks produced and neglected in Abakaliki Nigeria since the early 1960s were compared with pyramids in Egypt (Daily Trust News, 29th Sept., 2012). They portend great potential for the bioethanol industries. Managing these wastes by utilizing them in bioethanol production will imply clean environment, income and efficient energy generation.

Second generation bioethanol feedstocks are lignocellulosic bioethanol feedstocks that can be pretreated and hydrolysed to common fermentable sugars. Lignocellulosic complex is the most abundant biopolymer in the Earth comprising of about 50% of the world biomass (Claassen et al., 1999). Lignocellulosic biomass in the form of wood and agricultural residues (like rice husk) is virtually inexhaustible, since their production is based on natural photosynthetic process. It was estimated that terrestrial plants produce about 1.3×10^{10} metric tons per annum which is energetically equivalent to about two-thirds of the world's energy requirement (Kim and Yun, 2006). Lignocellulosic materials contain three primary constituents which are cellulose, hemicellulose and lignin. Cellulose and hemicellulose are carbohydrates that can be broken down by enzymes, acids, or other compounds to simple sugars, and then fermented to produce bioethanol (Graeme, 2010).

Four main steps are needed to make bioethanol from lignocellulosic materials. They are pretreatment, enzymatic hydrolysis, fermentation and distillation.

i. Pretreatment: This is where biomass is subjected to milling, heat and chemicals to make it more digestible. The goal of the pretreatment process is to break down the lignin structure and disrupt the crystalline structure of cellulose so that the hydrolysis of carbohydrate to monomeric sugars can be achieved rapidly with greater yields (Harmsen et al., 2010). This could be achieved using physical, physicochemical, chemical, or biological treatment.
ii. Enzymatic hydrolysis or saccharification: Here, the carbohydrate polymers (cellulose and hemicellulose) are broken into their monomer sugars. This can be achieved with acids (dilute or concentrated) or cellulase enzymes (White et al., 2008).
iii. Fermentation: This is where sugars are fermented into bioethanol. Apart from hexoses, pentoses are equally present since hemicellulose accounts for approximately 25 to 40 percent of lignocellulosic material (Graeme, 2010). Co-fermentation refers to the fermentation of both five-carbon and six-carbon sugars to ethanol.

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iv. Bioethanol recovery: This is achieved through distillation and concentration of the product. Although the boiling point of ethanol, 78.3°C, is significantly lower than the boiling point of water, 100°C, these materials cannot be separated completely by distillation. Instead, an azeotrope mixture (i.e. a mixture of 95% ethanol and 5% water) is obtained, and the boiling point of the azeotrope is 78.15°C. For blending with gasoline, purity of 99.5 to 99.9% is required, to avoid separation. Currently, the most widely used purification method is a physical absorption process using molecular sieves (Graeme, 2010).

This research work is aimed at (1) producing bioethanol as an environmentally friendly and cost effective energy source from the reduced sugar hydrolysate obtained from rice husk agro-waste and (2) applying two fermentation processing strategies, SHCF and SSCF to produce bioethanol from rice husk and comparing their outcomes.

2. MATERIALS AND METHODS

Sample Preparation for Pretreatment

Rice husk was obtained from a processing mill at Abakiliki, Ebonyi state, Nigeria. It was dried under the sun prior to its utilization.

Physico-Chemical Pretreatment

Chemical hydrolysis was performed concurrently with mechanical comminution. 15g of rice husk was mechanically comminuted in a high speed blender with 100ml of 1% NaOH solution for one minute. Thereafter, the hydrolysate sugar content was analyzed using a high precision brix refractometer. Further hydrolysis was recorded when the broth was incubated for 10 minutes at 120°C after the first step mechanical comminution.

Measurement of Sugar Level

A high precision refractometer (Grand-index with automatic temperature compensation) was used to analyze the substrate's hydrolysate sugar levels in degree brix (Maroulis et al., 2003).
In a typical measurement, a drop of the sample was put on the glass surface of the refractometer and the sugar level subsequently determined. The results in degree brix were subsequently converted to weight of sugar using equation (1):
Weight of hydrolysate sugar (g/L) = °Brix x Specific gravity x 10 (Robert, 2003). (1)

The pH Adjustment and Sterilization

Before addition of any micro-organism to the pretreated samples, their pH values were adjusted to prevent the micro-organism from dying in a hyper basic condition. The pH of each pretreated biomass was adjusted to 5.0 in a bowl by adding required amount of 2.5 M H_2SO_4. Subsequently, samples were sterilized in an autoclave at a temperature of 120°C for 20 min and cooled to appropriate temperature before the introduction of microorganisms.

Separate Hydrolysis and Co-Fermentation of Hydrolysate

Hydrolysis was done to further degrade the polysaccharides present in the pretreated substrates into monosaccharides subunits in other to enhance the fermentation product yield by S. cerevisiae and P. stipitis. The substrate in the conical flask was inoculated with 25 ml Trichoderma ressi innoculum and was incubated in a shaking incubator at an agitation rate of 150 rpm at 45°C for

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48 hr. It was then filtered and the soluble hydrolysate sugar yield in the filtrate was measured using a refractometer. The specific gravity was also measured using the Hydrometer before fermentation. The filtrate was sterilized in an autoclave and was then subjected to co-fermentation by inoculation with 25 ml of S. cerevisiae and 25 ml of P. stipitis inoculums under aseptic condition. It was properly covered with cotton wool and was incubated on a shaker at an agitation rate of 150 rpm at 30°C for 72 hr. The fermented broth sample was filtered and the total sugar and bioethanol content was determined. This experiment was triplicated under the same conditions.
Bioethanol yield was calculated based on the density of alcohol distillate at 20°C and expressed in weight % (w/v) by Hydrometer and Pycnometer measurements using equations 2 and 3 respectively (Park, 2000; Hadeel et al., 2011; Igwe et al., 2012).

$$\text{Ethanol \% (w/v)} = \frac{126.582(OSG - FSG)}{OSG} \quad (2)$$

Where OSG is original specific gravity (specific gravity before fermentation), FSG is Final specific gravity (specific gravity after fermentation) and 126.582 is from the Specific gravity of water / Specific gravity of pure ethanol.

$$\text{Specific gravity of ethanol sample} = \frac{(X_2 - X_1)}{(X_3 - X_1)} \quad (3)$$

Where: X_1 is weight (g) of empty pycnometer, X_2 is weight (g) of pycnometer + sample and X_3 is weight (g) of pycnometer + water.
After specific gravity values were calculated, the percentage ethanol of each sample was determined using a standard ethanol density table (IUPAC, 1985).

Plate 1: Hydrometer measurements

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Plate 2: A Pycnometer

Simultaneous Saccharification and Co-fermentation of Hydrolysate
The pretreated substrate was simultaneously inoculated each with 25 ml of Trichoderma ressi, 25 ml of S. cerevisiae and 25 ml of P. stipitis inoculums under aseptic condition. It was incubated on a shaker at an agitation rate of 150 rpm at 38°C for 72 hr. This experiment was triplicated under the same conditions.-+

Ethanol Recovery
Samples were distilled in a rotary evaporator at a temperature of 85^{o}C for 3 hr. Distillates were dried overnight using 3A molecular sieves to absorb water molecules. They were decanted, filtered and redistilled to remove sieve dust and achieve absolute bioethanol (Mackenzie et al., 2015).

3. RESULTS AND DISCUSSION

Table 1: Total Hydrolysate Sugars (g/L) Achieved from Substrates after Pretreatment and Enzymatic Hydrolysis

Substrate	Sugar Weight (g/L) After Physico-chemical Pretreatment	Chemical Reagent Involved	Sugar Weight (g/L) After Enzymatic Hydrolysis	Maximum Hydrometer Reading(OSG)
Rice Husk	104.0± 0.023*	1% NaOH	141.3 ± 0.016*	1.054 ± 0.0014*

*= Standard deviation, OSG = Original Specific Gravity

Data from Table 1 show that enzymatic hydrolysis equally made significant contribution to the hydrolysate sugar weight of substrates though the physico-chemical pretreatment step was more contributory to the total hydrolysate soluble sugar realized. This could be due to the inhibitory effects of the sugars released during physico-chemical pretreatment on cellulase activities (Sun and Cheng, 2002).

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Fermentation

The sugar hydrolysates obtained were fermented to bioethanol by microbial co-cultures of Saccharomyces cerevisiae and Pitchia stipitis. Separate hydrolysis and co-fermentation (SHCF) and Simultaneous Saccharification and co-fermentation (SSCF) processing strategies were adopted. These strategies were adopted because Saccharomyces cerevisiae, which is the most widely used microorganism for bioethanol production, does not utilize pentose sugars. Pichia stipitis is a promising microorganism for this bioprocess since it can transform both pentose and hexose sugars into bioethanol, which is an important advantage since both kinds of sugars are present in hemicellulosic hydrolysates (Graeme, 2010).

The bioethanol yields as obtained from the hydrometer and Pycnometer readings are presented in Table 2. The total sugar content of substrates during the fermentation process decreased. This was noticed by decrease in the substrate's hydrometer readings after fermentation. Higher bioethanol yield was obtained from the substrate with SSCF process when compared with yield from SHCF process. This may be due to the synergistic action involved when the three involved fermentative micro-organisms were inoculated at the same time in SSCF process. Additionally, it could be due to the degradation of monomeric sugars obtained during enzymatic hydrolysis in SHCF before yeasts were inoculated to act on them.

Table 2: Bioethanol Yield at 20^{o}C from Hydrometer and Pycnometer Measurements

Substrate	**Hydrometer Readings**		**Pycnometer Readings**		**Bioethanol yield (%w/v)**			
	FSG SHCF	FSG SSCF	SG SHCF	SG SSCF	SHCF Hydro meter	SHCF Pycno meter	SSCF Hydro meter	SSCF Pycno meter
Rice husk	1.018 ±0.00 14*	1.003±0.0 016*	0.9933± 0.0004*	0.9909±0. 0003*	4.32	4.65	6.12	6.45

*=Standard deviation, SHCF = Separate Hydrolysis and co-fermentation, SSCF = Simultaneous Saccharification and Fermentation, FSG = Final Specific Gravity.

The difference in bioethanol yield of substrates measured using Hydrometer and Pycnometer readings in Table 2 were not significant and they served as a check for each other. Rice husk bioethanol yield of 6.45% w/v was a little greater than 6.0% w/v yield reported by Prasad et al. (2013) using Trichoderma isolates and S. cerevisiae.

4. CONCLUSION

The result from this study provided evidence that readily available rice husk agro-waste of no or little commercial value can be utilized in bioethanol production. It also revealed that (1) mixed cultures of Trichoderma reesei, Saccharomyces cerevisiae and Pichia stipitis through SSCF process resulted to a good synergistic fermentation yield better than other results from other workers and (2) SSCF process gave a better bioethanol yield and was less time consuming than SHCF process.

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5. REFERENCES

1. Claassen, P.A., van Lier, J.B., Stams, A.J. and Weusthuis R.A. (1999). Utilization of biomass for the supply of energy carriers. Applied Microbiology and Biotechnology, 52:741-743.
2. Graeme, M. W. (2010), Bioethanol: Science and technology of fuel alcohol. Graeme M. Walker &Ventus Publishing ApS. Pp. 10-85.
3. Hadeel, A., Hossain, A.B., Latifa, K., Abear, J. and Norah, A. (2011). Bioethanol fuel production from rambutan fruit biomass as reducing agent of global warming and greenhouse gases. African Journal of Biotechnology 10: 10157-10165.
4. Harmsen, P.F., Huijgen, W.J., Bermudez, L.M., and Bakker, R.R. (2010). Literature review of physical and chemical pretreatment processes for lignocellulosic biomass. Energy Research Centre of the Netherlands. P. 50-54.
5. Igwe, C.J., Agbaeze, E.K., Obike, I.A., Christopher, U. and Sonde, U.C. (2012). Extraction, characterization and determination of ethanol fuel composite qualities of Saccharum officinarum, Pennisetum purpureum and Costus afer. Asian Journal of Plant Science and Research, 2: 643-649.
6. International Union of Pure & Applied Chemistry (1985). A Standardization of methods for determination of the alcohol content of beverages and distilled potable spirits. Ed. Butter W., London. pp. 1-4.
7. Mackenzie, A., Kathryna B., Ivette C. and Gabriella M. (2015). Removing water from an azotropic ethanol-water mixture through adsorption. Retrieved May, 2017 from: http://www.sciencedirect.com/science/article/pii/S09060852410004475#
8. Maroulis, Z. B., Krokida, M. K., Karathanos, V. T., and Marinos-Kouris, D. (2003).Drying kinetics of some vegetables. Journal of Food Engineering, 59: 391-393.
9. Park, L.S. (2000). How do you take a hydrometer reading and what is it used for anyway? Midwest Home brewing and Winemaking Supplies. Retrieved July 10, 2016 from: www.midwestsupplies.com
10. Prasad, M., Savani, K., Intwala, V. and Chirag, P. (2013). In-vitro optimization of bioethanol production from agro wastes using Trichoderma sps. International Journal of Pure and Applied Bioscience, 1(6): 86-95.
11. Riungu, A., Salim, M., Njenga, J., Yusuf, O. and Walyambillah W. (2014). Bioethanol production from waste crops and crop residues. Nairobi, Kenya.
12. Robert, P., (2003), Wine Concentrate, the Australian & New Zealand Grapegrower and Winemaker 2003 Annual Technical Issue, No. 473a, p. 127.
13. Sun, Y. and Cheng, J. (2002). Hydrolysis of lignocellulosic materials for ethanol production: A Review. North Carolina State University, Raleigh, USA.
14. White, J.S., Yohannan, B.K. and Walker, G.M. (2008). Bioconversion of brewer's spent grains to bioethanol. FEMS Yeast Research, 8: 1175-1184.