IARJSET

ISSN (Online) 2393-8021
ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Vol. 8, Issue 4, April 2021

DOI: 10.17148/IARJSET.2021.8420

Comparative Study on Bioethanol Production from Sugar, Starch, Cellulose and Algal Biomass Sources

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Abstract: In this era fossil fuel like coal, oil and natural gas are playing a major role and being the primary source of energy. It is expected to deplete within next 50 years. So scientists are in the urge of finding an alternative source of energy like electrically driven car, petrol from plastic, changing sea water to usable source of energy and bioethanol or biofuel production from renewable products. This article is based on the comparative study on the production of bioethanol from various raw materials like sugar, starch, cellulose and algal biomass. Basic four steps being followed in the process of bioethanol production. They are pretreatment, hydrolysis, fermentation and recovery. All these biomass used for bioethanol production are sugary material made of simple or complex saccharide compound. They are pretreated to get rid of any foreign particles and are hydrolyzed to breakdown the complex polysaccharide. In all these bioethanol production process, yeast plays a vital role and is preferred widely as they are commercially and easily available product. And bacterial species like *Zymomonas mobilis* is also potentially used now days. Various factors influence the process of formation of ethanol and these factors are needed to be optimized for efficient ethanol production.

Keywords: Bioethanol, hydrolysis, pretreatment, fermentation, biomass, sugar, enzyme, yeast, temperature, pH.

INTRODUCTION

Bioethanol which is referred as ethyl alcohol with chemical formula of (C_2H_5OH) is a clear colorless liquid, melts at -114.1ºC and boils at 78.5ºC. It is biodegradable, cause low environmental pollution and low in toxicity. When burning of ethanol it gives carbon dioxide and water. Ethanol is blend with gasoline in ratio of 1:19 with 5% ethanol and 95% petrol. By blending bioethanol with petrol, it will help to extend the life of diminishing oil supplies. Now only 5% ethanol can be blended with conventional fuel without modification of engine. For 10% ethanol blend, engine modification has to be done. Manufacturing companies are into this for making of engines that work for 10% ethanol blend with gasoline.

Bioethanol is renewable form of resource, which is being used as an alternative source of fuel to the current fossil fuel. Two major things which contributed to the production of bioethanol are that the fossil fuel rate in the Earth is getting depleted day by day and are expected to get vanished off in next 50 years and another reason is that the occurrence of global warming caused by the increase in carbon dioxide emission into the atmosphere from the combustion of fossil fuel. Bioethanol is largely produced through fermentation process, eventhough it can also be manufactured by the chemical process of reacting ethylene with stream. The sugar source for the production of ethanol comes from fuel or energy crops.

The largest bioethanol producer is U.S using corn. Starch in the corn kernel are used as a substrate for production of bioethanol, which is the costlier part of corn plant. But many countries in the world are in great food crisis. So they cannot go for a substrate like corn, which is a major food sources for many countries. These edible product, which are used for producing bioethanol may also lead to increase in food price and can also lead to depletion in the crop that are being used for consumption. So scientist are using an alternative source of energy of substrate like agriculture waste (eg. rice husk, rice straw, garden waste, paper waste, plastics etc.,).

In recent day's plastic are used for producing biofuel by using process of pyrolysis. Technologies and ideas are being developed for using of all non- renewable and other waste into usable form of energy. Bioethanol are being produced from various sources like sugary material (Eg., Beetroot, sugarcane, sweet sorghum and fruits), starchy materials (Eg., corn, wheat, rice, potato, cassava and sweet potato), cellulose (Eg., wood, used paper, crop residue, agricultural waste) and algal biomass (Eg., micro and macro-algae). Scientists are even trying to develop bioethanol form municipal solid waste.

When bioethanol are produced from lingo-cellulosic biomass, it includes 2 major steps. First step is pretreatment and second is hydrolysis and fermentation process. The pretreatment method is done to remove lignin and enhance the penetrating of hydrolysis agents without chemically destruction of cellulose and hemicellulose. Production of bioethanol from starch and sugar will violates the world's population is in great economic crisis and food. In order to find an alternative approaches, lingo-cellulosic biomass are used as cellulose is similar to starch and sugar because it is also a polymer of glucose.

DOI: 10.17148/IARJSET.2021.8420

Methods involved in Bioethanol production:

The method involved in bioethanol production includes four important processes. First is pretreatment, second is hydrolysis and third is fermentation used to convert sugar to ethanol, which is followed by distillation to separate and purify the ethanol.

Pretreatment:

There are different pretreatment method refer table 1, including dilute acid pretreatment (DAP), Steam explosion pretreatment (SEP), organosolv liquid hot water (LHW), ammonia fiber expansion (AFEX), soaking in aqueous ammonia (SAA), sodium hydroxide / lime pretreatment and ozonolysis are used to treat the samples like plant parts and waste biomass.

Table 1. Different pretreatment methods invoved in production of bioethanol

Physical Pretreatment	Physicochemical Pretreatment	Chemical Pretreatment
Mechanical comminution	Steam explosion method	Acid pretreatment
Pyrolysis	Liquid hot water method	Alkaline pretreatment
Microwave oven pretreatment	Ammonia Fiber Explosion	Organosolv preatment
Ultrasound pretreatment	Co_2 explosion	
Chemical pretreatment	Wet oxidation	

Hydrolysis:

Hydrolysis process occur either by acid hydrolysis or enzyme hydrolysis refer table 2. In enzyme hydrolysis, synthetic enzyme or enzymes which are isolated form microbes like lipase, cellulose, amylase, and xylanase are used to convert complex carbohydrates to simple sugar. Acid hydrolysis also promotes release of other substances which are present in the biomass other than release of sugar. Acid like sulfuric acid, hydrochloric acid, acetic acid, etc., are used in this process.

Table 2. Hydrolysis process obtained using various sources

Enzymes	Acids	Microorganism
Alpha amylase	Sulfuric acid	*Pichiastipitis*
Gluco amylase	Hydro chloric acid	*Aspergillusniger*
Xylanase	Nitric acid	*Pencillumsp*
Pectinase	Trifluoro acetic acid	*Saccharomyces cerevisiae*
Glucuronidase	Phosphoric acid	*Bacillus licheniformis*
Cellulase	Formic acid	*Zymomonasmobilis*

Fermentation:

In fermentation process the sugar (glucose, fructose or other monosaccharaides) is converted to ethanol by microbes. The major organism, which plays a vital role is yeast *Saccharomyces cerevisiae*. And also other bacteria like *Zymomonas mobilis* is the most common frequently employed for bioethanol production.

Purification:

After fermentation process less yield of ethanol is produced. Only after purification process, high percentage of ethanol is extracted. Like batch process, dehydration, rectification and evaporation of ethanol are employed. These processes are done as they have significantly some quantity of water, which has to be removed. Final product is the crude bioethanol.

Production of bioethanol from sugar biomass: used sweet sorghum stalk, which has high sugar content. It can be used as a feedstock for ethanol production under hot and dry climatic conditions because it has high tolerance to salt, drought and waterlogging. The water and fertilizer requirement are much lower than sugarcane. It can be harvested every four months twice a year. After washing, crushing and milling the grains to get the juice, the starchy material is gelatinized, liquefied and saccharified using alpha-amylase and gluco-amylase enzyme to produce glucose. Then using S.cerevisiae the fermentation process is done. Alcohol from fermented mash is concentrated up to 95% v/v. This is further concentrated to produce ethanol with 99.6% v/v minimum concentration (Almodares and Hardi, 2009).

Bioethanol production form rice water waste: Rice waste water after cooking was used as a source of bioethanol production, Acid hydrolysis using Hcl and Enzymatic hydrolysis using crude amylase which was extracted form *Bacillus liceniformis* present in the water sample collected from sewage water. $CaCo_3$ is added to help maintain the stability of the amylase. Saccharification was done and found that acid hydrolysis followed by enzyme hydrolysis showed high ethanol yield and most efficient process. Low quality rice used for high yield as better substrate. *Sachromyces cervisiae* grown aerobically high YPD medium used for fermentation. Flasks were incubated with yeast entrapped in Calcium alginate gel. Estimation was done after 12, 24, 36 & 48 hrs of fermentation using potassium

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dichromate assay. *Bacillus licineformis* at 60ºC of pH 7.5, the activity of crude amylase was achieved and maximum activity seen (Chethanas *et al*., 2011)

Bioethanol production from starch biomass:

Bioethanol production from sugarcane bagasse using fermentation process used sugarcane bagasse, which is a waste product after crushing of juice out of it. This is made use, to generate renewable source of energy. The sugarcane bagasse was pretreated, dried and grinded. 2 major enzymes are used for liquefaction and saccharification of sugarcane bagasse. First alpha-amylase was added to slurry along with phosphate buffer to breakdown the cellulose into smaller size called dextrin. Second gluco-amylase is added to the mixture, it hydrolyze the dextrin to fermentable sugar. In fermentation process *S.cerevisiae* was used to ferment the simple sugar to ethanol and carbon dioxide. The effect of pH and temperature were changed, to find which optimum pH and temperature is required for high yield from effective activity of *S. cerevisiae* on fermentation process (Wong and Sanggari, 2014).

Production of Bioethanol from waste potato: used potato tuber for bioethanol production. They were washed, peeled, sliced and milled. The *S. cerevisiae* percentage and sugar concentration was varied, like 2.6% and 4% *S. cerevisiae* solution and 7.5g, 105g sugar used. Potassium iodine and dichromate solution was used for acid hydrolysis and are titrated with sodium thiosulfate solution. 12 different of experiment design was prepared with potato, yeast solution and sugar concentration. High ethanol concentration was seen in group g, with 75g potato, 73mL distilled water, 2g yeast, 1% 105g sugar, 90min time with 9.17mL ethanol production (MerveDuruyurek *et al*., 2015)

Bioethanol production from waste office paper. Used waste paper collected form office and photocopy service waste. Acid hydrolysis with Hydrogen peroxide, autoclaved and samples filtered and centrifuged to obtain hydrolysate product. Fermentation done with *S. cerevisiae* with orbit shaking at 150rpm. Some physical properties such as acid value, turbidity, color, refractive index were determined and compared with standards. Ethanol concentration of the sample determined used refractometer ABBE – REF1. Maximum ethanol concentration is achieved at 120min when sulfuric acid of 5% weight is used for hydrolysis. During 24hrs at 30ºC and 150rpm, 0.1035mL of bioethanol/g waste office paper with purity of 9.67% was obtained after fermentation with *S.cerevisiae* (Maceriras and Alfonsin).

Biethanol production form algal biomass:

Algal bioethanol production technology: A trend towards sustainable development: using of algal biomass is more efficient as they cultivation rate is 10fold higher than other land based plants. First is the algal growth, for biomass production both in lab condition and open pond cultivation. Next is dewatering of the algae. Then in fermentation procedure, the sugar which is obtained is added with yeast and water. Further distillation process is done to remove unwanted materials from the ethanol. Supercritical fluid extraction could be one of the best ways to produce wet biomass of the algae. Using algal biomass as raw materials for bioethanol production is sustainable and eco-friendly source for renewable biofuel production (RiazBibil *et al*., 2016).

Effects of acid pre-treatment on the microbial fermentation process for Bioethanol production from micro algae - in this experiment the microalgae chlorella as a source of bioethanol production. For enzymatic hydrolysis alpha-amylase from *Bacillus liceniformis* and amyloglucosidase form *Aspergillus niger* were used as catalyst. Acetic acid and sulfuric acid were the type of acid used for pre-treatment. *Sacchromyces cervisiae* dry yeast was used by activating it. Simultaneous Saccharification and Fermentation (SSF) process was used. And after 84 hrs incubation, reducing sugar content in each samples were monitored through DNS method, FTIR spectroscopy, GC-FID. Ethanol extracted through distillation process using rotary evaporator. ANOVA results showed that there was significant different in concentration and type of acid towards ethanol production. The highest yield obtained was 0.28g ethanol/g microalgae, when pretreated with 5% (v/v) sulfuric acid (Chai Keepluwan,2019).

Bioethanol production from cellulose biomass:

Bioethanol production from Corn cob Hydrolyzed by cellulose of *Aspergillus niger* and *Zymomonas mobilis* and *Saccharomyces cerevisiae* isolated from palm wine: used decaying corn cob obtained from a dumpsite. A.niger was isolated from dumpsite corn cob by serial dilution the remnant of corncob water. And the cellulose activity of *A. niger* was assayed. The hydrolysate was used for fermentation with pure colonies of yeast and to it pure colonies of *Z.mobilis* obtained from palm wine was also inoculated. The bottles were removed at 8hrs interval for determining the amount of ethanol produced by acidified dichromate/thiosuphate titration method. The sugar amount in the medium was also determined after each period of fermentation by DNSA method of Miller. Highest cellulase activity was obtained on the 5th day (2.30mg/ml/sec). As there was high microbial cell density of *S. cervisiae* during 32nd hr. The result also showed an increase in alcohol concentration with decrease in sugar concentration and increase in microbial cell density when *Z. mobilis* was used (Orji Jerry *et al*., 2016)

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Status and perspectives in Bioethanol production from sugar beet: used sugar beet. These sugar beet are used for sugar production, around 20% of sugar is produced from sugar beet by around 50 countries. They are preferred as they have short growing season, the water requirement is four times less water and sugar content is 25% higher than sugarcane. The sugar beet root are cleaned with water and are cut and grinded, then to get rid of unwanted non sugar product, carbonation step is carried out. As sugar is seen in molasses, the thick juice is crystallized. Pretreatment process like mechanical commution, liquid hot water pretreatment and ultrasound pretreatment are carried out. Enzymatic and acid hydrolysis process is done. Fermentation is carried out using yeast at 30ºC at pH 5 for 27 days. Study was made using beet molasses, thick juice and raw juice. From that, raw juice produced higher ethanol content (CristinaMarzo *et al*., 2019)

Bioethanol productions from pineapple and watermelon peels are used. These samples were asusual washed, oven-dried and trounced to powder. No hydrolysis process was done, directly used the powdered peel to fermentation. The pH was adjusted to 4.5, 5.5, 6.5 in different sludge, with one normal Hcl and NaOH. Then 2ml of harvested *Aspergillus niger* from contaminated soil and *Penicillum sp.* from fresh palm wine were inoculated for fermentation to take place. Distillation process was carried at a higher temperature above 78º C and statistical analysis was taken. From this, it is known that pineapple peels fermented with *A. niger* recorded the highest volume of yield of ethanol with mean ISE of 1147±0.67 after distillation at the pH 4.5 with ethanol concentration 12.06±0.2%. More than *Penicillum sp.,* and *A. niger* showed effective yield (Agbor and Ukpong, 2019).

CONCLUSION:

The yield of bioethanol production is purely relies on the acids and enzymes which are used for acid hydrolysis and enzymatic hydrolysis process. In addition, the pre-treatment methods followed before processing includes the temperature, optimum pH for the yeast activity, sugar content present in the by-product and also purification method chosen. Now, the second generation bioethanol productions are being adapted all over the world and are preferred widely due to relatively inexpensive as well as readily and locally available.

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ISSN (Print) 2394-1588

International Advanced Research Journal in Science, Engineering and Technology

Vol. 8, Issue 4, April 2021

DOI: 10.17148/IARJSET.2021.8420

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