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Production of Bioethanol from Sugarcane Bagasse

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ABSTRACT: Bio-fuel has been a source of energy that human beings have used since ancient times. Increasing the use of bio-fuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities. Bio-fuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources. The aim of the project is the production of low cost ethanol by using lignocellulosic materials basically the agro wastes like sugarcane bagasse, rice husk, wheat straw, corn fiber, crop residues, grasses and other materials like saw dust, wood chips, solid animal wastes etc.

KEYWORDS: Bioethanol Production; Sugarcane Bagasse, fermentation process

I. INTRODUCTION

Bioethanol is simply ethanol is a renewable energy source which is made by fermenting the sugar and starch components of plant. It is produced from the agricultural product such as corn, sugarcane, potatoes, rice, beetroot and recently using grapes, banana, dates and other wastes. This is due to the decreasing amount of fossil fuels, alternative energy sources need to be renewable, sustainable, efficient, cost effective, convenient and safe [2]. The demand for oil is expected to increase to 57% from 2002 to 2030. The average price of gasoline in 2005 was \$2.56 per gallon, which was \$0.67 higher than the average price of gasoline in the previous year. Yet in June 2008, the average price of gasoline in the US reached \$4.10 per gallon [6]. Rise in energy demand in worldwide and the progressive demising of oil reserves motivate the search for alternative energy resources, especially for those derived from renewable materials such as biomass [8]. As a result, they come up ethanol production as substitutes to fossil fuel. The lower cost to produce bioethanol is come from biomass waste because the raw materials are available in abundance. The main objective of this thesis is to successfully produce bioethanol from sugarcane bagasse. Sugarcane bagasse is an agricultural waste which used to produce bioethanol using fermentation process. Next is to determine the yield of bioethanol produced from sugarcane bagasse. The bioethanol will be determined using HPLC. The highest peak shows the ethanol content. Last objective is to study the effects of temperature and pH of fermentation process in bioethanol production. Bio-fuel has been a source of energy that human beings have used since ancient times. Increasing the use of bio-fuels for energy generation purposes is of particular interest nowadays because they allow mitigation of greenhouse gases, provide means of energy independence and may even offer new employment possibilities. Bio-fuels are being investigated as potential substitutes for current high pollutant fuels obtained from conventional sources.

II. OBJECTIVE

1. The main objective of this thesis is to successfully produce bioethanol from sugarcane bagasse.
2. Sugarcane bagasse is an agricultural waste which used to produce bioethanol using fermentation process.
3. Next is to determine the yield of bioethanol produced from sugarcane bagasse.
4. The bioethanol will be determined using HPLC. The highest peak shows the ethanol content.

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5. Last objective is to study the effects of temperature and pH of fermentation process in bioethanol production.

III. PRODUCTION PROCESS

Processing of lignocellulosics to ethanol consists of four major unit operations: pretreatment, hydrolysis, fermentation, and product separation/purification. Pretreatment is required to alter the biomass macroscopic and microscopic size and structure as well as its sub-microscopic chemical composition and structure so that hydrolysis of the carbohydrate fraction to monomeric sugars can be achieved more rapidly and with greater yields. Hydrolysis includes the processing steps that convert the carbohydrate polymers into monomeric sugars. Although a variety of process configurations have been studied for conversion of cellulosic biomass into ethanol, enzymatic hydrolysis of cellulose provides opportunities to improve the technology so that biomass ethanol is competitive when compared to other liquid fuels on a large scale.

3.2 Pretreatment of lignocellulosic materials

The effect of pretreatment of lignocellulosic materials has been recognized for a long time. The purpose of the pretreatment is to remove lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the materials. Pretreatment must meet the following requirements: (1) improve the formation of sugars or the ability to subsequently form sugars by enzymatic hydrolysis; (2) avoid the degradation or loss of carbohydrate; (3) avoid the formation of byproducts inhibitory to the subsequent hydrolysis and fermentation processes; and (4) be cost-effective. Physical, physicochemical, chemical, and biological processes have been used for pretreatment of lignocellulosic materials.

3.2.1 Physical pretreatment

3.2.1.1. Mechanical comminution

Waste materials can be comminuted by a combination of chipping, grinding and milling to reduce cellulose crystallinity. The size of the materials is usually 10–30 mm after chipping and 0.2–2 mm after milling or grinding. Vibratory ball milling has been found to be more effective in breaking down the cellulose crystallinity of spruce and aspen chips and improving the digestibility of the biomass than ordinary ball milling. The power requirement of mechanical comminution of agricultural materials depends on the final particle size and the waste biomass characteristics.

3.2.1.2. Pyrolysis

Pyrolysis has also been used for pretreatment of lignocellulosic materials. When the materials are treated at temperatures greater than 300 °C, cellulose rapidly decomposes to produce gaseous products and residual char. The decomposition is much slower and less volatile products are formed at lower temperatures. Mild acid hydrolysis (1 N H₂SO₄, 97 °C, 2.5 h) of the residues from pyrolysis pretreatment has resulted in 80–85% conversion of cellulose to reducing sugars with more than 50% glucose. The process can be enhanced with the presence of oxygen. When zinc chloride or sodium carbonate is added as a catalyst, the decomposition of pure cellulose can occur at a lower temperature.

3.2.2. Physico-chemical pretreatment

3.2.2.1. Steam explosion (autohydrolysis):

Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. In this method, chipped biomass is treated with high-pressure saturated steam and then the pressure is swiftly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160–260 °C (corresponding pressure 0.69–4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure. The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis. Ninety percent efficiency of enzymatic hydrolysis has been achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips. The factors that affect steam explosion pretreatment are residence time, temperature, chip size and moisture content.

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Optimal hemicellulose solubilization and hydrolysis can be achieved by either high temperature and short residence time (270 °C, 1 min) or lower temperature and longer residence time (190 °C, 10 min). Recent studies indicate that lower temperature and longer residence time are more favorable.

3.2.2.2. Ammonia fiber explosion (AFEX)

AFEX is another type of physico-chemical pretreatment in which lignocellulosic materials are exposed to liquid ammonia at high temperature and pressure for a period of time, and then the pressure is swiftly reduced. The concept of AFEX is similar to steam explosion. In a typical AFEX process, the dosage of liquid ammonia is 1–2 kg ammonia/kg dry biomass, temperature 90 °C, and residence time 30 min. AFEX pretreatment can significantly improve the saccharification rates of various herbaceous crops and grasses. It can be used for the pretreatment of many lignocellulosic materials including alfalfa, wheat straw, wheat chaff, barley straw, corn stover, rice straw, municipal solid waste, softwood newspaper, kenaf newspaper, coastal Bermuda grass, switchgrass, aspen chips, and bagasse. The AFEX pretreatment does not significantly solubilize hemicellulose compared to acid pretreatment (to be discussed in the following section) and acid-catalyzed steam explosion compared the steam and ammonia pretreatment for enzymatic hydrolysis of aspen wood, wheat straw, wheat chaff, and alfalfa stems, and found that steam explosion solubilized the hemicellulose, while AFEX did not.

IV. MATERIALS USED

4.1.1 Chemicals Required

- (i) Dilute sulfuric acid = 0.2M, 0.3M, 0.5M
- (ii) Concentrated NaOH = dropwise
- (iii) DNS = 10 gm
- (iv) Phenol = 2 gm
- (v) Sodium sulphite = 0.5 gm
- (vi) Sodium hydroxide = 10 gm
- (vii) Na-K tartarate (40%)

4.2 EQUIPMENTS REQUIRED

4.2.1 Vertical autoclave

This equipment is primarily used for sterilization purpose. It is an enclosed space where steam bath is given to any equipment placed inside it. Water filled in it is heated by electric coils present at bottom. It has a vent at top, from where steam can be released to maintain the required pressure. For our case, sterilization is required after maintaining the pH and also for each filtration. Pressure around 2.02 kg/cm² is used. Once the pressure reaches 2.02 kg/cm², it is maintained for half an hour. The Equipments are allowed to cool down before removing from the autoclave.

4.2.2 Laminar flow chamber

- a) Firstly, the whole chamber is wiped on the inside by spirit solution or ethanol.
- b) UV light is turned on with the shutter completely closed and left for 15 minutes. After switching off the UV, we again wait for 15 minutes.
- c) Next the fan is turned on and shutter opened.
- d) Any transfer taking place in these conditions should be done close to a Bunsen burner flame.
- e) All flasks or jars should be properly capped inside the chamber itself.

4.2.3 Vacuum filtration

For this filtration, a filter having sintered base is fitted into a Buchner-type funnel which in turn is air tightly attached to a conical flask having a side outlet to provide vacuum. For every filtration, Whatman filter papers are attached onto the filter. Also after every filtration, the conical flask and filter media are cleaned and sterilized.

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4.2.4 UV-Spectrophotometer

To analyze the samples, UV-Spectrophotometer is used. For our analysis, we use the particular wavelength measurement. The equipment consists of two cuvettes, one of which acts as a reference while in the other cuvette the sample is kept. Once kept inside, a UV-light is used to know the absorbance of a particular sample at a particular wavelength.

4.2.5 Shaking incubator-

This instrument is used for fermentation. It is an enclosed space where given conditions are maintained at a particular rpm. Flasks are kept on a shaking platform moving at a particular rpm.

4.2.6 pH-meter

This instrument is used for measuring the pH of a given solution.

V. LITERATURE REVIEW

Charlie Marengo Dodo et al. Bioethanol production is one of the most promising possible substitutes for fossil-based fuels, but there is a need to make available cost-effective methods of production if it is to be successful. Various methods for the production of bioethanol using different feedstocks have been explored. Bioethanol synthesis from sugarcane, their tops and leaves have generally been regarded as waste and discarded. This investigation examined the use of lignocellulosic sugarcane leaves and tops as biomass and evaluated their hydrolysate content. The leaves and tops were hydrolysed using concentrated and dilute sulphuric acid and compared with a combination of oxidative alkali-peroxide pre-treatment with enzyme hydrolysis using the enzyme cellulysin® cellulase. Subsequent fermentation of the hydrolysates into bioethanol was done using the yeast *Saccharomyces cerevisiae*. The problem of acid hydrolysis to produce inhibitors was eliminated by overliming using calcium hydroxide and this treatment was subsequently compared with sodium hydroxide neutralisation.

W. Braide et al. This study investigates the potential of ethanol production from agro wastes. Agro waste from sugarcane *Saccharum officinarum* (sugarcane bagasse, sugarcane bark) and maize plant *Zea mays* (corn cob, corn stalk, corn husk) was subjected to a pretreatment process using acid hydrolysis was applied to remove lignin which acts as physical barrier to cellulolytic enzymes. Ethanol fermentation was done using *Saccharomyces cerevisiae* for 5 days and the ethanol yield, specific gravity, pH and total reducing sugar were also determined. From the results, the specific gravity, sugar content and pH decreased over time while the Sugarcane bagasse, Sugarcane bark, Cornstalk, Corn cob and Cornhusk gave maximum percentage ethanol yield of 6.72, 6.23, 6.17, 4.17 and 3.45 respectively at 72 hrs Fermentation. Maximum yields of ethanol were obtained at pH 3.60, 3.82, 4.00, 3.64 and 3.65. These findings show/prove that ethanol can be made from the named agricultural waste and the process is recommended as a means of generating wealth from waste.

F. A. F. Antunes et al. Biofuels have gained important place on the world stage, due to their sustainability and the fast depletion rate of fossil fuels. Brazil is the second largest ethanol producer (23.6 billion liters in 2012/2013) in the world by alcoholic fermentation directly from the juice or from the molasses obtained in sugar production facilities [1, 2]. During the extraction of sugarcane juice from the stem, sugarcane bagasse (SB) is generated in high amount. According to the Brazilian National Supply Company (CONAB) [1], the sugarcane production correspondent to the 2013/2014 harvest year is about 652 millions of metric tons. These values are correspondent to about 174 millions of metric tons of SB, considering the proportion indicated by Procknor [3]. Around 75–90% of the SB is used in heat and electricity generation in sugarcane processing industries. Remaining SB may serve as an excellent raw material for second generation (2G) ethanol production due to the presence of high amount of carbohydrates such as glucose and xylose [4]. In first generation ethanol production technology, *Saccharomyces cerevisiae* is the most widely used microorganism for the fermentation of sucrose available in the juice or molasses into ethanol. This yeast can also

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be used for 2G ethanol production from glucose solution obtained by pretreatment of cellulosic fraction of SB [4, 5]. For the economic ethanol production from SB, it is equally important to consider hemicellulosic fraction along with cellulosic part of cell wall. Hemicellulose represents about one-third of the carbohydrate fraction available in SB [4]. This macromolecular fraction is rich in pentose residues, mainly xylose, which are not fermented by native *S. cerevisiae*. However, there are some microorganisms able to ferment xylose to ethanol or other products [6]. The use of xylose metabolizing microorganism will increase in the global yield of ethanol in sugarcane based biorefineries.

Umamaheswari et al. In the present study, pretreated sugarcane bagasse is used as the substrate for bioethanol production using cellulase and various yeast strains including thermotolerant strains by simultaneous saccharification and fermentation method at the optimized process conditions. Various pretreatment techniques namely dilute and concentrated sulphuric treatment, sodium hydroxide combined with high pressure steam treatment and steam autoclaving treatment are conducted for mechanically pretreated sugarcane bagasse (milled ~ 100 mesh, 0.15 mm) for different time intervals (5, 10 and 15 min). The objective of the treatment step is to reduce the lignin and hemicellulose without altering the cellulose content because cellulase enzymes are highly specific in the cellulose hydrolysis reactions. From the results, the acid and alkali treatment techniques are not suitable for the enzymatic hydrolysis of pretreated sugarcane bagasse due to the decreased cellulose content and also the hemicellulose and lignin content was significantly reduced.

Felipe Scott et al. Bioethanol has been used as a biofuel in large scale since the implementation of the Brazilian alcohol program (Goldemberg et al. 2004). However, its production from sugar cane in Brazil and corn grains in the United States has been subject of debate regarding its effects on food prices and land use change (LUC). Production of bioethanol from lignocellulosic materials (LC), also called second-generation bioethanol (bioethanol 2G), is proposed as an alternative without such adverse effects. Bioethanol 2G has been an active field of research in the last decades and nowadays there are several companies scaling up their process in what seems to be the beginning of a learning process for having commercial scale production processes no later than 2020. Scott et al. DOI: 10.2225/vol16-issue3-fulltext-72 Compared to the accessibility of sucrose in sugar cane and starch in grains, cellulose and hemicellulose in LC are hardly available for saccharification and fermentation due to the presence of lignin, and a stage of "pre-treatment" is required to facilitate its conversion to fermentable sugars.

Beatriz Gutiérrez-Rivera et al. The bioethanol industry has developed rapidly in recent years to cope with the depletion of fossil fuel. Materials unsuitable for human consumption e.g. lignocellulose [1] are considered ideal substrates for bio-ethanol production. Lignocellulosic biomass represents a potential source of carbohydrates (cellulose and hemicellulose) for microbial fermentation into ethanol and other chemicals [2]. Dilute sulfuric acid has been widely used to pretreat sugar cane bagasse and this results in the hemicellulose fraction being released as a pentoses xylose and arabinose [3]. Moreover, soluble side products (furfural, 5 hydroxymethyl furfural, acetate, phenolics and others) formed during dilute acid hydrolysis inhibit microbial growth and retard fermentation [2,4]. The optimization of fermentation efficiency, with the aim of industrial scale production of ethanol from lignocellulosic biomass, has been the sugar mixture proceeded efficiently with a traditional glucose fermenting strain, xylose fermentation was often slow and inefficient due to the conflicting oxygen requirements between the two strains and/or catabolite repression on xylose assimilation caused by glucose [1].

Raquel L. Salomón et al. Hemicellulose is a major component of lignocellulosic materials and its utilization is essential for the development of any profitable procedure for transformation of this renewable resource. Xylan is the major constituent of sugar cane bagasse hemicellulose and it is hydrolyzed to xylose during the saccharification of lignocellulosics with the xylanases contained in some crude cellulase preparations (Dekker, 1985; Poutanen et al., 1987). If maximum ethanol production from lignocellulosics is intended, it would be essential to utilize the generated xylose but *Saccharomyces cerevisiae* cannot utilize this pentose. However it is possible to take advantage of alcoholic fermentation if this sugar is transformed to its keto-isomer, xylulose (Wang et al., 1980; Jeffries, 1981; Chiang et al., 1981; Gong et al., 1981; Hsiao et al., 1982; Hahn-Hagerdahl et al., 1986). D-Xylose can be isomerized with the same

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enzyme used for industrial production of fructose, xylose/glucose isomerase (E.C. 5.3.1.5.) (Chen, 1980). This paper reports the results of experiments carried out to produce ethanol from the whole polysaccharide fraction of sugar cane bagasse using the system of coupled columns described elsewhere (Blanco et al., 1982, 1984), with the addition of a unit containing immobilized xylose/glucose isomerase.

S. Vijayalaxmi et.al. *Exiguobacterium* sp. VSG-1 was isolated from the soil sample and characterized for the production of lignocellulolytic enzymes. Production of these enzymes by the strain VSG-1 was carried out using steam-exploded sugarcane bagasse (SCB) and found to secrete cellulase, pectinase, mannanase, xylanase, and tannase. The growth and enzyme production were found to be optimum at pH 9.0 and 37 °C. Upon steam explosion of SCB, the cellulose increased by 42 %, whereas hemicelluloses and lignin decreased by 40 and 62 %, respectively. Enzymatic hydrolysis of steam-exploded SCB yielded 640 g/l of total sugars. Fermentation of sugars produced from pretreated SCB was carried out by using *Saccharomyces cerevisiae* at pH 5.0 and 30 °C. The alcohol produced was calculated and found to be 62.24 g/l corresponding to 78 % of the theoretical yield of ethanol. Hence, the strain VSG-1 has an industrial importance for the production of fermentable sugars for biofuels.

Maria Carolina de Albuquerque Wanderley et.al. Lignocellulosic biomass, such as agricultural residues, potentially can be used for the biofuel production. Sugarcane bagasse, the major by-product of the sugarcane industry, is an economically viable and very promising raw bagasse for bioethanol and biomethane production (Rabelo et al., 2011; Badshah et al., 2012). Bioethanol production from lignocellulosic biomass, by using *Saccharomyces cerevisiae*, comprises the hydrolysis of cellulose and sugar fermentation. In order to obtain fermentable sugars from sugarcane bagasse, the cellulose can be saccharified using either acids or enzymes. However, somewhat acid enzymes are preferred rather acid because enzymatic hydrolysis is free from fermentation inhibitory products (Rivera et al., 2010; Galbe and Zacchi, 2002; Sun and Cheng, 2002).

Abdulkareem A Saka Member et.al. Biofuel can be referred to as fuels derived from biological carbon fixation leading to dissipation of energy. Biofuels can also be derived from conversion of various lignocellulosic biomasses into liquid fuels and biogases [1]. In recent times, biofuels have received constant attention from the general public and science fields inclusive. This new found interest build up is attributed to certain factors such as oil price hikes and the urgent need for increased security in the world's energy sector and most importantly Africa's. Some fuels which can be gotten from solid biomass include ethanol, methanol, biodiesel, and hydrogen and methane also known as gaseous fuels. Research and development in biofuel production is made up of three basic areas: production of the fuels, uses and applications of the fuels, and distribution of infrastructure [2].

VI. PROCESS FLOW DIAGRAM

- 1) Production of ethanol from corn will be discussed in the next section; this section will focus on sugarcane ethanol production. So, what needs to be done to get the sugar from sugarcane?
- 2) The first step is sugarcane harvesting. Much of the harvesting is done with manual labor, particularly in many tropical regions. Some harvesting is done mechanically. The material is then quickly transported by truck to reduce losses.
- 3) The cane is then cut and milled with water. This produces a juice with 10-15% solids from which the sucrose is extracted. The juice contains undesired organic compounds that could cause what is called sugar inversion (hydrolysis of sugar into fructose and glucose). This leads to the clarification step in order to prevent sugar inversion.
- 4) In the clarification step, the juice is heated to 115°C and treated with lime and sulfuric acid, which precipitates unwanted inorganics.
- 5) The next step for ethanol production is the fermentation step, where juice and molasses are mixed so that a 10-20% sucrose solution is obtained. The fermentation is exothermic; therefore, cooling is needed to keep the reaction under fermentation conditions. Yeast is added along with nutrients (nitrogen and trace elements) to

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keep yeast growing. Fermentation can take place in both batch and continuous reactors, though Brazil primarily uses continuous reactors.

- 6) Figure 7.4 shows a schematic of one process for ethanol production along with the option to produce refined sugar as well. Sugarcane contains the following: water (73-76%), soluble solids (10-16%), and dry fiber or bagasse (11-16%). It takes a series of physical and chemical processes that occur in 7 steps to make the two main products, ethanol and sugar.

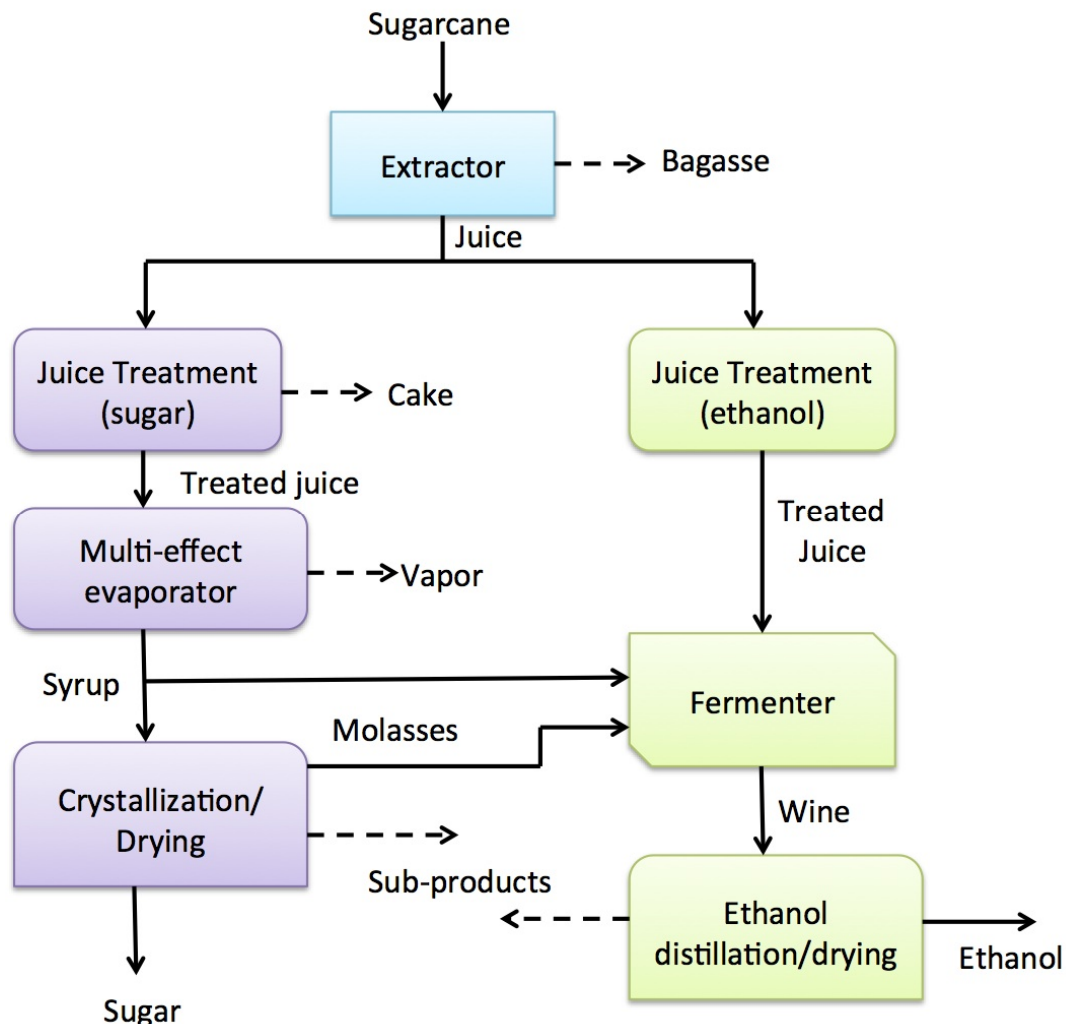


Figure 1 Schematic of process of sugarcane to produce ethanol and sugar.

So, why produce both sugar and ethanol? Both are commodity products, so the price and market of the product may dictate how much of each product to make. This is how Brazilian ethanol plants are configured. In order to have an economic process, all of the products, even the by-products, are utilized in some fashion. As noted previously, one of the major by-products is the dry fiber of processing, also known as bagasse. Bagasse is also a by-product of sorghum

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stalk processing. Most commonly, bagasse is combusted to generate heat and power for processing. The advantage of burning the bagasse is lowering the need for external energy, which in turn also lowers the net carbon footprint and improves the net energy balance of the process. In corn processing, a co-product is made that can be used for animal feed, called distillers grains, but this material could also be burned to provide process heat and energy. Figure 7.5 shows a bagasse combustion facility. The main drawback to burning bagasse is its high water content; high water content reduces the energy output and is an issue for most biomass sources when compared to fossil fuels, which have a higher energy density and lower water content.

Bagasse (see Figure 7.6) can have other uses. The composition of bagasse is: 1) cellulose, 45-55%, 2) hemicellulose, 20-25%, 3) lignin, 18-24%, 4) minerals, 1-4%, and 5) waxes, < 1%. With the cellulose content, it can be used to produce paper and biodegradable paper products. It is typically carted on small trucks that look like they have “hair” growing out of them.



Figure 2. The Usina Santa Elisa sugar mill in Sertãozinho, Brazil. Bagasse, a by-product of sugar production, can be burned for energy or made into ethanol.

VII. MATERIAL BALANCE

A necessary first step in assessing the feasibility and potential of new technologies is a techno-economic evaluation of various process scenarios. Such evaluations usually identify the critical parameters of the process and dictate minimal performance criteria for its feasibility. The objective of laboratory bench-scale or demonstration-scale work is then to provide experimental evidence for whether or not the assumed process performance is attainable. Therefore, the reliability of the experimental process data is critical for the unbiased assessment of the technology. In the biomass-to-ethanol process, the pretreatment of the lignocellulosic feedstock before enzymatic hydrolysis, and the subsequent enzymatic hydrolysis and fermentation of the resulting sugars to ethanol are the essential parts of the process.

Technoeconomic evaluation of the NREL biomass- to-ethanol process has identified the SSF step as being the most critical for overall process performance (4). Effective pretreatment, however, can have a large impact on the economics

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by improving the effectiveness of the enzymatic hydrolysis. Therefore, laboratory evaluations of the biomass- to-ethanol process need to consider pretreatment and SSF steps concurrently.

The efficacy of the various pretreatment methods is typically evaluated based on the following criteria:

1. The yield or recovery of the main sugars in soluble form;
2. The rate and extent of the pretreated substrate's enzymatic digestibility; and
3. The rate and degree of the solubilized and insoluble sugars fermentability through SSF for ethanol production. The last criterion is the most direct measure of the conversion process performance, and is designed to assess both the digestibility of the solids and the fermentability of the prehydrolysate (pretreatment liquor). This is typically limited by the presence of inhibitors, such as phenolic fragments from solubilized lignin, acetic acid from hemicellulose, and the sugar degradation products furfural and hydroxymethylfurfural (HMF). The concentration of inhibitors increases with the pretreatment severity and the degree of solubilization of the cellulosic, hemicellulosic, and lignaceous components of the feedstock.

The need for a general framework under which pretreatment and SSF experimental data for different feedstocks can be consistently evaluated is therefore of primary importance, as stated in the pretreatment literature (10,11). A comprehensive framework should include:

1. Material balance evaluation;
2. Sugar recovery information; and
3. SSF and overall process ethanol yields.

Material balance calculations are essential for verifying the integrity and consistency of the compositional analysis data both after pretreatment and after

SSF, especially in light of reported difficulties associated with the accurate determination of the composition of pretreated lignocellulosic feedstocks and SSF residues (9,12,13). The information on sugar yields and recoveries can then be readily obtained from the material balance calculations around the pretreatment process. Similarly, the SSF and overall process ethanol yields can be calculated from SSF and pretreatment mass balance information, and the produced ethanol. In an effort to adopt a consistent evaluation procedure to facilitate comparison of results between different groups throughout NREL's biomass-to-ethanol project, a set of EXCEL templates was developed to perform material balance calculations around the pretreatment, SSF, and overall conversion processes. These templates are described next. In addition, a set of formulas developed to calculate ethanol yields correctly for the overall conversion process involving pretreatment and SSF is described in the following section.

Pretreatment Carbon Balance

The material balance for the pretreatment process was followed both in terms of total mass and for the individual components, in terms of carbon. This choice for the individual component balances was made (1) to avoid complications with the water balance resulting from the various hydration and dehydration reactions, and (2) to illustrate clearly changes in the distribution of carbon in the liquid and solid phases that occur between the various carbon-containing substances during the course of the pretreatment process. All major components of the biomass hemicellulosic, cellulosic, and lignaceous fractions were considered for individual component balances. These include:

1. The hexose sugars, glucose, mannose, and galactose;
2. The pentose sugars, xylose and arabinose;
3. Lignin;
4. Acetic acid, which results from hydrolysis of the acetyl groups of hemicellulose (14);
5. The sugar degradation products furfural and HMF (15,16); and
6. 4-oxopentanoic or levulinic acid and formic acid, the former resulting from degradation of HMF (17), and the latter mainly from HMF (17) and to a lesser degree from furfural (18). The template for the pretreatment carbon balance is shown in Table 1. The type of pretreatment, pretreatment equipment, catalyst, feedstock, and pretreatment conditions are listed on the top of the template for easy reference. The first part of the spreadsheet consists of the total mass balance calculations. The information needed here is the dry solids loading and liquor volume (catalyst solution) used and the corresponding amounts recovered at the end of the pretreatment. From this data, the hydrolyzed biomass

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fraction (an important design parameter) is calculated." In addition to providing this information, the accuracy of the total mass balance is critical, since it affects the balance calculations on the individual components.

Molecular and C-Mol Weights

Component	C	H	O	N	OMe	Formula weight, g	C-mol weight, g
Glucose	6	12	6			180.16	30.03
Mannose	6	12	6			180.16	30.03
Galactose	6	12	6			180.16	30.03
Xylose	5	10	5			150.13	30.03
Arabinose	5	10	5			150.13	30.03
Glycerol	3	8	3			92.10	30.70
Acetic acid	2	4	2			60.05	30.03
Lactic acid	3	6	3			90.08	30.03
Succinic acid	4	6	4			118.09	29.52
Lignin	9	8.05	2.70		1.41	203.09	22.57
Furfural	5	4	2			96.09	19.22
HMF	6	6	3			126.11	21.02
Formic acid	1	2	2			46.03	46.03
Levulinic acid	5	8	3			116.12	23.22
Cellulose	6	10	5			162.14	27.02
Cellobiose	12	22	11			342.30	28.53
Cell mass (D5A)	3.874	6.657	2.081	0.752		97.07	25.06
Ethanol	2	6	1			46.07	23.03
Carbon dioxide	1		2			44.01	44.01

Therefore, extra attention needs to be given to obtaining the needed mass and moisture content of the untreated and pretreated solids. The second and main part of the spreadsheet consists of the balance calculations on individual components. Compositional analysis data for the raw solids, pretreated solids, and pretreatment liquor, together with the total mass balance information, are used in these calculations. The solids composition is typically reported on a dry-wt basis, with the sugar concentrations reported as monomeric equivalents. For the particular sample shown in Table 1, the analyzed components make up 91.8% of the raw dry biomass, after applying weight adjustments for anhydro-sugars and acetyl groups," ash is 0.6%, and the remaining 7.6% is most likely uronic acids and extractives. Total soluble sugar concentrations, including both monomeric and oligomeric forms, are measured in the liquid streams in grams per liter. To use a common basis for the calculations, the compositions of the solids and liquid streams were converted to carbon moles (C-mol), using information from the total mass balance and the C-mol weights of the components, which are summarized in Table 2. A C-mol is the amount of a substance containing 1 mol of the element carbon (27), i.e., it equals the molecular mass divided by the number of carbon atoms in the molecule. A compositional formula for hardwood (beech) lignin (19) was used to calculate the molecular weight of yellow poplar lignin. A slightly different formula has been reported for softwood lignin (19), resulting in a 7% lower molecular weight. An advantage of using C-mol as the basis for the calculations is that the C-mol ratio of the various components within a stream reflects the distribution of carbon within that stream. In the example of Table 1, more than 60% of the carbon in the pretreated solids is in the form of glucan, and about 38% of the carbon comes from lignin. Furthermore, the fraction of each component that becomes solubilized as a result of the pretreatment is calculated as the fraction of the total C-mol of that component appearing in the liquid stream. For example, the data of Table I show that 14-18% of the glucan, 94% of the xylan, and 33% of the initial lignin are solubilized. Furthermore, based on the summary carbon balance information, 57% of the initial carbon remains in the solids after pretreatment, which agrees well with the 42% solubilization that is calculated from the total mass balance data (see above, discussion on total mass balance calculations). For the nonreacting components, the total recovery is calculated as the fraction of the initial carbon recovered in the solids and liquid streams after pretreatment. An exception is glucose and xylose recovery, for which

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the carbon associated with HMF and furfural, respectively, is added to the amounts of glucose and xylose recovered in the pretreatment solids and liquor to calculate overall recoveries. We therefore assume that all HMF comes from glucose dehydration and similarly that all furfural comes from xylose.

This assumption is not entirely correct, however, since all hexoses have been reported to produce HMF when treated in dilute sulfuric acid and all pentoses to produce furfural, among other products, in acidic media (20). However, the reactivity of individual sugars in dilute acid varies according to the approximate order xylose > arabinose > mannose > glucose ~ galactose(21), and different sugars give different yields of HMF and furfural, with arabinose giving much lower furfural yield than xylose and mannose and galactose significantly lower yields of HMF than either glucose or fructose (20). Therefore, the above assumption is rather justifiable. Similarly, since levulinic acid and formic acid are degradation products of HMF, the carbon associated with these components is being added to the recovery of glucose. Finally, the overall carbon recovery for the pretreatment process is calculated as the percentage of the total initial carbon recovered in all components in both the liquid and solids streams. The individual component and overall carbon recoveries shown in Table 1 are typical of the percolation process (5). Larger losses on a percentage basis are observed with the minor sugars and are mainly attributed to analytical error.

The recoveries from batch dilute sulfuric acid pretreatment are comparable to those reported here, except for xylose, for which losses of 10-25% are usual. Such losses are mainly caused by the formation of polymeric humic compounds that result from furfural degradation (22), which can be analyzed as lignin and therefore upset the lignin balance as well.

VIII. CONCLUSION

1. The ethanol concentration in ml/lit of acid hydrolyzed bagasse increases with the increasing number of days while sugar concentration decreases. This shows that the sugar is being fermented by the help of the micro organism yeast for the production of ethanol.
2. The amount of sugar is maximum on the first day after fermentation i.e. 19.552 gms/lit and it is almost zero on the 10TH day of fermentation.
3. The maximum concentration of ethanol is 323 ml/lit of acid hydrolyzed bagasse which was found on the 10TH day of fermentation.
4. With increasing molar concentration of sulphuric acid, the concentration of ethanol and sugar decreases. More amount of ethanol and sugar was found for 0.2M than 0.3M and 0.5M for each day.

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