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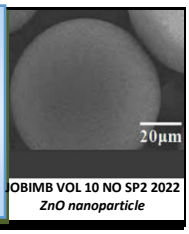

Production and Recovery of Succinic Acid from Oil Palm Frond (OPF) Fermentation

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ABSTRACT

This study aimed to determine the production of succinic acid by using different nitrogen sources in oil palm frond (OPF) fermentation and the recovery of succinic acid. A comparison between two nitrogen sources, i.e., yeast extract and peptone, was performed and a fermentation solution containing the highest concentration of succinic acid was used to carry out the recovery process. Activated carbon treatment at 4% to 6% (w/w) was performed to determine the best dosage that could be used for the recovery of succinic acid in the fermentation solution. Based on the results obtained, it was found that yeast extract was able to produce a higher concentration of succinic acid at 2.62 g/L with a yield of 1.53 g/g, compared to peptone which afforded a concentration of 1.89 g/L with a yield of 1.10 g/g. It was also found that the dose of 5% (w/w) activated carbon was the best to increase the concentration of succinic acid and adsorb other organic acids. After the activated carbon was fed into the fermentation solution, it was found that the succinic acid content increased from 2.62 g/L to 3.43 g/L with a percentage increase of 24.7%.

INTRODUCTION

Succinic acid is a C4 dicarboxylic acid with the molecular formula of $C_4H_6O_4$, a molecular weight of 118.09 g/mol, and a melting point of 185-190°C. Succinic acid is also one of the organic acids containing four carbons and two functional carboxyl groups (-COOH). Succinic acid is often applied in food, medicine, detergents, and resin production. Since 1877, the potential of succinic acid as an antibiotic and natural cure has been identified by scientists who have studied the geology of amber [1]. The use of succinic acid in the medical sector is said to improve cellular respiration and glucose metabolism which allows the body to function optimally [2].

Nowadays, succinic acid is produced for human consumption synthetically from maleic anhydride through the oxidation of butane or converted from biomass through fermentation catalyzed by microorganisms. The fermentation process for producing succinic acid is gaining popularity because it uses renewable raw materials as a substrate, making it a green technology. Through the fermentation process, glucose is converted to succinic acid via the reductive cycle of the tricarboxylic acid (TCA) [3].

Fermentable sugars, particularly glucose, can be recovered through pretreatment or pre-hydrolysis. Typically, pretreatment techniques were used to remove lignin and silica as well as to disrupt the cellulose-hemicellulose matrix structure to facilitate the conversion of cellulose and hemicellulose to simple sugars [4]. Hydrolyzed sugars in high concentrations have the potential to be used as feedstock in the production of succinic acid. Among the best succinic acid-producing bacteria, *Actinobacillus succinogenes* has already been used in the commercialization stage. Typically, succinic acid production involves the use of the rumen-type bacterium *A. succinogenes* [5].

In this study, the production of succinic acid from oil palm fronds (OPF) was investigated. The function of OPF as the main source of carbon during the fermentation process is one of the good measures to utilize the waste of the oil palm industry in Malaysia. In addition, this study will also identify differences in the yield of succinic acid by using different nitrogen sources in OPF fermentation. Finally, this study examined the recovery of succinic acid from OPF fermentation, before and after purification, at different activated carbon loadings.

MATERIALS AND METHODS

Pretreatment of oil palm frond using dilute acid

The milled and dried oil palm frond (OPF) was soaked in dilute sulfuric acid (H_2SO_4) solution overnight with a concentration of 2.0% (v/v). The mixture was further autoclaved at 121℃ for 20 minutes. Thereafter, the hydrolyzed OPF samples were infused with 4 M sodium hydroxide (NaOH) until the pH of the hydrolysate reached pH 6.08 for further processing.

Fermentation of OPF bagasse

The biocatalyst employed during fermentation was *A. succinogenes* which was purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ, Brunswick, Germany). The inoculum was prepared using Brain Heart Infusion (BHI) medium under aerobic conditions at 37℃ and 150 rpm for 18 h.

Following inoculation, the OPF hydrolysate was supplemented with (per litre medium): 0.2 g magnesium chloride hexahydrate ($MgCl_2.6H_2O$); 0.2 g calcium chloride dihydrate ($CaCl_2.2H_2O$); 3.0 g potassium dihydrogen phosphate (KH_2PO_4); 1.0 g NaCl; 15.0 g yeast extract, and 40.0 g magnesium carbonate ($MgCO_3$). The OPF hydrolysate was dispensed into the serum bottles as the main carbon source, capped with a butyl rubber stopper, and then clamped with an aluminium seal. Batch cultivation was carried out in 100-mL serum bottles by adding 10% (v/v) inoculum to a working volume of 50 mL under sterile conditions, and the culture was incubated at 37 ℃ and 200 rpm for 71 h. The steps were repeated using peptone as the sole nitrogen source. The best nitrogen source that yields the highest succinic acid in the fermentation broth was chosen to undergo further recovery process.

Recovery of succinic acid

Fermentation broth containing succinic acid was treated using activated carbon to remove residual glucose and impurities. Activated carbon were loaded at 4% (w/w), 5% (w/w), and 6% (w/w) into 100 mL of fermentation broth to remove impurities. The flask was then placed in an incubator shaker set at 200 rpm, 30℃ for 24 hours. The fermentation solution was centrifuged after the adsorption procedure to remove the activated carbon. To guarantee that all suspended residues in the succinic acid solution were removed, a vacuum filtering system (Whatman cellulose nitrate membrane filters, plain, sterile, 0.45 µm pore size, 47 mm circle) was used.

Metabolite analysis

Metabolite analysis was done on the fermentation before and after the recovery process. Accordingly, analysis of the fermentation metabolites was carried out using high-performance liquid chromatography, HPLC (UltiMate 3000 LC system, Dionex, Sunnyvale, CA) equipped with a refractive index (RI) detector (RefractoMax 520, ERC, Germany) set at 40 °C. Rezex ROA-Organic acid column (300 mm × 7.8 mm; Phenomenex, USA), with a guard column (50 mm × 7.8 mm) was used and was isocratically eluted with 0.005 N H_2SO_4. Calibration curves were established using succinic acid, formic acid, acetic acid and ethanol.

RESULTS AND DISCUSSION

Pre-treatment of oil palm frond (OPF)

The fermentable sugars in OPF are derived from homopolymer cellulose (i.e. its monomer is glucose) and also heteropolymer hemicellulose (i.e. its monomer is xylose) [6]. In this study, pretreatment with dilute acid was carried out as it is a commonly used method for the recovery and production of sugars from lignocellulose materials [7]. The acid often used for hydrolysis is sulfuric acid (H_2SO_4). This study incorporated dilute H_2SO_4 hydrolysis because this method is easier to perform and able to remove hemicellulose completely.

The composition of the hydrolysis-treated material found in the hydrolysate produced was used as the main substrate and carbon source for *A. succinogenes* fermentation. The concentration of glucose contained in the resulting hydrolysate was 1.707 g/L. Among critical factors leading to suboptimal sugar content is that lignin cannot be completely dissolved and this seemed to inhibit the production of sugar during hydrolysis. In addition, the broth component that is not detoxified before being fed in the fermentation medium is also one of the factors causing the low glucose content in hydrolysate [7].

Succinic acid production from OPF

Fermentation is carried out using two different types of nitrogen sources namely yeast extract and peptone. Both of these nitrogen sources are organic nitrogen sources that are able to aid the growth of *A. succinogenes* in fermentation solutions. However, the results of succinic acid and other by-products are different. Succinic acid (SA) is formed as a major product through a series of enzyme-catalyzed reactions in the site of the anaerobic reductive pathway of the TCA cycle [6]. Among the observed products, only formic acid (FA) and acetic acid (AA) were formed as by-products during fermentation in serum bottles and neither ethanol nor lactic acid can be detected. However, Kim et al. detected the formation of lactic acid possibly due to the presence of molecular oxygen during fermentation that alters metabolic pathways in *A. succinogenes* [8]. In this study, observations of SA, FA, AA and glucose were performed at 0 h and 71 h of fermentation.

Succinic acid production with yeast extract

Based on the HPLC analysis, the concentrations of succinic acid and other by-products can be determined. **Fig. 1** shows the concentrations obtained for SA, FA, AA and glucose for a total of t = 71 h by using yeast extract as a nitrogen source. The trend of metabolites formed in this study seems to consistent with previous studies on SA production using *A. succinogenes* [9].

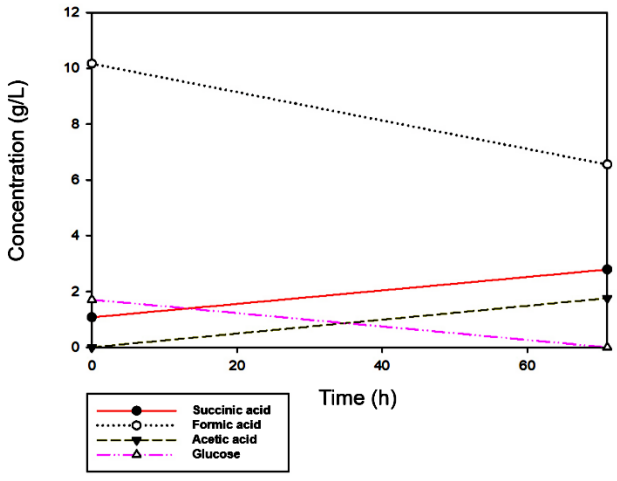

Fig. 1. Concentrations for SA, FA, AA and Glucose (Yeast extract) with respect to time.

The observation from the graph was an increasing trend in SA concentration from 1.064 g/L (at t = 0 h) to 2.60 g/L (at t = 71 h). In addition, an increase in by-product AA can also be seen which recorded concentrations of 0 g/L and 1.540 g/L at the initial and end of fermentation, respectively. FA, on the other hand, was formed initially due to the effect from the pretreatment and was eventually dropped to 6.130 g/L. This might be due to the utilization of formic acid to compensate energy deficiency within *A. succinogenes* cell [6]. Glucose also showed a declining pattern as it was fully utilized by *A. succinogenes*. This observation is consistent with the study of Luthfi et al. because there is an increase in the concentration of products and by-products at the end of fermentation. However, the SA concentration values obtained from this study were low compared to the study by Luthfi et al., due to several factors hindering the production of SA in fermentation solutions [6]. The low glucose value of 1.707 g/L in the OPF hydrolysate compared to the previous study (60 g/L) was the plausible factor hindering the high production of succinic acid in this study.

Succinic acid production with peptone

Fig. 2 shows the concentrations obtained for SA, FA, AA and glucose from t = 0 h to t = 71 h by using peptone as a nitrogen source.

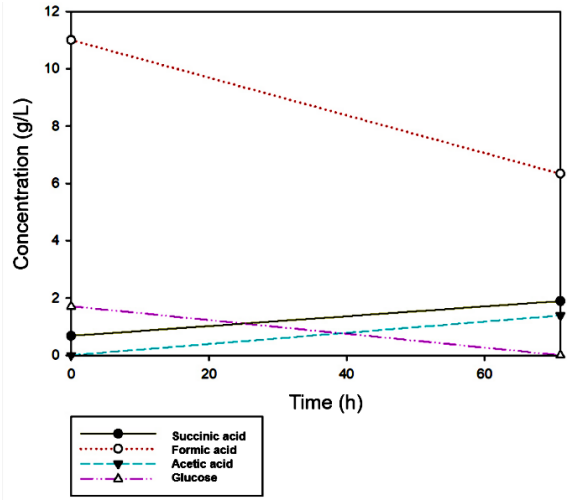

Fig. 2. Concentration for SA, FA, AA and glucose (Peptone) with respect to time.

As with yeast extract, the trend of fermentation with peptone showed increment in SA concentration from 0.662 g/L (at t = 0 h) to 1.885 g/L (at t = 71 h). In addition, an increase in by-products such as FA and AA can also be seen which recorded concentrations of 5.690 g/L and 1.364 g/L at the end of fermentation, respectively.

Comparison of yield

The average values of the findings obtained from fermentation are outlined in **Table 1**. Innoculation to increase cell density was carried out for 18 hours before being fed into the fermentation medium. As can be seen in **Table 1**, the highest yield of succinic acid obtained was 1.53 g/g with a concentration of 2.62 g/L resulting from 1.71 g/L of glucose contained in OPF hydrolysate. The results suggested that the yeast extract was able to produce more succinic acid than the peptone which produced succinic acid at a yield of 1.10 g/g with a concentration of 1.88 g/L at the end of fermentation. The results obtained from this study are in line with the results of the study conducted by Yu et al., where corn hydrolysate was used as the main carbon source [10]. The results showed that the yeast extract afforded the best succinic acid production compared to peptone.

From the results obtained, it is proved that yeast extract is a good source of nitrogen. This is because yeast extract is reported to have a high protein content, reasonable molecular weight distribution, high amino acid content, and rich in vitamins and minerals [11], which promoted microbial growth of *A. succinogenes* and the production of succinic acid.

Table 1. Concentration and yield of the components

Type of nitrogen source	SA (g/L)	FA (g/L)	AA (g/L)	$Y_{SA/S}$ (g/g)	$Y_{FA/S}$ (g/L)	$Y_{AA/S}$ (g/L)
Yeast extract	2.62 ± 0.1	6.13 ± 1.0	1.54 ± 0.3	1.53	3.59	0.90
Peptone	1.88 ± 0.1	5.69 ± 0.9	1.36 ± 0.0	1.10	3.33	0.80

Fermentation of OPF hydrolysate

The total sugar content in OPF hydrolysate consisting of glucose was 1.707 g/L. Based on the graph, lignocellulose sugars are perfectly utilized by *A. succinogenes* for cell growth. The source of carbon taken up by the cell serves as a substrate of the metabolic network, where it is broken down to supply amino acid groups and other components that make up the cell [12]. Therefore, for this study, the sugar supplied through OPF hydrolysate was relatively low and also influenced the concentration of succinic acid at the end of fermentation.

Recovery of succinic acid

The effect of activated carbon dosage on the composition of the fermentation solution is shown in **Table 2**. The concentration of succinic acid after activated carbon treatment was determined to ensure minimum loss of succinic acid during the treatment process. Observations of the results trend showed no reduction in succinic acid concentration despite an increase in activated carbon loading. From the results before and after adsorption, it was observed that the concentration of succinic acid increased by a percentage of 24.7%. Formic acid was reported to decrease as activated carbon loading increased. This is because the low concentration of formic acid as well as its smaller molecular size compared to other organic acids increase the rate of adsorption on the surface of activated carbon.

A previous study reported that small-sized molecules can reach the adsorbent surface faster than larger molecules during activated carbon adsorption [13]. From the results obtained, it can be concluded that activated carbon treatment at 5% (w/w) is suitable for treating fermentation broth for succinic acid recovery as the highest SA was preserved, while the by-product AA was considerably low compared to those obtained using 4% (w/w) and 6% (w/w).

Table 2. Concentration of the components in the broth

Component (g/L)	Activated carbon dosage (g activated carbon / g broth)			
	0%	4%	5%	6%
Succinic acid	2.62 ± 0.14	3.33 ± 0.03	3.43 ± 0.01	3.29 ± 0.01
Formic acid	6.13 ± 0.95	0.81 ± 0.01	0.76 ± 0.00	0.67 ± 0.01
Acetic acid	1.54 ± 0.34	5.54 ± 0.07	5.41 ± 0.02	5.48 ± 0.01
Glucose	n.d	n.d	n.d	n.d

n.d no data

Use of activated carbon as an adsorbent

The surface characteristics of activated carbon and morphology before and after adsorption were determined using a high-resolution field scanning electron microscope (FESEM). **Fig. 3** shows the FESEM results for a porous activated carbon block (a) magnified × 1000 (surface 1), (b) magnified × 5000 after adsorption was performed (surface 1), (c) magnified × 1000 (surface 2) (d) magnified × 5000 after adsorption was performed (surface 2). FESEM analysis allows direct viewing of surface morphology which provides important information about the properties of activated carbon such as pore shape and structure.

Observations on the surface morphology of activated carbon used as adsorbents showed that activated carbon particles were found in various shapes and sizes. **Fig. 3 (i)** and **(ii)** show the presence of various types of pore sizes which are mostly oval on the surface of activated carbon. Therefore, it can be concluded that the activated carbon pore structure is a cylindrical capillary-shaped pore. Each different pore size plays an important role to be an adsorption site for the adsorption of various types of molecules. From **Fig. 3 (iii)** and **(iv)**, it can be seen that the pores are densely filled with molecules adsorbed after being introduced into a fermentation solution.

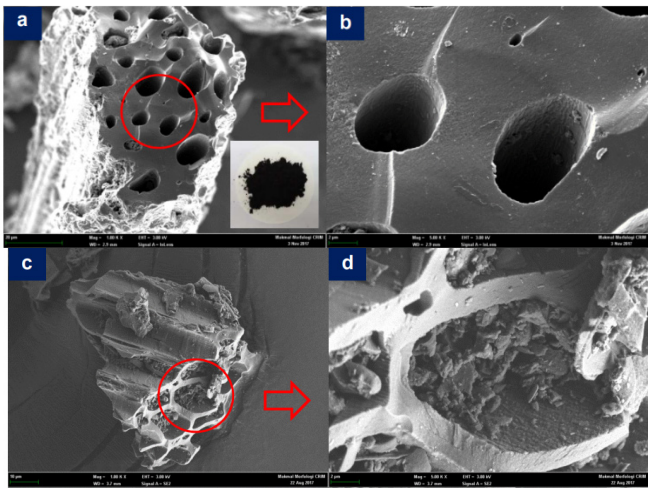

Fig. 3. FESEM results for a porous activated carbon before adsorption (a, b), and after adsorption (c, d).

CONCLUSION

In this study, the OPF was used as the main carbon source for the growth of *A. succinogenes* due to its low value and economic friendliness. The highest concentration of succinic acid when yeast extract was used as a source of nitrogen was 2.62 g/L with a yield of 1.53 g/g. The highest concentration of succinic acid when peptone was used as a source of nitrogen was 1.89 g/L with a yield of 1.10 g/g. It was determined that the yeast extract was capable of producing a higher yield of succinic acid than that of peptone. After the activated carbon was fed into the fermentation solution, it was found that the succinic acid content increased from 2.62 g/L to 3.43 g/L with a percentage increase of 24.7%. This study revealed that yeast extract was a better source of nitrogen regardless of the cost, while the use of activated carbon as an adsorbent was excellent to remove by-products and retain a higher concentration of succinic acid.

REFERENCES

1. Teodor EDB, Georgiana Ileana Teodor, Eugen Silviu. Qvo vadis? Current trends and challenges in succinite and other fossil resins (romanite) studies. AMBERIF 2016. 2016:11.
2. Saxena R, Saran S, Isar J, Kaushik R. Production and Applications of Succinic Acid. Current Developments in Biotechnology and Bioengineering: Elsevier; 2017. p. 601-30.
3. Zeikus JG, Jain MK, Elankovan P. Biotechnology of succinic acid production and markets for derived industrial products. Appl Microbiol Biotechnol. 1999;51.
4. Zakaria MR, Hirata S, Hassan MA. Hydrothermal pretreatment enhanced enzymatic hydrolysis and glucose production from oil palm biomass. Bioresour Technol. 2015;176:142-8.
5. Ahn JH, Jang Y-S, Lee SY. Production of succinic acid by metabolically engineered microorganisms. Curr Opin Biotechnol. 2016;42:54-66.
6. Luthfi AAI, Jahim JM, Harun S, Tan JP, Mohammad AW. Biorefinery approach towards greener succinic acid production from oil palm frond bagasse. Process Biochem. 2016;51(10):1527–37.
7. Zhang Q, He C, Ren J, Goodsite ME. Waste to Renewable Biohydrogen: Volume 1: Advances in Theory and Experiments: Academic Press; 2021.
8. Kim MI, Kim NJ, Shang L, Chang YK, Lee SY, Chang HN. Continuous production of succinic acid using an external membrane cell recycle system. J Microbiol Biotechnol. 2009;19.
9. Song H, Lee SY. Production of succinic acid by bacterial fermentation. Enzyme Microb Technol. 2006;39(3):352-61.
10. Yu J, Li Z, Ye Q, Yang Y, Chen S. Development of succinic acid production from corncob hydrolysate by *Actinobacillus succinogenes*. J Ind Microbiol Biotechnol. 2010;37(10):1033-40.
11. Li R, Jin M, Du J, Li M, Chen S, Yang S. The Magnesium concentration in yeast extracts is a major determinant affecting ethanol fermentation performance of *Zymomonas mobilis*. Front. Bioeng Biotechnol.. 2020;8:957.
12. Wang X, Xia K, Yang X, Tang C. Growth strategy of microbes on mixed carbon sources. Nat Comm. 2019;10(1):1-7.
13. Shimizu Y, Ateia M, Yoshimura C. Natural organic matter undergoes different molecular sieving by adsorption on activated carbon and carbon nanotubes. Chemosphere. 2018;203:345-52.