Archana D. et al. 2019, 7:4
ISSN (Online): 2348-4098
ISSN (Print): 2395-4752

International Journal of Science, Engineering and Technology

An Open Access Journal

Bio-ethanol Production from Sugarcane Bagasse using Pichia Stipitis

Archana D. **Jagadish H Patil**
Department of Chemical Engineering
RV College of Engineering
dhimakearchana@gmail.com

Abstract - In the present scenario, due to the increase in demand and supply of fuel consumption and fluctuation in cost of crude oil, the world is searching for alternative fuels. Renewable energy systems are rapidly becoming more efficient and cheaper such that their share of total energy consumption is increasing. Ethanol production from bagasse is often complex process which involves four main steps namely pretreatment, hydrolysis, fermentation and finally recovery with purification. In the pretreatment step, there will be moisture removal and size reduction. The second step involves acid hydrolysis of Sugarcane Bagasse using 0.1 to 0.5M sulphuric acid. The highest concentration of Ethanol produced was 331 ml/l using 12.5gm of SCB and 0.3M Sulphuric acid with the aid of Pichia stipitis.

Keywords- ***Pichia stipitis*****, Bio-ethanol, Sugarcane Bagasse, Fermentation**

I. INTRODUCTION

The increase demand and supply of the fossil fuels along with the depletion has created the concern to look for the alternative energy resources. The rate of consumption of the fuels has increased to such an extent that there would be severe crises in the future. In order to meet these demanding criteria, many researchers have been done in particular to develop an environmental friendly bio-ethanol.

In the past, it was projected that demand of bio-ethanol would grow annually between 2010 and 2030. Most of the natural fuel resources are imported from Middle East. The largest consumer of Energy in the world is United States followed by China, Russia and finally Japan. Based on the ranking of Energy consumption, India ranks 5th position and accounts for 4% of global energy consumption.

The growth rate of annual energy demand has steadily increase at 4.8% and by 2013 studies reveal that India becomes world's 3rd biggest energy consumer (Das & Singh, 2004) Brazil uses pure ethanol and blended ethanol as a vehicle fuel. It was noted that, 22% of blended fuel was used to run 6 billion petroleum vehicles, whereas pure ethanol was used for running 5 billion vehicles(Graham & Downing, 1993).

The largest producer of sugarcane in the world is Brazil, and it uses sugarcane as the raw material for the production of bio-ethanol (Shinoj, 2015).

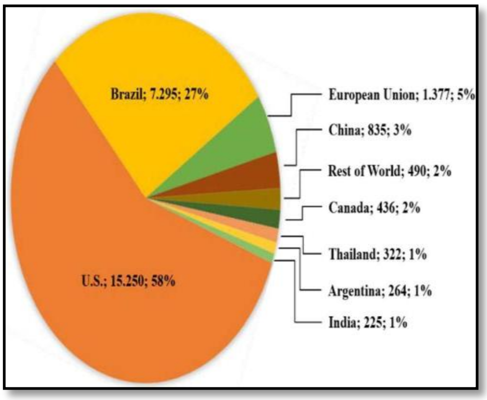

Figure 1 Pictorial Representation of Production of Bio-ethanol across various Countries (Vu & Ramaraj, 2018)

The maximum production of bio-ethanol in US is around 60% of entire world. Brazil contributes about 27% of production. India contributes about 1% production (225 million gallons) to the total production in 2016 as shown in Figure 1.India is also one among largest sugarcane producers in the world. About 60% of ethanol is produced from sugarcane and rest is produced from starchy grains. The production of sugarcane is about 285 million tons per annum (Das & Singh, 2004) . As a substitute to the fossil fuel, the research of bio-ethanol production has been undertaken since many years. By developing new energy forms using the renewable energy sources many countries have taken initiations to reduce the dependence on oil and gas imports. Major research and application of bio-ethanol for renewable energy purpose were initiated by US and Brazil. The corn and sugarcane are used as a major raw material mainly for the production of bio-ethanol in US and Brazil respectively.

1. Bio-ethanol – Lignocelluloses' is one of the most abundant raw materials that are present of cell wall of woody plant parts. So the production of ethanol from these wastes can act as a waste treatment and aid in conversion of its products into value added resources. Many researches also reveal that lignocellulose is an unique substitute used or production of bio-ethanol due to its low cost and high yield.

Kim *et al* (Kim & Dale, 2004) reported on the global potential involved in the production of bio-ethanol from crop residues. The studies reveal that about 49.1 gigalitre/year of bio-ethanol is produced using 73.9 Teragram of dry crop residue in the world and Asia produces up to 291 gigalitre/year of bio-ethanol. Furthermore it was stated that bio-ethanol obtained from sugarcane bagasse can generate about 3.6% total world's electricity. Table 1 presents the potential bio-ethanol production from bagasse.

Table 1 Production of Bio-ethanol from Sugarcane Bagasse

Country	Production in GL		Total bio-ethanol	Gasoline equivalent
	From SCB	From waste Sugarcane		
Asia	0.8200	21.300	22.100	15.900
Africa	0.2300	03.330	03.560	02.560
America	0.5501	24.870	25.450	18.290
Europe	-	00.004	00.004	00.003
world	1.5900	51.300	52.900	38.000

II. MATERIALS AND METHODOLOGY

1. Materials and Equipments

The raw material sugarcane bagasse was collected from RVCE Campus. The bagasse was washed with distilled water and sun dried for 24 hours. The standard strain *Pichia stipitis* with NCIM number 3497 was procured from National Chemical Laboratory, Pune. The chemicals used were as follows: Dilute Sulfuric acid, Sodium Hydroxide, Phenol, Sodium Sulfite, Sodium-potassium tartarate.

2. Methodology

The Sugarcane Bagasse was collected, washed with distilled water and sun dried for 24 hrs for the moisture removal. The dried bagasse was reduced in size using the grinder. This dried bagasse was further taken for acid hydrolysis. This process is mainly done to break the chains and liberate the sugar molecules before the fermentation process, which is involved in Ethanol production. Five conical flasks of 250 ml capacity were taken. 12.5 gram of SCB was added to each of them. Along with the addition of SCB, H_2SO_4 was added to each flask. The concentration of H_2SO_4 in each conical flask was increased from 0.1 M to 0.5 M. All the samples were left to be soaked in the acid for one day.

The pH of the solution was checked with the help of pH meter. As the samples are hydrolyzed with acid, Sodium hydroxide was added to neutralize the pH in the range of 5 to 5.5. For this purpose, 1N NaOH was added drop wise to each flask. After maintaining the pH value, the conical flasks along with the solution were cotton plugged and placed in a vertical autoclave for half an hour at 121°C and later it was allowed to cool at room temperature. The cotton plug was detached from the conical flask and 10ml of Pichia stipitis was added in Laminar Air Flow chamber. Once again the conical flasks were cotton plugged and placed in a shaking incubator at the 30˚C and 120 rpm for 24 hrs.

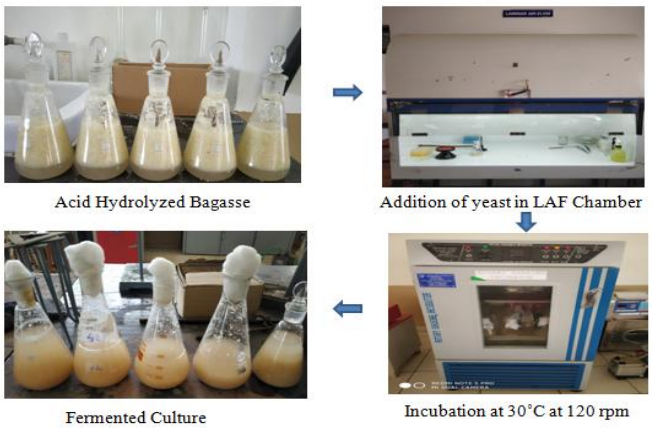

Fig. 2 Fermentation using Pichia Stipitis.

After the fermentation process, the ethanol was separated using distillation process carried out at 60°C. The collected filtrate was analyzed using UV-Spectrophotometer at the wavelength of 197nm.

III. RESULT AND DISCUSSION

Standard plot for ethanol was obtained using the various concentration of ethanol and analyzed in UV Spectrophotometer. The concentration of Ethanol was obtained using the calibration chart shown in Figure 3a. The concentration of Ethanol obtained by varying the time for 0.1M to 0.5M H_2SO_4 (Figure 3b). The comparison of the ethanol concentration obtained by various acid concentrations is depicted in Figure 3c. The acid concentration of 0.3M resulted in highest concentration of

Ethanol (331ml/l). The further increase in molarity of H_2SO_4 leads to reduction in the concentration of ethanol.

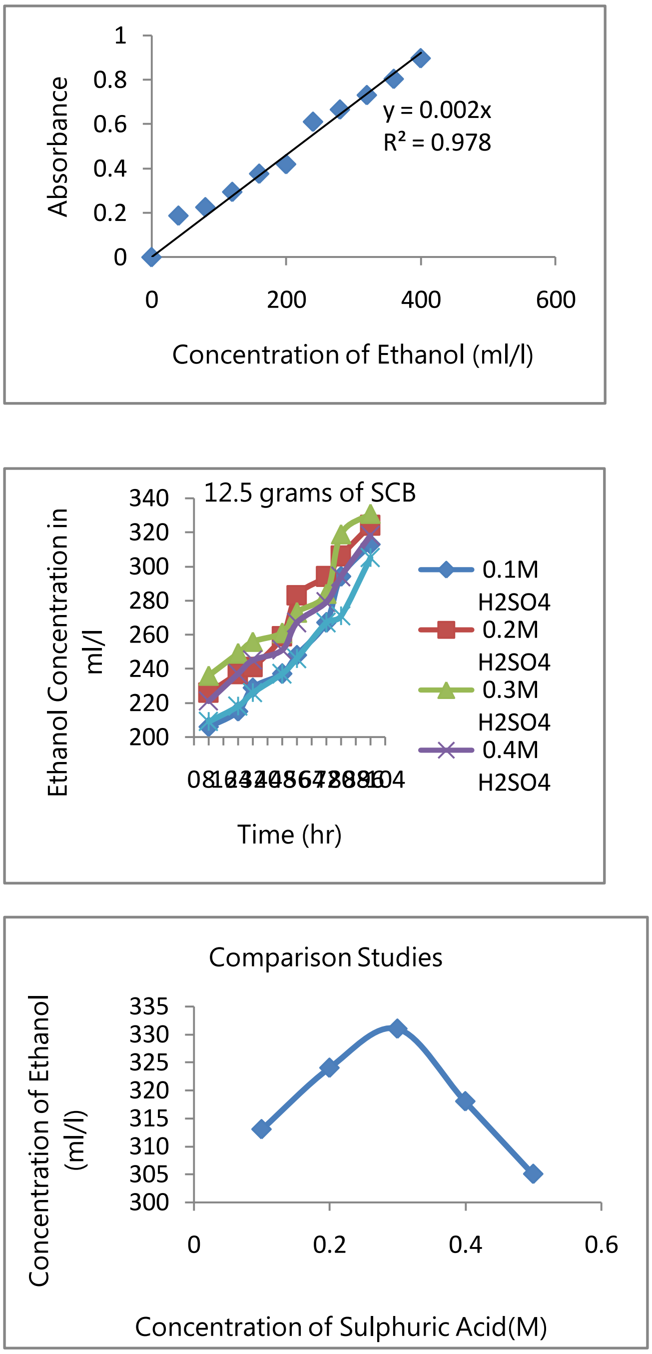

Fig. 3(a) Calibration Chart, (b)Ethanol Concentration obtained at 0.1M to 0.5M Sulphuric Acid and (c)Comparison Studies.

IV. CONCLUSIONS

An attempt was made to produce the Bio-Ethanol using Sugarcane Bagasse and Pichia stipitis. Sugarcane bagasse serves as promising lignocellulosic substrate for bio-ethanol production. Acid pretreatment has been found to be most effective method among the other methods. This pretreatment method gave an adequate amount of reducing sugar which was then subjected to fermentation using pichia stipitis to produce the bio-ethanol. The highest concentration of Ethanol produced was 331 ml/l using12.5gm of SCB and 0.3M Sulphuric acid with the aid of Pichia stipitis. Thus clear pathway was established to convert to Bio-Ethanol.

REFERENCES

[1] H. Das and S. K. Singh, "Useful Byproducts from Cellulosic Wastes of Agriculture and Food Industry - A Critical Appraisal," Crit. Rev. Food Sci. Nutr., vol. 44, no. 2, pp. 77–89, 2004.

[2] Graham and Downing, "Renewable biomass energy: Understanding regional scale environmental impacts," in Proceedings of the First Biomass Conference of the Americas: Energy, Environment, Agriculture, and Industry, 1993, pp. 1566–1581.

[3] P. Shinoj, S. . Raju, C. Ramesh, K. Praduman, and S. Msangi, "Biofuels in India: Future Challenges," Policy Br. No. 36, vol. 56, no. 2, pp. 179–179, 2015.

[4] P. T. Vu, Y. Unpaprom, and R. Ramaraj, "Impact and significance of alkaline-oxidant pretreatment on the enzymatic digestibility of Sphenoclea zeylanica for bioethanol production," Bioresour. Technol., vol. 247, no. January, pp. 125–130, 2018.

[5] A. Demirbas, "Biofuels sources, biofuel policy, biofuel economy and global biofuel projections," Energy Convers. Manag., vol. 49, no. 8, pp. 2106–2116, 2008.

[6] S. Kim and B. E. Dale, "Global potential bioethanol production from wasted crops and crop residues," Biomass and Bioenergy, vol. 26, no. 4, pp. 361–375, 2004.

[7] H. Hernawan et al., "Bioethanol production from sugarcane bagasse by simultaneous sacarification and fermentation using Saccharomyces cerevisiae," AIP Conf. Proc., vol. 1823, 2017.

[8] B. Yñiguez-Balderas, B. Ortiz-Muñiz, J. Gómez-Rodríguez, B. Gutierrez-Rivera, and M. Aguilar-Uscanga, "Ethanol production by Pichia stipitis immobilized on sugarcane bagasse," Bioethanol, vol. 2, no. 1, pp. 44–50, 2016.

[9] V. Ezebuiro and C. J. Ogugbue, "Bioethanol Production by an Ethanol-Tolerant Bacillus cereus Strain GBPS9 Using Sugarcane Bagasse

and Cassava Peels as Feedstocks," J. Biotechnol. Biomater., vol. 05, no. 04, 2016.

[10] A. Ajit, A. Z. Sulaiman, and Y. Chisti, "Production of bioethanol by Zymomonas mobilis in high-gravity extractive fermentations," Food Bioprod. Process., vol. 102, pp. 123–135, 2017.

[11] L. Tian et al., "Enhanced ethanol formation by Clostridium thermocellum via pyruvate decarboxylase," Microb. Cell Fact., vol. 16, no. 1, pp. 1–10, 2017.

[12] B. K. Bajaj, V. Taank, and R. L. Thakur, "Characterization of yeasts for ethanolic fermentation of molasses with high sugar concentrations," J. Sci. Ind. Res. (India)., vol. 62, no. 11, pp. 1079–1085, 2003.

[13] M. L. Skotnicki, K. J. Lee, D. E. Tribe, and P. L. Rogers, "Comparison of ethanol production by different Zymomonas strains," Appl. Environ. Microbiol., vol. 41, no. 4, pp. 889–893, 1981.

[14] L. Jamai, K. Ettayebi, J. El Yamani, and M. Ettayebi, "Production of ethanol from starch by free and immobilized Candida tropicalis in the presence of α-amylase," Bioresour. Technol., vol. 98, no. 14, pp. 2765–2770, 2007.

[15] D. Dahnum, S. O. Tasum, E. Triwahyuni, M. Nurdin, and H. Abimanyu, "Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch," Energy Procedia, vol. 68, pp. 107–116, 2015.

[16] Y. Tang, D. Zhao, C. Cristhian, and J. Jiang, "Simultaneous saccharification and cofermentation of lignocellulosic residues from commercial furfural production and corn kernels using different nutrient media," Biotechnol. Biofuels, vol. 4, pp. 1–10, 2011.

[17] Z. Yu and H. Zhang, "Ethanol fermentation of acid-hydrolyzed cellulosic pyrolysate with Saccharomyces cerevisiae," Bioresour. Technol., vol. 93, no. 2, pp. 199–204, 2004.

[18] Wafa A Baz, Lubna S Nawar, and Magda M Aly, "Production of Biofuel from Sugarcane Bagasse wastes using Saccharomyces cerevisiae," J. Exp. Biol. Agric. Sci., vol. 5, no. 6, pp. 871–877, 2017.

[19] W.-S. H. wen-Heng Chen, tTng-Shiang Lin, Gia-Luen Guo, "Ethanol Production from rice straw hydrolysates by pichia stipitis," in Energy Procedia, 2012, vol. 14, pp. 1261–1266.

[20] M. Jaqueline, M. Maciel, K. D. S. Barbosa, and L. A. Cassa-barbosa, "Ethanol production from sugarcane bagasse hydrolysate using newly adapted strains of Meyerozyma guilliermondi CBA-524 and Pichia kudriavzevii CBA-519," no. 17, pp. 182–186, 2017.

[21] H. K. Asif, A. Ehsan, Z. Kashaf, A. A. Abeera, N. Azra, and Q. Muneeb, "Comparative study of bioethanol production from sugarcane molasses by using Zymomonas mobilis and Saccharomyces cerevisiae," African J. Biotechnol., vol. 14, no. 31, pp. 2455–2462, 2015.

[22] I. Santosh, P. Ashtavinayak, D. Amol, and P. Sanjay, "Enhanced bioethanol production from different sugarcane bagasse cultivars using co-culture of Saccharomyces cerevisiae and Scheffersomyces (Pichia) stipitis," J. Environ. Chem. Eng., vol. 5, no. 3, pp. 2861–2868, 2017.

[23] S. Shrivastava, K. G. Tekriwal, A. C. Kharkwal, and A. Varma, "Bio-ethanol production by simultaneous saccharification and fermentation using microbial consortium," Int.J.Curr.Microbiol.App.Sci, vol. 3, no. 7, pp. 505–511, 2014.

[24] R. Giri, B. S. Kundu, P. Diwan, K. Raj, and L. Wati, "Ethanol production from direct sugarcane and juice by yeast," Agric. Sci. Dig. - A Res. J., vol. 33, no. 3, p. 188, 2014.

[25] R. Doppelbauer, H. Esterbauer, W. Steiner, R. M. Lafferty, and H. Steinmüller, "The use of lignocellulosic wastes for production of cellulase by Trichoderma reesei," Appl. Microbiol. Biotechnol., vol. 26, no. 5, pp. 485–494, 1987.

[26] K. Polman, "Review and analysis of renewable feedstocks for the production of commodity chemicals," Appl. Biochem. Biotechnol., vol. 45–46, no. 1, pp. 709–722, 1994.

[27] M. Asgher, Z. Ahmad, and H. M. N. Iqbal, "Alkali and enzymatic delignification of sugarcane bagasse to expose cellulose polymers for saccharification and bio-ethanol production," Ind. Crops Prod., vol. 44, pp. 488–495, 2013.

[28] M. Galbe and G. Zacchi, "Pretreatment of lignocellulosic materials for efficient bioethanol production," Adv. Biochem. Eng. Biotechnol., 2007.

[29] L. R. Lynd, "Overview and Evaluation of Fuel Ethanol from Cellulosic Biomass: Technology, Economics, the Environment, and Policy," Ssrn, 2011.

[30] P. D. Muley and D. Boldor, "Advances in Biomass Pretreatment and Cellulosic Bioethanol Production Using Microwave Heating," no. June, pp. 173–180, 2017.