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### **APPENDIX** A

### **PREPARATION OF 0.1 M CITRATE BUFFER SOLUTIONS**

0.1 M citrate buffer was prepared from the mixture of 0.1 M citric acid monohydrate  $(C_6H_8O_7 H_2O)$  solution and 0.1 M trisodium citrate dihydtrate  $(C_6H_5O_7Na_3 2H_2O)$  solution. To prepare both  $C_6H_8O_7 H_2O$  solution and  $C_6H_5O_7Na_3 2H_2O$  solution with molar concentration of 0.1 M, 21.01 grams of  $C_6H_8O_7 H_2O$  and 29.41 grams of  $C_6H_5O_7Na_3 2H_2O$  were added and dissolved well with separated 1 L of distilled water respectively. The next step of 0.1 M citrate buffer with pH 5.0 was prepared according to Table A-1 by mixing 35.0 mL of 0.1 M  $C_6H_8O_7 H_2O$  solution with 65.0 mL of 0.1 M  $C_6H_8O_7 H_2O$  solution with 65.0 mL of 0.1 M  $C_6H_5O_7Na_3 2H_2O$  solution to achieve a total volume of 100 mL of citrate buffer solution.

	(Arduengo, 2012).	
pHyalue	$0.1 \text{ M C}_{6}\text{H}_{8}\text{O}_{7} \text{ H}_{2}\text{O} (\text{mL})$	0.1 M C <sub>6</sub> H <sub>5</sub> O <sub>7</sub> Na <sub>3</sub> 2H <sub>2</sub> O (mL)
3.0	82.0	18.0
4.0	59.0	41.0
5.0	35.0	65.0
6.0	11.5	88.5

 Table A-1: Ouideline for 0.1 M citrate buffer preparation within a specific pH range (Arduengo, 2012).

### **APPENDIX B**

# PREPARATON OF 3,5-DINITROSALICYLIC ACID (DNS) REAGENTS USING MILLER METHOD

3,5-dinitrosalicylic acid (DNS) reagents was prepared according to the miller method which function to determine the present of reducing sugar – glucose (Miller, 1959). 5 grams of DNS powders were weighed and then added into 100 mL of 2 M NaOH solution. Simultaneously, 150 grams of Na-K-tartarate which function as colour stabilizer were dissolved in 500 mL of distilled water. Next, both of the prepared mixture solutions were mixed thoroughly before stored as DNS reagent solution in labelled dark bottle and kept in refrigerator when the time not in usage.

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### **APPENDIX C**

### PREPARATION OF GLUCOSE STANDARD CURVE FOR DATA ANALYSIS

A standard curve for glucose concentration analysis was constructed by initially preparing 0 g/L, 10 g/L, 20 g/L, 30 g/L, 40 g/L, 50 g/L, 60 g/L, 70 g/L, 80 g/L, 90 g/L, and 100 g/L of glucose solutions in distilled water and then 2 mL from each of the varying concentration of glucose solution was added with 2 mL of DNS reagents solution in test tube respectively. Further heating on the test tubes in water bath at 95 °C for 5 minutes was carried on and then the test tubes with mixture solutions were let to be cooled down with running tap water before addition of 8 mL of distilled water into the test tubes. Then, UV-vis spectrophotometer was used to determine the glucose concentration of samples at wavelength of 540 nm. Table C-1 and Figure C-1 respectively show the absorbance analysis and glucose standard curve with determined best fit line and linear regression with equation.



 Table C-1: Absorbance analysis for glucose standard curve.



### **APPENDIX D**

## RAW DATA FOR PRELIMINARY STUDIES: SELECTION OF BEST CHEMICAL PRETREATMENT ON BANANA TRUNK BIOMASS

The raw data of comparison studies between 2% (v/v)  $H_2SO_4$  solution (acidic) and 2% (v/w) NaOH solution (alkaline) in banana trunk biomass pretreatment are listed in Table D-1.

 Table D-1: Raw data of glucose production analysis for preliminary studies by using UV-vis spectrophotometer.

Chemical	Absorbance reading (∆540 nm)				Glucose	Standard	
type	Reading 1	Reading 2	Reading 3	Average Reading	(g/L)	deviation	
2% (w/v) NaOH	0.0516	0.0518	0.0499	0.0511	51.10	1.0440	
2% (v/v) H <sub>2</sub> SO <sub>4</sub>	0.0646	0.0646	0.0648	0.0647	64.47	0.1155	

### **APPENDIX E**

### **RAW DATA FOR ONE-FACTOR AT-A-TIME (OFAT) STUDIES**

opyright The raw data of One-Factor-at-A-Time (OFAT) studies for three influencing factors of substrate concentration, treatment duration, and treatment temperature are listed in Table E-2. In addition, Table E-1 shows the fixed parameters values for respectively OFAT studies parameters. Table E-1: Fixed parameters values for respectively OFAT studies parameters

Table E-1: Fixed parameters values for respectively OFAT studies parameters.					
OFAT studies Fixed parameter					
Substrate concentration $(0/(m/n))$	Treatment temperature	100 <b>C</b>			
(% (W/V))	Treatment duration	30 minutes			
Treatment duration	Substrate concentration	10% (w/v)			
(min)	Treatment temperature	100 °C			
Treatment temperature	Substrate concentration	10% (w/v)			
$\bigcirc$ (C)	Treatment duration	30 minutes			

Substrate	Absorbance reading ( $\Delta$ 540 nm)				Glucose	Standard	
conc. (% (w/v))	Reading 1	Reading 2	Reading 3	Average Reading	conc. (g/L)	deviation	
4	0.0290	0.0293	0.0291	0.0291	29.13	0.1528	
6	0.0363	0.0362	0.0361	0.0362	36.20	0.1000	
8	0.0518	0.0540	0.0542	0.0533	53.33	1.3317	
10	0.0646	0.0646	0.0648	0.0647	64.67	0.1155	
Treatment	Abso	orbance rea	ding (∆540	nm)	Glucose	Standard	
duration (min)	Reading 1	Reading 2	Reading 3	Average Reading	conc. (g/L)	deviation	
10	0.0358	0.0358	0.0358	0.0358	35.80	0.0000	
20	0.0513	0.0514	0.0514	0.0514	51.37	0.0577	
30	0.0646	0.0646	0.0648	0.0647	64.67	0.1155	
40	0.0551	0.0551	0.0551	0.0551	55.10	0.0000	
Treatment	Absorbance reading (∆540 nm)			Glucose	Standard		
temp. (℃)	Reading 1	Reading 2	Reading	Average Reading	conc. (g/L)	deviation	
40	0.0122	0.0122	0.0122	0.0122	12.20	0.0000	
60	0.0283	0.0283	0.0283	0.0283	28.30	0.0000	
80	0.0356	0.0355	0.0352	0.0354	35.43	0.2082	
100	0.0646	0.0646	0.0648	0.0647	64.67	0.1155	
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**Table E-2**: Raw data of glucose production analysis for OFAT studies by using UV-vis spectrophotometer.

### **APPENDIX F**

### RAW DATA FOR OPTIMIZATION STUDIES: RESPONSE SURFACE , copyright **METHODOLOGY (RSM)**

The raw data of optimization studies of pretreatment process with consideration on the

The raw data of optimization studies of pretreatment process with consideration on the parameters of substrate concentration and treatment duration via RSM are listed in Table F-1.

Run	Substrate	Treatment	Abso	Glucose			
	conc. (% (w/v))	duration (min)	Reading 1	Reading 2	Reading 3	Average reading	conc. (g/L)
1	30.00	25.00	0.0817	0.0821	0.0817	0.0818	81.83
2	10.00	25.00	0.0539	0.0540	0.0538	0.0539	53.90
3	20.00	22.93	0.0755	0.0756	0.0753	0.0755	75.47
4	20.00	30.00	0.0840	0.0841	0.0840	0.0840	84.03
5	34.14	30.00	0.0814	0.0819	0.0815	0.0816	81.60
6	10.00	35.00	0.0668	0.0669	0.0668	0.0668	66.83
7	20.00	30.00	0.0844	0.0846	0.0844	0.0845	84.47
8	5.86	30.00	0.0310	0.0317	0.0312	0.0313	31.30
9	30.00	35.00	0.0889	0.0887	0.0888	0.0888	88.80
10	20.00	30.00	0.0840	0.0846	0.0846	0.0844	84.40
11	20.00	37.07	0.0996	0.0995	0.0996	0.0996	99.57
12	20.00	30.00	0.0843	0.0843	0.0843	0.0843	84.30
13	20.00	30.00	0.0847	0.0842	0.0842	0.0844	84.37

 Table F-1: Raw data for glucose production analysis for optimization studies by using UV-vis spectrophotometer.

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### **APPENDIX G**

### PHOTOGRAPH FOR RESEARCH PROJECT

The photos of experimental preparation and equipment used are taken and shown in Plate G-1 and G-2 respectively.



Plate G-1: Preparation of raw material (banana trunk) in the form of (a) sliced pieces, (b) dried pieces in oven, and (c) powder after grinded, (d) pretreated in chemicals, and (e) dried after pretreatment.



Plate G-2: Equipment used in research experiment including (a) oven, (b) grinder, (c) sieve shaker, (d) pH meter, (e) water bath, and (f) UV-vis spectrophotometer.

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