A STUDY ON BIO-ETHANOL EXTRACTION FROM FRUIT WASTE BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION

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ABSTRACT

Fruit waste could be used for producing ethanol in an efficient way by means of mashing specialized enzymes. In this paper fruit waste of oranges, apples and tangerines was processed simultaneously by saccharification and fermentation in a laboratory-scale. The enzymes used for this study are commonly-found C-Tec2 and H-Tec and Wild-type Saccharomyces Cerevisiae was the yeast used for ethanol fermentation. The experimental analysis was conducted using the 1260 Infinity Quaternary LC System® of the Agilent Technologies Co. The experiment results showed the glucose and ethanol production from oranges, apples, and tangerines it could be concluded that most of fruits showed decreasing trends of the glucose production and increasing trends of the ethanol production in 2 hours. After twenty four of incubation the glucose produced from fruits was mostly converted to ethanol. The study results suggest that fruit waste could be utilized as economically- as well as environment-friendly substrate by simultaneous saccharification and fermentation.

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1. INTRODUCTION

Since the industrial revolution in the mid-18th century, fossil fuels have driven a great part of human society. However, because of air pollution and global warming [1], extensive researches on alternative energy source have been continuously in progress. Researches on biomass, geothermal and wind power would be good examples of them.

Biomass is the biological material derived from living, or recently living organisms. It mostly-often refers to plants or plant-based materials, which are specifically called lignocellulosic biomass. Biomass as an energy source can be used either directly via combustion to produce heat, or indirectly after converting it to various forms of biofuels such as methanol, ethanol, methane gas, hydrogen gas or electricity [2]. Among them bio-ethanol (biologically-driven ethanol) has drawn more attention than others especially as an alternative energy source for transportation.

Bio-ethanol is produced from starch plants that contain sucrose such as sugar cane, corn, wheat, potato and fruits by biological procedures. Leading countries of bio-ethanol technologies such as USA and Brazil are expanding bio-ethanol markets by large-scale cultivation of corn and sugar cane. However, in Korea, farmland is insufficient for large-scale cultivation, which has seemingly been one of the main causes to reduce the stakeholder’s interest in the relevant studies on using various biomasses [3].

Recently studies on extracting bio-ethanol from fruit waste are getting attraction especially in the developing countries of low income [4-9]. In Korea annual throughput of fruit waste from the domestic wholesale markets amounts to 131,746 tons, which costs ca. 5.5 billion Korean Won (ca. 5.5 million USD) for disposal [5]. The use of fruit waste as biomass would give great advantages considering the shortage of energy resources and increasing disposal costs.

In this paper, the author will introduce a study result on bio-ethanol extraction using selected fruit waste. The fruits used are orange, apple and tangerine and the extraction was made on the basis of simultaneous saccharification and fermentation procedure. The author expects the result could suggest a better alternative of fruit waste utilization solving environment and energy problem at the same time.
2. MATERIALS AND METHODS

2.1. Materials

2.1.1 Fruits

The fruits used in this experiment are orange, apple and tangerine. They show relatively high amounts of crop and consumption in Korea and accordingly it is expected that their wastes take high percentage numbers. The compositional characters of the fruits used are shown in the Table 1. The data were measured in 100g of each fruit.

<table>
<thead>
<tr>
<th>Content</th>
<th>Species</th>
<th>Orange</th>
<th>Apple</th>
<th>Tangerine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water (%)</td>
<td></td>
<td>86.84</td>
<td>86.67</td>
<td>85.14</td>
</tr>
<tr>
<td>Carbohydrates (g)</td>
<td></td>
<td>11.89</td>
<td>12.76</td>
<td>13.34</td>
</tr>
<tr>
<td>Cellulose (g)</td>
<td></td>
<td>2.5</td>
<td>1.3</td>
<td>1.8</td>
</tr>
</tbody>
</table>

2.1.2. Saccharomyces Cerevisiae (S. Cerevisiae)

The Saccharomyces Cerevisiae is the yeast that produces ethanol. Wild-type Saccharomyces Cerevisiae yeast was cultured for this study in the Korea Research Institute of Bioscience & Biotechnology (KRIBB). The cultivation was made at 30 °C for 48 hours in a shaking incubator (170 rpm).

2.1.3. Cellulase

The cellulase is the enzyme that decomposes glucose into monosaccharide. C-Tec2 and H-Tec known as common enzymes were used for this study.

2.1.4. Basic Broth Medium

The Yeast extract Peptone Dextrose (YDP) broth was used as the basic broth medium for seed inoculation of S. Cerevisiae. The YDP broth medium is composed of 10 g/L yeast extract, 20 g/L peptone, and 20 g/L glucose.

2.1.5. Minimum Broth Medium

Minimum broth medium was used for ethanol production of S. Cerevisiae. The minimum broth medium is composed of 5 g/L yeast extract, 5 g/L peptone, 0.4 g/L MgSO₄, and 5 g/L KH₂PO₄.
2.2. Methods

2.2.1. Pretreatment of Fruits

10 g of fruit peels and fruits in total were ground into the size of 0.420 mm (40 standard mesh; Sigma-Aldrich) and dried in 85 °C for 24 hours using a drying oven (Figure 1). The dried ground fruits were then pasteurized for 10 minutes at 120 °C using an autoclave.

Figure 1: It is showing the pre-processed fruits

2.2.2. Yeast Culture

*S. Cerevisiae* was inoculated on the YDP broth medium in a shaking incubator in the following condition: 48 hours, 30 °C, pH 5.5 and 170 rpm. 180 ml of cultured *S. Cerevisiae* were used for ethanol fermentation.

2.2.3. Ethanol Fermentation of Fruits

To shorten the ethanol fermentation time with *S. Cerevisiae* and mashing by C-Tec2 and H-Tec, the saccharification and fermentation processes were used simultaneously. A 100 ml-solution, which is composed of 30 % *S. Cerevisiae* culture fluid and 70 % minimum broth medium, 10 g of fruit peels and ground fruits, 100 μl C-Tec2 and H-Tec are mixed together in the triangle flask. The composition was placed in the shaking incubator in 35 °C and 100 rpm condition.
2.3 Analyzing Method

2.3.1 High Pressure Liquid Chromatography (HPLC)

A 1260 Infinity Quaternary LC System® (Agilent Technologies Co.) was used for HPLC to analyze the sample (Figure 3) with 0.005 M of H$_2$SO$_4$ and a column for sugar analysis. The samples were passed through microdisk filters and collected in 2 hours, 8 hours and 24 hours (Figure 4).

Figure 2: It is showing the fruit samples on a shaking incubator

Figure 3: It is showing the 1260 Infinity Quaternary LC System for High Pressure Liquid Chromatography (HPLC)

Figure 4: It is showing the micro disk filters used for this study
3. RESULTS AND DISCUSSION

3.1. Glucose Produced by Enzymes from Fruits

The amount of the glucose production by various fruits is shown in Figure 5. In two-hour incubation time, glucose was conspicuously produced from all types of fruit waste. The orange showed the highest value followed by the apple. After eight-hour incubation most of the glucose production was drastically decreased and within twenty four hours the glucose production in all kinds of fruits seemed deceased.

It is noteworthy that the orange and the apple showed the highest speeds of glucose production whereas the orange peel itself showed a relatively-slow but long-lasting trend. In addition the tangerine showed relatively low value (less than a half of the highest) compared to the other fruits. Considering the compositional characters of the fruits (Table 1) the result implies different procedure for saccharification would need to be applied to different types of fruit waste in order to achieve stable glucose production.
3.2. Ethanol produced by S. Cerevisiae from Fruits

The amount of the ethanol produced by S. Cerevisiae is in Figure 6. In two-hour incubation time, all fruits started to produce ethanol. In eight hours, apple, apple peels and tangerine peels showed the biggest amount of the ethanol production, whereas the orange peel showed the smallest. In twenty four hours, apple showed the highest ethanol production. Tangerine and tangerine peels showed no prominent differences during eight-hour incubation time.

Unlike the case of glucose production the ethanol production seems more diversified in speed depending on the types of fruit waste. In addition the orange and tangerine do not show different aspects of ethanol production whether the whole or the peel of the fruit is incubated. Considering the compositional characters of the fruits (Table 1) the main factor of various ethanol production rates seems governed by the composition of cellulose as seen the slight difference of ethanol production values between in apple as a whole and in apple peel (Figure 6).

![Figure 6: It is showing the amount of the ethanol produced by S. Cerevisiae along with the incubation time](image)

4. CONCLUSIONS

In this study, an experiment about ethanol production from orange, apple and tangerine with common enzymes was performed. The simultaneous saccharification and
fermentation process by C-Tec2 and H-Tec was also performed in order to produce high-density glucose and to shorten the time of ethanol production.

According to the result the glucose and ethanol production from oranges, apples, and tangerines it could be concluded that most of fruits showed decreasing trends of the glucose production and increasing trends of the ethanol production in 2 hours. After twenty four of incubation the glucose produced from fruits was mostly converted to ethanol. These results suggest the possibility of using fruit waste as a substrate for simultaneous saccharification and fermentation, which could be applied for bio-fuel development in a short period in an economically efficient as well as in an environment-friendly ways.

Despite the confirmed possibility of bio-fuel production using fruit waste it is suggested that more customized procedure depending on the types of fruit waste should be developed and applied. The production speeds of glucose and ethanol show different values among the fruits and their status of wasting. This situation would become more critical issue when large-scale production is demanded to be developed.

5. ACKNOWLEDGEMENTS

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6. REFERENCES


