ETHANOL IS THE 1ST GENERATION BIO-FUEL

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INTRODUCTION

It has always been in humankind's goal to find sources for sustainable alternative energy. Concern over price increases and environmental issues brought on by the use of gasoline and diesel is on the rise. Therefore, it is essential that we consider all potential sources of energy. Cellulosic ethanol, which is made from inexpensive and plentiful cellulosic feedstocks like wheat straw, switchgrass, and wood, may also be generated from rich sources like corn, sugar beet, sweet sorghum, and sweet potatoes. Agricultural wastes, such as corn cobs, stover, sorghum stalks, and by products from the sugar industry, such as sugarcane molasses, are thought of as less expensive sources of ethanol in order to avoid the usage of staple crops.

Finding sources of renewable alternative fuels has always been a concern of mankind. There is growing interest in rising prices and environmental problems due to the use of gasoline and light oil. That is why it is imperative to look to every possible source of energy. Ethanol can be made from abundant sources such as corn, sugar beets, sweet sorghum, and sweet potatoes, or from abundant and inexpensive cellulosic feedstocks such as wheat straw, switchgrass, and wood, known as cellulosic ethanol. to prevent

Agricultural waste such as corn cobs, rudders, sorghum stalks and sugar by-products Industries such as sugar cane molasses are considered cheap sources of ethanol. Corn, which requires little processing, was the substrate of choice for this project. In addition, the yield is also higher compared to cellulose substrates. Potato skin scraps were also used to make ethanol. Approximately 58% of dry weight as starch. Ethanol is mainly produced in India Cane molasses, a by-product of the sugar industry, contains Glucose is readily converted to ethanol by yeast. They are known as first generation fuels Because they are made from seeds, grains and sugar. Corn is the most commonly used, Goods from Western countries such as Brazil and the United States. lots of petrol and diesel Widely used fuel for automobiles in India with the exception of some places that use nature gas. Bioethanol can be used in vehicles by blending it with gasoline, known as gasohol or amount of pollution caused by fossil fuels produced by the product combustion of carbon dioxide, carbon monoxide, nitrogen oxides, sulphur, etc. compared to natural gas, bioethanol and biodiesel. Aside from the well-known use of ethanol as a fuel, about 45% of ethanol produced comes from agriculture. Used as drinking alcohol, 40% in industry, rest available mixed with gasoline. Ethanol is used in the chemical and pharmaceutical industries. It is also used in industries such as the production of ethyl tertiary butyl ether (ETBE). It has three methods for pre-treatment of biomass. physical, chemical, biological. On an industrial scale, biological processes are preferred. unwanted by-products. Only the targeted response required for is executed. The remaining biomass after product formation and thus product extraction can optionally be used. Other purposes such as animal feed. All biological reactions proceed optimally. The terms and production costs are also cheaper than other methods.

Starch is made up of amylose and amylopectin. It consists of Alpha 1.6 and Alpha 1.4 linkage. When starch is cooked at high pressure and high temperature, it gelatinizes and becomes effective. An enzyme that accesses and digests polymers. Dry grinding method was used for this project. High yields are obtained for the production of ethanol, mainly because it is economical, and because you can use whole corn kernels, as opposed to wet milling processes that involve it. Separation of starch, germ, fibber, etc.

Milling
↓
Liquefaction
↓
Simultaneous saccharification and Fermentation
↓
Centrifugation
↓
Distillation

Fig.1. Outline of the dry grind process

Yeast is the most common organism when it comes to fermentation processes. A simultaneous saccharification and fermentation (SSF) process was used. in a single reactor. Yeast is added along with saccharifying enzymes that produce glucose units. They were immediately converted to ethanol.

The yield of ethanol could be calculated using the following equation.

Glucose	—Yeast→	Ethanol +	Carbondioxide + Heat
1mole		2 moles	2 moles
180g		92g	88g

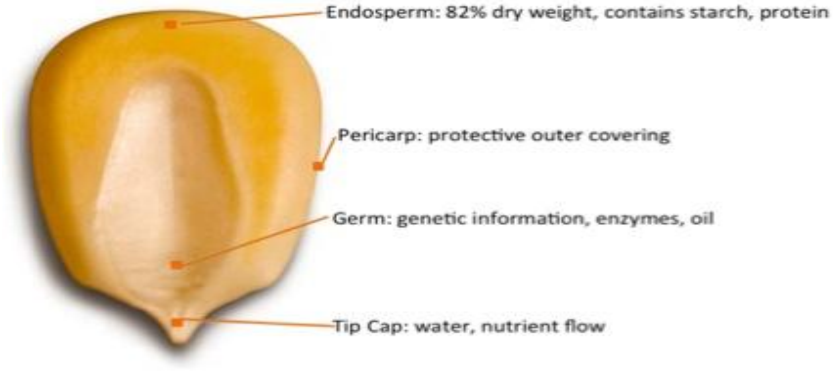
That way there is no chance of glucose. Concentration and produced ethanol prevent microbial contamination. Ethanol yield can be calculated using the following formula: Microorganism selection is one of the key factors for production. It can withstand osmotic pressure and resistance to ethanol. Yeast is the same ones were used because they were in frequent use for decades. Both solid-state and submerged fermentations were performed to confirm efficiency. Solid state fermentation was attempted because the organism is well-suited to producing ethanol fermentation like that. Yeast adapts very well to low humidity environments. it doesn't need to move or for ventilation, there is no energy requirement.

LITERATURE REVIEW

To satisfy the desire to improve people's lives through science and technology some were voted against given the potential ethanol has in this regard efforts to perfect fermentation technology and raw materials used.

2.1 Corn

Corn has the lowest total cost of ethanol production. What part of the corn is used for ethanol? Corn kernels are mainly used for ethanol production. The diagram shows the general composition of corn. An image of a yellow dent cone commonly used in ethanol production. The endosperm is mainly composed of starch, corn energy stores, and proteins for germination. Starch used to make fuel. The pericarp is the outer covering that protects the core and retains the nutrients it contains. The pericarp resists water and steam and protects against insects and microbes. The centre of life is the germ. It contains genetic information, enzymes, vitamins and minerals that help grains grow into corn plants. About 25% of the germ is corn oil, a valuable part of the grain. In the tip cap, the core is attached to the flask and water and nutrients flow through the tip cap. This part of the kernel is not covered with pericarp.



Composition of a Kernel of Corn

Starch is a polymer. It is made up of D-glucose units. Therefore, glucose content directly affects ethanol yield. Starch is the main component, accounting for 62%. Corn kernels are also composed of protein and fibber (19%), water (15%) and oil (4%). Minor amounts of other ingredients may also be present, but these are minor amounts compared to the major ingredients. Starch is composed of her two different polymer molecules, amylose and amylopectin. Considering these two carbons, starch can be broken down into these components. Amylopectin constitutes 50% (80% of starch) of yellow-pitted corn kernels and amylose constitutes 12% of kernel (20% of starch). One bushel (56 lbs) of corn yields multiple products.

1 bushel offers: 31.5 lbs. strength or 33 lbs. Sweetener or fuel 2.8 gallons of ethanol or 22.4 pounds of PLA fibber (a starch-based polymer called polylactic acid). Additionally, corn weighs 13.5 pounds. Gluten Food (20% Protein), 2.5 lbs. Gluten meal (60% protein) and 1.5 lbs. of corn oil. Based on this information, we can calculate the actual yield relative to the theoretical yield and determine the yield achievable in ethanol conversion.

This is shown below:

1 bushel of corn:

56 lbs/bu x 62% starch = 34.7 lbs of starch/bu

34.7 lbs starch x 1.11 lbs glucose/lb starch = 38.5 lbs glucose/bu

The reaction of glucose to ethanol:

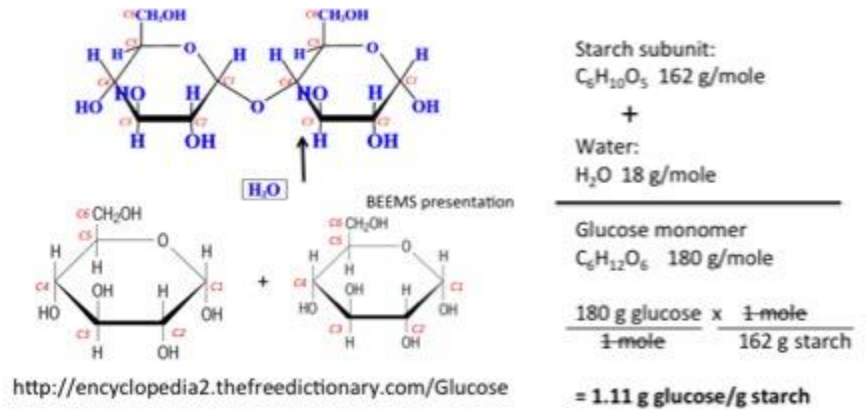
$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + CO_2$$
$$180 g/mol \quad 2*46\ g/mol$$

38.5 lbs glucose x 92 lbs EtOH/180 lbs glucose = 19.7 lbs EtOH/bu

19.7 lbs EtOH x 1 gal EtOH/6.6 lbs = 3.0 gal EtOH/bu theoretical

100 x 2.8/3.0 = 93% yield of ethanol

Hydrolysis is also required for the degradation of glucose, a pre-treatment of lignocellulosic biomass. When water ionizes into H+ and OH-, a molecule like maltose breaks into two glucose molecules. Without enzymes, acid, and heat, the reaction would not proceed quickly. This diagram shows the ratio of glucose monomers to glucose subunits in starch. When starch is broken down, water molecules are added to form glucose. This is where the pound glucose/pound starch value for the above calculation is derived.



Maltose Reacting in water to form two glucose molecules

2.1 Ethanol

Ethanol (ethyl alcohol, C2H5OH, (melting point -114°C, boiling point 78.4°C) is water-soluble, density of 789 g/l at 20°C. Produces catalytic hydration of petroleum products (ethylene) synthetic ethanol. Bioethanol is obtained by alcoholic fermentation of sucrose or simply sugar derived from biomass. Absolute ethanol and 95% ethanol are excellent solvents, it is used in many industrial products such as paints, perfumes and tinctures. solution from ethanol (70-85%) is used as a disinfectant in medicine. Synthetic ethanol has the largest market share in the industry, outside of its use in motor fuels. Since it is cheaper than biomass-derived ethanol, it can be used in a wider range of applications. However, bio-ethanol is conquering the alcoholic beverage market, accounting for a small share of vehicles fuel market. Ethanol intended for non-food uses is rendered unfit for human consumption addition of small amounts of toxic or unpleasant substances such as methanol or gasoline. The use of ethanol as a fuel has a long history, and in 1826 Samuel Morley Ethanol was used as fuel in the prototype of America's first combustion engine 1899. The federal government supports ethanol through subsidies and lowers its price of gasoline. In 1902, a car powered by ethanol was unveiled at the Alcohol Fuel Exhibition in Paris in 1908, the Ford Model T was launched and could run on either ethanol or gasoline. 1925, some countries (Brazil, France, Germany) impose petrol retailers. All gasoline sold is blended with ethanol. Ethanol fuels were competing until the late 1930s gasoline. But with the discovery of many cheap oil fields, ethanol became less and less important. Market share in the 1940s. The ethanol fuel update dates back to 1973 cheaper than gasoline, the Brazilian government in 1975 Pro-Alcohol is a nationwide strategic program to produce ethanol and use it as alcohol alternative fuel. In the United States, the Energy Tax Act of 1978 exempted E10. (Gasoline and 10% bioethanol blend, v/v) from excise duty. then another US state Program guaranteed loans for investments in the construction of ethanol plants. Brazil and America are still his two major producers and consumers of bioethanol in the world. The main reason here are the glories of bioethanol:

1. Use as an octane booster in unleaded petrol instead of methyl tert-butyl ether (MTBE)

2.Used as an oxygenator to clean gasoline combustion and improve air quality

3. Use as an alternative fuel to reduce CO2 emissions and mitigate climate change risks

4. Use renewable energy to partially replace oil and increase security of supply.

2.3 CORN ETHANOL

Corn ethanol is ethanol made from corn biomass and is the primary source of ethanol fuel in the United States, renewable fuel standards require it to be blended with gasoline. Corn ethanol is made by ethanol fermentation and distillation. It is disputed whether the production and use of corn ethanol reduces greenhouse gas emissions by gasoline. About 25% of the US corn acreage is used for ethanol production.

2.3.1 Usage

Since 2001, corn ethanol production has increased more than sevenfold. from 9:50 Billions of bushels of corn were produced in 2001 and 710 million bushels were used in production corn ethanol. In 2018 he was 5.60 compared to 14.62 billion bushels of corn. US reports billions of bushels were used to make corn ethanol energy authorities. Overall, 95% of ethanol is made from corn [5].

Today, corn ethanol is mostly used in blends with gasoline to create blends like E10, E15 and E85. Ethanol is formulated in more than 98% of US gasoline and reduces air. Environmental pollution. Corn ethanol is used as an oxygenator when blended with gasoline. E10 and E15 can be used on all engines without modification. However, blends like E85, with a much greater ethanol content, require significant modifications to be made before an engine can run on the mixture without damaging the engine. Some vehicles that currently use E85 fuel, also called flex fuel, include, the Ford Focus, Dodge Durango, and Toyota Tundra, among others. The future use of corn ethanol as a main gasoline replacement is unknown. Corn ethanol has yet to be proven to be as cost effective as gasoline due to corn ethanol being much more expensive to create compared to gasoline.[6] Corn ethanol has to go through an extensive milling process before it can be used as a fuel source. One major drawback with corn ethanol, is the energy returned on energy invested (EROI), meaning the energy outputted in comparison to the energy required to output that energy. In the future, as technology advances and oil become less abundant, the process of milling may require less energy, resulting in an EROI closer to that of oil another serious problem. The problem with corn ethanol as a gasoline substitute is standard engine damage vehicle. E10 contains 10% ethanol and is suitable for most road vehicles E15 contains 15% ethanol and is generally prohibited for use in automobiles before 2001. However, in hopes of replacing petrol containing E85 in the future 85% ethanol, engine modifications are required to allow the engine to sustain when handling high loads. Amount of ethanol over time. So mostly old and modern. Without proper engine modifications to accommodate the increase, the vehicle becomes obsolete. Corrosive with large quantities of ethanol. Most gas stations don't even offer it Refuelling of E85 vehicles. The United States Department of Energy reports that only 3,355 gas stations, out of 168,000, across the United States offer ethanol refuelling for E85 vehicles.

2.3.2 Environmental issues

Corn ethanol has fewer greenhouse gas emissions than petrol and is full tank. It is biodegradable, unlike some fuel additives such as MTBE. But as the energy spreads many US distilleries are predominantly coal-fired, and there has been much debate about this Sustainability of corn ethanol as an alternative to fossil fuels. Other controversies Vast arable land required for cultivation and direct impact on grain supply indirect impacts of land use change. Other issues are related to pollution and irrigation water use. Ethanol life cycle treatment, energy balance, emissions intensity manufacturing.

a. Greenhouse gas emissions

A corn processing plant near Columbus, Nebraska. Several full life cycle studies have revealed Corn ethanol reduces well-to-wheel greenhouse gas emissions by up to 50% compared to gasoline. However, other studies have found that corn produces ethanol. Considering the use of fertilizer, CO2 emissions per unit of energy are higher than gasoline, Change in land use.

b. Agricultural land

One of the major controversies in corn ethanol production is the need for arable land. Agricultural land for growing corn for ethanol. Animal consumption. In the United States, 40% of acreage is devoted to grain used for corn ethanol production, 25% converted to ethanol after balancing a by-product, only 60% of the harvest can be consumed by humans or animals.

2.3.3 Economic impact of corn ethanol

This is according to the Renewable Fuels Association (RFA), an ethanol industry lobby group. Ethanol production raises the price of corn due to increased demand. The RFA claims that ethanol production has positive economic effect for US farmers, but it does not elaborate on the effect for other populations where field corn is part of the staple diet. An RFA lobby document states that "In a January 2007 statement, the USDA Chief Economist stated that

farm program payments were expected to be reduced by some $6 billion due to the higher value of a bushel of corn production in 2009 reached over 13.2 billion bushels, and a per acre yield jumped to over 165 bushels per acre. In the United States, 5.05 billion bushels of corn were used for ethanol production out of 14.99 billion bushels produced in 2020, according to USDA data.

According to the U.S. Department of Energy's Alternative Fuels Data Centre, "The increased ethanol [production] seems to have come from the increase in overall corn production and a small decrease in corn used for animal feed and other residual uses. The amount of corn used for other uses, including human consumption, has stayed fairly consistent from year to

year. This does not prove there was not an impact on food supplies: Since U.S. corn production doubled (approximately) between 1987 and 2018, it is probable that some cropland previously used to grow other food crops is now used to grow corn that too insignificant land is or is likely to be converted or returned to agricultural use. That may have negative environmental impacts.

2.3.4 Alternative biomass for ethanol

Food production leftovers such as corn stalks can be used to make ethanol instead of corn. Ethanol made from sugar beet used in Europe and sugar cane in Brazil Up to 80% less carbon dioxide from the well to the wheels. Use of cellulosic biomass. Ethanol production is considered a second-generation biofuel. A solution to the food versus fuel debate, potentially reducing lifecycle greenhouse gases Up to 86% emissions compared to gasoline.

MATERIALS AND METHODS

2.1. Materials:

Corn was obtained from a local market.

2.2. Enzymes:

Alpha amylase and Glucoamylase.

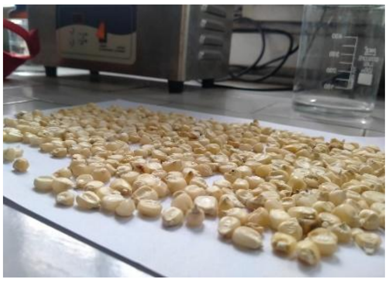
2.3. Microorganism:

Baker's yeast Saccharomyces cerevisiae was obtained from a local market.

2.4. Methods:

2.4.1. Milling:

Thoroughly dried Corn kernels were milled in a flour mill to obtain a coarsely milled powder. The corn flour and PPW powder were size separated using a sieve shaker. The flour and Powder retained in 3 different mesh sizes were collected and stored under air-tight conditions. The grinding helps to break the tough outer coatings of the corn kernel, which will increase the surface area of the starch. Once the corn is broken down, it is mixed/slurry with heated water to form a mash or slurry.



Corn Kernels **Corn Flour**

2.4.2. Liquefaction:

Once the corn slurry is made, it goes through cooking and liquefication. In a 250ml conical flask, 5-15g of corn flour of a chosen particle size was added along with 100 ml of distilled water. It was cooked at 121°C and 15 psi for 30 minutes in an autoclave. The cooking stage is also called gelatinization. Water interacts with the starch granules in the corn when the temperature is >60°C and forms a viscous suspension. The liquefaction step is actually partial hydrolysis that lowers the viscosity. It is essentially breaking up the longer starch chains into smaller chains. One way to measure this is to look at dextrose equivalents (DE), or a measure of the amount of reducing sugars present in a sugar product, relative to glucose, expressed as a percentage on a dry basis. Dextrose is also known as glucose, and dextrose equivalent is the number of bonds cleaved compared to the original number of bonds. The equation is:

$$\text{Equation 1:} 100 \times \frac{\text{number of bonds cleaved}}{\text{number of original bonds}}$$

Pure glucose (dextrose): DE = 100

Maltose: DE = 50

Starch: DE = 0

Dextrin: DE = 1 through 13

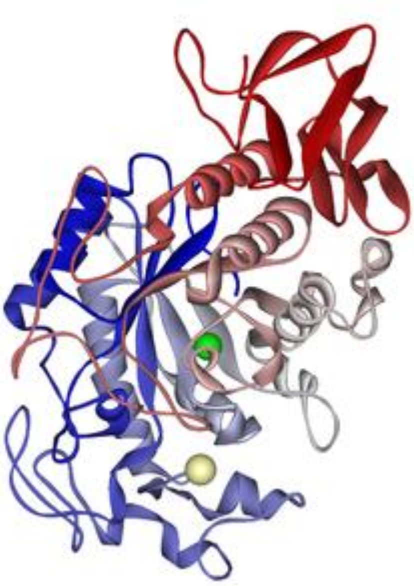
Dextrin are a group of low molecular weight carbohydrates produced by hydrolysis of starch or glycogen. Dextrin are mixtures of polymers of D-glucose units linked by α (1,4) or α (1,6) glycosidic bonds. Dextrin are used in glues and can be a crispness enhancer for food processing.

Maltodextrin: DE = 3 through 20

Maltodextrin is added to beer.

Recall that starch hydrolysis is where water reacts with the sugar to break the sugar down and form glucose. The water breaks into the H+ and OH- ions to interact with the starch as it breaks down. In order to accomplish liquefaction, the reaction must take place under certain conditions. The gelatinized corn was then allowed to cool down followed by the addition of alpha amylase (579U/g) enzyme (0.08g/g of corn). The flask was maintained in the stirrer for 2 hours at 90°C and at 150rpm [10]. At this step usually the pH is 6.0, if not it is adjusted using ammonia and sulfuric acid. At this stage, shorter dextrin are produced but are not yet glucose.

Alpha amylase



One type of α-amylase

The α-amylase for liquefaction acts on the internal α (1,4) glycosidic bonds to yield dextrin and maltose (glucose dimers). A type of α-amylase exists in the saliva of humans; a different α-amylase is utilized by the pancreas. The α-amylase works a little faster than the β-amylase, and the β-amylase works on the second α (1,4) glycosidic bond so that maltose is formed. β-amylase is part of the ripening process of fruit increasing the sweetness of fruit as it ripens.

2.4.3. Saccharification:

The next step in the process of making ethanol is saccharification. Saccharification is the process of further hydrolysis to glucose monomers. A different enzyme is used, called a glucoamylase (also known by the longer name amyloglucosidase). It cleaves both the α (1,4) and α (1,6) glycosidic bonds from dextrin ends to form glucose. The optimum conditions are different from the previous step and are at a pH of 4.5 and a temperature of 55-65°C.

Baker's Yeast

Protein structure of glucoamylase (aka a ϒ-amylase)

Some of the newer developed enzymes (granular starch hydrolysing enzymes – GSHE) allow skipping the liquefaction stage by hydrolysing starch at low temperatures with cooking. Advantages include:

1. reduced heat/energy
2. reduced unit operation (reducing capital and operating costs)
3. reduced emissions
4. higher DDGS. They work by "coring" into starch granules directly without the water swelling/infusion.

Disadvantages include:

1. enzymes cost more
2. contamination risks.

2.4.4.Fermentation:

The final chemical step in producing ethanol from the starch is fermentation. The chemical reaction of fermentation is where 1 mole of glucose yields 2 moles of ethanol and 2 moles of carbon dioxide. The reaction is shown in Equation below:

$$C_6H_{12}O_6 \rightarrow 2C_2H_6OH + 2\ CO_2$$

To cause fermentation to take place, yeast is added. A common yeast to use is saccharomyces cerevisiae, which is a unicellular fungus. The reaction takes place at 30-32°C for 2-3 days in a batch process. Supplemental nitrogen is added as ammonium sulphate ((NH4)2SO4) or urea. A Protease can be used to convert proteins to amino acids to add as an additional yeast nutrient. Virginiamycin and penicillin are often used to prevent bacterial contamination. The carbon dioxide produced also lowers pH, which can reduce the contamination risk. Close to 90-95% of the glucose is converted to ethanol. It is possible to do saccharification and fermentation in one step. It is called Simultaneous Saccharification and Fermentation (SSF), and both glucoamylase and yeast are added together. It is done at a lower temperature than saccharification (32-35°C), which slows the hydrolysis into glucose. As glucose is formed, it is fermented, which reduces enzyme product inhibition. It lowers initial glucose concentrations, lowers contamination risk, lowers energy requirements, and produces higher yields of ethanol. Because SSF is done in one unit, it can improve capital costs and save residence time.

2.4.5. Centrifugation and Distillation:

After fermentation, the broth was centrifuged at 6000rpm for 10 minutes. The supernatant was collected and fed into a simple distillation column. The boiling temperature of ethanol is 78°C hence distillation was carried out around that temperature to facilitate the evaporation of ethanol. The vapor was collected and got condensed by means of the circulation of cold water around the column. The distillate having ethanol was recovered in a conical flask at the other end of the column.

2.4.6. Solid state fermentation:

It is the type of fermentation which uses minimal amount of water. It is possible only if microorganisms could survive in low moisture content. Yeast is very much suitable for such conditions, thus solid state fermentation was carried out using corn. The amount of water to be added can be calculated using this formula:

$$Required\ moisture\ content = \frac{X}{X + \text{amount of substrate used}(g)}$$

where X- is the amount of water to be added (ml)

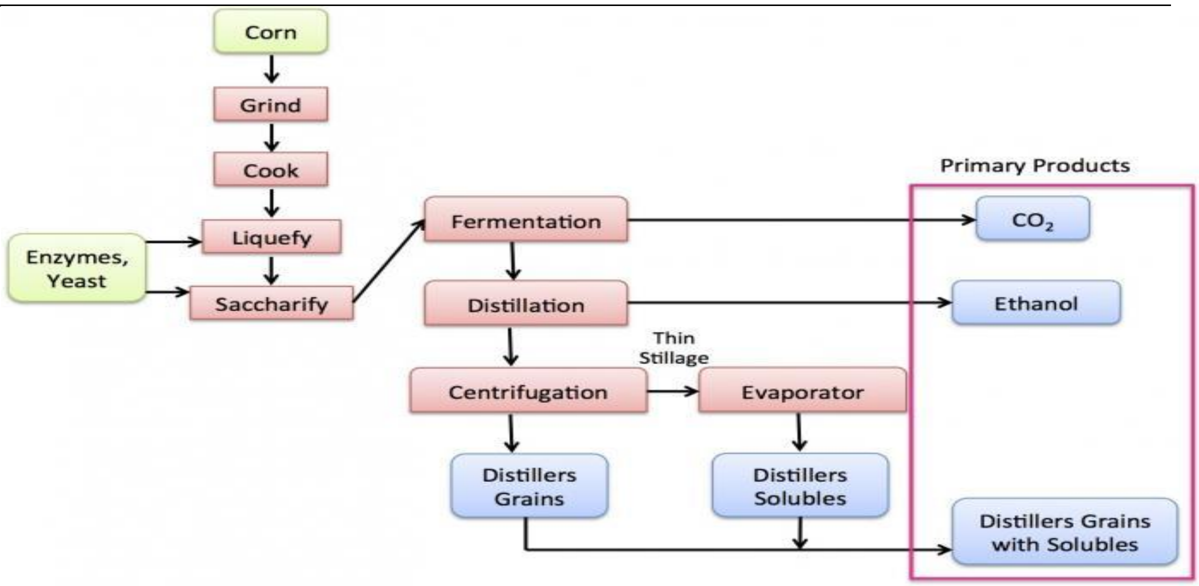
2.4.7. Analysis:

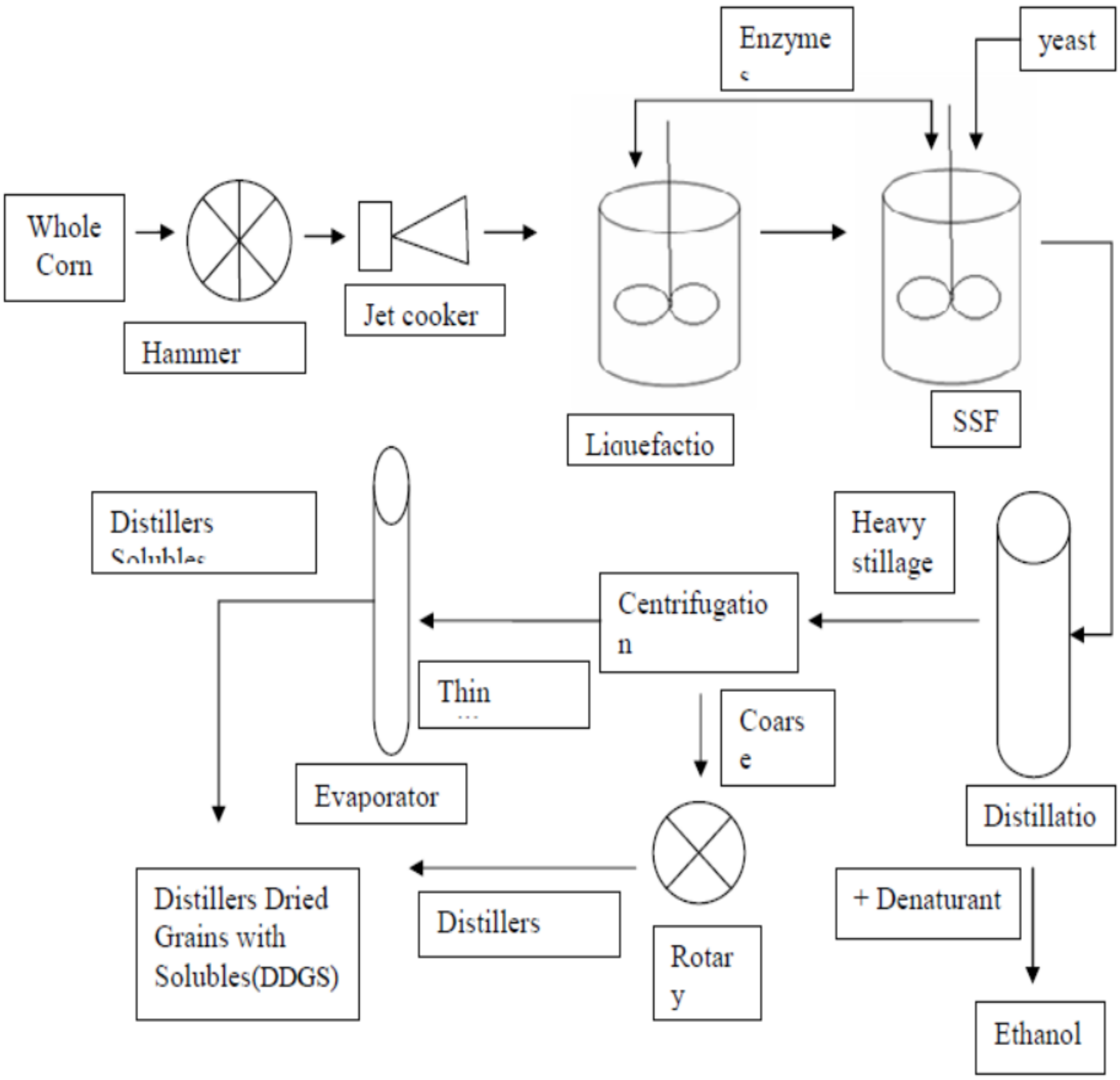
The compositional analysis of corn was done for estimation starch using anthrone reagent [11], protein using Lowry's assay [12], crude fibber [13], moisture [14] and ash content [15]. The reducing sugar concentration was found using DNS method.[16] The difference in sugars produced before and after the fermentation was used to find the amount of reducing sugars produced. The ethanol product confirmation was done using the potassium dichromate method.[17] It gave the percentage alcohol content of the sample.

2.4.8. Response Surface Methodology:

Response surface methodology was used for creating an experimental protocol using which the process variables were optimized. It determines the optimum conditions using the statistical techniques. It optimizes all the independent variables with a minimal number of experiments.[18] It combines special experimental designs with Taylor first order and second order equations. The experiments were designed using MINITAB 16 software in such a manner that several combinations of the process variables are taken into account. The important process variables which were included for optimization are:

1. Particle size
2. pH
3. Substrate concentration


Corn
Grind
Cook
Liquefy
Saccharify
Enzymes, Yeast
Fermentation
Distillation
Centrifugation
Thin Stillage
Evaporator
Distillers Grains
Distillers Solubles
Primary Products
CO_2
Ethanol
Distillers Grains with Solubles


Whole Corn
Hammer
Jet cooker
Enzyme
yeast
Liquefactio
SSF
Distillers
Heavy stillage
Centrifugatio n
Thin
Evaporator
Coars e
Distillatio
Distillers Dried Grains with Solubles(DDGS)
Distillers
Rotar y
+ Denaturant
Ethanol

CONCLUSION

The potential of maize as a feedstock for ethanol production was investigated. The choice of substrates, enzymes, microorganisms and the conditions under which they work have been shown to be critical for ethanol yield. The ethanol yield was done under optimized conditions of pH, particle size, and substrate concentration respectively. Potato skin waste was also used in production, but the yield was not comparable to that of corn. Process development was carried out when the entire production process was scaled up to the industrial level. This includes calculation of 1000liters of ethanol production, equipment selection and fermenter design.

REFERENCES

[1] M. L. Maurice, “Factors Effecting Ethanol Fermentation Via Simultaneous Saccharification and

Fermentation,” *International Journal of Environmental Science and Development,*, 2011.

[2] C. Israilides, A. G. Vlyssides, D. Arapoglou, R. Marchant, and A.A.Vlysides, “Integrated Management Of Potato Starch Wastes,” no.September 2008, pp. 16–17.

[3] S. Ray, A. Goldar, and S. Miglani, “Ethanol Blending Policy:Issues Related to pricing,” *ICRIER Policy Series*, 2011.

[4] M. Antonio and D. Neves, “Bioethanol Production from Wheat Milling Bioethanol Production from

Wheat Milling,” no. January, 2006.

[5] B. P. Lamsal, H. Wang, and L. A. Johnson, “Bioresource Technology Effect of corn preparation

methods on dry-grind ethanol production by granular starch hydrolysis and partitioning of spent beer

solids,” *Bioresource Technology*, 2011,vol. 102, no. 12, pp. 6680– 6686.

[6] S. Schwietzke, Y. Kim, E. Ximenes, N. Mosier, and M. Ladisch, “Ethanol Production from Maize,”

2009,vol. 63,

[7] C. Bradley and K. Runnion, “Understanding Ethanol Fuel Production And Use,” 1984.

[8] S. Nikolic, L. Mojovic, D. Pejin, M. Rakin, and V. Vesna, “Improvement of Ethanol Fermentation of Corn Semolina Hydrolyzates with Immobilized Yeast by Medium Supplementation,” *Food Technol.*

Biotechnol, 2009,vol. 47, no. 1, pp. 83–89.

[9] B.S.Mienda, A. Idi, and A. Umar, "Microbiological Features of Solid State Fermentationand its

Applications - An overview," *Research in Biotechnology*, 2011,vol. 2, no. 6, pp. 21–26

[10] D.A. Ramirez-cadavid, O. Kozyuk, and F. C. M. Jr, "Improvement in commercial scale dry mill corn ethanol production using controlled flow cavitation and cellulose hydrolysis,"*Biomass conversion,*

springer, 2013.

[11] Thayumanavan, B. and Sadasivam, S. *Qual. Plant Foods Hum. Nutr.*, 1984, 34, p.253.

[12] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and K. J. Randall, "Protein measurement with Folin phenol reagent," *J. Biol Chem.*, 1951, pp. 265–275

A.Meenakshi et al /Int.J. ChemTech Res.2014,6(5),pp 2843-2853. 2853

[13] Maynard, A.J. (Ed.), *Methods in Food Analysis*, 1970, Academic Press, New York, p. 176.

[14] AACC, "Materials and methods 3.1," 2000, pp. 32–40.

[15] A.Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, and D. Templeton, "Determination of Ash in

Biomass" Laboratory Analytical Procedure (LAP),2008.

[16] Miller, G.L. *Anal. Chem.*, 1972, 31, p. 426.

[17] N. Kumar, J. P. Singh, R. Ranjan, C. Subathradevi, and M. S. V, "Bioethanol production from weed plant (Cyperus rotundus) by enzymatic hydrolysis," *Advances in Applied Science Research*, 2013, vol.4, pp. 299–302

[18] C. C. AKOH, C. SHU-WEI, L. GUAN-CHIUN, and J.-F. SHAW, "Enzymatic Approach to Biodiesel Production," *Journal of Agricultural and Food Chemistry*, vol. 55, pp. 8995–9005, 2007.

[19] Y. Lin, W. Zhang, C. Li, K. Sakakibara, S. Tanaka, and H. Kong, "Factors affecting ethanol

fermentation using Saccharomyces cerevisiae BY4742," *Biomass and Bioenergy*, pp. 1–7, 2012.

[20] K. Naidu, V. Singh, L. D. B. Johnston, K. D. Rausch, and M. E. Tumbleson, "Effects of Ground Corn Particle Size on Ethanol Yield and Thin Stillage Soluble Solids," *Cereal chemistry*, vol. 84, no. 1, pp.2–5, 2007.

[21] M.L. Schuler, F. Kargi Bioprocess Engineering Basic Concepts Prentice Hall, Englewood Cliffs,1992.

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