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Two-steps microwave-assisted treatment on acid hydrolysis of sago pith for bioethanol production

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Abstract. Sago is a genus of palm that can be utilized to produce fermentable sugars as substrate for bioethanol. Sago pith is a heterogeneous substrate consists of starch and fiber. Acid hydrolysis by microwave heating radiation can break down starch and fibers together in a very short time, so it is considered to be very efficient process. The use of microwave energy (as power level) and variation of heating time can produce fermentable sugar with certain characteristics. This study included the preparation and analysis of sago pith flour; process of acid hydrolysis (0.3 M and 0.5 M H₂SO₄) using two steps microwave heating, first with power level 30% (1, 2 and 3 min) and second with power level 70% (3 min); and ethanol production. The conventional treatment (autoclaving at 121°C for 15 min) was carried for the comparison. The highest fermentable sugar (105.7 g/l) was resulted from microwave heating with power level 30% for 2 min followed by the power level 70% for 3 min. This hydrolyzate then used as substrate for bioethanol fermentation and partially neutralized (pH 3, 4, 5) by using yeast *Issatchenkia orientalis*, and the highest ethanol (2.8 g/l) was produced in pH 5.

1. Introduction

Sago palm (*Metroxylon sp.*) is a potential commodity for agroindustrial raw material, and considered as an extremely sustainable plant because of its ability to thrive in most soil conditions. This crop has a significant value to be utilized for some commercial products, such as modified starches, lactic acid, cyclodextrins, and ethanol [1]. It was also predicted that approximately 60 million tons of sago starch will be produced per year at the end of the twentieth century [2]. In addition, it has also been reported that Indonesia covered 1.25 million ha area of sago forest, and assumed its productivity 100 kg per stem with the population of 30 stems per ha, then 6.48 millions ton per year of sago starch could be produced [3].

Sago starch is derived from the pith of sago and accumulated in the pith of sago trunk. It has a high content of starch (81-85%) on the pith [4]. It was also stated that sago pith starch have a high potency as a raw material of ethanol production, since sugar and starch-based biomass are currently predominant and economically material in industry [5]. In addition, our previous study [6] found that



sago pith also containing fiber (cellulose and hemicellulose), and this lignocellulose was also able to be converted into ethanol. The conversion of starch, cellulose and hemicellulose into fermentable sugars using energy efficient, economic and faster way was the greatest concern for commercial fuel ethanol production [7]. The conversion includes two processes: hydrolysis of starch, cellulose and hemicellulose to fermentable sugar, and fermentation of the sugars to ethanol by yeast or bacteria. Hydrolysis is a critical stage in fermentable sugars production, before the fermentation process. Chemical and enzyme hydrolysis have been widely employed to breakdown the starch and cellulose into fermentable sugar [8, 9, 10], however the approaches still tend to be slow, expensive and of high dilutions that give poor yields of glucose [11]. Microwave irradiation is an alternative method for hydrolysis of biomass to simple sugars. Microwave heating presents a potentially faster, efficient and selective method for thermal treatment of biomass, compared with conventional heating [12], the reaction rate of starch hydrolysis to glucose was accelerated 100 times under microwave irradiation [13]. Therefore, it was considered as energy efficient approach for biomass pretreatment under low pressure and temperature [14, 15], and the microwave heating even improved the glucose selectivity [16]. Previously, it was reported that microwave treatment for saccharification in water and acid hydrolysis can produce oligosaccharides [17, 18].

In this study, a breakthrough system developed by assisting the acid hydrolysis with two-steps microwave treatment to improve the more efficient saccharification process of starch and fiber from sago pith. This research investigated the effect of microwave treatment and optimal acid concentration to sugar characteristics resulted by modifying the acid concentration, power level (energy) of radiation and radiation period. The hydrolysates then be fermented into bioethanol by using *Issatchenkia orientalis*. The typical yeast has a high tolerance in low pH [19] and this characteristic was important to against the acid condition due to the acid hydrolysis. In addition, the yeast also has an ability to convert both hexose and pentose sugar into bioethanol [20, 21], and this characteristic was significantly needed in bioethanol production from lignocellulose biomaterial that consisted of the two types of sugar.

2. Methods

2.1. Materials

The raw material used in this research was sago pith from *Metroxylon sp.*, provided from local sago starch industry in Cimahpar, Bogor, Indonesia. The hydrolysis used sulphuric acid (p.a), then assisted by heating of radiation from microwave oven Sharp type R-348 C output 1000 W High power and 32 L capacity. The *Isaatchenkia orientalis* was a collection of Biology Department, Faculty of Mathematic and Science, Bogor Agricultural University. This isolate was obtained from rotten fruits and has been identified and characterized its performance in bioethanol fermentation.

2.2. Preparation and Characterization of Sago Pith Flour

Native sago pith was cut into smaller size, rasped and dried in sun drying. The crude flour then pulverized and passed 35 mesh screens. Some chemical characteristics of the dried flour were analyzed as moisture, ash, protein, fat, crude fiber, and carbohydrate (by differences) contents, starch content (*Luff Schoorl*), and fibre component.

2.3. Acid Hydrolysis Assisted by Two-Steps Microwave Heating

Sago pith mainly consists of starch and limited amount of fiber. Sago pith slurries (10%) hydrolysed in aqueous sulphuric acid (0.3 M and 0.5 M) were poured into glass jar to get the first heating from microwave oven on 30% of power level (output 350 Watt) for a various time (1, 2 and 3 minute). The second heating was continued on higher power level (70%, output 650 Watt) for 3 minute. After that, the hydrolyzat was filtered and neutralized by NH_4OH , and the fermentable sugar resulted was then

analyzed its total sugar amount, reducing sugar amount, dextrose equivalent (DE), degree polymerization (DP), filtrat volume, syrup clarity, and HMF and furfural content. Microscopic analysis of the filtrat and residue was also observed. A conventional heating by autoclaving was carried out as a comparison.

2.4 Production of Bioethanol

Fermentable sugar produced from the best treatment was fermented by using *Isaatchekia orientalis* (10% starter) in a various pH (3, 4, and 5). The carbondioxide volume as the indicator of by-product in bioethanol production was measured in every 3h for the first 12 h fermentation period, then every 6h in the next 36 h period, and every 12 h in the last 36 h. After 72h of fermentation, the ethanol content was determined by using *Gas Chromatography*. The substrate of autocalving treatment and technical glucose were used as comparison.

3. Results and Discussion

3.1. Characteristics of Sago Pith Flour

Proxymate data (Table 1) explained that the major component of sago pith was carbohydrate (81.6%) and 55.86% of it was starch. It has been verified that sago pith, inner portion of the trunk after the removal of the outer bark-like layer, contained the starch which was accumulated in the pith of sago palm stem from the base upwards [1]. The starch content is influenced by the maturity of sago plant. The trunk is fully saturated with starch almost to the crown at the optimal maturity [22].

The second component of sago pith was fiber (7.93%), consisted of cellulose (2.93%) and hemicellulose (4.72%). The starch and two component of fiber are potential substrates to be hydrolyzed into fermentable sugar (glucose and xylose), since glucose is major components of starch and cellulose, whereas hemicellulose contains xylose and glucose as the major components, together with arabinose and galactose, and lower levels of rhamnose, mannose, fucose and uronic acids [23]. The content of lignin (6.86%) is less than reported by Ref. [1] that was about 9% to 22%, therefore the delignification process was not needed to be carried out in this study.

However, the moisture contents was high (14.5%), this will decrease its quality because of the microorganism growth. To solve the problem the sample has been dried in order to minimize the moisture content into safe condition for storage ($\leq 10\%$).

Table 1. Chemical characteristics of sago pith flour.

Components	Percentage (%)
Moisture (db)	14.52
Ash (db)	5.16
Fat (db)	3.72
Protein (db)	1.59
Crude fiber (db)	7.93
Carbohydrate (db)	81.6
Starch (db)	55.86
NDF (neutral detergent fiber)	14.51
ADF (acid detergent fiber)	9.79
Cellulose	2.93
Hemicellulose	4.72
Lignin	6.86

3.2. Acid Hydrolysis Assisted by Two-Steps Microwave Heating

The approach of hydrolysis can be chemically or enzymatically. The first technique is widely applied in industry because of its low cost, fast, and simple process, although it produces by-products that could inhibit the next process. Acid hydrolysis using sulphuric acid was usually used to convert starch or lignocellulose into intermediate products such as maltodextrin, oligosaccharide, and glucose syrup that have a wide application in food, feed, fuel, textile and pharmaceutical industries. The existence of acid could break down starch molecules at random and, theoretically it produced 60% glucose [24]. Furthermore, the glucose decomposition resulted some components that were identified as 5-hydroxymethylfurfural (HMF), 1,6-anhydroglucose, levulinic acid, and formic acid [25].

However, since the sago pith consisted starch and fiber which could not be soluble in cold water, the pre-treatment before the hydrolysis step was important to be developed. Microwave heating is one of the pre-treatment methods which has an important role in degrading some polysaccharides into their monomers and oligomers [18]. The heating energy of microwave penetrates directly and uniformly into materials, and is then absorbed by polysaccharides causing the cleavage of the glycosidic polysaccharide linkages. Figure 1 shows the change of fiber microscopic structure after microwave heating and acid hydrolysis treatment.

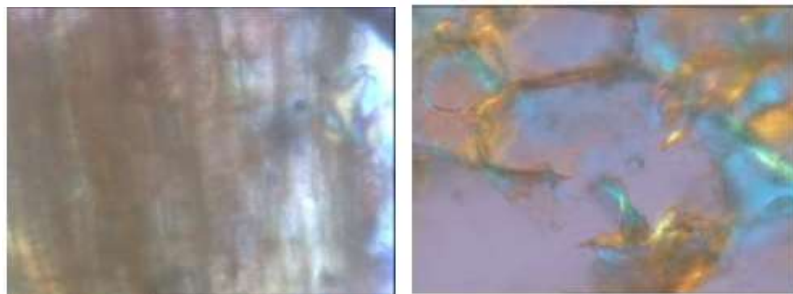


Figure 1. The microscopic fiber structure observed by light-polarized microscope: (1a) native sago pith starch and (2a) hydrolyzates of sago pith starch after two-stage microwave heating (power level 30% in 3' and power level 70% in 3') and hydrolysis (0.5 M sulphuric acid)

It can be seen that the two combined treatments convert a compact fibre (Fig 1a) into an amorphous structure (Fig 1b), and most of the native starch granules disappeared, and converted into gel form products. The blue color of the fiber fraction also indicated not completely degraded crystalline parts. The heating pre-treatment using autoclaving and microwave could increase the solubility and break down the crystallinity of starch and fiber, and caused the starch to gelatinize and hydrolyze directly into depolymerized products [6]. This degradation process can be proved by analyzing the soluble sugar contents in the hydrolyzates (Table 2).

Table 2. Parameter of hydrolizates after acid hydrolysis assisted by two-step microwave treatment of sago pith flour.

Treatment	Reducing sugar (g/l)	Total sugar (g/l)	Average DE value	Average DP value	HMF (mg/l)	Furfural (mg/l)	Filtrat clarity (%T)	Filtrat volume (ml)	Residue weight (%)
A1B0	83.17	90.50	91.90	1.08	8.10	0.06	89.85	75.75	24.01
A1B1	18.85	68.38	27.57	3.63	0.28	0.00	79.35	59.75	47.10
A1B2	24.40	80.81	30.20	3.32	0.45	0.00	87.05	56.50	42.48
A1B3	26.87	81.20	33.09	3.03	0.48	0.00	89.50	54.00	37.00
A1B4	32.24	82.93	40.08	2.49	0.63	0.00	76.30	49.00	35.25
A2B0	106.20	111.61	95.15	1.05	65.64	1.71	87.15	80.00	20.63
A2B1	23.34	80.69	28.93	3.46	0.96	0.04	65.60	67.50	30.62
A2B2	30.33	92.83	32.67	3.06	1.46	0.05	72.15	65.50	29.42
A2B3	48.86	105.56	46.24	2.16	1.56	0.05	76.15	64.00	28.14
A2B4	37.41	102.39	37.51	2.74	1.88	0.06	61.30	61.00	26.43

- A1 : 0.3M sulphuric acid
- A2 : 0.5M sulphuric acid
- B0 : autoclaving heating (comaprison)
- B1 : 70% power level (control)
- B2 : 30% power level (1') + 70% power level (3')
- B3 : 30% power level (2') + 70% power level (3')
- B4 : 30% power level (3') + 70% power level (3')

Table 2 described detail chemical characteristics of hydrolizates products. Total sugar is the important parameter of hydrolysis efficiency, since it determines the amount of soluble reducing sugar (monomer), dimer and its oligomers those the products of degradation process of sago pith starch and fiber. The amount of total sugar ranged from 80.81 g/L to 111.61 g/L was resulted by two-steps heating rather than one-step heating (68.38 g/L to 80,69 g/L) from microwave treatment in dilute acid. This due to the radiation energy obtained by the material was higher in two step heating (30% power level continuing to 70% power level) than the other, and made the more release of polysaccharides linkages. It drove to the increase of total sugar and reducing sugar amount, indicated by the decrease of average DP values (2.49-3.32). DP (degree of polymerization) expressed the number of monomer unit in the molecule, the more few number of DP value, the more high the content of reducing sugar. DP value up to one meaning there are much more monomers rather than disaccharides or oligosaccharides (DP value above 2), since glucose and xilose only contains 1 unit molecule.

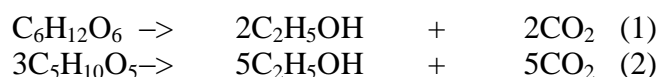
However, autoclaving treatment produced the highest total sugar and reducing sugar (111.61 g/l and 106.20 g/l) with the lowest DP value (1.05). It described that the hydrolysis process occuring perfectly, but the undesired product HMF (hydroxy methyl furfural) also accumulated in much more high amount as 65.64 mg/L rather than microwave heating (0.28-1.28). This by-products could inhibit the fermentation of bioethanol, therefore microwave heating was the better treatment than others.

In general, the acid concentration influence the degradation process. The hydrolysis in dilute acid of 0.5 M resulted a higher total sugar amount than 0.3 M in the same condition of microwave heating. The best combination treatment of acid concentrarion, hydrolysis time, and total amount of energy was obtained from 0.5 M acid concentration, first heating on 30% power level for 2 minute in the first and continuing to second heating on 70% of power level for 3 minutes. This best treatment resulted the high amount of total sugar (105.56 g/L) and reducing sugar (48.86 g/L), and also the minimal DP value (2.16). These three parameters could indicate the sufficient fermentable sugar to be converted into ethanol.

3.3. Production of Bioethanol

The fermentable sugar resulted from best treatment of hydrolysis assisted with two stages microwave was determined by the content of total sugar, reducing sugar and HMF. The high amount of total sugar and reducing sugar indicated the high availability of fermentable sugar as the substrate of bioethanol. In contrast, the HMF content should be minimized in order to avoid the inhibition of ethanol production, since this by product could decrease the yield of ethanol. The hydrolyzed sago starch from the best treatment then was converted into ethanol by *Isaatchenkia orientalis*. The typical hexose sugar, including glucose, galactose and mannose can be fermented by numbers of wild type microorganisms, yet pentose sugar such as xylose and arabinose can only be fermented by only few number of wild type microorganisms, and frequently resulting low ethanol yield. *Isaatchenkia orientalis* was one of a specific yeast strain, because it was low pH tolerance [20, 21, 26], . It is important to be noticed that the fermentable sugar from acid hydrolysis is acid condition. It was also underlined that its superior ability to ferment glucose to ethanol under high stress conditions, such as acid (H₂SO₄), salt (Na₂SO₄), or heat stress [27].

The observation toward CO₂ volume used as additional data for indicating the fermentation was running well. It was also suggested that the volume of CO₂ were equivalent with the ethanol content, shown by the chemical reaction as follows:



From the reaction above, the ethanol content which are resulted from either glucose (i) or xylose (ii) are equivalent with the yielded glucose. The alteration of CO₂ volume are not directly used for determining bioethanol content from hydrolysate [28]. It was mainly determined by multiplying the volume of CO₂ with coefficient 1.045 as Gay-Lussac equation. Figure 2 explained that as the time of fermentation increased, the level of CO₂ was also escalated, yet after 12 h of incubation the increasing level of CO₂ were not significant. This condition only occurred in pH 4 and 5. It can be confirmed by measuring the ethanol content using GCMS (Table 3).

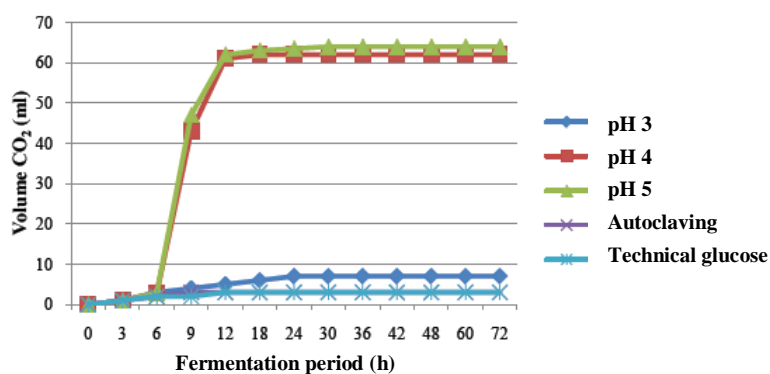


Figure 2. The accumulation or augmented CO₂ derived from 72 hours of sago pith hydrolysate fermentation in any various pH and the comparison (autoclaving and technical glucose).

Table 3. Ethanol yield and fermentation kinetics parameter of fermentable sugar resulted by acid hydrolysis assisted by two-step microwave treatment of sago pith flour.

Substrate	Parameter			
	Ethanol yield (%)	Ethanol yield (g/l)	$\Delta s/s$	Yp/s
Sample (pH 3)	0.220	1.783	0.126	0.022
Sample (pH 4)	0.314	2.481	0.180	0.032
Sample (pH 5)	0.361	2.852	0.250	0.036
Autoclaving pH 5 (comparison)	1.840	14.536	0.167	0.186
Technical glucose pH 5 (control)	1.611	12.727	0.118	0.175

Table 3 shown the higher yield of ethanol (2.852 g/l) resulted by fermentation in pH 5, rather than pH 3 and 4. This can be assumed that optimal condition for *Issatchenkia orientalis* growth was in pH 5. However, the ethanol yield produced was still lower compared to autoclaving treatment. This could be suggested that autoclaving has a sufficient time and energy to hydrolysed the sago pith. The average DP values of this treatment was 1.05 (Table 2). It means that the starch and fiber had been hydrolyzed perfectly into their monomers. The content of reducing sugar was about 106.20 g/l, and it was two times higher than microwave heating (48.86 g/l). The higher concentration of reducing sugar made an available substrate for fermentation, and increased ethanol yield.

4. Conclusion

Two-steps microwave-assisted on sulphuric acid hydrolysis of sago pith flour produced a better characteristics of fermentable sugar rather than one-step treatment. The higher acid concentration and longer hydrolysis time increased the total sugar and reducing sugar amount of the hydrolyzates. It also improved the hydrolysis process, indicated by the raise of DE value and the decrease of DP value. In addition, the sugar resulted has been proven able to be a substrate of bioethanol (0.22-0,36%) by *Issatchenkia orientalis* in pH 5. For further research, some improvements should be carried out to increase the hydrolysis efficiency and ethanol yields, such as : size reduction of sago pith, detoxification of fermentable sugar, and isolation of indigenous yeast.

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