Synthesis of Bioethanol from Coconut Husks as Organic Solvent for Grease Removal

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Abstract

The project aims to synthesise ethanol from coconut husks and investigate its properties as an organic solvent to remove grease and oil. This can provide an environmentally-friendly alternative to commercially-produced ethanol, as well as to make use of coconut husk waste to reduce its impact on the environment. This can also demonstrate an alternative use of ethanol as a cleaner for grease and oil removal. An investigation into the effectiveness of pretreatment before fermentation was done by carrying out pretreatment on coconut husk powder before enzymatic hydrolysis with cellulase. Using the DNS method, the amount of fermentable sugars was found and compared to find out the effectiveness of pretreatment. For the fermentation process, *Saccharomyces cerevisiae* and *Zymomonas mobilis* were compared based on ethanol yield. The project involves several characterisation tests on the ethanol solutions post-distillation. It was observed that *Zymomonas mobilis* is a more effective fermenting agent than *Saccharomyces cerevisiae* as using *Zymomonas mobilis* led to greater ethanol yields. Distilled ethanol solutions were found to be too diluted, rendering the characterisation tests inaccurate. It was found that it is viable and the more effective way to synthesise ethanol from coconut husks is by using pretreatment before enzymatic hydrolysis and by using *Zymomonas mobilis* as the fermenting agent. However, further studies can be conducted to obtain more concentrated ethanol and characterise the synthesised ethanol. Other variables during fermentation, such as pH and temperature, can also be tested to find the optimal conditions for the highest ethanol yield.

1. Introduction

Ethanol, or ethyl alcohol, has an increasing demand worldwide, predominantly as a more environmentally-friendly alternative to fossil fuels (Menetrez, 2014), but also for use in industries such as automotive, pharmaceuticals and cosmetics. However, existing ways of producing ethanol are not environmentally-friendly enough. Most are directly derived from sugars or oils of grown crops, such as corn and sugarcane, due to lower costs (Menetrez, 2014). The growth of such crops for ethanol production has resulted in much higher overall greenhouse gas emissions through causing deforestation, creating a “carbon debt” that cannot

be compensated by savings in greenhouse gas emissions from ethanol use (Fargione, Hill, Tilman, Polasky, & Hawthorne, 2008; Searchinger *et al.*, 2008; Gecan, Johansson, & FitzGerald, 2009). At the same time, it also increases food prices. One-third of the corn harvest in the United States is used to produce ethanol (Menetrez, 2014). Increased demand for corn to produce ethanol tripled corn prices and directly contributed to food prices increasing in 2008 (Gecan *et al*., 2009). Thus, current production methods harm the environment and affect food prices, creating a need for alternative sources and methods.

A promising alternative is cellulosic ethanol, which is produced from lignocellulose, including cellulose, hemicellulose and lignin. Cellulosic ethanol does not require land to grow crops (Gecan *et al.*, 2009), since the materials needed can be found in most plants. High contents of lignocellulose can also be found in some food waste. Several studies have thus concluded that many types of food waste are commercially viable for ethanol production, including cassava pulp (Valeriano *et al.*, 2018), jackfruit peel (Yuvarani & Dhas, 2017) and potato peel (Arapoglou, Varzakas, Vlyssides, & Israilides, 2010).

Coconut husks take up to eight years to decompose when thrown away, which is very long for a biomass (Carrijo, de Liz, & Makishima, 2002). At the same time, they are made up of 46% cellulose, 11% hemicellulose and 43% lignin (Umoh & Jonah, 2017), all of which are present in large amounts in other types of food waste that are proven to be viable for ethanol production and can be used for the production of cellulosic ethanol. This demonstrates a high potential of synthesising ethanol from coconut husks as an environmentally-friendly alternative that can also mitigate the impact of coconut husk waste.

Typically, acidic hydrolysis is carried out to convert lignocellulosic material into fermentable sugars before fermentation (Arapoglou *et al.*, 2010; Yuvarani & Dhas, 2017), but enzymatic hydrolysis using cellulase has been found to be more effective in doing so than acidic hydrolysis in various different biomasses, because it can work under mild conditions, reduce water and energy consumption and amount of by-products (Israilides *et al.*, 2008), and can give a higher yield of fermentable sugars (Arapoglou *et al.*, 2010; Amezcua-Allieri, Durán, & Aburto, 2017). Studies show that acidic and alkali pretreatment can degrade hydrogen bonds in hemicellulose and partially degrades cellulose and lignin, resulting in a higher yield of fermentable sugars from enzymatic hydrolysis using cellulase in other biomass (Amezcua-Allieri *et al.*, 2017; Imman, Arnthong, Burapatana, Champreda, & Laosiripojana, 2014). Therefore, to maximise yield, enzymatic hydrolysis would be used over acidic hydrolysis,

while pretreatment would be tested if it increases the effectiveness of hydrolysis in coconut husks.

Saccharomyces cerevisiae is most commonly used for fermentation, while the use of *Zymomonas mobilis* has been found to give a higher yield of ethanol in some biomasses (Sivasakthivelan, Saranraj, & Sivasakthi, 2015), due to higher productivity, alcohol tolerance and a broader pH range for production (Yang *et al*., 2016). However, in other biomass, *Z. mobilis* is found to produce a lower yield than *S. cerevisiae* (Behera, Mohanty, & Ray, 2010). Both *Z. mobilis* and *S. cerevisiae* would be used to find out which would produce a higher yield specifically from coconut husks.

In most studies, only the yield and sometimes the properties of ethanol as a biofuel are measured (Muhaji & Sutjahjo, 2018; Yuvarani & Dhas, 2017). However, due to the rise of electric vehicles and other factors, the growth in demand for ethanol as a biofuel is decreasing. This opens up opportunities to explore other purposes for ethanol. Ethanol is an amphipathic solvent, containing polar and nonpolar ends (shown in Figure 1). Grease and oil, on the other hand, are non-polar. When mixed with water, which is polar, dilute ethanol can be used to clean stains. However, few experiments have been done to evaluate the viability of ethanol's slight non-polar property as a solvent for grease and oil. Ethanol also displays antimicrobial properties against bacteria (Oh & Marshall, 1993; Valle, Cabrera, Puzon, & Rivera, 2016), which enhances its usefulness as a cleaner. Thus, the use of ethanol, given its nonpolar and antimicrobial properties, as a cleaner to remove grease would be investigated.

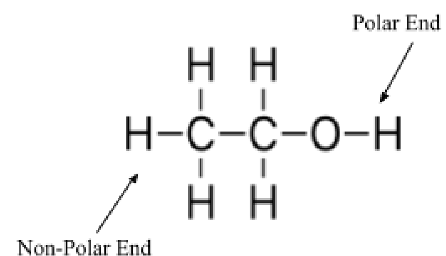

Figure 1: Structure of Ethanol

2.1 Objectives

a. To synthesise ethanol from coconut husks using acidic and alkali pretreatment and different fermenting agents to find out the most effective method of synthesising ethanol from coconut husks.

b. To investigate the use of ethanol synthesised from coconut husks as an organic solvent for grease and oil removal.

2.2 Hypotheses

a. Pretreatment before hydrolysis leads to greater yields than without pretreatment.

b. Fermentation using *Zymomonas mobilis* leads to greater yields than *Saccharomyces cerevisiae*.

c. Ethanol synthesised from coconut husks can dissolve grease and oil.

3. Methodology

3.1 Preparation of coconut husks

Coconut husks were ground into powder. Coconut husk powder was washed and dried, before being divided into portions of 10 g.

3.2 Pretreatment

To create a pretreated sample, 10 g of coconut husk powder was acidified by 100 ml of 0.0765 M aqueous sulfuric acid, warmed at 100°C and stirred at an intensity of 2 on a hot plate for 2 hours. The solution was then neutralised, vacuum-filtered and the residue was washed with deionised water. The residue was then alkalined with 100 ml of 0.375 M aqueous sodium hydroxide. The solution was warmed at 100°C and stirred at an intensity of 2 for 2 hours. The solution was then neutralised, vacuum-filtered and the residue was washed with deionised water. 200 ml of 0.1 M sodium acetate buffer solution was then added to the residue.

For samples without pretreatment, 100 ml of distilled water was added to 10 g of coconut husk powder and stirred to form a suspension.

3.3 Enzymatic hydrolysis

Cellulase powder was added to each sample in a 1:16 (cellulase:substrate) ratio in mass. 0.1 M sodium acetate buffer was also added to the coconut husk powder in a 1:20 (substrate:solution) ratio. The solution was then placed in the shaker for 24 hours at 35°C at 120 rpm. After hydrolysis, the solution was vacuum-filtered and the filtrate containing reducing sugars was collected.

3.4 Preparation of DNS reagent

DNS reagent was prepared to test for the concentration of reducing sugars. Dinitrosalicyclic acid reacts with reducing sugars in the solution, oxidising the reducing sugars into their carboxyl groups, while the yellow dinitrosalicyclic acid is reduced to red-brown amino-nitrosalicyclic acid. The intensity of the colour in the reagent is proportional to the concentration of reducing sugars, so it could be measured with a spectrophotometer at 540nm (Garriga, Almaraz, & Marchiaro, 2017). 1 g of dinitrosalicylic acid was dissolved in 20 ml of 2 M aqueous sodium hydroxide with stirring to form the first solution. 30 g of sodium potassium tartrate tetrahydrate was dissolved in 50 ml of distilled water with stirring to form the second solution. Both solutions were added together, heated and mixed to homogenise. Distilled water was added until the total volume reached 100 ml.

3.5 Glucose standard curve

Solutions with 20%, 40%, 60%, 80% and 100% concentrations of glucose were prepared by adding 0.2 mg, 0.4 mg, 0.6 mg, 0.8 mg and 1.0 mg respectively per 1 ml of distilled water. 1 ml of each solution was added to 1 ml of DNS reagent in a 10 ml centrifuge tube. It was put in a bath thermostatised at 100°C for 5 minutes before it was cooled to room temperature. The volume was made up to 10ml by adding distilled water. It was read at 540 nm in a spectrophotometer.

3.6 DNS testing

Two sets of samples were tested for their amount of reducing sugars, one with pretreatment done and the other without. Both sets of solutions were tested after enzymatic hydrolysis. 1 ml of sample solution and 1 ml of DNS reagent were added in a 10 ml centrifuge tube. The pH was brought to 10 using 0.375 M aqueous sodium hydroxide. It was put in a water bath thermostatised at 100°C for 5 minutes before it was cooled to room temperature. The volume was made up to 10 ml by adding distilled water. It was homogenised and read at 540 nm in a spectrophotometer.

3.7 Growth of fermenting agents

Saccharomyces cerevisiae and *Zymomonas mobilis* were grown in nutrient agar plates. They were then cultured in potato dextrose broth solution and left in the shaker at 35°C and 120 rpm for 24 hours.

3.8 Fermentation

Each sample was divided into two parts. *Saccharomyces cerevisiae* was added into the first sample. *Zymomonas mobilis* was added into the second sample. Nutrients for the fermenting agents were also added in the following concentrations: peptone (1 g/L), ammonium sulfate (0.5 g/L), magnesium sulfate (0.5 g/L) and yeast extract (0.5 g/L). The bottles containing the solutions were then left in the incubator at 35°C for 72 hours for fermentation. After fermentation, the concentration of ethanol was measured using an ethanol meter. The ethanol was then distilled.

3.9 Grease and oil solubility test

To 1 ml of ethanol solution obtained from the previous step, grease was added in 0.01 g intervals and mixed after each interval. Any observable loss in grease solid was taken to be the dissolution of grease. The mass of grease at which there was no observable dissolution of grease was taken to be the grease solubility threshold of the ethanol solution.

To another 1 ml of ethanol solution obtained from the previous step, 0.01 ml of oil was added dropwise using a micropipette and mixed after each drop. If the solution remained colourless, the oil was taken to be miscible. The volume of oil at which a distinct layer of oil formed above the ethanol was taken to be the oil miscibility threshold of the ethanol solution.

3.10 Zone of inhibition test

Plates of agar were prepared overnight. For the culture of *Escherichia coli*, centrifuge tubes of *Escherichia coli* and Lysogeny broth were placed in the shaker for 24 hours. *Escherichia coli* was then spread on the agar plates. Using a 0.1 ml micropipette tip, 3 well-diffusion holes were made in the agar plates: 1 for 10% bleach solution, 1 for distilled water, and 1 for the ethanol sample. The agar plates were then incubated at 35°C for 24 hours.

4. Results and Discussion

4.1 Effect of pretreatment on amount of reducing sugars

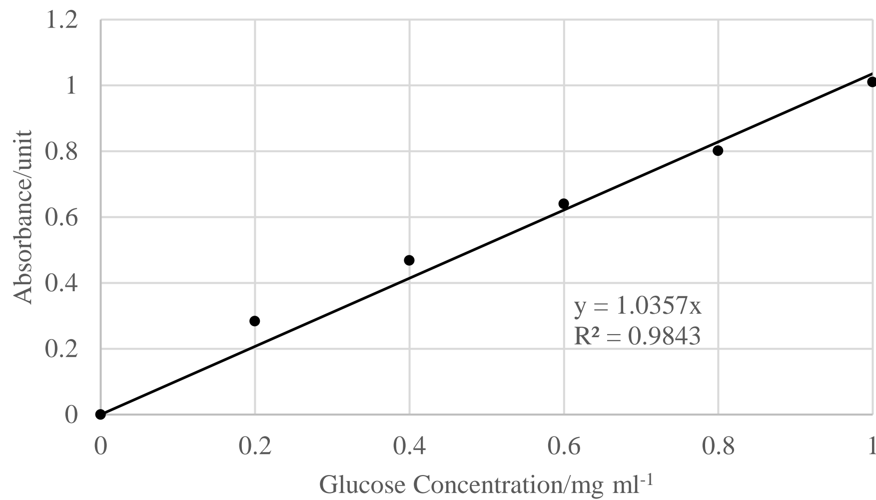

Figure 2: Glucose standard curve for DNS testing

Using the glucose standard curve, the concentration of reducing sugars in the sample solutions can be determined by dividing the absorbance by the gradient of the curve.

The absorbance of samples both with and without pretreatment after hydrolysis was divided by 1.0357 to find the concentration of glucose. The mean of each set of samples was taken and plotted in Figure 3.

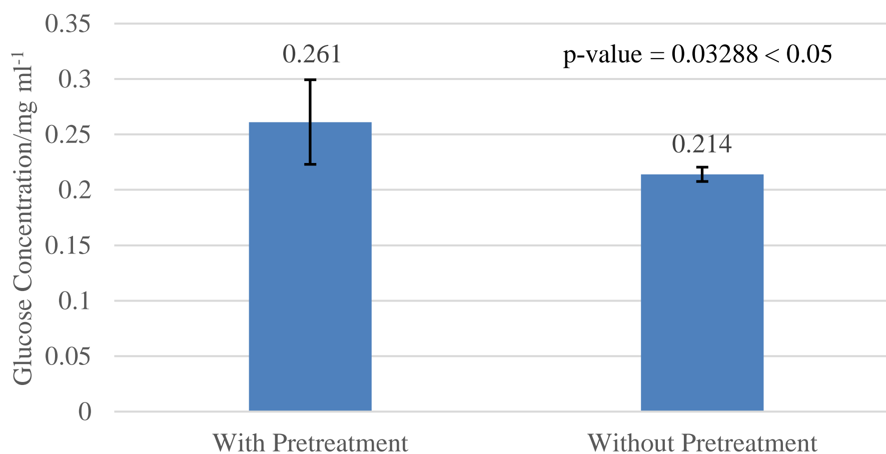

Figure 3: Graph of glucose concentration in samples with and without pretreatment

The Mann Whitney U test showed a p-value of 0.03288. As the p-value (0.03288) is less than 0.05, there is a significant difference between the glucose concentrations of samples with pretreatment and samples without pretreatment. Samples that were pretreated had a mean glucose concentration of 0.261 mg/ml, while samples that were not pretreated had a mean

glucose concentration of 0.214 mg/ml. Thus, it can be concluded that pretreatment leads to a higher yield of reducing sugars during enzymatic hydrolysis, specifically in coconut husk powder.

This is consistent with previous studies that have found acidic and alkali pretreatment led to a higher yield of reducing sugars from enzymatic hydrolysis. During acidic pretreatment, hemicellulose is dissolved and the structure of lignin changes, both of which are present in large amounts in coconut husks. Lignin hinders enzymatic hydrolysis as it interferes with the binding of the enzyme to the substrate (Kaya, Heitmann, & Thomas, 2000). Removing the lignin through acidic treatment will help to increase the efficiency of enzyme hydrolysis. During alkali pretreatment, the cell wall is disrupted through dissolving hemicellulose, lignin, silica, and hydrolyzing uronic and acetic acid esters. The crystallinity of cellulose is also decreased. This increases the digestibility of lignocellulosic material by enzymes, thus increasing the yield of reducing sugars (Kaya, Heitmann, & Thomas, 2000).

4.2 Ethanol yield

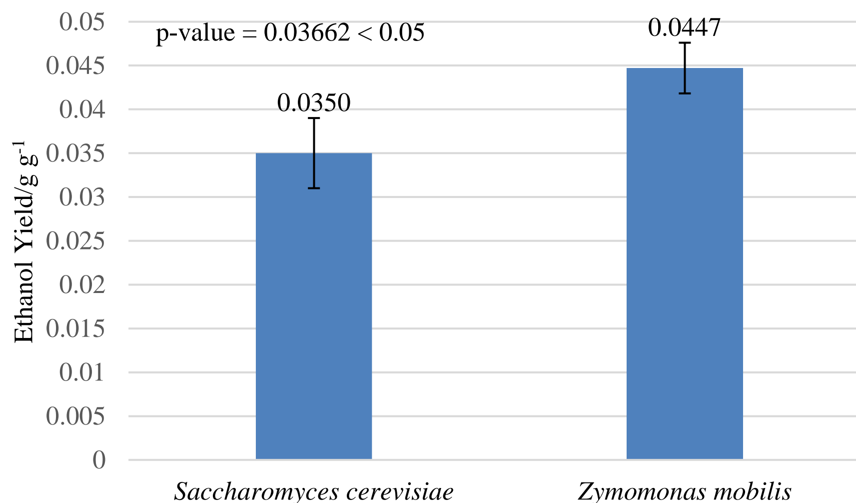

Figure 4: Graph of ethanol yield per gram of coconut husk powder

The ethanol yield was calculated by measuring the total volume of the solution and concentration of ethanol after fermentation to calculate the total mass of ethanol synthesised using the density of ethanol. The total mass was divided by the mass of coconut husk powder initially used to obtain the ethanol yield. Using *Saccharomyces cerevisiae* yielded 0.0350 g of ethanol per 1 g of coconut husk powder used. Using *Zymomonas mobilis* yielded a higher 0.0447 g of ethanol per 1 g of coconut husk powder used. According to the p-value obtained from the Mann Whitney U test, *Zymomonas mobilis* produced a statistically higher yield of ethanol than *Saccharomyces cerevisiae* (shown in Figure 4).

This implies that for coconut husks, *Zymomonas mobilis* produced ethanol more effectively than *Saccharomyces cerevisiae*. The results are in line with other studies that used lignocellulosic biomasses to synthesise ethanol with *Zymomonas mobilis* and *Saccharomyces cerevisiae* (Sivasakthivelan, Saranraj, & Sivasakthi, 2015). *Zymomonas mobilis* has higher ethanol tolerance and sugar uptake ability (Yang et al., 2016), so it is more effective in producing ethanol than *Saccharomyces cerevisiae*.

4.3 Zone of inhibition test

After two repeats of the zone of inhibition test, no observable ‘wells’ formed, with only minimal traces of wells being observed. The test showed that the ethanol solutions had minimal to zero antibacterial properties. However, this contradicted the literature review, which showed that ethanol should in fact possess antibacterial properties, and wells should have formed in the agar plates. One explanation for the poor results of the zone of inhibition test would be that the ethanol samples were too dilute as the ethanol concentrations were in the range of 1 - 2%. Most of the ethanol were left in the cooler and fractionating column. Because of this, when the samples were distilled after fermentation, the distillate had a low concentration of ethanol and consisted of mostly distilled water. Given the concentration, the results were consistent with previous studies, which found no growth inhibition against *Listeria monocytogenes* when 1.25% ethanol is used but found that 5% ethanol strongly inhibited bacteria growth (Oh & Marshall, 1993). As a result, the ethanol samples contained mostly water and had minimal antibacterial properties.

4.4 Grease and oil solubility test

For both tests, there were no observable dissolution of grease and oil. This showed that grease and oil were insoluble in the ethanol solutions. This contradicted the literature review. A possible explanation would be that the ethanol solutions were too diluted after distillation. Since a vast majority of the ethanol solutions were water, grease and oil would naturally be largely insoluble in the solutions. This is because grease and oil are non polar, whereas water is polar. Water molecules are unable to form hydrogen bonds with grease and oil molecules and in order to form dispersion forces with grease and oil molecules, water molecules have to overcome hydrogen bonds between themselves, which is not energetically-favourable. Hence, grease and oil would be insoluble in water and was not soluble in the diluted ethanol solutions.

5. Conclusion and Future Studies

Coconut shell and coconut husk could both be used to obtain bioethanol. It could also be concluded that pretreatment of the coconut husk waste in the form of acidic and alkaline hydrolysis had a significant impact on the amounts of reducing sugars present after enzymatic hydrolysis of the coconut husk waste. During the fermentation stage of the hydrolysed solution, it could be concluded that *Zymomonas mobilis* yielded a higher concentration of ethanol than *Saccharomyces cerevisiae*. Thus, coconut husk waste could be effectively utilised to synthesise ethanol with pretreatment, enzymatic hydrolysis and *Zymomonas mobilis* to reduce waste and also replace polluting methods of producing ethanol. This could benefit the environment by decreasing greenhouse gas emissions from producing ethanol and processing coconut husk waste.

Only a very small amount of coconut husk powder was used for each sample, so the concentration and net volume of ethanol after fermentation were very low. Thus, during the distillation process, not all the ethanol could be obtained as a large percentage of the ethanol was left in the cooler and fractionating column. When distilled out, most of the distillate comprised water, with a low concentration of ethanol. Hence, the ethanol collected was too dilute. Therefore, the characterisation tests did not accurately represent the feasibility of ethanol synthesised from coconut husk waste as a solvent for grease and oil removal. The characterisation tests also failed to accurately reflect any antibacterial properties of the ethanol solutions. The ethanol solutions could also not be compared against commercial ethanol as they were too diluted after distillation.

Future research could be done with more efficient distillation to obtain more ethanol for characterisation. Since fractional distillation resulted in loss of reducing sugars which were possibly stuck to or trapped within the solid powder, alternative methods like gas chromatography could be tried to obtain the ethanol. Alternatively, a greater mass of coconut husks could be used so that a greater net volume of ethanol would be yielded. This allows a greater volume of more concentrated ethanol to be obtained from distillation, so characterisation could be conducted more effectively and results would more accurately represent the feasibility of ethanol as a solvent for grease and oil removal.

The effect of other variables, such as temperature and pH, on the efficiency of fermentation can also be investigated to further improve the efficiency of ethanol synthesis.

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