Use of cellulose extract from durian husks for synthesis of fibres and biofuel

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Abstract

Food-waste disposal and the need for more environmentally-friendly and renewable alternative energy sources have been two major problems in today's world. The predominant method of disposing of food waste in Singapore is by incineration, which places pressure on Singapore's disposal facilities and Singapore's landfill. Singapore mostly relies on natural gas as its main energy source. However, due to its unsustainability, Singapore has been pressured to venture into possible alternative energy sources. Recently, methods to use fruit peels as an environmentally friendly and reliable energy source has been investigated due to their high cellulose contents. Durian, a popular fruit in Singapore and other Southeast Asian countries, has been found to contain a rather high cellulose content, even among other fruits. It can be hypothesized that the cellulose, if extracted and processed, can be used to produce biofuel or other useful products. This paper tests the viability of the method and also investigates the different factors that affect the efficiency and percentage yield of this process. Our results have shown that a large percentage of the cellulose found in durians can be efficiently abstracted and converted into glucose, thus proving the hypothesis true. Our results have also shown that a higher acid concentration and higher temperature can significantly increase the yield of glucose, as our final product.

1. Introduction

1.1 Literature review

Food waste has become a prevalent problem in Asia, whereby the agricultural sector in Asian countries continues to grow for self-sufficiency and export earning. It is caused by logistic flaws in the transportation of goods, poor infrastructure, or the high-quality standard of food in developed countries. (Gustavsson et. al, 2016). Statistics reveal the annual amount of urban food waste in Asian countries could rise from 278 to 416 million tonnes from 2005 to 2025 (Paritosh et al., 2017). On a local scale, Singapore, an urbanized country, produced about 744,000 tons of organic food waste with only 18% recycled (NEA,2021).

Durian is a popular fruit among Southeast Asia countries. In 2016, around 377,000 metric tons of durians were produced by Malaysia alone and 226,000 metric tons of durian husk were then improperly disposed of in Malaysia. Malaysia's Ministry of Agriculture and Food Industries (MAFI) has predicted 443,000 metric tons of durian production in 2030 (Malay Mail, 2020). Therefore, durian waste is especially severe in Asian countries with high durian production and consumption. Improper disposal of durian and food waste may result in environmental adversities such as wastage of land and capital and harmful gas emissions from incineration. (Marshall & Farahbakhsh, 2013). Even in a small nation like Singapore, the Agri-Food and Veterinary Authority of Singapore (AVA) reported 8,900 tonnes of fresh and chilled durians were imported into Singapore, more than the amount recorded at the halfway point in 2017 when a total of 14,300 tonnes were imported (Jun, 2018). Therefore, there is much necessity to research this field as durian waste is detrimental to the environment and economy and an issue of paramount importance.

Current methods of putting durian waste to good use are converting durian husks into ethylene absorber paper for prolonging the storage life of fruits (Tengrang, 2017) and creating biodegradable gel bandages for surgery (Ng, 2021). The durian husk is an abundant source of holocellulose and allows it to produce packages and paper and absorb ethylene gas which negatively affects the texture and taste of fruits. Besides, durian husk has a high glucose content, and the extracted glucose can be infused with glycerol to form a soft gel used in gel bandages. This application solved three problems with a solution, making surgery bandages cheaper, reducing durian waste, as well as eliminating bandage trash.

Recently, biofuel has become a popular alternative to fossil fuels globally, which provides for over 85% of all the energy we consume in 2016 (Rinkesh, 2016), and abstains the need to place a strain on the unrenewable source of energy. The production and consumption of biofuel are rather environmentally friendly and reduce harmful gas emissions by up to 86% compared to petroleum products, which could solve the issue of GHG emission, especially in Asian countries (Dixon, n.d.). Biofuel also has other industrial benefits compared to petroleum oil: being relatively less flammable compared to fossil diesel, easy to source for, and having significantly better lubricating properties and higher cetane (Rinkesh, 2020). Eventually, biofuel may even be cheaper in the future due to increased demand, and reduce countries' reliance on other forms of fuels. All these factors have contributed to the increased production of biofuel globally, requiring more land and capital to grow crops meant for biofuel production, eventually leading to higher food prices and deforestation, inducing negative economic and social impacts such as food insecurity (Guardian News and Media, 2017). Therefore, durian waste may prove handy in aiding the production of biofuel in Asia and diminish the need for growing extra crops to process into biofuel.

This is because studies have shown that durian waste (especially its husks) is a good source of cellulose and has the potential to produce biofuel and other products including food products or clothes. The durian husk consists of 60.45% of cellulose, 15.45% of lignin, and 13.09% of hemicellulose (Manshor et al., 2014), compared to another fruit having husks, coconut, composed of only 44.34% of hemicellulose and cellulose content (Sangian et al.,2018). Thus, durian husk may be a viable source of cellulose for allowing great efficiency of holocellulose production. Cellulose can then be converted into biofuel through the above steps of converting it to glucose to be then fermented in yeast. Pure glucose can also be used to make food products, and cellulose fibers can be used to make paper.

The main steps of biofuel production include removal of lignin from holocellulose using an oxidizing agent, solubilization and reaction of hemicellulose with a strong base and hydrolysis of cellulose into glucose which is catalysed by a strong acid. Afterwards, glucose can be then fed to yeast in order to produce bioethanol (biofuel). Firstly, bleaching of durian fibres involves a strong oxidising agent such as sodium hypochlorite or calcium hypochlorite (Admin, 2016). The hypochlorite ion decomposes into chloride and a highly reactive form of oxygen: $2ClO^- \rightarrow 2Cl^- + O_2$. The HOCl can then attack the chemical bonds in a coloured compound, either completely destroying the chromophore of lignin (the part of the molecule that gives it its colour), or converting the double-bonds in the chromophore into single bonds, thereby preventing the molecule from absorbing visible light(May, 2011). Hypochlorite reactions with lignin produce chloroform as a by-product (Hintz, 2003). Therefore, lignin is eliminated from the durian rind powders, leaving behind holocellulose. Next, the conversion of holocellulose into cellulose. Holocellulose is defined as the total polysaccharide fraction of lignocellulosic biomass which is composed of cellulose and mostly hemicelluloses. Hemicelluloses are linear or branched polymers and are essentially amorphous (does not exhibit crystalline structure), due to the many side groups attached to their heteropolysaccharide structures(Heredia et al., 1995). The second biggest constituent of plant fibres is the hemicellulose β-1→4-D-xylan constructed from β-1→4-linked d-xylopyranosyl residues forming the linear backbone chain. Studies have shown that hemicellulose can be solubilized when concentrated NaOH is added. This is because there is the saponification of the ester bonds that link hemicellulose to other lignocellulosic components and removal of acetyl and uronic acid substitutions on hemicellulose (Carvalheiro et al., 2008). Finally, remaining cellulose is converted into glucose. Cellulose is an organic compound with the formula $(C_6H_{10}O_5)$.

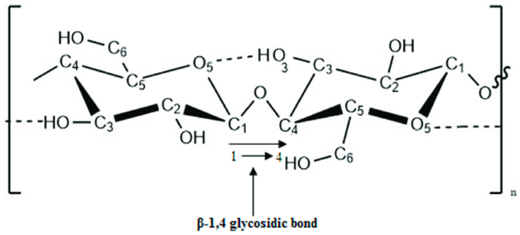


Figure 1.1.1: Structure of cellulose, indicating glycosidic bond (Sampath et al., 2016))

Acid hydrolysis is a hydrolysis process in which an acid is used to catalyze the cleavage of a chemical bond via a nucleophilic substitution reaction, with the addition of the elements of water (H_2O). This means that high concentration sulfuric acid catalyses the condensation reaction of the glycosidic bonds (reaction of C_1 and C_4 with water) in cellulose which can then be hydrolysed further into simple sugars such as glucose.

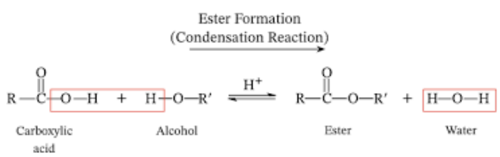


Figure 1.1.2: Condensation reaction equation (Nagwa, n.d.)

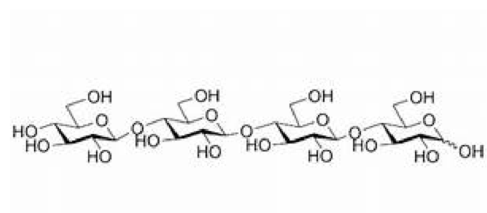
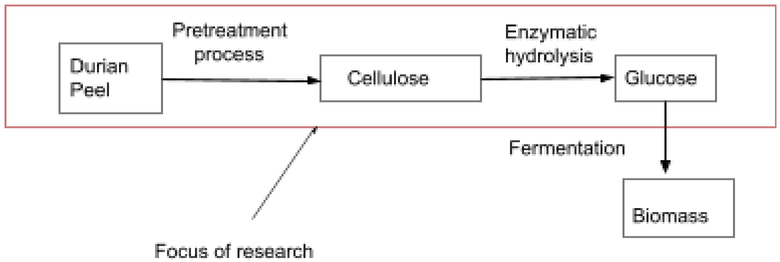


Figure 1.1.3: Cellotetraose structure (Dextrauk)

During this process, cellulose can be converted into several oligosaccharides containing glucose units, mainly cellotetraose (four-anhydroglucose polymers) by the breakage of the β-1,4-glycosidic bonds by acids. Excess water allows cellotetraose to hydrolyze to glucose (S.C. Bhatia, 2014).

1.2 Objective and hypotheses

The aim of the project is to deduce an optimal way to convert parts of durian husks to usable energy. Patpen Penjumras et al, (2014) reported that most of the plants contain about 40-55% cellulose, as it confers strength and stability to the plant cells. Durian rinds, specifically, have been found to be a good source of cellulose (Rachtanapun et al., 2012). However, there is a rather long process to convert cellulose into glucose or usable energy. Research has concluded the following method: 1.Extract cellulose from durian peels, 2.Convert cellulose into glucose using enzymatic hydrolysis, and 3. Ferment glucose into biofuel. Other research has also shown alternative ways of conducting each of the above steps. Although the general idea is consistent, different chemicals and conditions can be used to attempt to improve the results. This research will look into factors affecting the first 2 processes and in an attempt to maximize its efficiency of doing so.



Hypothesis:

1. *The 2 processes stated above can successfully convert durian peels into glucose which can be then used for biofuel and other purposes..*
2. *Varying independent variables will change the rate and output of glucose such that we can find the optimal conditions for the processes to occur in.*

Independent variables	Part 2: ● Concentration of acids used in hydrolysis ● Ratio of cellulose sample to acid solution ● Temperature of the setup ● Time and temperature of post-hydrolysis
Dependent variables	Glucose Yield
Constant variables	Type of durian used, ratio of cellulose sample to sodium hypochlorite used, concentration of sodium hypochlorite used, ratio of cellulose sample to sodium hydroxide used, concentration of sodium hydroxide used, duration of heating of set-ups

2. Methods and Materials

2.1 Materials

Apparatus used in this experiment include: grinder, glass rod, sieve, vacuum-filter, conical flask, oven, heating mantle, refractive index detector and RoA-Organic acid(8%) column, pH paper, beaker and coffee filter. Chemicals used in this experiment were sulfuric acid (10%, 20%, 30%), sodium hydroxide and sodium hypochlorite. The sample of durian rind used in this study was Musang king durian rinds taken from Ah Seng Durian Store in Singapore.

2.2 Extracting cellulose from durian peels

(i)Remove lignin from durian rinds

The following process is taken from Tawakkal et al. (2012), as cited in (Patpen Penjurmas et al.(2014)).

Durian husks of the species Musang king, were collected and washed thoroughly with water in a container before being chopped up with a knife on a chopping board (Figure 2.2.1) They were then grinded using a grinder until in powder form (Figure 2.2.2) Sodium hypochlorite was then added to 10g - 12g of durian rind powders at a 1g : 5ml ratio before being left overnight (Figure 2.2.3). The mixture was then filtered until there is no visible colour (Figure 2.2.4).


Figure 2.2.1: Chopping of durian rinds


Figure 2.2.2: Grinded durian rinds in a beaker


Figure 2.2.3: Mixture of NaOCL and durian rinds

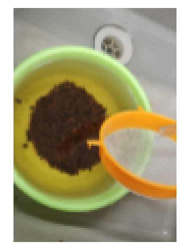


Figure 2.2.4: Filtration of mixture

(ii)Convert holocellulose to cellulose by mercerization

17.5% NaOH solution was added in a beaker and then heated in a water bath and stirred thoroughly with a glass rod at a 1g : 10ml ratio. The sample was left overnight. The sample was then filtered and the residue was washed with deionised water until pH neutral (by pH paper) before it was dried overnight in a vacuum oven at 80°C.

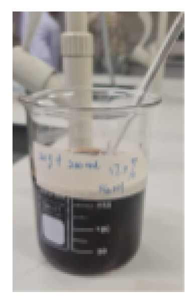


Figure 2.2.5 Adding of Sodium Hydroxide to holocellulose

2.3 Conversion of cellulose into glucose by acid hydrolysis

The following process is taken from (Kong-Win Chang et al., 2018)

Different concentrations (10%, 20% and 30%) of sulfuric acid were added to 2.5g - 3.5g of cellulose obtained from part 1 at a 1g : 8ml ratio at 100°C. The mixture was then neutralised with 1M sodium hydroxide until pH 7. The sample was then filtered using a pump filter and the concentration of glucose present was measured using a refractive

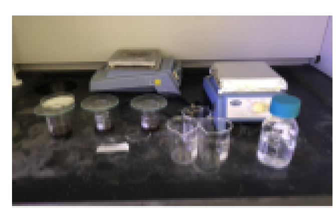


Figure 2.3.1 Sulfuric acid added into cellulose and heated

index detector and RoA-Organic acid column*. After the optimal concentration was found, the same procedure was repeated with optimal concentration of sulfuric acid used but was heated at temperatures (25°C, 60°C and 100°C).

Experiment was repeated three times to obtain average and ensure reliability

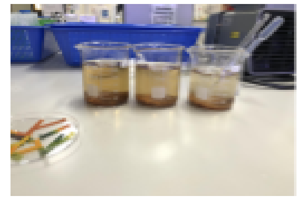


Figure 2.3.2 The mixture was neutralised

2.4 Testing and results definitions

The mixture of glucose and cellulose was vacuum-filtered (Figure 2.4.1). By using the refractive index detector and RoA-Organic acid column, in which the conditions are as follows: 1ml per minute (flow rate), 0.005 M sulfuric acid (mobile phase), the amount of glucose content was measured. The model of the refractive index detector and RoA-Organic acid column is Shimadzu LC20A (Figure 2.4.2). Glucose yield was defined in this experimentation as: mass of glucose/2.5g of cellulose sample.

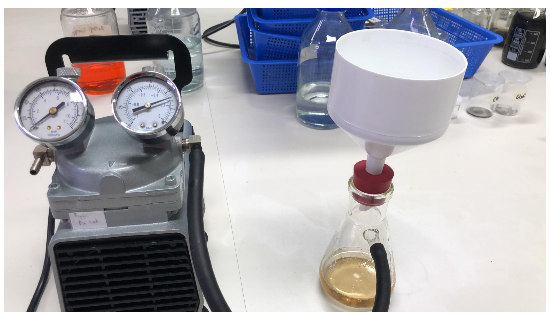


Figure 2.4.1: Vacuum filtration of mixture of glucose and cellulose

Figure 2.4.2: HPLC used to calculate glucose yield

3. Results and Discussion

3.1.1 Factor of concentration of sulfuric acid

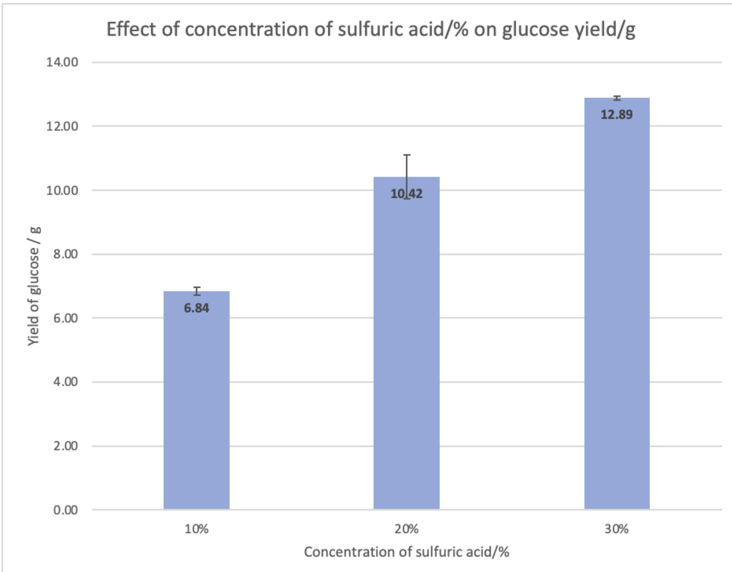


Figure 3.1.1: Graph showing effect of concentration of sulfuric acid/% on glucose yield/g.

According to Figure 3.1.1, the relationship between concentration of sulfuric acid used and the yield of glucose is shown. All the other variables were kept constant, mainly temperature of set-up. Concentration of sulfuric acid was the independent variable in which they were added to set-ups at different concentrations, 10%, 20% and 30%. The graph shows a general increasing trend, whereby when the concentration of sulfuric acid increases, glucose yield increases. Therefore, the highest amount of glucose yielded was achieved at the highest concentration of sulfuric acid used, thus showing that concentration of sulfuric acid used in acid hydrolysis has an effect on the amount of glucose yielded. However, the glucose yield is increasing at a slowing rate. This is shown as a 10% increase in sulfuric acid concentration from 10% to 20 % led to an increase of 52.3 % increase in glucose yield when the next 10% increase only led to 23.7% increase.

By using the one way anova test, the p value of 0.000117 is obtained. Our null hypothesis was that concentration of sulfuric acid does not affect the amount of glucose yielded. This is thus proven wrong by the p-value. Our alternative hypothesis is that concentration of sulfuric acid does affect the amount of glucose yielded and this is proven right by our p - value.

3.1.2 Discussion on the factor of concentration of sulfuric acid

This is most likely because when the concentration of sulfuric acid increase, there is a greater number of moles of sulfuric acid molecules that can dissociate in water to provide H^+ ions that accelerate the rate of the condensation reaction of glycosidic bonds in the cellulose by withdrawing electron density of the atom bearing the leaving group, thus making it more susceptible to nucleophilic attack by H_2O. However, the glucose yield is increasing at a slowing rate. This might be because there is not enough processed cellulose and water to react and be catalysed by the H^+ ions at any given time, indicating that the amount of durian cellulose is the limiting indicator.

3.2.1 Factor of temperature of set-ups

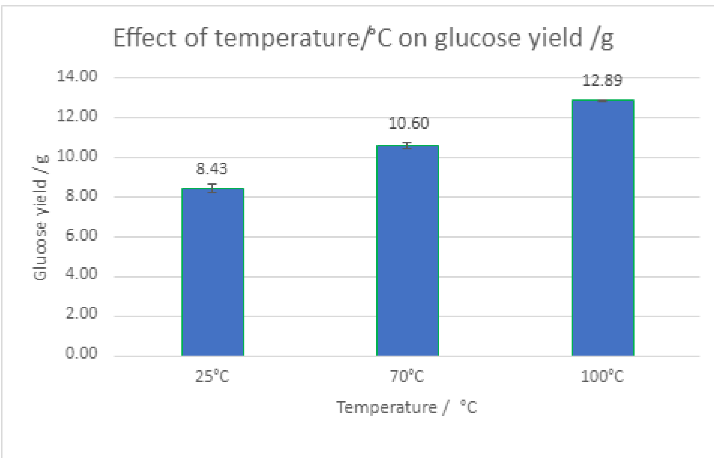


Figure 3.2: Graph showing effect of temperature of set-ups/°C on glucose yield/g

According to Figure 3.2, the bar graph shows the relationship between temperature of reaction and the yield of glucose. All the other variables were kept constant, mainly concentration of acid. Temperature change was the independent variable where the samples were heated at different temperatures, 25°C, 60°C and 100°C. The associated error bars are barely visible, showing that the standard deviation of the bar graphs are very small, thereby indicating that the results obtained were consistent. The graph shows an increasing trend, whereby when the temperature increases, the glucose yield increases. Therefore, the highest amount of glucose yielded was achieved at the highest experimental temperature, thus showing that temperature has an effect on glucose yield.

Using a one way anova test, the p-value of <0.001 was obtained. Our null hypothesis was that temperature does not affect glucose yield and this is proven wrong. Our alternative hypothesis was that temperature does affect glucose yield and there is a significant difference between the yield of glucose at different temperatures.

3.2.2 Discussion on factor of temperature of set-ups

There could be 2 reasons as to why increase in temperature may cause an increase in glucose yield. Firstly, this could be a result of temperature altering the structure of cellulose, allowing for hydronium ions (formed by H^+ ions from sulfuric acid and water molecules) to react with ester bonds in cellulose more easily. The rise in temperature also allows kinetic energy of water molecules and H^+ ions to move about faster, thus allowing increased frequency of effective collisions between them and cellulose ester bonds, thus increasing the rate of condensation reaction of glycosidic bonds of cellulose.

3.3 Limitations

As the oven temperature cannot be increased past 150°C, it was impossible to check the limits of the effect of temperature of reaction and glucose yield over this temperature, thus possibly not providing us the full picture or full trend of temperature against glucose yield.

High concentration heated at high temperatures were a safety hazard and thus could not be used, thus unable to prove trends at higher concentrations.

4. Conclusion and Recommendations for future work

Glucose was successfully synthesized from durian husks and the conclusion of temperatures and concentration of acid having a significant effect on the amount of glucose yielded can be made. More specifically, the higher the concentration of acid (up to 30%) and temperature (up to 100°C), the glucose yield increases. Although the idea of extracting glucose from fruit peels is not novel, the high cellulose content of durian husks have proven experimentation in this field to be especially prospective and efficient, while dealing also with the problem of durian waste and reducing usage of resources specifically for biofuel production.

In the future, more experimentation can be done with higher temperature and concentration of acids and bases used in the 3-step procedure to determine the exact optimal factors to achieve maximum glucose yield for effective biofuel production. It would be interesting to investigate the cost-efficiency of such a method and determine the practicality of the method compared to other fruit peels such as orange peels, or other durian types. If this method were to be used on a larger scale, further research can be conducted to show if the amount of durian used would affect the most suitable conditions.

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