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Comparative Study of Bioethanol Production from Waste Banana Fruits and Grape Fruits

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Petroleum consumption in the transportation sector causes severe damage to the environment. Bioethanol is used as a biofuel or fuel additive because of its properties that give clean burning, reducing air pollution and avoiding global warming. Still, Sri Lanka depends on petroleum fuel. As a result, Sri Lanka generates 270000 tons of fruit waste per year, including bananas, and it is time to investigate alternatives to fossil fuel. It would diminish environmental pollution and reduce the dependence on imported fossil fuels. In present study, the waste of bananas and grapes is used to produce bioethanol using *S. cerevisiae* (Baker's yeast). Temperatures of 30 ºC and pH 5.0 were maintained in the fermentation medium. This study shows that waste grapes have a higher ethanol concentration, 6.08% greater than bananas at 5.11%. The grape and banana ethanol yields are 46.77 g/L and 39.46 g/L, and the specific gravity shows 0.871 and 0.882, respectively.

Keywords: Bioethanol, Biofuel, Fermentation, Specific gravity.

INTRODUCTION

Nowadays, the world tent to use gasoline on a large scale for the transportation sector and industrial energy sources conventionally. Excessive use of fossil fuels generates harmful effects on the earth, mainly they affect to increase of global warming and grotesque climate change. Furthermore, due to the rapid consumption of conventional fossil fuels and their unpredictable demand, gasoline prices increase day by bay perishing to developing countries. Therefore, the world needs cleaner and renewable alternatives such as bioethanol and biodiesel, overcoming the conventional fossil fuels [1].

Overcoming the above challenges, researchers tend to produce biofuels that have a significant advantage over conventional fuels. Bioethanol is an ecologically sustainable biofuel having a higher-octane rate and clean and proper burning quality, improving the environment's air quality [2]. Bioethanol is renewable energy produced by fermentation of renewable sources like agricultural feedstocks, and it is used as biofuel or fuel additives in the transportation sector. Ethanol or ethyl alcohol is a volatile, colourless, flammable liquid biofuel with a molar mass of 46.07 g/mol, a density of 0.789 g/mL, a melting point of -114 ºC and a boiling point of 78.37 ºC. Likely most significant single ethanol is used as a crucial industrial ingredient, base chemical for organic compounds, antibacterial hand sanitizer and medical wipes; bioethanol can also be used [3]. World ethanol production for transport fuel is raising daily and mostly, bioethanol production is being done by several countries such as USA, Brazil, Ukraine, Pakistan, South Africa and some countries in Asia [4,5].

The result of reducing CO_2 emission and improving air quality, bioethanol is aimed to blend up to 5-20% with convention gasoline increasing octane number of blended fuels. Common ethanol blending ratios are E5 (5% ethanol and 95% gasoline), E10 (10% ethanol and 90% gasoline). This blending requires no engine modification is another advantage [6]. According to the researchers, bioethanol's important qualities make it suitable as renewable energy having ecofriendly combustion against fossil fuel. Bioethanol has high oxygen content (35% w/w). It helps to reduce hydrocarbon and CO emissions after burning. Bioethanol also has high octane number (107) and high latent heat of vaporization (0.912 MJ/kg) that prevents premature ignition and cylinder knocking and occurring spontaneous ignition in the engine. It also has low energy content (21.2 MJ/dm^3) by increasing compression ratio and power, decreasing burning time [7-10].

However, because the world consumes gasoline at many large scales of 95 million barrels per day, replacing bioethanol with conventional petrol is still a challenge. It is proposed to use crops such as oilseeds, wheat or rice starch and sugar canes as feedstock for bioethanol production is no longer agree because of the availability of feedstocks at that much scale. Furthermore, the use of convenience foods in the human food chain could lead to food arrear. However, the use of agro-residues, forest residues municipal wastes, *etc*. limit the amount of bioethanol production. Therefore, bioethanol production needs the most trust continuous and cheap feedstock consumed on a large scale to overcome fossil fuels. When exploring the above facts use of waste fruits and vegetables for bioethanol production is a much suitable and cheap method. Fruit contain sugars, and every fruit generates 50% of its weight as waste could be an as good source for bioethanol feedstock. The use of waste fruits may tend to solve the problem of waste management [1]. Also, using a comparatively cheaper feedstock of raw materials using efficient fermentative microorganisms is the possible way to get bioethanol demand when it is commercialized in the present energy crisis [11].

Still, in 2020, Sri Lanka has to fill their fuel requirement with imported fossil fuel. Due to the lack of large-scale agricultural land Sri Lanka cannot produce first-generation bioethanol. However, it has been estimated 270000 tons of harvested fruits (about 30% of banana, 46% of papaya, 18% of pineapple, 40% of lime, 41% of avocado and 90% of jackfruits) are wasted annually due to improper management of packaging, handling transportation and storage. Therefore, there is a big chance to study the possibility of producing bioethanol using fruit wastes as a possible fuel substitute [12]. According to Salam *et al*. [13] there are different types of waste fruits and different parts of the fruit can be used as energy sources for ethanol production. Apple juice is one of the typical drinks and a large amount of waste apple is generated by industries. Around 134g/Kg of ethanol can be produced using apple pomace. Banana occupies the 4th world rank and mango is the 5th rank in field production. It generates a significant amount of waste due to less management of harvesting, transporting, marketing and processing steps. Banana and mango peels, respectively, obtained a maximum ethanol yield of 13.84% and 19.98%. Also, citrus peels (~15.6-19.17% of ethanol) and date palm fruits (~19.7% ethanol) can be used as the energy source for bioethanol production [13]. Overripe grape waste can also be used as a substrate for ethanol production because grapes contain a naturally high sugar content level of around 26 g in 100 g of grape juice. It is higher than the natural sugar content in maize and beet-root [14]. Banana fruits and their associated residual biomass can convert into ethanol under the fermentation and distillation process, and also banana peel contains a low level of lignin, which could be a suitable substrate for the production of ethanol. The mixture of skin and pulp of rotten fruits is served as an energy source for ethanol production because it involve reducing initial production cost [15]. In this study, comparatively evaluate ethanol yield of waste banana and grape sample.

The typical way of bioethanol production is alcoholic fermentation of reducing sugars, which biological process by a microorganism that covers simple sugar into ethanol, releasing CO_2 and other byproducts. Low generation time, easy to operate downstream processes, low maintenance cost and ease of handling are significant advantages in the biological production of ethanol [16,17]. Basic steps in microbial fermentation; making fermentable sugars (hexoses and pentoses), fermentation of sugars by microorganisms under optimum conditions and purification of produced ethanol are usually achieved by distillation techniques [18]. Waste fruits, also known as first generation bioethanol feedstock which is the richest source of fermentable sugars [19]. In this study, *Saccharomyces cerevisiae* (Baker's yeast), which typical microorganism used in industrial ethanol production, is used to produce bioethanol from the waste of bananas and grapefruits. *S. cerevisiae* grows in high sugar concentration and produces a high yield of ethanol by providing an enzyme named zymase that converts the sugars present in the medium to produce ethanol [20]. The optimum conditions *viz*. pH of the medium (5.0), temperature (30 ºC) for the growth of *S. cerevisiae* and highest yield of ethanol were maintained.

EXPERIMENTAL

Preparation of samples: A fully ripened banana sample was collected from a local market in Gothatuwa town, Rajagiriya, Sri Lanka. A fully ripened spoiled grapefruits sample was collected from the market in Jaffna, Sri Lanka. From each fruit's samples (banana and grape), 250 g was weighed into different beakers and fruit samples were washed with 5% $KMNO_4$; after that samples were rinsed twice with distilled water separately.

Then, each 250 g of fruits samples were ground using a home grinding blender with the peels. Then ground fruits pulp was transferred into a 1000 mL Erlenmeyer flask and 300 mL of distilled water was added to dissolve the mixture pulp. The mixture was boiled for 15 min while stirring. After that the mixture was allowed to cool down to room temperature while stirring. After inoculum was added into the treated fruit sample, the Erlenmeyer flask was made upto the volume to 1 L with distilled water and the pH of the medium was adjusted to 5.0 pH using sulfuric acid. The duplicate sample was run for each banana and grape sample.

Inoculum preparation: About 100 mL of warm distilled water was added into another beaker. Sucrose (50 g) and Baker's yeast (10 g) was added into warmed distilled water. This mixture was added to the treated fruit mixture in the Erlenmeyer flask. Then the mixture was shaked well and made up the final volume up to 1000 mL by adding distilled water.

Fermentation process: The general fermentation process is a reaction in a dark, airtight bottle containing fruit content and yeast inoculum. After yeast injection into fruit content in the Erlenmeyer flask, the whole mixture was transferred into 2.5 L of a dark bottle with a small cap. Before the mixture was added into the dark bottle, about 10 mL of sample was taken and allowed to settle down the sediment for subsequent determination of the initial specific gravity of the sample and finally the bottleneck was sealed. The flexible tube was placed through the airtight. Another end of the tube was sunk in a water bath to release the CO_2 produced from microorganisms resulting in an anaerobic condition in the medium. The fermentation mixtures were placed at room temperature (30 ºC) for 7 days of the incubation period. After every 12 h, the specific gravity of medium was determined to ensure the production of ethanol and determine the end of ethanol production in the medium. Suppose the fermentation process is kept in the medium. In that case, the specific gravity of the medium reduces gradually, and when the fermentation process has no longer occurred in the medium, the specific gravity becomes constant.

Recovery of the product: After 7 days of the fermentation process, the fermented mixtures of waste banana and grapefruits were filtered using a beaker covered with a piece of folded muslin cloth separately. The filtrate was the liquid raw bioethanol. The concentration of liquid raw bioethanol was determined using the specific gravity method. Then rest of the whole sample was distilled using a lab distiller to collect the concentrated bioethanol. In this study, double distillation was followed to get more concentrated ethanol and the distillation was performed in a distillation assembly for about 4-6 h. The distillation process was optimized to obtain the maximum percentage of bioethanol in the final product. After the distillation process, the ethanol assay was done.

Estimation of specific gravity: The difference between the specific gravity of fruit juice before and after fermentation demonstrate the amount of ethanol produced.

Determination of ethanol concentration: The final distillate of ethanol sample was examined by the Chemical and Microbiological Laboratory, Industrial Technology Institute, Sri Lanka. The gas chromatography-FID technique was used to determine the concentration of ethanol using flame ionization detection technique (Reference No: SS2109903).

RESULTS AND DISCUSSION

In this study, ethanol was produced successfully using different fruit wastes such as banana and grape. Therefore, the comparative study has been carried out to compare the ethanol yield of each fruit's samples.

In fermentation, process temperature plays a significant role because when the temperature of the fermentation medium increases, the rate of alcoholic fermentation increases. According to the literature, optimum temperature ranges between 25 ºC to 40 ºC. When the temperature goes below the optimum range, the reaction rate will be decreased. At a high temperature above 40 ºC, most enzymes involved in the ethanol production pathway in microorganisms can denature or unfold. At the lower temperature, the rate of enzyme activity decreases usually. This study was done at room temperature 30 ºC to achieve the optimum yield of ethanol.

Yeast (*Saccharomyces cerevisiae*) survives in the slightly acidic medium to occur their metabolic pathway in optimum conditions. The optimum pH to achieve the maximum ethanol yield ranges from pH 4 to 6. According to the literature, a gradual increase in ethanol concentration from 4-6 of pH in the medium was shown. Beyond the optimum range, the rate of alcoholic fermentation decreases [18]. This study maintained the pH of both banana and grape fermentation medium at pH 5.

The specific gravity method was used to get an approximate value of the ethanol concentration produced in the medium. As the fermentation progressed, the specific gravity of medium considerably decreased and it indicated sugar fermentation producing ethanol. The specific gravity reaches the constant value after the incubation period, indicating the end of the fermentation process.

Table-1 shows the specific gravity values of both banana and grape samples after the fermentation process; 0.882 and 0.871, respectively. Using the specific gravity method, the concentration of ethanol produced in the medium was determined. It is shown as a percentage; the grape sample achieves an ethanol concentration 6.08% greater than banana 5.11%. This result shows that the over-ripened grapefruits contain more fermentable sugars than those in the over-ripened banana fruits. Janani *et al*. [21] also reported that the grapes waste sample achieved a higher ethanol yield among the other waste fruit samples; banana, apple and papaya. Similarly, Chitranshi & Kapoor [14] also reported that grape sample achieved 5.23% of ethanol concentration with room temperature 30 ºC and pH 4 in the fermentation medium.

When considering the specific gravities and concentrations of both grape and banana samples, the values are slightly lower than the values reported by Janani *et al*. [21]. The rate of shaking or agitation of the fermentation medium controls the entry of nutrients from the fermentation medium into cells.

The removal of ethanol produced in the cell to medium also depends on the shaking rate. The higher rate of simulation of nutrients and ethanol by shaking results in higher ethanol production and the typical agitating speed used in the experiment is 150-200 rpm [22]. However, it is not advisable to use an excessive rotation rate to reduce the cell's metabolic activity. Therefore, the production of ethanol is advisable with smooth agitation. In this study, continuous agitation was not given to the medium; thus, the rate of the alcoholic fermentation may be slow down resulting low level of ethanol concentration.

A distillation technique was used in this study to separate the produced ethanol from the medium. Using a higher grade of distillation assembly, a more concentrated product was achieved. After double distillation of the samples, 78 mL of distillate with 76% (v/v) ethanol concentration was obtained from the wastes grape sample. And 68.5 mL of distillate with 73% (v/v) concentration was obtained from the waste banana sample. The ethanol yield of waste banana and waste grape samples was calculated using ethanol concentrations values determined by gas chromatography (FID). Ethanol yield obtained by banana and grape; 39.46 g/L and 46.77 g/L, respectively. This result also shows that higher fermentable sugar contains in the grape sample than in the banana sample.

TABLE-1
SPECIFIC GRAVITY AND CONCENTRATION OF BIOETHANOL FROM WASTE SAMPLES OF BANANA AND GRAPE

Fruit sample	pH of the fermentation medium	Temperature of the fermentation medium (°C)	Specific gravity	Concentration of ethanol (%)
Banana sample	5	30	0.882	5.11
Grape sample	5	30	0.871	6.08

After the filtration step, the solid part of the waste sample can be used as the organic fertilizer for plants. This research followed the procedure to produce ethanol from fruit samples with less usage of chemicals. Therefore, there is no need for any detoxification steps before the solid part is released into the environment.

Conclusion

The massive utilization of fuel ethanol as an alternative for conventional fossil fuel requires that its production technology must be cost-effective, sustainable, renewable and environmentally friendly. A considerable amount of waste fruits generates by agricultural fields and industrial fields. The use of waste fruits as the feedstock reduces the production cost of the process and rice the demand for bioethanol. This study shows that waste banana and waste grape can serve as raw materials for ethanol production. About 6.08% of ethanol concentration was obtained from the grape sample. The banana sample obtained less amount of ethanol than grape is 5.11%. Ripened grapefruits contain more fermentable sugars than other fruits. Improving the pretreatment and hydrolysis methods to convert lignocellulosic materials into ethanol provides more yield and benefits.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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