Waste valorization by biotechnological conversion of seed pods into biobutanol

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Abstract: Biofuels produced from biomass are a clean and efficient technology that does not adversely affect the environment. It can be used in replacing fossil fuels to sustain growing energy demands. Recently, butanol is considered to be a relatively new biofuel, which has some advantages over ethanol for use as a transportation fuel. Furthermore, butanol can be blended with gasoline to any proportion for better engine performance. In this study, the golden rain tree (*Cassia fistula*) pods were used as a sole substrate for biobutanol production. Golden rain tree is cultivated as an ornamental shade tree, yielding dark brown and large leathery pods. These pods contain high sugar content; it was extracted by thermal extraction method for biobutanol production. Then, the raw materials have undergone thermal extraction in optimal condition and enzymatic hydrolysis were used for (ABE) fermentation via *Clostridium acetobutylicum* TISTR 2375. It was found that the sugar from extraction and enzymatic hydrolysis can be utilized by *C. acetobutylicum* TISTR 2375, which can produce butanol yield 0.0006 g butanol /g pods. Consequently, study results showed that efficient pretreatment is another vital parameter in the biobutanol production process. In conclusion, although the total sugar and fermentable yield from this study are adequate, the golden rain tree pods waste has the potential to be studied further to check its feasibility at large-scale production. This review will briefly update the recent trends for lignocellulosic biomass as a substrate and feasible pretreatment that can be used for the production of biobutanol.

Keywords: Golden rain tree pods, Sugars, Fermentation, Biobutanol.

1. Introduction

The rapid development of our society, the current environmental, economic and social concerns regarding sustainability energy have pushed researchers towards discovering cleaner, renewable and sustainable energy resource. Biofuels produced from living biomass (such as biodiesel, biogas, bioethanol, biobutanol, etc.) is a clean and efficient technology that does not adversely affect the environment. It can be used in replacing fossil fuels for sustaining growing energy demands [1]. At present, butanol is considered to be a relatively new biofuel, which has some advantages over ethanol for using as transportation fuel. Several researches showed that the use of butanol has a high calorific value, which is 29.2 MJ/L (melting point -89.5 °C, boiling point 117.2 °C, flash point 36 °C, the self-ignition 340 °C) that is closer to that of gasoline (32 MJ/L) and ethanol (19.6 MJ/L). Furthermore, butanol can be blended with gasoline to any proportion for better engine performance [2,3].

Biobutanol can be produced from fermentation route by means of bacteria of the genus Clostridium such as *C. acetobutylicum*, *C. beijerinckii*, *C. saccharobutylicum*, and C. *saccharoperbutylacetonicum*. This process occurs under anaerobic conditions through acetone-butanol-ethanol (ABE) fermentation. Aside from solvent, Clostridium bacteria can also produce acids (acetic and butyric acid) and gases (hydrogen and carbon dioxide) [4]. The first phase of ABE fermentation is the acidogenic phase in which the acid-forming trunks are stimulated, yielding acetic and butyric acids as main products. This commonly occurs during the exponential growth phase. The second phase is the solventogenic phase, the acids derived from the first phase are used as substrates for the production of acetone, butanol and ethanol [5-6].

Renewable materials of biofuel production are available in the country. It is further subcategorized into different generation biofuels which are based on raw material being utilized and technological processes done during the conversion into biofuels. First generation of biofuels, the source is derived from edible oil-bearing crop plants such as palm oil, corn, soybean, sunflower, etc. However, there is the high cost of production and the food with fuel dispute is the main constraints on using those materials for the production of biofuel. Issues over feedstock sourcing, impact on biodiversity, land availability for growing agricultural crops, and global food crisis are among the firm criticisms lambasted by environmentalists and non-government organizations. With that, lignocellulosic feedstock from plant biomasses came into the development of second-generation biofuels. Indeed, the second-generation biofuels cover a wider range of feedstock in the sense that they are mainly derived from non-edible feedstock such as lignocellulosic plant biomasses, agriculture residues (e.g., bagasses, straws, etc.), and waste products such as waste cooking oil. These feedstocks are advantageous because it can counter the food-versus-fuel issues present in the first-generation biofuels. To date, the exploitations of various oleaginous microorganisms (microalgae, bacteria, yeast, and fungi) have resulted in the rise of third generation biofuels. However, pretreatment of the material is needed to obtain enough six-carbon sugar before it can be fermented and use for the production of biobutanol. The easiest way to produce biobutanol is to covert six-carbon sugar through fermentation [5-7].

Rain tree (*Samanea saman*) and golden rain tree (*Cassia fistula*) is widely interspersed in the tropics. It is cultivated as an ornamental shade tree, yielding dark brown and large leathery pods. Rain tree pods have been normally used as a feed for goats and other ruminants because they comprise high amount of protein. The rain tree pod was reported to be having high total sugar and protein. It contains 10.00-17.30% total sugar and 15.31-18.00% protein [7-8]. Meanwhile, golden rain tree pods were reported to be having glucose (42.5%) and protein (11.94%). However, it was observed that most of the pods are unutilized and remain to the ground until they are putrid. Most of rain tree and golden rain tree pods are planted along roadside and university vicinities which defoliate and pounded to the ground and becomes glutinous that invites flies when rotting [8]. Therefore, rain tree and golden rain tree pods can be a promising material for biobutanol production because it contains appreciable amount of sugar and has a significant volume of production during its fruiting season in Thailand. This study aims to investigate of feasibility and thermal extraction method of golden rain tree pods for biobutanol production.

2. Materials and methods

This study aimed to produce biobutanol from rain tree and golden rain tree pods using *Clostridium acetobutylicum* TISTR 2375 through ABE fermentation. Golden rain tree pods were collected and dried to reduce the moisture content of the samples. The dried samples were milled using mortar and pestle and were used in thermal extraction method. The optimization of the factors such as temperature and time. The best condition for thermal extraction was applied in the enzymatic hydrolysis of the samples. Afterwards, ABE fermentation was done with *Clostridium acetobutylicum* TISTR 2375 to produce biobutanol.

Golden rain tree (*Cassia fistula*) were grown at Maejo University campus, Chiang Mai, Thailand). The ripened pods of rain tree and golden rain tree that were fell on the ground were collected durng March to April of 2018. Then, the samples were dried at 50 °C for 48 hours in an oven to prevent mold growth, seed germination, and rotting. Dried samples were stored in plastic bag until experiments are performed. Batch fermentation was carried out in 100 ml bottle. Rain tree and golden rain tree pods thermal extraction as carbon sources were rapidly inoculated with 10% (v/v) actively growing RCM cell suspension. The initial pH of the medium was adjusted to 6.5 using 2 M NaOH and 1 M HCl. To generate an anaerobic condition, the medium was spared with oxygen-free nitrogen gas for 4 min Before inoculation, the medium was sterilized at 121 °C for 15 min, followed by inoculation with inoculum culture ($OD660 = 1.5{\sim}2.0$) of *C. acetobutylicum* TISTR 2375 (4.5 ml cell suspension in 45 ml medium). The fermentation was operated at 35 °C for 120 hour [9-12]. The samples were withdrawn at time intervals of 24 hours for analysis. Prior to analysis, the samples were centrifuged at 8,000 rpm for 15 min.

3. Results and discussion

3.1. Feedstock characteristics and biomass yield

Golden rain tree (*Cassia fistula* Linn.) with a family name of Caesalpiniaceae commonly known as Amulthus is a deciduous tree with greenish grey bark, compound leaves, leaf lets are each 5-12 cm long pairs. A semi-wild tree known for its beautiful bunches of yellow flowers. The fruit pods are 40-70 cm long and 20-27 mm in diameter, straight or slightly curved, smooth but finely striated transversely, the striations appearing as fine fissures. The rounded distal ends bear a small point marking the position of the style. The dorsal suture appears as a single vascular strand and the ventral suture as two closely applied strands (Figure 1).

Figure 1 Rain tree and golden rain tree pods (A, B); Hot air drying (C, D); Storing samples (E, F)

Internally the pod is divided by thin, buff colored, transverse dissepiments at intervals of about 0.5 cm. Each compartment contains one seed which is flat, oval, reddish brown with a well-marked raphe. The seed contains a whitish endosperm in which the yellowish embryo is embedded (Table 1). The long pods which are green, when unripe, turn black on ripening after flowers shed. Pulp is dark brown in color, sticky, sweet and mucilaginous, odor characteristic, and somewhat disagreeable. It is reported that the golden rain tree pods consists 19.94% proteins, 31.3% sucrose, 26.2% fructose and 42.5% glucose [12]. It contains high sugar content. And there are still no reports of golden rain tree pods use for the production of biofuels.

3.2 Lab scale experiment of sugar extraction

Golden rain tree pods contained a sugar-rich juice that can be readily utilized for biobutanol production. Most of the sugar is stored inside the brownish pulp of the pods. Extraction of sugar from each agricultural source requires unique operating conditions developed based on sugar and water content, fiber structure and composition, and geometric size. The traditional method to extract sugar from sugar plant is to squeeze the stalks through a roller mill, releasing the sugar rich juice in a process derived from sugar cane sugar extraction. The main drawbacks of crushing are: 1) there is substantial fermentable sugar remaining after a single crushing (less than half of the total sugar in the stalks typically is recovered) and 2) it is labor and energy intensive [13].

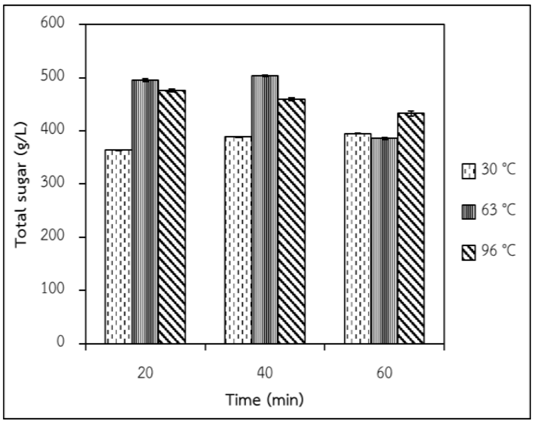

Figure 2. Total sugar production from rain tree pods with thermal extraction method

For example, to extract sugar from cashew apple bagasse, the optimum extraction conditions of liquid: solid 3.26 (mL/g), pH 6.42, extraction time 6.30 h and temperature 52.27 °C (Figure 2). This method of extraction is called the water extraction method. It has the draw backed is that the sugar concentration in the extraction water typically is fairly low making [14-15]. Therefore, improved water extraction methods were used the thermal in the process of extraction sugar which developed and assessed in this research to overcome the low sugar concentration. At present, the researches focus on the development of production processes that can increase the amount of acetone, butanol and ethanol, and can reduce the cost of production.

Table 1 Sugar yield after enzymatic hydrolysis

Feedstocks	**Total sugar (g/L)**	**Reducing sugar (g/L)**	**DP**
Rain tree pods			
Pretreated (control)	507.16±2.59	111.90±1.65	4.5
Eenzymatic	554.93±1.49	281.43±1.43	2.0
Golden rain tree pods			
pretreated (control)	271.84±2.28	57.62±2.18	4.7
Enzymatic	350.95±1.72	143.81±2.18	2.4

Note: DP means the degree of polymerization

Therefore, the study on factors affecting the growth and/or ability of each substrate and conversion of acid production to the solvents production of bacteria (Figure 3). The study on the factors affecting batch and continuous fermentation is aimed in order to enhance the production of biobutanol. The details are as follows. One of the most important factors to consider when developing an ABE solution is the cost of production. The choice of agricultural raw materials to cultivate in the country as a substitute for fermentation can reduce production costs. The production of acetone, butanol and ethanol, can be used for various substrates including starch and sugar, such as fermented rice straw, sugarcorn juice [15], etc. In addition, agricultural raw materials such as lignocellulose can produce biobutanol. However, it requires the pretreatment and hydrolysis of raw materials before the sugar can be used to ferment which resulted to higher production cost. The easiest way to produce biobutanol is to convert six-carbon sugar through ABE fermentation.

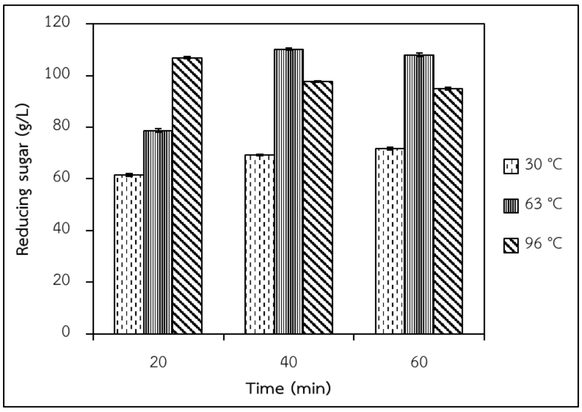

Figure 3. Reducing sugar production from rain tree pods with thermal extraction method

The success and sustainable operation of a commercial cellulosic biorefinery are highly dependent on price, quality, and availability of feedstocks. Currently, rain tree and golden rain tree pods are primary feedstock chose for cellulosic ethanol production and the same feedstock can be used for butanol production, which is a potential alternative biofuel [15]. In this study, rain tree and golden rain tree pods were characterized and evaluated as substrates for butanol production. The results of the thermal extraction in golden rain tree pods revealed that the optimal condition at temperature 63 °C for 40 min and 96 °C for 60 min, respectively. And 258.82 g/L total sugar and 57.22g/L reducing sugar from golden rain tree pods. These raw materials that have been thermal extracted and continued hydrolysis with cellulase enzymes. It was found out that the total sugar concentration were increased from 441.99±59.28 g/L to 525.07±51.72 g/L from rain tree pods and 272.34±5.65 g/L to 344.48±19.74 g/L from golden rain tree pods.

3.3 Biobutanol production

The reducing sugar concentration was increased from 115.71±4.95 g/L to 263.33 ±26.74 g/L from rain tree pods and 55.24±4.36 g/L to 144.29±30.74 g/L from golden rain tree pods. Then, the raw materials undergone thermal extraction in optimal condition and enzymatic hydrolysis were used for ABE fermentation via *C. acetobutylicum* TISTR 2375. It was found out that the sugar from extraction and enzymatic hydrolysis can be utilized by *C. acetobutylicum* TISTR 2375, which can produce butanol of 0.0002 g butanol /g pods from rain tree pods. For golden rain tree pods, sugar from extraction and enzymatic hydrolysis can be used by *C. acetobutylicum* TISTR 2375, butanol yields 0.0006 g butanol /g pods (Figure 4).

This study was produced small amount of butanol, indicating that the rain tree and golden rain tree pods juice contained a supernumerary amount of sugars. High initial sugar concentrations cause inhibition of the cells growth during the early stage, which then limits the fermentation. This result showed that the concentration of fermentation medium components including carbon source significantly affects the biobutanol fermentation. However, optimization in terms of sugar dilution for butanol production by *C. acetobutylicum* TISTR 2375 golden rain tree pods should be further investigated to incuse the biobutanol yield of addition.

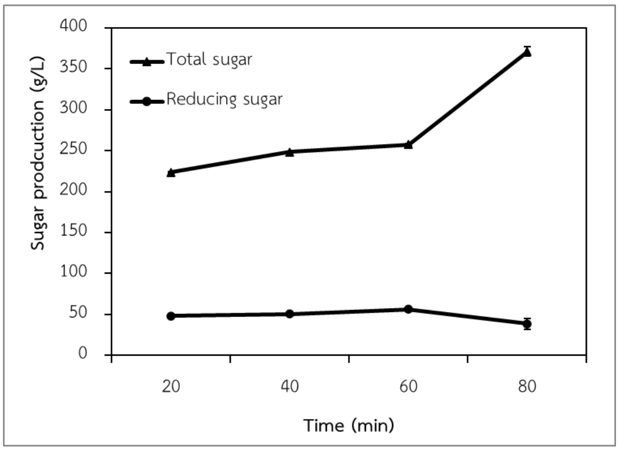

Figure 4. Total sugar and reducing sugar production from golden rain tree pods with thermal extraction method

4. Conclusions

Analysis of sugar production from golden rain tree pods showed that it contains moisture 4-10 %, total sugar 54.60±0.71 to 257.68±1.39 g/L and reducing sugar 6.57±0.18 to 56.30±1.16 g/L at 30-96 °C and 20-60 min condition. Statistical optimization for thermal extraction of total sugar and reducing sugar was successfully carried. A total experiment of 27 runs was conducted to study the effect of variables. All the responses were significantly affected by the independent factors. When the temperature and time of thermal extraction increase, the total sugar and reducing sugar are increased. The optimum conditions for extraction of sugar were determined as extraction time 60 min and temperature 96 °C. Under these optimum operating conditions the maximum sugar production was recorded as 260.31 g/L of total sugar and 56.29 g/L of reducing sugar.

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