Experimental study of bioethanol production using mixed cassava and durian seed

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Abstract. The production of biofuels using conventional fermentation feedstocks, such as sugar- and starch-based agricultural crops will in the long-term lead to a serious competition with human-animal food consumption. To avoid this competition, it is important to explore various alternative feedstocks especially those from inedible waste materials. Potentially, fruit wastes such as damaged fruits, peels and seeds represent alternative cheap feedstocks for biofuel production. In this work, an experimental study was conducted on ethanol production using mixed cassava and durian seeds through fermentation by \textit{Saccharomyces cerevisiae} yeast. The effects of pH, temperature and ratio of hydrolyzed cassava to durian seeds on the ethanol yield, substrate consumption and product formation rates were analyzed in the study. In flask-scale fermentation using the mixed cassava-durian seeds, it was found that the highest ethanol yield of 45.9\% and a final ethanol concentration of 24.92 g/L were achieved at pH 5.0, temperature 35{}^\circ\mathrm{C} and 50:50 volume ratio of hydrolyzed cassava to durian seeds for a batch period of 48 hours. Additionally, the ethanol, glucose and biomass concentration profiles in a lab-scale bioreactor were examined for the fermentation using the proposed materials under the flask-scale optimum conditions. The ethanol yield of 35.7\% and a final ethanol concentration of 14.61 g/L were obtained over a period of 46 hours where the glucose was almost fully consumed. It is worth noting that both pH and temperature have significant impacts on the fermentation process using the mixed cassava-durian seeds.

1. Introduction

Biofuels in general and bioethanol in particular have taken the stage in addressing energy security problem, decreasing fossil fuel dependency and most importantly in reducing greenhouse-gas (GHG) emissions. Bioethanol can be produced by fermenting sugars from biomasses and used as fuel for vehicular engine internal combustion [1]. Currently, most of the conventional ethanol fermentation feedstocks are relying on agricultural crops, such as starch-based crops and sugar-based crops. However, such a heavy reliance on agricultural crops will one day lead to a serious competition with the demand for livestock feed and human food consumption [2]. Interestingly, abundant agricultural wastes, such as lignocellulosic materials has been proposed to relieve the fuel versus food competition problems. However, the use of this kind of materials presently still requires costly pretreatment to decompose complex lignin structures predominantly encountered in the wastes [3]. For this reason, it is important to explore various other alternative feedstocks from inedible waste materials. Fruit wastes, e.g., damaged fruits, peels and seeds, represent a potential cheap alternative to be used as feedstocks for biofuel production.
In some tropical countries like Malaysia, durian (*Durio zibethinus*) seed is regarded as agricultural residue which accounts for about 20-25% of the total fruit mass [4]. Note that, durian seeds are rich source of carbohydrates in the form of starch, with the floured and peeled floured forms of the seed containing 73.9% and 76.8% of carbohydrates, respectively [5]. Meanwhile, fresh durian seeds contain high moisture (51.5%) and carbohydrate (43.6%) [6]. In this paper, an experimental study was conducted on ethanol production using mixed cassava and durian seed through fermentation by *Saccharomyces cerevisiae* yeast.

In the flask-scale fermentation, the effects of pH, temperature and ratio of hydrolyzed cassava to durian seeds on the ethanol yield, substrate consumption and product formation rates were analyzed. Enzyme-catalysed reactions are highly sensitive to changes in temperature [7]. According to [8, 9], the optimum temperature of saccharifying enzymes for fermentation and microbial growth is in the range of 25-35°C. Additionally, it has been widely recognized that the cultivation pH plays an important role in ethanol production [10]. *Saccharomyces cerevisiae* is an acidophilic organism by nature where the optimal range for this microorganism to grow lies between pH 4 and 6 [11]. A constant yeast intracellular pH has to be maintained at its optimum pH range. Maintaining the optimal intracellular pH is crucial as many pH-dependent enzymes are operating within the yeast cell during growth and metabolism [12]. In the present work, we also conducted the fermentation in a lab-scale bioreactor where the ethanol, glucose and biomass concentration profiles were studied using the proposed materials under the optimum conditions obtained from the flask-scale study. The lab-scale study was conducted in addition to the flask-scale study in order to gain a better understanding of the fermentation process using the complex mixed cassava-durian seeds.

2. Materials and methods

Hydrolyzed cassava and durian seeds were used as a carbon source for the fermentation process. The flask-scale fermentation was run for a period of 48 hours. Meanwhile, the lab-scale bioreactor experiment was executed in batch mode by using a BIOSTAT A-plus 2 L, MO-Assembly bioreactor, which was run for a period of about 48 hours until the substrate was used up.

2.1. Preparation of durian seeds

The durian seeds were washed to remove traces of durian fruit and slime and dried in dehydrator to remove water content within the seeds. Dried durian seeds were milled using a pulveriser.

2.2. Medium preparation

First, 100 g of cassava powder was mixed with 1 L of 0.5 mol/L H₂SO₄ solution. Meanwhile, 100 g of milled durian seeds was mixed with 1 L of 0.5 mol/L H₂SO₄ solution. Then, the cassava medium culture and durian seed medium culture were cooked at 121°C for 20 minutes in order to break down the starch into fermentable sugars. For the flask-scale fermentation, 200 mL of medium culture was prepared. The mixed medium cultures were prepared based on three ratios of hydrolysed cassava to durian seed where in this research the ratios: 85:15, 75:25 and 50:50. 1 g/L yeast extract, 2.5 g/L NH₄Cl, 2.91 g/L Na₂HPO₄, 3 g/L KH₂PO₄, 0.25 g/L MgSO₄, 0.08 g/L CaCl₂, 4.3 g/L citric acid and 3 g/L sodium citrate were dissolved in the mixed medium culture [13]. The medium culture was adjusted to desired pH by using solutions of 1 mol/L of NaOH and 0.5 mol/L of H₂SO₄. After adjusting the pH to a desired value, the medium culture was sterilized at 121°C for 20 minutes to avoid contamination and then cooled down to room temperature. Note that, the procedure for preparing 1.5 L of culture medium for a lab-scale bioreactor experiment was similar to that of the flask-scale experiment. The inoculum was prepared by using Baker’s yeast incubated in glucose solution for about 8-10 hours under room temperature. In the experiments, 8 mL and 60 mL of inoculums were added to the flask-scale and lab-scale bioreactor fermentation respectively, prior to the fermentation start-up.
2.3. Sample analysis

The UV spectrophotometer (Lambda 25, Perkin Elmer, USA) was used to analyze the glucose, ethanol, and cell concentrations at wavelength of 340nm under room temperature. The glucose and ethanol samples were prepared by using R-Biopharm test kits. For biomass cell concentration, the samples were first diluted to appropriate concentrations so that the corresponding values of absorbance were within the range between 0.1 and 0.4.

3. Results and discussion

3.1. Flask-scale fermentation

The effects of pH, temperature, and ratio of hydrolyzed cassava to durian seeds on the ethanol yield, substrate consumption, and product formation rates were analyzed in the flask-scale fermentation. Tables 1 and 2 show the experimental results for the flask-scale fermentation at 30°C and 35°C for a batch period of 48 hours, respectively.

| pH  | Ratio (cassava: durian seed) | Initial glucose (g/L) | Final glucose (g/L) | Final ethanol (g/L) | Glucose consumed (%) | Ethanol Yield (%) |
|-----|------------------------------|-----------------------|---------------------|---------------------|----------------------|------------------|
| 4.5 | 85:15                        | 50.89                 | 15.88               | 3.91                | 68.8                 | 11.2             |
|     | 75:25                        | 75.28                 | 12.81               | 7.11                | 83.0                 | 11.4             |
|     | 50:50                        | 92.62                 | 23.43               | 15.25               | 74.7                 | 22.0             |
| 5.0 | 85:15                        | 66.52                 | 48.63               | 4.41                | 25.8                 | 26.1             |
|     | 75:25                        | 76.10                 | 45.73               | 7.46                | 38.3                 | 26.3             |
|     | 50:50                        | 76.54                 | 30.59               | 15.74               | 60.0                 | 34.2             |
| 5.5 | 85:15                        | 67.83                 | 40.73               | 2.86                | 40.0                 | 10.5             |
|     | 75:25                        | 69.91                 | 47.02               | 3.40                | 32.7                 | 14.9             |
|     | 50:50                        | 73.92                 | 58.62               | 3.41                | 20.7                 | 22.3             |

Table 2. Experimental results for flask-scale fermentation at 35°C

| pH  | Ratio (cassava: durian seed) | Initial glucose (g/L) | Final glucose (g/L) | Final ethanol (g/L) | Glucose consumed (%) | Ethanol Yield (%) |
|-----|------------------------------|-----------------------|---------------------|---------------------|----------------------|------------------|
| 4.5 | 85:15                        | 48.14                 | 32.38               | 7.03                | 32.6                 | 44.6             |
|     | 75:25                        | 66.25                 | 13.63               | 21.46               | 79.5                 | 40.8             |
| 5.0 | 85:15                        | 56.04                 | 37.98               | 8.00                | 32.2                 | 44.3             |
|     | 75:25                        | 63.72                 | 39.43               | 10.76               | 38.1                 | 44.3             |
|     | 50:50                        | 72.93                 | 18.61               | 24.92               | 74.5                 | 45.9             |
| 5.5 | 85:15                        | 53.11                 | 20.64               | 7.00                | 61.2                 | 21.6             |
|     | 75:25                        | 62.86                 | 32.78               | 7.71                | 47.9                 | 25.6             |
|     | 50:50                        | 66.70                 | 16.34               | 13.17               | 75.6                 | 26.2             |

Results in Tables 1 and 2 show that the effects of ratio of cassava to durian seed on glucose and ethanol concentration profiles are significant. Notice that, the initial concentration of glucose increased when the amount of durian seeds introduced was getting larger. The culture medium with the ratio of 50:50 had more glucose content while the ratio of 85:15 had the lowest. This was due to the sugar content in durian seeds is higher than that in cassava powder. At the end of the
fermentation period, the glucose was substantially consumed and converted into ethanol. Notice that, the higher the glucose content in the medium, the higher the ethanol formation during the fermentation process, which was in agreement with the literature report [8]. Therefore, the fermentation at the ratio of 50:50 was considered to be the most appropriate condition because it can lead to the highest amount of initial glucose concentration and final ethanol concentration. 

As shown in Table 2, at temperature of 35°C, the percentages of glucose consumption under the ratio of 50:50 were more or less similar (about 75%) under all three different pH values. On the other hand, the percentages of glucose consumption under the ratio of 50:50 and temperature of 30°C (refer to Table 1) varied significantly across the three pH values. Under pH 5.5, the percentages of glucose consumption at temperature 30°C were lower than that at temperature 35°C. The situation was reversed under medium pH of 4.5. It can be noticed that at the ratio of 50:50, it seemed that the introduction of larger amount of durian seed into the mixed feedstock tended to increase the percentages of substrate consumption.

Apart from increasing the initial substrate concentration, the ethanol productivity can be improved by controlling a number of other parameters. One of these parameters is the medium pH in which the fermentation is taking place [14]. As investigated by [8], the ethanol production under fixed glucose concentration can strongly be affected by different pH values. A pH range of 4.0 - 5.0 is regarded as the operational limit for ethanol production. During the cell growing stage, it is crucial that yeast cells maintain its intracellular pH at an optimal value. The deviation of the extracellular pH from this optimal point requires the yeast cells to add in more effort by investing energy to either pump in or out hydrogen ions as to preserve the optimal intracellular pH [15]. Hence, the occurrence of substantial pH difference between the intracellular and extracellular environments can lead to the deactivation of enzymes, which consequently impedes the growth of yeast cells and then ultimately reduces the ethanol production efficiency [11]. In view of the importance of pH, the present studies investigated the ethanol formation at various pH values, which were 4.5, 5.0 and 5.5. From Table 1 and Table 2, it was observed that ethanol yield was at its highest when pH was fixed at 5.0 at temperature 30°C and 35°C. Interestingly, the ethanol yield was at its least at pH 5.5 among the three tested pH values. Apparently despite of diverse substrate concentrations due to different material ratios, the ethanol yields were quite comparable under the medium pH of 5.0, i.e., for the lab-scale case, the yields were within ±3% of the average value.

It should be noted that, yeasts are large globular proteins which act as biological catalysts. At a higher temperature, the rate of yeast activity is higher because the collisions between substrate and the enzymes active site are more frequent as molecules are moving even more rapidly at high temperature [16]. However because of several other biological limitations, the increment of yeast activity is only up to a point where the cells are at the maximum efficiency. Beyond this optimum temperature, yeast cells begin to denature due to excessive kinetic energy which breaks the bonds eventually alters the active sites shape. This results in the rapid decrease or even complete halt of the enzymatic reactions involved. On the other hand, at lower temperatures the cells specific growth rate is lower which is due to their low ethanol tolerance at lower temperatures [17]. In this study, the variation of fermentation temperatures between 30°C and 35°C revealed that the ethanol production was at its highest when the fermentation was operated at the temperature of 35°C (see Tables 1 and 2. This can be observed in all of the results obtained when comparing the experimental studies under similar pH and similar mixtures of cassava and durian seed. Moreover, it can be seen that the ethanol yield increased significantly at the temperature of 35°C compared to 30°C under all three different pH values and ratio of hydrolysed cassava to durian seed. At temperature of 35°C, the lowest ethanol yields were obtained under pH of 5.5 although with high percentages of glucose consumption. The consumption of glucose could be used for powering the tricarboxylic acid (TCA or Kreb’s) cycle leading predominantly to cell respiration [18]. Hence, the optimal trade-off between respiration and fermentation seemed to
occur when the highest ethanol yield was obtained under pH of 5.0.

In the flask-scale fermentation, it can be concluded that the highest ethanol yield of 45.9% and a final ethanol concentration of 24.9 g/L were achieved at pH 5.0, temperature 35°C and 50:50 volume ratio of hydrolyzed cassava to durian seed over a batch period of 48 hours. The optimum condition of the fermentation process from proposed material was used in the lab-scale bioreactor experiment.

The fermentation experiments by using solely durian seeds at pH 5.0 and temperature 35°C, over a batch period of 48 hours were conducted for a comparison purpose. Table 3 shows the experimental results for solely durian seeds fermentation.

| Experiments | Initial glucose (g/L) | Final glucose (g/L) | Final ethanol (g/L) | Glucose consumed (%) | Ethanol Yield (%) |
|-------------|-----------------------|---------------------|---------------------|----------------------|-------------------|
| A           | 65.39                 | 35.79               | 2.72                | 45.3                 | 9.2               |
| B           | 63.63                 | 31.16               | 3.29                | 51.0                 | 10.1              |

From Table 3, it was observed that the ethanol yields of 9.2% and 10.1% for both experiment A and B, with initial concentration of glucose 65.39 g/L and 63.63 g/L respectively. Notice that, the initial concentrations of glucose by using solely durian seeds were slightly lower compared to the culture medium with ratio of 50:50. The initial concentration of glucose did not increase when the amount of durian seeds introduced was getting larger. This might due to the overloaded durian seeds solid suspension in the fermentation medium which caused less room for water. Thus, the hydrolysis process of durian seeds might be limited leading to low initial concentration of glucose. This in turn results in a low ethanol yield. Also, the low ethanol yield could be caused by the osmotic stress experienced by yeast cells [19]. The limitation of available water might be responsible for the obstruction of cell growth, which resulted in low ethanol production. Such stress encountered caused yeast to divert the metabolic pathway producing ethanol to other pathways such as toward the glycerol formation in order to cope with the stress.

3.2. Microaerobic lab-scale bioreactor fermentation

The ethanol, glucose and biomass concentration profiles in the lab-scale bioreactor were examined for the fermentation using the proposed materials under the optimum flask-scale conditions: pH 5.0, temperature 35°C and 50:50 volume ratio of hydrolyzed cassava to durian seeds for a batch period of about 46 hours.

The delivery of small amount of oxygen to culture broth under anaerobic fermentation is important to maintain metabolite production and growth of microorganisms [20]. Thus, a controlled microaerobic condition, i.e., 0.5 LPM, was applied in bioreactor fermentation to improve the cell viability and enhance the ethanol formation by yeast. The glucose and ethanol concentrations profiles are shown in Fig. 1, whereas the biomass concentration profile is shown in Fig. 2.

In Fig. 1, the decrement in glucose concentration and increment in ethanol concentrations are expected due to the consumption of substrate to produce ethanol throughout the fermentation process. The initial concentration of glucose was at 41.95 g/L and it dropped to about 1.00 g/L at the end of fermentation process. Meanwhile, the ethanol was produced gradually and it reached up to 14.61 g/L after batch period of 46 hours. The initial glucose concentration differed from that given by the optimum condition from the flask-scale fermentation, which could be due to the insufficient hydrolysing process to break down the long chains of starch into fermentable
sugar with bigger amount of raw materials (i.e., cassava and durian seeds remained). Thus, it led to lower ethanol formation as less fermentable substrate was introduced.

In Fig. 2, a slow microbial growth phase was observed for about 20 hours during which the consumption of substrate and production of ethanol were small. After the slow microbial growth phase, it was followed by a rapid growth phase in a short period, during which the cells multiplied in an exponential order after the cells eventually adapted to the medium environment. It can be noticed that, the consumption of glucose and ethanol production were increased rapidly during this period. The biomass increased throughout the experiment, but dropped towards the end of experiment. It is interesting to note that, the decrease in cell viability could be due to the exhaustion of substrate and this led to the inhibition of ATP synthesis. Another reason for the drop in cell viability could be due to the yeast cells were metabolically inactive due to the higher rate of ethanol formation and this led to the leakage of intracellular metabolites into the growth medium [21]. Hence, these circumstances caused the cell death. The ethanol yield of 35.7% and a final ethanol concentration of 14.61 g/L were obtained over a period of 46 hours where the glucose was almost fully consumed.
4. Conclusion

As a conclusion, the experimental results showed that the durian seeds can be used to supplement or even replace the conventional feedstocks (e.g. cassava) for ethanol production, which produced higher fermentable sugar content than cassava powder. In the flask-scale fermentation, the results showed that the highest ethanol yield of 45.9% and final ethanol concentration of 24.92 g/L were obtained under the conditions of pH 5.0, temperature 35°C and 50:50 volume ratio of hydrolyzed cassava to durian seed, over a period of 48 hours. The ethanol yield is about 90% of theoretical maximum of glucose conversion by yeast. Under this optimum condition, the lab-scale bioreactor fermentation resulted in 35.7% of ethanol yield of and a final ethanol concentration of 14.61 g/L over a period of 46 hours. It is interesting to note that, the ethanol fermentation using mixed cassava and durian seed is highly affected by the values of pH, temperature and ratio of hydrolysed cassava to durian seeds.

Acknowledgments

This work is supported by a grant from the Curtin Sarawak Research Institute (CSRI), no. CSRI-6009.

References

[1] Walker G M 2010 Bioethanol: Science and Technology of Fuel Alcohol (Dundee: Ventus Publishing Aps)
[2] Balat M and Balat H 2009 Appl. Energ. 86 2273–82
[3] Cheng J (ed) 2010 Biomass to Renewable Energy Processes (Florida: CRC Press Inc.)
[4] Amid B T and Mirhosseini H 2012 Food Chem. 132 1258–68
[5] Amin A M and Arshad R 2009 JPTI 1 367–375
[6] Brown M J 1997 Durio - A Bibliographic Review (New Delhi: IPGRI)
[7] Lee S H, Jung J Y and Jeon C O 2014 Food Microbiol. 38 16–25
[8] Lin Y, Zhang W, Li C, Sakakibara K, Tanaka S and Kong H 2012 Biomass Bioenergy. 47 395–401
[9] Zhang W, Lin Y, Zhang Q, Wang X, Wu D and Kong H 2013 Fuel 112 331–337
[10] Wong C L, Yen H W, Lin C L and Chang J S 2014 Bioresource Technol. 152 169–176
[11] Narendranath N V and Power R 2005 Appl. Environ. Microb. 71 2239–43
[12] K C Thomas S H H and Ingledew W M 2002 Appl. Environ. Microb. 68 1616–23
[13] Thatipamala R, Rohani S and Hill G A 1992 Biotechnol. Bioeng. 40 289–297
[14] Fakruddin M, Quayum M A, Ahmed M M and Choudhury N 2012 Biotechnology 11 248
[15] Narendranath N V, Thomas K C and Ingledew W M 2001 J. Am. Soc. Brew. Chem. 59 187–194
[16] Eed J 2012 ESSA 10 19
[17] Torija M J, Rozius N, Poblet M, Guillamón J M and Mas A 2003 Int. J. Food Microbiol. 80 47–53
[18] Rao D G 2010 Introduction to Biochemical Engineering (New Delhi: Tata McGraw-Hill Education)
[19] Soufi B, Kelstrup C D, Stoehr G, Fröhlich F, Walther T C and Olsen J V 2009 Mol. Biosyst. 5 1337–46
[20] Garcia-Ochoa F and Gomez E 2009 Biotechnol. Adv. 27 153 – 176
[21] Cot M, Lloreta O, Francois J and Benbadis L 2007 FEMS Yeast Res. 7 22–32