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APPLICATION OF PINEAPPLE WASTE FOR BIOETHANOL PRODUCTION AS A CLEAN ENERGY SOURCE FOR PETROL-POWERED ENGINE

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ABSTRACT

Bioethanol derived from agricultural waste offers a sustainable alternative to conventional energy source such as fossil fuels as the demand for energy continues to increase and the supply depletes. In Malaysia, agricultural wastes are potential to be one of the feedstock for producing bioethanol due to low cost and readily available. The utilization of agricultural waste for bioethanol production also does not disturb the consumer food supply as it is based on waste-to-energy concept. Bioethanol produced is then blended with gasoline (petrol) to create a mixture that can be used in modern vehicles. In this study, pineapple waste has been chosen to investigate the feasibility of using pineapple waste as a substrate to produce bioethanol. Different parts of pineapple waste were treated with different acid concentrations (5, 10 and 15 %, v/v) at a constant high temperature and pressure which were later fermented for a time period of 48 hours. At the same time, one small-scale bioreactor was also fabricated to perform the fermentation of pineapple waste. It was found that the pineapple residue contained a good amount of glucose up to 455.84 g/L when 15% (v/v) H_2SO_4 used for acid hydrolysis was carried out. However, the fermentation of pineapple pulp had produced the highest bioethanol concentration (375.43 g/L) when 15% (v/v) H_2SO_4used for acid hydrolysis was performed. Bioethanol concentration obtained through the fabrication of small-scale reactor on the other hand was 374.78 g/L.

Keywords: Pineapple waste, Acid hydrolysis, Bioethanol production, Glucose consumption

INTRODUCTION

There has been an increasing interest worldwide in alternative sources of energy, due to the world's growing demand for energy and the inevitable depletion of world's energy reserve. Plus, negative environment impacts generated by burning of fossil fuels have incentivized the research and development of energy alternatives. Unlike fossil fuels, ethanol is a renewable energy source produced through fermentation of crops high in sugar or starch (Goldemberg 2007) . It is then blended with gasoline (petrol) to create a mixture that can be used in modern vehicles.

Pineapple is one of the most popular and highly grown fruits in tropical countries such as Malaysia. Pineapple waste is readily available agricultural waste which is rich in sugars, lignocellulosic components and other basic nutrients that can support yeast growth. The use of agricultural waste for bioethanol production has also the added advantage of transforming waste materials into a renewable energy source. José (Goldemberg 2007) has reported that ethanol yield could be produced from rotten pineapples waste as the substrate and the raw ethanol yield can be recommended to be used safely for petrol engine blending with pure petrol as it did not have any dangerous and higher value of element. Continuous ethanol production from pineapple cannery waste using immobilized yeast cells was studied in (Hossain and Fazliny 2010). Other researchers have used different approaches to generate ethanol using banana waste (Velásquez-Arredondo, Ruiz-Colorado, and De Oliveira 2010),(Oberoi et al. 2011; Gabhane et al. 2014; Bello et al. 2014). Life-cycle energy and environmental analysis of bioethanol production from cassava in Thailand (Papong and Malakul 2010). Production of ethanol from Carica papaya (pawpaw) agro waste: effect of saccharification and different treatments on ethanol yield (Akin-Osanaiye, Nzelibe, and Agbaji 2002). Watermelon juice is found to be a promising feedstock supplement, diluent, and nitrogen supplement for ethanol biofuel production (Fish, Bruton, and Russo, n.d.). Potential utilization of sorghum field waste for fuel ethanol production employing *Pachysolen tannophilus* and *Saccharomyces cerevisiae* was reported in (Sathesh-Prabu and Murugesan 2011).Generally, food waste are found to be valuable resource for the production of chemicals, materials and fuels, in the current situation and global perspective (Dung et al. 2014). Detailed review on how to use fruits waste for the production of ethanol for engine fuels can be found in [7-9]. In order to fill the gaps, this paper tries to investigate the feasibility of using pineapple waste to produce bioethanol; and to convert waste pineapple to biomass sugars; also to produce bioethanol from biomass sugars using yeasts. At the same time, factors that influence bioethanol production such as different fruit parts, acid concentrations used for autoclaved pre-treatment and fermentation period will be demonstrated in detail.

MATERIALS AND METHODS

In this paper, the parameters to be varied are different fruit parts (skin, pulp, mixture of skin and pulp, residue and juice), concentration of the sulphuric acid (5%, 10% and 15% v/v) and different fermentation period (24 hours and 48 hours).

Other variables of the ethanol fermentation such as temperatures, pH, amount of yeast, shaking period and different yeast strains are to be maintained constant.

Raw Materials

Getically modified and naturally grown pineapples were bought from fruit stalls from the fruit market located in Samarahan town Kuching, Malaysia. The pineapples selected included those with physical defects, as well as the fine pineapples which were then kept in the laboratory of Energy, University Malaysia Sarawak until they had fully ripen and later become rotten before being used.

Preparation of Substrates

Waste pineapples were thoroughly washed under running tap water, cut using a knife and blended using an electronic juice blender. Then they were subjected to different substrate preparations: pineapple mash (pulp, skin and mixture of both), pineapple juice after being sieved manually using folded clothes and last but not least, residue (cellulose) collected in the folded clothes after the extraction of the fruit juice. All the substrates were later dispensed into conical flasks that were labeled and covered with aluminum foil to be stored in refrigerator.

Acid Hydrolysis

The hydrolysis of pineapple waste were done using sulphuric acid (H_2SO_4). Pineapple substrates were treated with different acid concentrations (5, 10 and 15 %, v/v) in an autoclave at a constant high temperature and pressure for three hours. Hydrolyzed solutions were then filtered.

YPD Medium Preparation

YPD medium was prepared by mixing 10 grams of yeast extract, 20 grams of peptone, and 20 grams of glucose in 1 liter of water. After that, the solution was stirred, autoclaved and stored in the refrigerator.

Yeast Cultivation

Instant yeast was purchased and inoculated into YPD medium after rehydration before it was incubated at room temperature on an orbital shaker at 150rpm for 20 hours before inoculation into the fermentation medium.

Fermentation

Hydrolyzed solution of each substrate utilized was added to the YPD broth in a 250 ml conical flask. The ratio of the YPD broth to the hydrolyzed solution was maintained at 1:2. The conical flasks were then fixed on the orbital shaker with an agitation rate of 150 rpm at room temperature for 48 hours. Samples of the fermentation (about 0.5 ml) were withdrawn every 1 to 2 hours.

Glucose and Bioethanol Concentration

Glucose and bioethanol concentration were determined by using Atago digital handheld ‘pocket’ refractometer with a scale ranging between 0 and 45 % brix unit.

Bioethanol Production Using Small Reactor System

A small-scale reactor system was fabricated to perform the fermentation of the pineapple waste. Hydrolyzed solution, YPD medium which contained yeast culture and commercial sugar were added to the ferment tank and then stirred for 8 hours with the temperature maintained at 30^0C after being heated.

RESULTS AND DISCUSSION

Glucose Concentration Measurement

Table 1 shows that a difference in the glucose concentrations was obtained from the different types of acid hydrolysis substrates. The glucose concentration achieved with the use of 5 % H_2SO_4 was the highest (210.98 g/L) when the acid hydrolysis process utilized pineapple residue as the substrate, followed by pulp (200.20 g/L), mixture of pulp and skin (194.04 g/L), and lastly skin only (167.86 g/L). Pineapple juice, which was not being hydrolyzed recorded a concentration of 192.50 g/L which was higher than that of the fruit skin.

The pineapple pulp is rich in fermentable sugars and starch compared to its skin, which comprised of a large portion of lignocelluloses and a little amount of sugars. Therefore, the hydrolysis of pineapple pulp would produce a higher glucose concentration compared to the fruit skin and the mixture of both. Other than that, the number of contaminating microorganisms on the different fruit parts could lead to a difference in the glucose concentration obtained. According to (Obire and Anyanwu 2009) high fungal counts were due to the rich soil fungal flora of the field where fruits were collected and might also be attributed to the fact that the peels are the reception for all microorganisms before penetration into the pulp of the fruit.

The glucose concentrations of all substrates utilized (with the exception of pineapple juice) during the acid hydrolysis process showed an increase with regard to increasing acid concentration. For example, the glucose concentration readings for pineapple residue were increased from 27.4 g/L to 45.4 g/L and 59.2 g/L.

Table 1: Glucose Concentrations in Different Hydrolyzed Solution

Hydrolyzed Solution	Glucose Concentration (g/L)		
	5% (v/v)	5% (v/v)	5% (v/v)
Skin	167.86	329.56	397.32
Pulp	200.20	344.96	441.98
Mixture	194.04	340.34	438.90
Residue	210.98	349.58	455.84

Fermentation Output

From the perspective of smell, it was found that the fermented samples released an aroma cum with sour smell upon the removal of aluminum foil. In fact, the smell was getting stronger by the second day of fermentation compared to the first day. Also, it was observed that the fermented samples produced bubbles throughout the experiment. Figures 1-3 show the glucose consumption, as well as the bioethanol production, from all substrates utilized during the fermentation processes.

Effect of Different Fruit Parts on Ethanol Concentration

A difference in the bioethanol concentrations was obtained from different types of hydrolyzed pineapple waste substrates. It was found that the pineapple pulp had shown the best result in term of bioethanol concentration produced, which was 375.43 g/L (Figure 8). It was also found that the bioethanol concentration produced from pineapple juice was 116.24 g/L after 48 hours of direct fermentation (Figure 1). The pulp is richer in fermentable sugars and starch compared to its skin, which is comprised of 30–45 % cellulose, 20–35 % hemicellulose but no lignin and a little amount of sugars (Ruangviriyachai, 2010). In addition, the pineapple skin is prone to be contaminated by fungi and bacteria. These contaminating microorganisms would thereby compete for sugars that are available for yeast fermentation. Subsequently, smaller amount of sugars could then be converted into the bioethanol. As a result, the fermentation of pulp would produce the highest concentration of bioethanol compared to the fruit skin, the skin-pulp mixture and the cellulose residue for different acid concentrations.

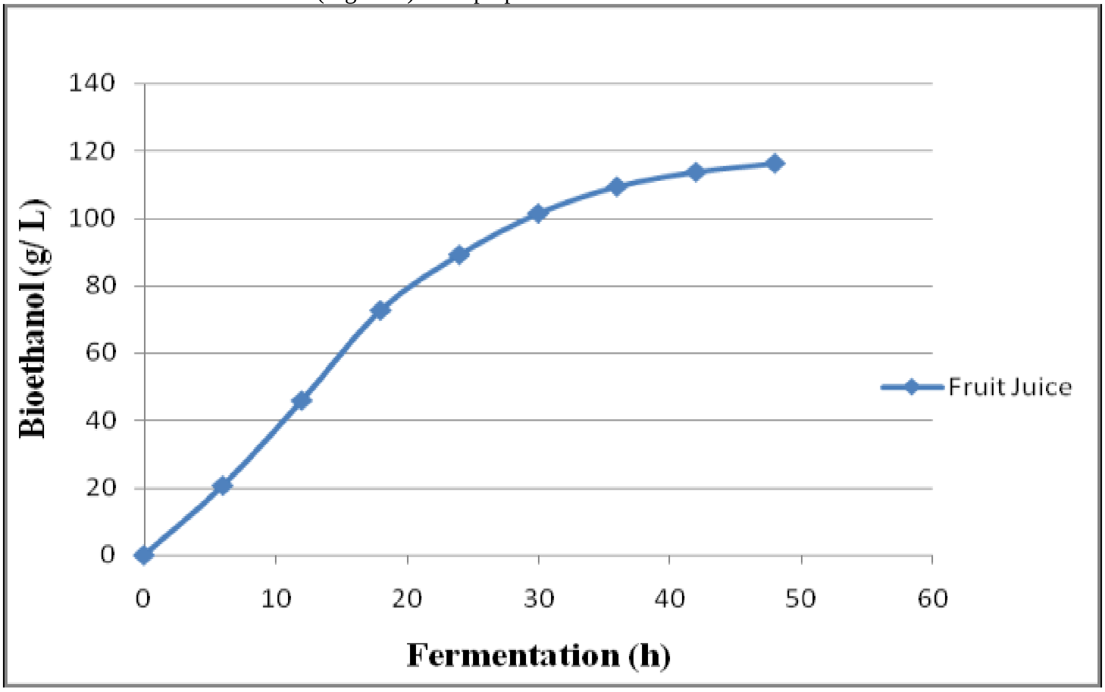

Fig. 1. Bioethanol (from Pineapple Juice) Production during Fermentation

Pineapple juice contains mostly water, as well as impurities, such as minerals, salts, acids, dirt and fiber, besides sugars. In order to be efficiently used as a raw material for ethanol production through fermentation, those impurities must be removed first. One of the ways to improve the bioethanol concentration was by submitting the fruit juice to physical and chemical treatments such as sterilizing the fruit juice high temperature to eliminate any wild yeast or treating it with either sodium or potassium metabisulphite to destroy or inhibit the growth of undesirable microorganisms such as acetic acid bacteria and moulds.

Effect of Different Acid Concentrations on Bioethanol Concentration

A difference in the bioethanol concentrations was obtained from different acid concentrations for all types of hydrolyzed pineapple waste substrates. Previously, acid hydrolysis of pineapple waste (except pineapple juice) showed an increase in glucose concentration from 5 to 15 % (v/v). The concentration of glucose was the highest for all fruit parts when a concentration of 15 % (v/v) H_2SO_4 was used. The same happened when glucose was being converted to bioethanol. Fermentation of pineapple waste at different acid concentrations showed an increase in bioethanol concentration with time. The concentration of bioethanol was the highest for all fruit parts when the concentration of 15 % (v/v) H_2SO_4 was used.

Angela and Koehler (Graf and Koehler 2000) noted that conversion efficiency is increased with the increase in acid concentration and the concentrated acids have more potential to break down the cellulose materials or the starch materials into subunits of sugars. These 6-carbon sugars are easily utilized by the yeast and converted it into ethanol. Hence, the amount of sugars released upon hydrolysis increases with the concentration of acids used in the acid hydrolysis. According to Tasi´c *et al.*(Wu et al. 2008) who had studied the acid hydrolysis of potato tuber mash in bioethanol production, the rate of hydrolysis and the maximal dextrose equivalent rose with increasing acid concentration, probably due to the increase in the activity of hydrogen ions participating in the reaction as catalyst.

Effect of Fermentation Time on Glucose and Bioethanol Concentration

Time duration was set from 0 to 48 hours. Figure 2 shows the consumption pattern of the glucose during the fermentation process of pineapple skin at three different acid concentrations. Initial concentration of glucose was 167.86 g/L for 5 % (v/v) H_2SO_4 of fermentation sample. There were

63.21 g/L and 9.0 g/L of glucose after 24 and 42 hours of fermentation respectively. It was found that as the fermentation period approached 48 hours, there was little glucose consumption and eventually the concentration of glucose was reduced to 0 g/L at the end of fermentation.

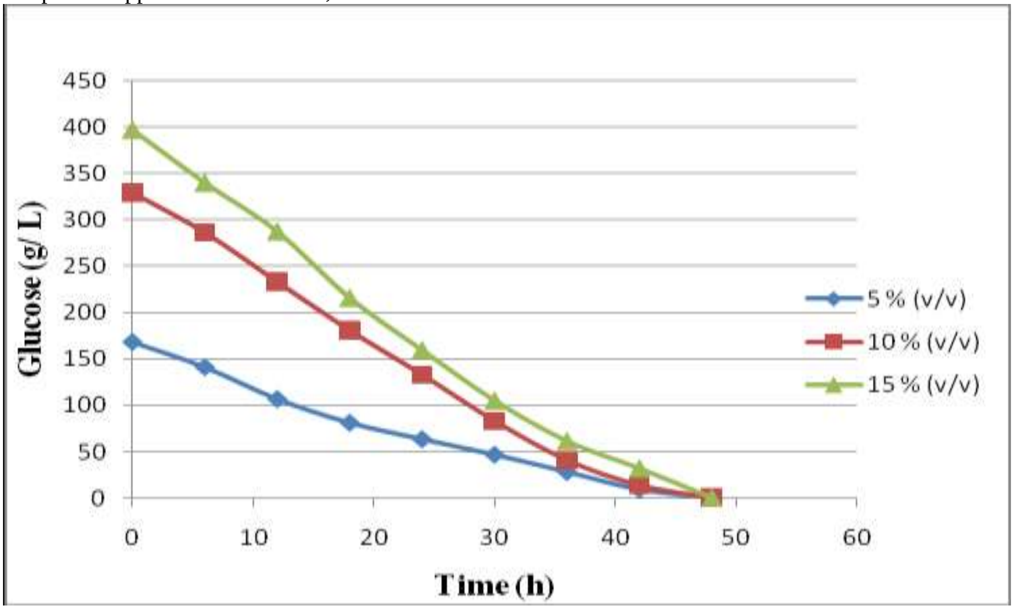

Fig. 2. Glucose (from Pineapple Skin with Different Acid Concentrations) Consumption during Fermentation

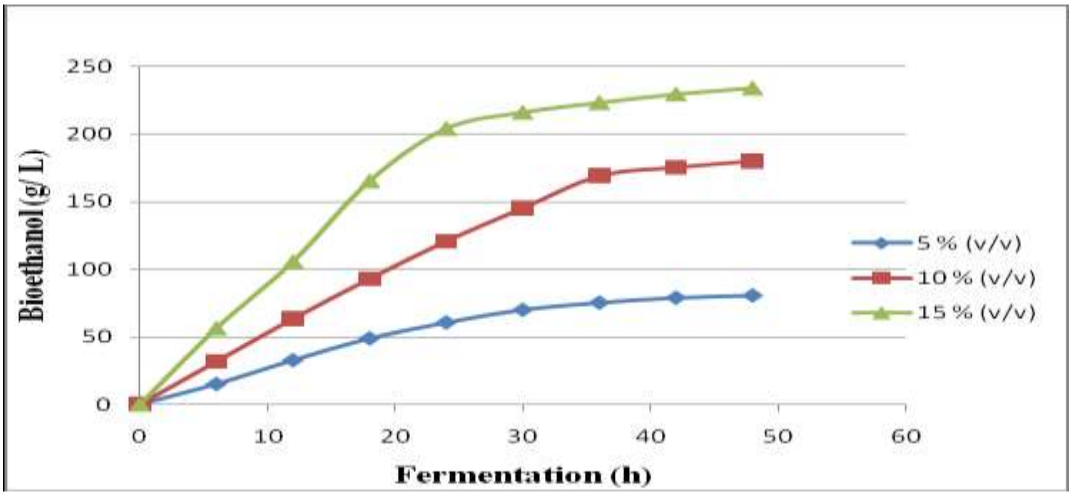

Fig.3. Bioethanol (from Pineapple Skin with Different Acid Concentrations) Production during Fermentation

Figure 3 shows that while the concentration of glucose decreased, bioethanol in the product increased from 0 to 32.65 g/L and 80.48 g/L respectively from5 % (v/v) H_2SO_4 of pineapple skin fermentation after 12 hours and 48 hours of fermentation. The decreasing trend of glucose concentration and increasing trend of bioethanol concentration were observed in all fermented samples, regardless of acid concentrations and fruit parts. This means that the reaction worked in the correct way. During fermentation, the yeast rapidly consumed the glucose, especially in the exponential phase as a source of carbon and energy. Above a certain level their amount began to deplete and the rate of conversion was slowed down. At the mean time, the glucose was converted to bioethanol as a result.

In addition, it was found that fermentation period affected the concentration of bioethanol as the longer the fermentation period was, the higher the concentration of the bioethanol produced. However, if the fermentation period was too long, nutrients and simple sugars would deplete and yeasts could no longer function.

Bioethanol Production Using Small Reactor System

Assuming all the sugar contents in the feedstock was 100% converted into sugar during hydrolysis and there was no sugar loss during the experiment, the bioethanol concentration was found to be 374.78 g/L. This is considered more efficient than the fermentation processes done without reactor system for the highest bioethanol concentration obtained after 48 hours of fermentation was 375.43 g/L using pineapple pulp as the substrate. The bioethanol concentration produced using the reactor system could have been higher if the fermentation period was pro-longed. The bioethanol collected still needs to undergo another distillation process before it can practically be used as a renewable energy source. Figure 4 shows a sample of collected bioethanol produced using small reactor system.

Fig. 4: Slurry Collected from the Reactor System

CONCLUSIONS

This paper reported the potential of using pineapple waste for the production of bioethanol. The experimental steps were to investigate the feasibility of using pineapple waste to produce bioethanol by evaluating the waste to ensure the availability of fermentable sugars needed to support the growth and consequently the production of bioethanol by using yeast. Other than that, experiments were performed to evaluate a few parameters that were taught to influence the bioethanol production using pineapple waste.

The results indicate that the amount of glucose present in the waste was sufficient for undergoing fermentation. Since the significant factors were different fruit parts, acid concentrations used for autoclaved pretreatment and fermentation period, experiments were carried out to study in detail the correlation between bioethanol concentration and these factors. The results, thereby revealed that that there is a fairly strong correlation between sulphuric acid concentration and bioethanol concentration. As the sulphuric acid concentrations were increased, the bioethanol concentrations also increased. For example, bioethanol production from the pineapple skin was increased from 80.48 g/L to 179.89 g/L and 234.33 g/L when 5 % (v/v) of sulphuric acid used was increased to 10 and 15 % (v/v). The bioethanol concentrations were also very affected by the fermentation time. Bioethanol production increased when the fermentation time was increased until it gradually stopped around 48-hours. Meanwhile, an increase in the fermentation period resulted in a decline in glucose concentration until it completely dropped to zero value at the 48th hour. As for the study of different fruit parts, different sugar contents (glucose) obtained results in a difference in bioethanol production. Fermentation of pineapple pulp at 15 % (v/v) sulphuric acid was found to show the highest bioethanol concentration, which was 375.43 g/L.

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