CHAPTER 3

METHODOLOGY

3.1 Flow Chart of Overall Experiment

This research study was divided into five main parts in which including the preparation of raw materials, preliminary studies for the selection of effective chemical in pretreatment, optimization studies on the chemical pretreatment conditions, enzymatic hydrolysis and data analysis on the glucose concentration of raw materials. Preparation of raw materials includes the processes of drying, grinding, and sieving of banana trunks. The next part of the research was the aim of the research which is to optimize the chemical pretreatment conditions after the selection of effective pretreatment chemical by involving the use of Design-Expert software to provide suggested data and factors determination through RSM. The data analysis was done after the enzymatic hydrolysis of the treated raw materials by determining the yield of extracted glucose after all treatment processes. Figure 3.1 illustrates the flow chart of the experimental work to give an overview of the experiments in this research study.

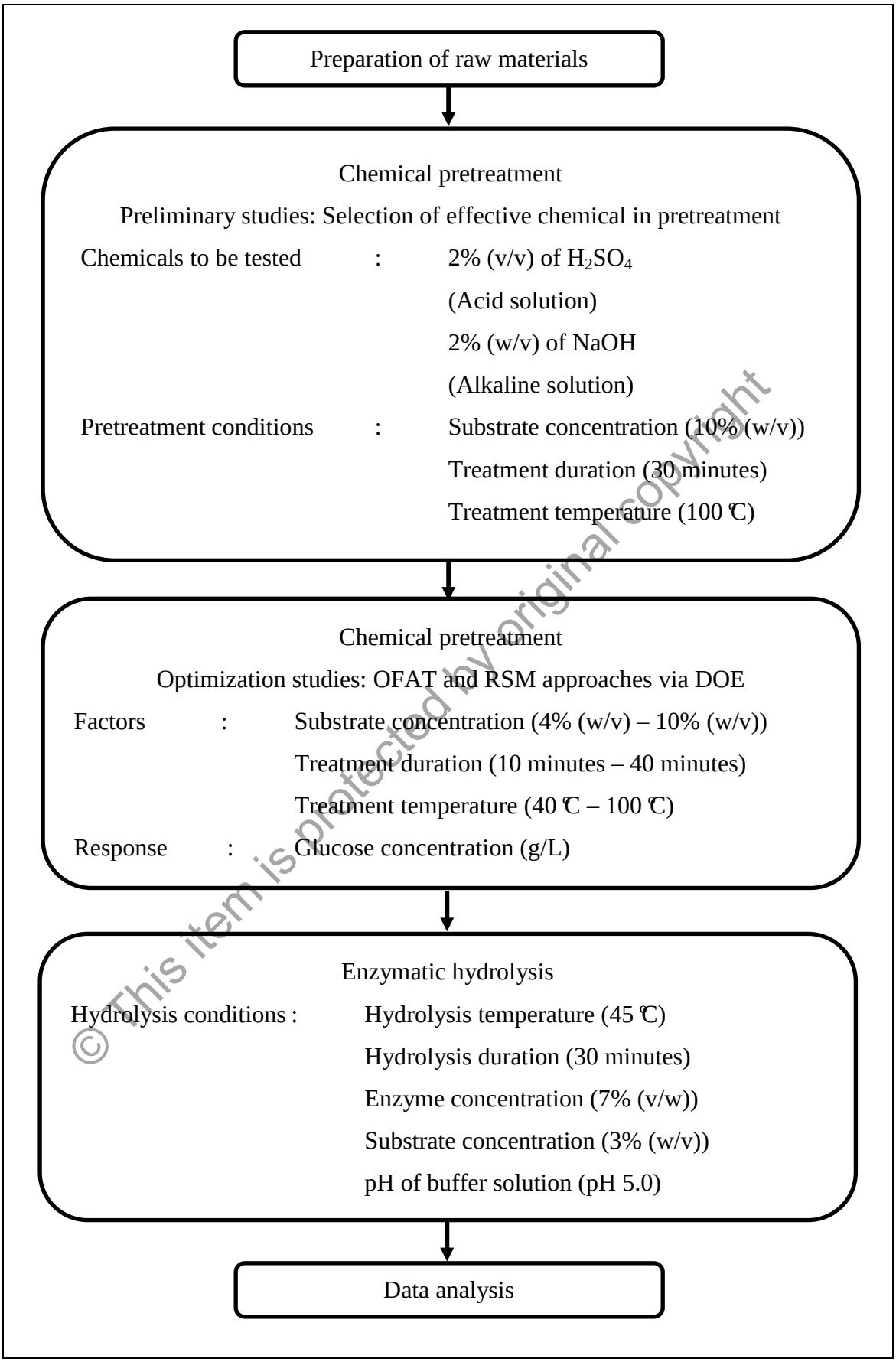

Figure 3.1: Flow chart of the steps involved in the optimization studies for glucose production

3.2 List of Chemicals Used

All the chemicals used in this study are analytical graded unless stated otherwise. The list of chemicals used in this research study is listed in Table 3.1.

Table 3.1: List of chemicals used in research study and its respective brands.

Chemicals	**Brands**
Sodium Hydroxide (NaOH)	HmbG Chemical
Sulphuric Acid (H_2SO_4)	Merck Chemical
Citric Acid Monohydrate ($C_6H_8O_7 \cdot H_2O$)	Merck Chemical
Trisodium Citrate Dihydrate ($C_6H_5O_7Na_3 \cdot 2H_2O$)	Merck Chemical
3,5-Dinitosalicylic Acid (DNS)	ACROSS Chemical
Sodium Potassium Tartarate (Na-K-tartarate)	HmbG Chemical
Cellulase Enzyme	Novozymes
Glucose ($C_6H_{12}O_6$)	HmbG Chemical

3.3 Preparation of Raw Materials

Fresh banana trunks were collected from banana plantation areas in Perlis and Penang states of Malaysia. The collected banana trunks were cleaned with washing water to remove the soil and dirt. The cleaned banana trunks were chopped into slices and further dried in oven at 80 ℃. After 24 hours of drying process, the dried banana trunks were grinded with grinder and sieved with sieve-shaker to obtain powder form of raw materials with the average particle sizes of 500 μm. The powders were kept in a sealed container with labelling to prevent from moisture contamination.

3.4 Preparation of Acid and Alkaline Solutions

To prepare 2% (v/v) of H_2SO_4 acid solution with total volume of 200 mL, 4 mL of H_2SO_4 was measured and then mixed with 196 mL of distilled water. To prepare 2% (w/v) of NaOH alkaline solution with total volume of 200 mL, 4 grams of NaOH was weighed and then dissolved in 200 mL of distilled water.

3.5 Selection of Most Effective Chemical Pretreatment Method

20 grams of grinded banana trunks powders as substrate were suspended in both 200 mL of 2% (v/v) H_2SO_4 acid solution and 2% (w/v) NaOH alkaline solution respectively. Both of the mixture solutions were incubated in 100 ℃ of water bath. After 30 minutes of incubation, the residues were filtered and washed with tap water extensively until pH turned neutral. The cleaned residues were dried overnight in oven at 70 ℃ for further comparison treatment. The most effective pretreatment chemical was selected according to its highest glucose yield after enzymatic hydrolysis treatment on the two different dried residues which pretreated with different types of chemical solutions.

3.6 Design of Experiment (DOE) for Optimization Studies

There are three parameters considered in this research study, namely substrate concentration, treatment duration, and treatment temperature. The research study was started with One-Factor-at-A-Time (OFAT) analysis to determine the optimum pretreatment condition point for each parameter range. Table 3.2 shows the three major tested parameters with its different parameter levels setting respectively.

Table 3.2: Experimental parameters with its sets of parameter levels.

Parameters	Parameter levels
Substrate concentration (%(w/v))	4, 6, 8, 10
Treatment duration (minutes)	10, 20, 30, 40
Treatment temperature (℃)	40, 60, 80, 100

Note: Each OFAT study was conducted under fixed parameters values respectively (refer Appendix E)

After determination of the optimum magnitude range for each single parameter in OFAT analysis, the only significant parameters were taken into consideration for further optimization process by using Design-Expert version 7.1.5 (Stat-Ease, Inc.) software which providing the application of Response Surface Methodology (RSM) coupled with Central Composite Rotatable Design (CCRD).

3.7 Enzymatic Hydrolysis of Pretreated Raw Materials

To prepare 7% (v/w) cellulose enzyme solution, 0.21 mL of cellulose enzyme solution was suspended in 100 mL of 0.1 M citrate buffer solution with pH 5.0 (refer Appendix A). 3 grams of pretreated raw materials as substrates were dissolved thoroughly in 7% (v/w) cellulose enzyme solution with total volume of 100 mL and incubated at temperature of 45 ℃ for 30 minutes by using water bath equipment. After the incubation step, the sample solution was filtered in order to separate the residues from the solution by using filter paper. The separated supernatant was kept for further glucose concentration determination by using DNS reagents (refer Appendix B).

3.8 Determination of Glucose Concentration of Hydrolyzed Samples

2 mL of DNS reagents was mixed well with 2 mL of samples in test tubes and further heated in 95 ℃ of water bath for 5 minutes. Next, the test tubes containing mixture solution was cooled down with running tap water after heating. Addition of 8

mL of distilled water to the 4 mL of cooled mixture solution to become total volume of 12 mL was carried out before the determination of glucose concentration by using UV-vis spectrophotometer at wavelength of 540 nm (Miller, 1959).

3.9 Statistical Analysis and Validation Test

All the experimental activities were conducted in triplicates and the average experimental data were statistically analyzed. Analysis of variance (ANOVA) was conducted at 5% significance level using the Design-Expert version 7.1.5 (Stat-Ease, Inc.) software. Validation test was run after RSM to validate the optimization results and verify the developed model in term of its capability and reliability which predicted from the Design-Expert software.