Concentration variation of sodium hydroxide (NaOH) pretreatment and its effect in the lignocellulosic compositions of dried ripe Carabao mango peels

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ABSTRACT

Alkaline pretreatment refers to the application of alkaline solutions such as NaOH, Ca(OH)2, or NH3 to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzymes to the cellulose. Mango peels are one of lignocellulosic waste materials containing a greater amount of cellulosic properties. In this study, the effect of varying the alkaline concentration in the lignocellulosic compositions of dried ripe Carabao mango peels was determined at varying NaOH pretreatment concentrations: 0.6 M, 0.8 M and 1.0 M. The compositions before and after the pretreatment were compared to determine the best condition to obtain the maximum delignification. Results showed that among the three concentrations, 1.0 M NaOH pretreatment gave the most favorable result. It decreased the amounts of acid soluble lignin (from 7.79% to 2.14%) and extractives (from 41.51% to 7.77%) and increased the amounts of holocellulose (from 32.10% to 72.11%), alpha-cellulose (from 26.27% to 50.47%) and hemicelluloses (from 6.16% to 21.62%). Further evidence showed that the least amount of reducing sugar present in the filtrate which is 3.85 mg glucose/mL was found at 1.0 M NaOH indicating that more cellulosic compositions in the substrate were converted to reducing sugar after pretreatment. The results suggest that at 1.0 M NaOH, mango peels would be best utilized by fermentative microorganisms in any downstream process.

Keywords: mango peels, NaOH pretreatment, delignification, reducing sugar, bioethanol production

1. Introduction

Global biofuel production has been increasing rapidly over the last decade, but the expanding biofuel industry has raised important concerns. The sustainability of many first-generation biofuels has been increasingly questioned because of its displacement of food-crops and its effects on the environment and climate change. This criticism has raised attention to the potential of second-generation biofuels. Second-generation biofuels are made from lignocellulosic biomass that is generally not edible.

Lignocellulosic materials are sufficiently abundant and generate very low net greenhouse emissions. Approximately 90% of the dry weight of most plant materials is stored in the form of cellulose, hemicellulose, lignin, and pectin [1]. Even though abundant, the commercialization of the process to produce bioethanol is limited due to insufficient research especially with relation to minimization of production cost. Pretreatment of lignocellulosic materials is not simple. For the last two decades, extensive research on the conversion of lignocellulosic materials to bioethanol has been completed.

Mango peels is one of the lignocellulosic waste materials which can be a source of bioethanol. In a recent study conducted by chemical engineering graduates [2], results showed that alkaline pretreatment is the best pretreatment for the delignification of mango peels. However, the alkaline concentration during pretreatment was not optimized in that investigation.

Pretreatment of lignocellulosic materials aims to decrease crystallinity of cellulose, increase biomass surface area, remove hemicellulose, and break the lignin barrier to facilitate conversion of carbohydrate polymers into fermentable sugars in a rapid way. Pretreatment is one of the most expensive processing steps for production of sugars from biomass.

One of the most common pretreatments used is the alkaline pretreatment. Alkaline pretreatment refers to the application of alkaline solutions such as NaOH, Ca(OH)$_2$ or NH$_3$ to remove lignin and a part of the hemicellulose, and efficiently increase the accessibility of enzymes to the cellulose.

This study aimed to determine the effects of variation in alkaline concentration in the lignocellulosic compositions of dried ripe Carabao mango peels. Specifically, it sought to: (1) determine the lignocellulosic compositions of untreated dried ripe Carabao mango peels; (2) compare the effect of varying alkaline concentration (0.6 M, 0.8 M, 1.0 M) during pretreatment on the aforementioned substrate; (3) determine the amount of reducing sugar present in the filtrate after each alkaline pretreatment; and (4) compare the lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment.

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2. Materials and methods

Carabao mangoes were obtained from Bolbok, Batangas City. The samples used were the ripe ones. Materials preparation and pretreatment processes were conducted in the Chemical Engineering Laboratory, Batangas State University -Alangilan. All the tests were done at the National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños, Los Baños, Laguna.

The collected ripe Carabao mango peels were air-dried, and placed in the cabinet dryer for further drying. Afterwards, peels were pulverized, weighed, and placed in a flask. The peels were soaked in diluted NaOH, with a pH of 11.5, in three various concentrations (0.6 M, 0.8 M, 1.0 M) for 48 hours. There were three trials for each concentration.

After soaking, the residue was removed from the solutions by filtering through a cheesecloth. The samples were rinsed until they reached a pH of 7.0. The water used in washing the samples was tested for the amount of reducing sugar. The samples were then oven dried at 100 °C. Upon conclusion of the alkaline pretreatment, analysis of variance was used to determine the pretreatment conditions that removed the most lignin [3].

These procedures were purely based on the Biomass Compositional Analysis Procedure from National Renewable Energy Laboratory.

One-way Analysis of Variance (ANOVA) was used to evaluate the effects of alkaline on the lignocellulosic compositions of dried ripe Carabao mango peels and to determine the pretreatment conditions that removed the most lignin. Paired t-test was used to determine if there were significant differences in the lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment.

3. Results and discussion

3.1. Lignocellulosic compositions of untreated dried ripe Carabao mango peels

Table 1 presents the amount of lignocellulosic compositions of untreated dried ripe Carabao mango peels. Based on the analysis conducted at Biotech, UPLB, the substrate contained 41.51% extractives, 7.79% acid soluble lignin, 32.10% holocellulose, 25.94% alpha-cellulose and 6.16% hemicellulose.

Table 1. Lignocellulosic compositions of untreated dried ripe carabao mango peels.

Compositions	Results (%w/w)
Extractives*	41.51
Acid Soluble Lignin**	7.79
Holocellulose**	32.10
Alpha-Cellulose**	25.94
Hemicellulose**	6.16

*Moisture free basis, **Moisture and extractive free basis

It is clear that the dried ripe Carabao mango peels are a potential source of bioethanol because of its greater amount of cellulosic properties. High amount of lignin in the substrate shows that it should undergo several pretreatment methods to break down the linkages between hemicellulose and lignin [4].

Lignin forms a protective shield around cellulose and hemicellulose to protect the polysaccharides from enzymatic degradation. Afterwards, it converts the biomass into ethanol. Then, the cellulose must be readily available for cellulase enzymes [5]. Thus, by removing the lignin, the cellulose becomes vulnerable to enzymes and allows the yeast to convert the glucose into ethanol during fermentation [6].

Therefore, pretreatment must be applied to remove lignin in the substrate, decrease cellulose crystallinity and increase the surface area for enzymatic activity.

The goal of all pretreatments is to break the lignin seal and to disrupt the crystalline structure of cellulose in order to make the cellulose more accessible to enzymes that convert the carbohydrate polymers into fermentable sugars [7].

3.2. Effect of varying alkaline concentration in the lignocellulosic compositions of dried ripe Carabao mango peels

Three different NaOH concentrations (0.6 M, 0.8 M and 1.0 M) were used to determine their effect in the lignocellulosic compositions of dried ripe Carabao mango peels.

Table 2 shows the amount of lignocellulosic compositions before and after the pretreatment. Extractives and acid soluble lignin showed a significant decrease while the concentration increased. On the other hand, the amounts of holocellulose, alpha-cellulose and hemicellulose increased while the concentration increased.

Table 2. Lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment.

Compositions	Before pretreatment (%w/w)	After pretreatment (%w/w)		
		0.6 M	0.8 M	1.0 M
Extractives*	41.51	12.57	11.10	7.77
Acid Soluble Lignin**	7.79	2.37	2.21	2.14
Holocellulose**	32.10	67.43	69.95	72.11
Alpha-Cellulose**	25.94	38.59	46.21	50.47
Hemicellulose**	6.16	28.84	23.91	21.62

*Moisture free basis, **Moisture and extractive free basis

The first composition that had a significant decrease was the extractives. The amount of extractives decreased from 41.51% to 12.57% (0.6 M), 11.10% (0.8 M), and 7.77% (1.0 M). As the concentration of alkaline increases, there is a significant decrease in the amount of extractives.

Further removal of extractives was very important in the accuracy of the lignin composition. It was apparent that the extractives present in the substrate were most influential in limiting the accuracy of a more representative material balance. This was particularly evident when trying to determine the lignin content, due to the incomplete removal of the extractives, even after pretreatment. Despite the minor dissolution of hemicellulosic sugars, extraction with mild alkaline removed most of the extractives from the peels. After pretreatment, the extent of extractive removal and their reaction and or precipitation with lignin was heavily dependent on the pretreatment conditions used.

Alkaline pretreatment was simply a delignification process. The amount of acid soluble lignin decreased from 7.79 to 2.37% (0.6 M), 2.21% (0.8 M), and 2.14% (1.0 M).

Based on the study of [8], which utilized rice straw as their substrate and underwent pretreatment using alkaline pretreatment with varying concentrations, the untreated rice straw contained 18.9% of lignin. After pretreatment, the concentration of lignin was found to decrease as the concentrations of the alkaline increase. The results were 17.9 % (0.1 M), 16.1% (0.25 M), 8.4% (0.5 M), 8.3% (1.0M), 8.2% (1.5 M), 8.1% (2.0 M) and 8.1% (2.5 M).

In the study conducted by [9], that utilized wheat straw and barley straw and pretreated with 1% and 2% NaOH, the amount of the lignin after pretreatment decreased from 8.33% to 7.82% (1% NaOH) and to 7.09% (2% NaOH) for wheat straw. Meanwhile, for barley straw, the drop was from 11.95% to 6.65% (1% NaOH) and to 4.52 (2% NaOH). This means that the rice straw delignified and the expected cellulose increased. The changes of lignin content effectively overcame biomass resistance to enhance the accessibility of cellulose and hemicellulose to enzymatic attack, which could potentially result in improved cellulosic bioethanol production.

Lignocellulosic biomass cannot be saccharified by enzymes to high yields without a pretreatment, mainly because the lignin in fruit cell walls forms a barrier against enzymatic attack [10]. An ideal pretreatment would reduce the lignin content and crystallinity of the cellulose and increase the surface area [5].

Lignin is degraded in nature by various organisms, but the mechanism of natural degradation is largely unknown. It is thought that oxidants such as NaOH may play an important role. Under certain conditions, NaOH is known to react with lignin and has been widely used to bleach high-lignin pulps [11].

Under suitable conditions, H_2O_2 will delignify wheat straw [9] and other crop residues to a point where the cellulose can be enzymatically converted to glucose with near quantitative yield. NaOH treated lignocellulosic materials could be rapidly fermented to ethanol with greater than 90% efficiency in the presence of cellulose [11].

In the present study, fruit biomass peel residue pretreated with 1.0 M NaOH (pH of 11.5 soaked for 48 h) removed most of the lignin. Lignin is also responsible for integrity, structural rigidity, and prevention of swelling of lignocelluloses. Thus, lignin content and distribution constitute the most recognized factor which is responsible for recalcitrance of lignocellulosic materials to enzymatic degradation by limiting the enzyme accessibility; therefore, the delignification processes can improve the rate and extent of enzymatic hydrolysis.

The third composition was the holocellulose. Based on the result obtained, there was an increase in the holocellulose amount from 32.10% to 67.43% (0.6M), 69.95% (0.8 M), and 72.11% (1.0 M). It was clear that as the concentration of the alkaline increases, the amount of holocellulose increases.

This means that the bond that surrounds holocellulose was broken down due to pretreatment. The said bond prohibits the enzymatic attack to the cellulose. Holocelluloses were produced by delignification of wood or other biomass materials and may retain substantial amounts of various hemicelluloses.

Moreover, it was also clear that there was an increase in the amount of alpha-cellulose from 26.27% to 38.59% (0.6 M), 46.21% (0.8 M) and 50.47% (1.0 M). As the concentration of the alkaline increases in this study, the amount of alpha-cellulose also increases.

In the study of [6], it showed that there was an increase in the amount of alpha-cellulose in straw during alkaline pretreatment with varying NaOH concentration. The pretreatment resulted in 60% (2% NaOH), 69.4% (4% NaOH), 80% (8% NaOH) from 35% before pretreatment.

Alpha-cellulose is a highly refined form of cellulose and is the most abundant in dried ripe Carabao mango peels. It reportedly exhibited similar crystallinity and porosity with wood biomass and shows the natural structure of cellulose fibers.

A high increase of alpha-cellulose only indicates that it follows the purpose of pretreatment which increases the accessible surface area and the size of pores and on the other hand decreases its crystallinity and its polymerization degree.

Lastly, hemicellulose increased from 6.16% to 28.84% (0.6 M), 23.91% (0.8 M) and 21.62% (1.0 M). As the concentration of the alkaline increases, the amount of hemicellulose decreases. Also, hemicellulose can be dissolved in strong alkaline solutions.

Hemicellulose provides structural integrity to the cell. As hemicellulose can cover the surface of cellulose and prevent the access of cellulases, the removal of hemicellulose may be essential to facilitate complete cellulose hydrolysis. However, of the three main components of lignocellulose, hemicelluloses are denoted to be the most sensitive to changes in pretreatment conditions [12]. Therefore, compared to the removal of lignin, the removal of hemicelluloses during pretreatment has not been a major issue.

Presented in Figure 1 are the lignocellulosic compositions of dried ripe Carabao mango peels before and after 0.6 M NaOH pretreatment. Using 0.6 M NaOH in alkaline pretreatment, the walls that hold the lignocellulosic materials had been broken down and the lignin had been removed. Whereas, the extractives decreased, this is due to the proportional increase in the lignocellulosic compositions of the mango peels.
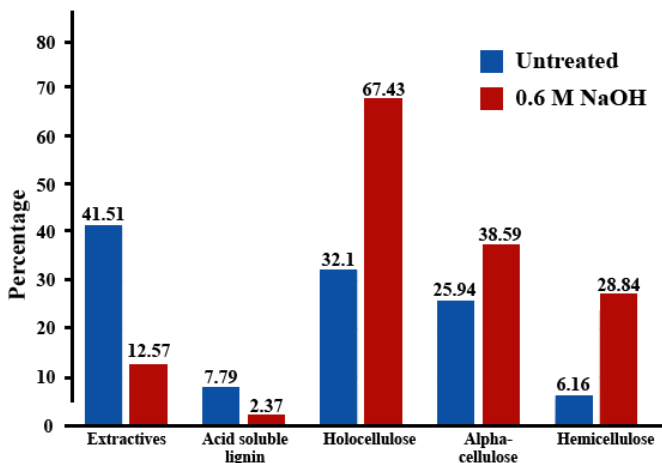

Figure 1. Lignocellulosic compositions of dried ripe Carabao mango peels before and after 0.6 M NaOH pretreatment.

Presented in Figure 2, are the lignocellulosic compositions of dried ripe Carabao mango peels before and after 0.8 M NaOH pretreatment. With the aid of alkaline pretreatment, most of the extractives and the lignin were removed which was comparable to the increase of alpha-cellulose, holocellulose and hemicellulose.
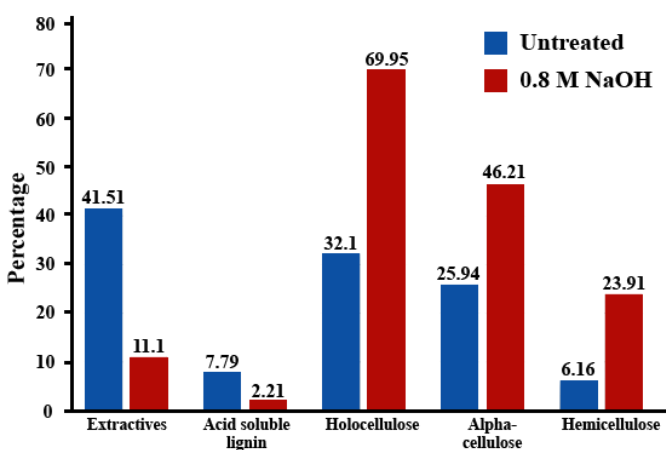

Figure 2. Lignocellulosic compositions of dried ripe Carabao mango peels before and after 0.8 M NaOH pretreatment.

Figure 3 presents the lignocellulosic compositions of dried ripe Carabao mango peels before and after 1.0 M NaOH pretreatment. It is evident that there was a high increase in the cellulosic components of the dried ripe Carabao mango peels. This is due to the delignification of the alkaline being used specially with a higher concentration of alkaline which leads to the increase in the alpha-cellulose, hemicellulose and holocellulose.
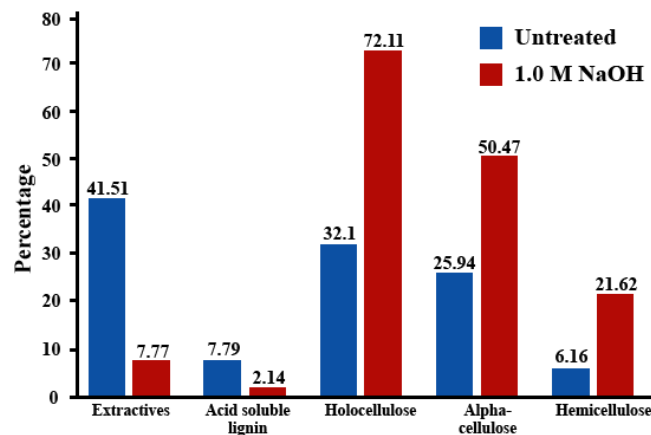

Figure 3. Lignocellulosic compositions of dried ripe Carabao mango peels before and after 1.0 M NaOH pretreatment.

Analysis of variance (ANOVA) was used to determine the effects of the pretreatments on the lignocellulosic compositions of dried ripe Carabao mango peels. Table 3 presents the summary of P-values and F-values for the comparison of the lignocellulosic compositions among the treated dried ripe Carabao mango peels. The P-values of the gathered data were obtained using ANOVA.

Table 3. Analysis of variance for the comparison of the lignocellulosic compositions among treated dried ripe Carabao mango peels.

Compositions	P-Values	F-Values	Interpretation	Decision on H_o
Extractives	P0.001	93.0202	Significant	Do not accept H_o
Acid Soluble Lignin	0.0940	3.5975	Not Significant	Accept H_o
Holocellulose	P0.001	29.8318	Significant	Do not accept H_o
Alpha-cellulose	P0.001	22.976	Significant	Do not accept H_o
Hemicellulose	P0.001	8.716	Significant	Do not accept H_o

$\alpha=0.050$, n=3 df=2, $F_{critical} = 5.143$

It was found out that the P-values of extractives, holocellulose, alpha-cellulose and hemicellulose are less than 0.05 level of significance thus, the null hypothesis was rejected. This indicates that the lignocellulosic compositions of treated dried ripe Carabao mango peels differ significantly using different concentrations.

However, the P-value for acid soluble lignin is 9.40×10^{-2} which is greater than 0.05 level of significance. This led to the acceptance of the null hypothesis.

3.3. Reducing sugar in the filtrate after alkaline pretreatment

The reducing sugar concentration in the filtrate after alkaline pretreatment was analyzed. Table 4 shows the average concentration of the reducing sugar present in the filtrate after alkaline pretreatment.

Table 4. Average concentration of reducing sugar in the filtrate after alkaline pretreatment.

Alkaline concentration	Reducing sugar (mg glucose/mL)
0.6 M	9.95
0.8 M	5.27
1.0 M	3.85

As seen on Table 4, 1.0 M alkaline concentration has resulted in a lower yield of reducing sugar in the filtrate of 3.85 mg glucose/mL than that 0.6 M concentration in which 9.95 mg glucose/mL was obtained.

With respect to alkaline concentration as shown in the table, 1.0 M concentration caused partial starch hydrolysis in the substrate, resulting in lower yield of reducing sugar in the filtrate. We can deduce that using a higher concentration, starch hydrolysis will be more complete. Furthermore, this implies that most of the cellulosic compositions in the substrate were converted to reducing sugar after pretreatment and complete hydrolysis is preferable for maximum fermentable sugars. In comparison with other concentrations, it means that this could give the highest result of reducing sugar that could be converted. Thus, the highest concentration of reducing sugar in the filtrate gives the lowest fermentable sugar present in the treated mango peels.

3.4. Comparison of lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment

The lignocellulosic compositions of the filtrate were compared before and after treatment. Paired t-test was used to statistically analyze its difference. Table 5 shows the comparison of the compositions of the substrate before and after pretreatment. The sample mean of treatment untreated exceeds the sample mean of treatment 1.0 M by an amount

that is greater than would be expected by chance, rejecting the hypothesis that the population mean of treatment 1.0 M is greater than or equal to the population mean of treatment untreated. ($P = <0.001$)

It was shown that all compositions have a significant difference after pretreatment. This result was the same with the results obtained using 0.6 M and 0.8 M of NaOH.

Figure 4 shows the comparison of the lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment. 1.0 M NaOH pretreatment gave the highest increase of alpha-cellulose, hemicellulose and holocellulose, which were 50.47%, 21.62% and 72.11% respectively.
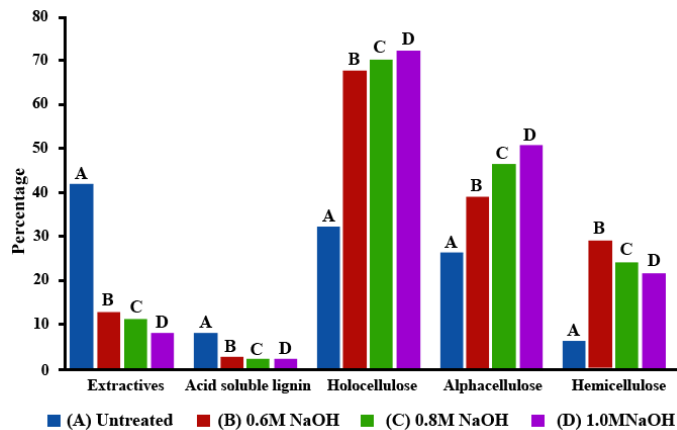

Figure 4. Comparison of lignocellulosic compositions of dried ripe Carabao mango peels before and after pretreatment.

This increase showed that it was easier to utilize with fermentative microorganisms. This also gave the best result in the removal of lignin. From the acid soluble lignin of the untreated samples which was 7.79%, this pretreatment gave the highest removal of acid soluble lignin which was 2.14%. Removal of lignin improves the reactivity of the remaining polysaccharides. It also shows that the percentage of hemicellulose in 1.0 M NaOH is lower compared to the other concentration. This means that some of the hemicellulose was removed.

In this case, removal of lignin and hemicellulose from the microfibrils is thought to expose the crystalline cellulose core, which can then be hydrolyzed by cellulose enzymes. It is probable that systematic removal of lignin and hemicellulose

Table 5. Paired t-test on the comparison of the lignocellulosic compositions of dried ripe Carabao mango peels between untreated and treated with 1.0 M NaOH.

Compositions	t_{calc}	P-Values	Interpretation	Decision on H_o
Extractives	-54.109	0.000341	Significant	Do not accept H_o
Acid Soluble Lignin	-45.388	0.000485	Significant	Do not accept H_o
Holocellulose	90.103	0.000123	Significant	Do not accept H_o
Alpha-cellulose	16.977	0.00345	Significant	Do not accept H_o
Hemicellulose	10.812	0.00845	Significant	Do not accept H_o

$\alpha=0.050$, n=3 df=2, $t_{tab1} = -3.182$; $t_{tab2} = 3.182$

will result in the marked reduction of cellulose loadings required to convert cellulose to cellobiose or glucose. Based on these facts, 1.0 M NaOH can be considered the best concentration among the three concentrations used.

Figure 5 shows the comparison of the different alkaline concentrations that were used in the pretreatment for obtaining the amount of reducing sugar that was present in the filtrate.
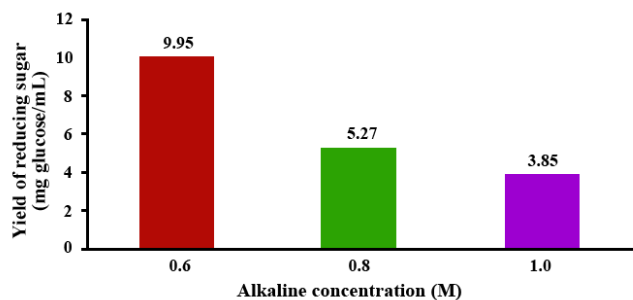

Figure 5. Comparison of the amount of reducing sugar in the filtrate after pretreatment of each alkaline concentration.

It is clear that 0.6 M alkaline concentration yields the highest reducing sugar in the filtrate of 9.95 mg glucose/mL. It implies that more fermentable sugars were present in the filtrate, thus giving a lower yield of bioethanol during fermentation. Therefore, 1.0 M concentration was considered the best concentration among the three concentrations used in terms of reducing sugar compositions in the filtrate. It indicates that more cellulosic compositions in the substrate were converted to reducing sugar after pretreatment and at 1.0M concentration; a partial starch hydrolysis occurred during pretreatment.

4. Conclusions

The present study generalizes that Carabao mango peels exhibit potential as feedstock for bioethanol production.

Among the three concentrations varied, 1.0 M NaOH had the greatest effect in the lignocellulosic compositions of the dried ripe Carabao mango peels. It gave the highest increase in the amount of hemicellulose, alpha-cellulose and holocellulose while there was a great decrease in the concentration of hemicellulose, acid soluble lignin and extractives.

Furthermore, 1.0 M NaOH gives the smallest amount of reducing sugar in the filtrate, amounting to 3.85 mg glucose/mL.

Optimization of pretreatment parameters such as pretreatment time and temperature are recommended to know if there is a significant effect in the lignocellulosic composition of dried ripe Carabao mango peels.

Percentage recovery of the sample after pretreatment is suggested to determine if there is enough substrate for enzymatic saccharification.

A parallel study using ripe Indian mango peels or unripe Carabao mango peels may be conducted.

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