Simultaneous saccharification and fermentation of sago hampas into biobutanol by *Clostridium acetobutylicum* ATCC 824

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**Abstract**
Simultaneous saccharification and fermentation (SSF) by *Clostridium acetobutylicum* ATCC 824 was conducted to produce biobutanol from sago hampas. Sago hampas is a waste generated from the processing of sago starch. This waste is composed of 54.6% starch and 31.7% of cellulose and hemicellulose, with only 3.3% of lignin. In order to fully utilize the starch and cellulosic materials, saccharification using a mixture of amylase (Dextrozyme) and cellulase (Acremonium cellulase) was conducted using 0.09 g/mL sago hampas, producing 67.0 g/L of fermentable sugar. The SSF and delayed SSF (DSSF) were conducted using 0.07 g/mL sago hampas with the optimized enzyme loading of Dextrozyme amylase (71.4 U/g substrate) and Acremonium cellulase (20 FPU/g substrate). The SSF of sago hampas generated 6.12 g/L of solvents with biobutanol concentration of 3.81 g/L and the yield of 0.11 g-biobutanol/g-sugar. In order to improve biobutanol concentration and productivity, DSSF was introduced. In DSSF, the inoculum was introduced into the system after 24 hour of fermentation to allow the optimal saccharification process for sugar production. This process generated 4.62 g/L of biobutanol which was 18% higher than normal SSF since the saccharification and fermentation were operated at their optimal condition.

**KEYWORDS**
ABE fermentation, biobutanol, *Clostridium acetobutylicum*, enzymatic saccharification, sago hampas, simultaneous saccharification and fermentation

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**INTRODUCTION**

The recent increase in energy demand and the depletion of fossil fuel have diverted the attention of researchers toward the utilization of various renewable resources for the production of biofuel such as biobutanol worldwide.¹ Biobutanol has been reported as a better liquid biofuel than bioethanol with promising potential to replace gasoline without modification.
on the current distribution and engine system. It has high hydrophobicity property which makes it safe and suitable to be applied directly or blended with gasoline as fuel. However, the major challenges in producing biobutanol from the renewable biomass are the cost of the fermentation substrate, multiple processing steps, strain capability, product inhibition, and multiple end products.

In order to make biobutanol more feasible for industrial application, a suitable substrate for fermentation has been investigated by many researchers. Some of the substrate used was starch-based materials such as corn, cassava, and konjac waste, and lignocellulosic-based materials such as king grass, oil palm empty fruit bunch, corn stover, oil palm decanter cake, wheat straw, and other substrates. Sago hampas, which is a waste produced in the processing of sago starch can be used as a substrate for biobutanol production. This substrate is produced at approximately 110 tonnes/year. In current practices, most of the sago wastes produced in sago mill are dumped in open land or directly disposed to the nearby river, which contributes to a serious environmental problem due to its slow degradability and high starch content.

Sago hampas is composed of 49.5% starch and 40.5% of cellulose and hemicellulose with only 7.5% of lignin. The processing of sago hampas into sugar does not require any pretreatments prior to the saccharification process. There is no inhibitor compounds were released after the saccharification of sago hampas, eliminating the detoxification process required prior to the fermentation process. This is notably important to reduce the operational cost as compared to other types of lignocellulosic biomass. The sago pith residues (SPR) obtained after saccharification of sago hampas by amylase is a lignocellulosic material that can be further hydrolysed by cellulase into a mixture of fermentable sugars. Most of the studies that have been conducted only focused on the utilization of the starchy component of sago hampas or lignocellulosic component of SPR as fermentation substrate.

There are limited studies on the utilization of both starch and lignocellulosic biomass have been reported especially on the simultaneous saccharification and fermentation (SSF) process to produce biobutanol. Therefore, the feasibility of this substrate for biobutanol production through SSF involving the combination of enzymatic saccharification by amylase and cellulase to work together is depicted in this paper.

The acetone-butanol-ethanol (ABE) fermentation is the most commonly known process for the production of biobutanol by solventogenic *Clostridium* sp. usually using sugars from biomass. In the conventional biobutanol production from biomass, the separate hydrolysis and fermentation (SHP) process is commonly used which involve a separate process of (1) saccharification of biomass into sugar and (2) the ABE fermentation of the sugar produced into biobutanol. The SSF that combines process (1) and (2) simultaneously shows the potential for biobutanol production since this process could reduce the number of processing steps and process duration, which could improve process productivity while maintaining biobutanol production yield. However, performing SHF ensures that both the enzyme and the microbe function under their optimal conditions, whereas SSF requires an optimal condition for saccharification and fermentation to be conducted simultaneously.

### 2 | MATERIALS AND METHODS

#### 2.1 | Microorganism and inoculum preparation

*Clostridium acetobutylicum* ATCC 824 was purchased from American Type Culture Collection (ATCC, USA). The inoculum was prepared by transferring 1 mL of the *C. acetobutylicum* ATCC 824 stock culture into 99 mL of commercial Reinforced Clostridial Medium (RCM) (Merck, Germany). The RCM was sparged with nitrogen gas for 15 minute and autoclaved at 121°C for 15 minute prior to the inoculation. The culture was grown at 37°C for 24–30 hour, depending on the growth profile until log phase was reached. The inoculum final OD₆₂₀ was set at 1.0 before it was used in the fermentation stage.

#### 2.2 | Substrate preparation

Sago hampas was collected from River Link Sago Resources Sdn. Bhd. in Mukah, Sarawak, Malaysia. The collected sago hampas was sun-dried for 1–2 days to eliminate excess water from the wet sago hampas. The sago hampas was then oven dried at 60°C for 24 hour. Next, the dried sago hampas was ground and kept at room temperature in a sealed plastic bag prior to use.

#### 2.3 | Saccharification of sago hampas

Dried sago hampas was gelatinized by boiling at 100°C for 15 minute using a water bath before cooling at room temperature to 60°C. The saccharification process was conducted by adding amylase (Dextrozyme DX 1.5X, Novozymes, Denmark) and/or cellulase (Acremonium, Meiji Seika Co, Japan) in each saccharification flask. The mixture was then incubated in a shaker incubator (Tuff, Malaysia), in their respective saccharification conditions. Saccharification by Dextrozyme amylase was conducted in 0.1 mol/L acetate buffer pH 4.2 and incubated at 60°C, 200 rpm for 60 minute. Meanwhile, saccharification by Acremonium cellulase was conducted in 0.05 mol/L acetate buffer pH 4.8 and incubated at 50°C, 200 rpm for 3 days. The saccharification mixture was then incubated in a mixture of enzymes (amylase + cellulase), was conducted in 0.1 mol/L acetate buffer pH 4.2 and incubated at 60°C, 200 rpm for 3 days. All the saccharification processes were carried out in 250-mL shake flasks with 100 mL working volume.
The saccharification conditions of sago hampas with the highest sugar yield was used in the subsequent experiment.

2.4 \ \textbf{ABE fermentation}

The ABE fermentation process was conducted and modified based on the study by Ibrahim et al.\textsuperscript{11} and the National Renewable Energy Laboratory (NREL) on SSF Experimental Protocols-Lignocellulosic Biomass Hydrolysis and Fermentation.\textsuperscript{28} The fermentation media consist of P2 medium components, yeast extract (BD, USA), acetate buffer, Dextrozyme amylase and/or Acremonium cellulase and distilled water (for dilution purpose). The P2 medium components are buffer, vitamin and mineral solution which were filter-sterilized as described by Monot et al.\textsuperscript{29} The yeast extract, 0.05 mol/L acetate buffer (pH 5.5) and distilled water were sparged with nitrogen gas for 15 minute and autoclaved at 121°C for 15 minute before used. Dextrozyme amylase and Acremonium cellulase were sparged with nitrogen gas for 15 minute and filter sterilized using a 0.2 μm nylon driven filter prior to fermentation.

2.4.1 \ \textbf{Separate hydrolysis and fermentation}

The SHF process was performed in 125-mL serum bottles with 100 mL working volume. The SHF medium was composed of 6 g/L of yeast extract, 50 g/L of sago hampas hydrolysate or commercial glucose (as control) prepared in 0.05 mol/L of acetate buffer, pH 5.5. The SHF medium was sparged with nitrogen gas for 15 minute before autoclaved at 121°C for 15 minute.\textsuperscript{28} Then, 2 mL of each P2 medium (buffer, vitamin and mineral solution) were added into the fermentation flask before precultured with 10 mL of \textit{C. acetobutylicum} ATCC 824. Fermentation was carried out at 37°C for 120 hour with 150 rpm shaking speed. Samples were collected every 24 hour and kept at −20°C in the freezer prior to analysis.

2.4.2 \ \textbf{Simultaneous saccharification and fermentation}

Sago hampas at 0.07 and 0.09 g/mL were weighed into serum bottles and added with 6 g/L of yeast extract dissolved in 0.05 mol/L of acetate buffer pH 5.5 before sparging with nitrogen gas at 37°C for 120 hour with 150 rpm shaking speed. Samples were collected every 24 hour and kept at −20°C in the freezer prior to analysis.

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|l|l|}
\hline
\textbf{Substrate} & \textbf{Substrate collection} & \textbf{Starch} (%) & \textbf{Cellulose} (%) & \textbf{Hemicellulose} (%) & \textbf{Lignin} (%) & \textbf{Others} (%) & \textbf{References} \\
\hline
Sago Hampas & Narathiwat, Thailand & 49.7 & 20.2 & 7.9 & 3.3 & – & 56 \\
 & Pusa, Sarawak & 49.5 & 26.0 & 14.5 & 7.5 & – & 19 \\
 & Pusa, Sarawak & 58.0 & 23.0 & 9.2 & 3.9 & – & 44 \\
 & Kuching, Sarawak & 52.0 & 16.0 & 9.8 & 5.2 & – & 57 \\
 & Sibu, Sarawak & 37.0 & 40.0 & 13.0 & 4.5 & – & 58 \\
 & Pusa, Sarawak & 45.0 & – & – & – & – & 26 \\
 & Mukah, Sarawak & 54.6 & 21.4 & 10.3 & 3.3 & 10.4 & This study \\
\hline
Sago Pith Residue (SPR) & Pusa, Sarawak & Nd & 44.0 & 14.5 & 4.9 & 4.7 & 19 \\
 & Pusa, Sarawak & Nd & 37.0 & 20.0 & 6.0 & – & 44 \\
 & Narathiwat, Thailand & Nd & 23.0 & 9.2 & 3.9 & – & 56 \\
 & Seratok, Sarawak & Nd & 38.0 & 53.0 & 6.0 & – & 59 \\
 & Mukah, Sarawak & Nd & 52.2 & 13.9 & 6.2 & 27.7 & This study \\
\hline
\end{tabular}
\caption{Chemical characteristics of sago hampas and sago pith residue (SPR) in % dry weight basis}

Nd, not detected.
150 rpm shaking speed. Samples were collected every 24 hour and kept at −20°C in the freezer prior to analysis.

2.5 Analytical procedures

The determination of starch content was done using iodine starch colorimetric methods reported by Nakamura, while the determination of lignocellulosic content was conducted based on the gravimetric method described by Goering and Van Soest. The ABE and organic acids were analysed using gas chromatography (Model GC-17A, Shimadzu, Japan) equipped with column BP21 and flame ionization detector following the methods by Ibrahim et al. The sugar monomers were quantified using a HPLC (Jasco, Japan) equipped with a refractive index (RI) detector and a column (Shodex KS-801, Japan) for ligand exchange chromatography. The mobile phase used was 100% ultrapure water with a flow rate of 0.6 mL/min and the temperature of the column was fixed at 80°C using an oven column. The reducing sugar concentration was determined using the dinitrosalicylic acid method by Miller. Glucoamylase assay for Dextrozyme amylase was done using the methods by Leaes et al. Cellulase assay for Acremonium cellulase was performed based on National Renewable Energy Laboratory-Measurement of Cellulase Activities.

3 RESULTS AND DISCUSSIONS

3.1 Sago hampas characteristics

To evaluate the quality of the substrate for fermentation, the chemical compositions of sago hampas and SPR were determined as shown in Table 1. The starch content in sago hampas was 54.6% on a dry weight basis, which is slightly higher as compared to the previously reported studies. The value for cellulose, hemicellulose and lignin was 21.4%, 10.3%, and 3.3%, respectively, with the remaining compositions are ash and other components. Starch possesses the highest percentage composition in sago hampas as compared to other chemical compositions. SPR, which is the solid residue left after the saccharification of sago hampas into α-glucose. The solid residue (SPR) obtained after the saccharification of sago hampas by Dextrozyme amylase contained 66.1% of cellulose, 13.9% of hemicellulose, 6.2% of lignin, and other components such as ash and extractive. The higher carbohydrate composition (starch, cellulose, and hemicellulose) of more than 86.3% in sago hampas as compared to other types of biomass provide beneficial advantages of sago hampas to be used as fermentation feedstock including for biobutanol production.

Besides, this substrate composed of a very low percentage of lignin as compared to other types of lignocellulosic biomass, and therefore no pretreatment process was needed prior to saccharification or fermentation, reducing the number of steps and cost of operation. Table 1 also shows the tabulated locations of the sample collection. It should be noted that the difference of the chemical compositions of sago hampas might be due to the different location and batch of the plantation, in which the soil possesses different nutrient composition.

3.2 Saccharification of sago hampas

The aim of performing the enzymatic saccharification of sago hampas is to obtain a high concentration of sugar for biobutanol production. The optimum sugar concentration for ABE fermentation by Clostridia was around 60-80 g/L. Therefore, it is important to generate enough sugar for ABE fermentation, especially for SSF. Low sugar concentration of below than 40 g/L could cause the Clostridium sp. to produce more acids than solvents. In order to obtain a high sugar concentration from sago hampas, saccharification using a mixture of amylase and cellulase was conducted.

3.2.1 Effect of Dextrozyme amylase loadings

For the saccharification of sago hampas using Dextrozyme amylase at 285.7 U/gsubstrate it was observed that a runnier solution was formed after 1 hour of the saccharification process as compared to a thick viscous solution before adding the Dextrozyme amylase. This process generated 33.6 g/L of glucose after 1 hour of saccharification time. The hydrolysis yield of glucose per starch content was 79.1%. A complete degradation of starch in sago hampas was due to the presence of amyloligosidase and pullulanase in Dextrozyme amylase. These two enzymes are necessary to fully hydrolyze α-1,4 and α-1,6 linkages in the starchy component of sago hampas into α-glucose.

In order to optimize the Dextrozyme amylase loading in the saccharification of sago hampas into glucose, a set of Dextrozyme amylase loading range of 0.5-20.0 U/mL was tested, which is equivalent to 7.1-285.7 U/gsubstrate. From this experiment, it was found that the optimal Dextrozyme amylase loading was 71.4 U/gsubstrate that had produced 31.6 g/L of glucose. Increasing the Dextrozyme amylase loading up to 285.7 U/gsubstrate did not significantly affect the glucose release as shown in Figure 1A.

3.2.2 Effect of Acremonium cellulase loadings

The solid residue (SPR) obtained after the saccharification of sago hampas by Dextrozyme amylase contained 66.1% of cellulose and hemicellulose, with 6.2% lignin, which was comparable to the findings by Linggang et al. The SPR was further hydrolysed by Acremonium cellulase which produced sugar monomers of 10.2 g/L of glucose, 1.6 g/L of xylose, 0.3 g/L of arabinose, and 1.6 g/L of cellobiose (disaccharide). The hydrolysis yield of total sugar over cellulose and hemicellulose...
content was equivalent to 65.4%. The saccharification process took 72 hour, a longer process duration as compared to saccharification by Dextrozyme amylase. This is because the structure of cellulose and hemicellulose is more complex than starch and required a mixture of cellulases (endoglucanase, exoglucanase, and β-glucosidase) to fully hydrolyse the cellulosic components.45

In order to determine the optimal cellulase loading, an experiment using a range of 10-80 FPU/gsubstrate of Acremonium cellulase was conducted. According to Ouyang et al,46 the cost of cellulase is highly contributed to the total cost of the hydrolysis process, therefore it is important to keep the enzyme dosage as minimal as possible. From this experiment, it was found that the Acremonium cellulase loading above 20 FPU/gsubstrate did not significantly affect the sugar released. The saccharification using 20 FPU/gsubstrate produced 24 g/L of total sugars, which is equivalent to 65.4% of hydrolysis yield.

3.2.3 | Effect of substrate loading

A higher sugar concentration could be obtained by increasing the substrate loading in the saccharification process which could meet the sugar concentration required for ABE fermentation. Therefore, sago hampas at concentrations of 0.05-0.13 g/mL were saccharified using the optimized enzyme loading of Dextrozyme amylase (71.4 U/gsubstrate) and Acremonium cellulase (20 FPU/gsubstrate). It was observed that sugars production was significantly increased when substrate concentration has been increased from 0.05 to 0.09 g/mL. Substrate loading of 0.11 and 0.13 g/mL showed no significant difference in sugar produced as compared to 0.09 g/mL, resulted in the reduction of hydrolysis yield.

In this study, although the level of reducing sugars produced was 77.2 g/L at sago hampas concentration of 0.13 g/mL, however, the hydrolysis efficiency decreased to 61.95% due to the failure of the hydrolysis process. This situation happened when the sago hampas concentration increased more than 0.09 g/mL, there are more solid materials present in the mixture, which cause high viscosity and thus prevent efficient mixing, limit mass transfer and reduce the adsorption of the enzyme to substrate. Therefore, it can be concluded that 0.09 g/mL of substrate loading is optimal to produce sugar from sago hampas. Besides, this substrate loading was generated 66.9 g/L of total sugar concentration which is enough to meet the sugar concentration required for ABE fermentation.

3.2.4 | Effect of saccharification temperature

Since saccharification will be conducted simultaneously with ABE fermentation, an effect on the saccharification temperature of 37°C (optimal temperature for ABE fermentation)11 and 60°C (optimal temperature for saccharification)47 by a mixture of Dextrozyme amylase and Acremonium cellulase.
was conducted. From this experiment, it was found that the total sugars produced was 63.2 and 66.9 g/L, at 37 and 60°C respectively (Figure 1). There is only 5.5% difference in sugar produced showing that the saccharification can be conducted at 37°C. In typical enzymatic saccharification, the temperature of 50–60°C was found to be optimum, however, in this study, the results showed that the saccharification between 37 and 60°C did not show any significant difference. This finding is also supported by Krishna et al, where saccharification by cellulase conducted in the range of 35-50°C, only a negligible decrease (maximum 8% for 35°C) in sugar yields was observed, however, they had tested on the saccharification using cellulase only. This result is useful for the SSF process as the optimum temperature is needed in both hydrolysis and fermentation, and high temperature is the limiting factor for Clostridia to grow, which subsequently caused a low yield of biobutanol. In addition, the glucose concentration determined was 51.2 g/L, with the presence of less than 2 g/L of each of maltose, arabinose, and cellobiose.

3.3 | ABE fermentation

3.3.1 | Separate hydrolysis and fermentation

Separate hydrolysis and fermentation (SHF) was conducted using sugar produced from the saccharification of sago hampas by a mixture of Dextrozyme amylase and Acremonium cellulase. The initial sugar concentration was set at 50 g/L and was compared with the SHF using 50 g/L of glucose. From this experiment, it was observed that SHF using glucose had produced a higher biobutanol (7.57 g/L) as compared to SHF using sago hampas hydrolysate (5.99 g/L). This observation might be due to the presence of a variety of sugars existed in the sago hydrolysate. Although Clostridia can consume both hexose and pentose sugars for their growth and products formation, these microorganisms show diauxic behavior, by which they consume glucose first followed by xylose. Ezeji et al also reported that Clostridia prefer glucose than C5-sources when tested in ABE fermentation model. This trend of sugar profile can also be seen in other SHF studies using lignocellulosic biomass as substrate. Because of this situation, the trend of solvents production showed a distinct behaviour in SHF using glucose over SHF using hydrolysate. The highest biobutanol production was obtained after 48 hour of glucose fermentation, and 72 hour of sago hampas hydrolysate fermentation. Most of the sugar in SHF has been consumed after 120 hour of fermentation and it was utilized as a source of energy for cell growth, acids, and solvents production. In the first 24 hour, the log phase of the cell growth is identified before it continues with ABE production during the stationary phase which was similar to the study by Ibrahim et al that employed similar microorganism but using different lignocellulosic hydrolysate.

Figure 2 also shows the acids profile of both SHF using glucose and sago hampas hydrolysate. A higher acetic acid (3.19 and 7.27 g/L) was produced as compared to butyric acid (1.05 and 2.37 g/L) for both fermentations using glucose and hydrolysate respectively. A higher acid produced in

**FIGURE 2** SHF by *Clostridium acetobutylicum* ATCC 824 using (A & B) 50 g/L glucose and (C&D) 50 g/L sago hampas hydrolysate. Symbols represented X, cell; o, pH; +, reducing sugar; c, acetone; □, butanol; △, ethanol; ◊, acetic acid; ■, butyric acid. All the fermentations were conducted in triplicates for reproducibility check, and representative profiles are presented.
SHF using hydrolysate than glucose might be the reason for a low biobutanol produced in SHF using hydrolysate. In SHF using hydrolysate, the cells need to adapt with different kinds of sugar composition which limits the cells to re-assimilate the acids to be converted into solvents. It should be noted that the amount of acid might be different intra- and extracellular as has been reported by Maddox et al.\textsuperscript{51} and Ibrahim et al.\textsuperscript{32}

### 3.3.2 Simultaneous saccharification and fermentation

Simultaneous saccharification and fermentation using sago hampas as substrate was performed under the similar condition of SHF, except, the substrate used was dried sago hampas at the concentration of 0.07 g/mL as shown in Figure 3A,B and 0.09 g/mL as shown in Figure 3C,D. From this experiment, only 28 g/L of sugar was consumed, from 35.6 to 59.2 g/L of total sugar produced using 0.07 and 0.09 g/mL of sago hampas respectively. The sugars from both SSF processes do not completely utilized especially in fermentation using 0.09 g/mL sago hampas that had left about half of the sugar concentration (29.16 g/L). This situation resulted with a lower biobutanol production in fermentation using 0.09 g/mL sago hampas (2.77 g/L) as compared to 0.07 g/mL sago hampas that produced 3.81 g/L of biobutanol, with the yield of 0.05 g-biobutanol/g-sugar and 0.11 g-biobutanol/g-sugar respectively.

The sugar was not completely consumed during SSF due to a high solid presence and low water content that have caused poor mixing and less space for cell growth.\textsuperscript{52} In order to supply a high sugar concentration for SSF process, high solid content was needed. However, high solid concentration has an adverse effect on the \textit{Clostridium} sp. since the cells have a low tolerance to fluid shear stress in high solid content,\textsuperscript{52} resulting in low biobutanol yield and concentration as compared to SHF. Although the biobutanol yield in SSF is slightly lower than SHF, SSF could still provide a better productivity than SHF besides its advantages in reducing the number of steps, time, and apparatus.\textsuperscript{11} The initial pH was set at pH 5.5 based on previous studies.\textsuperscript{11,53,54} The pH was dropped to 4.9 after 24 hour of fermentation due to the formation of acids indicating the acidogenic phase of the ABE fermentation and slightly increased during the solventogenic phase.

### 3.3.3 Delayed simultaneous saccharification and fermentation

The delayed simultaneous saccharification and fermentation (DSSF) process was done to reduce the viscosity of the slurry at high solid loadings and increase the efficiency of saccharification and fermentation since the enzyme and the microbe were operated at their optimal conditions.\textsuperscript{11} The reducing sugars produced after 24 hour of saccharification was 43.61 g/L, which should be enough for cells to initiate the biobutanol production from ABE fermentation. This result was in agreement with Ibrahim et al.\textsuperscript{11} who had suggested that the ABE fermentation should be supplemented with at least 40 g/L of sugar concentration for the whole process to work. From this experiment, 36.51 g/L of sugar was consumed from 43.61 g/L of total

![Figure 3](image-url)
sugar produced in the system using 0.07 g/mL of sago hampas (Figure 4). The sugars produced by DSSF was almost completely utilized by the cells with the remaining sugar detected was 7.10 g/L. A significant amount of biobutanol production was achieved at approximately 4.62 g/L with the yield and productivity of 0.11 g-biobutanol/g-sugar and 0.06 g/L·hour, respectively. This yield is 50% higher than the normal SSF. According to Paulová et al.55 a typical SSF process has limited sugar availability due to a different optimum temperature for saccharification and fermentation, and DSSF could enhance the productivity and shorten the process as compared to normal SSF. This is because the cells were introduced into the system after 24 hour of saccharification where the presaccharification step help to prepare readily sugar to be consumed by the cells right after the cells were introduced into the system.

4 | CONCLUSIONS

In conclusion, this study has shown the potential of biobutanol production from sago hampas using both SHF and SSF, involving a mixture of amylase and cellulase. The hydrolysis of starch and lignocellulosic components of sago hampas was successfully conducted using a mixture of Dextrozyme glucoamylase and Acremonium cellulase at optimum enzyme loading of 71.4 U/g-substrate of amylase and 20 FPU/g-substrate of cellulase, substrate concentration of 0.09 g/mL, with no pretreatment involved. The optimum sugar concentration for ABE fermentation (60-80 g/L) was obtained from this hydrolysis which was equivalent to 67.0 g/L. SHF process of sago hampas produced significantly high biobutanol titter of 5.99 g/L while the SSF process produced 2.77 g/L of biobutanol. The SSF was improved by introducing DSSF that generated higher biobutanol concentration of 4.62 g/L, which is 1.5-fold higher productivity as compared to normal SSF.

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REFERENCES

1. Mamman AS, Lee J-M, Kim Y-C, et al. Furfural: hemicellulose/xylose- derived biochemical. Biofuels Bioprod Bioref. 2008;2:438-454.
2. Mascal M. Chemicals from biobutanol: technologies and markets. Biofuels Bioprod Bioref. 2012;6:483-493.
3. Ndaba B, Chiyanzu I, Marx S. n-Butanol derived from biochemical and chemical routes: a review. Biotechnol Rep. 2015;8:1-9.
4. Ibrahim MFMF, Ramli N, Kamal Bahrin E, Abd-Aziz S. Cellulosic biobutanol by Clostridia: challenges and improvements. Renew Sustain Energy Rev. 2017;79:1241-1254.
5. Ezeji TC, Qureshi N, Blaschek HP. Bioproduction of butanol from biomass: from genes to bioreactors. Curr Opin Biotechnol. 2007;18:220-227.
6. Lee SY, Park JH, Jang SH, Nielsen LK, Kim J, Jung KS. Fermentative butanol production by Clostridia. Biotechnol Bioeng. 2008;101:209-228.
7. Pfromm PH, Amanor-Boadu V, Nelson R, Vadlani P, Madl R. Bio-butanol vs. bio-ethanol: a technical and economic assessment for corn and switchgrass fermented by yeast or Clostridium acetobutylicum. Biomass Bioenerg. 2010;34:515-524.
Clostridium saccharoperbutylacetonicum N1-4. Appl Biochem Biotechnol. 2010;161:157-170.

9. Shao M, Chen H. Feasibility of acetone-butanol-ethanol (ABE) fermentation from Amorphophallus konjac waste by Clostridium acetobutylicum ATCC 824. Process Biochem. 2015;50:1301-1307.

10. Gallego LJ, Escobar A, Peñuela M, Peña JD, Rios LA. King Grass: a promising material for the production of second-generation butanol. Fuel. 2015;143:399-403.

11. Ibrahim MF, Abd-Aziz S, Yusoff MEM, Phang LY, Hassan MA. Simultaneous enzymatic saccharification and ABE fermentation using pretreated oil palm empty fruit bunch as substrate to produce butanol and hydrogen as biofuel. Renew Energy. 2015;77:447-455.

12. Wang L, Chen H. Increased fermentability of enzymatically hydrolyzed steam-exploded corn stover for butanol production by removal of fermentation inhibitors. Process Biochem. 2011;46:604-607.

13. Abdul Razak MN, Ibrahim MF, Yee PL, Hassan MA, Abd-aziz S. Statistical optimization of biobutanol production from oil palm decanter cake hydrolysate by Clostridium acetobutylicum ATCC 824. BioResources. 2013;8:1758-1770.

14. Qureshi N, Saha BC, Hector RE, Hughes SR, Cotta MA. Butanol. A national laboratory 23 of the U.S. Department of Energy; 2008, p. 20.

15. Awg-Adeni DS, Bujang KB, Hassan MA, Abd-Aziz S. Recovery of glucose from residual starch of sago hampas for bioethanol production. Biomed Res Int. 2013;2013:Article ID 935852.

16. Malaysian Department of Statistic. Statistics of Sago: External Trade in Sago 27 Flour and Starches of Sago Palm, 2012, 28-31 Statistics of Sago. 2012;28-31.

17. Abd-Aziz S. Sago starch and its utilisation. J Biosci Bioeng. 2002;94:526-529.

18. Apun K, Jong BC, Salleh MA. Screening and isolation of a cellulolytic and amylolytic Bacillus from sago pith waste. J Gen Appl Microbiol. 2000;46:263-267.

19. Jenol MA, Ibrahim MF, Yee PL, Abd-aziz S. Sago biomass as a sustainable source for biohydrogen production by Clostridium butyricum A1. BioResources. 2014;9:1007-1026.

20. Guan W, Xu G, Duan J, Shi S. Acetone–butanol–ethanol production from fermentation of hot-water-extracted hemicellulose hydrolysate of pulping woods. Ind Eng Chem Res. 2018;57:775-783.

21. Liew ST, Arbakaria A, Rosfarizan M, Raha AR. Production of solvent (acetone-butanol-ethanol) in continuous fermentation by Clostridium saccharobutylicum DSM 13864 using gelatinised sago starch as a carbon source. Malays J Microbiol. 2006;2:42-50.

22. Madihah MS, Ariff AB, Khalil MS, Suraini AA, Karim MIA. Anaerobic fermentation of gelatinized sago starch-derived sugars to acetone-1-butanol-ethanol solvent by Clostridium acetobutylicum. Folia Microbiol. 2001;46:197-204.

23. Linggang S, Phang LY, Wasoh H, Abd-Aziz S. Acetone–butanol–ethanol production by Clostridium acetobutylicum ATCC 824 using sago pith residues hydrolysate. Bioenergy Res. 2013;6:321-328.

24. Sasaki C, Kushiki Y, Asada C, Nakamura Y. Acetone–butanol–ethanol production by separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) methods using acorns and wood chips of Quercus acutissima as a carbon source. Ind Crops Prod. 2014;62:286-292.

25. Guan W, Shi S, Tu M, Lee YY. Acetone-butanol-ethanol production from Kraft paper mill sludge by simultaneous saccharification and fermentation. Bioresour Technol. 2016;200:713-721.

26. Awg-Adeni DS, Bujang KB, Hassan MA, Abd-Aziz S. Recovery of glucose from residual starch of sago hampas for bioethanol production. Biomed Res Int. 2013;2013:935852.

27. Jenol MA. Biohydrogen production from sago hampas by Clostridium butyricum A1. Selangor: Universiti Putra Malaysia; 2014.

28. Dowe N, McMillan J. SSF experimental protocols—lignocellulosic biomass hydrolysis and fermentation. Golden, CO: National Renewable Energy Laboratory; 2008.

29. Monot F, Martin J-R, Petidemange H, Gay R. Acetone and butanol production by Clostridium acetobutylicum in a synthetic medium. Appl Environ Microbiol. 1982;44:1318-1324.

30. Nakamura LK. Lactobacillus amylovorus, a new starch-hydrolyzing species from cattle waste-corn fermentations. Int J Syst Bacteriol. 1981;31:56-63.

31. Georing HK, Van Soest PJ. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and some Applications). Charlottesville, VA: U.S. Agricultural Research Service, U.S. Agricultural Research Service, The University of Virginia; 1970, p. 20.

32. Ibrahim MF, Linggang S, Jenol MA, Yee PL, Abd-Aziz S. Effect of buffering system on acetone-butanol-ethanol fermentation by Clostridium acetobutylicum ATCC 824 using pretreated oil palm empty fruit bunch. BioResources. 2015;10:3890-3907.

33. Denko S. Food Analysis with Shodex Columns. Kanagawa: Canadian Life Science; 2006.

34. Miller GL. Use of dinitrosaiicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31:426-428.

35. Leaes EX, Lima D, Miklasevicius L, et al. Effect of ultrasound-assisted irradiation on the activities of α-amylase and amyloglucosidase. Biocatal Agric Biotechnol. 2013;2:21-25.

36. Adney B, Baker J. Measurement of Cellulase Activities. Colorado: A national laboratory 23 of the U.S. Department of Energy; 2008.

37. Kim M, Day DF. Composition of sugar cane, energy cane, and sweet sorghum suitable for ethanol production at Louisiana sugar mills. J Ind Microbiol Biotechnol. 2011;38:803-807.

38. Bellido C, Loureiro Pinto M, Coca M, Gonzalez-Benito G, Garcia-Cubero MT. Acetone-butanol-ethanol (ABE) production by Clostridium beijerinckii from wheat straw hydrolysates: efficient use of penta and hexa carbohydrates. Bioresour Technol. 2014;167:198-205.

39. Bledzki AK, Mamun AA, Volk J. Physical, chemical and surface properties of wheat husk, rye husk and soft wood and their polypropylene composites. Compos Part A Appl Sci Manuf. 2010;41:480-488.

40. Awang Adeni DS. Bioethanol Production from Residual Starch of Sago (Metroxylon Sago) Hampas. Universiti Putra Malaysia; 2015.

41. Qureshi N, Saha BC, Hector RE, et al. Production of butanol (a biofuel) from agricultural residues: Part II – Use of corn stover and switchgrass hydrolysates. Biomass Bioenerg. 2010;34:566-571.
SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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