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Total Sago Effluent Concentrate (TSEC) for Bioethanol Production via Fed-Batch Simultaneous Saccharification and Fermentation (SSF)

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Abstract

Sago processing waste stream, commonly called sago effluent, consists of a mixture of wet solid biomass and liquid hydrolysate. This industrial byproduct is rich in carbohydrates, particularly lignocellulosic materials, making it a promising feedstock for bioethanol production. In this study, sago effluent was subjected to physical pretreatment involving mechanical pulverisation and centrifugation to obtain a dense starch-rich wet biomass, termed total sago effluent concentrate (TSEC). The TSEC was subsequently used as a substrate for bioethanol production via fed-batch Simultaneous Saccharification and Fermentation (SSF), catalysed by amylolytic enzymes (α-amylase and glucoamylase) and Saccharomyces cerevisiae. SSF experiments were conducted in both non-buffered and citrate-buffered media, with buffer concentrations of 0, 25, 50 and 100 mM. Maximum ethanol production was achieved in the non-buffered medium, with a concentration of 23.44 ± 4.16 g/L and a theoretical ethanol yield (TEY) of 62.94 ± 11.18%.

No statistically significant differences were observed in ethanol production across the different buffered conditions. The highest ethanol concentration among the buffered systems was recorded at 23.22 ± 2.44 g/L (62.33 ± 6.57% TEY) in the 25 mM citrate buffer. This was followed by 20.67 ± 2.85 g/L (55.33 ± 4.80% TEY) in the 50 mM buffer and 20.61 ± 1.79 g/L (55.33 ± 4.82% TEY) in the 100 mM buffer. Overall, the findings demonstrate that TSEC is an effective and viable feedstock for bioethanol production using the fed-batch SSF process, with optimal performance observed in a non-buffered fermentation system.

Keywords: Bioethanol, Simultaneous Saccharification and Fermentation (SSF), Total Sago Effluent Concentrate.

Introduction

The transportation sector currently accounts for approximately 60% of global oil consumption[14]. Projections indicate that this demand will continue to rise over the coming decades, posing risks to global supply and price stability[3]. Consequently, extensive efforts are being made to develop and implement more sustainable biofuel alternatives[10,23]. Among these, bioethanol has emerged as a promising supplement to fossil fuels due to its renewability and environmentally friendly combustion, which produces only water and carbon dioxide as by-products[3,8,31,32]. From an economic perspective, bioethanol offers several advantages including the reduction of greenhouse gas emissions, improved energy security and the generation of employment opportunities, thereby providing financial benefits for both urban and rural communities[13].

However, the increasing global demand for bioethanol, combined with the food-feed-fuel competition, has necessitated the exploration of alternative carbohydrate sources, particularly lignocellulosic biomass[5,19,34]. Lignocellulosic biomass, composed primarily of cellulose, hemicellulose and lignin, is a non-edible and renewable resource. This category includes sewage sludge, municipal solid waste (MSW) and various forms of agricultural and industrial residues[22]. Its abundance and non-competition with food sources make lignocellulosic biomass an attractive and ethical option for large-scale bioethanol production[17,22].

Metroxylon sagu, commonly known as the sago palm, is recognised for its high starch content within its biomass[5]. In Malaysia, particularly in the state of Sarawak, approximately 200,000 tons of sago are produced annually, with about 47,000 tons exported to Peninsular Malaysia, Japan, Taiwan and Australia[1]. However, sago starch production generates substantial amounts of industrial waste, notably in the form of sago hampas, which is often improperly disposed of in nearby streams or factory compounds[4,21]. Such practices contribute to elevated biological oxygen demand (BOD) and chemical oxygen demand (COD), posing a significant threat to aquatic ecosystems, raising serious environmental concerns[2].

Sago waste typically contains approximately 65.7% starch, making it a promising feedstock for bioethanol production. To enhance its utility, the waste was subjected to mechanical pulverisation and centrifugation to isolate a starch-rich concentrate, designated as total sago effluent concentrate (TSEC). TSEC is characterised by a higher solid content enriched in starch and fibrous components, making it a suitable substrate for bioethanol fermentation. In this study, TSEC was employed as a feedstock for bioethanol production through enzymatic hydrolysis using simultaneous saccharification and fermentation (SSF) under fed-batch conditions. Experiments were conducted in both non-buffered and buffered media with varying concentrations of citrate buffer and different biomass loadings.

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SSF is widely regarded as a preferred method for bioethanol production from lignocellulosic feedstocks[31], as it combines enzymatic hydrolysis and fermentation in a single vessel, thereby reducing process time and contamination risk. According to Vincent et al[28], SSF offers advantages such as higher reaction rates, increased ethanol yields and improved final ethanol concentrations.

Material and Methods

Preparation of glycerol stock and working culture of *Saccharomyces cerevisiae*: *Saccharomyces cerevisiae* (ATCC 24859) was obtained from the Microbiology Laboratory 2 collection at the Faculty of Resource Science and Technology, Universiti Malaysia Sarawak (UNIMAS). The yeast culture was initially revived in yeast malt broth (YMB) (Himedia Laboratories, India) at ambient temperature on a rotary shaker (Daihan Scientific, South Korea) set at 150 rpm for 24 hours. The culture was then subcultured on Rose Bengal chloramphenicol agar (RBCA) (Himedia Laboratories, India) and incubated at room temperature for 48 hours. Colonies were observed microscopically and further subcultured overnight in YMB for glycerol stock preparation. For long-term storage, the overnight culture was aliquoted into YMB supplemented with 20% (v/v) glycerol (R and M Chemicals, United Kingdom) and stored at –20 °C in an ultra-low temperature freezer (Sanyo MDF-U500VX-PE, Wolf Laboratories Ltd., United Kingdom)[26].

***S. cerevisiae* inoculum preparation:** *S. cerevisiae* inoculum was prepared by inoculating the glycerol stock culture into fresh YMB and incubating overnight at ambient temperature on an orbital shaker at 150 rpm. After incubation, *S. cerevisiae* cells were harvested by centrifugation (BK-1032J Low Speed Centrifuge, Biobase, China) in two 50 mL centrifuge tubes at 4000 rpm for 10 minutes[27]. The supernatant was discarded and the resulting *S. cerevisiae* cell pellets were transferred into the fermentation broth for use in subsequent experiments.

Pretreatment of substrate: Sago effluent was collected from Herdsen Sago Mill in Pusa, Sarawak. The effluent was ground to obtain finely homogenised sago hampas fibrous residue. The solid portion of the sago hampas was separated from the liquid effluent by centrifugation in four 50 mL centrifuge tubes at 4000 rpm for 10 minutes. Both the sedimented solids and supernatant were collected and stored in sterilised bottles for further analysis and long-term storage.

Characterisation of sago hampas and total sago effluent concentrate (TSEC): The moisture content of sago hampas was determined gravimetrically after oven drying at 105 °C for 72 hours using a laboratory oven (Shel Lab, USA), as calculated using equation 1. Total sago effluent concentrate (TSEC) was subsequently destarched, oven-dried and ground into a fine powder for compositional analysis. The powdered sample was subjected to starch and fibre analysis using methods such as phenol-sulfuric acid (PSA) assay, acid detergent fibre (ADF), neutral detergent fibre (NDF) and Klason Lignin determination. Ash content was determined using a furnace incineration method where the sample was dry ashed at 550 °C for 6 hours by an ashing Muffle furnace (KC 40/13, ThermoConcept, Germany) until ash was obtained.

$$\text{Moisture content \%} = \frac{(\text{weight of wet hampas} - \text{weight of dry hampas})}{\text{weight of dry hampas}} \text{ X } 100\% \tag{1}$$

Fermentation broth preparation: Fermentation broths were prepared as outlined in table 1, following modified methods based on Vincent et al[29]. Each batch culture consisted of 100 mL of fermentation broth, with the pH maintained between 4.8 and 5.4. The broths comprised of a mixture of total sago effluent concentrate (TSEC), sago effluent and 2 M citrate buffer, except for the control, which was prepared without buffer. All fermentation broths were sterilised by autoclaving at 121 °C for 15 minutes using a 25X electric pressure steam steriliser (All American, USA).

Simultaneous saccharification and fermentation (SSF): Following sterilisation, 75 µL each of α-amylase and glucoamylase was added to the fermentation broths and incubated at ambient temperature for one hour. Subsequently, harvested *S. cerevisiae* cells were inoculated into the broths. Fermentations were carried out in triplicate (n = 3) for five days at ambient temperature on a magnetic stirrer (48-I, Biobase Industry, China) set to 150 rpm. During the fed-batch SSF process, an additional 5 g of TSEC along with 20 µL of α-amylase and 20 µL of glucoamylase were aseptically added to each fermentation broth at 24, 48 and 72-hour intervals.

Sample collection: Fermentation samples (2.0 mL) were aseptically collected at 0, 12, 24, 36, 48, 60, 72, 84, 96 and 120 hours into sterile centrifuge tubes.

Table 1
The ratio of fermentation broth preparation

	Control (mL)	25 mM (mL)	50 mM (mL)	100 mM (mL)
2M Citrate Buffer	-	1.25	2.5	5.0
Total Sago Effluent Concentrate (TSEC)	20.0	20.0	20.0	20.0
Sago Effluent	80.0	78.75	77.5	75.0
Total	100.0	100.0	100.0	100.0

For High-Performance Liquid Chromatography (HPLC) and phenol-sulfuric acid (PSA) assays, samples were centrifuged at 13,500 rpm for 10 minutes using a microcentrifuge (WiseSpin CF-10, Daihan Scientific, South Korea). The resulting supernatants were filtered through 0.45 µm nylon membrane filters (Whatmann, NJ, USA) to remove residual solids. Filtered samples were stored at –20 °C until further analysis.

High-Performance Liquid Chromatography (HPLC): Ethanol, residual glucose, lactic acid and acetic acid concentrations were analysed using an HPLC system (Waters 2695 Separations Module, Alliance HPLC System, USA), equipped with a column heater, refractive index detector and computer interface. Fermentation products were separated using a Bio-Rad Aminex HPX-87H column (150 × 7.8 mm; Bio-Rad, USA), with 5 mM sulfuric acid (H_2SO_4) as the mobile phase at a flow rate of 0.8 mL/min and a column temperature of 65 °C[13,27]. Concentrations were calculated based on standard calibration curves corresponding to the retention times of standard reference compounds.

Phenol-Sulfuric Acid (PSA) assay: Sample supernatants were diluted 1:5 and thoroughly mixed using a vortex mixer (VX-200, Labnet International Inc., USA). A 0.2 mL aliquot of each dilution was transferred into clean test tubes in triplicate ($n = 3$). Subsequently, 0.2 mL of 5% phenol was added and mixed thoroughly, followed by the addition of 1.0 mL of 98% sulfuric acid. After mixing, 5.6 mL of distilled water was added to bring the total volume to 7.0 mL. The final mixture was vortexed and absorbance was measured at 490 nm using a spectrophotometer (SP-880, Metertech Inc., Taiwan). Equivalent sugar concentrations (g/L) were calculated based on a glucose standard curve generated before the assay.

Statistical analysis: Tukey's post hoc tests were conducted using SPSS Statistics Software version 21 to evaluate statistically significant differences in theoretical ethanol yield across fermentation broths containing different citrate buffer concentrations.

Results

Characterisation of total sago effluent concentrate (TSEC): The compositional analysis of total sago effluent concentrate (TSEC) before simultaneous saccharification and fermentation (SSF) is summarised in tables 2 and 3. The percentages of starch and cellulosic components were consistent with previous studies[13,24,28]. As shown in table 2, the TSEC comprised of 88.11 ± 2.09% sago hydrolysate and 1.89 ± 0.14% dry sago hampas. Table 3 provides the primary components of dried sago hampas, which contained 3.56 ± 0.80% starch, 0.32 ± 0.08% cellulose, 1.07 ± 0.08% hemicellulose, 0.28 ± 0.04% lignin and 0.03 ± 0.02% ash on a dry weight basis.

Phenol-Sulfuric Acid (PSA) assay: The results of the PSA assay are presented in figure 1. Initial carbohydrate concentrations were 39.83 g/L in the non-buffered broth and 34.08, 36.91 and 34.18 g/L in the 25, 50 and 100 mM buffered broths respectively. A marked decline in total carbohydrate concentration was observed across all broths during the first 48 hours of fermentation. At 24, 48 and 72 hours, 5 g of TSEC and 20 µL of amylolytic enzymes (α-amylase and glucoamylase) were added, leading to slight increases in carbohydrate concentrations at 60 and 84 hours. However, residual carbohydrates remained detectable in all broths at 120 hours, indicating incomplete conversion into ethanol.

Simultaneous saccharification and fermentation (SSF): Ethanol production profiles over the SSF period are shown in figure 2. All broths exhibited similar trends, with ethanol production initiating at 12 hours and peaking at 96 hours. Maximum ethanol concentrations ranged from 20.61 g/L in the 100 mM buffered broth to 23.45 g/L in the non-buffered broth. A slight decline in ethanol concentrations was observed after 96 hours. Figure 3 shows the glucose concentration profiles. Initial glucose levels were 37.30 g/L in the non-buffered broth and 35.43, 31.31 and 33.05 g/L in the 25, 50 and 100 mM buffered broths respectively. In the non-buffered broth, glucose declined rapidly to 4.80 g/L within 36 hours and was undetectable from 48 to 72 hours, with transient increases at 60 and 84 hours.

Table 2
Component analysis of Total Sago Effluent Concentrate (TSEC) comprising of sago hydrolysate and dried sago hampas

Composition			Amount (%)
Total Sago Effluent Concentrate (TSEC)	Sago effluent hydrolysate (Water Component)	Water	88.11 ± 2.09
		Free starch	1.31 ± 0.05
	Dried Sago Hampas (Solid Component)	Starch	3.56 ± 0.80
		Cellulose	0.32 ± 0.08
		Hemicellulose	1.07 ± 0.08
		Lignin	0.28 ± 0.04
		Ash	0.03 ± 0.02
		Others	5.33 ± 0.01

Table 3
Component analysis of dried sago hampas. The percentage was based on dry weight, n=3

Compositions	**% w/w**
Starch	65.80 ± 4.25
Cellulose	5.95 ± 1.53
Hemicellulose	18.20 ± 1.60
Lignin	5.25 ± 0.70
Ash	0.58 ± 0.40
Other	4.22 ± 0.01

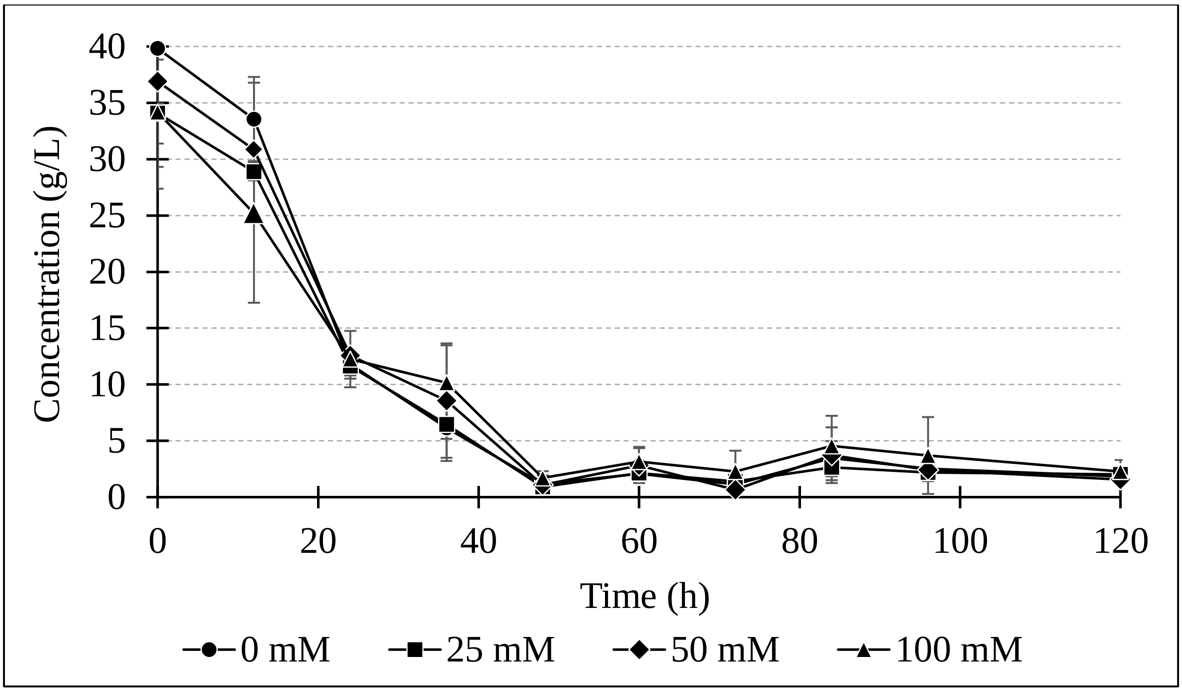

Figure 1: Time course of total carbohydrate content in broths containing TSEC with different strengths of citrate buffer during fed-batch bioethanol fermentation, as determined by the Phenol-Sulfuric Acid assay.
[Note: The data points represent the average of three independent experiments (n=3)]

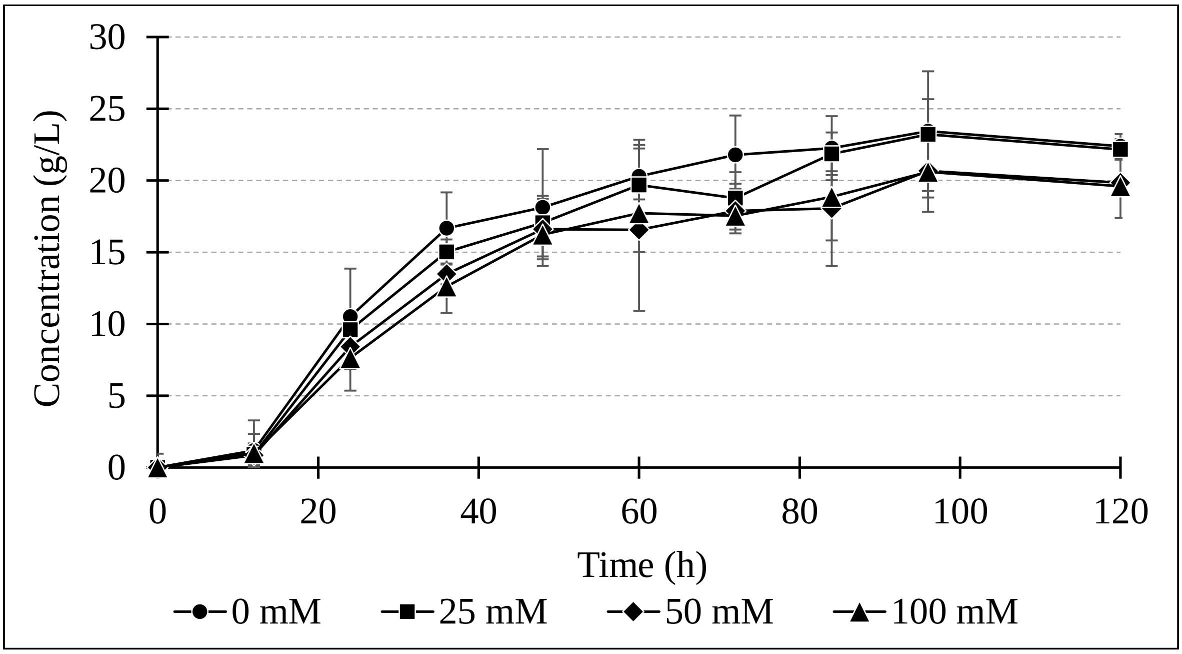

Figure 2: Time course of ethanol concentration in broths containing TSEC with different strengths of citrate buffer during fed-batch bioethanol fermentation, as determined by HPLC.
[Note: The data points represent the average of three independent experiments (n=3)]

A similar trend was observed in the buffered broths, though glucose consumption rates and completeness varied with buffer strength. In the 25 mM buffered broth, glucose concentration dropped to 5.08 g/L by 36 hours and was undetectable from 48 to 72 hours. A slight increase occurred at 84 hours before declining again. In the 50 mM broth, glucose decreased to 0.91 g/L at 60 hours and was fully consumed by 72 hours. A minor increase was observed at 84 hours. In the 100 mM buffered broth, glucose dropped to 1.64 g/L by 48 hours, but residual glucose remained detectable throughout, indicating incomplete utilisation by the end of fermentation.

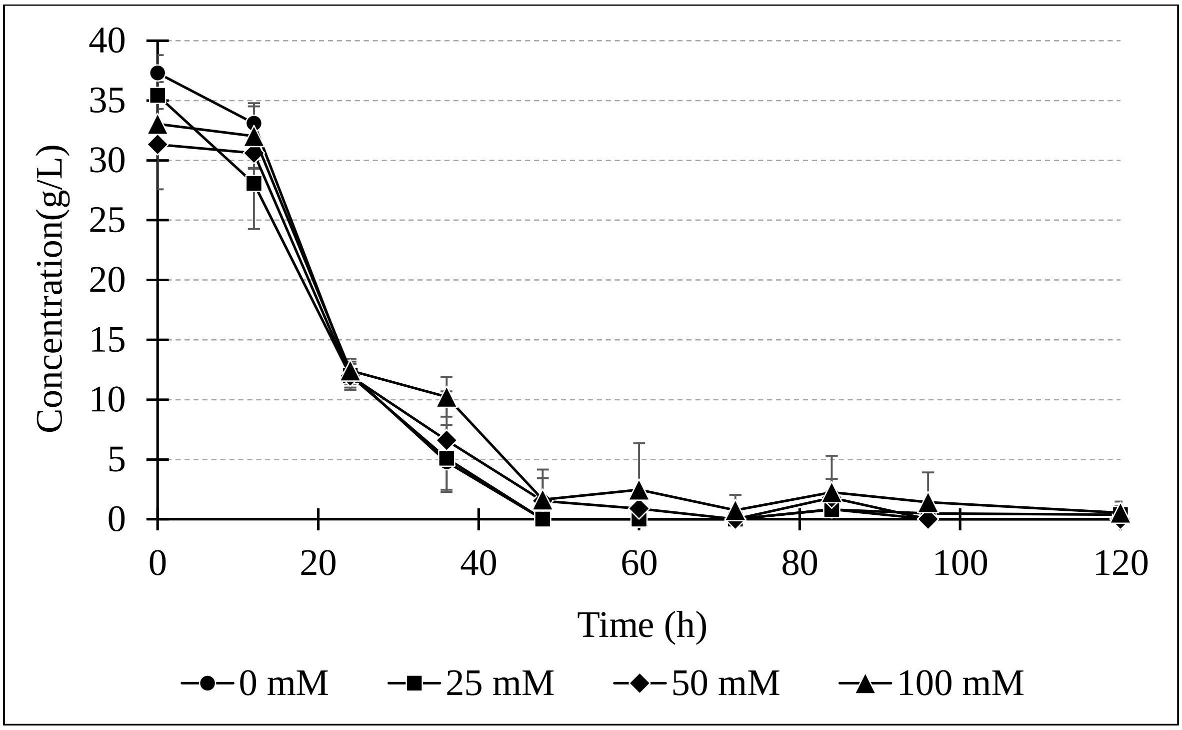

Figure 3: Time course of glucose concentration in broths containing TSEC with different strengths of citrate buffer during fed-batch bioethanol fermentation, as determined by HPLC. [Note: The data points represent the average of three independent experiments (n=3)]

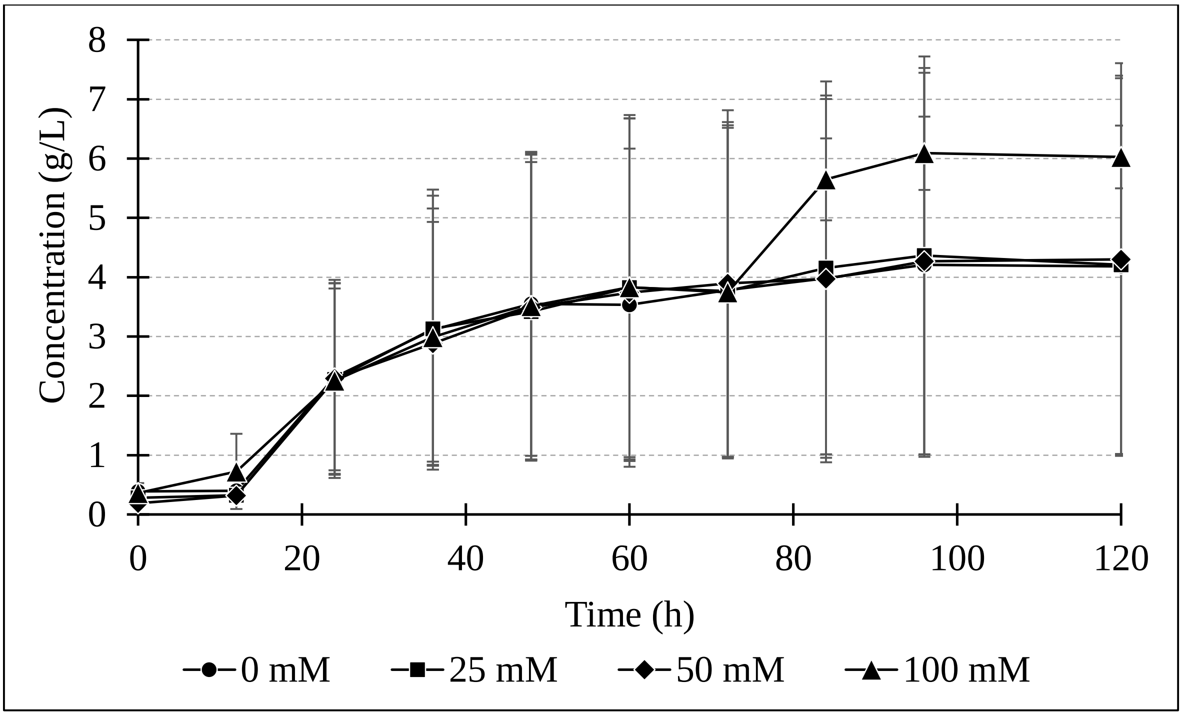

Figure 4: Time course of lactic acid concentration in broths containing TSEC with different strengths of citrate buffer during fed-batch bioethanol fermentation, as determined by HPLC. [Note: The data points represent the average of three independent experiments (n=3)]

The concentrations of fermentation by-products, such as lactic acid and acetic acid, were also quantified using HPLC. Results indicated that lactic acid levels increased progressively after 24 hours and peaked at 120 hours across all fermentation broths. Notably, lactic acid production was highest in the 100 mM buffered broth compared to the other conditions (Figure 4). At 120 hours, the maximum lactic acid concentrations in the non-buffered, 25 mM and 50 mM buffered broths were 4.18, 4.21 and 4.30 g/L respectively. In contrast, the 100 mM buffered broth reached a peak concentration of 6.03 g/L. Overall, lactic acid concentrations ranged from 0.19 to 6.03 g/L. According to Hung et al[11], ethanol production is significantly inhibited when lactic acid concentrations exceed 30 g/L (3.0% w/v). Therefore, the lactic acid levels observed in this study were below the inhibitory threshold and did not adversely affect yeast metabolism or ethanol yield.

Acetic acid profiles are presented in figure 5. The non-buffered broth produced the highest acetic acid concentration, peaking at 0.99 g/L at 120 hours. In contrast, the 25, 50 and 100 mM buffered broths showed final acetic acid concentrations of 0.86, 0.83 and 0.80 g/L respectively, following a steady increase throughout the fermentation period. According to the results shown in table 4, the non-buffered broth produced the highest ethanol concentration (23.44 g/L), corresponding to a theoretical ethanol yield (TEY) of 62.94%. This was followed closely by the 25 mM buffered broth (23.22 g/L; TEY: 62.33%). The 50 mM buffered broth yielded 20.67 g/L ethanol (TEY: 55.49%), while the lowest production was observed in the 100 mM buffered broth (20.61 g/L; TEY: 55.33%).

Discussion

In this study, bioethanol was produced from total sago effluent concentrate (TSEC) via simultaneous saccharification and fermentation (SSF) in non-buffered and buffered broths (25, 50 and 100 mM citrate buffers). TSEC refers to the high-starch solid fraction of total sago effluent (TSE) that has undergone mechanical pulverisation and centrifugation.

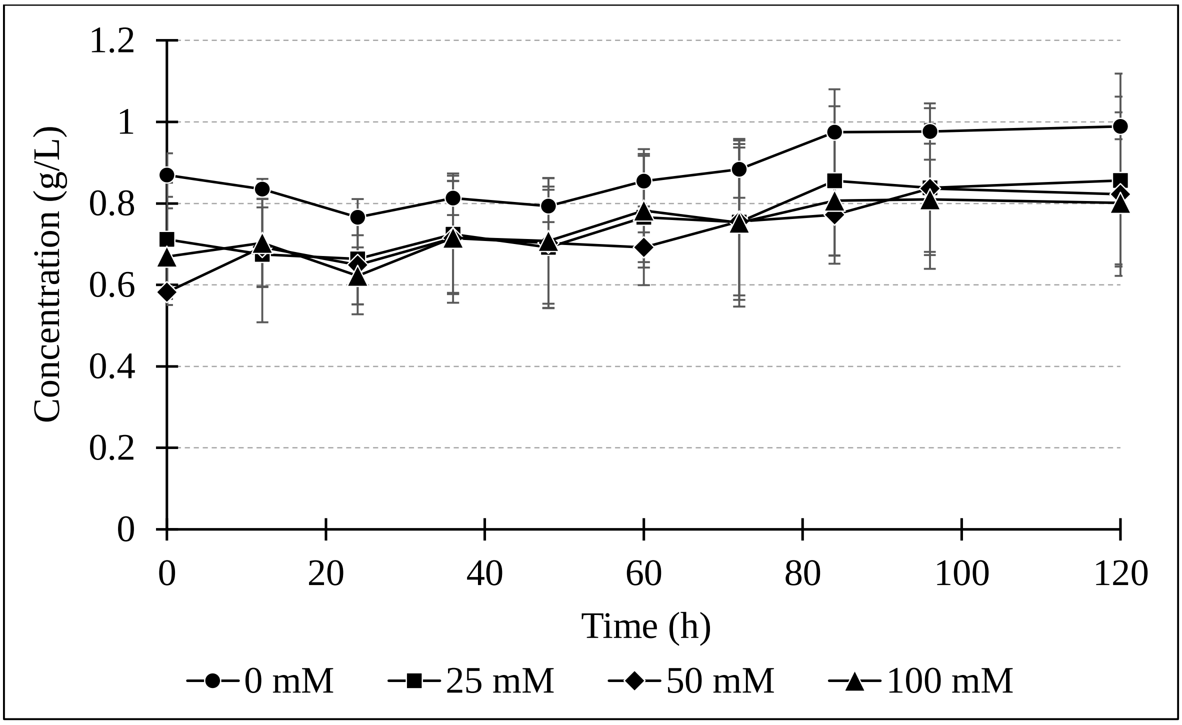

Figure 5: Time course of acetic acid concentration in broths containing TSEC with different strengths of citrate buffer during fed-batch bioethanol fermentation, as determined by HPLC.
[Note: The data points represent the average of three independent experiments (n=3)]

Table 4
The maximum production and ethanol yield produced during fed-batch fermentation of bioethanol usingTotal Sago Effluent Concentrate (TSEC) of different buffer strengths

Fermentation broth	Maximum production of ethanol (g/L)	Experimental theoretical yield (%)
Non-buffered	23.44 ± 4.17	62.94 ± 11.18
25 mM	23.22 ± 2.45	62.33 ± 6.57
50 mM	20.67 ± 2.85	55.49 ± 7.66
100 mM	20.61 ± 1.79	55.33 ± 4.82

*Data are mean triplicates ± S.D (Different superscript letters in the same column show significant differences

It consists of sago effluent hydrolysate and sago hampas. The SSF process integrates enzymatic hydrolysis and fermentation in a single step. As reported by Vincent et al[28], SSF is a promising method for bioethanol production, offering advantages over separate hydrolysis and fermentation (SHF) such as improved reaction rates, higher ethanol yields and reduced enzyme inhibition.

Compositional analysis of TSEC was performed before SSF. These analyses are essential, as the efficiency of the saccharification process depends on the content of cellulose, hemicellulose and lignin in lignocellulosic biomass[25]. As shown in table 2, TSEC contained 88.11% moisture and 1.31% soluble starch. The dry solids were composed of 65.80% starch, 5.95% cellulose, 18.20% hemicellulose and 5.25% lignin (Table 3). These values are comparable to, though slightly different from, those reported previously by Hung et al[13] and Vincent et al[28]. Notably, the starch content in this study exceeded compared to the yield reported by Awg-Adeni et al[5], which ranged between 30% and 50%, indicating the suitability of TSEC as a substrate for amylolytic hydrolysis and subsequent fermentation by *Saccharomyces cerevisiae*[13].

The SSF process was conducted in fed-batch mode, with periodic additions of 5 g TSEC and amylolytic enzymes at 24, 48 and 72 h to enhance ethanol yield[25]. High-performance liquid chromatography (HPLC) analyses showed that ethanol production began at 12 h in all broths. The highest initial ethanol concentration was recorded in the non-buffered broth (1.19 g/L), followed by 100 mM (1.02 g/L), 25 mM (0.92 g/L) and 50 mM (0.87 g/L) buffered broths (Figure 2). The rapid conversion of glucose into ethanol confirms the efficiency of SSF. The initial increase in ethanol corresponded to a sharp decrease in glucose concentration, driven by *S. cerevisiae* activity. Ethanol production peaked at 96 h, indicating sustained yeast activity and nutrient availability. This contrasts with the findings of Hung et al[11] who reported ethanol peaks at 48 h in batch SSF using sago hampas.

According to Bolmanis et al[6], fed-batch fermentation mitigates metabolic overflow, balancing ethanol production and biomass growth. In this study, *S. cerevisiae* produced 0.51 g ethanol per g glucose consumed, aligning with reported theoretical yields of 64%–90%[12,20]. The observed yield of 62.94% (non-buffered broth) was statistically analysed using Tukey's post hoc test and was considered fair. SPSS analysis indicated no significant differences in ethanol yields across all buffer conditions as shown in figure 6 ($p < 0.05$).

Initial glucose concentrations were highest at 0 h in all broths, reflecting efficient enzymatic hydrolysis of TSEC. Glucose levels sharply declined during the first 48 h due to yeast uptake. Slight increases in glucose concentration at 36, 60 and 84 h coincided with enzyme additions (Figure 3), indicating successful starch hydrolysis. After 84 h, glucose levels declined again, confirming conversion into ethanol. However, residual sugars remained detectable in 25 and 100 mM broths, suggesting incomplete conversion. No glucose was detected at 120 h in the non-buffered and 50 mM broths, likely due to concentrations falling below the HPLC detection threshold. Similar findings were reported elsewhere[2,13,28], that noted the presence of other hexose sugars in similar systems.

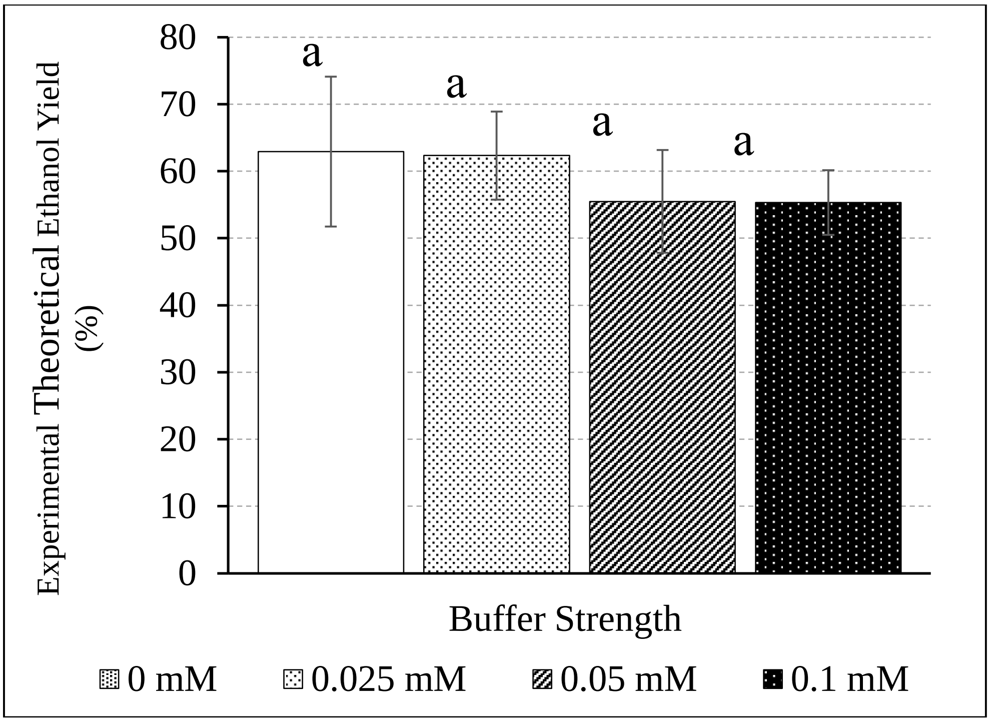

Figure 6: Experimental theoretical yields of bioethanol in different buffer strengths during fed-batch fermentation. [Note: Letters on top of column indicate statistically significant differences via Tukey's post hoc test, α=0.05]

To complement the HPLC analyses, the phenol-sulfuric acid (PSA) assay was conducted to quantify oligosaccharides and short-chain polysaccharides that are not distinguishable by the current HPLC setup. PSA is a reliable colorimetric method for total carbohydrate estimation[16]. As shown in figure 1, residual carbohydrates were still present at 120 h, suggesting the presence of non-fermentable or gradually fermentable saccharides.

Lactic acid was monitored as a fermentation by-product, as its presence may indicate contamination by the presence of *Lactobacillus spp*.[28] These bacteria tolerate ethanol, low pH and temperatures, ranging from 30 to 45 °C[7,15] and can compete with yeast for glucose, producing toxins that disrupt fermentation. The highest lactic acid concentration was observed in the 100 mM buffered broth (6.02 g/L), compared to 4.18, 4.20 and 4.30 g/L in the non-buffered, 25 mM and 50 mM broths, respectively (Figure 4).

Although elevated, these concentrations were below the inhibitory threshold of 30 g/L[28], indicating that ethanol production was not significantly impacted. In industrial ethanol production, sterile conditions are often not maintained and low-level microbial contamination is common[11,25]. However, the relatively low lactic acid levels observed in this study suggest minimal contamination.

Acetic acid, another marker of biomass hydrolysis, was also measured. Its accumulation during SSF suggests ongoing lignocellulose degradation[31]. Results in this study (Figure 5) showed that acetic acid concentrations increased gradually, reaching 0.80–0.99 g/L by 120 h. Hung et al[11] attributed acetic acid formation to ethanol oxidation and its levels correlate with fermentable sugar availability[33]. Low concentrations of acetic acid can stimulate ethanol production by increasing ATP demand and glycolytic flux. However, excessive acetic acid can inhibit yeast growth when undissociated molecules cross the cell membrane at low pH[11,18]. In this study, acetic acid levels remained well below the inhibitory concentration (0.80% w/v)[11] and no adverse effects on ethanol production were observed.

Conclusion

This study demonstrates that bioethanol can be efficiently produced from TSEC using fed-batch SSF. The highest theoretical ethanol yield (62.94%) was achieved in the non-buffered broth followed by 62.33%, 55.49% and 55.33% in the 25, 50 and 100 mM buffered broths respectively. These results confirm the feasibility of using TSEC as a low-cost, high-starch feedstock for bioethanol production, with SSF offering efficient conversion when supported by amylolytic enzymes and *S. cerevisiae*.

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References

1. Ahmad M.N., Awg-Adeni D.S., Suhaili N. and Bujang K., Optimisation of pre-harvest sago frond sap for the production of l-lactic acid using *Lactococcus lactis* IO-1, *Biocatal. Agric. Biotechnol.*, **43**, 102435 **(2022)**

2. Amin N., Sabli N., Izhar S. and Yoshida H., Sago wastes and its applications, *Pertanika J. Sci. Technol.*, **27(4)**, 1841–1862 **(2019)**

3. Assan T.M., Sahari E.S., Suhaili N., Awg-Adeni D.S., Kanakaraju D. and Vincent M., Evaluation of generic fertiliser as an alternative inorganic nitrogen source for ethanolic glucose fermentation by *Saccharomyces cerevisiae*, *Borneo J. Resour. Sci. Technol.*, **13(1)**, 32–41 **(2023)**

4. Awg-Adeni D.S., Abd-Aziz S., Bujang K. and Hassan M.A., Bioconversion of sago residue into value-added products, *Afr. J. Biotechnol.*, **9(14)**, 2016-2021 **(2010)**

5. Awg-Adeni D.S., Bujang K., Hassan M.A. and Abd-Aziz S., Recovery of glucose from residual starch of sago hampas for bioethanol production, *Biomed. Res. Int.*, **2013**, 935852 **(2013)**

6. Bolmanis E., Dubencovs K., Suleiko A. and Vanags J., Model predictive control - A standout among competitors for fed-batch fermentation improvement, *Fermentation*, **9(3)**, 206 **(2023)**

7. Brexó R.P. and Sant'Ana A.S., Impact and significance of microbial contamination during fermentation for bioethanol production, *Renew. Sustain. Energy Rev.*, **73**, 423–434 **(2017)**

8. Chin K.L. and H'ng P.S., A real story of bioethanol from biomass: Malaysia perspective, Biomass now - Sustainable growth and use, In Matovic M.D., eds., InTech, United Kingdom **(2013)**

9. Graves T., Narendranath N.V., Dawson K. and Power R., Effect of pH and lactic or acetic acid on ethanol productivity by *Saccharomyces cerevisiae* in corn mash, *J. Ind. Microbiol. Biotechnol.*, **33(6)**, 469–474 **(2006)**

10. Guo Y., Liu G., Ning Y., Li X., Hu S., Zhao J. and Qu Y., Production of cellulosic ethanol and value-added products from corn fiber, *Bioresour. Bioprocess*, **9(1)**, 81 **(2022)**

11. Hung H.C., Awg-Adeni D.S., Johnny Q. and Vincent M., Production of bioethanol from sago hampas via simultaneous saccharification and fermentation (SSF), *Nusantara Biosci.*, **10(4)**, 240–245 **(2018)**

12. Inbathamizh L., Rakshi A.D., Sandhya S., Usha Nandhini S. and Sudha S., Microbial Technologies in Wastewater Treatment: A Positive Step towards Sustainable Environment, *Res. J. Chem. Environ.*, **27(3)**, 100-107 **(2023)**

13. Lucena B.T., dos Santos B.M., Moreira J.L., Moreira A.P.B., Nunes A.C., Azevedo V., Miyoshi A., Thompson F.L. and de Morais M.A., Diversity of lactic acid bacteria of the bioethanol process, *BMC Microbiol.*, **10(1)**, 1-8 **(2010)**

14. Masuko T. et al, Carbohydrate analysis by a phenol–sulfuric acid method in microplate format, *Anal. Biochem.*, **339(1)**, 69–72 **(2005)**

15. Nair L.G., Agrawal K. and Verma P., An overview of sustainable approaches for bioenergy production from agro-industrial wastes, *Energy Nexus*, **6**, 100086 **(2022)**

https://doi.org/10.25303/214rjbt1640172

16. Ndukwe J.K., Aliyu G.O., Onwosi C.O., Chukwu K.O. and Ezugworie F.N., Mechanisms of weak acid-induced stress tolerance in yeasts: Prospects for improved bioethanol production from lignocellulosic biomass, *Process Biochem*., **90**, 118–130 **(2020)**

17. Robak K. and Balcerek M., Current state-of-the-art in ethanol production from lignocellulosic feedstocks, *Microbiol. Res*., **240**, 126534 **(2020)**

18. Ruchala J., Kurylenko O.O., Dmytruk K.V. and Sibirny A.A., Construction of advanced producers of first- and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (*Schefferso mycesstipitis*, *Ogataea polymorpha*), *J. Ind. Microbiol. Biotechnol*., **47(1)**, 109–132 **(2020)**

19. Santoso B., Recovery of starch from sago pith waste and waste water treatment, In Ehara H., Toyoda Y. and Johnson D., eds., Sago Palm, Springer, Singapore **(2018)**

20. Srivastava N., Srivastava M., Mishra P.K. and Gupta V.K., Eds., Biofuel production technologies, Critical analysis for sustainability, Singapore, Springer **(2020)**

21. Tan K.T., Lee K.T. and Mohamed A.R., Role of energy policy in renewable energy accomplishment: The case of second-generation bioethanol, *Energy Policy*, **36(9)**, 3360–3365 **(2008)**

22. Thangavelu S.K., Ahmed A.S. and Ani F.N., Bioethanol production from sago pith waste using microwave hydrothermal hydrolysis accelerated by carbon dioxide, *Appl. Energy*, **128**, 277–283 **(2014)**

23. Tse T.J., Wiens D.J. and Reaney M.J.T., Production of bioethanol: A review of factors affecting ethanol yield, *Fermentation*, **7(4)**, 268 **(2021)**

24. Vincent M., Sequential saccharification and fermentation of corn stover for the production of fuel ethanol using wood-rot fungi, *Saccharomyces cerevisiae* and *Escherichia coli* K011, Dissertation, Iowa State University, United States of America **(2010)**

25. Vincent M., Jabang E., Nur N.M., Esut E., Unting L.B. and Awg-Adeni D.S., Simultaneous co-saccharification and fermentation of sago hampas for bioethanol production, *Agric. Eng. Int. CIGR J*., **17(2)**, 160–167 **(2015b)**

26. Vincent M., Johnny Q., Awg-Adeni D.S. and Suhaili N., Potential of *Candida glabrata* from ragi as a bioethanol producer using selected carbohydrate substrates, *Nusantara Biosci*., **13(1)**, 1-10 **(2021)**

27. Vincent M., Pometto A.L. and van Leeuwen J.H., Simultaneous saccharification and fermentation of ground corn stover for the production of fuel ethanol using *Phanerochaete chrysosporium*, *Gloeophyllum trabeum*, *Saccharomyces cerevisiae* and *Escherichia coli*, *J. Microbiol. Biotechnol*., **21(7)**, 703-710 **(2011)**

28. Vincent M., Pometto A.L. and van Leeuwen J.H., Ethanol production via simultaneous saccharification and fermentation of sodium hydroxide-treated corn stover using *Phanerochaete chrysosporium* and *Gloeophyllum trabeum*, *Bioresour. Technol*., **158**, 1–6 **(2014)**

29. Vincent M., Senawi B.R., Esut E., Muhammad Nor N. and Awg-Adeni D.S., Sequential saccharification and simultaneous fermentation (SSSF) of sago hampas for the production of bioethanol, *Sains Malays*, **44(6)**, 899–904 **(2015a)**

30. Yamashita Y., Sasaki C. and Nakamura Y., Development of efficient system for ethanol production from paper sludge pretreated by ball milling and phosphoric acid, *Carbohydr. Polym*., **79(2)**, 250–254 **(2010)**

31. Yang S., Land M.L., Klingeman D.M., Pelletier D.A., Lu T.Y.S., Martin S.L., Guo H.B., Smith J.C. and Brown S.D., Paradigm for industrial strain improvement identifies sodium acetate tolerance loci in *Zymomonas mobilis* and *Saccharomyces cerevisiae*, Proc. Natl. Acad. Sci. USA, **107(23)**, 10395–10400 **(2010)**

32. Zakaria S., Ahmadzadeh A. and Roslan R., Flow properties of novolak-type resin made from liquefaction of oil palm empty fruit bunch (EFB) fibres using sulfuric acid as a catalyst, *Bioresour*., **8(4)**, 5884-5894 **(2013)**.

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