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Response surface methodology optimization study on corncob pretreatment: reduction of sodium hydroxide usage and enhancement in pulpzyme HC biobleaching efficiency

[1]Tang, T.-K., [2,3]Lee, Y.-Y., [4]Phuah, E.-T., [5]Tan, C.-P., [6]Kanagaratnam, S., [7]Wang, Y., [8]Cheong, L.-Z., [1]Jamalullail, N.A., [1]Yap, K.-L., [1]Lee, C.-M., [10]Tan, J.S. and [1,9,*]Lai, O.-M.

[1]International Joint Laboratory on Plant Oils Processing and Safety (POPS) JNU-UPM, Institute of Bioscience, Universiti Putra Malaysia, 43400, Serdang, Selangor.
[2]Monash-Industry Palm Oil Education and Research Platform, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia.
[3]School of Science, Monash University Malaysia, 47500 Bandar Sunway, Selangor, Malaysia.
[4]Department and Agriculture and Food Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak.
[5]International Joint Laboratory on Plant Oils Processing and Safety (POPS) JNU-UPM, Department of Food Technology, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[6]Food and Feed Technology Unit, Malaysian Palm Oil Board, 6 Persiaran Institusi, Bandar Baru Bangi, 43000 Kajang, Selangor, Malaysia.
[7]International Joint Laboratory on Plant Oils Processing and Safety (POPS) JNU-UPM, Department of Food Science and Engineering, College of Science and Engineering, Jinan University, Guangzhou 510632, China.
[8]Department of Food Science and Engineering, College of Food and Pharmaceutical Sciences, Ningbo University, Ningbo 315211, China.
[9]Department of Bioprocess Technology, Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
[10]School of Industrial Technology, Universiti Sains Malaysia, 11800, Gelugor, Pulau Pinang, Malaysia.

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Abstract

Lignocellulosic rich corncob biomass possesses less complex structure, lignin and pigment content. As compared to wood pulp, it is considered to be a better alternative for the production of cellulose fibre. The present study was conducted to optimize both the alkaline (using sodium hydroxide) and biobleaching (using Pulpzyme HC) pretreatment process of corncob to promote lignin removal and cellulose swelling. It was the aim of this work to achieve mild processing conditions for corncob pretreatment in order to minimize the chemical usage. Results demonstrated that the mild pretreatment approach employed was found to successfully increase cellulose swelling and lignin removal from the corncob biomass. In alkaline pretreatment process, reaction temperature showed to be the most prominent effect in enhancing lignin removal and cellulose swelling as compared to sodium hydroxide concentration and reaction time. RSM optimized conditions for alkaline pretreatment process: 0.5 M NaOH, reaction temperature of 80°C and reaction time of 30 mins manage to increase the sedimentation index (indicate swelling of cellulose) from 0 to 30 and reduce the kappa number (represent lignin removal) from 82 to 32, respectively. Meanwhile, for biobleaching pretreatment using Pulpzyme HC, reaction time play a more significant role than the Pulpzyme HC concentration in promoting lignin removal and increasing cellulose swelling. RSM optimized conditions showed that the kappa number was reduced from 32 to 18 whereas the sedimentation index increased from 30 to 60 when the alkaline pretreated corncob was biobleached with Pulpzyme HC.

1. Introduction

Sustainable and environmental-friendly product has become the subject of interest in recent years. However, to be in line with this global sustainability trend, there are several obstacles that need to be overcome by the industries. For example, in the pulp and papermaking industry, the conventional method employed in pulp processing often utilizes chlorine-containing bleach as a

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*Corresponding author.
Email: *omlai@upm.edu.my*

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bleaching agent to whiten the pulp as part of the approach to isolate the increasing demand for value-added cellulose. This often generates toxic compounds that cause a huge impact on the environment. Sometimes, it is common that an extra oxygen delignification step is implemented to reduce the consumption of the bleaching agent. Nonetheless, this method has its drawback of requiring high capital and maintenance cost. Besides, the major source of pulp in the pulp and paper industry is obtained from wood logging. This non-sustainable activity has led to an intense debate over the years. It is the major cause of concern for rainforest depletion that supplies not only oxygen but also the biodiversity and gene pools to the earth. As such, not only that conditions used for the pretreatment process to isolate cellulose needs to be environmental-friendly, seeking alternative sustainable sources of cellulose to replace the wood pulp is imperative too.

Today, the enzymatic bio-bleaching method which offers a lower energy consumption and considered more eco-friendly, has gained popularity in effectively reducing and phasing out the usage of chlorine bleaching. The lignocellulosic enzymes such as xylanase, laccase and manganese peroxidase are the most common example of enzymes used for bio-bleaching. The benefits of utilizing the bio-bleaching approach in the pulp and paper industry are well published. For example, laccase used in oxygen bleaching of kraft pulp enhanced the delignification process by removing 50% of lignin (Tavares *et al.*, 2004). Apart from laccase, xylanase is another enzyme widely used for bio-bleaching. It hydrolyzes xylan in hemicellulose, resulting in a loosened structure of hemicellulose in the cell wall of lignocellulosic biomass (complex structure of lignin and hemicellulose). Numerous studies showed that the usage of xylanase managed to reduce the consumption of chemicals in the bleaching process (Sharma *et al.*, 2015; Kumar *et al.*, 2017). Dai *et al.* (2016) reported that the bio-bleaching carried out using xylanase is able to reduce Cl_2 usage up to 29.8%. Additionally, Sridevi *et al.* (2016) reported there is a decrease of 3.5 points in kappa number and 3.1 points increase in brightness in xylanase biobleached paper pulp. Recently, actinobacterium fermentation broth that contains xylanase was used as a biobleaching agent to bleach paper pulp. It was found that 60 mins of biobleaching treatment using the 5-day fermentation broth significantly reduced 12.5% usage of the chemical bleach and increased 55% of the brightness of the pulp (Hamedi *et al.* 2020). A similar study was conducted by Sharma *et al*. (2020) demonstrated that utilization of a cocktail of the enzyme (xylanase, pectinase, amylase, protease and lipase) reduced 50% of chemical usage without compromising the brightness of sample.

Corncob, the food waste obtained after the corn kernels are removed is rich with lignocellulose. It possesses several advantages as compared to the wood pulp as a raw material source or fibre. The less complex structure, lower lignin and pigment content of corncob, suggested that a lesser treatment (chemical or physical) is needed to extract the cellulose fibre. Additionally, corncob, as the by-product of corn commodity is easily available at a cheaper price. The aforementioned advantages of corncob ultimately reduce the operation cost used for its pretreatment. The present study aimed to develop a simple, feasible and optimized approach that involves the pretreatment of agricultural waste corncob using sodium hydroxide in a reduced dosage manner prior to subsequent Pulpzyme HC (commercial thermostable xylanase) pretreatment to bio-bleach the pulp. NaOH was utilized as the first step of pretreatment as it can create a porous structure that allows the enzyme to further react to the NaOH treated corncob to perform the biobleaching process. The condition for both of the pretreatment steps is determined using the response surface methodology approach. The influence of sodium hydroxide concentration, reaction time and temperature on the physicochemical attributes of the corncob pulp treated were investigated. Further, the bio-bleaching ability of Pulpzyme HC in the colour improvement and its help in reducing chlorine bleaching usage was also investigated.

2. Materials and methods

2.1 Materials

The corncob used in this research was obtained from Nelson’s Franchise (M) Sdn Bhd (Selangor, Malaysia) and Pulpzyme HC (xylanase) were purchased from Novozyme (Bagsvaerd, Denmark). All the reagents and chemicals used were of analytical grade or HPLC grade. The corncob was first ground using a coconut grater machine. The juice was then removed from the ground corncob using a coconut milk extractor. The corncob was then dried in the oven at 55°C, sieved with a 1 mm size sieve, and stored in a dry place prior to the experiment.

2.2 Proximate analysis of corncob

The proximate analysis including moisture content, ash, crude fat, crude fibre and carbohydrate were determined based on AOAC method (984.25:2000, 940.26:2000, 989.05:2000, 962.09:1997) and method of analysis for nutrition labeling (AOAC, 1993). Crude protein was determined by using Kjeldahl standard method MS 1194:1991. Total dietary fibre, insoluble dietary fibre and soluble fibre content were analyzed according to AOAC 993.19, 991.42 and 993.19 method, respectively.

2.3 Pretreatment of corncob by Response Surface Methodology (RSM)

2.3.1 Sodium hydroxide pretreatment of corncob

For the pretreatment of corncob, 15 g (6%, w/v) of corncob was mixed with 250 mL of sodium hydroxide solution in a conical flask under the stirring speed of 350 rpm. After the reaction, the pretreated pulp was filtered. Filtrates were collected and residue (we denoted it as the pretreated pulp) were washed thoroughly with RO water. Face centered central composite design with an alpha of 1.0 was chosen for the optimization of the three pretreatment processing parameters namely: sodium hydroxide concentration (NaOH), reaction time (t), and reaction temperature (T). The response variables determined were corncob's Kappa number, sedimentation index, filtrates' lignin and chromophore content. Table 1a shows the independent variables code and units for alkaline pretreatment. The range of pretreatment conditions: sodium hydroxide concentration, reaction time and reaction temperature were selected based on previously published literature. There was a total of 15 experimental runs with five replicates at the center point generated by Design Expert 7.0.0 software (Stat-Ease Inc., Minneapolis, USA). The 15 reactions were conducted in random manner. Data obtained from the experiment were analyzed using ANOVA and multiple regression analysis at a 95% significance level. Three-dimensional response surface plots were generated. Numerical optimization was used to optimize the response of interest. Sodium hydroxide amount was set to the lowest for numerical optimization. Corncob was then pretreated using the optimized alkaline pretreatment process prior to the subsequent bio-bleaching step.

Table 1a. Independent variables code and unit for alkaline pretreatment

Independent variables	Symbol	Variable codes		
		-1	0	1
Sodium hydroxide concentration (M)	NaOH	0.5	1.24	2
Reaction time (hrs)	t	0.5	2.25	4
Reaction temperature (°C)	T	25.0	52.5	80

2.3.2 Verification production

The optimized model generated by RSM for alkaline pretreatment of corncob was verified. Corncob pulp was produced on large scale by treating the corncob with the optimized alkaline pretreatment conditions generated by RSM: 0.5 M NaOH, 30 mins reaction time and temperature of 80°C. Corncob pulp was analyzed for the kappa number and sedimentation index.

2.3.3 Bio-bleaching pretreatment of corncob

Bio bleaching was conducted on the sodium hydroxide pretreated pulp using an enzyme known as Pulpzyme HC. A two factors and three levels Face Centre Composite Design (FCCD) consisting of 13 experimental runs with five replicates at the center point generated by Design Expert 7.0.0 software (Stat-Ease Inc., Minneapolis, USA) was employed in this study. The independent variable studied were: Pulpzyme HC activity per sample weight (u/g) and reaction time (t). Table 1b shows the independent variables code and units.

Table 1b. Independent variables code and unit for biobleaching

Independent variables	Symbol	Variable codes		
		-1	0	1
Pulpzyme HC activity/g of sample (u/g)	E ratio	0.5	5.25	10
Reaction time (h)	t	0.5	2.25	4

Bio bleaching process was conducted as follows: 3.0% (w/v) pretreated pulp of were top up with pH7 buffer solution (0.11 M KH_2PO_4 + 0.09M Na_2HPO_4) till 250 mL. Samples were stirred with a magnetic stirrer hot plate for 10 mins for adaptation and homogenization purpose prior to the addition of Pulpzyme HC. The mixture was stirred throughout the reaction. The conditions of biobleaching were set according to FCCD design while reaction temperature was set at 50°C and stirring speed at 350 rpm, respectively. The enzymatic reaction was terminated by denaturing the enzyme at 80° C for 30 mins. Subsequently, the enzymatically treated pulp was filtered and analyzed for the following responses such as Kappa number, sedimentation index, while filtrate was collected to determine the content of lignin released and chromophore.

2.3.4 Chemical bleaching of sodium hydroxide treated corncob fibre and xylanase treated

The 3% w/v xyalanase-treated pulp were bleached with different concentration sodium hypochlorite namely 0%, 0.5% and 1.0% (w/v) for 15 mins. The colour and brightness of the bleached pulp were determined using UltraScan Pro Spectrophotometer (Hunter Associates Laboratory, Inc, USA) at 457 nm wavelength.

2.3.5 Kappa number of corncob fibre

Kappa number analysis was conducted according to the ES ISO 302:2012 method for the determination of lignin content in the pulp.

2.3.6 Sedimentation index of corncob fibre

Pulp containing approximately 0.1 g of the dry weight of each sample was weighed and placed in a measuring cylinder. Then, distilled water was added until 10 mL and the mixture was mixed well. After mixing, the measuring cylinder was placed on the flat surface for exactly 10 mins to allow the sample to sediment. The sedimentation index was then calculated:

$$\text{Sedimentation index} = \frac{\text{Height of sedimented fibre}}{\text{Height of total volume}} \times 100\% \quad (1)$$

2.3.7 Particle size analysis of corncob fibre

The particle size distribution of NaOH pre-treated and bio-bleached corncob pulp was determined by light scattering using Mastersizer 2000 (Malvern Instrument Ltd, UK) with distilled water as the dispersant (Zhang *et al.*, 2013). Samples were diluted to 0.06 % (w/v) to avoid multiple scattering effects and dispersed in distilled water at 1200 rpm until an obscuration rate of 15% was obtained. The reflected index of dispersant (water) and disperser (corncob pulp) was set at 1.33 and 1.46, respectively.

2.3.8 Release of chromophoric material and lignin in filtrate

The filtrate obtained was measured spectrophotometrically at 280 nm to determine the lignin content and 465 nm for analyzing the chromophoric material (Bissoon *et al.*, 2002). Chromophoric materials were represented by the absorbance value while lignin content was calculated based on the equation (2):

$$\text{Lignin content} = \frac{\text{Absorbance} \times \text{Dilution}}{\text{Absorbtivity}} \quad (2)$$

2.4 Statistical analysis

All measurements were performed in triplicates unless stated. Data were presented as mean±SD. Significant differences ($P<0.05$) of the samples were analyzed using one-way analysis of variance (ANOVA) by LSD test using SPSS (IBM Corporation, USA).

3. Results and discussion

3.1 Corncob composition

The proximate analysis and the fibre content of corncob are shown in Table 2. The cellulose content of corncob was 43.8% which was similar to raw wood having around 40% to 50% of cellulose (Mohomane *et al.*, 2017; Silvy *et al.*, 2018). Meanwhile, the hemicellulose content was about 47.7% which is considered relatively high. In terms of lignin content, corncob showed a very minimal amount of lignin which was around 9.1% as compared to raw wood which usually has up to 30% lignin. Therefore, corncob can be considered as a good alternative source of cellulose. Additionally, with its loosen structure as compared to wood, corncob require less pretreatment steps, making it easier to be converted into pulp.

Table 2. Composition of dried corncob

Composition	Amount g/100 g
Moisture content	11.5±0.02
Carbohydrate	43.7±0.04
Crude Protein	3.2±0.03
Ashes	1.9±0.01
Crude Fat	1.4±0.02
Crude fibre	38.4±0.03
Acid detergent lignin (% dry weight)	9.1±0.30
Cellulose content (% dry weight)	43.8±1.40
Hemicellulose content (% dry weight)	47.7±0.80

Data were performed in triplicate. Mean±standard deviation values

3.2 Sodium hydroxide pretreatment

Sodium hydroxide pretreatment is a common treatment used for cellulose extraction. It induces fibre/cellulose swelling. The process allows the complex structure of fibre to loosen up and make it easier for subsequent treatment. The level of pretreatment was quantitated by measuring four important responses namely kappa number of corncob fibre, swelling effect toward corncob fibre (sedimentation index), lignin and chromophore released into the filtrate. Kappa number is a direct representation of lignin content. The reduction of the kappa number is important as it indicates a lesser amount of lignin present in the wood or pulp. The swelling effect of pulp (sedimentation index) is crucial for the subsequent bio bleaching pretreatment process as it enhances enzyme accessibility toward the pulp. A higher sedimentation index is preferable as the pulp are easier to be treated during the bleaching process. These are the two most important responses since it determined the efficiency of pretreatment process. Besides that, the lignin and chromophore released into the filtrate are complementary data to counter check that the lignin reduced in pulp were successfully released into the filtrate.

Since NaOH pretreatment on fibre is a well-known method, the range of pretreatment parameters such as NaOH, reaction time and reaction temperature was acquired from previous reports (Zhang *et al.*, 2010; Winuprasith and Suphantharika, 2013; Kim *et al.*, 2016). However, the literature search showed that the range of pretreatment various across different studies. Hence, in the present study, response surface methodology was employed to minimize the NaOH usage for the pretreatment of the corncob. An FCCD design having 3 level and 3 factors were selected for this design. Table 3 shows the effect of sodium hydroxide pretreated corncob (Kappa number and sedimentation index) as well as the

Table 3. FCCD for 16 experimental runs and experimental data of the responses (Kappa number, sediment rate, lignin in filtrate, chromophore in filtrate) for the study on effect of the sodium hydroxide pretreatment on corncob

Run	Factors			Responses			
	NaOH (M)	Temp (°C)	Time (h)	Kappa number reduction	Sediment index	Lignin in filtrate (g)	Chromophore in filtrate (abs)
1	2.00	25.00	4.00	44.22230	26	2.6490	0.2488
2	1.25	80.00	2.25	72.00126	38	3.2305	0.3798
3	1.25	25.00	2.25	39.96313	29	2.6693	0.2294
4	0.50	80.00	4.00	71.31873	33	3.4990	0.4629
5	2.00	80.00	0.50	65.63697	36	3.2291	0.3805
6	1.25	52.50	2.25	63.49170	37	3.0550	0.2773
7	2.00	52.50	2.25	61.99892	34	2.8293	0.2676
8	1.25	52.50	2.25	61.85306	36	3.1248	0.2895
9	1.25	25.00	0.50	20.02251	19	1.6043	0.1823
10	1.25	52.50	2.25	59.10881	40	3.1755	0.2921
11	1.25	52.50	2.25	59.78751	35	3.0380	0.2872
12	1.25	52.50	0.50	44.13616	34	2.7323	0.2546
13	1.25	52.50	2.25	60.02132	36	3.1083	0.2836
14	1.25	52.50	4.00	63.39109	36	3.0713	0.2735
15	0.50	52.50	2.25	53.99481	28	2.8658	0.2912

filtrate (lignin and chromophore) for the 15 experimental runs generated from response surface methodology. A backward elimination with the best multiple correlation coefficient was used to determine the best-fitting model for each of the four studied responses. The backward elimination was conducted by including all the terms first before further removing the least significant term (factors/interaction). All the models were best fitted on a quadratic model. The models' equations are listed as followed:

Kappa number reduction = $59.98+4.42A+17.46B+8.19C-3.78B^2-6.00C^2$

Sedimentation index = $3.63+0.27A+0.55B-0.52A^2-0.27B^2$

Lignin released = $3.05-0.018A+0.28B+0.17C-0.16AB-0.34AC-0.21BC-0.19A^2-0.13C^2$

Chromophore released = $0.28-.0066A+0.075B+0.0095C-0.028AB-0.028AC+0.034B^2$

For a model to be deemed as a good model, a few criterias need to be met. The model itself must be significant. The lack of fit of the model must not be significant. R2 value should be high. From the result, all four models have high R2 and adjusted R2 value of above 90% while the predicted R2 has a slightly lower value but is still under the acceptable range of above 80%. The 3 models which are Kappa number reduction, sedimentation index and lignin released fulfil all the above criteria. It shows that the quadratic model with backward elimination is sufficient to demonstrate the relationship between the responses and the independent variables. Nevertheless, the model for chromophore released shows a significant lack of fit indicating that the poor fit of the model. Therefore, the chromophore model is not feasible to be used.

3.3 Single factor response (Kappa number)

Kappa number and swelling effect are two important parameters to determine the effectiveness of NaOH pretreatment. Kappa number is an indication of lignin presence in fibre which have a direct influence on the colour of the pulp as lignin contributes to the browning of the pulp. All NaOH concentration was found to be able to reduce for kappa number which is a direct indicator for lignin removal. The single factor effects on the kappa number of corncob fibre are shown in Figure 1. As can be seen, kappa number reduction increases with NaOH concentration but the reduction is not as obvious as compared to reaction time and reaction temperature. An increase in the NaOH concentration from 0.5 M to 2.0 M has only a slight effect on the kappa number reduction. As for the reaction time, it seems to have a prominent effect on the reduction of the kappa number but reached the maximum kappa number reduction at around 1.25 hrs. A further increase in reaction time to 2 hrs only slightly reduce the kappa number. This is because the NaOH pre-treatment reaction has reached its steady state. Meanwhile, the most obvious kappa number reduction can be seen from reaction temperature where the kappa number changes from ~40 to ~73. This indicated that the reaction temperature is the major contributor to the kappa number reduction of the fibres. Every 10°C rise in temperature resulted in an increase in the rate of reaction by double. Hence, it elevated the rate of the pretreatment process as well. Today, many studies used various ranges of reaction times and reaction temperatures for NaOH pretreatment on fibre. For example, mangosteen was pretreated with 1 M NaOH at 90°C for 2.75 hrs (Winuprasith and Suphantharika, 2015). Similarly, cellulose was extracted from corncob using 1 M NaOH pretreatment at 80°C for 2 hrs in which the NaOH pretreatment process was repeated twice (Shogren *et al.*, 2011). Also, similar work pretreated sorghum with 0.81 M of NaOH at 120°C for 60 mins. On the other hand, it was found out that the usage of NaOH could be reduced by increasing the pretreatment temperature. Hence, with response surface methodology, the statistical tool was able to minimize the chemical usage by manipulating a more significant factor such as temperature to obtain similar kappa number reduction for corncob pretreatment. Thus, the response surface methodology can be considered as a versatile tool to produce a fibre with a low kappa number using the minimum amount of NaOH where other factors can then be increased to achieve this. At the same time, it is also an effective tool to allow the user to have the flexibility to switch the factors to fix its need. For example, if the labour cost is high or the user intend to deliver the product faster, then the process can be adjusted with response surface methodology to be completed within a shorter time frame, to cut down the labour cost.

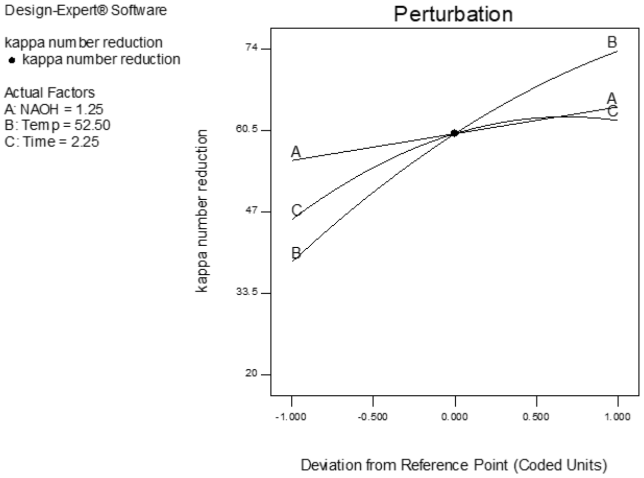

Figure 1. Perturbation curve for kappa number reduction

3.4 Single factor response (Sedimentation index)

The sedimentation index is an indicator of cellulose swelling. The higher the sedimentation index, the higher the swelling capacity of the fibre and vice versa. Cellulose swelling is an important step as it opens up the fibre bundle which increases the accessibility and penetrability of the corncob fibre for the subsequent enzymatic reaction - bio bleaching. The single factor effect on the sedimentation index of fibre is shown in Figure 2. Only 2 factors have a significant effect on the sedimentation index which were the sodium hydroxide concentration and reaction temperature. Even though reaction time was insignificant, it should be noted that it does not mean the reaction time is not required for the reaction to take place. The insignificant result was because the selected range of the reaction time used in the study is insignificant. In terms of NaOH concentration, the cellulose swelling initially increased but reduced with a further increment of NaOH concentration. The function of NaOH in wood and pulp pretreatment is to dissolve unwanted compound in the fibre in order to get the cellulose. At the same time, it also breaks the intermolecular hydrogen bond inside the cellulose structure (amorphous and crystalline) causing the decrease in cellulose crystallinity. This resulted in the fibrils' size to increase in the radial direction and thus led to swelling of the internal structure and increased the sedimentation index. Nevertheless, a further increase in NaOH concentration might dissolve hemicellulose and sometimes cellulose (Isogai and Atalla, 1998). Therefore, the fibrous length shortened and the ability to swell decreased. In order to have pulp with better strength, a higher swelling is preferable. Therefore, again, it indicated that the concentration for NaOH pretreatment does not necessarily need to be more than 1 M. In fact, using a higher concentration of NaOH may give an undesirable result. Meanwhile, for the reaction temperature, an increase in temperature increased the sedimentation index.

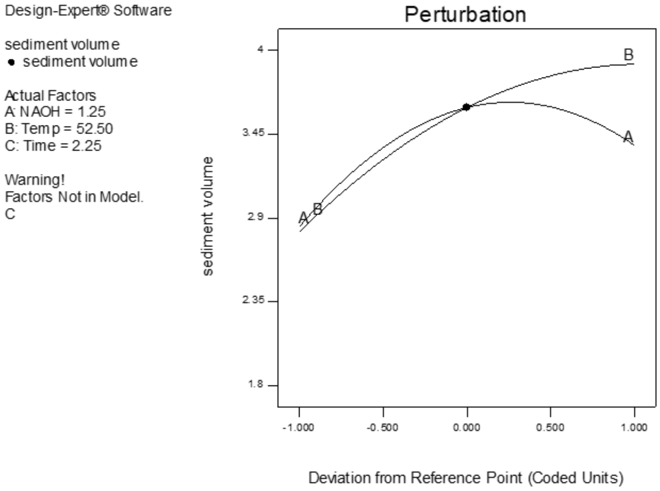

Figure 2. Perturbation curve for sedimentation index

3.5 Single factor response (Lignin released into filtrate)

Lignin determination in the filtrate was used as complementary data for the determination of the presence of lignin in the fibre after pretreatment. It refers to the lignin amount in the filtrate which is released and collected after the NaOH pretreatment. As can be seen from the perturbation graph in Figure 3, an increased in sodium hydroxide promoted the released of lignin into the solution. Nevertheless, the NaOH concentration showed a reverse lignin release when the concentration increased from 1 M to 2 M. In fact, it causes a drop in lignin content to in filtrate especially when treated at the highest 2 M concentration. This was an unexpected phenomenon as lignin released was expected to be in parallel with sodium hydroxide concentration since it enhanced the delignification process. One of the plausible causes is because higher sodium hydroxide concentration may increase the fibre porosity causing the fibre to be a good absorber for the entrapment of lignin monomer that is supposed to be released in the filtrate (Karthikeyan *et al*., 2013). It should be noted that even though lignin has been released from the intact fibre structure it was attached to the surface fibre. Furthermore, higher NaOH concentration for fibre pretreatment might not be a good choice as it increases the water consumption as more water is needed to wash out the pretreated fibre in order to completely remove the held lignin. The kappa number reduction as mentioned in the earlier section does not show a similar trend as the sedimentation index. This is because the lignin was held by the fibre and washed out during the washing step. Meanwhile, the temperature was also one of the significant factors in this response whereby the filtrate lignin showed a directly proportional relation to it. Also, the reaction time reached a steady state at around 1 hour.

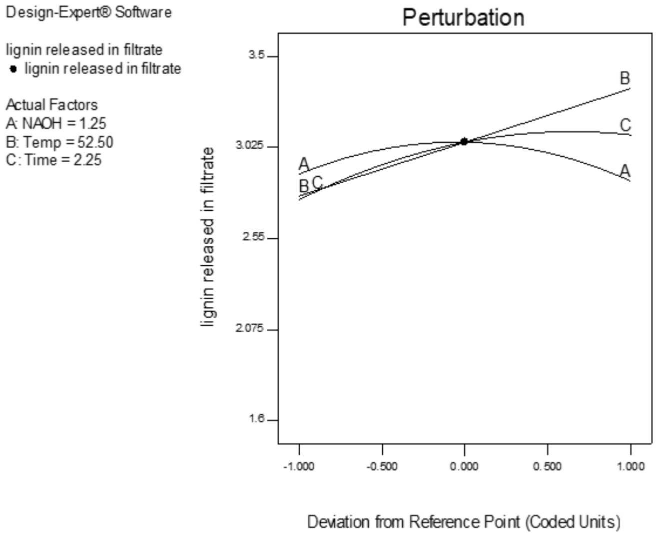

Figure 3. Perturbation curve for lignin released into filtrate

3.6 Single factor response (Chromophore released into filtrate)

The coloured pigment produced from pretreated corncob pulp is shown in filtrate as chromophore material (Figure 4). The chromophore released basically are brown in colour, which was a reaction product between lignin and NaOH. The only factor that has a significant effect on the chromophore content in filtrate was the reaction temperature. As the temperature increased, the amount of chromophore increases as well. However, the other two factors did not show any significant effect on the chromophore content. Since the model obtained for this response showed a significant lack of fit therefore this response can be ignored.

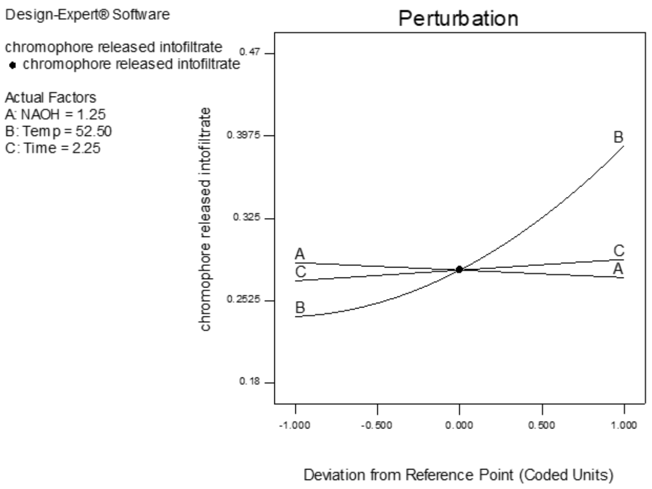

Figure 4. Perturbation curve for chromophore released into filtrate

3.7 Interaction between factors (3 –D contour plot)

A higher temperature promoted the delignification process and subsequently resulted in a lower kappa number of the fibre. The reduction of kappa number could be achieved at high reaction temperature while the sodium hydroxide concentration has less effect on the kappa number. This can be observed in Figure 5. It was proven through various studies that treatment of lignocellulosic biomass with liquid at the higher temperature would help in delignification up to 85%, along with hydrolysis of hemicellulose (Yan *et al*., 2014). Hobden (1956) state that "temperature is the most important factor affecting the dissolution of lignin". According to Sing *et al*. (2013), during the high temperature pretreatment, lignin decomposition, coalescence and mobility occurred which can be observed through scanning and transmission electron microscopy. Besides, the higher temperature also affects the delignification by breaking the covalent bond and formation of intermolecular and intramolecular

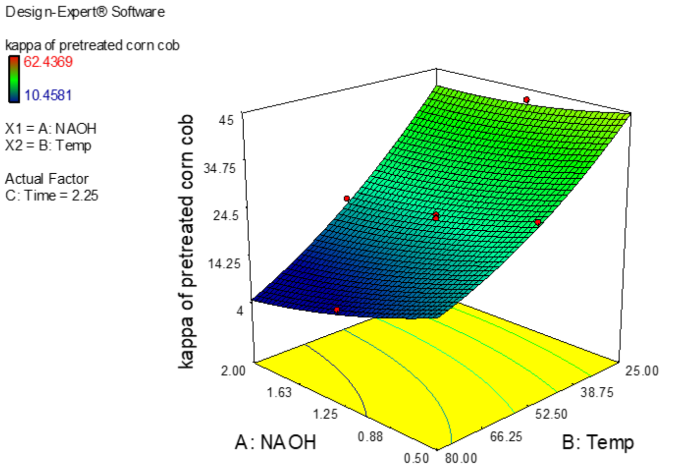

Figure 5. 3D contour graph showing the effect of sodium hydroxide and reaction temperature on the kappa number of pretreated corncob

interaction. In addition, reaction time also significantly affect the delignification of the corncob. As shown in Figure 6, at the reaction temperature of 52.5°C, an increase in reaction time reduced the kappa number from ≈45 to ≈17. On the other hand, sodium hydroxide concentration showed the least effect on the kappa number. This can be seen through the slope of sodium hydroxide in Figure 5 and Figure 6 which was less steep. It can be deduced from the graph that kappa number reduced when the reaction time was extended regardless of sodium hydroxide concentration.

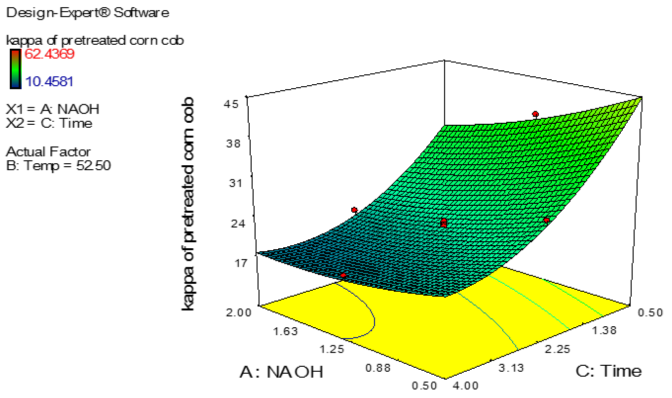

Figure 6. 3D contour graph showing the effect of sodium hydroxide and reaction time on the kappa number of pretreated corncob

Meanwhile, in terms of cellulose swelling, an increase in sodium hydroxide concentration and reaction temperature caused an increase in the cellulose swelling which is indicated from the higher sedimentation index (Figure 7). The reaction performed at a much faster rate at higher temperature as compared to low temperature.

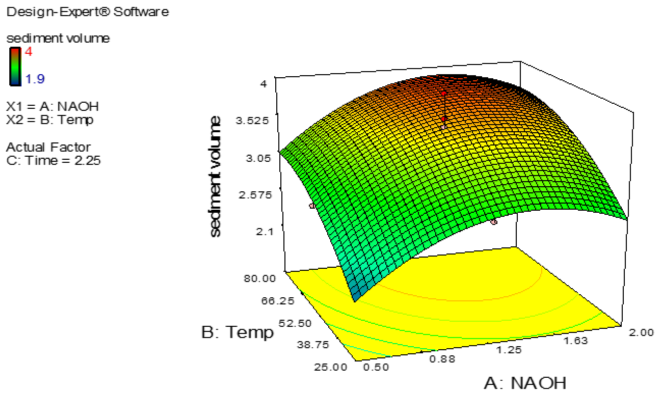

Figure 7. 3D contour graph showing the effect of sodium hydroxide and reaction temperature on the sedimentation volume of pretreated corncob

As for the amount of lignin content present in filtrate, decreased sodium hydroxide concentration promoted the release of lignin into the filtrate at high temperature but reduces it at high NaOH concentration (Figure 8). Lignin content in the filtrate increases with reaction temperature but reduces with increasing sodium hydroxide concentration. Sodium hydroxide with the lowest concentration enhanced the lignin release rather than the high concentration. A low sodium hydroxide concentration may reduce swelling of cellulose which can then decrease the chances of lignin monomer to be entrapped to it. Hence, a low sodium hydroxide concentration is more preferable as it will reduce the water needed to wash off the lignin monomer that was entrapped in the fibre. Since the current study was to minimize the usage of chemical like sodium hydroxide as a prior step to prepare the corncob for further bio-bleaching, maximum delignification of corncob is not necessary. Therefore, we decided that the pretreatment be carried out using a lower concentration of sodium hydroxide (to enable easier washing) with a shorter period of the time frame and at a higher temperature.

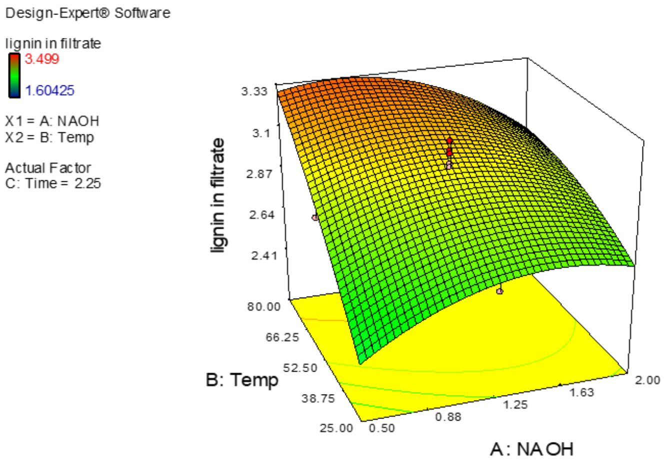

Figure 8. 3D contour graph showing the effect of sodium hydroxide and reaction temperature on the lignin content in filtrate

3.8 Verification of the production

The optimized condition generated from the response surface methodology which were 0.5 M NaOH, reaction temperature of 80°C and reaction time of 30 mins resulted in corncob with kappa number reduction of 55.04 and sedimentation index of 31.2. A series of 5 experimental runs using the optimized conditions was performed to verify the model estimated from the response surface methodology (Table 4). Kappa number reduction have a 10% deviation from the predicted value, 49.92 compared to 55 whilst sedimentation index is well

Table 4. Verification of selected parameter for sodium hydroxide pretreatment

Sample	Kappa number reduction	Sedimentation index
1	48.48	31
2	53.45	31
3	48.81	29
4	50.89	32
5	47.97	30
Mean	49.92	30

fit to the predicted value generated by the model which is 30 compare to 31.2.

3.9 Biobleaching

Bio bleaching is the process of bleaching the pulp in replacement of chemical bleaching as a move to reduce the chemical used to whiten pulp prior to further application. Pulpzyme HC was used in the present study to perform the bio bleaching for corncob. For bio bleaching, only parameters such as enzyme load and reaction time were studied as the optimum temperature and pH for Pulpzyme HC are known to be 50°C and pH 7, respectively based on manufacturer product specification. An FCCD with 2 factors and 3 levels was used to optimize the condition of biobleaching. The 13 experimental runs with the independent and dependent variables are shown in Table 5. Kappa number fit well with linear model whereas both the sedimentation index, lignin and chromophore content in filtrate fit the quadratic model well.

Kappa number was not reduced to ≈10 after bio-bleaching even with the usage of 0.5 u/g of enzyme. Based on Figure 9, the kappa number decreased alongside the increase of reaction time. This showed that a longer time was better for the enzymatic reaction as it will provide sufficient time for the complete action of Pulpzyme HC on the pretreated corncob. Meanwhile, the enzyme load amount did not significantly affect the Kappa number. Thus, to reduce the consumption of enzyme and lower the operation cost, bio bleaching was conducted for 2.75 hrs, using 0.5 u/g of enzyme. Our results were consistent with the prediction data where the kappa number of pretreated corncob was reduced from 32.54 to 17.97 after undergoing enzymatic bio bleaching (Table 5). Similar studies also showed that bio-bleaching using xylanase managed to reduce the kappa number of pulp by 25%. The usage of commercial xylanase (Novozyme 473) resulted in a 31% reduction of chlorine consumption, 30% reduction in total organic carbon in the extraction stage effluent and at the same time increased the brightness, tensile strength and burst factor of treated fibre (da Silva *et al.*, 1994).

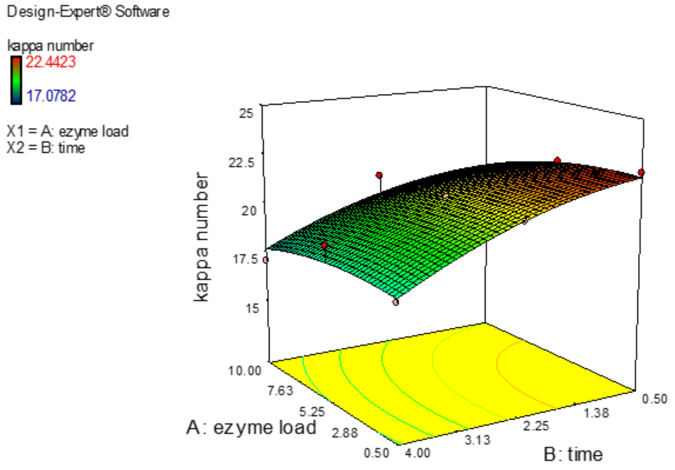

Figure 9. 3D contour graph showing the effect of enzyme load and reaction time on the kappa number of bio-bleached corncob

3.10 Properties of the corncob after pretreatment

Kappa number showed an obvious decrease from 82.45 to 32.54 when the corncob was pretreated with NaOH. Subsequent pretreatment of the NaOH pretreated corncob with Pulpzyme HC also reduces the kappa number from 32.54 to 17.97 (Table 6). It indicated the possibility that NaOH created porous structures that allow the accessibility of the Pulpzyme HC to further remove the lignin from the corncob. At the same time, particle size also decreased with the pretreatment process indicating the reduction of the size of the fibre. The sedimentation index also increased after treatment NaOH and enzymatic pretreatment. All these properties are required for treated corncob to be used for micro fibrillated cellulose production which can then be

Table 5. FCCD for 13 experimental runs and experimental data of the responses (Kappa number, sediment rate, lignin in filtrate, chromophore in filtrate) for the study on effect of the biobleaching on corncob

Run	Enzyme (U)	Time (hrs)	Kappa number reduction	Sedimentation index	Lignin in filtrate (abs)	Chromophore in filtrate (abs)
1	10	4	13.64	65	0.1245	0.1746
2	0.5	2.25	9.79	37	0.0962	0.1113
3	5.25	2.25	9.88	42	0.1178	0.1459
4	5.25	2.25	10.05	42	0.1091	0.1294
5	5.25	2.25	10.19	58	0.1105	0.1221
6	5.25	2.25	9.94	42	0.1027	0.1224
7	5.25	2.25	10.15	46	0.1065	0.1331
8	10	0.5	10.28	42	0.1090	0.1455
9	0.5	0.5	8.2	36	0.0587	0.0738
10	5.25	0.5	8.78	42	0.0898	0.1100
11	0.5	4	12.54	46	0.0971	0.1164
12	5.25	4	11.46	58	0.1153	0.1569
13	10	2.25	9.97	51	0.1272	0.1475

Table 6. Properties (kappa number, sedimentation rate and particle size) of raw, NaOH pretreated and bio-bleached corncob

Sample	Kappa number	Sedimentation index	Particle Size (µm)
Raw Corncob	82.46 ± 0.06^{a}	2.00 ± 0.60^{a}	NA
NaOH pretreatment	32.54 ± 0.03^{b}	30.0 ± 0.50^{b}	365.12 ± 15.67^{a}
BioBleach	17.97 ± 0.03^{c}	61.0 ± 0.50^{c}	235.42 ± 6.92^{b}

Values are expressed as mean±SD of triplicate. Values with different superscript within the same column are significantly different ($p<0.05$).

Table 7. Colour and brightness of corncob fibre treated with NaOCl after chemical and enzymatic treatment

Sample	NaOCl concencetration (%)	Colour			Brightness (%)
		L	a	b	
NaOH pretreated fibre bleached with NaOCl	0	46.2 ± 0.6^{a}	6.2 ± 0.7^{c}	36.1 ± 2.2^{e}	7.1 ± 0.2^{a}
	0.5	62.0 ± 0.4^{c}	-3.2 ± 0.4^{a}	17.7 ± 0.6^{d}	13.7 ± 0.7^{b}
	1	63.7 ± 0.5^{c}	-2.4 ± 0.5^{a}	14.4 ± 0.2^{c}	14.9 ± 0.4^{b}
Enzymatic treated fibre bleached with NaOCl	0	58.0 ± 1.0^{b}	-1.3 ± 0.3^{b}	40.1 ± 0.5^{f}	15.9 ± 0.5^{b}
	0.5	84.7 ± 1.6^{d}	-0.9 ± 0.4^{b}	4.7 ± 0.4^{b}	26.8 ± 1.2^{c}
	1	83.9 ± 0.8^{d}	-0.7 ± 0.2^{b}	-1.0 ± 0.3^{a}	29.3 ± 1.3^{d}

Values are expressed as mean±SD of triplicate. Values with different superscript within the same column are significantly different ($p<0.05$).

utilized as an emulsion stabilizer. The treated corncob's pulp/fibre needs to be soft enough to pass through the high pressure homogenizer without blockage.

3.11 Colour and brightness of corncob fibre treated with NaOCl after enzymatic and chemical pretreatment

The colour of corncob fibre obtained from different pretreatment processes are shown in Table 7. Corncob fibre treated with NaOH and Pulpzyme before chemical bleaching using NaOCl have L, a, and B value of 46.2, 6.27, 36.1 and 58, -1.3 and 40.1, respectively. Corncob fibre showed an increase in the L-value from 46.2 to 58.0 after treated with Pulpzyme which indicated the increment of a whitish colour. Similarly, the positive "a" value which indicated redness colour was reduced to -1.3 while the yellowish b value was slightly increased from 36 to 40.1. Both the NaOH and enzymatic-treated corncob went through different concentrations of NaOCl chemical bleaching. However, when enzymatically treated fibre underwent chemical bleaching (0.5% NaOCl), it showed a drastic increase in the whitish colour to 84.7 while yellow colour was reduced to colourless at around 4.7. Further increase in the NaOCl concentration to 1%, caused the yellowish colour to almost vanish which can be seen from the b value that was close to 0 (-1.0). The L value of NaOH treated fibre improved but plateaued at 63.7. As compared to enzymatic-treated corncob, at the concentration of 0.5% NaOCl, the enzymatic- treated fibre have an L value of 84.7. In contrast, NaOH treated fibre have little improvement in reducing yellow colour even NaOCl was increased from 0.5 % to 1%, b value only reduced from 17.7 to 14.4, respectively. As compared to enzymatically treated fibre where there was a drastic drop of the b value from 40 to 4.7 and finally to -1.0. The results showed that enzymatic bleaching has a significant influence on colour enhancement. Based on the results, it can significantly help to reduce the usage of chlorine-based bleaching. An increase in the bleaching efficiency generally comes from hydrolysis of xylan by the Pulpzyme HC that loosened up the complex structure of the fibre. With that, the bleaching agent had easier access to the inner side of fibre bundle structures. Meanwhile, the NaOH treated fibre was still tough and the compact structure can be hardly accessed by the NaOCl, and therefore higher dosage of NaOCl was needed for bleaching. In addition, by looking at the particle of enzymatic-treated fibre it showed that the size was smaller, therefore, more surface area can come into contact with the bleaching agent which increased the bleaching efficiency. It truly proved that the enzymatic treatment was not only able to reduce the colour of fibre but more importantly, it helps to reduce the amount of chlorine-based bleach needed and further decreased the chemical usage.

We also investigated the brightness of the Pulpzyme-treated corncob in comparison with the untreated corncob after NaOCl bleaching. With 0.5% sodium hypochlorite, the brightness of Pulpzyme treated corncob pulp were significantly enhanced from 15.89 to 26.82 ($P<0.05$), whereas the brightness of corncob pulp untreated with Pulpzyme only increased from 7.12 to 13.74. Corncob pulp brightness increased due to the removal of chromophore structure connected to the surface of xylan. The brightness was achieved around 30 when 1% of sodium hypochlorite was used for bio-bleaching corncob pulp. This showed that Pulpzyme HC treatment can promote the brightness of corncob pulp which directly reduced the consumption of chlorine-based bleach.

4. Conclusion

Lignocellulosic rich corncob biomass serves to be a better alternative for the production of cellulose fibre as compared to wood pulp attributed to its less complex structural property, lignin and pigment content. The mild alkaline and biobleaching pretreatment process using sodium hydroxide as well as Pulpzyme HC employed for the pretreatment of corncob in producing corn pulp was found to successfully increase both the cellulose swelling and lignin removal from the corncob biomass. In the alkaline pretreatment process, reaction temperature demonstrated to be the most prominent effect in enhancing lignin removal as well as cellulose swelling as compared to other parameters like sodium hydroxide concentration and reaction time. Optimized conditions for alkaline pretreatment generated by RSM: 0.5 M NaOH, reaction temperature of 80°C and reaction time of 30 mins increase the sedimentation index (indicate swelling of cellulose) from 0 to 30 and reduce the kappa number (represent lignin removal) from 82 to 32, respectively. Meanwhile, biobleaching pretreatment reaction time plays a more significant role than the Pulpzyme HC concentration in promoting lignin removal and increasing cellulose swelling. RSM optimized conditions showed that the kappa number was reduced from 32 to 18 whereas the sedimentation index increase from 30 to 60 when the alkaline pretreated corncob was biobleached with Pulpzyme HC.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgments

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References

Bissoon, S., Christov, L. and Singh, S. (2002). Bleach boosting effects of purified xylanase from *Thermomyces lanuginosus* SSBP on bagasse pulp. *Process Biochemistry*, 37(6), 567-572. https://doi.org/10.1016/S0032-9592(01)00259-X

da Silva, R., Yim, D.K. and Park, Y.K. (1994). Application of thermostable xylanases from *Humicola* sp. for pulp improvement. *Journal of Fermentation and Bioengineering*, 77(1), 109-111. https://doi.org/10.1016/0922-338X(94)90221-6

Dai, Y., Song, X., Gao, C., He, S., Nie, S. and Qin, C. (2016). Xylanase-aided chlorine dioxide bleaching of bagasse pulp to reduce AOX formation. *BioResources*, 11(2), 3204-3214. https://doi.org/10.15376/biores.11.2.3204-3214

Hamedi, J. Vaez Fakhri, A. and Mahdavi, S. (2020). Biobleaching of mechanical paper pulp using *Streptomyces rutgersensis* UTMC 2445 isolated from a lignocellulose-rich soil. *Journal of Applied Microbiology,* 128(1), 161-170. https://doi.org/10.1111/jam.14489

Isogai, A. and Atalla, R.H. (1998). Dissolution of Cellulose in Aqueous NaOH Solutions. *Cellulose*, 5 (4), 309-319. https://doi.org/10.1023/A:1009272632367

Karthikeyan, A., Balamurugan, K. and Kalpana, A. (2014). The effect of sodium hydroxide treatment and fibre length on the tensile property of coir fibre-reinforced epoxy composites. *Science and Engineering of Composite Materials*, 21(3), 315-321. https://doi.org/10.1515/secm-2013-0130

Kim, J.S., Lee, Y.Y. and Kim, T.H. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource Technology*, 199, 42-48. https://doi.org/10.1016/j.biortech.2015.08.085

Kumar, D., Kumar, S. S., Kumar, J., Kumar, O., Mishra, S.V., Kumar, R. and Malyan, S.K. (2017). Xylanases and their industrial applications: a review. *Biochemical and Cellular Archives*, 17(1), 353-360.

Mohomane, S.M., Linganiso, L.Z., Buthelezi, T. and Motaung, T.E. (2017). Effect Of Extraction Period On Properties Of Sugarcane Bagasse And Softwood Chips Cellulose. *Wood Research*, 62(6), 931-938.

Sharma, P., Sood, C., Singh, G. and Capalash, N. (2015). An eco-friendly process for biobleaching of eucalyptus kraft pulp with xylanase producing *Bacillus halodurans*. *Journal of Cleaner Production*, 87, 966-970. https://doi.org/10.1016/j.jclepro.2014.09.083

Sharma, A., Balda, S., Gupta, N., Capalash, N. and Sharma, P. (2020). Enzyme cocktail: An opportunity for greener agro-pulp biobleaching in paper industry. *Journal of Cleaner Production*, 271, 122573. https://doi.org/10.1016/j.jclepro.2020.122573

Shogren, R.L., Peterson, S.C., Evans, K.O. and Kenar, J.A. (2011). Preparation and characterization of cellulose gels from corncobs. *Carbohydrate Polymers*, 86(3), 1351-1357. https://doi.org/10.1016/j.carbpol.2011.06.035

Silvy, N., Reza, S., Uddin, N. and Akther, M. (2018). Comparison between Different Components of

Some Available Hardwood and Softwood in Bangladesh. *IOSR Journal of Biotechnology and Biochemistry*, 4(1), 1-5.

Sridevi, A., Sandhya, A., Ramanjaneyulu, G., Narasimha, G. and Devi, P.S. (2016). Biocatalytic activity of *Aspergillus niger* xylanase in paper pulp biobleaching. *3 Biotech*, 6(2), 165. https://doi.org/10.1007/s13205-016-0480-0

Tavares, A.P., Gamelas, J.A., Gaspar, A.R., Evtuguin, D.V. and Xavier, A.M. (2004). A novel approach for the oxidative catalysis employing polyoxometalate–laccase system: application to the oxygen bleaching of kraft pulp. *Catalysis Communications*, 5(9), 485-489. https://doi.org/10.1016/j.catcom.2004.06.002

Winuprasith, T. and Suphantharika, M. (2013). Microfibrillated cellulose from mangosteen (*Garcinia mangostana* L.) rind: Preparation, characterization, and evaluation as an emulsion stabilizer. *Food Hydrocolloids*, 32(2), 383-394. https://doi.org/10.1016/j.foodhyd.2013.01.023

Winuprasith, T. and Suphantharika, M. (2015). Properties and stability of oil-in-water emulsions stabilized by microfibrillated cellulose from mangosteen rind. *Food Hydrocolloids*, 43, 690-699. https://doi.org/10.1016/j.foodhyd.2014.07.027

Zhang, Liu, Y., Huang, Y.D. and Liu, L. (2013). Effect of particle size and distribution of the sizing agent on the carbon fibres surface and interfacial shear strength (IFSS) of its composites. *Applied Surface Science*, 287, 423-427. https://doi.org/10.1016/j.apsusc.2013.09.174

Zhang, M., Wang, F., Su, R., Qi, W. and He, Z. (2010). Ethanol production from high dry matter corncob using fed-batch simultaneous saccharification and fermentation after combined pretreatment. *Bioresource Technology*, 101(13), 4959-4964. https://doi.org/10.1016/j.biortech.2009.11.010