Microwave-assisted Acid Pretreatment of Sago Wastewater for Fermentable Sugar Production

Microwave-assisted Acid Pretreatment of Sago Wastewater for Fermentable Sugar Production

Noor Illi Mohamad Puad[1*], Ummul Madihah Mohd Rashidi[1], Azlin Suhaida Azmi[1], Mohd Firdaus Abd Wahab[1]

[1]Department of Biotechnology Engineering, Kulliyyah of Engineering, International Islamic University Malaysia (IIUM), P.O Box 10, 50728, Kuala Lumpur, Malaysia

*e-mail: illi@iium.edu.my

Abstract. Sago wastewater (SWW) is one of by-products from sago starch industry. In most practice, SWW is discharged to the nearby rivers. As it contains high amount of organic compounds, usual practice of disposing the wastewater will cause a detrimental impact to the environment. The organic compounds found in SWW such as starch can be utilized as substrate for biofuel and biohydrogen fermentations. However, this starch needs to first undergo pretreatment process to breakdown its complex sugars to the simpler ones. This study aimed to investigate the effect of microwave-assisted acid pretreatment of SWW for fermentable sugar (glucose) production using sulphuric acid (H_2SO_4). Three parameters that influenced microwave-assisted acid pretreatment were chosen for optimization which are residence time (3-9 min), acid concentration (0.2-0.6 M) and solid loading (5-15% w/v). Faced Centered Central Composite Design (FCCCD) tool under Response Surface Methodology (RSM) in Design Expert v9.0.6.2 software was used for designing the experiments and optimization purposes. The response was the amount of glucose converted (g/L). The maximum fermentable sugar produced was 3.920 g/L, obtained at 9 minutes with 15% solid loading using 0.6 M of H_2SO_4. Then, the kinetic study of acid hydrolysis was performed by following the first order reaction kinetics. The rate constant of reaction obtained was 0.1684 min^{-1}. It can be concluded that microwave-assisted acid pretreatment of SWW is able to produce high amount of fermentable sugar within a short residence time.

1. Introduction

Sago wastewater (SWW) is an effluent that is produced from sago starch extraction process. In each process, large quantities of water is required which resulting to high amount of solid and liquid materials in the wastewater being produced. These materials can be broken down into monosaccharide form which is fermentable sugar to be used as the substrate in the production of bio-based products such as biofuel and biogas [1]. It is reported that about 5000-6000 L of sago effluent per day was produced in each process [2]. In most practice, SWW that contains high Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD) is channelled into the nearby water bodies which may lead to water pollution. In addition, SWW is acidic due to the chemicals used during starch extraction process. Normally, the wastewater produced has an obnoxious odour, cloudy colour, acidic in nature. Due to this reasons, it might affect the soil fertility, natural ecosystems and environment. An efficient pretreatment is required to produce fermentable sugars and increase the accessibility of the carbohydrates structure [3].

There are various types of pretreatment available including chemical, physical, biological and physicochemical method. The pretreatment selected for biomass should be effective and low cost. In relation to these two priveleges, physical and chemical pretreatments are widely being used for processing lignocellulosic materials [4]. These methods are important in breaking down the structure of

starch in SWW and release fermentable sugars. Hence, the combination of pretreatment methods is a promising method to produce high yield of fermentable sugars.

Microwave heating is a pretreatment process that utilizes thermal and non thermal effects generated by microwave irradiation. Previous study reported that the reaction rate of starch hydrolysis to glucose was accelerated 100 times under microwave irradiation which is better than the conventional heating [5]. Therefore, combination of microwave heating with acid is a good alternative for pretreatment of SWW due to its high efficiency. Previous studies have reported on microwave-assisted pretreatment of different substrates including sago palm bark [6], oil palm empty fruit bunch [7], miscanthus [8] and sugarcane bagasse [9]. Although the parameters used were varied among these studies, the general principle of microwave heating is to disrupt the matrix structure of lignocellulose and enhance the production of reducing sugar.

Despite of many pretreatment methods of wastewater have been developed and reported, there are still lack of studies on the optimization of pretreatment process parameters. The important parameters for the microwave-assisted acid pretreatment process which are concentration of acid, solid loading and residence time were chosen in order to increase the production of fermentable sugars. Hence, this study aimed to pretreat SWW for fermentable sugar production using microwave-assisted acid pretreatment which is an alternative to utilize the waste. Respond Surface Methodology (RSM) with a Face Centered Central Composite Design (FCCCD) in Design Expert v9.0.6.2 software was employed for the design of experiments and optimization purposes. Then, kinetic study of acid hydrolysis was performed for pretreatment parameters under the maximum condition obtained by applying the first order kinetic reaction.

2. Materials and Methods

2.1 SWW collection, preparation and characterization

SWW was collected from a sago processing factory, in Batu Pahat, Johor, Malaysia. The collected SWW was in brownish color with approximately composed of 60% water and 40% sediment. The wastewater was stored in a cold room at 4 °C to avoid unwanted bio-degradation by the microorganisms while its sediment was sun-dried for 9 hours and then blended into the powder form.The characterization of SWW was performed according to the standard method for examination of wastewater [10] and the characteristics of SWW are as described in [11].

2.2 Optimization of microwave-assisted acid pretreatment process parameters

Microwave-assisted acid pretreatment was performed in a domestic microwave oven (Panasonic, NN-SM322M). The microwave oven has a maximal operation power of 800 W. In every pretreatment proces, a 500 mL of conical flask was placed inside the microwave. The top of conical flask was covered with cotton wool and gauze to keep the contents from spilling, evaporating or getting contaminated.

Three parameters namely concentration of H_2SO_4 (0.2 -0.6 M), solid loading (5-15%) and residence time (3-9 min) were chosen to be optimized by using FCCCD in Design Expert v9.0.6.2 software (Table 1). These parameters and ranges were chosen because of their significant effects to the glucose conversion as reported by the literature. H_2SO_4 is preferred than other types of acid due to its easy availability and will not give strong effect compared to hydrochloric acid (HCl) [10]. Furthermore, temperature was not being considered in this study because of the equipment limitation to measure the temperature of samples after pretreatment. The pretreatment experiments were consistently performed at 100 W microwave power to avoid volume losses of samples [12].

The response of each run which is the amount of glucose converted was recorded by measuring the concentration of glucose using Dinitrosalicylic acid (DNS) method before and after pretreatment as shown in Table 1. The design matrix with 17 experimental runs were executed and the results were fitted to the quadratic model. In each run, different amounts of sediment were mixed with the wastewater to form a mixture. The solid loading was varied to 5%, 10% and 15%, respectively. For example, for 5% solid loading, 5 g of sediment was mixed in 100 mL of wastewater.

2.3 Kinetic study of acid hydrolysis

Hydrolysis of starch and its kinetic study were carried out by analyzing the reaction process during the chosen residence time. In one minute interval, 0.5 mL of sample was taken to be used for starch analysis.

The concentration of starch over time was obtained by measuring the concentration of starch that was hydrolyzed in each interval. The pretreatment condition for kinetic study was selected based on the result from the optimization study. The kinetic study was performed by following the first order reaction. Starch hydrolysis reaction is expressed as shown in equation (1) [13].

$$\underset{\text{Starch}}{(C_6H_{10}O_5)_n} + \underset{\text{Acid}}{nH_2SO_4} + \underset{\text{Water}}{nH_2O} \rightarrow \underset{\text{Glucose}}{nC_6H_{12}O_6} \quad (1)$$

2.4 Analytical method

2.4.1 Glucose analysis using Dinitrosalicylic Acid (DNS) assay.
DNS analysis was conducted in order to determine the amount of glucose converted after the pretreatment process. In DNS method, DNS will react with free carbonyl group of reducing sugar to form 3-amino-5-nitrosalicylic acid which has a maximum absorption at 540 nm. Preparation of DNS reagent and DNS analytical method were performed according to [9]. The amount of glucose in the samples were determined from a glucose standard curve.

2.4.2 Starch analysis using phenol sulphuric acid method.
Phenol sulphuric acid method is a colorimetric test to determine the total carbohydrates content in a sample. This method will detect all classes of carbohydrates such as monocarbohydrates, oligo carbohydrates and polysaccharides. In this method, glucose is dehydrated to hydroxymethyl furfural which then formed a yellow brown coloured product with phenol and has maximum absorption at 490 nm [14]. Phenol at 5% was prepared by dissolving 50 g of crystallized phenol in water and diluted to one litre. For the stock solution, 100 mg of D (+) glucose monohydrate was dissolved in 100 mL of distilled water. Then, 10 mL of stock solution was further diluted to 100 mL with distilled water which was used for working standard [14].

For the analysis of sample, 0.2 mL of the sample solution was pipetted into two separate test tubes. Then, distilled water was added into each for a final volume of 1 mL. After that, 1 mL of phenol solution and 5 mL of H_2SO_4 were added to each tube. The test tubes were shaken well and set aside for 10 minutes. Then, the test tubes were placed in a water bath for 25-30℃ for 20 minutes. The absorbance of sample was taken at 490 nm. The amount of total carbohydrates present in the sample solution was calculated by using the standard graph prepared [14].

3. Results and Discussion

3.1 Effects of acid concentration, solid loading and residence time on glucose production
RSM is used to establish an explicit functional relationship between input variables and output response through regression analysis. It is a tool that comprises of statistical techniques for modelling and analysis of experiments [15]. A total of 17 runs were carried out as proposed by FCCCD in Design Expert v9.0.6.2 for different ranges of H_2SO_4 concentration (0.2, 0.4 and 0.6 M), residence time (3, 6 and 9 mins) and solid loading (5, 10 and 15%) (Table 1). The experiments were carried out in triplicates and the amount of glucose converted (g/L) was calculated by subtracting the concentration after pretreatment with the initial concentration of samples.

From Table 1, the highest amount of glucose converted is 3.920 g/L (Run 7) achieved at 0.6 M H_2SO_4 with 15% solid loading for 9 minutes of residence time. Further analysis was performed in order to develop the regression model by analyzing the values of regression coefficient, analysis of variance (ANOVA), P-values and F-values. The value of R^2 is used to express the quality of fit of the model equation. In addition, several experiments were also proposed by the software in order to validate the developed model. Table 2 summarizes the data from ANOVA analysis.

Table 1: FCCCD matrix for amount of glucose converted after pretreatment for 17 runs.

Run	Acid concentration (M)	Residence time (min)	Solid loading (%w/v)	Amount of glucose converted (g/L)
1	0.4	6	15	0.358
2	0.4	3	10	0.138
3	0.6	3	15	0.389
4	0.6	9	5	1.802
5	0.6	6	10	0.292
6	0.2	9	15	1.300
7	0.6	9	15	3.920
8	0.4	6	10	0.503
9	0.2	3	5	0.069
10	0.4	9	10	2.137
11	0.4	6	10	0.496
12	0.2	3	15	0.175
13	0.2	6	10	0.195
14	0.6	3	5	0.063
15	0.4	6	10	0.439
16	0.4	6	5	0.499
17	0.2	9	5	0.170

Table 2: ANOVA for response surface quadratic model for glucose production.

Source	Sum of Square	Mean Square	F-value	P-value >F	
Model	2.03	0.23	40.49	<0.0001	***Significant***
Acid concentration (M)	0.27	0.27	48.39	0.0002	
Residence time (min)	1.11	1.11	202.60	< 0.0001	
Solid loading (%)	0.14	0.14	26.20	0.0014	
Lack of fit	0.031	0.00628	1.79	0.3967	***Not significant***

In this study, the value of R^2 calculated by ANOVA analysis was 0.9814. By refering to the R^2 value, the correlation between experimental and predicted values is good when the value of R^2 is closer to unity. From the value, it shows a high degree of correlation between the experimental and predicted values. By refering to Table 2, it shows that the quadratic model developed was highly significant which is <0.0001 since (P-value > F < 0.05) and the lack of fit is 0.3967 where it was not significant (P-value >F >0.1). It is desirable to obtain not significant lack of fit since it indicates the adequacy of the model. From the analysis, the highly significant factors that affect the amount of glucose converted is the residence time where the value of Prob >F was <0.0001 which is less than 0.05. Then, it was followed by concentration of acid and solid loading which are 0.0002 and 0.0014, respectively. Based on the ANOVA analysis, it can be concluded that all parameters are significant since the P-value was less than 0.05.

The result can be supported by previous study in which microwave-assisted acid pretreatment of switchgrass was performed at the residence times of 5, 10, 15 and 20 minutes. The higher yield of reducing sugar was obtained at the residence time of 15 minutes [16]. In [17], the highest yield of reducing sugar from microwave-assisted pretreament of sweet sorghum was obtained at 15 minutes of pretreatment time. The increase of reaction time to 20 minutes resulted in loss of sugars due to formation of inhibitors such as furfural. For the effect of acid concentration, it was reported that 5 wt% H_2SO_4 concentration allows the cellulose chains of sweet sorghum to break and then allowing contact for the pretreatment reaction [17]. Further degradation of sugars resulted to the formation of other compounds such as furfural. This concludes that residence time can be increased up to certain time in order to obtain high amount of glucose converted. In this study, the maximum range of residence time chosen was 9 minutes due to safety concern and limitation of experimental set-up.

For predicting the optimal values of glucose converted within the selected ranges, a second order of polynomial model was fitted to the experimental results by Design Expert software. The model developed is shown in equation (2).

$$Amount\ of\ glucose\ converted = (+0.99 + 0.16A + 0.33B + 0.12C - 0.052A^2 + 0.17B^2 + 0.045C^2 + 0.18AB + 0.025AC + 0.12BC)^2 - 0.55 \quad (2)$$

where A is acid concentration (M), B is residence time (min) and C is solid loading (%).

The 3D response surface and 2D contour plot are the graphical representation of the regression equation which is used to determine the optimum condition within the selected ranges. The 3D and 2D plots for the interactions between two parameters are presented in figures 1, 2 and 3. The aim of the response surface is to find for the optimum condition such that the response is maximized. Figure 1 shows the 3D plot illustrating the response surface from the interaction between residence time and concentration of acid on the amount of glucose converted. It can be observed that at 10% of solid loading, the amount of glucose converted increased when both parameters (A and B) were set at the highest level.

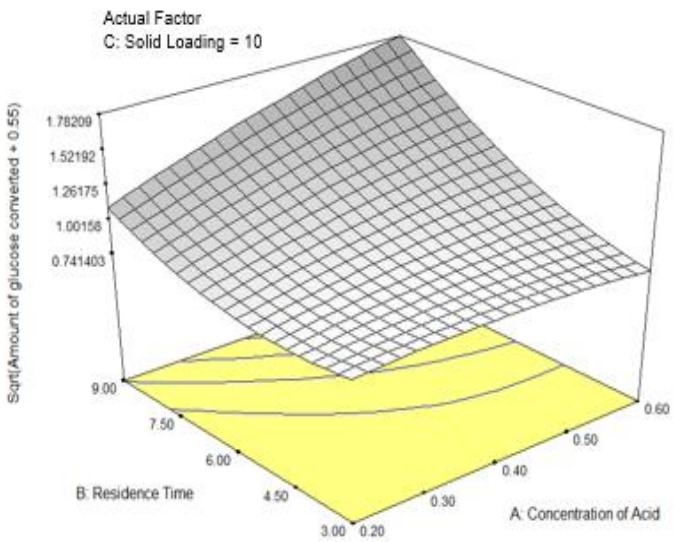

Figure 1: Response surface plot showing the effect of residence time (B) (min) and concentration of acid (A) (M) on amount of glucose converted (g/L).

Meanwhile, figure 2 represents the relationship between solid loading and concentration of acid. The actual factor for residence time was set at 6 minutes. It can be observed that solid loading did not give significant effect on the glucose converted since the response were nearly constant. From the plot, the amount of glucose converted increased when the concentration of acid was at the high level. Figure 3 illustrates the variation of the amount of glucose converted, as a function of solid loading and residence time by keeping the concentration of acid constant at 0.4 M. The plot shows that as both solid loading and residence time increased, amount of glucose converted increased.

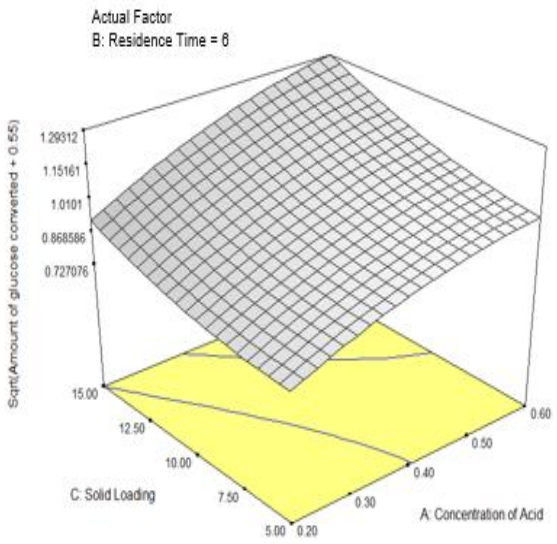

Figure 2: Response surface plot showing the effect of solid loading (C) (%) and concentration of acid (A) (M) on amount of glucose converted (g/L).

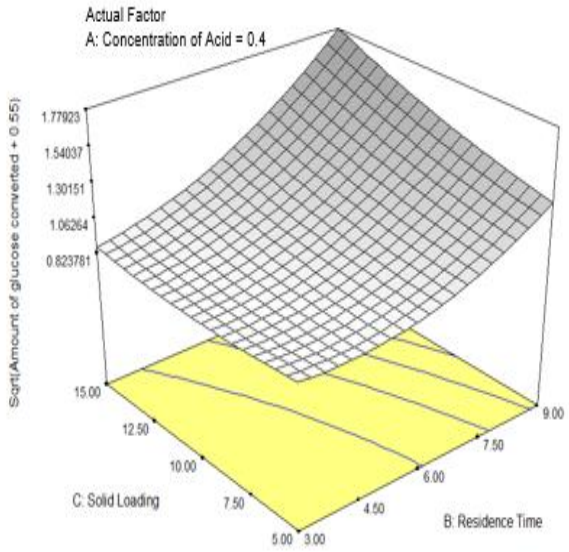

Figure 3: Response surface plot showing the effect of solid loading (C)(%) and residence time (B) (min) on amount of glucose converted (g/L).

Although the maximum amount of glucose converted was obtained at Run 7 (3.920 g/L), the microwave-assisted acid pretreatment process condition has yet to be optimized. Moreover, the optimal point has yet to be found since glucose yield was kept on increasing with respect to the selected ranges for all parameters. Basically, microwave-assisted acid pretreatment has the potential to produce high concentration of glucose in a short period of time. From this study, it was observed that the maximum glucose obtained was 3.920 g/L in only 9 minutes of residence time. This value can be compared with the conventional acid pretreatment method. It was reported that the highest glucose concentration achieved was 9.13 g/L at 100℃ reaction temperature for 60 minutes of incubation time which is comparatively higher than in microwave-assisted acid pretreatment. Besides that, [18] concluded that high sugar of 13.61 g/L was obtained under an optimal condition at 140℃ for 10 minutes of reaction time. It showed that even though the conventional acid pretreatment lead to high glucose concentration, a longer pretreatment time and high reaction temperature were needed. However, it is important to

identify the suitable temperature since higher temperature of pretreatment would cause in the sugar degradation and thus lead to the formation of inhibitors [3].

In order to validate the model, a validation experiment was carried out as proposed by Design Expert v9.0.6.2. The empirical value of glucose converted was 3.820 g/L which is acceptable as the value is closed to the predicted value of 3.839 g/L. Hence, the values obtained confirmed the adequacy of model with the percentage difference of 0.49%.

3.2 Kinetic study of starch hydrolysis

The kinetic equations used for the hydrolysis of starch are shown (equations 3 and 4):

$$\ln C_0/C = kt \quad (3)$$

$$\ln C = -kt + \ln C_0 \quad (4)$$

where C_0= initial concentration of starch (g/L), C = concentration of starch at time t (g/L) and k = rate constant.

It can be observed that the concentration of starch was decreased over time (figure 4). This is because the molecules of starch present in the samples have been hydrolyzed to glucose during the pretreatment process. It shows that the rate constant for the microwave-assisted acid pretreatment of SWW was 0.1634 min^{-1} which displays how fast the reaction occurs which is affected by the concentration of the reactant [19]. The linearity of plots in figure 4 demonstrates that acid hydrolysis of SWW follows the first order reaction with the R^2 value of 0.8669 which indicates how close the data are to the fitted regression line. Previous study has reported that the concentration of reducing sugar was increased over time which resulted in the declination of starch concentration [13]. This is because more starch was hydrolyzed into reducing sugars over period of time.

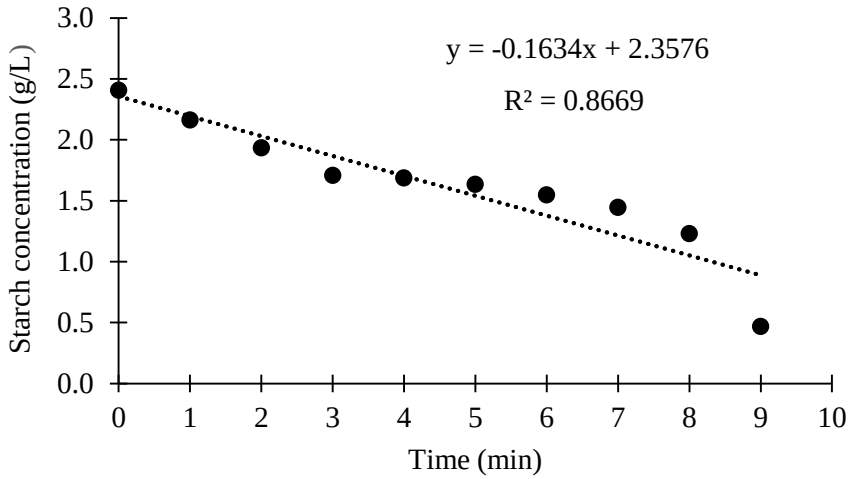

Figure 4: Plot of ln C versus residence time (min) using 0.6 M H_2SO_4 with 15% solid loading.

4. Conclusion

In this study, the optimization of pretreatment process parameters for maximum amount of glucose converted was conducted by using FCCCD. The chosen parameters were concentration of acid (M), solid loading (%) and residence time (min). The selected ranges were 0.2-0.6 M of H_2SO_4, 5-15% of solid loading and 3-9 minutes residence time. The maximum amount of glucose converted (3.920 g/L) was achieved from Run 7 (0.6 M of H_2SO_4, 9 minutes and 15% of solid loading). With the R^2 value of 0.9814, it confirmed the significance of the process parameters and the response of the experiments. Moreover, it can be concluded that the kinetic study followed the first order reaction kinetics. It was found that the rate constant value obtained was 0.1684 min^{-1} which indicates how fast the hydrolysis reaction of SWW occur. The R^2 value of 0.8669 obtained from the plot shows the adequacy of the kinetics model.

Acknowledgments
The authors would like to thank Department of Biotechnology Engineering, Faculty of Engineering, IIUM for kindly provided the financial support and laboratory facilities.

References

[1] Chen H 2014 Applications of Lignocellulose Biotechnology in Bioenergy. *Biotechnology of Lignocellulose: Theory and Practice* (Dordrecht: Springer Netherlands) p 213-45
[2] Anbukumar S, Prasad NM and Ashok Kumar M 2014 Effluent Treatment for Sago Industry Using Zeolite and Activated Carbon. *Open Journal of Water Pollution and Treatment*. **2014** 18-26
[3] Yang B and Wyman CE 2008 Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels, Bioproducts and Biorefining*. **2** 26-40
[4] Ethaib S, Omar R, Mustapa Kamal SM and Biak DR 2015 Microwave-assisted pretreatment of lignocellulosic biomass: A review. *Journal of Engineering Science and Technology*. **10** 97-109)
[5] Kunlan L, Lixin X, Jun L, Jun P, Guoying C and Zuwei X 2001 Salt-assisted acid hydrolysis of starch to d-glucose under microwave irradiation. *Carbohydrate Research*.**331** 9-12
[6] Ethaib S, Omar R, Mustapa Kamal SM, Awang Biak DR, Syam S and Harun MY 2017 Microwave-Assisted Pretreatment of Sago Palm Bark. *Journal of Wood Chemistry and Technology* **37** 26-42
[7] Nomanbhay SM, Hussain R and Palanisamy K 2013 Microwave-Assisted Alkaline Pretreatment and Microwave Assisted Enzymatic Saccharification of Oil Palm Empty Fruit Bunch Fiber for Enhanced Fermentable Sugar Yield. J*ournal of Sustainable Bioenergy System*s. **3** 7-17
[8] Zhu Z, Macquarrie DJ, Simister R, Gomez LD and McQueen-Mason SJ 2015 Microwave assisted chemical pretreatment of Miscanthus under different temperature regimes. *Sustainable Chemical Processes*. **3** 15
[9] Zhu Z, Rezende CA, Simister R, McQueen-Mason SJ, Macquarrie DJ, Polikarpov I, et al 2016 Efficient sugar production from sugarcane bagasse by microwave assisted acid and alkali pretreatment. *Biomass and Bioenergy*. **93** 269-78
[10] Mohamad Puad NI, Abd Rahim NF and Azmi AS 2018 Acid Pretreatment of Sago Wastewater for Biohydrogen Production. *E3S Web Conf.* **34** 02012
[11] Ulhiza TA, Mohamad Puad NI and Azmi AS 2018 Optimization of culture conditions for biohydrogen production from sago wastewater by Enterobacter aerogenes using Response Surface Methodology. *International Journal of Hydrogen Energy*. **43** 22148-58
[12] Nomanbhay SM and Hussain R 2014 Microwave Assisted Alkaline Pretreatment and Microwave Assisted Enzymatic Hydrolysis of Palm Oil Mill Effluent (POME) for Optimum Fermentable Sugar Yield. *Chemical Science Transaction*s. **3** 350-8
[13] Bej B, Basu RK and Ash SN 2008 Kinetic studies on acid catalysed hydrolysis of starch. *Journal of Scientific and Industrial Research*. **67** 295-8
[14] Agrawal N, Minj DK and Rani K 2015 Estimation of Total Carbohydrate Present In Dry Fruits. IOSR Journal of Environmental Science, *Toxicology and Food Technology*. **1** 24-7
[15] Morshedi A and Akbarian M 2014 Application of Response Surface Methodology: Design of Experiments and Optimization: A Mini Review. *Indian Journal of Fundamental and Applied Life Sciences*. **4** 2434-9
[16] Keshwani DR Cheng JJ, Burns JC, Li L, and Chiang V 2007 Microwave Pretreatment of Switchgrass to Enhance Enzymatic Hydrolysis. *2007 ASAE Annual Meeting*; St. Joseph, MI: ASABE
[17] Ndaba B 2013 *Microwave assisted pretreatment of sweet sorghum bagasse for bioethanol production*. South Africa: North-West University
[18] Satimanont S, Luengnaruemitchai and Wongkasemjit S. 2012 Effect of temperature and time on dilute acid pretreatment of corn cobs. *International Journal Chemical Biological Engineering*. **6** 333-7
[19] Muhaimin, Sudiono S 2017 Kinetic study of hydrolysis of coconut fiber into glucose. *AIP Conference Proceedings*. **20092** 1823