

# Potentials of sago fibre hydrolysate (SFH) as a sole fermentation media for bioethanol production

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**Abstract.** Sago wastewater which contains starchy fibres from sago starch processing mills is commonly discharged directly to nearby stream thus contribute to serious environmental pollution. Sago fibre which is known to be a local agricultural waste mainly contains residual starch of about (50 – 60 %) together with cellulosic component. These contribute to high carbohydrate contents which suitable to be used as substrate for ethanol production. Initially, sago fibre (SF) was converted into sago fibre hydrolysate (SFH) via enzymatic hydrolysis using commercial enzymes; Liquezyme SC DS and Spirizyme Fuel HS. This study emphasized on batch ethanol fermentation by commercial baker's yeast utilizing 50 g/L and 80 g/L glucose of SFH as the sole fermentation medium. The results indicate that 50 g/L glucose from SFH media is capable of generating maximum ethanol concentration at  $20.33 \pm 0.15$  g/L, with highest glucose consumption efficiency (97.78 %) during 24 hours of fermentation. Similar concentration of bioethanol was obtained in 50 g/L glucose of commercial glucose (CG) media which is at  $20.04 \pm 0.06$  g/L. However, lower ethanol concentration was obtained in both 80 g/L glucose from SFH ( $13.32 \pm 0.12$  g/L) and CG ( $12.98 \pm 0.04$  g/L media), respectively. Addition of yeast extract at 3 g/L into 80 g/L SFH as well as CG significantly improve ethanol fermentability (SFH:  $41.04 \pm 0.04$  g/L and CG:  $33.96 \pm 0.04$  g/L). Based on statistical analyses, 50 g/L glucose of SFH media exhibit the highest ethanol yield ( $0.42 \pm 0.003$  g/g) and highest fermentation efficiency ( $81.35 \pm 0.572$  %) compared to 80 g/L glucose ( $0.24 \pm 0.008$  g/g;  $46.65 \pm 1.50$  %). Conclusively, this study demonstrated that glucose in SFH was metabolized efficiently by commercial baker's yeast during ethanol fermentation, thus suggesting the capability of SFH to be a feasible and alternative substrate with less expensive nitrogen source for the renewable bioethanol production.

## 1. Introduction

The waste to wealth concept research has been practiced actively over the last few years, which widely focusing on the bioethanol production utilizing starchy lignocellulosic residue. Bioethanol (C<sub>2</sub>H<sub>5</sub>OH) or ethyl alcohol is a promising alternative source which is both renewable and environmental friendly [1]. Over the years, bioethanol production has increased dramatically due to high demand on green biofuel and crude oil shortage [2]. At present, global bioethanol production has reached about 5,340 million litres in 2017 and slightly increased to 5,380 million litres in 2018. This demand is met by using first generation bioethanol crops such as sugarcane and corn [3]. The research into new and alternative carbon sources such as industrial by-products and agricultural residues for uses in bioethanol fermentation is needed to sustain for the successful development of fermentation industries against traditional grain-based processes. Thus, a



renewable raw material, which is locally abundant, can be considered as an excellent feedstock for bioethanol production by virtue of high carbohydrate content, would be more preferable. In Malaysia, Sarawak is recognized as having the largest sago-growing areas, which is currently the world's biggest exporter of sago starch, exporting annually about 44,000 tonnes of starch mainly to peninsular Malaysia, Japan, Singapore and other countries [4]. It was estimated that starch contributed to about 15-16% (dry weight) of sago palm log [5]. Hence, for every 1 ton of sago starch produced, 6.5 ton palm's log was processed that leads to 20 ton of wastewater and 1 ton of sago fibre were discharged [5]. Approximately, 60,000 tonnes of sago fibre are produced daily from eight sago starch processing mills in Mukah [6]. Currently, these wastes are being washed off into nearby rivers or deposited in the factory's compound daily. This condition potentially deteriorates the rivers and the environment [7]. Sago fibre, which is free and abundant - is derived as a starchy lignocellulosic by-product from extraction of starch from the pith of the sago palm. On a dry basis, it contains 58 % residual starch together with high content of cellulosic components [8]. Previously, the sugar hydrolysate obtained from enzymatic hydrolysis of sago starch has been revealed by our group to be feasible as a cheap substrate for ethanol fermentation using *Zymomonas mobilis* [9]. Later, additional of trace elements as well as nitrogen and phosphorus would enhance the production of ethanol from sago fibre [10]. In any ethanol fermentation process, the greater substrate load would lead to increase in ethanol concentration, and improve the efficiency of downstream processing. Based on previous study, hydrolysate of sago fibre cannot be promoted as the sole carbon source if higher concentration of glucose (>100 g/L) was employed [11]. Thus, lower concentration of substrate was implemented in order to obtain maximum ethanol concentration. This project attempts to study the potentials of sago fibre hydrolysate (SFH) as the sole carbon source in bioethanol production utilizing 50 g/L and 80 g/L of initial glucose concentration by commercial baker's yeast.

## 2. Materials and Methods

### 2.1 Sago fibre

Sago fibre samples are obtained from the Ubom Sago Mill Sdn. Bhd. in Mukah, Sarawak in dried condition. The fibre was ground to pass 1 mm screen prior to produce sago fibre powder (SFP); to be used as raw material for glucose production and compositional analysis.

### 2.2 Enzymatic hydrolysis of sago fibre powder (SFP)

A suspension of sago fibre, 7 % (w/v) was prepared in 0.1 M monopotassium phosphate ( $\text{KH}_2\text{PO}_4$ ) buffer solutions at pH 4. The suspension was then boiled and gelatinized at 85 - 90 °C for about 30 minutes. After gelatinization, commercial enzymes of Liquozyme® SC DS and Spirizyme® Fuel HS (Novozymes, Denmark) were added into the suspension during liquefaction and saccharification stage. Upon completion of the hydrolysis process, the slurry obtained was filtered from its solid residual fibre, producing sago fibre hydrolysate (SFH). In order to maximize glucose recovery, recycle method was adopted in this study [10].

### 2.3 Inoculum preparation

An instant dry baker's yeast, labelled as Mauripan; *Saccharomyces cerevisiae* was used throughout this study. The yeast which was cultured on potato dextrose agar was transferred into sterile 100 mL inoculum media containing 20 g/L glucose and 5 g/L yeast extract. The pH for inoculum was adjusted at 5.5 before being incubated at 30 °C for 9 - 10 hours. Once the optical density (OD) of yeast cell reached to 0.5 to 0.6, which indicates log phase of the yeast growth profiles, the baker's yeast was developed and yeast was ready to be harvested. 10 mL of inoculum media was then removed and centrifuged in order to obtain the yeast pellet which then ready to be transferred into sterile fermentation media.

#### 2.4 Fermentation media and its process

SFH with initial glucose concentration of 50 g/L and 80 g/L was used as the main medium for ethanol fermentation and commercial glucose (CG) was used as a control throughout this study. The fermentation media was supplemented with nitrogen source; yeast extract, (3 g/L) [10]. The fermentation process was conducted via batch system, working volume of 100 mL shake flask, agitated at 100 rpm, 30 °C for 24 hours. In every 4 hours interval, the samples withdrawn were centrifuged at 10, 000 rpm for about 10 minutes at 4 °C. The brown clear supernatant was then used for determination of ethanol concentration and glucose consumption by High Performance Liquid Chromatography (HPLC) system (Shimadzu, Kyoto, Japan).

#### 2.5 Analytical procedures

Moisture content of sago fibre was determined using standard oven drying method at 105 °C to constant weight [12]. Analyses of crude fibre, crude fat, total ash and energy were sent to the Veterinary Diagnosis Laboratory, Sarawak. The residual starch content of sago fibre was measured by iodine starch colorimetric method [13]. Total reducing sugars was determined by using DNS method [14], whereas glucose and ethanol were analysed by using HPLC system (Shimadzu, Kyoto, Japan) equipped with a Refractive Index (RID-10A) detector with a column of prominence CTO - 20A (Aminex HPX – 87H column, 150 x 7.8 mm, BioRad Laboratories, Inc). Mobile phase used was 5 mM H<sub>2</sub>SO<sub>4</sub> with a flow rate set at 0.8 mL/min and column temperature was fixed at 60 °C.

#### 2.6 Data analysis

The data obtained from a series of fermentation process was compiled and analysed for ethanol yield (Y, g/g), ethanol fermentation efficiency (E<sub>f</sub>, %) and ethanol volumetric productivity (Q<sub>p</sub>, g/L/hr). Ethanol yield was calculated as the actual ethanol produced and expressed as gram ethanol per gram of glucose consumed (g/g). Theoretical yield of maximum ethanol was based on value 0.51. The fermentation efficiency (%) was calculated based on ethanol yield divided by theoretical yield of 0.51. Ethanol volumetric productivity was calculated as the maximum ethanol concentration obtained divide by the fermentation time (hrs).

#### 2.7 Statistical analyses

Data obtained from all of experiments were compared and analysed using a One Way ANOVA. LEAD Technologies, MINITAB Version 17.1.0 was used to perform the statistical analyses.

### 3. Results and Discussion

#### 3.1 Characterization of the sago fibre powder (SFP)

The composition of sago fibre powder is presented in Table 1. It was observed that the amount of residual starch in sago fibre was highest from CL Nee (Sibu) at 70.00 %, followed by Hup Guan (Johor, 65.70 %) and Ubom (Mukah, 59.00 – 60.00 %).

**Table 1.** Compositional analyses of the sago fibre powder (as percentage dry weight)

	This study (2017)	Abd - Aziz (2002)	Kumoro <i>et al.</i> (2008)	Awg - Adeni <i>et al.</i> (2013)
Sources of sago fibre	Ubom, Mukah	Hup Guan, Johor	CL Nee, Sibu	Herdson, Pusa
Residual starch	59.00 – 60.00	65.70	70.00	30.00 – 45.00
Moisture content	10.43	5.91	6.10	5.00 – 7.00
Crude Ash	3.35	4.10	4.16	3.00 – 4.00
Crude Fibre	22.94	14.80	18.76	30.00 – 35.00

Note: Apart from Hup Guan in Johor, all other factories are in Sarawak.

In this study, the residual starch content in SFP was 59.00 – 60.00 % (on a dry weight basis), which almost comparable to the starch contents reported by previous studies, which is in the range of 30 to 70 % [10, 15, 16]. The amount of residual starch (59.00 – 60.00 %) observed in sago fibre was quite high as compared to other studies due to several factors. The sago fibre is sourced from different sago mills, thus different methods or degree of starch extraction together with different facilities contribute to different quality of sago extraction process [10]. Most large sago mills have between 10 to 12 extractors which efficiently separate dissolved starch from the fibres. Smaller mills would have lesser number of extractors, which leads to higher percentage of residual starch in the effluent and attached to the fibres. Higher percentages of residual starch in sago fibre (SF) exhibited a less efficient starch extraction procedure in these factories, which leads to profit loss, disposal of large quantities of starch into the river and eventually leads to environmental pollution. This demands prompt and effective utilization of the sago fibre to minimize pollution and processing it into beneficial products to generate side-incomes for these sago mills. Moreover, the amount of residual starch and other composition including free sugars differ from every sago processing mill, depends on the effectiveness of the starch harvesting, extraction, filtration and sedimentation system used [17]. Thus, the amount of residual starch that still trapped within sago fibre would also being influenced by all the differences practices or techniques applied in sago factories.

### 3.2 Enzymatic hydrolysis of residual starch in SF for glucose production

Theoretically, in any chemical reaction, higher substrate concentration would have resulted in higher concentration of the end-product. Based on this study, recycled method was adopted in order to maximize the glucose production from SF. In every cycle, 7 % (w/v) suspension was prepared, thus total substrate load was accounted to be 21 % (w/v). The conversion of glucose was expressed in terms of glucose concentration (g/L) by using HPLC analysis. Table 2 represents the glucose concentrations (g/L) of sago fibre hydrolysate (SFH) for three-cycle enzymatic hydrolysis process. The glucose concentration after cycle I of enzymatic hydrolysis was 46.24 g/L. When SFH from cycle I was added with new substrate load for cycle II, 100.10 g/L glucose was generated. At the end of cycle III, maximum amount of glucose obtained was increased up to 149.36 g/L.

**Table 2.** Glucose production of three-cycle enzymatic hydrolysis of SFP

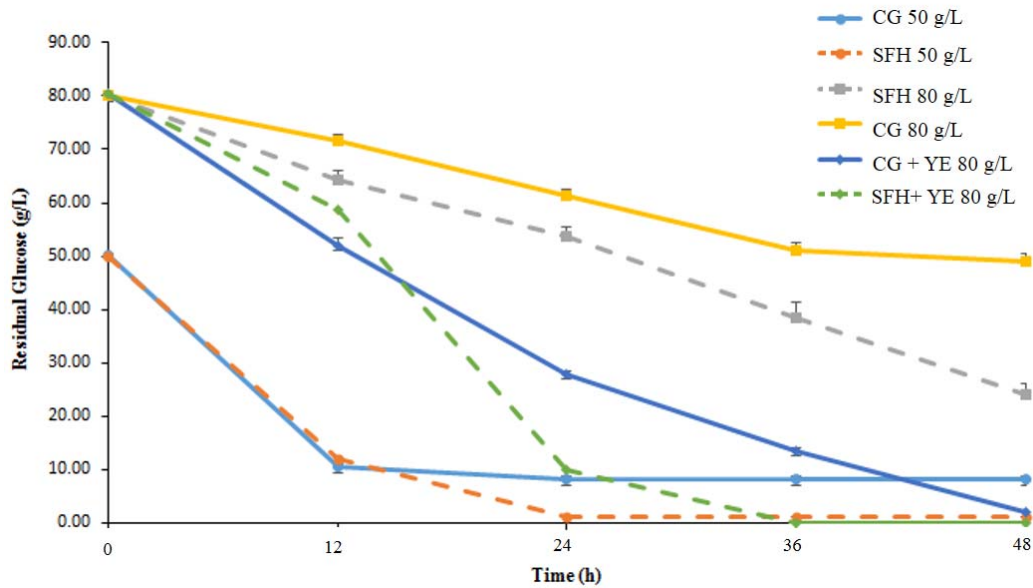
Cycle	Glucose Concentration, (g/L)
I	46.24 <sup>c</sup> ± 2.27
II	100.10 <sup>b</sup> ± 2.96
III	149.36 <sup>a</sup> ± 1.53

\*Means with the same letter are not significant at  $p < 0.05$ .

From the results, 7 % (w/v) SFP shows significantly higher glucose concentration as the total substrate load accounted, 21 % (w/v) upon completion of three-cycle enzymatic hydrolysis process. It was revealed that conducting hydrolysis process was much easier for cycle II and cycle III due to better solubilisation properties [10]. Addition of enzyme in subsequent hydrolysis cycle would maintain the substrate and enzyme ratio [18]. Repeating and boiling during enzymatic hydrolysis process will assists the starch granules dispersion, thus contribute to higher recovery of starch into glucose [19].

### 3.3 SFH as the sole fermentation medium for bioethanol production

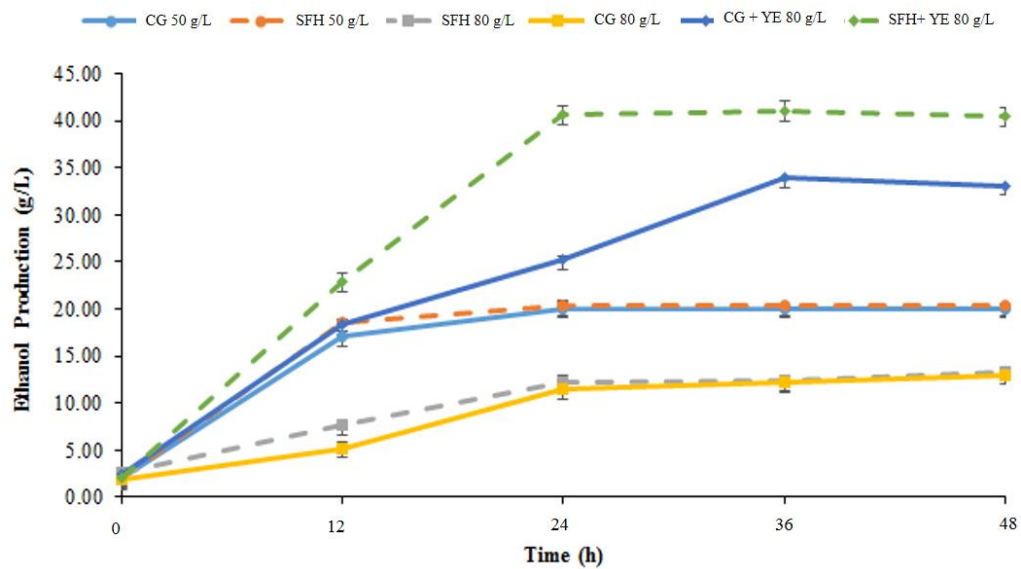
In this study, SFH (without addition of any nitrogen source) was used as the sole fermentation medium for generating alcohol utilizing 50 g/L and 80 g/L of initial glucose concentration, compared to commercial glucose. Figure 1 represents the fermentation profiles, indicating glucose consumption in SFH and CG media by baker's yeast during 24 until 48 hours of fermentation.



**Figure 1.** The glucose consumption profiles in SFH fermentation by commercial baker's yeast (CBY) under 50 g/L and 80 g/L initial glucose concentration.

Based on the fermentation profiles, it was observed that, 50 g/L of glucose in SFH media was almost consumed by the commercial baker's yeast (CBY) during 24 hours of fermentation, with remaining glucose at  $1.11 \pm 0.01$  g/L. Thus, the total glucose consumption in 50 g/L SFH media was 97.78 %, which indicates the fastest glucose consumption, compared to 50 g/L glucose in CG media, with total glucose consumption of 83.81 %, at 24 hours of fermentation. In contrast to 80 g/L of initial glucose concentration, it was observed that, 80 g/L of glucose in SFH media was not completely consumed by the yeast during 48 hours of fermentation, with remaining glucose of  $24.155 \pm 1.40$  g/L, thus indicates 69.83 % of glucose consumption. Similar trend was also observed under 80 g/L of glucose in CG media. The remaining glucose was at  $(48.99 \pm 1.40)$  g/L, with lower total glucose consumption of 38.75 %. In this fermentation condition, 80 g/L glucose in SFH media demonstrated slightly better performance on glucose consumption rather than in 80 g/L glucose of CG media.

Figure 2 represent the fermentation profiles, indicating ethanol production in SFH fermentation by CBY during 24 until 48 hours of fermentation. Based on the results obtained, it was observed that, 50 g/L glucose from SFH media can generate maximum ethanol during 24 hours of fermentation, which at  $20.33 \pm 0.15$  g/L. Similar ethanol concentration was obtained in 50 g/L glucose of CG media, which is at  $20.04 \pm 0.06$  g/L. However, in 80 g/L glucose of SFH, lower ethanol was produced ( $13.32 \pm 0.12$  g/L, 7 % lower) than in 50 g/L SFH media. Similar ethanol profile was obtained under 80 g/L glucose in CG fermentation, which is about  $12.98 \pm 0.04$  g/L. Based on the ethanol production profiles, higher and maximum concentration of ethanol was achieved when lower initial glucose concentration (50 g/L) was introduced, whereas lower concentration of ethanol was generated when higher initial glucose concentration was introduced (80 g/L). In this condition, the yeast might be unprotected to severe conditions such as high osmotic pressure, which increased the by-product inhibition and nutritional limitations [20]. Normally, inadequate of nitrogen source may also cause inhibition towards the yeast growth, thus reduce the ethanol conversion, as well as ethanol yield [21, 22].



**Figure 2.** The ethanol production profiles in SFH fermentation by baker's yeast under 50 g/L and 80 g/L initial glucose concentrations.

Table 3 represent the fermentation performance of SFH media utilizing 50 g/L and 80 g/L glucose as the sole carbon source for production of bioethanol. Based on the kinetic parameters, 50 g/L of initial glucose concentration in SFH media shows the highest glucose consumption efficiency (97.78 %), higher fermentation efficiency ( $81.35 \pm 0.572$  %), with respective ethanol yield of  $0.42 \pm 0.003$  g/g, indicating that CBY was well tolerates with SFH as the sole carbon source for ethanol production. However, when glucose concentration increased up to 80 g/L either in SFH or CG media, the ethanol fermentability decreased tremendously. Hence, selected yeast extract had been added into the fermentation media in order to improve ethanol fermentation process. Throughout the trial of adding yeast extract in fermentation media containing 80 g/L of initial glucose concentration, improved ethanol concentration were obtained in SFH ( $41.04 \pm 0.04$  g/L) and CG ( $33.96 \pm 0.04$  g/L). Both media able to generate maximum ethanol concentration during 36 hours of fermentation, much earlier than expected time for the fermentation to complete. This result indicates that glucose in SFH media (amended with YE) was fully metabolized by the CBY after 24 hours of fermentation, with 100 % glucose consumption efficiency. However, in 80 g/L glucose of CG media, the glucose was almost utilized during 48 hours of fermentation, indicates 97.33 % of glucose consumption efficiency, slightly slower compared in 80 g/L glucose in SFH media.

Interestingly, both ethanol yield ( $Y_{p/s}$ , g/g) and fermentation efficiency ( $E_f$ , %) were improved from  $0.24 \pm 0.01$  g/g to  $0.51 \pm 0.002$  g/g and  $46.65 \pm 1.50$  % to  $99.71 \pm 0.46$  % in 80 g/L SFH media only and 80 g/L SFH media (amended with YE), respectively. Clearly, the addition of YE as nitrogen source into hydrolysate with 80 g/L glucose, significantly assisting in fermentation process, thus enhanced the ethanol fermentability towards the CBY. In this fermentation condition, once higher initial glucose ( $>50$  g/L) was introduced into the same process, both ethanol yield and fermentation efficiency shows slight decline on their value, although the fermentation time was prolonged in order to achieve maximum ethanol concentration. Usually, the extension of fermentation time is a common practice in high gravity (HG) or very high gravity (VHG) fermentation, which also could decrease the ethanol productivity correspondingly [23]. Supplementation of correct amount and sufficient nitrogen

sources are crucial in order to increase the ethanol yield, as well as to enhance the fermentation efficiency and ethanol volumetric productivity of the fermentation process.

**Table 3.** Kinetic parameters of ethanol fermentation utilizing SFH (with addition of yeast extract) at initial glucose of 50 g/L and 80 g/L

Kinetic Parameters	50 g/L		80 g/L			
	SFH	CG	SFH	CG	SFH + YE	CG+ YE
Initial Glucose (g/L)	50.00 ± 0.00	50.28 ± 0.14	80.06 ± 0.04	79.98 ± 0.08	80.54 ± 0.44	80.51 ± 0.41
Glucose Consumption Efficiency (%)	97.78	83.81	69.83	38.75	100	83.24
Ethanol (g/L)	20.33 ± 0.15	20.04 ± 0.06	13.32 ± 0.09	12.98 ± 0.05	41.04 ± 0.04	33.96 ± 0.04
$Y_{p/s}$ (g/g)	0.42 ± 0.00	0.48 ± 0.01	0.24 ± 0.01	0.42 ± 0.01	0.51 ± 0.00	0.51 ± 0.00
$Q_p$ (g/L/h)	0.85 ± 0.01	0.84 ± 0.00	0.28 ± 0.00	0.27 ± 0.00	1.14 ± 0.00	0.94 ± 0.00
$E_f$ (%)	81.35 ± 0.57	93.07 ± 0.10	46.65 ± 1.50	82.00 ± 2.25	99.71 ± 0.46	99.15 ± 0.11

Notes: Parameters studied:  $Y_{p/s}$  ethanol yield (g/g),  $Q_p$  ethanol volumetric productivity (g/L/h),  $E_f$  fermentation efficiency (%). All data presented are the average of duplicate experiments with standard errors. Theoretical ethanol yield is 0.511 g/g glucose.

## 4. Conclusion

Sago fibre hydrolysate (SFH) obtained via enzymatic hydrolysis of sago fibre powder has been discovered to be an alternative substrate for producing bioethanol using commercial baker's yeast. This study suggested that ethanol fermentation in SFH as a sole fermentation media was limited at 50 g/L initial glucose. The addition of yeast extract however able to boost ethanol fermentability when initial glucose had been increased up to 80 g/L.

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