Sustainable valorization of sugarcane leaves for succinic acid and biochar production

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Abstract

   The leaves of sugarcane (Saccharum officinarum) are agricultural waste that is burnt before harvesting. This project aims to find an alternative way to increase the value of sugarcane leaves and decrease air pollution by using the leaves as raw material to produce succinic acid and biochar. Reducing sugars were extracted from the leaves by H_2SO_4 hydrolysis. The sugars were then fermented by *Yarrowia lipolytica* TBRC 4417 to produce succinic acid. The solid residue was used as the raw material for biochar production by pyrolysis. The effects of pyrolysis temperature (350, 400, and 450°C) and nitrogen gas flow rate (5, 10, and 15 L·min⁻¹) on the specific surface area of biochar were determined. The adsorption capacity of mixed nitrogen, phosphorus, and potassium compound solution at various concentrations by biochar was also investigated. The hydrolysis condition was at 1%v/v of H_2SO_4, 100 g·L⁻¹ of sugarcane leaves, and hydrolysis time of 60 min. The hydrolysate yielded sugar monomers at a concentration of ca. 13.00 g·L⁻¹ of xylose and 2.00 g·L⁻¹ of glucose. The fermentation process of extracted reducing sugar from sugarcane leaves by *Yarrowia lipolytica* TBRC 4417 was studied at 30°C for 84 h. with 120 rpm shaking. It was found that *Yarrowia lipolytica* TBRC 4417 produced succinic acid in glucose, mixed glucose and xylose, and extracted reducing sugars. The maximum succinic acid yield of 0.061 g succinic acid /g sugar consumption was obtained. For biochar production, the maximum specific surface area of 301.19 m²·g⁻¹ was found at a pyrolysis temperature of 400°C and the N_2 gas flow rate of 10 L·min⁻¹. The maximum adsorption capacity of the mixed solution was 28.45 wt%. The adsorption capacity of biochar was N>P>K at a total concentration of 100 mg·L⁻¹. This study demonstrates the agricultural waste's potential value as a useful feedstock for the biological generation of succinic acid and biochar.

1. Introduction

Sugarcane (*Saccharum officinarum*) is an important economic plant used for industrial purposes in Thailand [1]. Sugarcane plants are burnt to remove their leaves before harvesting resulting in air pollutants such as carbon monoxide (CO), carbon dioxide (CO_2), sulphur oxides (SO_x), nitrogen oxides (NO_x) and particulate matter (PM) [2-4]. In addition to the resulting air pollution, this problem can also damage soil microbial diversity. Sugarcane leaves contain 44% cellulose, 28% hemicellulose and 10% lignin [5]. These can serve as a potential feedstock for generating renewable energy. Acid hydrolysis can be used to extract reducing sugars from biomass. H_2SO_4 hydrolysis has widely used to extract the reducing sugar from biomass due to its fast reaction time and high efficiency [6]. The reducing sugars can be a sugar source for producing fine chemicals. It has been reported by Jutakridsada Saengprachatanarug [7] that ethanol could be produced from reducing sugars extracted from sugarcane leaves. Succinic acid is a dicarboxylic acid widely used in chemical industries to produce food and pharmaceutical products, surfactants and detergents, green solvents and biodegradable plastics, and ingredients to stimulate animal and plant growth. [8] Succinic acid can be derived from the fermentation of glucose and xylose [9]. In addition, the residue from the extracted leave can be used as a raw material for biochar. The biochar can be employed to uptake fertilizers during sugarcane cultivation by the thermochemical conversion process. Pyrolysis is a thermochemical conversion commonly used to convert organic materials into a solid residue containing ash and carbon, small quantities of liquid and gases. There are three types of pyrolytic reactions differentiated by the biomass's processing time and temperature: slow, flash and fast. In slow pyrolysis, tar and char are released as main products in low heating rate 0.10°C·s⁻¹ to 2.00°C·s⁻¹ at a range of 300°C·s⁻¹ to 600°C·s⁻¹ [10]. Fast pyrolysis occurs at rapid heating rates and moderate temperatures between 400°C and 600°C, which produces fewer amounts of gas and tar than slow pyrolysis. Bio-oil and gas are a primary product for plash pyrolysis, which is rapidly heated in a range of 650°C to 1000°C [11].

Therefore, in this research work, sugarcane leaves were a source of raw material to extract reducing sugars by H_2SO_4 hydrolysis. The fermentation of the reducing sugars by *Yarrowia lipolytica* yeast TBRC 4417 for succinic acid production was carried out. The sugarcane leaves residual from H_2SO_4 hydrolysis was used as a raw material for biochar production.

The slow pyrolysis was utilized in a range of 350°C to 450°C to produce biochar. The adsorption capacity of biochar for N, P, and K at various concentrations was investigated This research work was an alternative way for the bio-circular-green economy system to use sugarcane leaves to produce fine chemical and fertilizer.

2. Experimental

2.1 Materials

Sugarcane leaves were obtained from a local sugarcane farm in Khonkaen, Thailand. *Yarrowia lipolytica TBRC 4417* was purchased from BIOTEC Culture Collection (Thailand), H_2SO_4 (96%). All the chemicals were purchased from ACI Labscan (Thailand), HIMEDIA (India), KEMAUS (Australia).

2.2 Sugarcane leave pretreatment

Sugarcane leaves were washed and dried at 70°C for 12 h. After that, the dried leaves were milled using a blender until the particles passed through a 100 mesh sieve H_2SO_4.

2.3 Sulfuric acid hydrolysis of sugarcane leaves

The hydrolysis method used in this study followed that of Jutakridsada Saengprachatanarug. Briefly, 100 g of sieved sugarcane leaves were mixed with 1% H_2SO_4 aqueous solution. The total solution volume was 1000 mL. The mixed sample was autoclaved at 121°C for 1 h. The hydrolysate was separated from the residue solid by vacuum filtration with Whatman qualitative filter paper no. 4. The filtrate and the solid residue were used as raw materials for succinic acid and biochar, respectively, in the next experiment.

2.4 Production of succinic acid from sugarcane leaves

2.4.1 Microorganism and growth media

Yarrowia lipolytica TBRC 4417 was cultivated on yeast and malt extract agar (YMA) plate (10 $g·L^{-1}$ of glucose, 3 $g·L^{-1}$ of yeast extract, 5 $g·L^{-1}$ of malt extract, 5 $g·L^{-1}$ of peptone and 20 $g·L^{-1}$ of agar). The agar plate was incubated at 30°C for 72 h. After that, the microorganism was pre-cultivated in 100 mL Yeast and malt extract broth (YM). It consisted of 10 $g·L^{-1}$ of glucose, 3 $g·L^{-1}$ of yeast extract, 5 $g·L^{-1}$ of malt extract and 20 $g·L^{-1}$ of peptone to autoclave at 121°C for 30 min. Then put the single colony in YM, the media was incubated at 30°C, 120 rpm for 48 h.

2.4.2 Fermentation experiment

Before fermentation, the filtrate was added into erlenmeyer flasks 200 mL. The hydrolysate was adjusted to pH 5 before placing in a fermenter by 10 M NaOH solution. Nutrients were added before the fermentation process. The fermentation broth contains 3 $g·L^{-1}$ yeast extract, 5 $g·L^{-1}$ of malt extract, and 20 $g·L^{-1}$ of peptone. Subsequently, the hydrolysate sugarcane leave was sterilized through a 0.22 µm

membrane filter. The fermentation was done at 30°C with shaking at 120 rpm for 84 h. The samples were collected every 12 h until 84 h. The sample was centrifuged at 10000 rpm for 15 min and filtered through a 0.22 µm membrane filter before sugar and succinic determination by HPLC.

2.4.3 Pyrolysis process

The solid residue from the hydrolysis process was used to produce biochar. In brief, the solid was washed by DI water until pH 7.0 and dried at 70°C for 12 h. Then, 40 g of the solid residue was packed in the pyrolysis chamber. After that, the pyrolysis chamber was purged by nitrogen gas with a flow rate ranging from 5 to 15 $L·min^{-1}$ for 30 min. The pyrolysis was carried out at 350°C to 450°C for 1 h.

2.4.4 Adsorption testing for N, P, and K solution

In this experiment $(NH_4)_2S_2O_8$, H_3PO_4, KCl were used as N, P, and K sources, respectively. The nutrient solution was prepared from $(NH_4)_2S_2O_8$, H_3PO_4, KCl at a total concentration of 100, 200, and 400 $mg·L^{-1}$ in a total volume of 500 mL in the ratio of 1:2:1, respectively. In the adsorption experiment, 1 g of biochar was added to the nutrient solution with a magnetic stirrer at room temperature. The sample nutrient solution was collected at interval 0, 5, 10, 15, 30, 60, 90, and 120 min.

2.4.5 Characterization of the sugarcane leaves

The physical structures of the sugarcane leaves and the hydrolyzed sugarcane leaves were observed using Thermogravimetric analysis, and the sample was carried out at 600°C with a heating rate of 10°C·min⁻¹ under the nitrogen flow of 10 $mL·min^{-1}$. Surface characteristics of the sugarcane leaves, hydrolyzed sugarcane leaves and biochar were analyzed by Scanning electron microscope (SEM) (LEO 1450VP, USA). Fourier-transform infrared spectroscopy (FTIR) analysis was used to confirm a different chemical structure on sugarcane leave, hydrolyzed sugarcane leave, and biochar surface samples. The samples were analyzed by FTIR spectrometer (Bruker, Germany) using wavelengths between 600 cm^{-1} and 4000 cm^{-1} and a resolution of 4 cm^{-1}. The surface area and porosity of biochar from the pyrolysis process were observed using the Brunauer-Emmett-Teller (Micromeritics ASAP 2460, USA).

2.4.6 Cell growth analysis

The growth of yeast was determined by optical density (OD). The 1.5 mL sample was taken from each flask and centrifuged at 12000 rpm for 15 min. The supernatant was discarded, and the sediment cells washed twice with distilled water. The OD was measured using a UV-Vis spectrometer (Agilent 8453, USA) at a wavelength of 600 nm. The quantity of the cells was determined using a hemocytometer (BOECO, GERMANY) [7,12-14].

2.4.7 Sugar and succinic concentration analysis

The sample from the fermentation process was analyzed for sugar and succinic concentration using high-performance liquid

chromatography (RI, Waters e2695 separations model) with a refractive index detector (RI, waters 2414). The sugar concentration was analyzed with an RPM-monosaccharide column (300 mm × 7.8 mm; Phenomenex, Torrance, CA, USA). The mobile phase contained pure distilled water, and the flow rate was set at 0.6 mL·min⁻¹.

The succinic concentration was analyzed with the RPM-Mono-saccharide P column (300 mm × 7.8 mm; Phenomenex, Torrance, CA, USA). The mobile phase contained A: 0.03%v/v H_3PO_4, B: pure acetonitrile and the flow rate was set at 0.4 mL·min⁻¹. All samples were filtered through a 0.22 μm membrane filter prior to injection.

2.4.8 Concentration of N, P, K aqueous solution

The concentrations of nitrogen concentration (N), phosphorous concentration (P), and potassium (K) in the solution were determined by the methods that follow.

Nitrogen concentration (N) was determined following Kjeldahl procedure [15]. The sample was prepared in a total volume of 50 mL. The solution was added 10 g of Selenium reagent mixture ($CuSO_4$, Na_2SO_4), then the amount of 10 mL 0.1 N H_2SO_4 was mixed in the solution to complete digestion. After that, the solution was left to cool down. Distillation was performed by adding DI water to dilute the solution and adjust the pH to 9.5 with 6 N NaOH. During distillation, H_3BO_3 was used for NH_3 adsorption. The adsorption reached equilibrium within 3 min. The distillate solution was collected in a 75 mL in erlenmeyer flask and titrated with 0.02 N H_2SO_4 standard solution.

Phosphorous concentration (P) was determined following the Stannous chloride method [16]. Initially, 50 mL of sample was prepared in an erlenmeyer flask with phenolphthalein indicator. Then, 300 mL of H_2SO_4 mixed with 4 mL of HNO_3 in the total volume of 1 L were added to the erlenmeyer flask. One mL of this solution and 0.4 g of $(NH_4)_2S_2O_8$ was later added to the 50 mL sample. The solution was boiled until the total volume was reduced to 10 mL and cooling by adding 30 mL of water. Before using NaOH to adjust the pH to 7.0,

a phenolphthalein indicator was added to the sample. Finally, the volume of the solution was adjusted to 100 mL by adding DI water. The solution was mixed with an additional 4 mL of ($NH_{46}Mo_7O_{24}4H_2O$) and 0.5 mL of ($SnCl_25H_2O$). The mixed solution was measured absorbance by UV-Vis spectrometer (Agilent 8453, USA) at the wavelength (λ) = 690 nm.

Potassium concentration (K) was analyzed by Atomic Absorption Spectrophotometer (Analyst 100, USA). The standard potassium solution was prepared in the range of 0 ppm to 1 ppm. The solution was diluted to a total volume of 10 mL by adding 1% HNO_3.

3. Results and Discussion

3.1 Thermogravimetric analysis (TGA) of sugarcane leaves and hydrolyzed sugarcane leaves

TGA of sugarcane leaves and hydrolyzed sugarcane leaves were shown in Figure 1, which illustrates the thermal degradation in 4 stages. The first stage was a dehydration stage of material in a temperature range of 27°C to 110°C; the moisture in the biomass was removed, resulting in a slight weight loss. The second stage was active pyrolysis at 110°C to 340°C. The mass loss was mainly volatile matter. At the end of the stage, approximately the volatile matter was 59.07 wt% for sugarcane leaves and 58.61 wt% for hydrolyzed sugarcane leaves. The third stage was passive pyrolysis in a temperature range of 340°C to 480°C. The mass loss was mainly from the degradation of lignin and fixed carbon which accounted for approximately 31.66 wt% for sugarcane leaves and 33.73 wt% for hydrolyzed sugarcane leaves. Finally, during the final stage, oxygen was allowed into the reaction zone to combust with the biomass in a temperature range of 480°C to 700°C for sugarcane leaves and hydrolyzed sugarcane leaves. The complete combustion took place with the ashes consisting of 4.38 wt% of sugarcane leaves and 3.59 wt% of hydrolyzed sugarcane leaves.
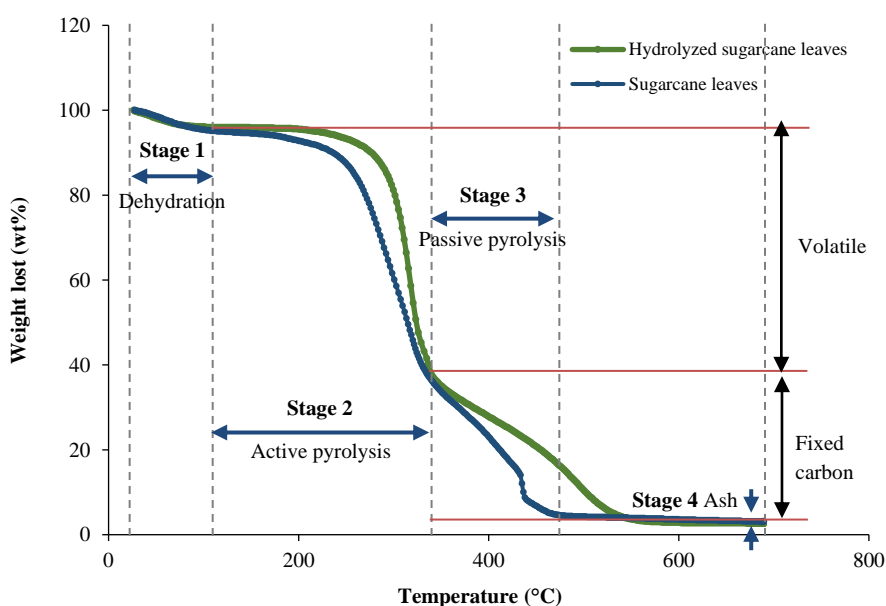

Figure 1. The thermogravimetric analysis of sugarcane leaves and hydrolyzed sugarcane leaves.

The SEM analysis (magnification 200x at 10 kV) of sugarcane leaves before and after the hydrolysis reaction is shown in Figure 2(a) and (b), respectively. The treated sugarcane leaves were activated carbon which was prepared at the condition given by N_2 flow rate of 10 L·min^{-1} and carbonization temperature of 400°C for 1 h. From these figures, improvements in the number and sizes of porous sites can easily be observed. Figure 2(a), (b) and (c) shows the SEM (magnification 200x at 10 kV) of sugarcane leaves before and after the hydrolysis reaction and biochar, respectively. The biochar was prepared at a carbonization temperature of 400°C for 1 h with N_2 flow rate of 10 L·min^{-1}. Figure 2(a) presents flattened surfaces of sugarcane leave, while the improvements in the number and sizes of porous sites on hydrolyzed sugarcane leaves can easily be observed as in Figure 2(b). Figure 2(c) illustrations biochar from pyrolysis, displaying a rougher surface than sugarcane leaves and hydrolyzed sugarcane leaves. The biochar from our work was compared with the other research; it was figured out that biochars derived from different feedstocks possess various physicochemical properties, exhibited different surface and porous. This may be biochars retain some feedstock features such as the cell structure of various kind of biomass (such as hardwood [17], softwood [18], and glass wood [19]).

FTIR analysis of sugarcane leaves, hydrolyzed sugarcane leave, and biochar is shown in Figure 3 The FTIR result shows the region of the spectrum at 500-4000 cm^{-1}. The spectrum peak at 1040 cm^{-1} indicates that C-OH stretching. The peak at 1600 cm^{-1} was associated with the stretching vibration of the C-O. The vibration stretching band around 1740 cm^{-1} were assigned to C=O [20]. The region of spectrum around 2800 cm^{-1} to 3400 cm^{-1} was attributed to CH_2, CH, and OH stretching group, respectively [21]. Besides, the different peaks of sugarcane leaves, hydrolyzed sugarcane leaves, and biochar. Sugarcane leave and hydrolyzed sugarcane leave were an observed region of spectrum at 1740, 2890, 2900, and 2400 cm^{-1}, indicating C=O, CH_2, CH and OH, respectively. In the case of biochar, its intensity peak was absent on the biochar spectrum. This may be due to the volatile organic compounds (VOCs) of sugarcane leaves and hydrolyzed sugarcane leaves removed during pyrolysis reaction [22]. Moreover, the C-O functional group on the biochar surface provide a high positive charge potential to absorb negative P ions (Phosphorus ions) [23]. The negative adsorption ability of biochar can be adsorbed and stored nutrition which can release during the plant lack of nutrition.
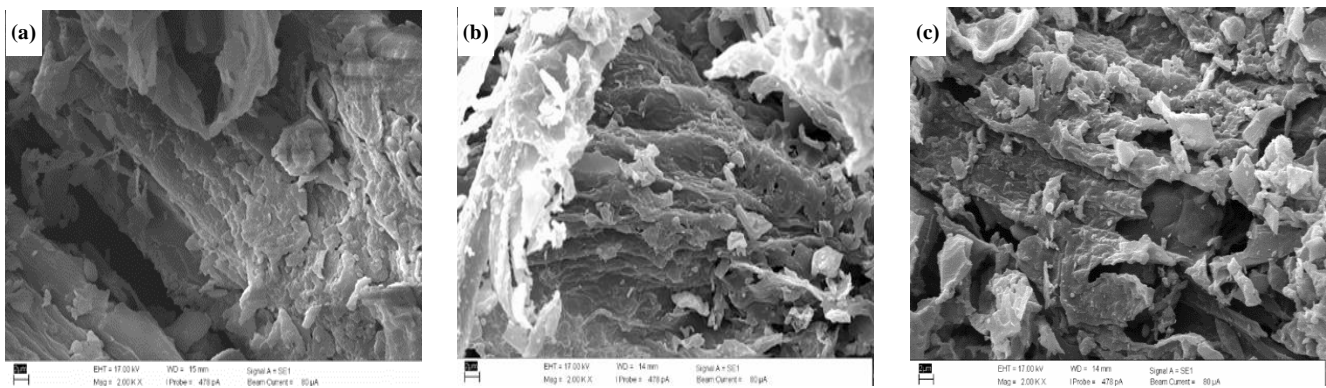

Figure 2. SEM photograph of sugarcane leaves after hydrolysis reaction (a), sugarcane leaves (b), and biochar (c).
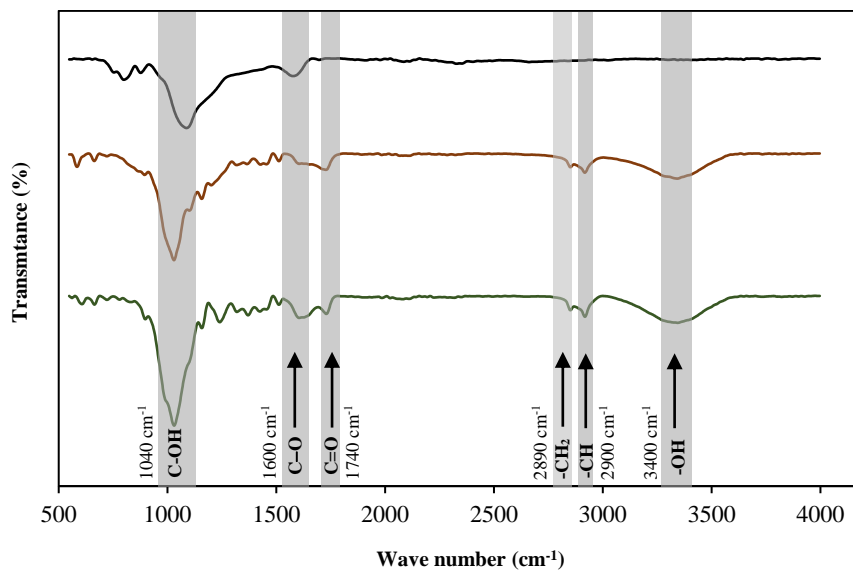

Figure 3. FTIR result of sugarcane leaves (green line), sugarcane leaves after hydrolysis reaction (dark brown line), and biochar (black line).

3.2 Succinic acid production

Figure 4 shows the yield of succinic acid production from the hydrolysate *and Yarrowia lipolytica* TBRC 4417. The hydrolysate was composed of glucose and xylose at the beginning of fermentation at 356±0.10 g·L^{-1} and 17.07±0.01 g·L^{-1}, respectively. During the fermentation process, glucose and xylose concentration gradually decreased. The final concentration of glucose and xylose was 3.06±0.70 g·L^{-1} and 15.93±0.72 g·L^{-1} at 84 h, respectively. The accumulation of succinic acid concentration was ca. 100.63±0.17 mg·L^{-1} at 84 h. A similar result was reported by Ong Li [9], who claimed that *Yarrowia lipolytica* could utilize both glucose and xylose during the fermentation process. However, the succinic acid yield from this study was less than that of their work. This result may be due to the difference in yeast strains. Yeast was obtained from a natural source in our work, while Ong Li [9] used modified yeast to produce succinic acid.

3.3 Biochar production

3.3.1 Characteristic of biochar

Table 1 shows the specific surface area of biochar at various preparation conditions. The maximum specific surface area of 301.189 m^2·g^{-1} was obtained at a carbonization temperature of 400°C, and N$_2$ flow rate of 10 L·min^{-1}. The surface area of biochar slightly increased with temperature from 350°C to 400°C and then dropped at increased carbonization temperature to 450°C. An increase in the surface area could cause the thermal decomposition of some impurity. However, at a higher temperature, the microporosity of biochar could collapse, resulting in decreased surface area. Furthermore, the surface area gradually increased with an increase of N$_2$ flow rate from 5 L·min^{-1} to 10 L·min^{-1}. after that, the surface area slightly decreased when the N$_2$ flow rate increased from 10 to 15 L·min^{-1}. This may be attributed to short residence time at the reaction zone during the pyrolysis process [24].

In addition, the biochar obtained from sugarcane leaves without the hydrolysis process at a carbonization temperature of 400°C, and the N$_2$ flow rate of 10 L·min^{-1} had a specific surface area of 116.28 m^2·g^{-1}. The specific surface area of the hydrolyzed biochar was 160% more than that of untreated biochar. This result was because the Hydronium ion from H$_2$SO$_4$ solution accessed the pores of the raw precursor, the cell wall in sugarcane leaves was degraded. This tended to increase the porosity of the raw precursor. Comparison, the obtained biochar in this work had a higher specific surface area (301.189 m^2·g^{-1}) than that of sugarcane-biochar (185.6 m^2·g^{-1}) and ricks-biochar (154.7 m^2·g^{-1}) from the work of El-Gamal Saleh [25].

Figure 5 shows the N$_2$ adsorption-desorption isotherms of biochar produced from hydrolyzed sugarcane leaves at a carbonization temperature of 400°C, and the N$_2$ flow rate of 10 L·min^{-1}. The biochar was the typical type IV isotherm based on IUPAC (International Union of Pure and Applied Chemistry) classification, which corresponded to the monolayer–multilayer adsorption [26]. Initially, it had the same path as the corresponding part of a Type II isotherm obtained with the given adsorptive on the same surface area of the adsorbent non-

porous form (IUPAC 1985). However, it presented a hysteresis loop, the lower branch of which represented measurements obtained by the progressive addition of gas of the adsorbent. The upper branch represented measurements by progressive withdrawal. The hysteresis loop was associated with the filling and emptying of the mesopores by capillary condensation [27]. This could prove to be the benefit to use this biochar as a fertilizer adsorber.
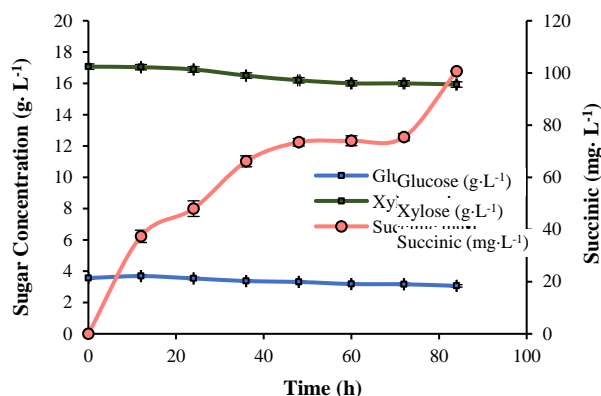

Figure 4. Succinic acid yield by *Yarrowia lipolytica* TBRC 4417 from hydrolyzed sugarcane leaves.
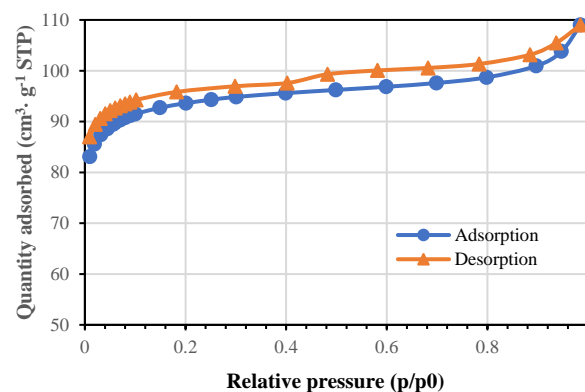

Figure 5. Adsorption/desorption isotherms of N$_2$ at 77 K on biochar derived from sugarcane leaves after H$_2$SO$_2$ hydrolysis reaction at carbonization temperature of 400°C, N$_2$ flow rate of 10 L·min^{-1}.

Table 1. specific surface area of biochar produced from sugarcane leaves after 1% H$_2$SO$_4$ hydrolysis at various preparation conditions.

Sample		Surface area (m^2·g^{-1})
Temperature (°C)	N$_2$ flow rate (L·min^{-1})	
350	5	103.82
400	5	202.58
450	5	96.18
350	10	297.44
400	10	301.19
450	10	199.77
350	15	210.90
400	15	242.35
450	15	115.90
Biochar from non hydrolysis sugarcane leaves		
400	10	116.28

3.3.2 Adsorption capacity test

Figure 6(a), (b), and (c) shows the adsorption capacity of biochar produced from hydrolyzed sugarcane leaves on NPK mixed aqueous solution at a ratio of $1:2:1$ with a total concentration of 100, 200, and 400 mg·L^{-1}, respectively. It can be noticed that at the beginning to 5 min, the adsorption of NPK on biochar significantly increased. Then, the adsorption capacity of all elements sharply dropped from 5 min to 15 min. It reached equilibrium after 20 min. The adsorption capacity was N>P>K for every mixed concentration. This could be due to the size and molecular weight of N, which is the smallest.
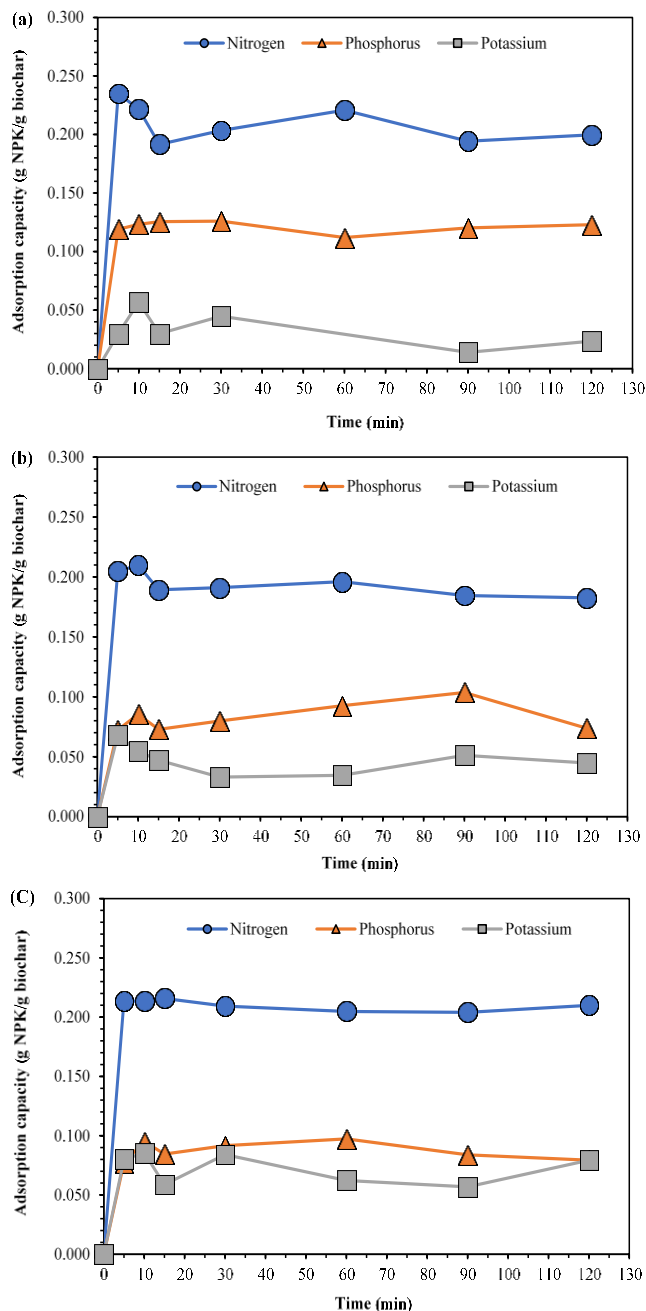

Figure 6. Adsorption capacity of bio char produced from hydrolyzed sugarcane leaves on NPK mixed aqueous solution at a ratio of $1:2:1$ with the total concentrations of 100 (a), 200 (b), and 400 mg·L^{-1} (c).
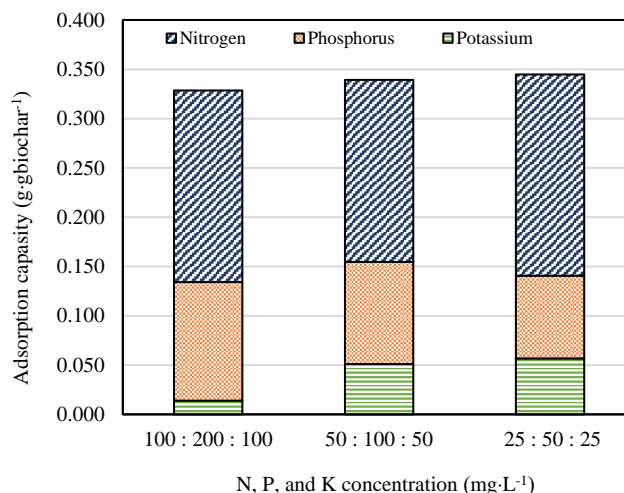

Figure 7. The effect of N, P and K concentration on biochar adsorption at adsorption time of 90 min.

The adsorption capacity of N, P and K by biochar at 90 min was tested. Figure 7 illustrates the adsorption capacity of biochar at various total N, P and K concentrations (100, 200 and 400 mg·L^{-1}) with the ratio of 1:2:1 at equilibrium time (90 min). The maximum adsorption efficiency was found at 28.45 wt% with the total concentration of N, P, and K at 100 mg·L^{-1}. The adsorption efficiency at 27.99 wt% and 27.10 wt% was obtained at the total concentrations of 200 mg·L^{-1} and 400 mg·L^{-1}. The adsorption efficiency at 27.99 wt% and 27.10 wt% was obtained at the total concentrations of 200 and 400 mg·L^{-1}. Nitrogen and Potassium compounds are in the form NH^{4+}-N and K^{+}, which is a positive ion charge, while Phosphate compound in the form of PO$_4^{2-}$ is a negative charge. It could be a negative charge at the surface and pore of biochar, attracting the positive iron [28]. This adsorbed phenomenon has been supported by FTIR result, as mentioned above. The biochar has the C-O functional group on the biochar surface provide a high positive charge potential to absorb negative ions. Also, the Nitrogen compound was adsorbed in a more incredible amount than phosphorus and potassium compounds, with the potassium compound being the least adsorbed. This could be due to the effect of the molecular size of nitrogen, the smallest, while potassium has the largest molecule [29].

4. Conclusions

In this work, succinic acid and biochar production from sugarcane leaves were successfully carried out. The reducing sugars in the hydrolysate were fermented for succinic acid by *Yarrowia lipolytica* TBRC 4417, which produced the maximum succinic acid concentration of 100.63±0.17 mg·L^{-1}. Furthermore, the maximum specific surface area of 301.189 m^2·g^{-1} was obtained from residue leaves by hydrolysis reaction at 400°C, N$_2$ flow rate of 10 L·min^{-1}. The maximum adsorption efficiency of 28.45 wt% was found at a total N, P, and K concentration of 100 mg·L^{-1} with the ratio of 1:2:1. Nitrogen compound shows the highest absorptivity on the obtained biochar. This study demonstrated the potential value of this agricultural waste as a useful feedstock for the biological generation of succinic acid and biochar.

Acknowledgements

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