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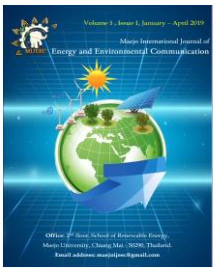

ARTICLE

Influential degree of polymerization of sugar extraction on alkali pretreatment from sunflower stalk wastes by applied statistical modelling

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ABSTRACT

This paper presents application of influence of degree of polymerization (DP) on optimally pretreated using a response surface methodology (RSM) approach for decreased DP level of optimal chemical and biological pretreatments from sunflower stalk. All experiments in this paper are applied by statistical designs for developing a statistic multifunction analysis model that focus on the effect of dissimilar factors for describing of the optimum values of the changed surface response on any variables. The process parameters of chemical model (Sodium Hydroxide concentration and Time) to pretreat for DP. The chemical pretreatment model was certified by 13 runs, at two factors, NaOH (1, 1.5, 2%) and Day (1, 2, 3) by central composite design (CCD). DP value of the chemical model was estimated by a Design Expert program version 11 trial: chemical model of DP highest and lowest of 25.80 and 6.16, consecutively. The aim of this experiments to investigate only DP from the chemical model of pretreatment. The procedure there are effective on sugar conversion and DP of the lignocellulosic biomass. Which pretreatment is a challenge for cost and competitive technology on large-scale of fermentable sugar in the step of hydrolysis.

1. Introduction

In addition energy has been needed for the growing demand and the development of the world Fossil fuels are the one source of energy the world. However, the use of fossil fuels is related with numerous environmental problems that affect the entire countries and the people (Vu et al., 2017). Nowadays, due to the high consumption, it is close to natural limits and takes a long time to be created (Vu et al., 2017). In consequence, biofuel from biomass therefore is an important alternative to substitute for primary energy. Lignocellulosic is a talented bioprocess substrate for the invention of biofuels because agricultural biomass are organized a possible source of low-cost material for the bioenergy construction (Manmai et al., 2019a). It is one of the most plentiful renewable resources for value-added chemicals. The construction of bioethanol from lignocellulosic has involved worldwide interest (Manmai et al., 2017a).

The three classifications of biomaterials for being bioethanol substrates are sugar (monosaccharides and disaccharides), starchy (reserve polysaccharides), and lignocellulosic (structural polysaccharides) crops (Bautista et al., 2019). Bioethanol is a type of bioenergy with good for environmental, signifies a reassuring biofuel in current which is ordinarily used in blending with oil (Manmai et al., 2017b).

Nomenclature and Abbreviation

RS	Reducing sugar
TS	Total sugar
RSM	Response surface methodology
CCD	Central composite design
SS	Sunflower stalk
DP	Degree of polymerization

It can be produced from different types of agricultural renewable materials such as sunflower stalk, sorghum stalk, sugarcane leaf and con stalk in a fiber part (Manmai et al., 2017c; Manmai et al., 2018a; Manmai et al., 2018b) and for weedy feedstocks are gooseweed and small-flowered nutsedge (Vu PT et al., 2017; Vu et al., 2018; Ramaraj and Unpaprom 2019a; Ramaraj and Unpaprom, 2019b) . In the same direction corn stalk juice and pineapple fruit peel wastes used for ethanol producing (Bautista et al., 2019; Casabar et al., 2019).

The major benefits of biofuels are presented in (Balat, 2011). The using of biofuels can help to reduce greenhouse gas emissions (Tran et al., 2019), provide clean and sustainable energy sources, and increase the agricultural profits for poor people from developing countries. However, there may be other socio-economic and environmental impacts that affect the potential of developing countries that will benefit from the increased demand for biofuels worldwide.

Carbohydrates consists of carbon, hydrogen and oxygen. The minutest carbohydrate structures are monosaccharides, it is different types of polymerization, forming complex sugars, starches, and fibers. The carbohydrates are classified agreeing to chemical-structural properties, considered of degree of polymerization, in addition to classification of the carbohydrates by physiological properties. The degree of polymerization relates the numeral of monomers in a molecule. The classification is categorized carbohydrates into four groups: sugars, oligosaccharides, polysaccharides, and polyols. The chemical structural classification was presented in Table 1 from (Scapin et al., 2017).

Table 1. Chemical structural classification of the carbohydrates by the degree of polymerization (Scapin et al., 2017).

Classification	DP	Subgroup	Components
Sugars	1	Monosaccharides	Glucose, galactose, fructose
	2	Disaccharides	Sucrose, lactose, maltose, trehalose
Oligosaccharides	3-9	Malto-oligosaccharides	Maltodextrin
		Other oligosaccharides	Raffinose, stachyose, fructooligosaccharide
Polysaccharides	>9	Starch	Amylose, amylopectin, modified starches
		Non-starch polysaccharides	Cellulose, hemicellulose, pectins, ydrocolloids
Polyols	1->9	-	Erythritol, xylitol, mannitol, sorbitol, maltitol, isomalt, lactitol, polyglycol.

Sodium hydroxide (NaOH) pretreatment is the one of the most standard procedures and it is widely studied in bioconversion of lignocellulose, it is extremely effective to increase the degradability of hardwoods and agricultural waste materials with low lignin concentrations. (Bali et al., 2015).

Response surface methodology (RSM) is frequently organized for model to evaluate a procedure to study the relation among several independent factors and one or more response and the optimization of a process (Ramaraj and Unpaprom, 2019a). The design of new trials and utilizing concurrent optimization protocols, including the central composite design (CCD) that the two-level factorial design with additional points (star points) and at least one central point. The efficiency of the method with a minimum run of trials (Bagheri et al., 2019).

In this study investes the optimized DP value that changes with time and the concentration of NaOH by using statistical modelling to explane changed value. This study focused on using diluted NaOH pretreatment procedures which embraces time and NaOH concentration. The accountable properties of input parameters on the product of DP value are inspected. Furthermore, an initial assessment of the use of these DP predictes condition for hydrolysis and bioethanol production in the future.

2. Materials and methods

2.1 Resources preparation

Sunflower Stalks (SS) with Leave were gathered after seed harvesting in Figure 1(c) from the farm of Program in Agronomy, Faculty of Agricultural Production, Maejo

University, Chiang Mai, Thailand (18° 8' 98" N 99°0' 13" E) in Figure 1(a). Fished SS in Figure 1(b) were dried in a solar drying house was showed in Figure 1(d) among ambient temperature at Chiang Mai (30–32 °C) for 3 days, the first time of drying. The resources were reduced size two steps by cutting and blending machines. Dried and powdered SS was dehydrated again for evaporation moisture and water in resources by a hot air oven at 60 °C for 48 h and stored in plastic bags at room temperature until further using. All resources preparation was adopted from (Manmai et al., 2019a; Manmai et al., 2019b; Manmai et al., 2020).

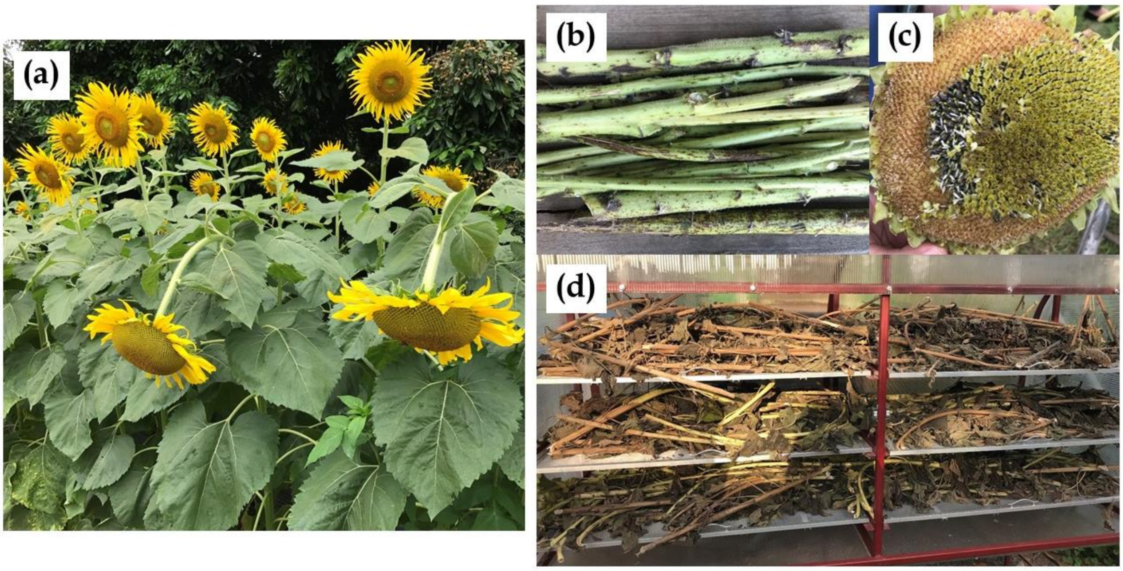

Figure 1. (a) Sunflower farm, (b) Fished sunflower stalk, (c) Seed and inflorescence (d) Dried stalk

2.2 Experimental design

In the prevent researches in 2019 and 2020, Manmai et al. presented the model for total sugar (TS) and reducing sugar (RS) were pretreated by NaOH (1, 1.5 and 2 % w/v) and T. reesei (1, 1.5 and 2 % v/v), which these research is series of two researches. The parameters of these study are used for DP of biomass pretreatment by chemical method consists of concentration of NaOH (%w/v, A_{NaOH}) and time (day, B_{NaOH}). All of factors were used as independent factors and three investigational levels: − 1, 0, 1. The range and center point values of the three independent factors were chosen after a series of preliminary single factor experiments in Table 2. A standard responsive surface design (RSM) is the central composite design (CCD). The RSM method is suitable for quadratic surface adjustment to optimize process factors with a minimum run of experiments, including analyzing the collaboration between parameters.

Table 2. Degree of polymerization parameters and their values used for experiment (Manmai et al., 2020)

Factors	Symbols	Unit	-1	0	1
NaOH	A_{NaOH}	$\%_{(w/v)}$	1	1.5	2
Time	B_{NaOH}	days	1	2	3

2.3 Analytical methods

Reducing sugar concentration was estimated by 3,5-dinitrosalicylic acid (DNS) method The absorbance was measured with a UV-Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 540 nm, using blank as control (Miller, 1959).

Total sugar concentration was analysed by phenol sulfuric methods at Spectrophotometer detector DV-8000 (Drawell, Osaka, Japan) at 490 nm (Dubois et al., 1956). Colorimetric method for determination of sugars and related substances. Degree of polymerization is calculated by total sugar concentration devises reducing sugar concentration.

3. Results and discussion

3.1. Composition of sunflower stalk

In this research, sunflower stalks contain the following composition: (dry basis), glucan 33.99 ± 0.15%, xylan 24.1 ± 0.11%, arabinan 0.96 ± 0.00%, and lignin 25.9 ±0.17%. Almost similar results of untreated sunflower stalks were reported in Hesami et al..

3.2. The equation in terms of coded

$$DP= 11.913 - 7.4799A - 2.2669B + 1.147AB + 3.180A^2 + 0.068B^2 \quad (1)$$

The coded in Equation 1 is used to predict about the response for given levels of each factor. The coded equation is beneficial for classifying the relative impact of the factors by comparing the factor coefficients.

3.3. ANOVA of NaOH pretreatment

The suitability of the models was observed by analysis of variance (ANOVA) and the results are showed in Table 3. The model, A-NaOH, B-Time, AB and A^2 *F*-values of 91.26, 378.2, 34.74, 5.92, 31.47of DP respectively, it means that the model is significant. Only B^2 is not signified of 0.0144. There are only a 0.01% accidental that an *F*-value this large could occur due to noise.

P-values less than 0.05 are designated model terms are fit. In this model A, B, AB, A^2 are significant model terms. And in this model there is only one value greater than 0.1 indicate the B^2 terms are not significant.

The Lack of Fit *F*-value of DP from NaOH pretreatment is 0.89. It indicates the Lack of Fit is not significant relation to the pure error. It means that the model to fit.

The model of DP there are identical percent of C.V., R^2, adjusted R^2 and predicted R^2 as 7.02, 0.9849, 0.9741 and 0.9453, respectively. The Predicted R^2 of 0.9453 is in realistic arrangement with the Adjusted R^2 of 0.9741 because the difference value is less than 0.2.

Adeq Precision is measured the signal to noise ratio. A ratio greater than 4 is desirable. The model ratio of 30.456 indicates an acceptable signal. This model can be used to navigate the design space.

In Table 4 showed to finish the number of experimental runs of alkali pretreatment beside the predicted and observed responses of DP.

Table 3. ANOVA model for optimization of DP from NaOH pretreatment of sunflower stalk

Source	Sum of Squares	df	Mean	*F*-value	*p*-value	
Model	405.01	5	81	91.26	< 0.0001	significant
A-NaOH	335.7	1	335.7	378.2	< 0.0001	
B-Time	30.83	1	30.83	34.74	0.0006	
AB	5.26	1	5.26	5.92	0.0452	
A^2	27.93	1	27.93	31.47	0.0008	
B^2	0.0128	1	0.0128	0.0144	0.9079	
Residual	6.21	7	0.8876			
Lack of Fit	2.48	3	0.8271	0.8864	0.5203	not significant
Pure Error	3.73	4	0.933			
Cor Total	411.22	12				
Pure Error	0.1705	4	0.0426			
Cor Total	12.45	12				
Std. Dev.	0.9421		R^2	0.9849		
Mean	13.41		Adjusted R^2	0.9741		
C.V. %	7.02		Predicted R^2	0.9453		
			Adeq Precision	30.4562		

Table 4. Designed runs with actual and predicted values of NaOH pretreatment of sunflower stalk

Std.	Run	Factor 1	Factor 2	Respond 1: DP		
		A:NaOH (%)	B:Time (Days)	Actual Value	Predicted Value	Residual
8	1	1.5	3	8.33	7.61	0.7129
10	2	1.5	2	11.83	11.91	-0.0862
1	3	1	1	10.45	9.71	0.7354
9	4	1.5	2	6.16	6.56	-0.3994
5	5	1	2	10.96	11.91	-0.9544
6	6	2	2	10.4	11.91	-1.51
12	7	1.5	2	18.89	19.23	-0.336
4	8	2	3	8.49	8.8	-0.3136

2	9	2	1	23.16	22.57	0.5862
7	10	1.5	1	12.73	11.91	0.8202
3	11	1	3	12.35	11.91	0.4346
13	12	1.5	2	14.81	14.25	0.5637
11	13	1.5	2	25.8	26.05	-0.2502

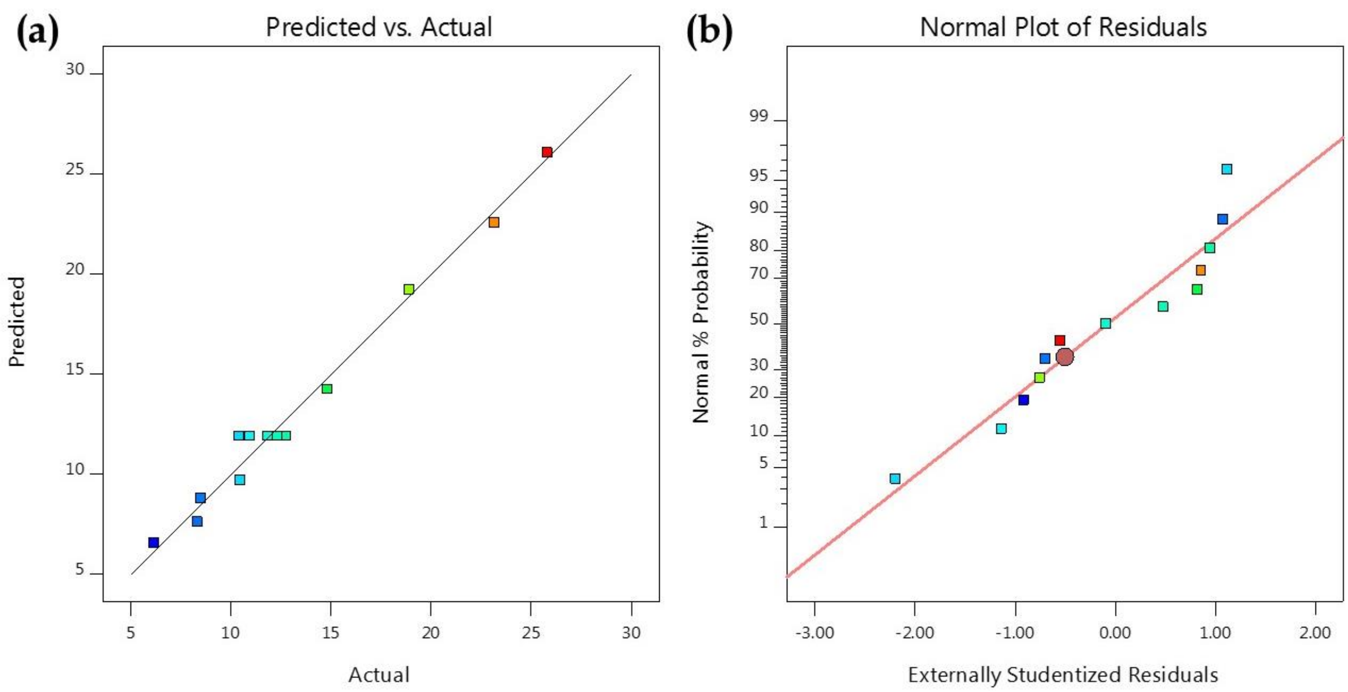

Figure 2. DP analytical plots of (a) Predicted versus actual plot; (b) Normal plot of residuals

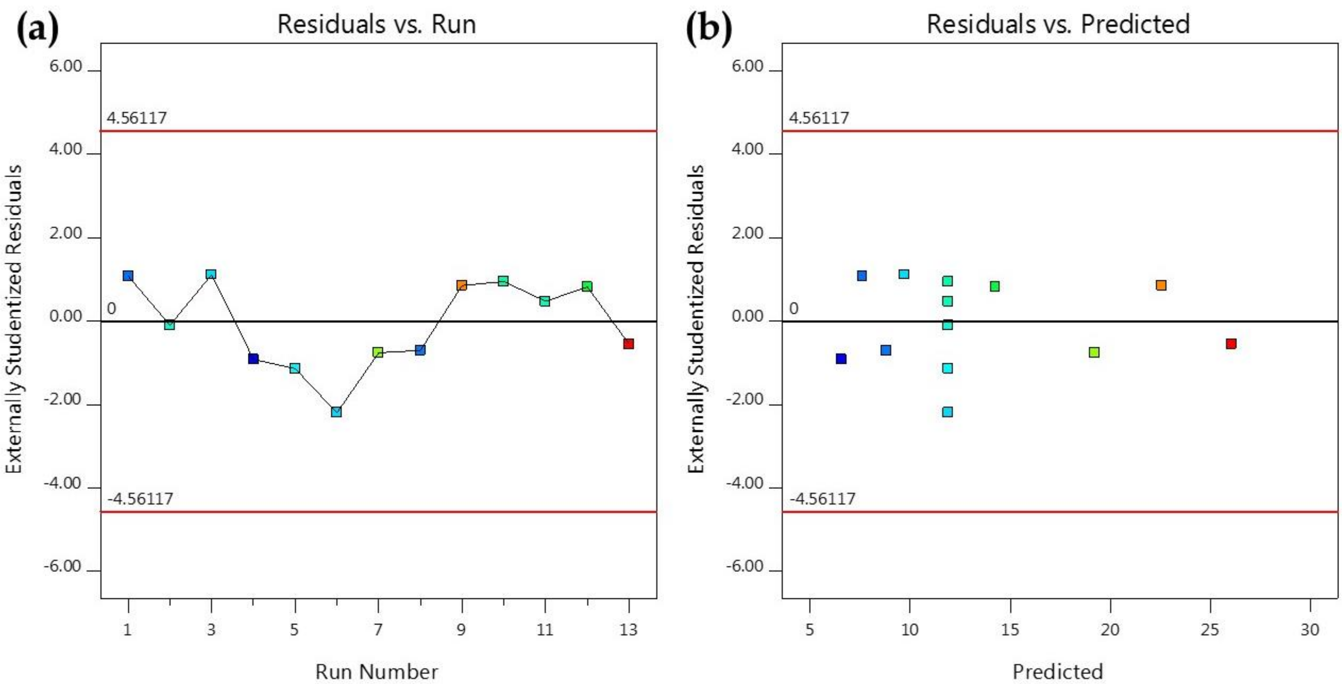

Figure 3. DP analytical plots of (c) Residuals versus the experimental run; and (d) pretreatment Residuals versus predicted

3.4 Optimization of NaOH pretreatment using CCD

Provisionally, Figure 2(a) was presented in a graph of the relationship between predicted versus actual plot in result of R^2 of 0.9849. And Figure 2(b) was presented in a graph of the externally studentized residuals versus predicted values to solution the standard deviations for normal probability. A finalizing of plots were showed in Figure 3 (c) and (d) of the residuals versus experimental run numbers were utilized to estimate the fit of the model using an internally studentized construction of SS model.

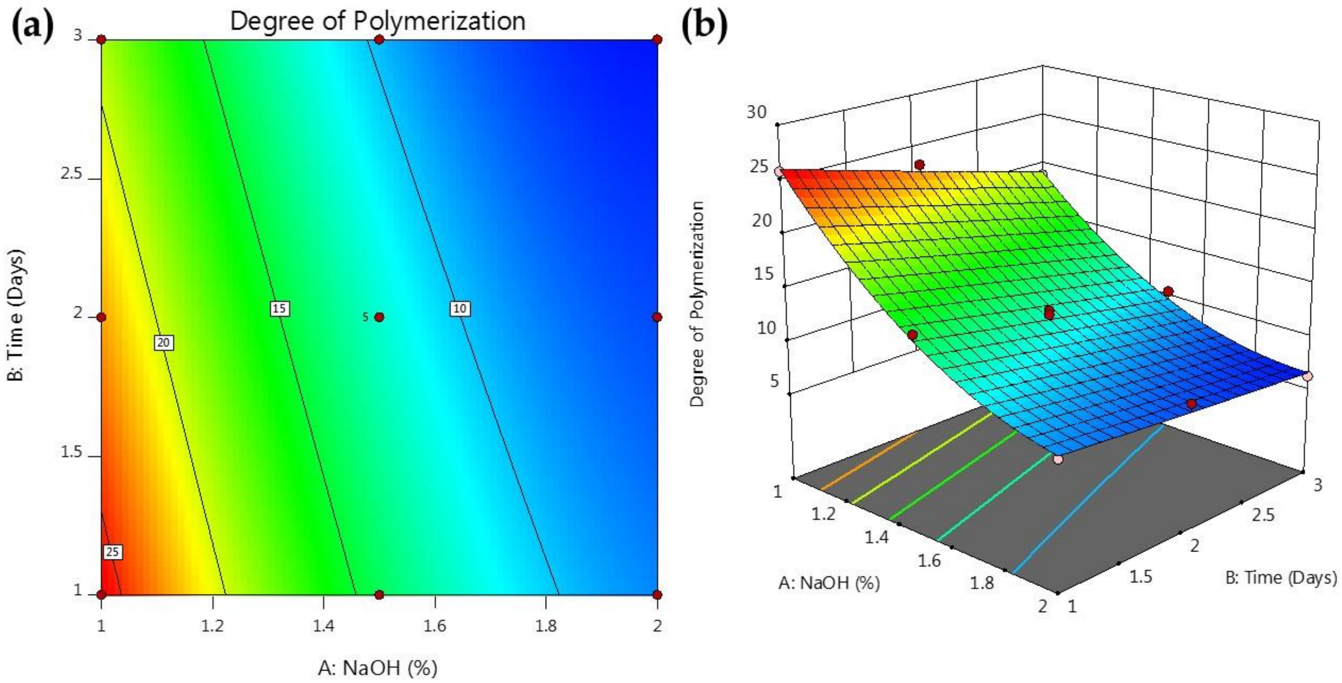

Figure 4. Plots attained from SS model of DP (a) Contour plot NaOH-Time interaction, (b) 3D plot NaOH-Time interaction

In these results, Contour and 3D plots are utilized to indicate the interactions of two independent factors and the simultaneous significance. Therefore, two factors are measured as study variables with the interaction of variable factors being evaluated on the response value. The results from Figure 4 (a) and (b) of SS model at pretreatment by 2% NaOH for 3 days, DP is 6.16, it operates with Table 1 that in the sample consists of oligosaccharides, malto-oligosaccharides, Maltodextrin, other oligosaccharides, Raffinose, stachyose and fructooligosaccharide. Which is good for enzymatic degradation in the next process, in the same direction as past research of Manmai et al. The highest TS and RS at the sample of pretreatment by 2% NaOH for 3 days (Manmai et al., 2019b), (Manmai et al., 2020). But on the other hands in sample was pretreated by 1%NaOH for 1 day, DP of 25.80, it can be predicted in this sample contains polysaccharides as lignocellulose.

Conclusion

In this study indicates that concentration of NaOH and time of reaction are impotent on the process of sugar producing for lignocellulose plants because in low concentration of NaOH (1%) and shot time (1 day) of pretreatment can be seen that example, lignocellulose has not yet been converted to oligosaccharides as the sample was pretreated by 2% NaOH for 3 days. Which oligosaccharides are good for the step of hydrolysis by enzyme to prepare fermentable sugar. Consequently, Sunflower is an agricultural waste for the renewable and low-cost biomass for biofuel and it can be the alternative materials of the future.

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