Investigation of Ethanol Production Potential from Lignocellulosic Material without Enzymatic Hydrolysis Using the Ultrasound Technique

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Abstract: This research investigates ethanol production from waste lignocellulosic material (sugarcane bagasse). The bagasse was first pretreated using chemicals and ultrasound techniques. These pretreatment techniques were applied separately and combined. The pretreated bagasse was then fermented anaerobically for biofuel production without enzymatic hydrolysis. The results showed higher ethanol production than those reported in the literature. The maximum ethanol production of 820 mg/L was achieved with a combination of ultrasound (60 amplitude level, 127 W) and acid (3% H_2SO_4 concentration). The combination of two-step pretreatment such as an ultrasound (50 amplitude level, 109 W) with acid (3% H_2SO_4 concentration) and then an ultrasound with alkaline (23% NaOH concentration) generated 911 mg/L of ethanol.

1. Introduction

The use of lignocellulose biomass to produce liquid biofuels such as bioethanol can overcome the problem of greenhouse gas (GHG) emissions, reduce reliance on foreign oil, enhance the agricultural economies, create more employment opportunities and ensure the sustainability of the world transportation systems. Agricultural waste such as wheat straw, corn stalks and sugarcane bagasse are abundant and inexpensive natural resources. The worldwide estimate production of lignocellulosic materials is around 1×10^{10} t annually [1]. Lignocellulosic biomass is mainly composed of cellulose, hemicellulose, and lignin [2].

Lignocellulosic materials can be sourced from plant and wood materials. The conversion of lignocellulose materials to biofuels is complex and needs pretreatment and enzymatic hydrolysis steps to enhance the efficiency of biofuel production. The enzymatic hydrolysis of lignocellulosic materials such as sugarcane bagasse is an important process for the production of second generation bioethanol. However, this step is slow, expensive and consumes energy. In a study by Verardi et al. [3] showed

that the yields of glucose, xylose, arabinose and mannose increased by 9% for glucose and were as high as 90% for the other monosaccharides when the stirring rate increased to 300 rpm [3].

According to the Department of Resources, Energy and Tourism, the Australian production of biofuels in 2006 was 106 million liters (ML) from edible substrates, of which ethanol comprised 62 ML and biodiesel 44 ML. In 2007–2008, production of biofuels reached 199 million liters (ML)/year, of which ethanol comprised 149 ML and biodiesel 50 ML [4]. Almost any plant-based material can be an ethanol feedstock. All plants contain sugars in different forms, and these sugars can be fermented to make ethanol. There are many processes that convert the plant material to sugar. The conversion process can be done using heat, chemicals and irradiation techniques [4]. Lignocellulosic materials are non-food based feedstocks such as crop, wood and plant residues. Lignocellulosic feedstocks offer many advantages over starch and sugar-based feedstock because they are waste products with low economical value. However, conversion of this feedstock into ethanol is a challenging process [5]. The process of converting lignocellulosic materials to ethanol is more tedious than current ethanol production from corn and sugarcane, and the technology of the conversion process is not yet feasible. Corn and sugarcane include high percentages of starch and sugar, respectively. Starch and sugar are easily digested by the yeast (yeast's favorite food). In contrast, cellulose is a sugar polymer that needs to be broken down to sugar monomers before the yeast can digest it, and it was also shielded from the yeast by lignin and hemicellulose [4].

The structure of lignocellulosic biomass is very complex in nature and is not suitable to be used directly in a fermentation process. In order to make it more degradable, pretreatment is necessary to increase the efficiency of the fermentation process [6]. Pretreatment is the most expensive step in the process of converting lignocellulosic biomass to ethanol. Thus, identifying and developing cost-effective pretreatment methods of lignocellulosic biomass is a major challenge [7].

Currently, the main aim of the pretreatment is to remove the lignin and hemicellulose, reduce cellulose crystalline, increase porosity and increase the internal surface area to alter the visible structure into a microscopic size. This process changes the biomass structure and improves downstream processing. Different pretreatments lead to different chemical reactions and characteristic interaction with the components of lignocellulosic biomass. The feedstocks ranging from grasses to soft and hard woods have a different sugar to lignin ratio. These variations create the need for various pretreatments of the biomass [8].

During the pretreatment, the cell structure and chemical composition of the bagasse is ruptured. This increases the surface area of the biomass and makes it more biodegradable, which helps to enhance the efficiency of the fermentation process. There are many types of pretreatment configurations that have been studied for the conversion of lignocellulosic biomass to ethanol. Pretreatment methods are broadly classified into physical, chemical and irradiation. Physical pretreatments include comminution (mechanical size reduction), ultrasound, steam explosion and hydro thermolysis [2]. In chemical pretreatment, acid or base is added to the bagasse to remove certain components. The acid and the alkaline pretreatment processes are aiming to remove lignin and leave the cellulose intact [9].

After the pretreatment step of lignocellulosic materials, an expensive enzymatic treatment is required to convert the separated cellulose to its sugar monomers. For example, in a study by Manzoor et al. [10], bagasse was pretreated with various concentrations of sulfuric acid at different time intervals from 0 to 3 h. Before adding the acid to the bagasse, it was autoclaved at 121 °C for 30 min. Then, 10 g of bagasse was put in a conical flask and different concentrations of sulfuric acid (0.5%–5%) were added. After pretreatment with acid, the enzymatic hydrolysis enhanced and the achieved yield of sugar was around 90%.

The main objective of the acid pretreatment is to degrade the hemicellulose of the bagasse, and this increases the surface area of the materials and will be more suitable for fermentation. In the concentrated acid treatment process, sulfuric acid (H_2SO_4) is used at a concentration greater than 40% at room temperature for 1 h [8]. The acid pretreatment can be divided into two types: high temperature greator than 160 °C with 5%–10% acid concentration and low temperature less than 160 °C with a sulfuric acid

concentration of 10%–40% [6]. According to Njoku [11], the dilute acid treatment is not effective in dissolving the lignin, but it can rupture the lignin and increase the digestibility of the cellulose, and 100% of hemicellulose can be removed in this method. It has been observed that hydrochloric acid was less active for the degradation of lignocellulosic materials when compared to sulfuric acid [12].

The main aim of the alkaline pretreatment is delignification of lignin. The removal of lignin is critical for the enzymatic hydrolysis [13]. The effects of the alkaline solution depend on the lignin content present in the biomass. In the sugar industry, caustic soda and sodium sulphite are combined in the pretreatment process for the effective removal of lignin [9]. Alkaline pretreatments require low temperature and pressure conditions compared to the other treatment methods [6]. It can be carried out at room temperature, and the reaction time can be a few minutes to several days. Adding NaOH to hardwood increases its digestibility from 14% to 55% with the decrease in lignin content up to 55%. For softwoods, the lignin removal rate can be greater than 26% [6]. In a study by O'Hara [9], lignin is removed up to 75% through the removal of a *p*-hydroxyphenyl lignin using alkaline pretreatment. Alkaline pretreatment is less corrosive in nature, and the cost of the reactor is reduced when comparing it with the acidic treatments [9].

The effect of passing high-frequency ultrasound waves into the biomass solution causes cavitation, and, as a result, disruption of the lignocellulosic structure. The C-H bonds are also affected by the cavitation [14]. Several factors should be considered during the sonication process to get an effective treatment of lignocellulose biomass. The duration of the sonication has the greatest impact on the pretreatment. The increase of the duration of the sonication increases the delignification of the biomass. The power level, frequency and amplitude are also important parameters. When the amplitude level is increased correspondingly the power level is also increased, which directly affects the intensity of the cavitation. It has been found that increasing the power level by more than 150 W does not improve the delignification of biomass in practice [14]. The use of ultrasound pretreatment with various lignocellulose materials has shown an increase in the glucose yield of 21.3% [8]. Rehman et al. [14] reported that pretreatment of sugarcane bagasse, by mixing it with distilled water (DI) and treating it with 22 kHz and 100 W power for 5–35 min under 55 °C temperature, has resulted in 90% extraction of hemicellulose and lignin. The efficiency of the pretreatment also depends on the particle size of the lignocellulosic biomass. It is reported that the smaller the particle size, the better separation is achieved [7].

Table 1 shows a comparison between different methods of pretreatments, possible changes to the structure of the lignocellulosic materials and some advantages/disadvantages of the processes. It is obvious from the table that chemical/physicochemical pretreatments are the most efficient and more favorable for the industry. The only disadvantage is the amount of chemicals required for the process, which can be reduced in case it is combined with some other physical processes.

Table 1. Typical methods for pre-treatment of lignocellulosic materials [15].

Pre-Treatment Methods	Processes	Possible Changes in Biomass	Notable Remarks
Physical	Milling Irradiation	- Increase in accessible surface area and pore size - Decrease in cellulose crystallinity - Decrease in degrees of polymerization	- The highly efficient methods are highly energy demanding - Cannot remove the lignin
Chemical and Physicochemical	Explosion Alkali Acid	- Increase in accessible surface area - Harsh environment - Delignification - Decrease in cellulose crystallinity - Decrease in degrees of polymerization - Hydrolysis of hemicelluloses	- The most effective processes for industrial applications - Rapid treatment rate
Biological	Fungi and Actinomycetes	- Delignification - Reduction in degree of polymerization cellulose - Partial hydrolysis hemicellulose	- Low energy requirement - Very low treatment rate - Did not consider for commercial application

Almost any organic material including lignocellulosic material, such as bagasse, can be processed with anaerobic digestion to produce biogas, but this does not apply to bioethanol. In recent years, a lot of development has been done in the area of waste to biogas [16,17]. Research also showed the feasibility to upgrade the biogas for biomethane production. In many studies [18–20], the feasibility of integrating an anaerobic digestion plant with an onsite polymeric membrane purification system to upgrade biogas to biomethane has been shown. Biomethane purity was achieved at 94% for a biogas fed at 50 kg/h with a methane content of about 55%. However, there is still uncertainty in regards to which is more economical to produce biogas or bioethanol from bagasse. Note that both products require pretreatment for the substrate (bagasse) before fermenting in an anaerobic digester.

Sugarcane bagasse was used in this research because it represents almost pure lignocellulosic material after the sugarcane went through crushing and hot water washing. Many studies have been carried out related to pretreatment of bagasse with acidic and alkaline solutions and ultrasound as sole processes [9,13,14]. Some other research combined steam explosion with chemical pretreatment [8]. To our best knowledge, no study has combined chemical pretreatment with ultrasound in one process. This research is investigating ultrasound in conjunction with chemicals as a pretreatment method to enhance degradability of bagasse. In addition, the aim is to identify suitable levels of ultrasound/chemicals to improve ethanol production. The novelty of this research is combining ultrasound with a chemical pretreatment in one step to enhance the effectiveness of both methods.

2. Methodology

2.1. Materials and Equipment

The lignocellulosic material used in the experiments is bagasse, which was collected from the Bundaberg sugarcane mill, Queensland, Australia. The collected bagasse was then stored at 4 °C in the fridge until use. The yeast used for fermenting the bagasse was baker's yeast (*Saccharomyces Cerevisiae*), which was bought from the local market. The material and the bottles (0.5 L) used as reactors were first autoclaved. The reactors were filled with the bagasse and distilled water and then placed in the autoclaving chamber; the temperature was set to 120 °C and the reaction time to 30 min. The reactors were taken out from the chamber and cooled down for 1 h and then used in the fermentation process. A Shimadzu QP2010 Gas Chromatograph Mass Spectrophotometer (GCMS) (SHIMATZU, Duisburg, Germany) was utilised in this research to measure the ethanol percentage in the solution. In addition, the accumulated CO_2 from the reaction was used to calculate the ethanol percentage in the solution to confirm the results.

An ultrasound digital sonifier (Branson, Emerson Electric Co., Danbury, CT, USA) was used for pretreating the bagasse with high-frequency waves of around 20 kHz (Figure 1). The amplitude and reaction time were set in the digital human-machine interface (HMI). Then, the transducer horn was immersed in the liquid to pretreat the bagasse.
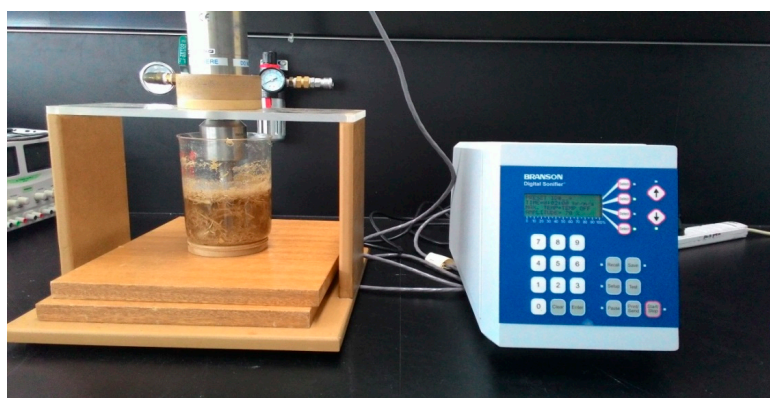

Figure 1. Branson digital sonifier.

Figure 1 shows the Branson digital sonifier, which is used for pretreating the bagasse in this experiment. The HMI is interfaced with the ultrasound transducer, and the ON and OFF time and amplitude level are selected accordingly.

2.2. Procedures

The collected bagasse was pretreated with sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), and ultrasound both separately and combined. The experiments were carried out in duplicates.

Table 2 shows a scheme to sum up the various experimental conditions tested in this research.

Table 2. Various experimental conditions tested.

Techniques/Chemicals	Test Conditions
sulfuric acid (H_2SO_4), sodium hydroxide (NaOH) and ultrasound	H_2SO_4 H_2SO_4 + Ultrasound NaOH NaOH + Ultrasound H_2SO_4 then with NaOH Ultrasound H_2SO_4 + Ultrasound then with NaOH + Ultrasound

In the acid (H_2SO_4) pretreatment, thirty grammes of bagasse were added to 500 mL glass reactors. Different sulfuric acid concentrations (1%–5%) were diluted in 400 mL of distilled water and added into the reactors. The reactors were covered with lids; all the samples were pretreated for 12 h. In the alkaline pretreatment (NaOH), about 21%–26% of sodium hydroxide was diluted in a similar amount of distilled water (400 mL) and then added to the reactors. The reactors were covered with lids and kept at room temperature for 24 h. After the acid and alkaline pretreatments, the bagasse was rinsed with water thoroughly and then fermented.

In the case of ultrasound pretreatment, a similar amount of bagasse and distilled water was used and then the sample was placed in the pretreating chamber where the ultrasound horn was immersed into the chamber. The reaction time and amplitude were set, and the frequency was adjusted to 20 kHz. Then, the ultrasound waves were continuously passed through the reactor for around 5 min. The above steps were repeated for different power levels for both continuous and pulsed frequency waves.

After the pretreatment, the bagasse was fermented with yeast for six days. The pretreated bagasse with acid/alkaline was washed with water to remove the acid/alkaline molecules present in the bagasse. After washing the bagasse, 400 mL of distilled water was added into the reactor along with 3–5 g of *Saccharomyces Cerevisiae* commonly used bakers/brewer's yeast. The yeast and the solution were stirred well, and it was ensured that they were covered tightly with the lid. The exhausted CO_2 was collected in upside-down plastic beakers (Figure 2), and GCMS and the collected CO_2 were used to estimate the quantity of the ethanol produced in each reactor. The reactors were placed in a shaker water bath (Figure 2), the temperature was set to 37 °C, and the shaking speed set to 100 rpm. Then, the biomass (bagasse) was fermented for six days.

The experiments were terminated after day six; samples were taken from the solution using syringes and filtered using Whatman filter paper (0.45 µm) (Sigma-Aldrich, St. Louis, MO, USA). The quantity of the ethanol produced measured with the help of a GCMS. In addition, the CO_2 collected was used to estimate the ethanol produced in each of the reactors. The pH and the temperature were measured after the fermentation (Figure 2). The samples were stored in the fridge for further analysis.

Figure 2. Bagasse fermentation and collection of CO_2 gas equipment.

3. Results and Discussion

The structure of the bagasse is visualised using an SEM microscope; Figure 3 shows the structure of bagasse before (Figure 3a) and after pretreatment with diluted acid (3%) and an ultrasound (Figure 3b). It is noted that the complex structure of bagasse is destroyed, and the cellulose fibers are now exposed.
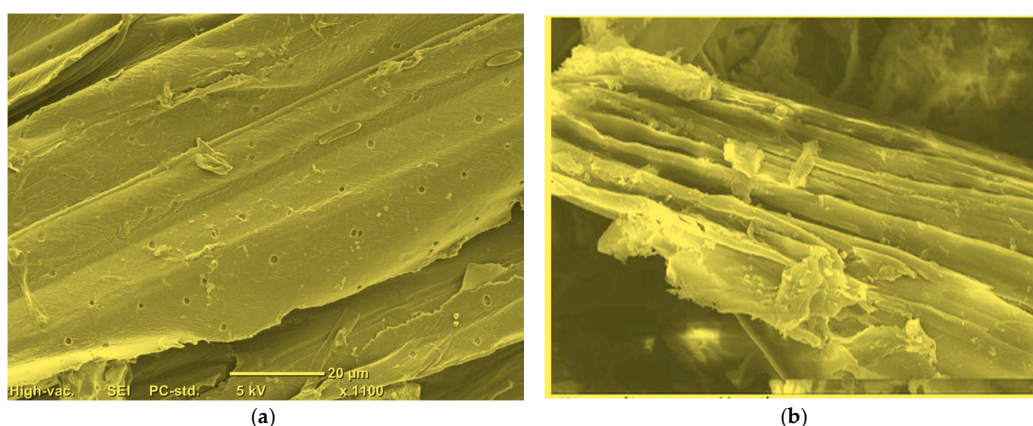

| (a) | (b) |

Figure 3. Bagasse structure before (**a**) and after acid (3%) and ultrasound pretreatment (**b**).

The sulfuric acid concentration was chosen between 1% and 5%. Minitab (Version 17, Minitab Inc., State College, PA, USA) was used for experimental design and optimisation purposes. In the first experiment, bagasse underwent a pretreatment with acid, which was followed by an ultrasound separately. The bagasse was treated for 12 h. During the pretreatment, the pH level varied from 2.6 to 4.2 and the temperature ranged from 21 to 49 °C. After 12 h of pretreatment with acid, the bagasse was rinsed with water and then treated with an ultrasound. Continuous ultrasound waves were used and the reaction time was set to 5 min. After pretreatment with the ultrasound, the bagasse was fermented using 3 g of baker's yeast (*Saccharomyces Cerevisiae*) for six days. The generated CO_2 was collected in upside-down plastic beakers. The final products in each reactor were also analysed with the Shimadzu GCMS, which showed that, apart from the ethanol, there was a small amount of butyric acid, most likely produced by *Clostridia* bacteria naturally present in the sugar cane bagasse. Additionally, some CO_2, acetic acid, valeric acid and other compounds stemming from the metabolism of the yeast and other bacteria in the solution were detected.

Figure 4 shows a surface plot of ethanol yield against the acid % and amplitude level. The optimum level of the acid % and the amplitude level were identified as 3% and 40–50, respectively. The corresponding ethanol produced is close to 638 mg/L.

Another experiment was carried out where acid and ultrasound pretreatments were combined in one process. After 12 h of acid pretreatment, the bagasse was then directly pre-treated with the contentious ultrasound waves. The acid concentrations used in this experiment were 2.5%, 3% and 3.5% (dilute H_2SO_4) and the ultrasound amplitude levels were 50, 60 and 70. After the pretreatment stage, the bagasse was rinsed thoroughly with water before fermentation. Yeast was added into the reactors (3 g) and then fermented for six days. The result shows that, as the pretreatment time and amplitude level increases, the ethanol production increases. By combining both pretreatment methods in one process, ethanol production was about 820 mg/L. This result is 22% higher than the results gained from the pretreatment experiment of acid and the ultrasound, which carried out separately.
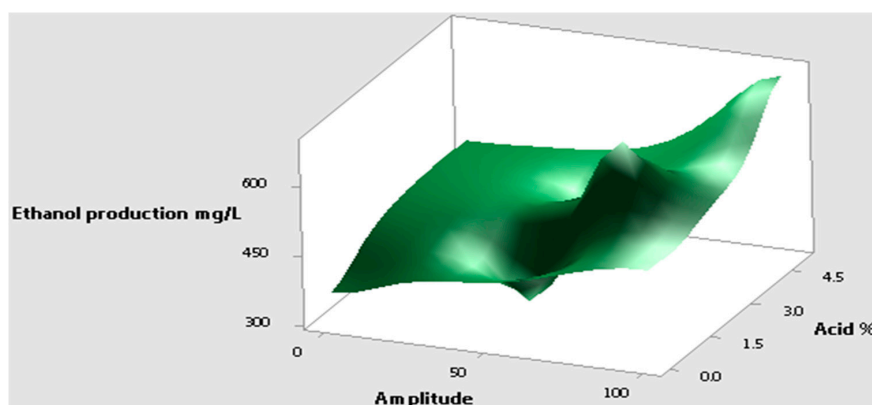

Figure 4. Surface plot for ethanol (mg/L) produced vs. amplitude and acid %.

Another set of experiments were conducted to investigate alkaline and ultrasound pretreatment separately and combined. Bagasse was first pretreated with the alkaline (NaOH) solution at different concentrations (21%, 22% and 23%). The pretreatment period was 24 h at room temperature. After 24 h of pretreatment with alkaline, the bagasse was thoroughly rinsed with water for 15 min. Then, the bagasse was fermented with 3 g of *Saccharomyces Cerevisiae* yeast for six days. The optimum concentration of NaOH was between 22% and 24%. Figure 5 shows ethanol production at different NaOH concentrations. It is evident from the graph, when the result is compared with the acid pretreated, that NaOH pretreatment results in remarkably less ethanol production. The difference is around 400 mg/L, and it is concluded that acid pretreatment is more efficient than alkaline pretreatment.
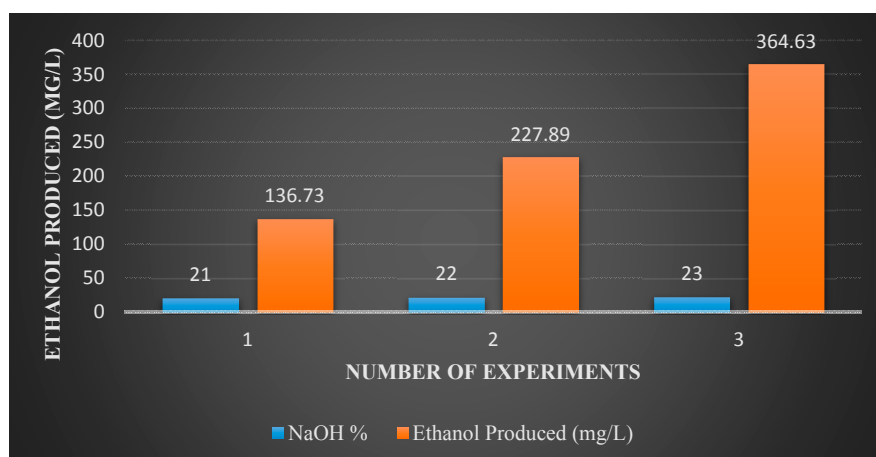

Figure 5. Ethanol production from NaOH pretreated bagasse.

When alkaline and an ultrasound are combined to pretreat the bagasse, the bagasse was first treated with the alkaline solution and then treated with the ultrasound directly. After 24 h of alkaline pretreatment, the bagasse was directly treated with the ultrasound. The alkaline concentrations used were 21%, 22% and 23%, and the ultrasound amplitude levels were 50, 60 and 70. After the pretreatment, the bagasse was rinsed thoroughly with water before fermentation. Baker's yeast *Saccharomyces cerevisiae* (3 g) was added into each of the reactors and fermented for six days. Figure 6 shows ethanol production from the pretreated bagasse with alkaline solution and ultrasound combined. The figure indicates that, at a higher amplitude level of 70 and at NaOH concentration of 23%, ethanol production is around 774.3 mg/L.
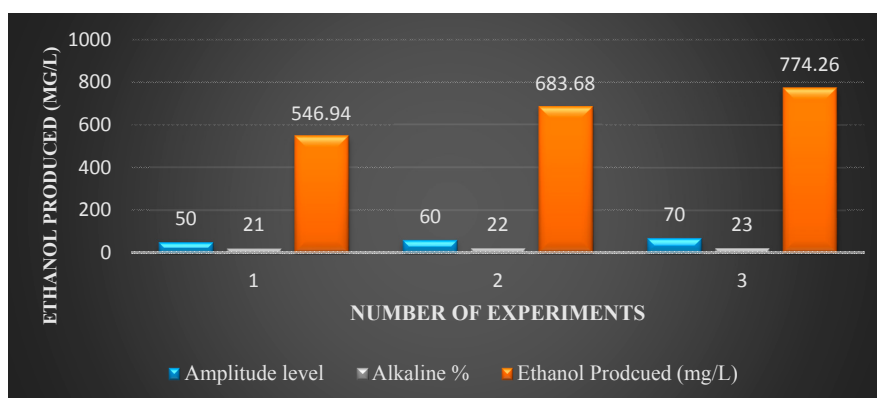

Figure 6. Ethanol production (mg/L) using NaOH and ultrasound pretreatment combined.

Bagasse was also investigated undergoing three subsequent pretreatments. Bagasse was first pretreated with chemicals: acid (H_2SO_4) for 12 h, then rinsed with water and pretreated with alkaline (NaOH) solution for another 24 h, and then rinsed with water. After the chemical treatments, the bagasse was pre-treated with an ultrasound with amplitude levels of 40, 50 and 60 for five minutes. Finally, the pre-treated bagasse was added to a 500 mL reactor and 400 mL of distilled water was added. The bagasse was then fermented with 3 g of *Saccharomyces Cerevisiae* yeast for six days. Figure 7 shows ethanol production for various pretreatment methods of acid and alkaline solutions and an ultrasound. By treating the bagasse with all of the methods one after another, 3% sulfuric acid, 23% of NaOH and an amplitude level of 50, and ethanol production reached 911.6 mg/L.
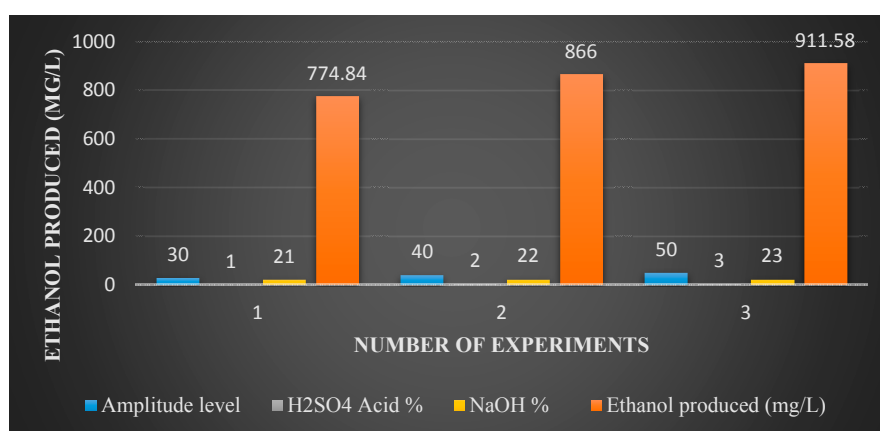

Figure 7. Ethanol production for subsequence pretreatment methods: acid, alkaline and ultrasound.

The results of the pretreatment methods carried out in this research were compared with literature results [13]. Acid pretreatment from literature showed ethanol production of 390 mg/L with a 3% acid

pretreatment, whereas for the experiment conducted in this research, by combining both pretreatment methods (Acid + Ultrasound) in one process, ethanol production was about 820 mg/L. For the alkaline pretreatment, the literature result is 220 mg/L of ethanol. The ethanol produced in this research is around 774.3 mg/L when combining both alkaline and ultrasound in one process. Pretreating bagasse with acid, alkaline and ultrasound in subsequent processes resulted in 911.6 mg/L of ethanol.

4. Economics of Lignocellulose to Bioethanol Conversion

A proper use of lignocellulosic biomass can potentially lead to a significant increase in the production of ethanol per hectare without extending the cultivated area. In addition, using lignocrellulosic biomass will avoid competing in an unsustainable way on the basis of food-based feedstock like corn, sugarcane or wheat as a raw material. This will eliminate any major nutritional and economic concerns.

With this background, this study reports the usefulness of the application of the ultrasound for the pretreatment of lignocellulosic biomass. A combined intensification approach of ultrasonic irradiation with other chemicals (without enzymatic hydrolysis) that can offer maximum yields and produce the lowest residues/by-products needs to be considered for economic analysis.

4.1. Choice of Technology

The choice of the best technology for lignocellulose to bioethanol conversion should be decided by overall economic analysis and not on financial feasibility ground. Although both economic and financial analyses aim at appraising profitability of an investment project based on a specific technology. The concept of cost-benefit in an economic analysis differs significantly from the financial analysis [21,22]. For example, to be financially acceptable, the cost for lignocellulose to bioethanol conversion must be lower than the equivalent amount of current gasoline extraction cost. Therefore, irrespective of any existing technology for lignocellulosic material, ethanol is not feasible to compete with fossil gasoline [23]. This is not only for the technology reason but also for policy reasons, e.g., bio-ethanol is competing with heavily subsidized fossil fuel, and, therefore, it decreases financial incentives for researchers for improvisation of the existing technology.

The baseline production cost of the enzyme was found to be $10.14/kg, the production cost of cellulase was found to be $0.2 per liter of ethanol produced and both are much higher compared to the commercial price of 0.03 $/L of ethanol [24]. Therefore, at the current technological level, enzyme production is not competitive. While enzymes contribute significantly to the cost of producing lignocellulosic ethanol [25], use of sonication can reduce processing costs compared to an alternative treatment by greatly reducing the cellulase requirement by about 50% [26]. Our analysis shows that, compared to high enzyme costs for biofuel production, the ultrasonic irradiation with other chemicals (without enzymatic hydrolysis) can lower feedstocks, reduce the fermentation times, and lessen the complexity of the process, and, therefore, the process has a lot of potential to reduce capital costs.

4.2. Subsidy Policy and Imperfect Competition

The latest estimate from the International Energy Agency (IEA) indicates that fossil-fuel consumption subsidies worldwide amounted to $548 billion in 2013, an increase from $523 in 2011. Global post-tax subsidies at $2 trillion were substantially bigger than pre-tax subsidies of $492 billion (6.5 percent of world gross domestic product (GDP)), and this situation represents a reflection of underestimating environmental damage associated with energy consumption [27]. Fossil fuel subsidies distort markets and impede research investment in lignocellulose to bioethanol conversion.

Future research is essential to isolate the economic analysis from financial analysis, which does consider environmental/social externalities, private cost-benefit and value environment as well as new green job creation [21]. The burning of fossil fuels at the current subsidised rate is likely to create an environmental crisis through emission of carbon dioxide, carbon monoxide and a significant quantity of nitrous oxide. However, ethanol production through bagasse with ultrasounds and chemicals

without enzymatic hydrolysis uses energy from renewables; no net carbon dioxide is added to the atmosphere, making it an environmentally beneficial energy source. Ethanol contains 35% oxygen that may result in a complete combustion of fuel and thus reduces tailpipe emissions. In addition, the toxicity of the exhaust emissions from ethanol is lower than that of fossil fuel. The reduction of GHG pollution and its associated effects on health and the environment are the main component of positive externalities that has to be considered for economic valuation of biomass conversion into ethanol.

Because of the low price and availability throughout the year, lignocellulosic waste is considered the most abundant biomass on earth and the most feasible option for biofuel production. However, without the proper use of lignocellulosic waste, open field burning is the most common practice of handling this waste in Asian regions [28]. Air emissions from the burning process of lignocellulosic waste are not only a threat to public health but are also wasting a great resource. The burning process reduces the local air quality, creating a variety of health concerns from the discharge of carcinogenic oxides (NO_x, SO_2 and CO_x) into the atmosphere leading to asthma or pulmonary morbidity in humans. It has been estimated that the activity of open burning of biomass in Asia leads to the emission of 0.37 Tg of SO_2, 2.8 Tg of NO_x, 1100 Tg of CO_2, 67 Tg of CO, and 3.1 Tg of CH_4 (million metric ton (mmt), equivalent to the unit of Teragram (Tg)) [29].

4.3. Policy Implications

A study by Zhao et al. [23] proposed a commercialization policy for ethanol, such that consumption tax should be exempted, the value added tax (VAT) be refunded upon collection, and a feed-in tariff for excess electricity (by-product) be implemented to facilitate the industrialisation of the technology. A minimum direct subsidy of $1.20/gal ethanol (2500 yuan/t ethanol) is also proposed for consideration [17]. An important factor for reducing the cost of bioethanol production is to use larger industrial facilities with the integrated approach (process engineering, fermentation and enzyme and metabolic engineering) rather than smaller ones. By increasing the plant size, the investment per unit output of product falls off for better economies of scale.

Furthermore, fuel ethanol from lignocellulosic biomass (crop residues, forestry and fruit and vegetable waste and weeds) may also open new employment opportunities in rural areas, and thus make a positive socio-economic impact and reduce global climate change [30]. However, producing cellulosic bioethanol from lignocellulosic waste presently is not financially feasible due to unclear economic policy that should be corrected through a Pigouvian approach.

4.4. Comparison Bioethanol to Biogas

In a study by Hamawand et al. [31], bioethanol and biogas from lignocellulosic materials has been addressed to show the economical and environmental advantage of bioethanol compared to biogas. Tables 3 and 4 show that bioethanol can produce more revenue due to its value as a liquid product. In addition, the amount of CO_2 generated during the production and due to the consumption of these products show that bioethanol generates 0.21 kg CO_2-e/kg processed and consumed less than biogas. The price of energy in Table 3 is based on the price of coal, which is $110 per tonne. This means $3.93 per gigajoule (GJ).

Table 3. Revenue generated from ethanol and biogas.

Waste Solid	Utilization Method	Amount	Energy	Total Energy, GJ	Revenue, $
Lignocellulosic	Ethanol	1000 kg 1000 kg ($1/L)	29 MJ/kg 29 MJ/kg	29 29	114 * 1000 **
	Biogas	1000 m^3 (1150 kg)	39 MJ/m^3	39	153

Notes: * based on the price of energy from coal; ** based on the price of ethanol in the market.

Table 4. CO_2 produced from production and usage of ethanol and biogas.

Wastes	Bio-Fuel Products	Energy, MJ/kg	Produced in the Process kg CO_2-e/kg Waste	Produced While Consumed, kg CO_2-e/kg Product	Total, kg CO_2-e/kg Processed and Consumed
Lignocellulosic	Ethanol	29	0.108	0.22	0.328
	Biogas	34	0.227	0.311	0.538

5. Conclusions

In this research, experimental works were conducted to investigate ethanol production through various pretreatment methods. Acid (H_2SO_4), alkaline (NaOH) and ultrasound were separately and in combinations tested as pretreatments for bagasse. The results from this study were compared with literature to show the impact of an ultrasound when combined with chemical pretreatments. The results from the experiments were processed through Minitab software for optimization purposes.

The optimum level of acid, alkaline and ultrasound amplitude were obtained as the following: the optimum level of acid was shown to be in the range of 2.5%–3.5%, for alkaline solution it was in the range of 22%–25% and, for the ultrasound, the amplitude level range was 45–60 (76–127 W).

The results show higher ethanol production than that reported in literature. The optimum ethanol production value of 820 mg/L was achieved when ultrasound (60 amplitude level or 127 W) and acid (3% H_2SO_4 concentration) pretreatments were combined. In addition, a value of 911 mg/L was achieved when all the pretreatments were used, and bagasse was treated with acid (3%) and ultrasound (50 or 109 W) and then treated with alkaline (23%) and ultrasound (50 or 109 W).

References

1. Hekkert, M.P.; Negro, S.O. Functions of innovation systems as a framework to understand sustainable technological change: Empirical evidence for earlier claims. *Technol. Forecast. Soc. Chang.* **2009**, *76*, 584–594. [CrossRef]

2. Sai, S.K. A Study of Ionic Liquids for Dissolution of Sugarcane Bagasse. Ph.D. Thesis, Queensland University of Technology, Brisbane, Australia, 2012.

3. Verardi, A.; Blasi, A.; Molino, A.; Albo, L.; Calabrò, V. Improving the enzymatic hydrolysis of *Saccharum officinarum* L. bagasse by optimizing mixing in a stirred tank reactor: Quantitative analysis of biomass conversion. *Fuel Process. Technol.* **2016**, *149*, 15–22. [CrossRef]

4. Warden, A.C.; Haritos, V.S. *Future Biofuels for Australia: Issues and Opportunities for Conversion of Second Generation Lignocellulosics*; Rural Industries Research and Development Corporation: Canberra, Australia, 2008.

5. Zafar, S. Biofuels from Lignocelluloses Biomass. Bio-Energy Consult, Power Clean Energy Future, 2014. Available online: http://www.bioenergyconsult.com/what-is-lignocellulosic-biomass/ (accessed on 20 October 2016).

6. Pradhan, R. *Production of Ethanol from Bagasse*; National Institute of Technology Rourkela: Rourkela, India, 2007.

7. Hamelinck, C.N.; van Hooijdonk, G.; Faaij, A.P. Future prospects for the production of ethanol from ligno-cellulosic biomass. *Biomass Bioenergy* **2005**, *28*, 384–410. [CrossRef]

8. Liyakathali, N.A.M. Ultrasonic Pretreatment of Energy Cane Bagasse for Biofuel Production. Master's Thesis, Anna University, Chennai, India, 2014.

9. O'Hara, I.M. Cellulosic Ethanol from Sugarcane Bagasse in Australia: Exploring Industry Feasibility through Systems Analysis, Techno-Economic Assessment and Pilot Plant Development. Ph.D. Thesis, Queensland University of Technology, Brisbane, Australia, 2011.

10. Manzoor, A.; Khokhar, Z.U.; Hussain, A.; Uzma; Ahmad, S.A.; Syed, Q.U.A.; Baig, S. Dilute sulphuric acid: A cheap acid for optimization of bagasse pre-treatment. *Sci. Int. (Lahore)* **2012**, *24*, 41–45.

11. Njoku, S.I. Optimization of the Production of Cellulosic Biofuels. Ph.D. Thesis, Aalborg University, Copenhagen, Denmark, 2012.

12. Lavarack, B. Estimates of ethanol production from sugar cane feedstocks. In Proceedings of the Australian Society of Sugar Cane Technologists, Townsville, Australia, 6–9 May 2003.

13. Boopathy, R.; Dawson, L. Cellulosic ethanol production from sugarcane bagasse without enzymatic saccharification. *BioResources* **2008**, *3*, 452–460.

14. Rehman, M.S.U.; Kim, I.; Chisti, Y.; Han, J.-I. Use of ultrasound in the production of bioethanol from lignocellulosic biomass. *Energy Educ. Sci. Technol. Part A Energy Sci. Res.* **2013**, *30*, 1391–1410.

15. Taherzadeh, M.J.; Karimi, K. Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int. J. Mol. Sci.* **2008**, *9*, 1621–1651. [CrossRef] [PubMed]

16. Hamawand, I. Anaerobic digestion process and bio-energy in meat industry: A review and a potential. *Renew. Sustain. Energy Rev.* **2015**, *44*, 37–51. [CrossRef]

17. Hamawand, I.; Baillie, C. Anaerobic digestion and biogas potential: Simulation of lab and industrial-scale processes. *Energies* **2015**, *8*, 454–474. [CrossRef]

18. Molino, A.; Nanna, F.; Ding, Y.; Bikson, B.; Braccio, G. Biomethane production by anaerobic digestion of organic waste. *Fuel* **2013**, *103*, 1003–1009. [CrossRef]

19. Molino, A.; Nanna, F.; Migliori, M.; Iovane, P.; Ding, Y.; Bikson, B. Experimental and simulation results for biomethane production using peek hollow fiber membrane. *Fuel* **2013**, *112*, 489–493. [CrossRef]

20. Molino, A.; Nanna, F.; Iovane, P. Low pressure biomethane production by anaerobic digestion (AD) for the smart grid injection. *Fuel* **2015**, *154*, 319–325. [CrossRef]

21. Sayan, C.; Muktadir, B.F.I.M.; Arpita, C. Economic viability of biogas and green self-employment opportunities. *Renew. Sustain. Energy Rev.* **2013**, *28*, 757–766.

22. Sayan, C.; Tawhidul, I. Financial viability and eco-efficiency of the solar home systems (SHS) in Bangladesh. *Energy* **2011**, *36*, 4821–4827.

23. Zhao, L.; Zhang, X.; Xu, J.; Ou, X.; Chang, S.; Wu, M. Techno-economic analysis of bioethanol production from lignocellulosic biomass in China: Dilute-acid pretreatment and enzymatic hydrolysis of corn stover. *Energies* **2015**, *8*, 4096–4117. [CrossRef]

24. Klein-Marcuschamer, D.; Oleskowicz-Popiel, P.; Simmons, B.A.; Blanch, H.W. The Challenge of enzyme cost in the production of lignocellulosic biofuels. *Biotechnol. Bioeng.* **2012**, *109*, 1083–1087. [CrossRef] [PubMed]

25. Kwiatkowska, B.; Bennett, J.; Akunna, J.; Walker, G.M.; Bremner, D.H. Stimulation of bioprocesses by ultrasound. *Biotechnol. Adv.* **2011**, *29*, 768–780. [CrossRef] [PubMed]

26. Ingram, L.O.; Wood, B.E. Ethanol Production from Lignocellulose. Patent US 6333181 B1, 25 December 2001.

27. Coady, D.; Parry, I.; Sears, L.; Shang, B. *How Large Are Global Energy Subsidies?* IMF Working Paper; International Monetary Fund (IMF): Washington, DC, USA, 2015.

28. Liu, H.; Polenske, K.R.; Xi, Y.; Guo, J.E. Comprehensive evaluation of effects of straw-based electricity generation: A Chinese case. *Energy Policy* **2010**, *38*, 6153–6160. [CrossRef]

29. Streets, D.; Yarber, K.; Woo, J.H.; Carmichael, G. Biomass burning in Asia: Annual and seasonal estimates and atmospheric emissions. *Glob. Biogeochem. Cycles* **2003**, *17*, 1099. [CrossRef]

30. Tew, T.L.; Cobill, R.M. Genetic Improvement of Sugarcane (*Saccharum* spp.) as an Energy Crop, in Genetic Improvement of Bioenergy Crops. In *Genetic Improvement of Bioenergy Crops*; Vermerris, W., Ed.; Springer: New York, NY, USA, 2008; pp. 273–294.

31. Hamawand, I.; Sandell, G.; Pittaway, P.; Chakrabarty, S.; Yusaf, T.; Chen, G.; Seneweera, S.; Al-Lwayzy, S.; Bennett, J.; Hopf, J. Bioenergy from cotton industry wastes: A review and potential. *Renew. Sustain. Energy Rev.* **2016**, *66*, 435–448. [CrossRef]