A Project on New Approach for the Production of Bio-ethanol from Lignocellulosic Biomass

Muthukkannan N.[1], Ayyappan. S.[2]

[1]Assistant Professor, Department of Biotechnology, Vanavarayar Institute of Agriculture, Manakkadavu, Pollachi-642 103, Tamil Nadu, India

[2]Associate Professor, Department of Mechanical Engineering, Dr.Mahalingam College of Engineering and Technology, Pollachi-642003, Tamilnadu, India

Abstract: ***The need for fossil fuels is increasing rapidly whereas the fuel resources are depleting day by day. Therefore, finding an alternative for fossil fuels is of great importance. Ethanol is a low carbon compound and the use is being encouraged in many countries, including India, to reduce the dependence on imported fossil fuels also to reduce the air pollution due to greenhouse gas emissions Agricultural residues especially lignocellulosic biomasses are the suitable choice and it is used for long term feed stock for the Bio-ethanol production due to its low cost and higher availability. It includes all plants and plant derived materials, including agricultural crops and trees, wood and wood residues, municipal residues, and other residue materials. In this project approach a low cost and reliable model is exhibited to produce Bio-ethanol from lignocellulosic biomass to meet the future challenge for fuel.***

Keywords: Bio-ethanol, Lignocellulose, biomass, Agriculture residue

1. Introduction

The need for fossil fuels is increasing rapidly whereas the fuel resources are depleting day by day. Therefore, finding an alternative for fossil fuels is of great importance. Ethanol is a low carbon compound and the use is being encouraged in many countries, including India, to reduce the dependence on imported fossil fuels also to reduce the air pollution due to greenhouse gas emissions (Aggarwal, P.K.2007).

In 2016, India imported 400 million liters of ethanol from foreign nations especially 80% of imported ethanol (worth $173 million) was sourced from the United States and others 18% were from Brazil and 2% from Bhutan and Pakistan (Statistica, 2018).

Presently in India, ethanol obtained from molasses is being blended, though to a limited quantity, in gasoline to reduce our dependency on imported oil (Tuli. D. K., Ravi P Gupta, 2016). Though up to 10% ethanol blending in gasoline has been allowed and mandated, the actual blending is extremely low due to limited availability of ethanol since its production is depend upon the sugarcane molasses. Therefore, alternate feedstocks to produce ethanol are essentially required for constant production of the second generation Bio-ethanol (Nisha Sharma & Nivedita Sharma, 2018).

Agricultural residues especially lignocellulosic biomasses are used for long term feed stock for the Bio-ethanol production due to its low cost and higher availability (Edgard Gnansounou and Arnaud Dauriat, 2005). It includes all plants and plant derived materials, including agricultural crops and trees, wood and wood residues, municipal residues, and other residue materials (Ayeni. A. O. *et al*.2013).

According to the report from Manoel Regis Lima Verde Leal, *et al*.2014, the global level bio-ethanol production utilizes the corn and wheat as a major Lignocellulosic biomass to produce largest quantity of Bio-ethanol around 15.8 billion gallons at 2017 in United States of America due to the presence of around 57-60% of fermentable materials in its. So the percentage of fermentable materials in the Lignocellulosic feed stock is determined the ethanol recovery (Ping Li. *et al*.2016).

Lignocellulosic biomass are mainly consist of cellulose (43%), hemicelluloses (20%), and lignin (27%), which are strongly bonded together, making it resistant to induced hydrolysis process and also insoluble in water (Augustine.O, *et al*.2015, E. N. Ali and M. Z. Jamaludin, 2015). Pretreatments like enzyme, chemical, thermal and their combinations followed by alcoholic fermentation with potential organisms on Lignocellulosic biomass yields high quantity of Bio-ethanol from sugar cane beets (18.8%), fruit extracts (15.6%) and sweet potato (13.6%) (Prasad M.P. (2014).

India is a third position in the globe around 7,770 hectors of land is utilized for corn production after United States of America and Brazil (FAO, 2008). The biomass contains 57.8 % fermentable material that occupied around 71.95 % m/m carbohydrates (DOSON, 2001).The corn stalk includes flower, stem, cob, husk and leaf. Among them corn cob have highest glucose content around 94.2% after hydrolysis process and the ethanol production of 24.0 $g\,L^{-1}$ were achieved (Ping Li. *et al*.2016). But cob is a commercial important food supplement used for various preparations .Since; the other parts are utilized for ethanol production in this project.

2. Methodology

In this project Lignocellulosic Bio-ethanol production is given in four steps process like Pretreatment, hydrolysis, fermentation, separation / distillation. So that high attention has to be given for all four major steps to increase the ethanol yield.

2.1 Physical treatment

After selection, the biomass is pre-washed with running tap water to remove the sticky impurities followed by chopped into small pieces by manually or mechanical cutter (Braide.W. *et al*.2016) and dried to reduce the moisture content of sample should be 10 ± 1% before milling(Marti tutt,2015**)**. Then ground the pieces into powder form (2mm size particle) using pulverizer (Muhammad Irfan.*et al*. 2014). The powder biomass is then boiled under autoclave for 121° C for 15 minutes and after removes the moisture by drying under oven at 40ºC for 2 hrs.

2.2 Chemical Treatment

1) Dilute acid pretreatment

In this treatment, initially the dried biomass is mixed with 1% H_2SO_4 (w/w) in 1:10 ratio (Marti tutt, 2015**)** after that, the sample mixer is heated up to 200-240ºC in an oven (Spyridon Achinas & Gerrit Jan Willem Euverink, 2016) for one hour. This process is helps to remove lignin content in biomass which acts as physical barrier to enzymatic treatment (Braide.W. *et al*.2016).Then the treated sample is cooled down to 50ºC and the pH mixture is neutralized by using $Ca(OH)_2$ before enzymatic hydrolysis.

2) Alkaline Pretreatment

In this treatment, initially the dried biomass is mixed with 1% NaOH (w/w) in 1:10 ratio after that, the sample mixer is heated up to 200-240ºC in an oven for one hour (Marti tutt,2015**)**. Then the treated sample is allowed to cool down for 50ºC and the pH mixture is neutralized by using HCL before enzymatic hydrolysis.

2.3 Hydrolysis Process

As per the Prasad M.P. (2014), instead of using pure enzymes in hydrolysis process, we can recover enzymes from microbes, which are producing both amylase and cellulase (*Aspergillus niger* and *Trichoderma viride) enzymes* in potato dextrose broth under 30ºC incubation for seven days. After incubation, the media is filtered and centrifuged for enzyme extraction; also the supernatant of both are used for enzyme assay before hydrolysis.

After that, the pretreated biomass is mixed with of pure water in 1:10 ratio and boiled as a whole and kept for sterilization. After cooled to 40ºC, 5% of microbial enzyme is added for hydrolysis at 37ºC for 3 hours of incubation and filtered aseptically.

2.4 Fermentation

It is a third phase in Bio-ethanol production. One of the most commonly used microorganisms are *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pachysolen tannophilus*, *Pichia striptis* and *Escherichia coli* (Andersen et al., 2011; Maitan-Alfenas et al., 2015). As per the Nisha Sharma and Nivedita Sharma (2018), both *S. cerevisiae* and *Zymomonas mobilis* are recognized as safe for producing ethanol in co-fermentation of both pentose and hexose sugars.

In this project, aseptically produced cells of both S. *cerevisiae* and *Zymomonas mobilis* (3g/L) are added to the medium (enzymatic hydrolyzed samples) and it kept at 30° C incubation for 48 hours (Thangavelu et al., 2014). In the course of fermentation more over 90% of the 6-carbon sugars are usually fermented and converted into ethanol. After complete fermentation the samples are collected by distillation to achieve fuel grade quality ethanol.

2.5 Analysis of fermentation broth

Concentration of reducing sugar is analyzed by spectrophotometer UV-Visible (Elico- SL-210) using DNS method (Okta bani,*et al,2015)* and is expressed as equivalent glucose concentration against calibration curve. Ethanol concentration is analyzed by GC using static head space analysis Shih, C. (2010) at adjusted salt concentration of 0.1 mM MgSO4 against calibration curve. Iso-propanol is used as an internal standard. Density, viscosity, and pH are measured by using pycnometer, Oswald viscometer, and pH meter respectively.

In this proposed project, the simplified unit operation for Bio-ethanol from corn waste biomass is furnished in Fig.1.

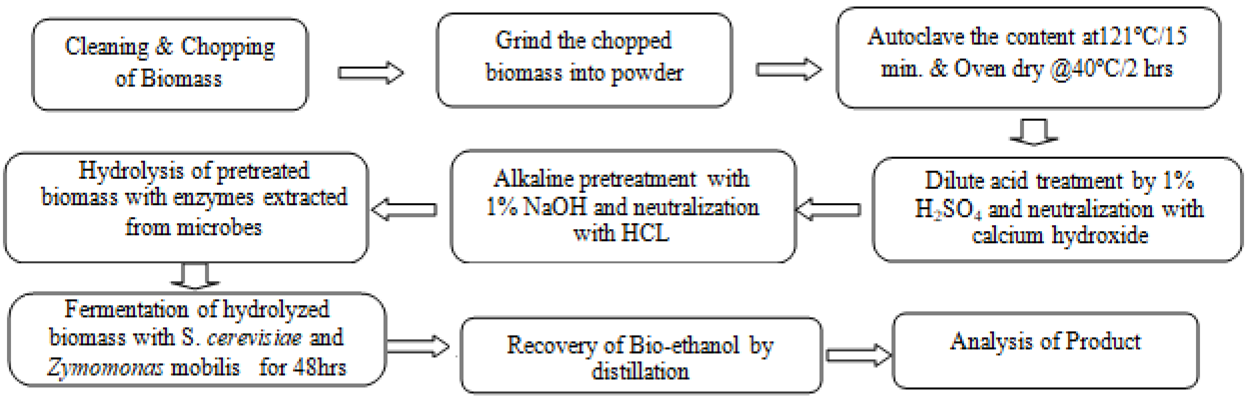

Figure 1: Flowchart for the production of Bio-ethanol from corn waste biomass

Instruments required

S.No	Name and specification of the Instrument	Cost in Rs.(Approx.)
1.	Weighing Scale- 0.1mg accuracy and heavy load (up to 500kg)	9500/-
2.	Hot air oven (multi tray) ,maximum heat load 300ºC	65,000/-
3.	Pulverizer –Stainless steel (5 kg/hr capacity)	30,000/-
4.	Auto clave (vertical model for industrial use)	75,000/-
5.	Cattle feed chopper machine	25,000/-

Chemicals and Glassware required:

S. No	Name and specification of the items	Cost in Rs.(Approx.)
1.	Borozil bottles 5 liter capacity (10 numbers)	5,000/-
2.	Glass funnel (big size) – 10 numbers	4500/-
3.	Plastic tubs (big size – 10 kg capacity)- 10 numbers	3000/-
4.	H_2SO_4 (1 Litre bottles) – 10 numbers	5500/-

Analytical charges required

S. No	Name of the analysis	Cost in Rs.(Approx.) /sample
1.	GC Analysis of fermented liquid	5,000/ -
2.	Ethanol density	500/ -
3.	Viscosity analysis	250/ -
4.	pH analysis	100/ -

3. Conclusion

This production economy is based on different factors like feedstock availability, bio processing technology efficiency, and end-products characteristics. There is a wide variety of sources (corn starch, sugarcane Lignocellulosic biomass, etc.) with low cost and high availability which can be used as a raw material for bio-ethanol production. But Lignocellulosic-based ethanol is not yet widely demonstrated because of its high costs especially in enzyme involved hydrolysis process. In this project the existing process is slightly modified by means of employing of micro-organisms (fungi, yeast, bacteria) instead of enzyme application. The choice of micro-organisms has to be made in terms of type and quantity as this has an impact on conversion rates and process stability.

Bio-ethanol production will be probably the most successful biofuel because it has plenty of usable forms (heat, power, electricity & vehicle fuel).The main credits of the usage of Bio-ethanol are: (i) It is an alternative fuel for reducing the risk of Carbon dioxide emission in to the environment thereby climatic change is reduced; (ii) It is partially substitute the oil needs by using as a renewable energy; (iii) Because of their oxygenated capability, it is blended with regular fuel to reduce the air pollutant emission in automobiles by means of complete combustion of the gasoline.

So this kind of project approach is helpful to produce Bio-ethanol in efficient way to meet the future challenges.

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