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Effect of storage condition on the viability of sago effluents as carbon source in fermentation medium for bioethanol production

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Abstract. In Sarawak, Malaysia, approximately 237 tons/day of sago effluent is commonly discharged into nearby river due to the sago starch extraction process. Due to the high concentration of polymeric compounds, particularly starch, in sago wastewater, which petrifies easily, this condition severely pollutes the environment in the affected area. This study was conducted to determine the viability of using sago effluent as a carbon source and fermentation medium for bioethanol production which indirectly help to minimize the environmental impact as well as the economics of the sago industry. The sago effluent obtained from the local sago mill was analysed for starch content and pH profile while stored at room and cold (4°C) temperature facility. Enzymatic hydrolysis was conducted to convert the residual starch into glucose as carbon source for bioethanol fermentation. Fresh sago effluent can be stored for up to 5 days in cold temperature where the starch content remains constant. The highest starch concentration in sago effluent was 61.33 g/L, in which 50.57 g/L glucose was obtained through the enzymatic hydrolysis process. Hence 82.5% of the starch to glucose conversion yield is revealed. Then, the sago effluent hydrolysate which acts as a carbon source as well as a fermentation medium able to generate 23.14 g/L of bioethanol, displays a 91% theoretical yield of glucose to ethanol. In conclusion, the utilization of sago wastewater as feasible alternative to cheap and locally available and sustainable source of raw materials to produce bioethanol.

Keywords: bioethanol, commercial bakers' yeast, fermentable sugar, residual starch, sago effluent

INTRODUCTION

Sago starch is produced in Sarawak from the sago palm industry and contributes to the Gross Domestic Product (GDP) Sarawak produces about 200,000 tons of sago starch and exports 47,000 tons annually, making it one of the largest producers of starch in the world (Ahmad *et al.*, 2022). The Javanese word "sago" refers to palm pith that contains starch. Its scientific name is *Metroxylon sago*, where metra stands for the parenchyma or pith and xylon for the xylem (Singhal *et al.*, 2008). The sago palm is the ideal starch crop, as it can produce 20 to 25 tons more starch per hectare per year than cassava, rice, or corn (Flores, 2008). The pith of the sago trunk needed to be pulverized for starch extraction. The tremendous procedure including tree cutting, log shattering, sago pith crushing, starch extraction, starch filtering, drying process, and packing must be completed, according to Nishimura *et al.* (2010). Sontoso *et al.* (2015) estimate that the pith's actual content of starch is about 65%; the remaining 35% is made up of fiber. In the sago pith waste, the leftover starch is still encased in parenchyma cells or fibers (Mohd *et al.*, 2001; Lai *et al.*, 2013; Awg-Adeni *et al.*, 2013). Alias *et al.*, (2021) stated that the composition of raw sago hampas consist of 56.0% starch, 20.7% cellulose, 11.2% hemicellulose, 3.1% lignin, 6.35% moisture, 3% extractives and 6.1% others.

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Sago effluent is discoloured and susceptible to putrefaction. It contains finely milled fibres, inextricable starch, and polyphenols (Santoso, 2018). Existing treatment methods, including sedimentation ponds, have negative consequences on the surrounding communities, like foul odor. In this regard, sago effluent was discovered to be a suitable substrate for the production of bioethanol since it does not interfere with the production of food or animal feed (Limayem & Ricke, 2012). The reliance on food crops to produce biofuel had raise multiple ethical concern on food security, fuel disputes, and significant soil resource loss. (Balat & Balat,. 2009; Kennes *et al.*, 2015). In a number of studies, sago waste has been used to produce enzymes and adsorbents as a substrate, including those involving the production of glucose syrup (Asben *et al.*, 2011), fructose syrup (Mishima *et al.*, 2011; Linggang *et al.*, 2012), bioethanol (Peristiwati *et al.*, 2011; Awg-Adeni *et al.*, 2013; Muradi *et al.*, 2020;), biobutanol (Linggang *et al.*, 2011;), lactic acid and animal feed (Ahmad *et al.*, 2023) and animal feed (Ahmad *et al.*, 2024) Sago industry is often created on the banks of creeks or rivers, or in locations adjacent to water sources, as transported to production locations are sago logs from plantations or forest using water transport. Large-scale sago processing can cause a buildup of sago starch leftovers in rivers, which can cause pollution.

Sago waste has been investigated for use as animal feed, a planting medium for mushrooms, and a source of organic fertilizer (compost). Several existing researches shows the potential use of sago hampas and waste water as adsorbent (Singal *et al.*, 2008; Farith *et al.*, 2012; Sim *et al.*, 2012), food and animal feed (Ahmad *et al.*, 2023), bioethanol (Dhiputra *et al.*, 2015), sago waste water as a potential source of alternative energy (Mel *et al.*, 2012; Yunus & Raof, 2015; Zulkharnain & Jerim, 2015).

The processing of 20 tons of sago starch will release a minimum of 400 tons of sago effluent causing pollution to the environment due to its high organic content. They also have a lot of organic components in them (such as lipids, protein, and carbohydrates). The marine life and water quality are impacted by the wastewater's high demand for biological oxygen (about 5820 mg/L) and chemical oxygen (about 10,220 mg/L) by consuming the dissolved oxygen in the water (Rosli *et al.*, 2018). Hence, this research was conducted to determine the potential of sago effluent as a carbon source and fermentation media for bioethanol production.

MATERIALS AND METHODS

Sago effluent

The sago effluent was collected from Hedsen Sago Mill located in Pusa, Sarawak, Malaysia. The sago effluent was filled up into the 16 L bottle and kept at a cold temperature (4°C) and room temperature (25-30°C) before further analyses.

Effect of storage temperature on starch content in sago effluent

Once the sago effluent reached the laboratory, it was stored at two different temperatures, room temperature (25°C – 30°C) and 4°C. This study was carried out to study the effect of storage temperature on the starch content of the sago effluent, resulting in selected parameters such as the pH profile, starch and sugar concentrations for both storage conditions being recorded every 5 days over a period of 25 days.

Commercial enzymes for hydrolysis of sago effluents' residual starch

For the enzymatic hydrolysis procedure, two types of enzymes (NOVOZYME, Denmark), were used. Liquozyme SC DS and Spirizyme Fuel HS, were used. Liqiozyme SC DS is an α-amylases enzyme, used for the liquefaction process (Zhao *et al.,* 2009; Lareo *et al.,* 2013), whereas Spirizyme Fuel HS is a mixture of glucoamylases for the saccharification stage. The Spirizyme enzyme was used for hydrolysing 1, 4 and 1, 6 alpha linkages in order to liberate glucose for subsequent fermentation by yeast (Ramchandran *et al.,* 2015).

Commercial baker's yeast (CBY)

A commercial baker's yeast, *Saccharomyces cerevisiae* in the form of active dried yeast pellets, was used in this study. The activation process of the dried yeast occured in Yeast Malt Broth (YMB) medium and maintained in glycerol stock at -20°C. Potato dextrose agar and yeast peptone glucose agar were used to culture the yeast before being added to

100 mL of inoculum media that included 20 g/L glucose and 5 g/L yeast extract. According to Awg-Adeni *et al.* (2013), before the cell pellet could be added to the fermentation media, the inoculum was incubated at 30°C for nine hours and then centrifuged at 8000 rpm for five minutes.

Enzymatic hydrolysis of sago effluent residual starch

Firstly, the raw sago effluent was boiled for 30 minutes (Janggu and Bujang, 2009). Then, the iodine test was performed to determine the starch concentration. The analysis of residual starch was determined using the colorimetric iodine-starch method (Nakamura, 1981). After pre-treatment, Liquozyme® SC DS enzyme at 3.4 U/mL was added into the suspension and allowed for another 30 minutes in the liquefaction stage. The suspension was stirred constantly to ensure proper mixing of the substrate and enzymes. After that, the suspension was let to cool to between 50 and 60ºC. Spirizyme Fuel HS enzyme was added to the mixture for the saccharification process, and the mixture was shaken in the incubator for a further 5–6 hours. The cooling process of suspension was performed in a water bath at 20ºC to inhibit additional hydrolysis once the hydrolysis was complete. The reducing sugar obtained from the hydrolysis process was analysed using dinitrosalicyclic acid (DNS) (Miller, 1959).

Preparation of inoculum and fermentation media for bioethanol production

All media were prepared in triplicates and performed aseptically in a laminar air flow cabinet (ERLA Series). All inoculum was prepared using one loop of the single yeast colony from the PDA agar and inoculated into 100mL of sterile inoculum media which contains 20 g/L of glucose (commercial glucose) and 5 g/L of yeast extract. The inoculum flask was then incubated in the Gyromax$^{TM\ 706}$ Orbital Shaker Incubator (Hotech Instruments Corp.) at 30ºC at 100 rpm with controlled agitation for 6-7 hours. Once the OD of yeast cell's was between 0.5 and 0.6, the baker's yeast had reached maturity and was prepared for centrifugation (10,000 rpm, 5 minutes), which produces the yeast pellet. Finally, it was time to inoculate the yeast pellet into sterile fermentation media. Sago Effluent Hydrolysate (SEH) was autoclaved at 121ºC for 15 minutes before being used as a carbon source and fermentation medium for bioethanol production in the batch fermentation system. The fermentation was conducted triplicate at a 100 mL working volume, an initial pH 5.5–5.6, an agitation speed of 100 rpm and a controlled temperature at 30 ºC. In this trial, the fermentation medium was also added with 5 g/L of yeast extract as a supplement.

Analysis of sugar and bioethanol

The presence of glucose and ethanol in the fermentation broth, as well as starch from the sago effluents, which is hydrolyzed to sugars by enzymes, were analysed using a Shimadzu chromatographic system (Shimadzu, Kyoto, Japan), outfitted with a Shimadzu LC-20AT and Shimadzu RID- 10A, for high performance liquid chromatography (HPLC). Prior to analysis, the sample was centrifuged at 10,000 rpm for five minutes, filtered with a 0.2 µm Puradisc filter membrane and maintained at 4 ºC before being injected into an Aminex Fermentation Monitoring Column, (Aminex HPX-87H HPLC column, 150 × 7.8 mm, BioRad Laboratories, Inc) maintained at 60 ºC. 5 mM H_2S0_4 was used as the mobile phase, and a flow rate of 0.8 mL/min was employed.

Biomass concentration (Dry Cell Weight)

Each sample was centrifuged at 10,000 rpm for 15 minutes while being stored at 4°C. The clear supernatant was then collected and its glucose and ethanol content were examined. The yeast cells were suspended in sterilized distilled water and centrifuged again at 10,000 rpm for 15 minutes at 4 ºC following which the cell-free water was removed and the pellet were oven-dried at 60ºC overnight before the weight stays the same. The centrifuge tube was weighed again after drying (Lin *et al.*, 2012), and the dry cell weight (DCW) was determined as follows:

$$\frac{(weight\ of\ dried\ centrifuge\ tube + cells)\ g\ -\ (weight\ of\ centrifuge\ tube)\ g\ \times\ 10^3}{Sample\ volume\ (mL)}$$

RESULTS AND DISCUSSION

Effect of storage in cold temperature (4ºC)

Figure 1 shows the effect of storage time on the pH profiles, starch concentration and sugar recovery of sago effluent when stored at cold temperature (4ºC). From the graph, the highest starch concentration was detected on the first 5-day interval, which is between 47.35 g/L and 46.33 g/L. After day 5, the starch concentration starts to decrease tremendously until day 25. The sugar recovery also shows the highest concentration on day 0 and day 5, which are 39.85 g/L and 40.11 g/L, respectively. After day 5, the sugar started to decrease until day 25, whereas pH was observed to start to decline after day 5. Sugar recovery recorded for this study refers to sugar concentration obtained from available starch after undergoing the enzymatic hydrolysis process.

The enzyme used for the liquefaction process is Liquozyme SC DS. The pH range for Liquozyme SC DS is pH 5.0-6.0. When pH declined, the enzyme activity also declined resulting the lower concentration of starch. This statement supported the starch concentration on day 0 and day 5, in which the pH is not significantly different from the pH range for Liquozyme SC DS to work effectively. After day 5 until day 25, pH starts to extensively decline further from the pH range for Liquozyme SC DS, which result in a lower sugar recovery. Based on Figure 1, day 0 and day 5 show a higher percentage of sugar recovery, which is 84.21% and 86.54%, respectively. After day 5, the percentage of sugar recovery starts to decline until day 25.

Effect of storage in room temperature (25-30ºC)

Under this study, the effect of fresh sago effluent stored at room temperature (25-30ºC) was studied. Selected parameters such as pH value, starch concentration and sugar recovery were analysed every five days, as shown in Figure 2. The highest starch concentration is 47.35 g/L, which was observed at day 0. After that, the starch concentration starts to decrease tremendously until day 25. The starch concentration profiles indirectly influence the sugar recovery (39.85 g/L) which shows the highest production on day 0 and decreases sharply until day 25. On the other hand, the pH also depicts a similar trend, which was declined drastically from day 0 until day 25. After day 0 until day 25, pH starts to decline far from the pH range for Spirizyme Fuel SH, which result in a lower sugar recovery. Moreover, sugar recovery is also different between day 0 until day 25. Based on Figure 2, the highest percentage of sugar recovery is 84.15%. After day 0, the percentage of sugar recovery starts to decline until day 25. pH is one of the factors that play a role in the conversion of starch into sugar.

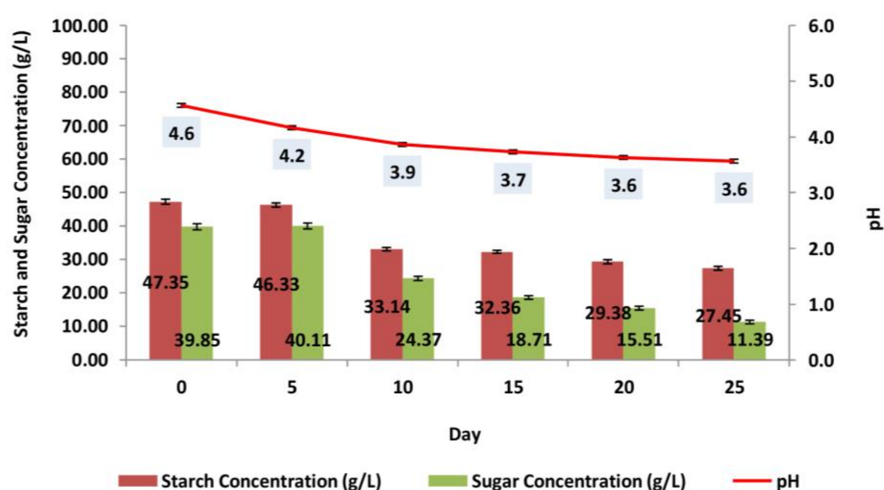

Figure 1. Effect of storage time for sago effluent based on pH value, starch concentration and sugar concentration at cold temperature (4 °C). One Way ANOVA method and Tukey tests method were employed in the statistical analysis. Every value is the mean SE of three replicates. At $p < 0.05$, means that share the same letter are not considered significant

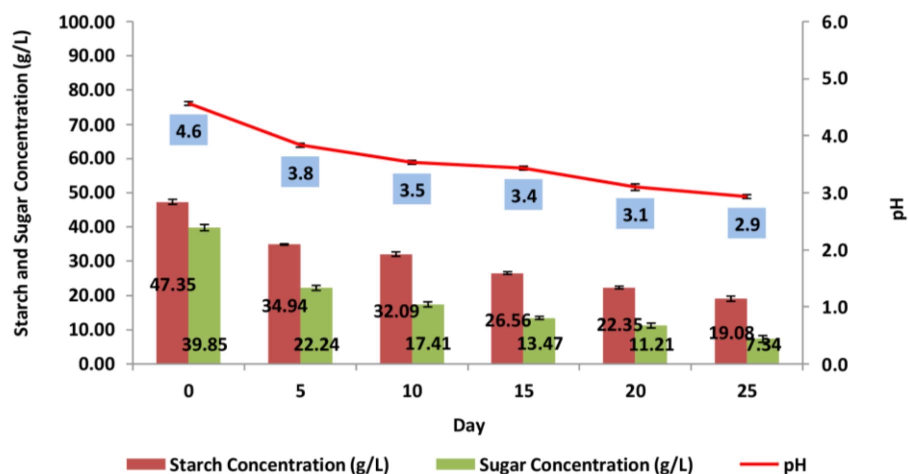

Figure 2. Effect of storage time for sago effluent based on pH value, starch concentration and sugar concentration at room temperature (25-30 °C). One Way ANOVA method and Tukey tests method were employed in the statistical analysis. Every value is the mean SE of three replicates. At $p < 0.05$, means that share the same letter are not considered significant.

Effect of pH reduction due to storage timeline and enzyme activity

The storage temperature for sago effluent plays an important role in controlling the pH value. When pH starts to decline, starch and sugar recovery also starts to decrease. Because dissolved particles, starch and trace elements are present, the pH of sago effluent was initially acidic and ranged from pH 3.5 to pH 5.6 (Kumar *et al.*, 2003). Microbial activity easily occurs at room temperature and hence will affect the pH value of sago effluent. Decomposition processes occur in sago effluent, resulting in the release of hydrogen ions in the wastewater and the production of carbon dioxide. Higher hydrogen ions cause the pH of the effluent to decrease (Bujang *et al.*, 2005). When pH declined, the enzyme activity also declined that resulting a lower concentration of starch. This statement supported the starch concentration on day 0, in which the pH is not significantly different from the pH range for Liquozyme SC DS. After day 0 until day 25, pH starts to decline tremendously, which eventually leads to lower starch concentration.

However, the enzyme used for the saccharification process is Spirizyme Fuel HS. Spirizyme Fuel HS is working within a broad operating range from pH 4.5 to 5.5. The ideal pH levels were 5.0, 4.5, and 4.0 for the liquefaction process, saccharification process, and simultaneous liquefaction and saccharification process, respectively (Zhao *et al.*, 2009). When pH declined, the enzyme activity also declined, that resulted in the lower recovery of sugar. This statement supported the sugar recovery on the day which the pH was not significantly different from the pH range for Spirizyme Fuel HS.

As we can see from the figure, when the pH dropped, the percentage of sugar recovery continuously declined. This situation returns to the effectiveness of the enzyme during the liquefaction and saccharification process. The enzyme will function well if the pH in the specified range. It is ideal to store sago effluent for five days in cold temperatures to maintain the starch content. The retrogradation of starch, which influences its gelatinization, digestibility, and rheology, can be impacted by lowering temperature (Chakraborty *et al.*, 2023). After being stored or chilled after gelatinization, eventually reassociate the disorganized amylose and amylopectin chains or reorganize them into a new organized structure (Chang *et al.*, 2021).

Enzymatic hydrolysis of sago effluent for glucose production

The sago effluent was prepared at 1 L scale after boiling pre-treatment step. Enzymatic hydrolysis of sago effluent was then conducted to convert starch that still exist in the effluent for glucose

production. On top of that, the hydrolysate obtained was also used as a fermentation medium for bioethanol production.

Enzymatic hydrolysis consists of two basic processes which are the liquefaction and saccharification process. "Gelatinization" refers to the melting of crystalline structures to produce starch. Gelatinization is a crucial process. Essential treatments include saccharification and liquefaction. A glucose polymer having 1,4- and 1,6-glycosidic linkages is starch. After being gelatinized, starch is easily hydrolyzed by enzymes or acids. The addition of Liquozyme SC DS (2 µL/g of SFP) to the suspension of gelatinized sago resulted in a runnier solution within 15-30 minutes of the liquefaction reaction. Then, Spirizyme Fuel HS (1 µL/g of SFP) was added during the saccharification stage in order to accomplish the glucose conversion process. Then, partly hydrolysed amylose and amylopectin molecules are depolymerised in the saccharification stage by glucoamylase action which eliminates the glucose units from non-reducing chain ends (Hii *et al*., 2012).

According to the total starch found in sago effluent (61.33 g/L), it is expected to obtain 6.13 grams of starch for every 100 grams of sago effluent. Theoretically in starch conversion, 1.0 gram of starch and water would generate about 1.11 g of glucose. The rate of starch-to-glucose conversion was determined using sago effluent hydrolysate and was quantified in terms of the amounts of glucose (g/L) and the percentage of conversion. Table 1 shows the glucose concentration and conversion yield after the enzymatic hydrolysis process.

Based on the result, the highest glucose concentration was 50.57 g/L obtained at 6 hours of enzymatic hydrolysis process. The starch-to-glucose conversion yield was 82.46%. Further enzymatic hydrolysis won't increase the sugar production indicating the respected procedure used in this study. In the enzymatic hydrolysis process, maltodextrin was initially released by lysozymes. Maltodextrin was obtained during the liquefaction process and contains mainly different oligosaccharides and dextrins (Sunaryanto *et al*., 2013). After all, the maltodextrin was then converted into glucose units by glucoamylase, which is an exozyme that cleaves off single glucose molecules by hydrolyzing α- (1, 4) linkage from the non-reducing ends of dextrins. During the saccharification stage, Spirizyme Fuel HS eventually hydrolyse starch completely to glucose along with, a little maltose and isomaltose, e.g maltotriose. Finally, the sugar hydrolysate produced from the hydrolysis process, which mainly contains 92% glucose was used as the carbon source for bioethanol production using commercial baker's yeast (CBY), Mauripan.

Table 1. Glucose concentration and starch to glucose conversion yield using 61.33 g/L starch of sago effluent.

Time (h)	Glucose Concentration (g/L)	Conversion Yield (%)[r]
0	5.75 [e] ± 0.04	9.37 [e] ± 0.07
2	15.42 [d] ± 0.03	25.14 [d] ± 0.04
4	30.04 [c] ± 0.06	48.98 [c] ± 0.10
6	50.57 [a] ± 0.13	82.46 [a] ± 0.22
8	46.19 [b] ± 0.14	75.31 [b] ± 0.22

Statistical analysis was determined using One ANOVA and Tukey tests. Each value is the mean ± SE of 3 replicates. Means with the same alphabet are not significant at $p < 0.05$. Notes: r = {glucose (g/L)/ starch (g/L)} x 100%. The data are the mean and ± standard errors (SE) of experiments performed in triplicate. Different alphabets mean significantly different values at a type one error rate of 0.05.

SEH as a carbon source and fermentation medium for bioethanol production

In this section, the sago effluent hydrolysate was further utilised as a carbon source and fermentation medium for bioethanol production. Bioethanol fermentability was analysed using three important parameters, namely glucose consumption profile, biomass concentration and ethanol production.

Based on the result shown in Figure 3, it was observed that from 12 hours into the 24-hour fermentation process, the yeast cells devoured nearly all of the glucose in the SEH media (91.18%). The residual glucose was found to be 4.46 ± 0.20 g/L at the end of fermentation. This indicate that the yeast cells actively metabolized glucose and were able to survive in SEH. Figure 3 shows that the DCW of the yeast cells has exhibited a consistent concentration at 12, 16, 20 and 24 hours of fermentation. Yeast, like all other microorganisms, goes through different growth stages (lag phase, logarithmic phase, stationary phase and death phase) (Bellissimi & Ingledew, 2005). There was a lag period, sometimes referred to as the latency phase, during the first four hours (Zamora, 2009). The lag phase, also known as the adaption stage, is the early growth phase during which the number of cells is largely steady before rapid expansion. The yeasts are metabolically active during this phase, producing enzymes and ribosomes acclimating to their new physicochemical environment (Bellissimi & Ingledew, 2005). This occurs before the cell starts to divide. The capacity of a living thing to change its population size and environmental factors such as temperature, pH, alcohol, oxygen, salt concentrations, nutrition, etc. will determine how long the lag phase lasts. After 6 hours, the log phase was then started and remained until 12 hours, exponential growth took place. The yeast starts to grow most quickly in the log phase because has been exposed to the new environmental conditions. At this point, 10^7–10^8 yeast cells per milliliter were added to the population (Zamora, 2009). After an incubation period of 12 hours, the growth rate slowed down and hardly any change or static conditions were observed. The baker's yeast was therefore in the stationary phase, or exponential phase. During this stage, cell metabolisms become slow and cells stop their' rapid cell division. High cell density was achieved by keeping cells growing for longer time in order to maximize the ethanol production (Russell, 2003). The ethanol production was obviously observed starting at 4 hours and continuously increasing until 16 hours of fermentation process. The highest ethanol concentration was 23.14 ± 0.03 g/L, which was detected at 16 hours of fermentation. Based on theoretical yield, the ethanol concentration shows a conversion of 91%.

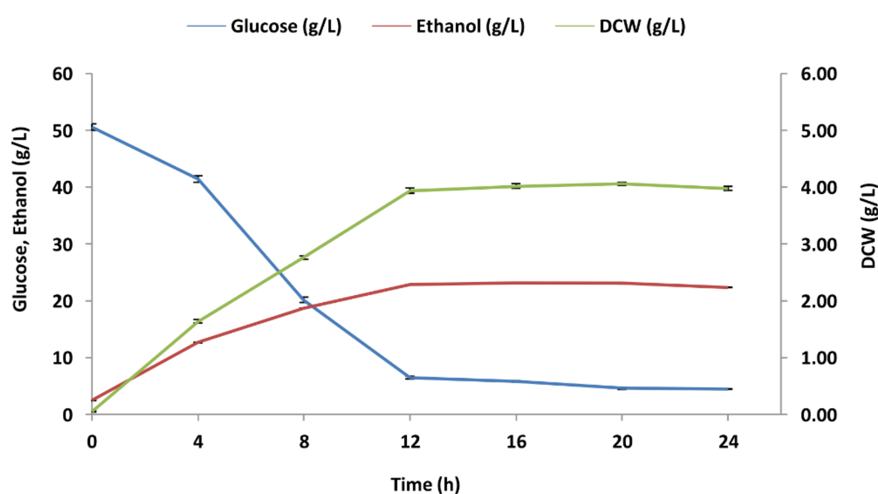

Figure 3: Fermentation profiles glucose consumption, yeast dry cell weight profile and ethanol production using sago effluent hydrolysate as a carbon source and fermentation medium. One Way ANOVA method and Tukey tests method were employed in the statistical analysis. Every value is the mean SE of three replicates. At $p < 0.05$, means that share the same letter are not considered significant.

According to the kinetic parameters (Table 2), the initial glucose concentration of SEH media shows high glucose consumption (92.92 ± 0.353 %) and high fermentation efficiency (96.38 ± 1.010 %), corresponding to an ethanol output of (0.49 0.005 g/g), indicating that SEH functions effectively as a carbon source and fermentation medium for the yeast cells. Based on the kinetic properties, the previous work by Mohamad *et al.* (2020) that ethanol was produced using sago fibre hydrolysate (SFH) as fermentation medium found that SFH media with an initial glucose concentration of 50 g/L had a glucose absorption of 97.78%, a fermentation efficiency of 81.35 ± 0.572 % and an ethanol yield 0.42 ± 0.003 g/g. By comparison between SEH and SFH, showed that SEH had a slightly higher fermentation efficiency (96.38% vs 81.35%) and a higher ethanol yield (0.49 g/g vs 0.42 g/g) compared to SFH. In contrast, glucose consumption was slightly lower in SEH compared to SFH (92.92 % vs 97.7 %). Nevertheless, both hydrolysates generated from sago effluent and sago fibre capable of being used as a carbon source as well as a fermentation medium for renewable ethanol production. The addition of yeast extract as a nitrogen source in this study also reveals a proper nitrogen supplement was needed for metabolism during fermentation and suggested 70% of the C/N ratio (Kalil *et al.*, 2008).

Table 2. Kinetic parameters of ethanol production using sago effluent hydrolysate as a carbon source and fermentation medium.

Kinetic parameters	SEH Media
Initial Glucose (g/L)	50.57 ± 0.556
Glucose Consumption Efficiency (%)	92.92 ± 0.353
Ethanol (g/L)	23.14 ± 0.027
$Y_{p/s}$ (g/g)	0.49 ± 0.005
Q_p (g/Lh)	1.45 ± 0.002
E_f (%)	96.38 ± 1.010

According to Junior *et al.* (2012), inadequate sources of nitrogen would also cause a reduction in ethanol yield, low biomass yield, slower rate of fermentation and increase risk of sluggish fermentation. The best organic source, according to their research, was yeast extract at a concentration of 13 g/L, which produced 85% of the 308 mL of metabolites per g of glucose used. In comparison to this study, 5 g/L of yeast extract has resulted in 96.38% of ethanol yield of 44.18 g/L glucose utilization. The nitrogen naturally present in sago wastewater contributes to the growth of the baker yeast and reduce dependency on nitrogen supplementation to produce bioethanol (Nururrahmah *et al.*, 2018).

CONCLUSION

This study examines the possibility of using sago wastewater as a carbon source and fermentation medium for the production of bioethanol. The initial study has focused on the effect of storage temperature on the starch content of fresh sago effluent. The finding shows that fresh sago effluent can be stored for up to 5 days at cold temperature (4°C) compared to room temperature (25°C - 30 °C) as the starch content can be maintained, hence improving the glucose yield. The highest glucose concentration was 50.57 g/L, which depicts 82.5% of starch to glucose conversion yield through enzymatic hydrolysis process. The 96.38 % bioethanol fermentability also proved that sago effluent capable of being used as a carbon source and fermentation medium for bioethanol production. Hence, this study reveals the possibility of turning huge sago effluent into an alternative source for producing value added products which indirectly can minimize the cost for waste water treatment plant at sago starch processing mills.

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CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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