RECOVERY OF BIOETHANOL FROM FOOD WASTE USING *SACCHAROMYCES CEREVISIAE*

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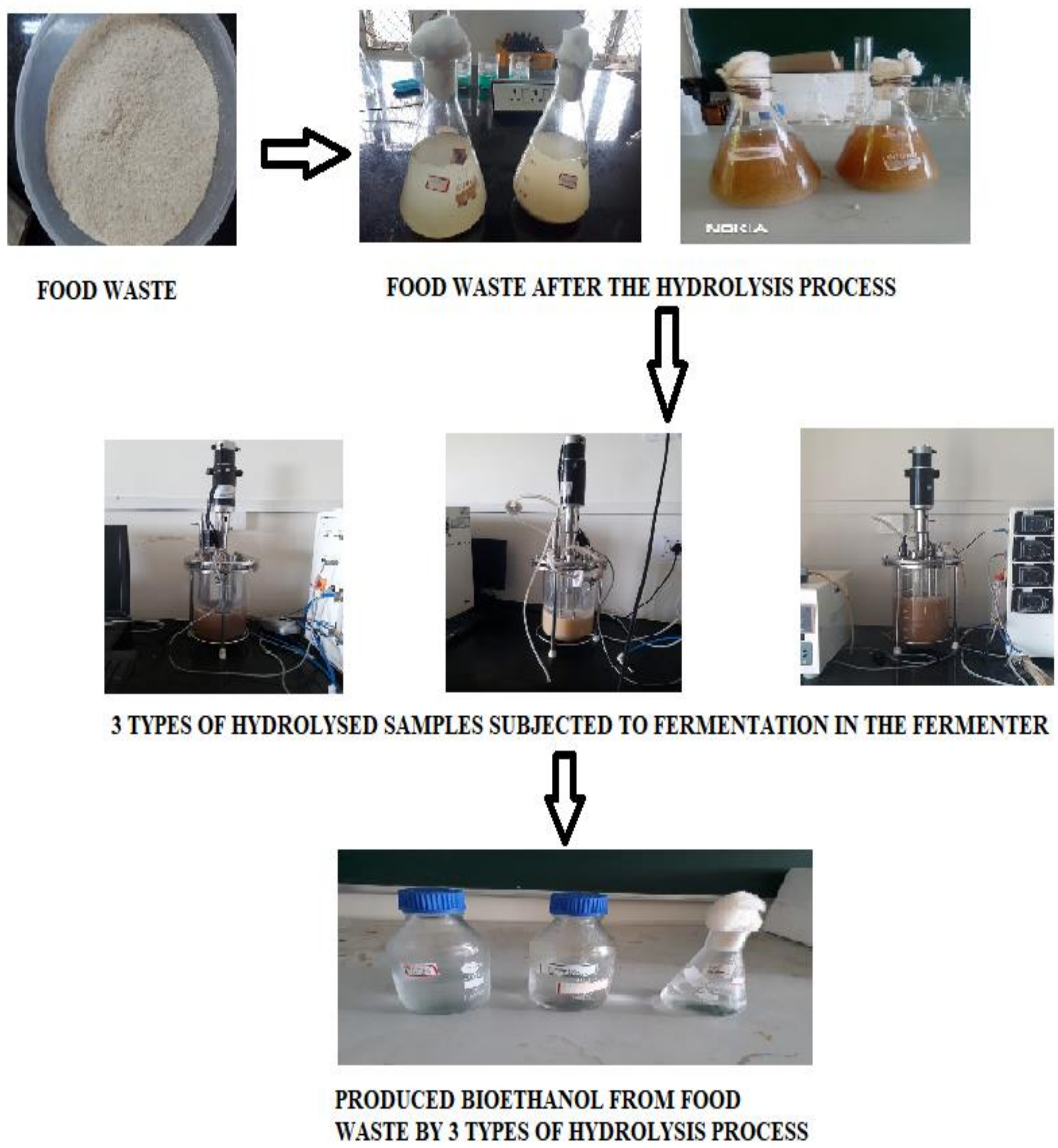
GRAPHICAL ABSTRACT



ABSTRACT

Excessive use of fossil fuels results in the rapid depletion of non-renewable fossil energy resources, a rise in fuel cost, and an uncontrolled emission of greenhouse gases which causes a severe threat to the environment. Bio-fuels are being scrutinized as substitutes for current high-pollutant fuels obtained from conventional sources. To meet the global demands, it becomes necessary to find an alternate source of fuel which is bioethanol.

In this work, a strategy to promote ethanol production from Leftover Cooked Rice (LCR) by comparing the different types of hydrolysis was proposed. Process integration comprised of mechanical pretreatment of the leftover cooked rice followed by hydrolysis which was then followed by fermentation. The food wastes of weight 50g taken in each of the 3 fermenters were subjected to acid hydrolysis, enzyme hydrolysis, and combined hydrolysis respectively. Commercially available Baker's yeast (*Saccharomyces cerevisiae*) was used for the fermentation process. The fermented samples were subjected to distillation to separate the bioethanol from them. The amount of bioethanol obtained from combined hydrolysis, acid hydrolysis, and enzyme hydrolysis was 400ml, 150ml, and 350ml respectively. Qualitative analysis of ethanol was done by using the Jones reagent. Hence, bioethanol can be produced from leftover cooked rice using the yeast *Saccharomyces cerevisiae*.

Keywords: bioethanol, leftover cooked rice, hydrolysis, *Saccharomyces cerevisiae*, qualitative analysis.

1. Introduction

Bio-fuel has been an energy source for human beings since ancient times. Excessive utilization of fossil fuels results in the rapid depletion of non-renewable fossil energy resources, a rise in fuel cost, and an uncontrolled emission of greenhouse gases, which causes a severe threat to the environment. This critical state has made it necessary to explore the substitutes for the high-pollutant fuels obtained from conventional sources. To meet the global energy demands, it becomes necessary to find an alternate source of fuel which is bioethanol.

The bioethanol that is produced from agricultural residues and forest residues is termed second-generation (2G) ethanol whereas the first-generation ethanol is produced from sucrose which is the juice extracted from sugarcane, sugarbeet, or sorghum [1]. Since the demand for food is not yet satisfied, second-generation ethanol production has gained more interest since these biofuels are produced from agricultural residues and waste biomass. The bioethanol produced from the waste emits a low amount of greenhouse gases when compared to conventional fuels [2]. The processes that are involved in bioethanol production are: pretreatment, hydrolysis, fermentation, and distillation

In Asia, rice is considered one of the important staple foods. Rice is wasted more than any other food and it accounts for 34% of the total wasted food [3]. The leftover cooked rice is rich in carbohydrates and therefore, it is considered a good feedstock for bioethanol production [4]. Starch in cooked rice is formed of glucose called amylase and amylopectin.

The main objective of the pretreatment is to rupture the lignin structure to enhance the ease of access of enzyme to the cellulose during the hydrolysis [5-8]. The natural structure of the lignocellulosic material cannot be affected efficiently by enzymatic hydrolysis. Therefore, the pretreatment step is essential for efficient hydrolysis of cellulose by removal of lignin and hemicelluloses, and reduction of the crystalline structure of cellulose.

Hydrolysis is the process where the cellulosic feedstocks are converted to ethanol [9]. Dilute acid hydrolysis is used to degrade the hemicelluloses which improves the enzymatic hydrolysis of cellulose and also help in weakening the glycosidic bond in the hemicelluloses and lignin-hemicellulose bond and lignin bond [10]. Enzyme hydrolysis is used to convert cellulose to glucose with the help of cellulose enzymes. Its efficiency is dependent on enzyme loading, lignin removal, and the duration of the process [11].

Fermentation process was carried out by using the dry yeast available commercially and is also known as *Saccharomyces cerevisiae* [12]. This was chosen because of its increased ethanol productivity and can withstand high concentrations of ethanol. It can also withstand a wide range of pH which in turn protects the substrate from infection [12, 13]. The process that takes place in ethanol production is explained through the equation.

$$C_6H_{12}O_6 \text{ (Glucose)} \rightarrow 2CH_3CHO \text{ (Acetaldehyde)} + 2CO_2 \rightarrow 2C_2H_5OH \text{ (Ethanol)}$$

The present study aimed at producing bioethanol from the leftover cooked rice by finding the optimum hydrolysis process and also aimed at analyzing the bioethanol qualitatively.

2. Materials and methods

2.1. Feedstock preparation

The leftover cooked rice for the study was collected from the hostel of the Government College of Technology, Coimbatore. The cellulose enzymes for the study were purchased commercially. The dry yeast which is also known as baker’s yeast was bought from a nearby supermarket. The sample was dried under the sun to remove the moisture content present in the rice and then it was subjected to mechanical pretreatment by grinding it to a coarse powder. The sample was then analyzed for total carbon to find the chemical composition using the TOC Analyzer of Make and Model: SHIMADZU & TOC-LCPH and total ash content to estimate the mineral content and inorganic material [14]. The ash content was measured by placing 1g of sample in the muffle furnace at 525ºC for 30 minutes where the sample gets converted to ash. The weight difference before and after the process gives the total ash content. The characteristics of the leftover cooked rice were presented in table 1.

Table 1. Characteristics of the leftover cooked rice

Parameter	Value
Total Carbon (ppm)	229.2
Total Organic Carbon (ppm)	228.8
Inorganic Carbon (ppm)	0.3955
Ash content (%)	4.5
Carbon (%)	42
Nitrogen (%)	2.5
Hydrogen (%)	6.2
Sulphur (%)	0.2
Oxygen (%)	30

2.2. Hydrolysis of leftover cooked rice

50 grams of food waste was taken for ethanol production and the ethanol production was analyzed under 3 categories: Sample subjected to acid hydrolysis alone, Sample subjected to enzyme hydrolysis alone, and Sample subjected to combined hydrolysis. The entire ethanol production process took place in the fermenter of Make & Model: BIOSPIN-OSA & BIO-AGE EQUIPMENT SERVICE for a period of 48 hours with a stirring speed of 120 rpm at a temperature of 27ºC. The purpose of using a fermenter for the entire ethanol production process is to maintain anaerobic conditions.

For the acid hydrolysis process, the sample was dissolved in 1% H_2SO_4 (10ml H_2SO_4 dissolved in 1000ml of distilled water) and was then taken in the fermenter. For the enzyme hydrolysis, to the sample, 33ml of cellulase enzyme dissolved in 1000ml of distilled water was added and was then taken in the fermenter. For the combined hydrolysis, the sample was dissolved in 1% H_2SO_4 and to the solution, 33ml of cellulase enzyme dissolved in 1000ml of distilled water was added and was then taken in the fermenter.

2.3. Fermentation of leftover cooked rice

For the fermentation process of three types of hydrolysed samples, the growth media was prepared by adding 10g/l of yeast extract, 20g/l of peptone, and 20g/l of dextrose in distilled water. It was then autoclaved and after cooling, baker's yeast was added and kept in a shaking incubator at 120rpm at 35ºC for 24 hours. After 24 hours, the growth media was added to the hydrolysed sample in the fermenter with a stirring speed of 120 rpm. For every 24 hours, the samples were analyzed for the presence of ethanol using a Double beam UV-Vis Spectrophotometer of Make and Model: PERKIN ELMER & LAMBDA 35 at a wavelength of 600nm till the absorbance started to decline.

2.4. Distillation of the fermented samples

Distillation was done to separate ethanol from the fermented sample. Before the distillation process, the samples were centrifuged at 10,000 rpm for 15 minutes to remove the solid residue from the liquid sample since the liquid will be subjected to distillation. The distillation process was carried out by Rotary Vacuum Evaporator of Make & Model: EQUITRON & ROUND BATH RUTEUA and after the distillation process, the ethanol was analyzed qualitatively.

3. Results and Discussion

3.1. Spectrophotometric Determination of Ethanol

Stock solutions were prepared by varying the amount of ethanol and the standard chart (Absorbance vs Concentration) was plotted as shown in figure 1.

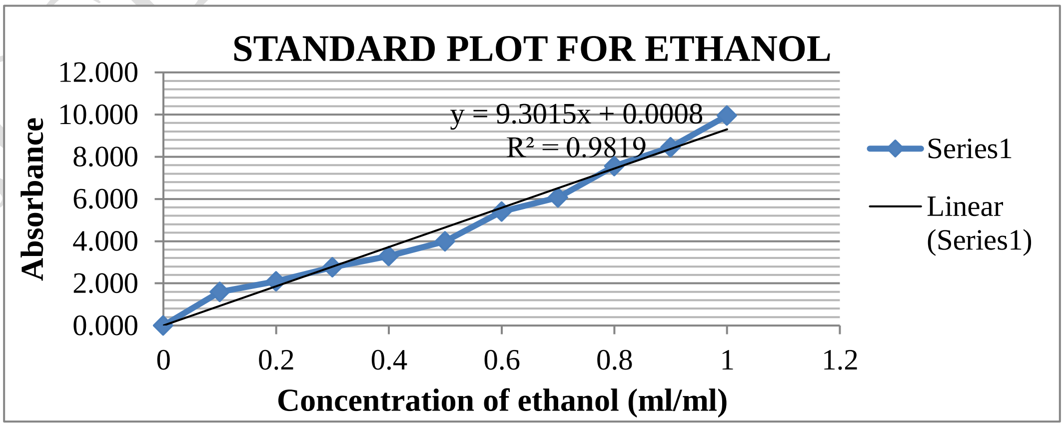


Figure 1. Standard Plot of Ethanol

3.1.1. Spectrophotometric Determination of acid hydrolysed fermented sample

The fermented sample obtained after the acid hydrolysis was analyzed using Double Beam UV-Vis Spectrophotometer. The absorbance of the fermented sample analyzed at 600nm daily was obtained and is shown in figure 2.

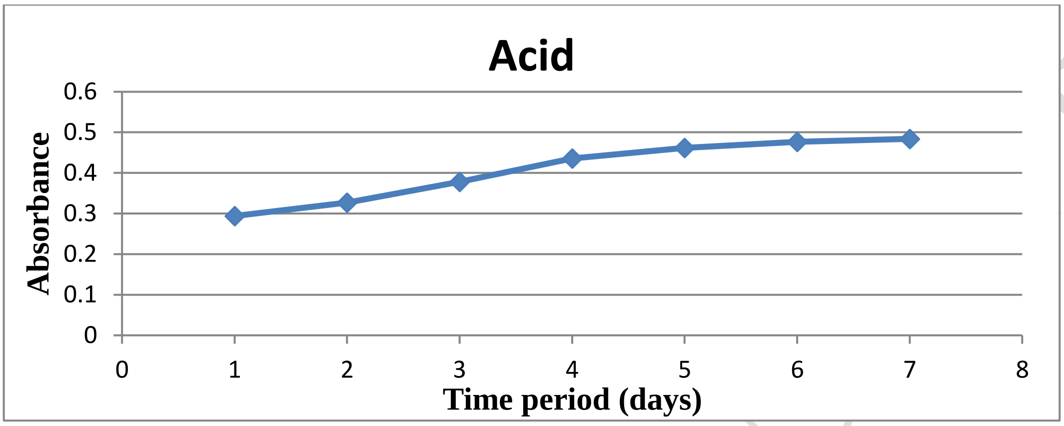


Figure 2. Absorbance of the acid hydrolysed sample

It is inferred from the figure that the absorbance value started to decrease from the 5th day. At the end of the 5th day, the net amount of ethanol that can be produced from the 50 grams of leftover cooked rice using the standard plot was found to be 204ml.

3.1.2. Spectrophotometric Determination of enzyme hydrolysed fermented sample

The fermented sample obtained after the enzyme hydrolysis was analyzed using Double Beam UV-Vis Spectrophotometer. The absorbance of the fermented sample analyzed at 600nm daily was obtained and is shown in figure 3.

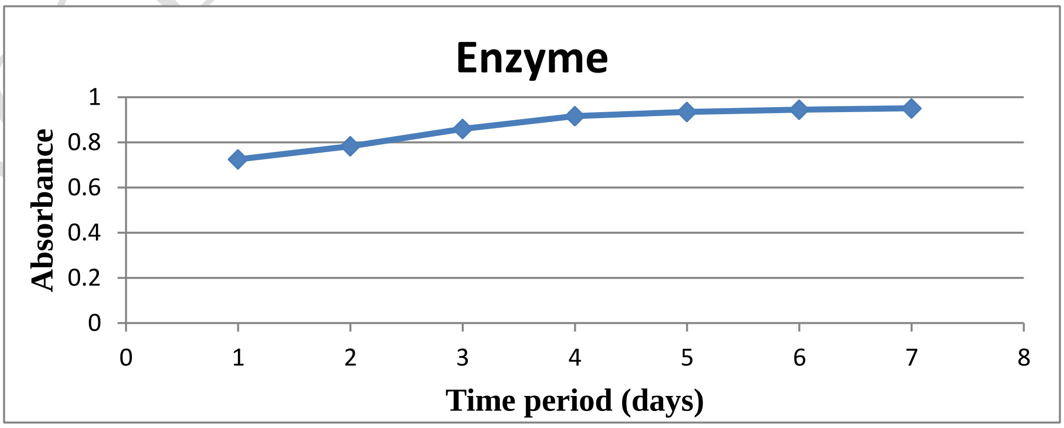


Figure 3. Absorbance of the enzyme hydrolysed sample

It is inferred from the figure that the absorbance value started to decrease from the 4th day. At the end of the 5th day, the net amount of ethanol that can be produced from the 50 grams of leftover cooked rice using the standard plot was found to be 454ml.

3.1.3. Spectrophotometric Determination of combined hydrolysed fermented sample

The fermented sample obtained after the combined hydrolysis was analyzed using Double Beam UV-Vis Spectrophotometer. The absorbance of the fermented sample analyzed at 600nm daily was obtained as shown in figure 4.

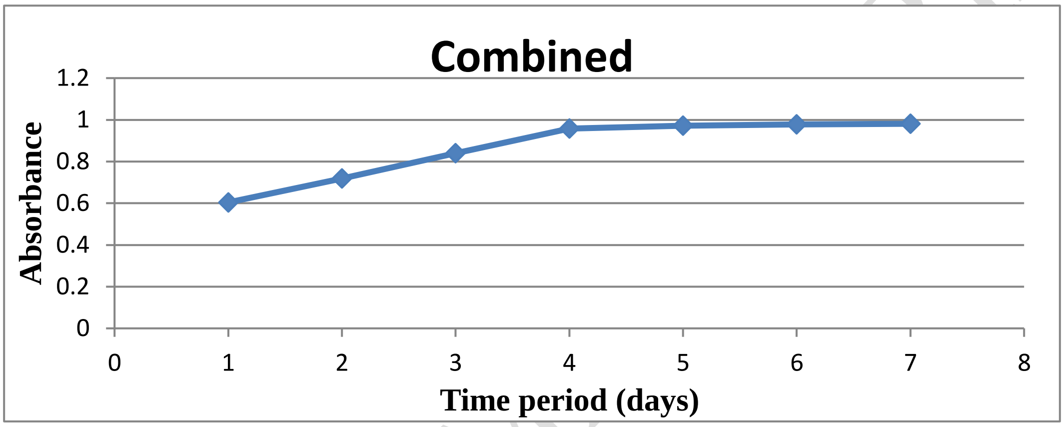


Figure 4. Absorbance of the combined hydrolysed sample

It is inferred from the figure that the absorbance value started to decrease from the 5th day. At the end of the 5th day, the net amount of ethanol that can be produced from the 50 grams of leftover cooked rice using the standard plot was found to be 440ml.

3.2. Extraction of ethanol from the fermented samples

After the centrifugation of the fermented samples, the solution was taken in the round-bottomed flask provided in the Rotary Vacuum Evaporator. The sample containing the flask was lowered to the water bath and once it reached the temperature of 75ºC, the ethanol started to evaporate and was collected on the other flask provided in the instrument. The amount of ethanol distilled from the acid hydrolysed, enzyme hydrolysed, and combined hydrolysed was 150ml, 350ml, and 400ml respectively. The percentage difference of ethanol that can be produced using the standard plot and the ethanol distilled is 30.51% in the case of acid hydrolysed sample, 25.87% in the case of enzyme hydrolysed sample, and 9.5% in the case of combined hydrolysed sample. As the raw material taken for the study is leftover cooked rice, the amount of ethanol produced from 70g of leftover cooked rice by enzyme hydrolysis in literature (Wie Han *et al.*, 2021) was found to be 370.3ml. In our study, a comparison was made between different types of hydrolysis processes and based on the ethanol productivity, the combined hydrolysis was found to be more effective.

3.3. Qualitative analysis of ethanol

This was done to confirm the ethanol produced [15]. The bioethanol was examined by Jones reagent ($K_2Cr_2O_7$+H_2SO_4). 2% Potassium dichromate solution was prepared by dissolving 2 grams of $K_2Cr_2O_7$ in 100ml of distilled water. 1ml of this solution was added to 3ml of distilled ethanol which resulted in the formation of orange colour. To this solution, 5ml of H_2SO_4 was added which resulted in the formation of blue-green colour as shown in figure 5. The colour change was because of the oxidation of ethanol to acetic acid.

Figure 5. Colour change from orange to blue-green

4. Conclusion

This study involves the collection of leftover cooked rice from the source, mechanical pretreatment of the food waste, hydrolysis of the food waste, fermentation, and then subjecting it to distillation. The leftover cooked rice was subjected to three types of hydrolysis processes: acid, enzyme, and combined hydrolysis. As a result of the hydrolysis, cellulose in the substrate was exposed to more degradation. The yeast *Saccharomyces cerevisiae* was cultured in the YPD media and was used for the fermentation of food waste. The amount of ethanol produced from the 50 grams of leftover cooked rice when subjected to combined hydrolysis, acid hydrolysis, and enzyme hydrolysis was 400ml, 150ml, and 350ml respectively. It is more prominent that the ethanol yield from the leftover cooked rice was more in the case of food waste subjected to combined hydrolysis. The colour change from orange to blue-green confirmed the ethanol produced. Therefore, ethanol can be produced from the leftover cooked rice using the yeast *Saccharomyces cerevisiae*. This would be a substitute for conventional fuels.

References

Dionisio S.R., Santoro D.C.J., Bonan C.I.D.G., Soares L.B., Biazi L.E., Rabelo S.C., Ienczak J.L., 2021, Second-generation ethanol process for integral use of hemicelluloses and cellulosic hydrolysates from diluted sulfuric acid pretreatment of sugarcane bagasse, Fuel, **304**, 121290.

Senkevich S., Ntaikou L., Lyberatos G., 2012, Bioethanol Production from Thermochemically Pre-treated Olive Mill Solid Residues using the yeast *Pachysolen tannophilus*, Global NEST, **14**, 118-124.

Abdullah Bilal Ozturk, Tulin Arasoglu, Jala Gulen, Shou Cheng, Najeeb Kaid Naseer Al-Shorgani, Hiroaki Habaki, Ryuichi Egashira, Mohd Sahaid Kalil, Wan Mohtar Wan Yusoff, Jeffrey Scott Cross, 2021, Techno-economic analysis of a two-step fermentation process for bio-butanol production from cooked rice, Sustainable Energy & Fuels, **5**, 3705-3718.

Xikai Chen, Yujia Zhao, Boyang Chen, Wang Su, Zhengxian Zhang, Yanxu Liu, Xiaobin Xu, Junhong Tang, Pingzhi Hou, Wei Han, 2021, Effects of Enzyme Volumes on Hydrolysis and Fermentation for Ethanol Production from Leftover Cooked Rice, Frontiers in Bioengineering and Biotechnology, **9**, 1-6.

Kretzschmar J., Majer S., Kroeger M., 2012, International Overview on Waste to Biofuel options with a focus on Waste Potentials in Germany and Funding Incentives in the EC, Global NEST, **14**, 183-191.

Roni Maryana, Dian Marifatun, Wheni A., Satriyo K.W., Angga Rizal W., 2014, Alkaline Pretreatment on Sugarcane Bagasse for Bioethanol Production, Energy Procedia, **47**, 250-254.

Li-Qun Jiang, Zhen Fang, Xing-Kang Li, Jia Luo, Suet-Pin Fan, 2013, Combination of dilute acid and ionic liquid pretreatments of sugarcane bagasse for glucose by enzymatic hydrolysis, Process biochemistry, **48**, 1942-1946.

Amrita Verma, Santhosh Kumar, Jain P.K., 2011, Key pretreatment technologies on cellulosic ethanol production, Journal of Scientific Research, **55**, 57-63.

Charilaos Xiros, Evangelos Topakas, Paul Christakopoulos, 2012, Hydrolysis and fermentation for cellulosic ethanol production, WIREs Energy and Environment, **2**, 633-654.

Swati Tyagi, Kui-Jae Lee, Sikandar I. Mulla, Neelam Garg, Jong-Chan Chae, 2019, Production of Bioethanol from Sugarcane Bagasse: Current Approaches and Perspectives, Applied Microbiology and Bioengineering, 21-42.

Katja Vasic, Zeljko Knez, Maja Leitgeb, 2021, Bioethanol Production by Enzymatic Hydrolysis from Different Lignocellulosic Sources, MDPI-Molecules, **26**, 1-23.

Raharja R., Murdiyatmo U., Sutrisno A., Wardani A.K., 2019, Bioethanol production from sugarcane molasses by instant dry yeast, International Conference on Green Agro-industry and Bioeconomy, **230**, 1-8.

Siti Hajar Mohd Azhar, Rahmath Abdulla, Siti Azmah Jambo, Hartinie Marbawi, Jualang Azlan Gansau, Ainol Azifa Mohd Faik, Kenneth Francis Rodrigues, 2017, Yeasts in sustainable bioethanol production: A review, Biochemistry and Biophysics Reports, **10**, 52-61.

Beatriz Salustiano Pereira, Raissa Nobre Castrisana, Caroline de Freitas, Jonas Contiero, Michel Brienzo, 2021, Chemical composition determines the bioenergy potential of food waste from pre-and post-production, Journal of Material Cycles and Waste Management, **23**, 1365-1373.

Esmil Beliya, Subhra Tiwari, Shailesh Kumar Jadhav, Kishan Lal Tiwari, 2013, De-oiled rice bran as a source of bioethanol, Energy Exploration & Exploitation, **31**, 771-782.