ISSN No:-2456-2165

Bioethanol Production from Lignocellulosic Agricultural Waste

N. Zidan
Department of Chemical Engineering, Faculty of Engineering
Cairo University, Egypt

Abstract:- The aim of this paper is to produce bioethanol from lignocellulosic agricultural wastes as rice straw. Various variables was studied and investigated to find out the optimum conditions for highest sugar (and hence ethanol) yield such as: Type of fungi/bacteria used, Incubation time, Temperature of medium and PH of medium. Hydrolysis and fermentation experiments were made to produce bioethanol, other experiments were made to estimate optimum conditions needed for the production of bioethanol with high purity and yield. It was found that the best type of bacteria for fermentation is penicillium. Calorific value of spent rice straw was also investigated and was found to 14.88 MJ/Kg and 36.56 MJ/Kg before and after biosorption respectively showing a high calorific values.

I. INTRODUCTION

Bioethanol is the principle fuel that substitutes petrol for vehicles. It can be produced by many ways. Bioethanol fuel is mainly produced by the sugar fermentation process which is a process used to extract energy by making changes in organic substrates through the action of enzymes. It can also be manufactured chemically through the reaction of both ethylene and steam. (Ibeto, Ofoefule, and Agbo, 2011)

In their paper, A Global Overview of Biomass Potentials for Bioethanol Production: A Renewable Alternative Fuel. *Trends in Applied Sciences Research,* Ibeto, Ofoefule, and Agbo mentioned that: “The main sources of sugar required to produce ethanol come from energy crops. These crops are grown specifically for energy use and include corn, maize and wheat crops, rice straw, willow and popular trees, sawdust, reed canary grass, cord grasses, miscanthus and sorghum plants.” (Ibeto, Ofoefule, and Agbo, 2011)

Bioethanol has a lot of advantages compared to conventional fuels. It comes from a renewable resource. Another benefit is that greenhouse gases will be reduced since the crops will absorb the carbon dioxide they produce while growing. Also, blending bioethanol with petrol will enhance the security of oil and solve the problem of the nonrenewable fuel. Bioethanol can also be biodegraded and it is less toxic than fossil fuels. In addition, Air quality will be improved by using bioethanol in older engines hence reducing the amount of carbon monoxide produced by the vehicle. (Ethanol and the environment, 2001)

There are more than a biofuel generation. The first-generation biofuels are the fuels that were extracted directly from plant and animal sources as starch, sugar, animal fats and vegetable oil. Some of the most popular types of first generation biofuels are: biodiesel, vegetable oil, bio-gas and bio-alcohols. While the second-generation biofuels refer to the fuels that have been derived by biological processes.

First generation biofuels has a serious problem concerning the global food supply. It is always important to ask if it is the right thing to produce fuel from food when the world already have one billion hungry people. Another problem is that farmers might grow crops for biofuel production which means that the food supply problem would grow bigger and more serious. Hence, the production of first generation biofuels should be avoided regardless its high profits because food production is more important than fuel production.

The second generation biofuels are more environmental friendly. In this generation, biofuels are produced by biological pre-treatment and enzymatic hydrolysis of lignocellulose followed by sugar fermentation. The second generation hence, has the highest potential for improvement so that it can replace the other production methods.

The aim of this paper is to produce bioethanol from lignocellulosic agricultural wastes as rice straw so various variables will be studied to find out the optimum conditions for highest sugar (and hence ethanol) yield such as: Type of fungi/bacteria used, Incubation time, Temperature of medium and PH of medium.

II. METHODS

A. Design of Experiment and Analysis

Biochemical conversion of lignocellulosic biomass to ethanol uses enzymes to break down the cellulose and hemicellulose found in plant cell walls into sugars, which are transformed into alcohol by microorganisms in fermentation.

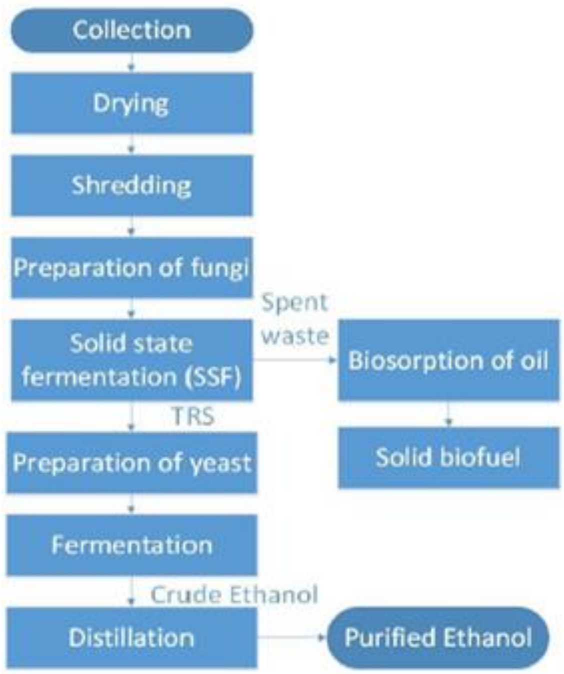

Fig 1:- Conversion of Lignocellulosic Biomass into Bioethanol

In this paper, studies were made on rice straw as feedstock. Fig. (1) Shows the major steps in converting biomass to biofuel. It starts with collection of rice straw, drying and shredding it. Pretreatment is then made followed by hydrolysis, fermentation and finally distillation to extract a pure bioethanol.

B. Preparation of Rice Straw

The rice straw is brought from the agricultural waste after harvesting the rice. It is then dried using sunlight for about 3 days to evaporate any liquids and moisture. Rice straw is shredded randomly to ease the transportation from farms to desired location. It is then dried again if required to make sure it is completely dry. A shredder is then used in order to make the rice straw of a maximum length of 1 cm to enhance the extraction of cellulose.

C. Preparation of Medium

A Sabouraud medium has to be prepared to provide an energy source for the growth of microorganisms. Using a mass balance, measure 3.5g peptone, 1.75g yeast extract and 7g glucose. Peptones are excellent natural sources of amino acids and proteins in growth media.

The peptone, yeast, and glucose are poured in a large beaker and then 350 ml of tap water is added to the beaker (only 300 ml needed, 50 ml excess). The Sabouraud medium is stirred using a magnetic stirrer in order for the powders to dissolve in water. The pH has to be adjusted to 5.5 as it is the ideal pH for the multiplication of the bacteria. The solution is distributed in 6 flasks where each flash contains 50 ml to act as a broth medium for the growth of bacteria. The flasks are put in an autoclave for sterilization at 121°C, 1.5 bar for 20 minutes and are incubated at 30°C. Another sample of the Sabaraud medium is prepared having the same concentrations and volume as the previous one. 15g of agar -a solid jelly-like substance derived from the polysaccharide agarose- is added to support the structure in the cell walls of certain species of algae. The second large flask of solution with agar prepared is distributed onto 6 plates. Also some of the broth medium of the six 50ml flasks are added to the plates.

D. Pretreatment of Rice Straw

Using the digital balance 2g of 1 cm rice straw is added to each of 6 empty flasks and then moistened by water. The flasks were then steam treated by autoclaving at 121˚C and 1.5 bars for 20 min.

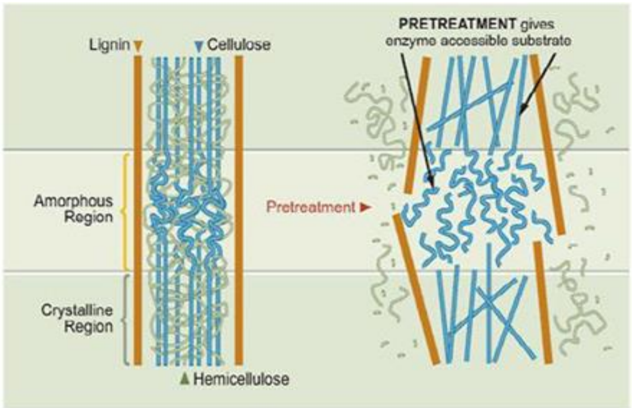

Fig 2:- Effect of Pretreatment on Biomass Structure (NAS,2009)

Maximizing the use of all lignocellulosic material that is capable of yielding simple sugars is essential for improving ethanol yield and lowering the cost of ethanol production. Hence, pretreatment of lignocellulosic material is required to improve the hydrolytic efficiency of cellulose by removing and hydrolyzing hemicellulose as shown in Fig. (2) Which shows the effect of pretreatment on biomass structure by separating the cellulose from the lignin, and by loosening the structure of cellulose and thereby increasing its porosity. (National Academy of Sciences)

E. Propagation and Hydrolysis

Several strains of fungi are grown in order to determine the fungi that produces the highest sugar yield during the hydrolysis stage.

ISSN No:-2456-2165

In a biosafety cabinet, a rod is used to sweep fungi onto the agar plates; a specific strain for every 2 plates. The biosafety cabinet provides a decontaminated environment to ensure that only the desired fungi grows on the plates and no other microorganisms contaminate the plates. After propagation, a cork borer is used to inject 10 discs from each plate onto a flask containing rice straw which have been previously pretreated in an autoclave, then 10 ml of water is added and the flask is shook for 1 minute. The flasks are incubated for 11 days for hydrolysis to take place. In the hydrolysis step, the cellulose polymers (long chains of sugar) are broken down by hydrolysis into five-carbon and six-carbon sugars (xylose and glucose) for fermentation into alcohol.

F. Sugar Extraction

The objective of this step is to find the best type of fungi that gives the highest percentage of sugar in order to ferment it to get the highest possible yield of ethanol. The sugar solution from each flask was divided equally into 2 small bottles; half of the sugar solution was taken for HPLC testing while the other half was used in the DNS test described elsewhere. The bottles were labeled with the same flask label in order to recognize the fungi type after the two tests.

G. Fermentation

The composition of the biomass feedstock determines the amount of ethanol that can be produced per ton of biomass. The ultimate yield is determined by the maximum yield of sugars that can be obtained from a given biomass type, and the yield of sugars is determined by the combined cellulose and hemicellulose content of the biomass. The ethanol yield can range from 105 to 135 gal/ton (on a dry-weight basis) if all bioprocessing steps occur at 100 percent efficiency, that is, all the structural carbohydrates—starch, cellulose, and hemicellulose—are used to produce ethanol. Because the efficiency is typically less than 100 percent, the actual yields are lower than this. (National Academy of Sciences).

Components of the Wickerham medium are added to the hydrolysate (sugar solution obtained from hydrolysis) in the fermentor in order for yeast (Saccharomyces cerevisiae) to grow and ferment the sugars into ethanol. Its components include: yeast extract, malt extract, peptone and glucose. After the medium is prepared, it is sterilized in an autoclave at 121˚C for 20 minutes. The yeast is then inoculated onto the medium and the mixture is incubated for 48 hours at 30C and 150 rpm where the fermentation process takes place.

III. RESULTS

A. Sugar Extraction

As mentioned before, two test were performed to detect the amount and type of sugars present. The objective of the tests was also detecting the presence of formic acid and acetic acid since those acids are very toxic to microorganisms; hence organisms might not be able to complete the fermentation process. The tests helps as well in detecting the type of yeast that will be used in the later fermentation process to get the highest amount of ethanol in the least time possible. Table (1) shows the HPLC results showing the amount, type and name of present sugars.

Sugars	Sugars concentration (g/l)
Xylose	3.10×10^{-1}
Glucose	1.25
Maltose	2×10^{-1}
Lactose	4.66×10^{-2}
Total Sugar	**1.706**

Table 1:- HPLC Results

Results shows that the main types of sugar are from the reducing sugar category which is able to act as reducing agent due to the presence of aldehyde or ketone groups. Glucose and Xylose forms the large amount of present sugars which will help later in the fermentation and conversion of those sugars to ethanol.

B. Fermentation

The glucose and xylose that result from the saccharification step are fermented into ethanol by microorganisms. Although Saccharomyces cerevisiae (type of yeast) has been used for fermentation in ethanol production from corn grain, it cannot ferment xylose sugars obtained from lignocellulose unless it is genetically modified. Organisms that ferment xylose and glucose have been developed through metabolic engineering. (National Academy of Sciences).

The concentration of ethanol after the fermentation process was found to be 92%, which could be increased to up to 99% purity by distillation.

The yield of ethanol from the entire process is approximately 100 gal/ton of rice straw.

C. Biosorption

In order to achieve zero wastes in the biological conversion of biomass into bioethanol, the spent rice straw (which mainly consists of lignin) could combusted to generate steam that could be used in the pretreatment stage or during distillation. Another useful application of the spent rice straw is that it can be used in cleaning up oil spills by the process of biosorption.

An experiment was carried out with the objective of finding the concentration and the absorption time at which the highest removal of oil can be achieved. Different oil concentrations were used and the volume of oil is measured once for each concentration used. The highest removal of oil was found to be at an oil concentration of 5% and at 5 hours.

Since the objective is zero wastes, the spent straw was analyzed after biosorption experiment to know its calorific value in order to know if it can be used in other applications.

	Calorific Value (MJ/kg)
Before biosorption	14.88
After biosorption	36.56

Table 2:- Calorific Value Test Results

Table (2) shows the results of calorific value test. The solid spent waste formed 14.88 MJ/Kg and 36.56 MJ/Kg before and after biosorption respectively the results show a high calorific value since every kg rice straw contain 36.56 MJ energy. Depending on these results, it was obvious that if the experiment was repeated again on industrial scale, Spent rice straw might be used as fuel source in plants to save the high energy costs in furnaces and distillation reboilers. The remaining spent straw could also be sold to plants to use it for fuel and electricity as asphalt industries…etc.

IV. CONCLUSION

Second generation bioethanol production is an encouraging solution to solve the energy and environmental crisis. The flexibility it offers, which is seen from the various possible routes of the production and various existing production technologies, endorses more development to obtain the better efficient production, feasible cost production and lesser national emission. Moreover, second generation bioethanol is beneficial from the perspective of industry since agricultural wastes practically have zero value for the industry as well as for the food, which helps to decrease the feedstock cost in the total production cost. We are running out of fuel and we must face it sooner or later so we should start now and think of other energy sources specially those which are renewable and cheap. Government must display the strong level of support, willingness and consistency for the program. The policies should aid the R&D of production of bioethanol, as well as trying to invite the shareholders to put their shares into production of renewable source. Bioethanol as a fuel is promising that more attention must be drawn to it, if this is done, success and efficiency will be guaranteed. That project not only solve an energy problem but also solve one of the greatest problems in some countries like Egypt as burning of rice straw accounts for 42% of air pollution in Egypt. If the project could be implemented in countries like Egypt, it would solve part of the pollution problem providing better health care too.

RECOMMENDATIONS

In order to make the process more efficient and save additional cost, many things should be applied before implementation of the process.

An interesting fact was that, pretreatment is mainly used to break the bonds between lignin and cellulose and hemicellulose. That lignin forms around 30% of lignocellulose. It was found that this lignin can be heated and used as energy source in boilers or as a raw material for lignin based chemical industries. It can also be used as copolymer or burnt in thermal power plant to produce electricity.

If this was taken into consideration during implementation on large scale, the process could save a lot of many and may obtain additional cost too.

Another interesting fact was the water used in process; it was found that through the process lot of water had been used for the furnace and for hydrolysis…etc. Distillation columns and molecular sieves produce large amount of water too on trial to obtain high purity bioethanol; so treating this water and using it again could help decrease the cost of water and hence decrease the utility cost and that's what is called zero liquid discharge system. It is true that treatment unit will have a high cost but at the end, its highest cost will be at the beginning (fixed capital) and at the end it should be much cheaper to add water treatment unit today to the plant than buying water many times during the plant years of operation. Anyway, this idea is still under studies and development.

REFERENCES

[1]. Arnold, K., & Stewart, M. (2008). *Design of Oil Handling Systems and Facilities* (Third ed., Vol. 1, Surface Production Operation
[2]. Adams F. V., Niyomugabo A., Sylvester O.P., Bioremediation of Crude Oil Contaminated Soil by Agricultural Wastes, 2016
[3]. Amarasekara, A. S. (2014). Handbook of Cellulosic Ethanol. John Wiley & Sons, Inc.
[4]. Abdeen, F. R., Mel, M., Al-Khatib, M., & Azmi, A. S. (2011). Dehydration of Ethanol on Zeolite Based Media Using Adsorption Process. *CUTSE International Conference* (pp. 1-12). Sarawak: Research gate.
[5]. • Biofuels: The fuel of the future. (2016, December). Retrieved from Biofuels site: http://biofuel.org.uk/first-generation-biofuels.html
[6]. Bajpai, P. (2011). Advances in Bioethanol. New Delhi, India: Springer.
[7]. Bioethanol looking to become the driving force in transport fuels", Akzo Nobel's homepage, 2001.

[8]. C.N. Ibeto, A.U. Ofoefule and K.E. Agbo, 2011. A Global Overview of Biomass Potentials for Bioethanol Production: A Renewable Alternative Fuel. *Trends in Applied Sciences Research, 6: 410-425*

[9]. Chorom M., Sharifi H. S., Motamedi H., Bioremediation of Crude Oil Polluted Soil, 2010

[10]. Clark, J. (2005). NON-IDEAL MIXTURES OF LIQUIDS. Retrieved from Chemguide website: http://www.chemguide.co.uk/physical/phaseeqia/nonideal.html

[11]. Davis, R. Z., "Design and Scale-Up of Production Scale Stirred Tank Fermentors" (2010). All Graduate Theses and Dissertations. Paper 537

[12]. Erbaum, J. B. (Ed.). (2009). *Bioethanol Production, Benefits and Economics*. Nova Science Inc .

[13]. Ethanol and the environment", Renewable Fuels Association's homepage, 2001, http://www.ethanolrfa.org/factfic_envir.html

[14]. ENERGY IN EUROPE, European Energy to 2020, A scenario approach, SPECIAL ISSUE - SPRING 1996", DIRECTORATE GENERAL FOR ENERGY (DG XVII), http://europa.eu.int/en/comm/dg17/e2020fd.pdf

[15]. Funk, J. R. (2008). *Boiler Basics*. Retrieved from Hughes Machinery: https://www.dol.ks.gov/Registration/Handouts2015/BoilerBasics-JasonFunk-ShConf2015.pdf

[16]. *First Generation Biofuels.* (2016, December). Retrieved from Biofuel Site: http://biofuel.org.uk/first-generation-biofuel.html

[17]. Funk, J. R. (2008). *Boiler Basics*. Retrieved from Hughes Machinery: https://www.dol.ks.gov/Registration/Handouts2015/BoilerBasics-JasonFunk-ShConf2015.pdf

[18]. Haluzan, N. (2010, November). *Biofuels Advantages and Disadvantages.* Retrieved from Renewable Energy Info: http://www.renewables-

[19]. info.com/drawbacks_and_benefits/biofuels_advantages_and_disadvantages.html

[20]. Harriott, P., Mcabe, W. L., & Smith, J. C. (1993). *unit operations of chemical engineering.*

[21]. Hitesh Jagani, Karteek Hebbar, Sagar S. Gang, P. Vasanth Raj, Raghu Chandrashekhar H. and J.Venkata Rao, An Overview of Fermenter and the Design Considerations to Enhance Its Productivity, 2010

[22]. Karl. (2010, August 31). Biofuel FAQ. Retrieved December 29, 2016, from http://www.makebiofuel.co.uk/biofuel_faq/

[23]. K, B., & M, B. (2001). *Ethanol Plant Development Handbook.* .

[24]. Lees, F.P. (1996). Loss prevention in the process industries, 2nd edn., Oxford

[25]. Ltd., T. E. (2010). *Emedicalprep.* Retrieved from https://www.emedicalprep.com/study-material/chemistry/surface-chemistry/factor-affecting-adsorption/

[26]. Mabee W., Sadler J., Sims R., Taylor M., From First to Second Biofuel Technologies: An Overview of Current Industry and R&D Activities, International Energy Agency, 2008

[27]. Ma, J. (1999, April 4). *Alibaba Group*. Retrieved from Alibaba.com Site: https://www.alibaba.com/product-detail/3A-molecular-sieves-powder-desiccant-size_60445713034.html

[28]. National Academy of Sciences (2009). Liquid Transportation Fuels from Coal and Biomass.

[29]. Ofoegbu, R. U., Yusuf O. L., Nwaogazie I. L., Bioremediation of Crude Oil Contaminated Soil Using Organic and Inorganic Fertilizers, Petroleum & Environmental Biotechnology, 2015

[30]. Perlmutter, B. A. (n.d.). *chemical processing.* Retrieved from chemical processing: http://www.chemicalprocessing.com/assets/wp_downloads/pdf/bhs_wpa_090914.pdf

[31]. Parameswaran Binod, R. S. (2010). Bioethanol Production from rice straw. In *Biotechnology resources* (pp. 4767-4768). Delhi: Centre for Biofuels, National Institute for Interdisciplinary Science and Technology

[32]. Patrik R. Lennartssona, P. E. (2013). *Bioresources Technology*. Stockholm: Swedish Centre for Resource Recovery, University of Boras.

[33]. Report PE A4-0096/1997, The Legislative Observatory of the European parliament, 1997, http://wwwdb.europarl.ep.ec/