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Extraction of Lignin from Agro-Waste Coir Fiber by Mild Alkali Treatment: A Statistical Approach for Process Optimization through Response Surface Methodology

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Lignin, a biomass with a wide range of applications, can be produced from agro-industrial waste, which can contributes to a reduction in the costs of production as well as the pollution load from the environment. The lignin content in coconut coir fiber is fairly high (40-45%) compared to other biomass. In current study, lignin is extracted from coconut coir fiber using a mildly alkaline solution and moist heat under pressure in an autoclave. Three factors were taken into consideration while designing the experiments: the variation in NaOH concentration, the variation in time and the variation in temperature. The optimal lignin extraction of 305.9742 mg/100 mL was achieved at 107 ºC and a heating time of 130 min by using a central composite design (CCD) of the response surface methodology (RSM).

Keywords: Lignin, Coir fiber, Alkali treatment, Optimization, Response surface methodology.

INTRODUCTION

The continuously growing global population demands some modifications to the current socio-economic structure in order to address several challenges. Supplementary concepts and emerging solutions can help with pollution, global warming, water quality, finite energy supplies, food security and environmental conservation to name a few of the problems. Traditional petroleum-based polymers portray perfect examples of major environmental pollutants due to their extensive usage through varied applications. This environmental awareness and concern currently attracted a significant research interest of scientists and technologists towards inexpensive, environmentally benign materials [1]. Certain properties of biopolymers including biocompatibility, biodegradability and low production cost make them suitable substitutes for various applications of petroleum based polymeric materials.

Agricultural waste is very resourceful for the extraction of a variety of biopolymers. Environmental pollution load and production costs can be significantly reduced by treating these discarded agro-industrial as raw materials. Lignin, a key structural material and main constituent of plants, follows cellulose to top the list of most affluent biopolymer sources. It can be a great replacement for inexhaustible sources of aromatics and petroleum-derived chemicals. In comparison to synthetic polymers, competitive pricing, and non-polluting features in lignin, attracts a lot of attention toward its potential use as a biopolymer [2]. In spite of having great possible applicability, owing to its many advantages *e.g.* abundant availability, renewability, recyclability, cost-effectiveness, environmental friendliness, presence of a large number of functional groups, *etc.* lignin is still a lesser explored biopolymer. This pushes lignin isolation to be studied and developed further in order for it to be recognized in material science and other practical applications. Literature states a considerable amount of lignin is contained in various biomass, such as woody materials, softwoods, bagasse, barley hay, rice straw, coir, different agricultural waste, *etc.* [3]. Coir or coconut fiber is a versatile natural fiber extracted from the coconut fruit and classified as a significant agricultural waste product. Coir is a lignocellulose material majorly containing hemicellulose (0.25%), cellulose (43.44%) and a substantially higher percentage of lignin ((35-45%) compared to various other fibers [4].

Extraction of lignin from agricultural waste has witnessed numerous advances. The industry has adapted to certain widely popular methods such as soda, kraft, organosolv and sulfite for

extraction of pure lignin from agricultural biomass [5]. But as a part of the pre-treatment process, the biomass is disintegrated into the structural components along with the segregation of lignin from carbohydrates. Some other well-known methods include steam explosion grinding/milling, autohydrolysis/hot water), acid treatment and alkali treatment [6-9]. The lignocellulose pretreatment utilizes both organic acids like acetic, oxalic, maleic and formic acid, as well as several concentrated or diluted mineral acids such as sulphuric, nitric, hydrochloric, phosphoric, hydrofluoric acid *etc.* The major downside to using acids is their toxicity and corrosiveness. While diluted acids demand high temperatures, organic acids are high-cost reagents which give them both a disadvantage as well. Apart from using acids in the pretreatment process, organosolv is widely applied for lignin extraction [10,11]. But this method demands a higher extraction cost because of the usage of expensive solvents as well as the leaching of toxic organic solvents into the environment and the high recovery cost of byproducts makes it unfavourable [12]. The methods involving the use of an alkaline reagent transpire as one of the most feasible processes mainly because of its vigorous pretreatment effect and relatively straight-forward scheme. Compared to other pretreatment technologies, pretreating the biomass with alkali can be achieved lower temperatures and pressures. Alkaline reagents essentially interact with lignin making it much more coherent to remove lignin. The affiliation between lignin and hemicellulose in the cellulose matrix of the lignocellulosic biomaterials is constructively attacked by NaOH [13]. Specifically, the ether and ester bonds in the lignocellosic composition are cleaved. Also, the lignin extraction by alkaline solution has an added advantage, as it selectively eliminates lignin without discrediting carbohydrate structure while increasing porosity, crystallinity and surface area as well. These methods commonly employ alkaline chemicals such as NH_3, NaOH, Na_2CO_3 and $Ca(OH)_2$. These processes are executed under less severe conditions than that of an acid-based procedure. Applying drastic methods for the extraction of lignin alters its native structure [14].

In present study, low alkali concentration was maintained to minimize the profound changes in the structure of isolated lignin. It was found to be more economical, non-corrosive and environment benign compared to other pretreatment methods. In this method, the recalcitrant structure of lignin was found to be more easily rendered by moist heat under pressure from the autoclave. The presence of moisture helps in the efficient heating process by distributing heat in the autoclave chamber uniformly. The experiments were designed by considering three factors, *viz.* (i) varying the concentration of NaOH, (ii) varying the time of extraction and (iii) varying the temperature of extraction. In the second step, a central composite design (CCD) of response surface methodology (RSM) was applied to deduce the optimized condition for lignin extraction process and to investigate the effects of three independent variables, concentration of NaOH, temperature and time of heating.

EXPERIMENTAL

The coconut coir used in the study was collected from Hanuman Agro Products, Tumkur District, India. Analytical grade sodium hydroxide and 98% sulphuric acid were procured from Merck, India.

Preparation of raw material: The coir fiber, procured from the local agro-industry, was cleaned and washed thoroughly with distilled water followed by acetone. It was afterward dried and conditioned according in a hot air oven and cut into small pieces of uniform length (~2 cm). The pre-conditioned coir fibers were then stored in an airtight container.

Extraction of lignin using alkaline solution: The preconditioned coir fibers were taken in 100 mL glass beaker and immersed in NaOH solution maintaining a 1:20 (w/v) ratio. It was then tightly sealed, mixed thoroughly and autoclaved (equitron digital autoclave) at 3 kPa pressure and specific temperature. After heating the suspension for the desired time, the sealed flasks were removed carefully from the autoclave. While the resulting dark brown colour suspension was still warm, it was filtered to separate the solid residue and then it was cooled to the ambient temperature of 27 ºC by placing it in a cold water bath at 10 ºC. The dark brown liquor, containing extracted lignin, was stored in an airtight glass container and refrigerated.

To optimize the conditions of lignin extraction from coir fiber, different trials were carried out by varying one factor at a time, while keeping the other factors fixed, at a constant pressure (3 kPa). The parameters chosen to be varied for achieving the maximum amount of lignin at optimal extraction conditions were (i) NaOH concentration (1%, 5%, 10%, 15%, 20% w/v) at constant time of heating (60 min) and temperature (110 ºC); (ii) process temperature (100, 105, 110, 115 and 120 ºC) maintaining uniform time of heating (60 min) and NaOH concentration (1%); (iii) time of heating (30, 60, 90, 120 and 150 min) at a fixed temperature (110 ºC) and NaOH concentration (1%).

Experimental design and statistical analysis for process optimization by response surface methodology: Response surface methodology (RSM) was used to optimize the process parameters in order to maximize the amount of lignin that could be extracted from coconut coir. Full factorial central composite design (CCD) of RSM was used to examine the individual and combined effects of the independent test factors, such as the concentration of NaOH, temperature for heating, and time of heating, on the amount of lignin extracted (Table-1). The findings of 20 experiments were analyzed using JMP software.

TABLE-1
INDEPENDENT PROCESS VARIABLES AND THEIR LEVELS FOR CENTRAL COMPOSITE DESIGN

Variable	Symbol	Variable level		
		–1	0	+1
NaOH concentration (%)	A	0.25	0.875	1.5
Temperature (°C)	B	100	110	120
Time (min)	C	30	90	150

Determination of total lignin content in coir fiber: A two-step extraction process was performed to determine the total lignin content in the procured coir fiber. Initially, the water and alcohol extractives were removed by refluxing 10 g of clean coir in a Soxhlet apparatus for 8 h with 200 mL of distilled

water followed by ethanol. Next, the extractive-free coir fiber was kept in an oven at 105 ± 3 ºC for drying [15]. After oven drying the sample to constant weight, it was subjected to two-step acid hydrolysis. In the first step, the hydrolysis was carried out with 72% H_2SO_4 for 1 h in a water bath and in the second step, the acid was diluted to a 4% concentration, subsequently autoclaving for 1 h. The hydrolyzates were used for the determination of total lignin in terms of acid-insoluble and acid-soluble lignin. The acid-insoluble and acid-soluble lignin was estimated by gravimetric and UV-VIS spectroscopic analysis respectively and the percentage of acid-insoluble and acid-soluble lignin was determined [16].

FTIR analysis: FTIR spectroscopic study of coir fiber (before and after lignin extraction) and extracted lignin was performed by FTIR spectrophotometer (Cary 600, Agilent Technologies) in the spectral range of 4000-400 cm^{-1}.

SEM analysis: The surface morphology of coconut coir before and after extraction of lignin was studied using SEM (Hitachi, 3400, Japan).

Quantification of lignin in liquor obtained by alkali extraction: The amount of lignin present in dark brown liquor after alkali extraction from coir fiber was quantified using a UV-VIS spectrophotometer (Systronics PC based double beam spectrophotometer 2202) at 280 nm [17].

RESULTS AND DISCUSSION

Determination of total lignin content in coir fiber: The percentage of acid-insoluble and acid-soluble lignins in the coir sample was found to be 26.50% and 23.25%, respectively.

FTIR spectral studies: FTIR spectra of coir fiber before and after extraction of lignin are shown in Fig. 1a-b. The peaks appearing in the range 3400-3200 cm^{-1} are assigned to the –OH stretch and the peak at 2938 cm^{-1} and 1600 cm^{-1} represent the C–H stretch from methyl and methylene groups and C=O bonds, respectively. The peak at 2922 cm^{-1} signify the presence of C–H stretching in the methyl group. After alkaline extraction, the removal of lignin and hemicellulose from the coir fiber is indicated by the absence of peak at 2842 cm^{-1} (Fig. 1b) [18].

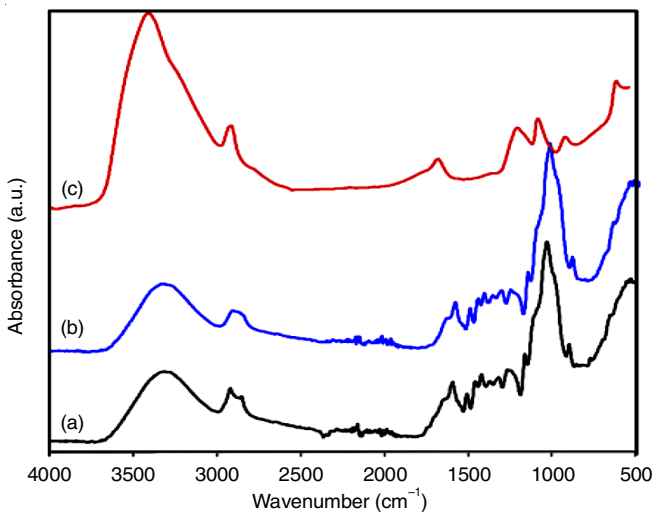

Fig. 1. FTIR spectra of (a) raw coconut coir, (b) coconut coir after lignin extraction by alkali (c) extracted lignin

The presence of aliphatic and aromatic hydroxyl groups in the lignin sample obtained after alkaline extraction is indicated by the broad absorption band assigned to the –OH stretching in the region 3400-3100 cm^{-1} (Fig. 1c). The peak at 1750-1500 cm^{-1} indicate the presence of carbonyl C=O group as unconjugated carbonyl esters, acetyl groups or conjugated carbonyl groups. The presence of phenols and ethers in lignin leads to the appearance of peaks in the 1300-1000 cm^{-1} region, which can be attributed to symmetric and asymmetric C-O stretching. Additionally, the aromatic C–H deformations was also apparent in the range of 1070-1030 cm^{-1} [19].

SEM studies: Fig. 2 represents the SEM images of the coir fiber before and after the extraction of lignin by mild alkali treatment. Before lignin extraction, the raw coir was found to possess a smooth and uniform topography, whereas, after lignin extraction, the surface of alkali-treated coir was more rough and ruptured with edges and cracks, which indicates the leaching out of lignin from the surface of coir fiber [18].

Quantification of lignin in extracted alkaline liquor

Effect of NaOH: The effect of NaOH concentration on the alkaline extraction of lignin from coir fiber was studied with varying NaOH concentrations (1-20% w/v) at constant 3 kPa pressure, 110 ºC temperature and fixed time of 60 min. It was observed that the lignin yield decreased with increase in NaOH concentration and maximum amount of lignin in the extracted liquor was achieved by using 1% NaOH solution (Fig. 3a).

Effect of temperature: The temperature was varied within the range of 100-120 ºC during the autoclaving of coir fiber to observe its effect on extraction process. The amount of lignin in the extracted liquor initially increased with augmenting the temperature up to 110 ºC and then gradually decreased as the temperature was increased (Fig. 3b).

Effect of time: Fig. 3c depicts the effect of time on the lignin extraction process at definite NaOH concentration (1%, w/v), temperature (110 ºC) and pressure (3 kPa) while varying the time of autoclaving the coir fiber from 30-150 min. The amount of lignin in the extracted liquor enhanced with increase in the duration of the process and maximized at 120 min. After 120 min of duration, the lignin yield was observed to be reduced.

Experimental design and statistical analysis for response surface methodology: Table-2 presents the actual and predicted values of the amount of lignin in the extracted liquor acquired by performing 20 experimental runs, according to CCD design with three independent variables, *viz.* concentration of NaOH, time and temperature of autoclaving the coir fiber in alkaline medium. The obtained data were analyzed using JMP software. The lack-of-fit test was applied to review the suitability of the model. The two important parameters, F-ratio and Prob > F of the lack-of-fit test that decides the quality of fit were observed to be 4.7838 and 0.06 (> 0.05), respectively. A very low lack-of-fit F ratio and insignificant Prob > F value signify the well-fitting of the model towards experimental variables (Table-3). The analysis of variance (ANOVA) was also performed to appraise the competency of model as well as to evaluate the linear and interactive effect of the variables on the response value.

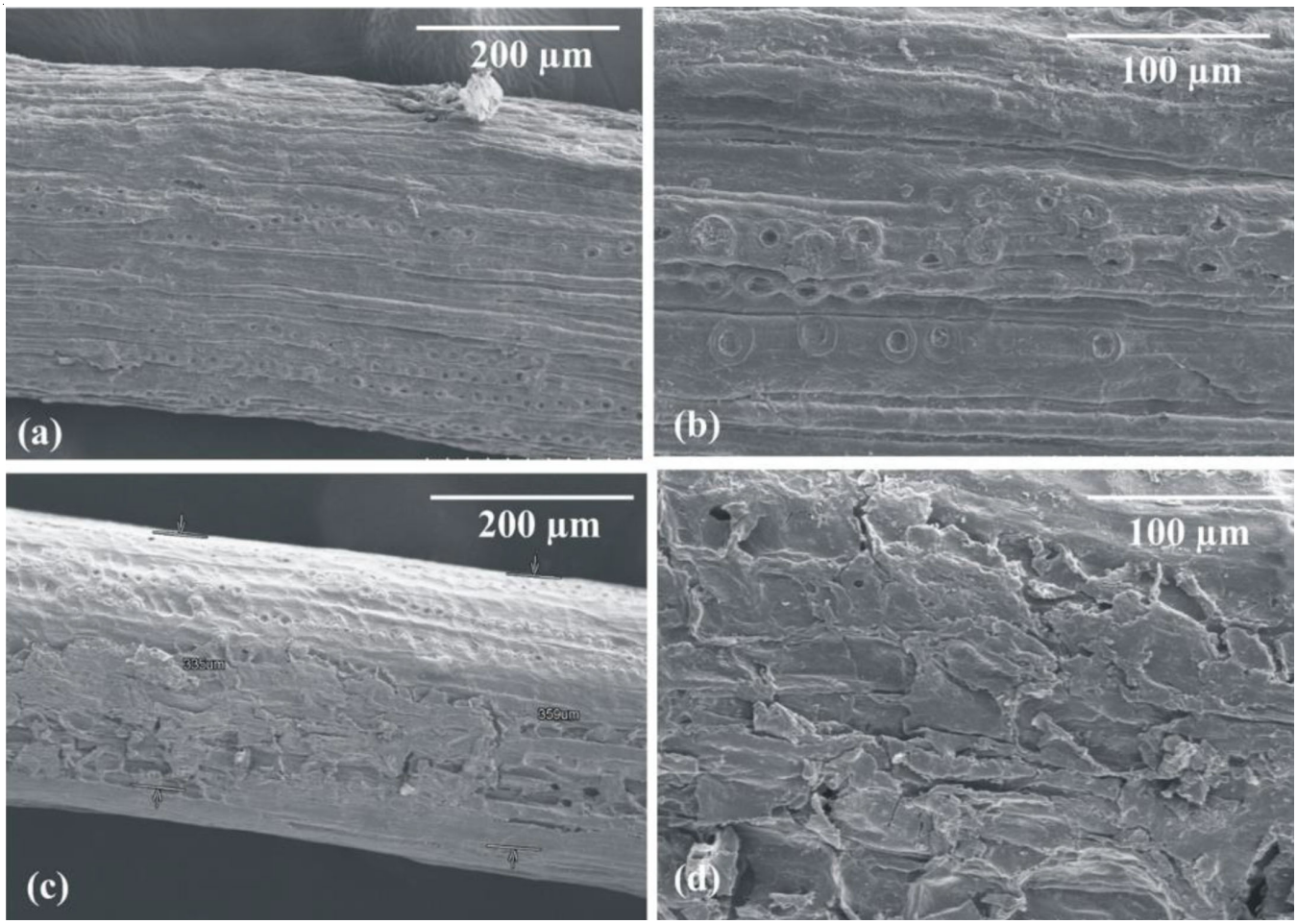

Fig. 2. SEM images of (a) raw coconut fiber (200 µm), (b) raw coconut fiber (100 µm), (c) coconut fiber after alkaline lignin extraction (200 µm), (d) coconut fiber after alkaline lignin extraction (100 µm)

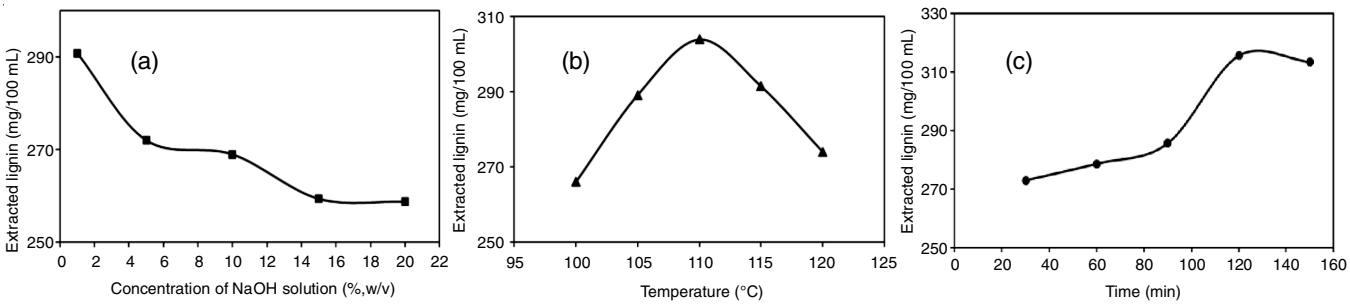

Fig. 3. (a) Effect of NaOH concentration, (b) effect of temperature, (c) effect of time on lignin extraction

The adequacy of the model is indicated by high F ratio (26.3353) and a desirable significant Prob > F value (< 0.0001). The terms A, B, C, A^2 and C^2 were found to be significant and thus suggests their profound effect on the response. According to the summary of fit report, R^2, adjusted R^2 and predicted R^2 values are 0.9595, 0.9231 and 0.9211, respectively (Table-4). High R^2 value in compliance with the predicted R^2 value (Fig. 4) as well as a comparatively lower adjusted R^2 value than R^2 value confirms the appropriateness and reliability of the model.

The 3D response surface plots were used to visualize the individual and interaction effects of process factors on the response within the experimental range (Fig. 5). The 3D plots were generated using JMP software by varying two variables within the experimental range while keeping the other constant. Fig. 5a demonstrates the interactive effect of NaOH concentration and temperature on the response at constant time. The results indicated that higher lignin extraction was achieved at lower NaOH concentrations. The 3D response surface plot, Fig. 5b portrays the combined effect of NaOH concentration and time on the response while the other variable *viz.*, temperature, held constant. Higher extraction of lignin was obtained at higher duration and lower NaOH concentration. The surface plot of response as a function of time and temperature at constant NaOH concentration is illustrated in Fig. 5c, which indicates that the lignin extraction from coir fiber increased with increasing temperature and then decreased at higher temperature

TABLE-2
EXPERIMENTAL DESIGN MATRIX OF CCD AND CORRESPONDING RESPONSE (CONCENTRATION OF LIGNIN EXTRACTED FROM COIR FIBER BY MILD ALKALI SOLUTION)

Run order	Experimental variables (coded values)			Response (lignin concentration, mg/100 mL)	
	A (%)	B (°C)	C (min)	Actual	Predicted
1	+1	+1	-1	246.8430	241.8077
2	0	0	0	297.0972	293.1445
3	0	0	0	290.7480	293.1445
4	0	0	-1	260.6237	267.0568
5	+1	0	0	268.7958	274.6084
6	+1	+1	-1	248.3276	248.8336
7	0	0	+1	296.9116	296.8529
8	0	0	0	294.9903	293.1445
9	-1	+1	+1	290.2367	288.1371
10	0	-1	0	292.5600	294.1563
11	-1	-1	+1	294.4856	297.9273
12	-1	+1	-1	256.2945	257.3178
13	-1	-1	-1	272.1123	269.1852
14	0	0	0	298.5465	293.1445
15	-1	0	0	291.9619	292.5237
16	+1	-1	+1	280.2235	277.6066
17	0	0	0	293.5897	293.1445
18	+1	+1	+1	271.3245	272.658
19	0	0	0	296.6438	293.1445
20	0	+1	0	280.9701	285.7482

TABLE-3
SUMMARY OF FIT AND LACK OF FIT

Summary of fit				Lack of fit	
Root mean square error	R^2	Adj. R^2	Predicted R^2	F ratio	Prob > F
4.7684	0.9595	0.9231	0.9211	4.7838	0.06

range. In Fig. 5, it can be observed that the independent process variables and their combined interaction had a significant effect on response [20].

The optimum condition to gain the maximum amount of extracted lignin from coir fiber was predicted with the aid of JMP software, which generated a set of solutions using the developed model equation. According to the process optimization modelling, the optimum condition with the desirability of 1, for obtaining the maximum amount of extracted lignin (305.9742 mg/100 mL) can be accomplished at 0.55 (%) of NaOH concentration, 107 ºC temperature and 130 min time.

TABLE-4
ANALYSIS OF VARIANCE

Source	Degree of freedom	Sum of squares	F ratio	Prob > F
Model	9	5389.1171	26.3353	<0.0001
A	1	802.3967	35.2901	0.0001
B	1	176.7378	7.7731	0.0192
C	1	2219.5279	97.6166	<0.0001
AB	1	11.7203	0.5155	0.4892
AC	1	0.0004	0.0002	0.9964
BC	1	2.1575	0.0949	0.7644
A2	1	252.3025	11.0965	0.0006
B2	1	28.0234	1.2325	0.2929
C2	1	344.3216	15.1435	0.0030

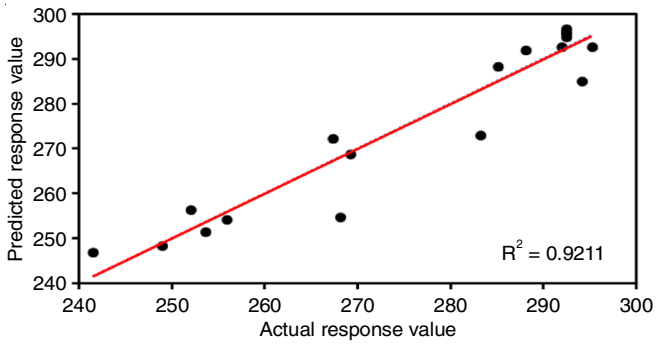

Fig. 4. Plot of actual and predicted amount of lignin (mg/100 mL) at different process conditions as designed by JMP software

Conclusion

The process of extraction of lignin from coir fiber had been studied and statistically optimized by response surface methodology. The NaOH concentration affected the lignin extraction inversely, while an escalation in the duration of the process, enhanced the amount of extracted lignin in liquor. An increase in temperature initially enhanced the extraction of lignin, but this effect decreased afterwards. The optimum operating conditions for the alkaline lignin extraction process to yield the maximum amount of lignin of 305.9742 mg/100 mL, was obtained applying CCD of RSM as NaOH concentration: 0.55% (w/v), temperature: 107 ºC and time: 130 min.

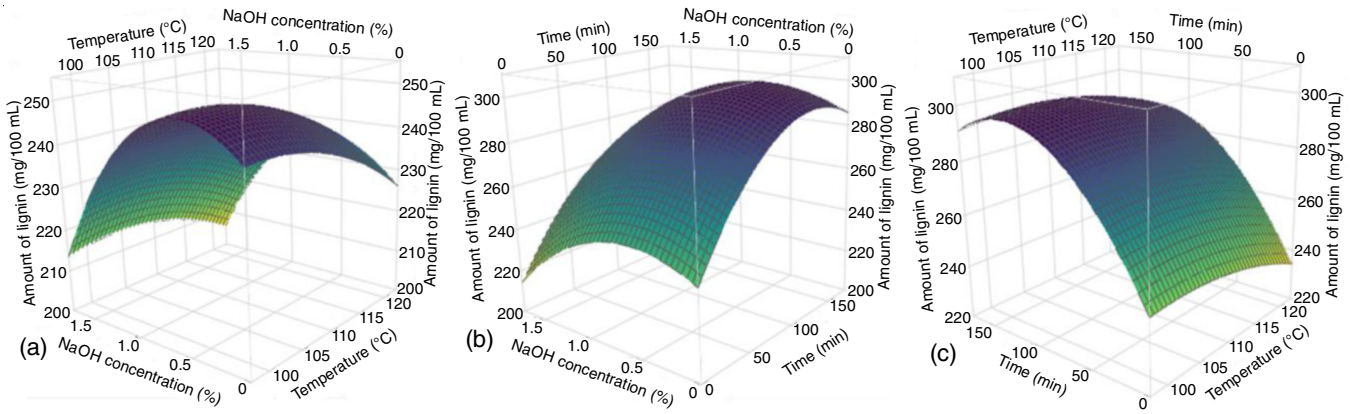

Fig. 5. Three-dimensional response surface plots for the interactive effects of (a) concentration of NaOH and temperature, (b) concentration of NaOH and time, (c) temperature and time on amount of extracted lignin from coconut fiber

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

REFERENCES

1. S. Ramakrishna, J. Mayer, E. Wintermantel and K.W. Leong, *Compos. Sci. Technol.*, **61**, 1189 (2001); https://doi.org/10.1016/S0266-3538(00)00241-4
2. D. Watkins, M. Nuruddin, M. Hosur, A. Tcherbi-Narteh and S. Jeelani, *J. Mater. Res. Technol.*, **4**, 26 (2015); https://doi.org/10.1016/j.jmrt.2014.10.009
3. P. Duarah, D. Haldar and M.K. Purkait, *Int. J. Biol. Macromol.*, **163**, 1828 (2020); https://doi.org/10.1016/j.ijbiomac.2020.09.076
4. A. Adewuyi, *Front. Energy Res.*, **10**, 741570 (2022); https://doi.org/10.3389/fenrg.2022.741570
5. S.A. Arni, *Ind. Crops Prod.*, **115**, 330 (2018); https://doi.org/10.1016/j.indcrop.2018.02.012
6. X. Zhang, J. Zhu, L. Sun, Q. Yuan, G. Cheng and D.S. Argyropoulos, *Ind. Crops Prod.*, **133**, 241 (2019); https://doi.org/10.1016/j.indcrop.2019.03.027
7. N. Mosier, C. Wyman, B. Dale, R. Elander, Y.Y. Lee, M. Holtzapple and M. Ladisch, *Bioresour. Technol.*, **6**, 673 (2005); https://doi.org/10.1016/j.biortech.2004.06.025
8. J.S. Kim, Y.Y. Lee and T.H. Kim, *Bioresour. Technol.*, **199**, 42 (2016); https://doi.org/10.1016/j.biortech.2015.08.085
9. K. Wörmeyer, T. Ingram, B. Saake, G. Brunner and I. Smirnova, *Bioresour. Technol.*, **102**, 4157 (2011); https://doi.org/10.1016/j.biortech.2010.11.063
10. L. Mesa, E. González, C. Cara, M. González, E. Castro and S.I. Mussatto, *Chem. Eng. J.*, **168**, 1157 (2011); https://doi.org/10.1016/j.cej.2011.02.003
11. D. Haldar and M.K. Purkait, *Biomass Convers. Biorefin.*, **12**, 1125 (2022); https://doi.org/10.1007/s13399-020-00689-y
12. S.M. Bhatt and Shilpa, *Biofuels*, **5**, 633 (2014); https://doi.org/10.1080/17597269.2014.1003702
13. J.J. Liao, N.H.A. Latif, D. Trache, N. Brosse and M.H. Hussin, *Int. J. Biol. Macromol.*, **162**, 985 (2020); https://doi.org/10.1016/j.ijbiomac.2020.06.168
14. A. Tejado, C. Pena, J. Labidi, J.M. Echeverria and I. Mondragon, *Bioresour. Technol.*, **98**, 1655 (2007); https://doi.org/10.1016/j.biortech.2006.05.042
15. A. Sluiter, B. Hames, R. Ruiz, C. Scarlata and D. Templeton, Determination of Extractives in Biomass, NREL Laboratory Analytical Procedures, TP-510-42619 (2005).
16. A. Sluiter, B. Hames, D. Hyman, C. Payne, R. Ruiz, C. Scarlata and J. Sluiter, Determination of Structural Carbohydrates and Lignin in Biomass, Laboratory Analytical Procedures (LAP) (2008).
17. R.A. Lee, C. Bédard, V. Berberi, R. Beauchet and J.-M. Lavoie, *Bioresour. Technol.*, **144**, 658 (2013); https://doi.org/10.1016/j.biortech.2013.06.045
18. A. Roy, S. Chakraborty, S.P. Kundu, R.K. Basak, S. Basu Majumder and B. Adhikari, *Bioresour. Technol.*, **107**, 222 (2012); https://doi.org/10.1016/j.biortech.2011.11.073
19. C.G. Boeriu, D. Bravo, R.J.A. Gosselink and J.E.G. van Dam, *Ind. Crops Prod.*, **20**, 205 (2004); https://doi.org/10.1016/j.indcrop.2004.04.022
20. A. Roy, *Environ. Sci. Pollut. Res. Int.*, **28**, 12011 (2021); https://doi.org/10.1007/s11356-020-08820-2