Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

FFNN-GA-Assisted Process Modeling and Optimization of Banana Fiber Pretreatment for Retrieval of Cellulose fiber and Other Value-Added Byproducts

Priya Kaithvar
Motilal Nehru National Institute of Technology
Sangeeta Negi
sn5@mnnit.ac.in

Motilal Nehru National Institute of Technology

Research Article

Keywords: Banana fiber, Genetic Algorithm, ANN, Feed Forward Neural Network, Alkali Pretreatment

Posted Date: November 13th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11589518/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

FFNN-GA-Assisted Process Modeling and Optimization of Banana Fiber Pretreatment for Retrieval of Cellulose fiber and Other Value-Added Byproducts

Priya Kaithvar[1] and Sangeeta Negi[1*]

[1]Department of Biotechnology, Motilal Nehru National Institute of Technology, Allahabad-211004, Uttar Pradesh, India

*Corresponding author

Dr. Sangeeta Negi

Professor

Department of Biotechnology

Motilal Nehru National Institute of Technology, Allahabad-211004, Uttar Pradesh, India-211004

Email- sn5@mnnit.ac.in, sangy20012001@yahoo.com

Phone: +919415015811

Keywords: Banana fiber, Genetic Algorithm, ANN, Feed Forward Neural Network, Alkali Pretreatment

Abstract

Present work deals with the alkaline pretreatment of pseudo stem fibers of banana and critical process parameter optimization through artificial neural network (FFNN) followed by genetic algorithm (GA) to maximize lignin retrieval, simultaneous reduction of sugar loss and derivatization into inhibitors, keeping cellulose fiber intact for pharmaceutical and textile industries. Critical process parameters like NaOH concentration, pretreatment time & temperature were considered as input variables and reducing sugar and lignin concentrations as response variables. A total of 32 experimental observations were obtained using a one-variable-at-a-time (OVAT) approach. Quadratic regression and a feed-forward artificial neural network (FFNN) were used to model the relationship between the pretreatment variables and responses. The final FFNN employed a 3-15-2 architecture and was trained using the Levenberg-Marquardt back-propagation algorithm. The model showed strong agreement between target and predicted responses, with overall correlation coefficients (R) of 0.99137 for lignin and 0.99864 for reducing sugar, with overall R^2 values of 98.28% and 99.73%. Based on the trained model, 43 additional combinations were generated, expanding the dataset to 75 observations for computational optimization. The GA identified an optimum condition of 3.42% NaOH, 11.10 h, and 71.15 °C, with predicted reducing sugar and lignin concentrations of -0.240 and 1.037 mg/mL**.** The Optimized condition was experimentally validated, which resulted in 0.15 mg/mL reducing sugar and 1.05 mg/mL lignin. These concentrations corresponded to 9.40 mg/g reducing sugar and 65.80 mg/g lignin released per gram of initial fiber, while the released sugar and lignin were equivalent to 0.94% and 6.58% of the initial fiber mass. The treatment also resulted in 17.33% weight loss, indicating the removal of non-cellulosic components while retaining the cellulose rich fiber structure. FE-SEM, XRD and FTIR and showed a homogenous structure with desirable fiber characteristics. Hence, the FFNN-

GA approach was found to be an efficient tool for optimizing the removal of non-cellulosic components from banana pseudostem with the released lignin, reducing sugar, furfural and phenolic as value added products for various industry oriented biorefinery processes such as pharmaceuticals, textile, biofuel, biomedical and filtration industries etc.

1. Introduction

Banana (Musa spp.) is widely cultivated worldwide in tropical and subtropical regions and India accounts for 26-28% of its global production. As per a report of ICAR-NRCB, 60 tonnes of biomass waste are generated per hectare of cultivation and disposing of this huge amount creates big problems for farmers, therefore, pseudostem of banana which is a major portion of biomass considered as alternative resource for cellulosic material and other byproducts nationwide [1, 2].

Banana pseudostem is mainly composed of cellulose, approximately 31.3-64.0%, followed by hemicellulose at 10.2-19.0%, lignin at 5.0-19.1%, and extractives at up to approximately 5.2%. However, its composition varies considerably depending on cultivar, plant part, geographical origin, maturity stage, and fiber extraction method. These structural components are organized within the plant cell wall, where cellulose microfibrils are embedded in a matrix of hemicellulose and lignin [3, 4]. Cellulose is an important structural component with potential for utilization in agricultural and industrial applications. Therefore, pretreatment is generally required to disrupt the lignin-carbohydrate matrix, to increase the accessibility of the cellulose-rich fraction, and facilitate subsequent fractionation or conversion [5].

Several pretreatment strategies have been developed to overcome the recalcitrance of lignocellulosic biomass, including conventional chemical methods such as acid and alkali

pretreatment, as well as physical and emerging approaches such as microwave-assisted pretreatment and ionic liquid based pretreatment. Among these methods, alkaline pretreatment using NaOH is widely used for lignocellulosic material because it promotes biomass swelling and disrupts ester linkages associated with lignin and hemicellulose, thereby facilitating lignin solubilization and increasing carbohydrate accessibility [6, 7].

The effectiveness of alkaline pretreatment is therefore governed by a trade-off between lignin solubilization and carbohydrate preservation. Increasing NaOH concentration, treatment time, or temperature generally increases pretreatment severity, which can enhance disruption of the lignocellulosic matrix and the release of lignin and carbohydrate derived components that serves as potential value added byproducts. Under harsh pretreatment conditions the released sugars may undergo further degradation, leading to the formation of degradation products such as furfural & 5-hydroxymethylfurfural (HMF), which can act as inhibitors during subsequent microbial or enzymatic conversion processes. Therefore, controlling pretreatment severity is important not only for effective lignin removal but also for minimizing undesirable carbohydrate degradation and inhibitor formation. The condition that promotes maximum lignin release doesn't necessarily correspond to the condition that minimizes carbohydrate solubilization [8, 9].

In the present study, this relationship was represented as a multivariable response system in which the pretreatment variables: NaOH concentration (X_1), treatment time (X_2), and temperature (X_3), jointly determine the reducing sugar (Y_1) and lignin (Y_2) responses is given in equation 1 and 2.

$$Y_1 = f(X_1, X_2, X_3) \quad (1)$$

$$Y_2 = f(X_1, X_2, X_3) \quad (2)$$

The effects of the three process variables depend on their combined levels, which can result in nonlinear changes in the responses. Hence, experimental evaluation of each process variable is necessary to understand its effect on the two responses & to identify suitable conditions for subsequent modelling and optimization [10, 11].

For optimization, a one-variable-at-a-time (OVAT) approach is useful for preliminary investigation because it allows the effect of an individual process variable to be examined while the other variables are maintained at selected levels. OVAT method provides an experimental information for identifying relevant operating ranges and response trends. However, it does not directly characterize the combined interaction among multiple process variables across the complete experimental domain. Hence, the experimental observations obtained through OVAT can provide the basis for subsequent modelling, while a nonlinear multivariable approach is required to describe the combined influence of the process variables more effectively [12].

Artificial neural network (ANN) modelling provides a useful approach because it learns nonlinear relationships between input variables and response directly from experimental data and accounts for the combined effects of multiple variables without requiring a predefined mathematical model. ANN modelling has increasingly been explored for lignocellulosic pretreatment and related bioprocesses [13]. However, ANN provides a predictive representation of the process, while prediction alone doesn't identify the combination of operating conditions that best meets a defined objective; therefore, optimization is required to identify suitable process conditions from the mode predicted response space. Furthermore, Genetic algorithms (GAs) are population based optimization methods that search complex solution spaces using evolutionary

operations such as selection, crossover, and mutation. Thus, ANN and GA serve complementary roles, with ANN learning the nonlinear relationship between process variables and responses, while GA searches the predicted response space to identify conditions that meet the predefined optimization criteria. ANN-GA frameworks have been successfully applied to nonlinear bioprocess optimization, including fermentation and biomass residue conversion systems [14, 15, 18].

Although extensive research has been conducted on banana pseudostem pretreatment and the growing application of ANN-based modelling in bioprocesses, whereas, the simultaneous computational optimization of alkaline pretreatment conditions such as, NaOH concentration, treatment time, and temperature for lignin release and controlled carbohydrate solubilization in banana fiber remains comparatively limited.

Therefore, current work focused on developing an FFNN-GA for modelling and optimizing the alkaline pretreatment of banana fiber. OVAT experiments were initially performed to investigate the effects of the selected input parameters on reducing sugar concentration and lignin concentration in the neutralized alkaline filtrate. Further, the experimental data were used to develop and train a FFNN for predicting the two responses, followed by GA optimization to identify suitable pretreatment conditions. The proposed optimized method can be used to support its utilization of lignocellulosic biomass in biorefinery, biocomposite, and bio-based material applications and related industries.

2. Materials and Methods

2.1 Raw Material Collection and Preparation

Banana pseudostem fibers were commercially purchased from online method that was unprocessed fiber, and used as the lignocellulosic raw material for the present study. The collected fibers were thoroughly washed with distilled water to remove dust, and other surface impurities. Further, fibers were initially air dried in hot air oven at 50 °C until a constant weight was achieved. The dried fibers were subsequently stored in airtight box under dry conditions for further studies.

2.2 Chemicals and Reagents

All chemicals used in this study have procured from SRL and Merck for accuracy and repeatability, these are sodium hydroxide (NaOH, ≥98% purity; CAS No. 1310-73-2), glacial acetic acid (CH_3COOH, 99.9% purity, ACS grade; CAS No. 64-19-7), 3,5-dinitrosalicylic acid (DNS, 98% purity; CAS No. 609-99-4), L-cysteine (≥98% purity; CAS No. 52-90-4), concentrated sulfuric acid (H_2SO_4, 95-98% purity; CAS No. 7664-93-9) and distilled water was used throughout the experiments.

2.3 Alkali Pretreatment of Banana Fiber

Alkaline pretreatment of the collected banana fibers was performed using a one-variable-at-a-time (OVAT) approach to investigate the individual effects of process parameters on lignin removal and reducing sugar release. Three critical pretreatment variables, such as, NaOH concentration (X_1), pretreatment time (X_2), and temperature (X_3), were selected for the OVAT experiments. The experimental ranges investigated were 1-6% NaOH, 2-12 h, and 30-80 °C (Table 1) [16]. Which were established based on preliminary experimental observations and previously reported studies on comparable lignocellulosic biomass.

Table 1: Process Parameters and Search Space for Pretreatment Optimization

Variable Code	Variable Name	Lower Bound	Upper Bound
X_1	NaOH concentration (%)	1	6
X_2	Pretreatment time (h)	2	12
X_3	Pretreatment temperature (°C)	30	80

During the OVAT experiments 1.5 g of banana fiber was treated with NaOH solutions of difference concentrations. One process variable was varied while the remaining variables were maintained at fixed levels, allowing the individual effect of each parameter on lignin removal and reducing sugar release to be evaluated. Following alkaline treatment, the pretreated fibers were separated from the alkaline liquor and washed with distilled water until the neutral pH was achieved and alkaline filtrate was subsequently neutralized using 1% (v/v) acetic acid to obtain a suitable pH for subsequent analytical determination. The neutralized filtrate was then used for the estimation of reducing sugar, lignin, HMF, and furfural concentrations [17].

2.4 Quantification of Reducing Sugar, Lignin, HMF, and Furfural

Reducing sugar concentration was determined by the 3, 5-dinitrosalicylic acid (DNS) method, a series of glucose standard solutions of known concentrations was prepared using glucose as the standard, whereas distilled water was used as the blank. For the estimation of reducing sugar in the samples, an appropriate aliquot of the unknown alkaline filtrate was taken. The standards and unknown samples were treated with DNS reagent, mixed thoroughly, and heated in a boiling water bath for 15 min. After heating, the tubes were cooled, and the reaction mixtures were diluted with distilled water. The absorbance was measured at 540 nm using a Hach DR 5000 UV-Vis Spectrophotometer (Refurbished). A standard calibration curve was prepared by plotting

absorbance against the corresponding glucose concentration, and the reducing sugar concentration of the unknown samples was determined from the calibration curve and expressed as mg/mL [19].

Lignin concentration was determined using the cysteine-assisted sulfuric acid (CASA) method. For this an appropriate aliquot of the neutralized alkaline filtrate was reacted with 72% H_2SO_4 containing L-cysteine at 0.1 g/mL and maintained at approximately 24 °C with continuous agitation for 60 min. The reaction mixture was diluted with distilled water, and absorbance was measured at 283 nm against an appropriate reagent blank. Lignin concentration was calculated and expressed as mg/mL of alkaline filtrate [20]. Hydroxymethylfurfural (HMF) was quantified based on its reaction with p-touluidine and barbituric acid, followed by measurement of absorbance at 550 nm [21]. Further, furfural which is an indicative of pentose degradation, was determined using a phenylhydrazine based method, with absorbance measured at 446 nm [22].

2.5 Experimental Design and Dataset Generation

The experimental data set consisted of 32 unique observations from the OVAT experiments as summarized in Table 2. These observations provided the main dataset for FFNN modelling and evaluation. While, the three process variables were input and the reduction sugar concentration and concentration of lignin in the neutralized alkaline filtrate were the response variables. Further, the trained FFNN was then applied to predict the responses for 43 additional combinations in the ranges of the investigated process variables. These observations were calculated computationally and considered as FFNN-predicted data, not experimentally measured. Then, the experimental and predicted observations were combined to form a larger data set of 75 observations (Table 3), which was used for further response analysis and GA optimization based on the FFNN [23].

190 Table 2. Experimental process parameters and measured responses obtained using the OVAT
191 approach

S. No.	NaOH (%)	Time (h)	Temperature (°C)	Reducing sugar (mg/mL)	Lignin (mg/mL)	HMF (mg/mL)	Furfural (mg/mL)
1	1.0	4	40	0.215	0.250	0.002	0.123
2	1.5	4	40	0.213	0.371	0.122	0.218
3	2.0	4	40	0.211	0.482	0.242	0.312
4	2.5	4	40	0.221	0.490	0.247	0.329
5	3.0	4	40	0.231	0.523	0.252	0.345
6	3.5	4	40	0.221	0.567	0.338	0.366
7	4.0	4	40	0.211	0.651	0.424	0.387
8	4.5	4	40	0.333	0.665	0.481	0.376
9	5.0	4	40	0.454	0.675	0.538	0.365
10	5.5	4	40	0.628	0.725	0.570	0.377
11	6.0	4	40	0.801	0.770	0.602	0.389
12	4.0	2	40	0.1625	0.534	0.220	0.220
13	4.0	3	40	0.156	0.471	0.322	0.269
14	4.0	4	40	0.192	0.430	0.424	0.318
15	4.0	5	40	0.212	0.465	0.473	0.343
16	4.0	6	40	0.231	0.523	0.522	0.367
17	4.0	7	40	0.271	0.514	0.526	0.399

18	4.0	8	40	0.310	0.529	0.530	0.430
19	4.0	9	40	0.435	0.556	0.555	0.450
20	4.0	10	40	0.560	0.580	0.580	0.470
21	4.0	11	40	0.628	0.593	0.595	0.490
22	4.0	12	40	0.695	0.609	0.610	0.510
23	4.0	2	30	0.119	0.452	0.160	0.012
24	4.0	2	35	0.162	0.488	0.190	0.019
25	4.0	2	45	0.330	0.564	0.270	0.035
26	4.0	2	50	0.455	0.608	0.320	0.045
27	4.0	2	55	0.485	0.624	0.365	0.054
28	4.0	2	60	0.515	0.627	0.410	0.062
29	4.0	2	65	0.584	0.633	0.435	0.069
30	4.0	2	70	0.652	0.638	0.460	0.075
31	4.0	2	75	0.776	0.640	0.480	0.080
32	4.0	2	80	0.899	0.651	0.500	0.085

192

193 2.6 Artificial Neural Network (ANN) Modelling

194 Among the experimentally measured responses, reducing sugar and lignin concentrations were
195 selected as the principal response variables for ANN modelling and subsequent GA optimization
196 because they directly represented carbohydrate release and delignification, which were the main
197 objectives of the pretreatment process. Whereas, HMF and furfural were also quantified as
198 indicators of carbohydrate degradation and potential inhibitor formation under increasing
199 pretreatment severity. Hence, these compounds were considered supporting analytical responses

rather than primary optimization variables and were not included in the ANN-GA modelling framework.

2.6.1 Quadratic Regression Analysis

Prior to FFNN development, quadratic regression models were fitted to the experimental data to describe the relationships between the three pretreatment variables and the two response variables. The models included linear, interaction, and quadratic terms of NaOH concentration (N), pretreatment time (t), and temperature (T), and for reducing sugar, the fitted regression equation was:

$$Y_{\text{Sugar}} = 0.313085 - 0.279207N + 0.045601t + 0.005251T + 0.011699Nt + 0.000365NT - 0.003496tT + 0.046452N^2 + 0.005876t^2 + 0.000100T^2 \quad (3)$$

The regression model showed R = 0.9759 and R^2 = 0.9525, indicating that approximately 95.25% of the variation in reducing sugar was explained by the fitted model whereas, for lignin, the corresponding regression equation was:

$$Y_{\text{Lignin}} = 0.434926 + 0.029428N - 0.193118t + 0.009323T + 0.028729Nt - 0.003576NT + 0.001153tT + 0.014267N^2 + 0.002618t^2 + 0.000032T^2 \quad (4)$$

The lignin model showed R = 0.9363 and R^2 = 0.8767, while the quadratic models explained 95.25% and 87.67% of the variability in reducing sugar and lignin responses. Following the regression analysis, an FFNN architecture was developed to model the nonlinear relationships between the three process variables and the two responses.

2.6.2 FFNN Architecture and Model Development

A feed-forward artificial neural network (FFNN) was developed using the Neural Network Toolbox in MATLAB R2024a (The MathWorks Inc., Natick, MA, USA). The final network consisted of three input neurons, 15 hidden neurons, and two output neurons (3-15-2) (Fig. 1), and the network was trained using the Levenberg-Marquardt back-propagation algorithm (trainlm), with mean squared error (MSE) as the performance function. The trained network weights and biases for the hidden and output layers are mentioned in Table 4 and 5, respectively.

Table 4. Final connection weights and biases of the hidden layer of the developed 3-15-2 FFNN

Hidden neuron	NaOH (W1)	Time (W2)	Temperature (W3)	Hidden bias
H1	-0.5730	0.9381	-3.0966	3.5944
H2	1.9414	-1.3170	-1.3271	-2.7038
H3	-3.6633	-0.2386	2.0846	2.6812
H4	-1.5680	-2.9450	0.3957	1.6842
H5	-2.5848	-1.9524	1.1946	1.7100
H6	-2.9918	1.4349	-1.6167	1.3431
H7	-1.5017	2.9192	0.7535	0.7491
H8	-1.1474	-1.5931	2.7708	-0.0589
H9	-0.8413	1.3596	-3.0139	-0.6547
H10	0.1382	2.8865	1.6522	0.9408
H11	-0.4249	2.1337	2.6291	-1.5903
H12	1.5948	-2.4593	1.6638	1.8835
H13	-1.8531	-2.5788	-1.2628	-2.4711
H14	1.7220	-2.1613	1.6610	3.1552

H15	-2.4649	1.7049	-2.1263	-3.0540

Table 5. Final connection weights and biases of the output layer of the developed 3-15-2 FFNN

Output neuron	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12	H13	H14	H15	Output bias
Reducing sugar	0.37302	0.32422	0.25333	-0.01011	-0.47344	-0.21867	-0.26655	-0.21411	-0.09655	-0.02655	-0.28881	-0.15455	0.01136	-0.14631	-0.08211	0.5477
Lignin	0.00070	-0.32111	-0.28522	-0.40888	-0.09466	-0.05933	-0.08711	-0.02222	-0.10033	-0.06069	0.31334	0.4015	0.0287	-0.36623	-0.44123	0.3477

Where H1-H15 represent the neurons of the hidden layer, and the corresponding weights represent connections between the hidden and output layers.

2.6.3 FFNN Performance Evaluation

The FFNN was evaluated using the training, validation, and testing datasets generated during network training, however, model performance was assessed using the correlation coefficient (R) between target and predicted values. Mean squared error (MSE) was used to monitor network performance during training, while the error histogram was used to assess the distribution of prediction errors.

2.6.4 Genetic Algorithm (GA) Optimization

GA optimization was performed using the trained FFNN model in the Global Optimization Toolbox of MATLAB R2024a (The MathWorks Inc., Natick, MA, USA). The GA searched within the predefined ranges of 1-6% NaOH, 2-12 h pretreatment time, and 30-80 °C temperature to minimize reducing sugar concentration while maximizing lignin concentration in the alkaline filtrate and the fitness function was defined as equation 5:

$$F = 3Y_1 - Y_2 \quad (5)$$

where Y_1 is the FFNN-predicted reducing sugar concentration and Y_2 is the FFNN-predicted lignin concentration in the alkaline filtrate. The GA was operated with a population size of 50, crossover fraction of 0.8, mutation probability of 0.01, and a maximum of 500 generations and five independent GA runs were performed to assess the consistency of the predicted solution [24], further experiments were performed to experimentally validate the GA optimized conditions.

2.7 Characterization of Experimentally Optimized Banana Fiber

The experimentally optimized banana fiber was characterized to evaluate the morphological, functional, mechanical, and structural changes caused by alkaline pretreatment. The cross-

sectional morphology of untreated and alkali-pretreated banana fibers was examined using optical microscopy to observe changes in the cellular structure. The surface morphology was further examined using a JEOL JSM-IT800 Field Emission Scanning Electron Microscope (FE-SEM). The tensile strength of untreated and experimentally optimized banana fibers was measured using a Universal Testing Machine (UTM) (10 kN capacity, Tinius Olsen, USA) to evaluate the effect of alkaline pretreatment on the mechanical properties of the fibers. Fourier-transform infrared (FTIR) spectroscopy was performed using a Shimadzu IRSpirit Fourier Transform Infrared Spectrophotometer to identify changes in the major functional groups of the banana fiber following alkaline pretreatment.

Additionally, X-ray diffraction (XRD) analysis was performed to evaluate changes in the crystalline structure of the banana fiber before and after alkaline pretreatment. The analysis was carried out using the Rigaku Smart Lab 3 kW system (RIGAKU3), to determine the changes in the crystalline characteristics of the treated fiber.

3. Result and Discussion

3.1 Experimental and FFNN-Predicted Response Behavior

The experimental results obtained by varying NaOH concentration, pretreatment time, and temperature showed that reducing sugar and lignin concentrations in the alkali filtrate didn't change proportionally with increasing pretreatment severity, indicating nonlinear relationship between the process variables and the responses. For example, increasing the temperature from 30 to 80 °C increased reducing sugar from 0.119 to 0.899 mg/mL and lignin from 0.452 to 0.651 mg/mL, whereas increasing treatment time from 2 to 12 h increased reducing sugar from 0.1625 to 0.695 mg/mL but caused only a smaller change in lignin (0.534-0.609 mg/mL) (Table 2). These

differences in response behavior indicates the nonlinear relationships and support the use of FFNN modelling.

The trained FFNN was then used to predict responses for additional combinations of the process variables within the defined experimental range whereas, the correlation analysis showed close agreement between the target and FFNN-predicted responses, with overall correlation coefficients of 0.99137 for lignin (Fig. 2) and 0.99864 (Fig. 3) for reducing sugar. The high correlation coefficients indicate that the FFNN was able to satisfactorily represent the observed response patterns. The stronger correlation obtained for reducing sugar was consistent with the relatively systematic variation of this response under the different experimental conditions. Similarly, Parkhey et al. (2020) [25] reported that ANN can be effectively used for nonlinear modelling of lignocellulosic pretreatment and also found that ANN accurately predicted pretreatment and saccharification responses of rice straw, with high predictive correlations during the training, validation, and testing stages.

3.1.1 Effect of NaOH Concentration, Pretreatment Time, and Temperature on Lignin and Reducing Sugar

Experimentally, increasing the NaOH concentration from 1 to 6% increased the lignin concentration in the alkali filtrate from 0.250 to 0.770 mg/mL at 4 h and 40 °C. Whereas, reducing sugar remained relatively constant between 1 and 4% NaOH (0.215-0.211 mg/mL) but increased sharply to 0.801 mg/mL at 6% NaOH (Table 2). This increase in lignin concentration can be attributed to enhanced lignin solubilization through disruption of lignin-carbohydrate linkages. At higher NaOH concentration, the greater disruption of the lignocellulosic matrix also promotes carbohydrate solubilization, resulting in increased reducing sugar release. Similarly, Xu et al.

297 (2020) [26] reported that alkaline pretreatment facilitates lignin modification and its separation
298 from carbohydrate fractions.

299 A similar was observed with increasing NaOH concentration and treatment duration in the
300 study by kaithvar et al. (2025) [27] on *Tinospora cordifolia* aerial root fiber. Increasing NaOH
301 concentration and treatment duration increased reducing sugar concentration from 0.013 to 0.080
302 mg/mL, while lignin concentration in the alkali filtrate also increased from 0.361 mg/mL under
303 mild treatment to 1.23 mg/mL at 4% NaOH and 12 h. Moreover, HMF and furfural reached 2.1
304 and 5.0 mg/mL, respectively, at 4% NaOH at 60 °C.

305 Furthermore, increasing the pretreatment time from 2 to 12 h at 4% NaOH and 40°C
306 increased reducing sugar from 0.1625 to 0.695 mg/mL, whereas lignin changed from 0.534 to
307 0.609 mg/mL (Table 2) after an initial decrease up to 4 h. Thus, treatment time had a greater effect
308 on reducing sugar than on lignin release. Similarly, temperature had a stronger effect on both
309 responses, increasing the temperature from 30 to 80 °C at 4% NaOH and 2 h increased reducing
310 sugar from 0.119 to 0.899 mg/mL and lignin from 0.452 to 0.651 mg/mL. Liu et al. (2021) [28]
311 also reported that pretreatment of banana pseudostem improved cellulose accessibility and altered
312 the lignin and hemicellulose fractions. Therefore, the different responses observed with NaOH
313 concentration, treatment time, and temperature indicate that lignin release and reducing sugar
314 formation do not increase at the same rate. Hence, both responses were considered together in the
315 subsequent FFNN modelling and optimization.

316 Furthermore, HMF and furfural were also measured to monitor carbohydrate degradation
317 during pretreatment. Increasing the NaOH concentration from 1 to 6% at 4 h and 40 °C increased
318 HMF from 0.002 to 0.602 mg/mL and furfural from 0.123 to 0.389 mg/mL. Likewise, increasing

the temperature (30 to 80 °C), increased HMF from 0.160 to 0.500 mg/mL and furfural from 0.012 to 0.085 mg/mL. With increasing treatment time from 2 to 12 h at 4% NaOH and 40 °C, HMF increased from 0.220 to 0.610 mg/mL, while furfural increased from 0.220 to 0.510 mg/mL (Table 2). Thus, HMF and furfural increased with the pretreatment conditions and were used as supporting indicators of carbohydrate degradation, whereas reducing sugar and lignin were selected as the principal responses for FFNN-GA optimization.

3.2 FFNN Model Performance

A FFNN model was developed to describe the relationship between the three pretreatment variables and the two responses based on the nonlinear behavior observed in the experiments. The final network consisted of three input neurons, 15 hidden neurons, and two output neurons, resulting in a 3-15-2 architecture (Fig. 1). The network was trained using the Levenberg-Marquardt back-propagation algorithm (trainlm).

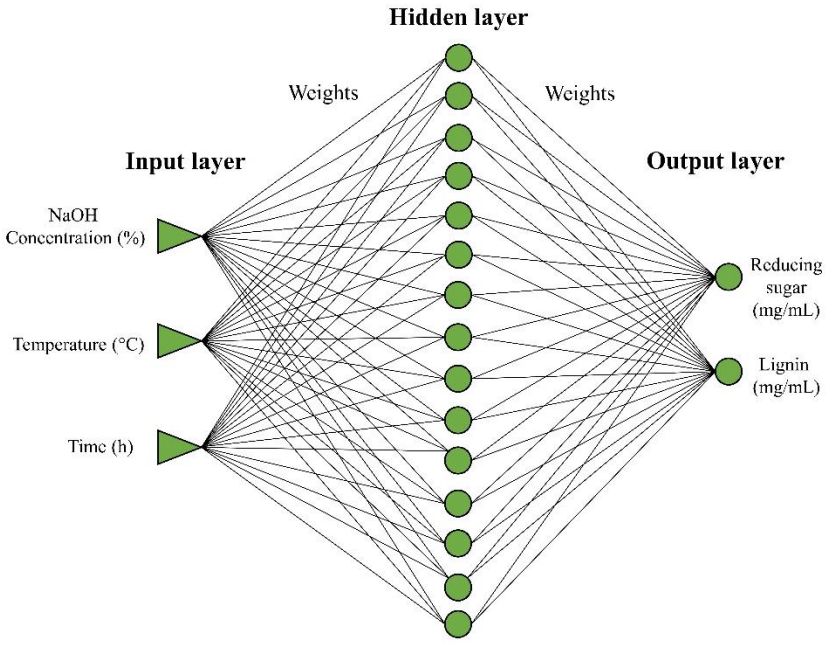


Fig. 1. Schematic representation of the developed 3-15-2 FFNN showing the three input variables, 15 hidden neurons, two output responses, and the interconnection weights between the network layers.

The FFNN showed good agreement between the target and predicted values for both responses. For lignin, the correlation coefficients for the training, validation, testing, and overall datasets were 0.99120, 0.99586, 0.99121, and 0.99137 (Fig. 2), and the corresponding values for reducing sugar were 0.99914, 0.99830, 0.99706, and 0.99864 (Fig. 3).

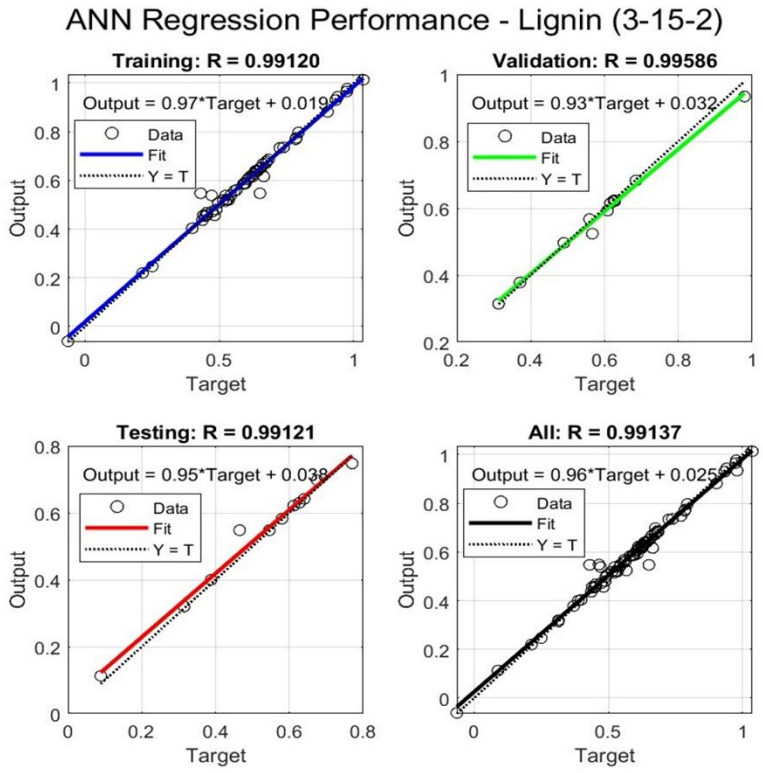


Fig. 2. Correlation plots between target and FFNN-predicted values for lignin during training, validation, testing, and overall datasets.

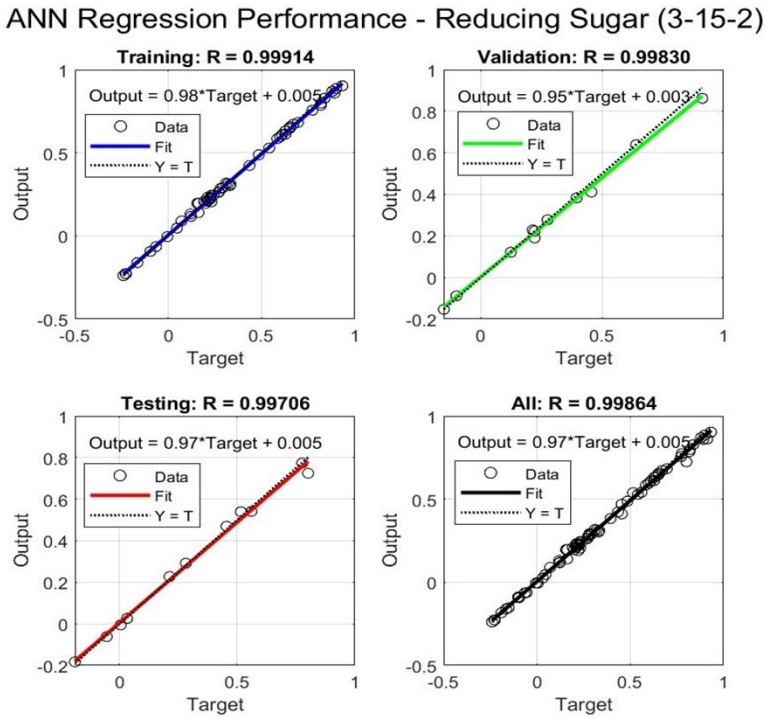


Fig. 3. Correlation plots between target and FFNN-predicted values for reducing sugar during training, validation, testing, and overall datasets.

The correlation plots also show the close agreement between the targets and predicted reducing sugar values across the different datasets. These consistently high R values indicate that the FFNN was able to capture the nonlinear behavior of the two responses. The slightly lower R values for lignin may be related to the greater variation observed in the experimental lignin values. Similar results were reported by Moodley et al. (2019) [29], who used ANN modelling to predict sugar yields from inorganic salt-based pretreatment and obtained R^2 values above 0.97. Likewise, Luo et al. (2021) [30] showed that ANN modelling could effectively predict multiple responses during lignocellulosic pretreatment.

Based on this good predictive performance, the trained network was further evaluated using the MSE performance plot (Fig. 4) and error histogram (Fig. 5). The minimum validation MSE was 0.000470021 at epoch 3, after which no clear improvement in validation performance was observed. The training, validation, and testing errors decreased during the initial epochs and then gradually stabilized.

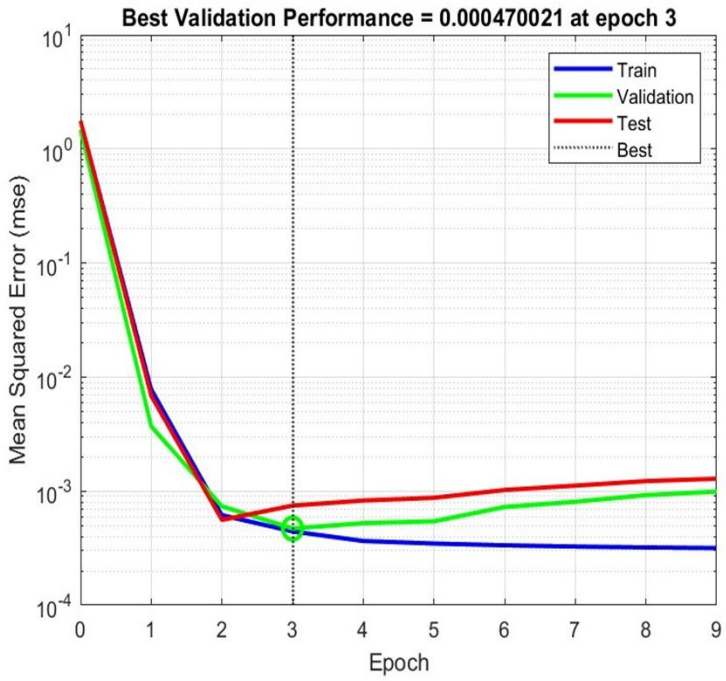


Fig. 4. MSE performance plot of the FFNN showing training, validation, and testing errors and the best validation performance

The error histogram showed that most prediction errors were concentrated around zero, with fewer errors at larger positive or negative values, indicating that the predicted values were generally close to the target values. Following this evaluation, the final connection weights and biases obtained after FFNN training are presented in Tables 4 and 5. These parameters define the learned relationship between the three pretreatment variables and the two responses and were used for

subsequent prediction and GA-based optimization. The different magnitudes and signs of the weights reflect the nonlinear relationships learned by the network and are not intended as a direct ranking of input-variable importance.

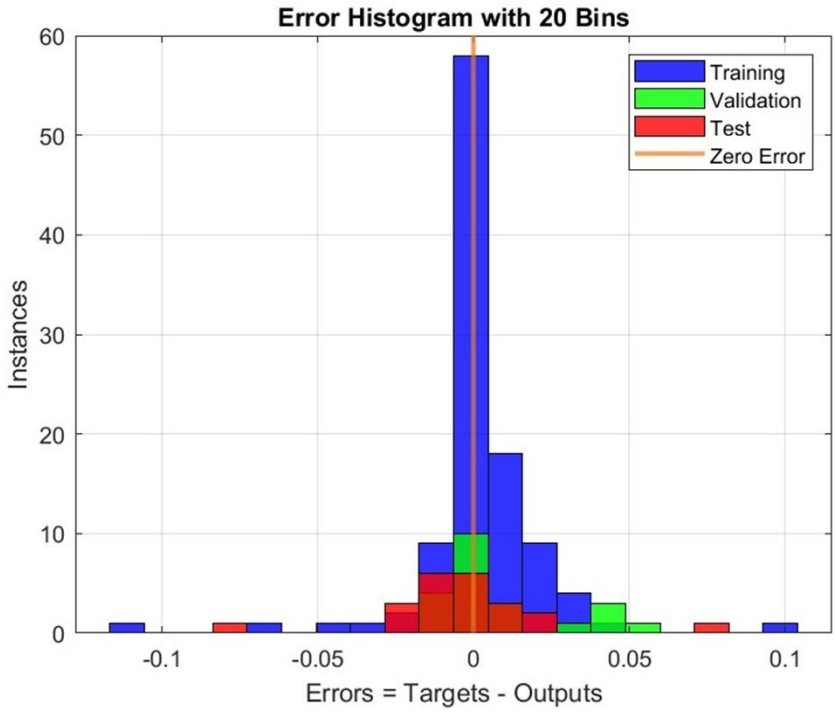


Fig. 5. Error histogram of the FFNN showing the distribution of prediction errors for the training, validation, and testing datasets.

3.3 GA Optimization and Response Surface Analysis

The trained FFNN was next coupled with a genetic algorithm (GA) to identify pretreatment conditions that minimized reducing sugar release while maximizing lignin release within the investigated ranges of 1-6% NaOH, 2-12 h, and 30-80 °C. The fitness function was defined as $F = 3Y_1 - Y_2$, where Y_1 and Y_2 represent the FFNN-predicted reducing sugar and lignin responses, therefore, lower fitness values favored lower reducing sugar and higher lignin release. The GA was run with a population size of 50, a crossover fraction of 0.8, a mutation probability of 0.01,

411 and a maximum of 500 generations. Five independent runs were performed to assess the
412 consistency of the predicted optimum.

413 Further, the five GA runs produced very similar results (Table 6), with temperature ranging
414 from 71.144 to 71.167 °C, treatment time from 11.097 to 11.100 h, and NaOH concentration from
415 3.4236 to 3.4238%. The predicted reducing sugar values ranged from -0.24029 to -0.24021
416 mg/mL, while lignin ranged from 1.0364 to 1.0367 mg/mL, with a fitness value of approximately
417 -1.7573 across all runs. This close agreement indicates that the GA consistently converged towards
418 the same region of the FFNN-predicted response space, indicating good stability of the
419 optimization process. Accordingly, the representative model-derived optimum was identified as
420 3.42% NaOH, 11.10 h, and 71.15 °C. At these conditions, the FFNN predicted a lignin
421 concentration of 1.0367 mg/mL and a reducing sugar concentration of -0.2402 mg/mL. The
422 negative reducing sugar value is a mathematical output of the FFNN and doesn't represent a
423 physically measurable concentration.

424 Table 6. GA optimization results from five independent runs for the simultaneous optimization of
425 reducing sugar and lignin responses.

GA Run	NaOH concentration (%)	Pretreatment time (h)	Temperature (°C)	Predicted reducing sugar (mg/mL)	Predicted lignin (mg/mL)	Fitness value
1st	3.4236	11.100	71.167	-0.24021	1.0367	-1.7573
2nd	3.4236	11.099	71.164	-0.24024	1.0366	-1.7573
3rd	3.4238	11.098	71.153	-0.24027	1.0365	-1.7573

4th	3.4237	11.098	71.153	-0.24027	1.0365	-1.7573
5th	3.4238	11.097	71.144	-0.24029	1.0364	-1.7573

The optimized conditions were experimentally evaluated using banana fiber. The experimental lignin concentration was 1.05 mg/mL, which was close to the predicted value, indicating good agreement. The experimentally obtained reducing sugar concentration was 0.15 mg/mL, indicating limited sugar degradation and thereby helping to preserve the cellulose-rich fraction for further modification and applications. Thus, the optimized pretreatment effectively promoted lignin removal while minimizing cellulose loss and inhibitor formation. The optimized banana fiber was subsequently selected for FE-SEM, FTIR, and XRD characterization.

3.4 GA convergence

The GA convergence behavior over the first 150 generation indicates that during the initial generations, the best fitness value decreased rapidly from approximately -1.48 to -1.70, indicating progressive improvement in the objective function as the algorithm explored different candidate solutions (Fig. 6). The mean fitness showed a similar decreasing trend, although with some fluctuations, indicating continued exploration of the candidate solutions. As the optimization progressed, the best fitness approached approximately -1.75, and the improvement between successive generations gradually became decreased. The best-fitness curve then became nearly constant, while small fluctuations in the mean fitness continued. Finally, the best and mean fitness values reached approximately -1.7573, indicating that the optimization had converged to a stable region of the objective-function space.

The optimization converged before reaching the maximum limit of 500 generations set for the GA, and therefore, Fig. 6 presents the convergence history over 0-150 generations to show the region where the fitness values approached a stable solution. The close final values of the best and mean fitness further indicate that the population converged towards similar candidate solutions.

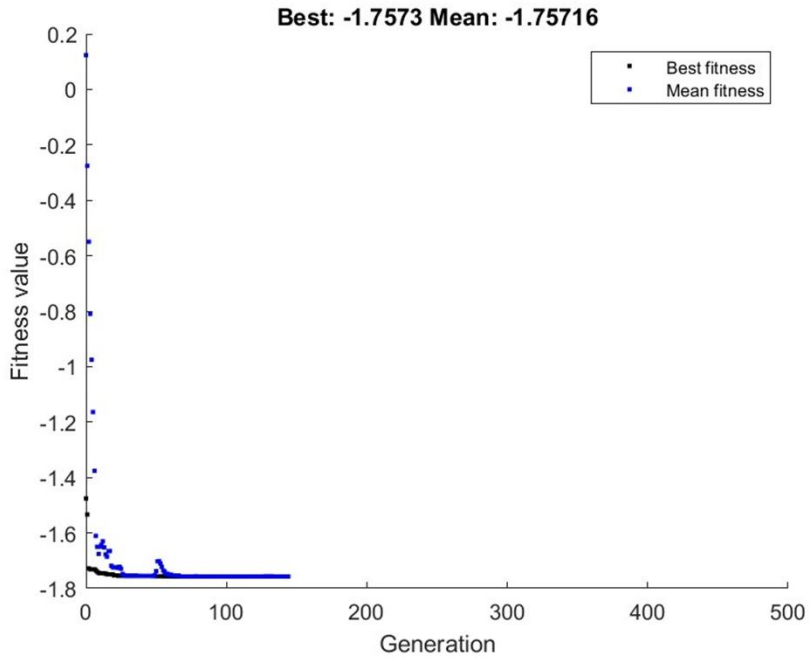


Fig. 6. Convergence behavior of the genetic algorithm showing the best and mean fitness values over successive generations.

The consistent convergence of the ANN-GA approach is supported by previous studies, in which ANN was combined with GA to optimize xylitol production in a bioreactor [31], while Shi et al. (2022) [32] applied a BPNN-GA framework to optimize aerobic composting of *Ganoderma lucidum* residue. Similarly, Haldar et al. (2023) [33] developed regression and ANN models to predict sugar yields from lignocellulosic pretreatment and enzymatic hydrolysis, with the reduced-interaction regression model achieving an R^2 of 0.891 and an adjusted R^2 of 0.849, while the GA-optimized ANN showed a strong fit with the experimental data. These findings, together with the

present results, support the application of ANN-GA approaches for modelling and optimizing nonlinear biomass-conversion processes.

3.5 Response surface and contour analysis

To further examine the combined effects of the pretreatment variables, FFNN-predicted three-dimensional response surfaces and contour maps were generated for reducing sugar and lignin concentrations (Figs. 7-10). The plots represent the pairwise interactions among NaOH concentration, pretreatment time, and temperature, with the third variable maintained at its reference level.

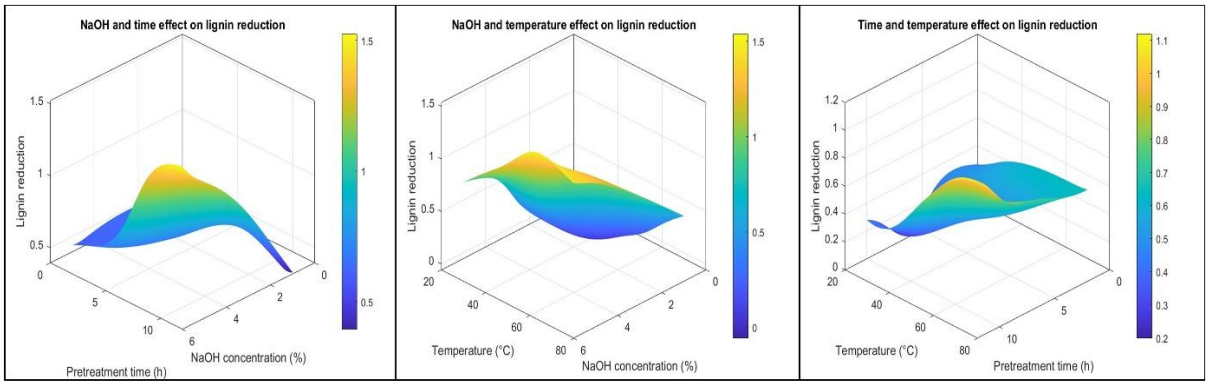


Fig. 7. FFNN-predicted response surfaces showing the effects of NaOH concentration, pretreatment time, and temperature on lignin concentration in the alkaline filtrate.

For reducing sugar, the response generally increased with increasing pretreatment severity (Fig. 8), consistent with the OVAT results. Experimentally, reducing sugar ranged from 0.119 to 0.899 mg/mL as temperature increased from 30 to 80 °C and from 0.1625 to 0.695 mg/mL as treatment time increased from 2 to 12 h, accordingly, the FFNN described the reducing sugar response with an overall R of 0.99864, with a similar trend observed in the corresponding contour map (Fig. 9).

Whereas, the lignin response showed a different pattern (Fig. 7), with experimental values ranging from 0.250 to 0.770 mg/mL. Among the pretreatment variables, NaOH concentration produced the greatest change in lignin release, whereas treatment time had a comparatively smaller effect. Furthermore, increasing the temperature from 30 to 80 °C increased the lignin concentration from 0.452 to 0.651 mg/mL.

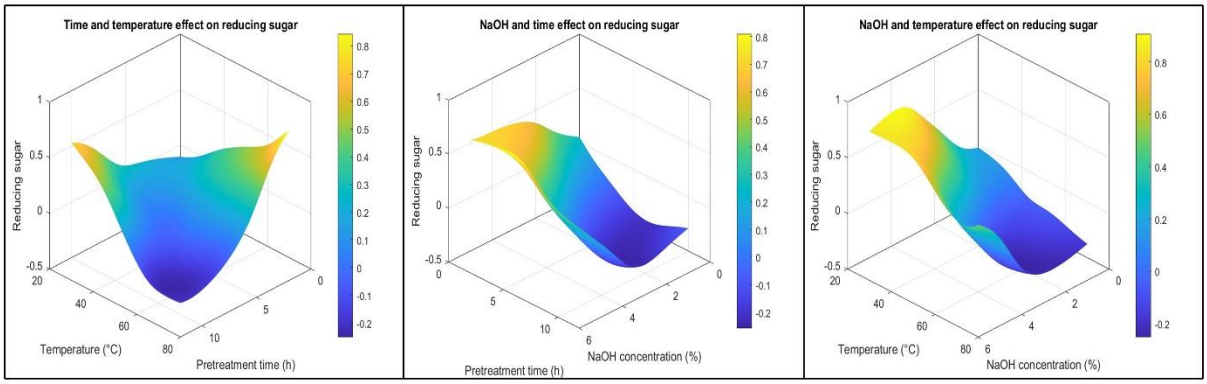


Fig. 8. FFNN-predicted response surfaces showing the effects of NaOH concentration, pretreatment time, and temperature on reducing sugar in the alkaline filtrate.

The FFNN described the lignin response with an overall R of 0.99137, while the contour map (Fig. 10) indicated that higher lignin release was not always associated with lower reducing sugar production.

Therefore, the response surfaces indicate a trade-off between lignin solubilization and carbohydrate release during alkaline pretreatment. From a biorefinery perspective, the resulting fractions may also have potential downstream value; however, commercial prices vary with purity, grade, processing, and application. For comparison, industrial lignin powder is listed at approximately ₹83/kg for a minimum order of 25 kg [3], whereas beechwood xylan for biochemical applications is listed at approximately ₹6200 per 25 g [35]. These prices are provided

only as commercial references and can't be directly assigned to the fractions obtained in the present study, as the measured lignin and reducing sugar represent recovered fractions rather than purified commercial products. Their actual economic value depend on recovery, purification, separation, processing costs, and production scale.

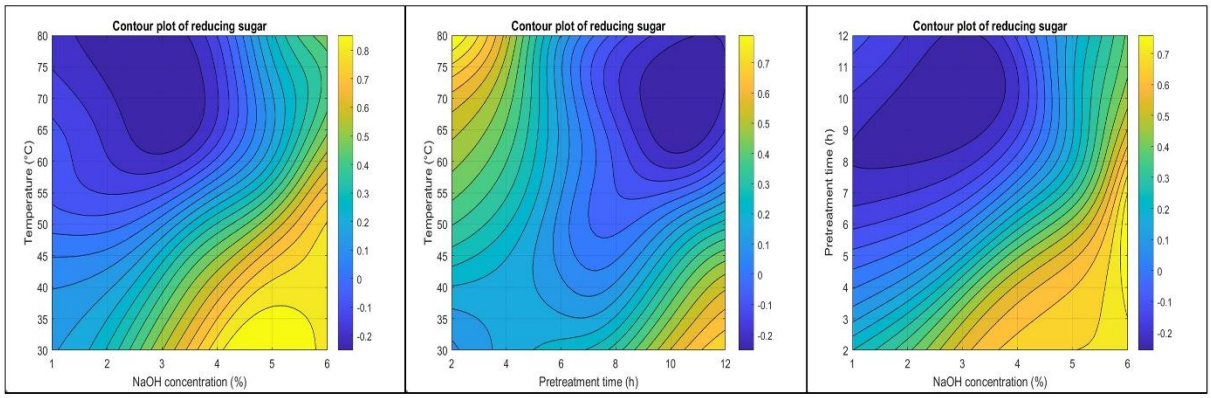


Fig. 9. FFNN-derived contour maps illustrating the predicted variation in reducing sugar concentration across the investigated pretreatment conditions.

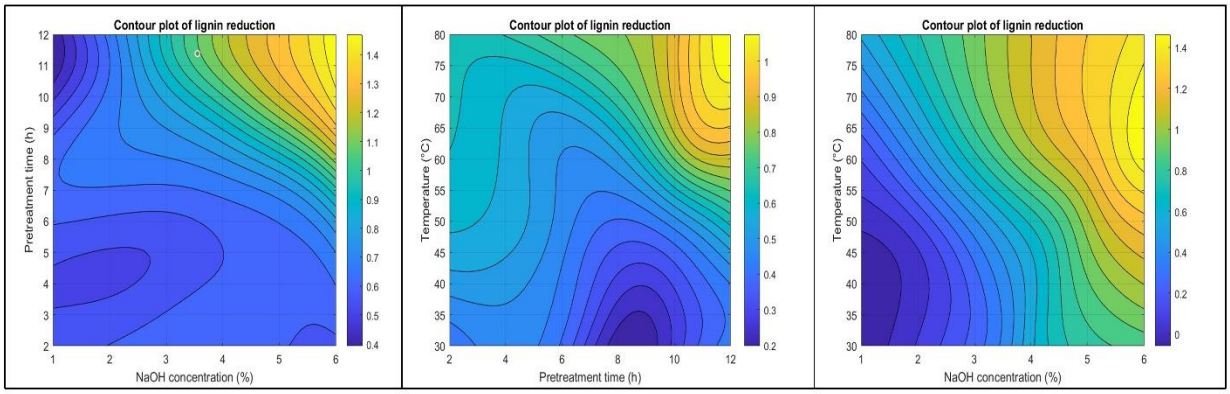


Fig. 10. FFNN-derived contour maps illustrating the predicted variation in lignin concentration across the investigated pretreatment conditions.

3.6 Characterization of Optimized Banana Fiber

Optical microscopy showed clear differences in the cross-sectional morphology of the untreated and alkali pretreated banana fibers (Fig. 11). The untreated fiber was found a relatively compact cellular structure, whereas the pretreated fiber showed more prominent cellular lumens and a comparatively loosened structure. This change resulted from the disruption and partial removal of non-cellulosic components during alkaline treatment, which exposed the internal cellular structure.

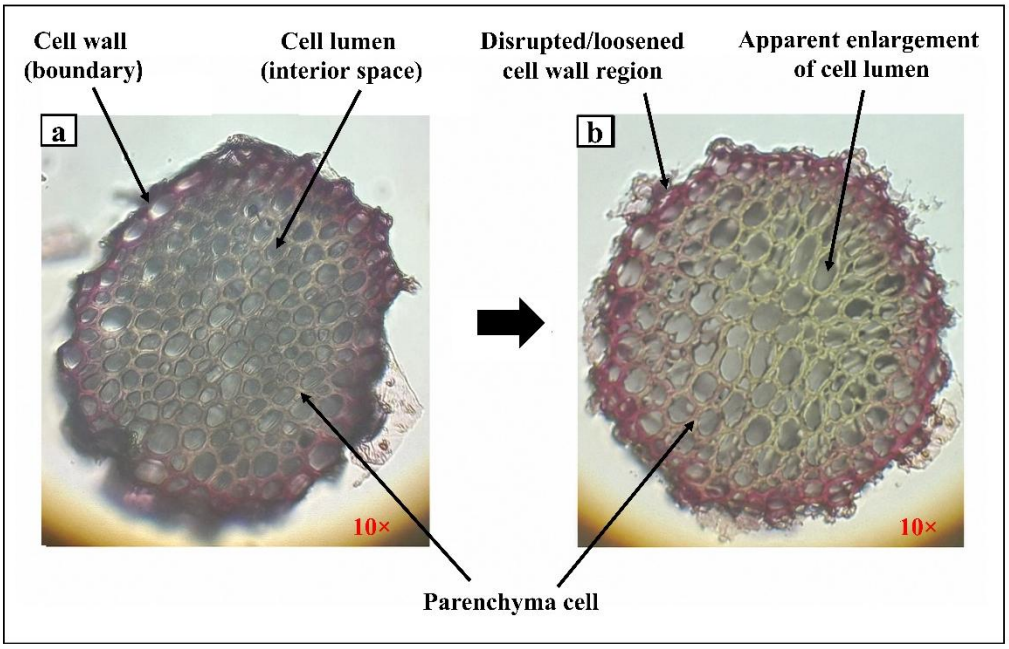


Fig. 11. Cross-sectional microscopic morphology of (a) untreated and (b) alkali-pretreated banana fiber, showing the cell wall, cellular lumen, parenchyma cells, and apparent structural changes following pretreatment. Images were obtained using a 10× objective lens.

Similarly, FE-SEM images showed a rough and irregular surface in the untreated fiber, whereas the pretreated fiber exhibited a comparatively smoother and more uniform surface with clearer fibrillar features (Fig. 12). The rough surface of the untreated fiber was associated with surface

deposits and non-cellulosic components, while their partial removal during alkaline treatment resulted in a smoother surface and clearer fibrillar features.

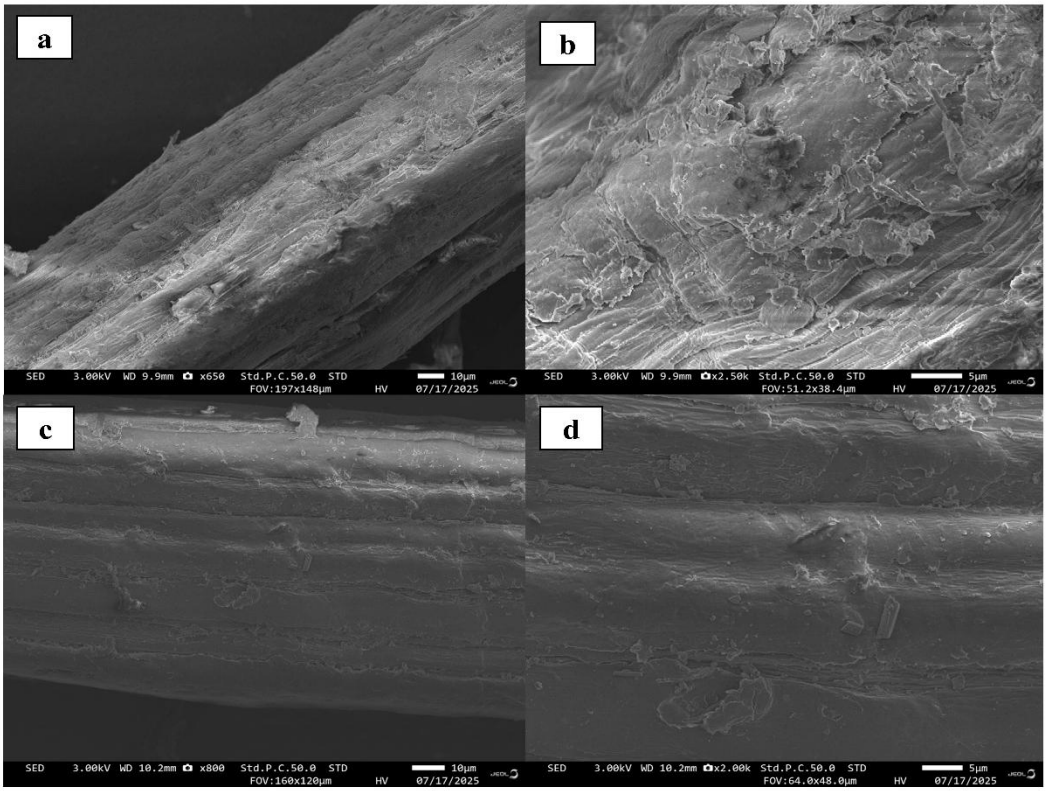


Fig. 12. FE-SEM images of (a, b) untreated banana fiber and (c, d) GA-optimized banana fiber treated with 3.42% NaOH for 11.10 h at 71.15 °C. Scale bars: 10 µm in (a, c) and 5 µm in (b, d).

Further the tensile strength of the untreated and optimized fibers was recorded 350±3 MPa and 400±3 MPa. The higher strength after alkaline treatment may be the removal of lignin and hemicellulose, which exposed the cellulose-rich structure and improved fiber strength. FTIR analysis showed changes in the characteristic absorption bands of untreated banana fiber and alkali treated banana fiber after alkaline pretreatment (Fig. 13). The bands around 3300 and 2920 cm^{-1} were related to O-H and C-H stretching. Changes around 1730 and 1510 cm^{-1} indicated

modification of carbonyl and lignin-related groups after treatment. The band around 1050 cm^{-1} was associated with C-O/C-O-C stretching of the cellulose-rich structure. Therefore, the changes in the FTIR bands confirmed modification of the fiber components after alkaline pretreatment.

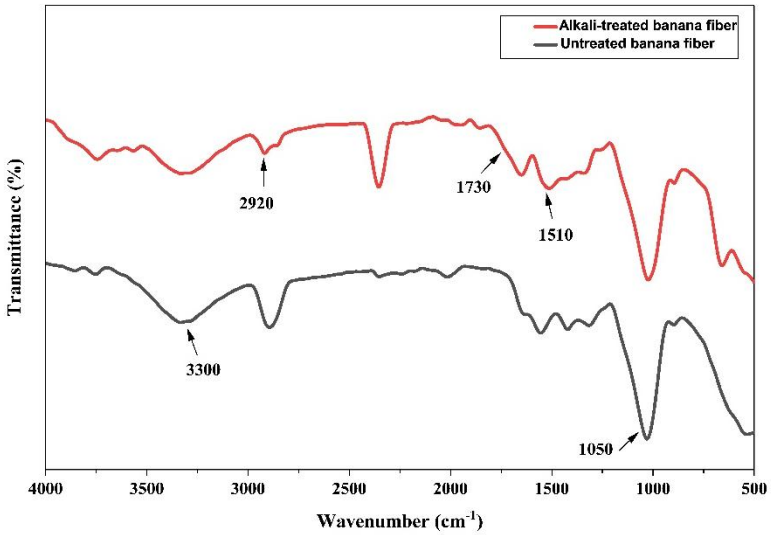


Fig. 13. FTIR spectra of untreated banana fiber (UTBF) and alkali-treated banana fiber (ATBF).

Whereas, XRD analysis also showed changes in the crystalline structure of the fiber after alkaline treatment (Fig. 14).

The main diffraction peaks were observed at approximately 15.2°, 16.5°, and 22.4°, which are characteristic of cellulose. The changes in the XRD pattern indicate modification of the fiber structure after treatment. The increased contribution of the crystalline region is associated with the removal of amorphous non-cellulosic components during alkaline pretreatment.

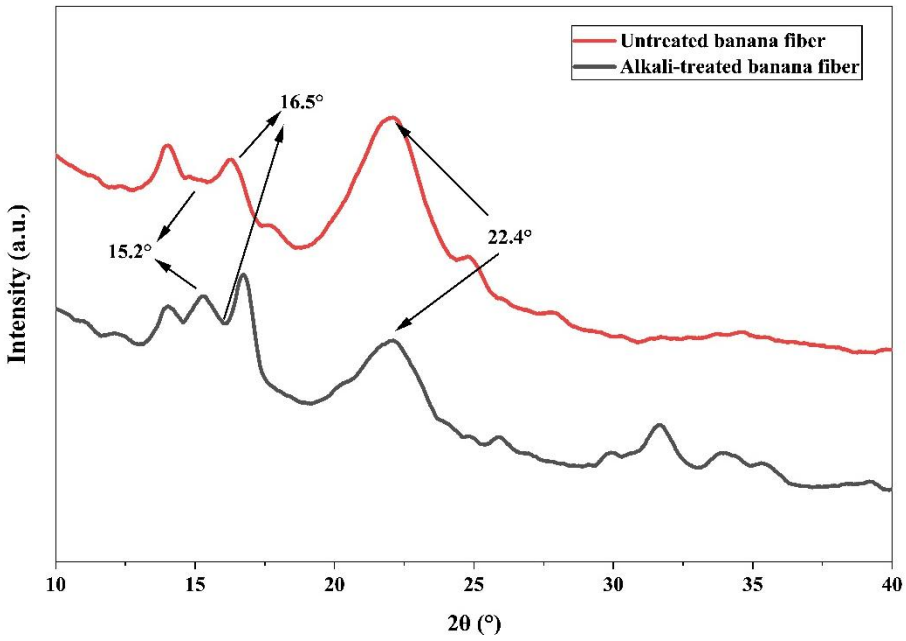


Fig. 14. XRD patterns of untreated banana fiber (UTBF) and alkali-treated banana fiber (ATBF), showing the characteristic diffraction peaks at approximately 15.2°, 16.5°, and 22.4°.

4. Conclusion

The study demonstrated that NaOH concentration, pretreatment duration, and temperature jointly influenced lignin solubilization and carbohydrate release during the alkaline pretreatment of banana fiber. Based on these responses, the FFNN-GA framework identified an optimized condition of approximately 3.42% NaOH, 11.10 h, and 71.15 °C. At these conditions, optimum release of lignin and minimum loss of sugar could be achieved, with lignin converted into HMF and furfural, while keeping the cellulose fiber intact and homogeneous, with high tensile strength and high crystallinity (based on FE-SEM, FTIR, and XRD analysis). The optimized condition resulted in 65.80 mg/g lignin and 9.40 mg/g reducing sugar released per gram of initial fiber, showing good agreement between the predicted and experimental responses. The optimized treatment resulted in a final fiber weight of 1.24 g from an initial 1.5 g, corresponding to 17.33%

591 weight loss. The entire process developed for the pretreatment of banana pseudostem resulted in
592 lignin and reducing sugar as byproducts. Making the process potentially sustainable and
593 economical. Lignin has demand as bulk chemical in industry and pretreated liquor with high sugar
594 and other nutrients considered as suitable low-priced media for biomanufacturing of many other
595 value-added products such as antibiotics, algal biomass, bioethanol etc. Cellulose fiber has
596 immense applications in various industries like pharmaceuticals, textile, biofuel, biomaterial,
597 fabrication of membranes etc. Hence, current study is highly impactful in the area of process
598 optimization for sustainable product recovery from agro-wastes.

599

600

601 List of Abbreviations

602 ANN: Artificial Neural Network

603 FFNN: Feed-Forward Neural Network

604 GA: Genetic Algorithm

605 OVAT: One-Variable-at-a-Time

606 NaOH: Sodium Hydroxide

607 DNS: 3, 5-Dinitrosalicylic Acid

608 CASA: Cysteine-Assisted Sulfuric Acid

609 H_2SO_4: Sulfuric Acid

610 MSE: Mean Squared Error

R: Correlation Coefficient

R^2: Coefficient of Determination

HMF: 5-Hydroxymethylfurfural

MATLAB: Matrix Laboratory

Acknowledgements

The authors are thankful to the Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, for providing financial support, and to Motilal Nehru National Institute of Technology (MNNIT), Allahabad, for providing the space and basic facilities to carry out the research work.

Author contributions

The study was conceptualized by Prof. Sangeeta Negi, and all experiments were conducted by Ms. Priya Kaithvar under the supervision of Prof. Sangeeta Negi. All authors contributed to reviewing and finalizing the manuscript.

Funding

The authors are thankful to Council of Scientific and Industrial Research (CSIR), Government of India, New Delhi, for providing the financial support for the experimental work.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest.

Author details

Department of Biotechnology, Motilal Nehru National Institute of Technology Allahabad, Prayagraj, Uttar Pradesh, India[1*]

Corresponding author: Sangeeta Negi

References

1. Badanayak P, Jose S, Bose G. Banana pseudostem fiber: A critical review on fiber extraction, characterization, and surface modification. J Nat Fibers. 2023;20(1):2168821. https://doi.org/10.1080/15440478.2023.2168821.
2. Castañeda Niño JP, Mina Hernandez JH, Valadez González A. Potential uses of Musaceae wastes: Case of application in the development of bio-based composites. Polymers. 2021;13(11):1844. https://doi.org/10.3390/polym13111844.
3. Arora A, Nandal P, Singh J, Verma ML. Nanobiotechnological advancements in lignocellulosic biomass pretreatment. Mater Sci Energy Technol. 2020;3:308–318. https://doi.org/10.1016/j.mset.2019.12.003.
4. Joshi M, Manjare S. Chemical approaches for the biomass valorisation: A comprehensive review of pretreatment strategies. Environ Sci Pollut Res. 2024;31(36):48928–48954. https://doi.org/10.1007/s11356-024-34473-6.
5. Jamaldheen SB, Kurade MB, Basak B, Yoo CG, Oh KK, Jeon B, et al. A review on physico-chemical delignification as a pretreatment of lignocellulosic biomass for enhanced bioconversion. Bioresour Technol. 2022;346:126591. https://doi.org/10.1016/j.biortech.2021.126591.
6. Negi S, Singh SP, Das S. Exploring hostel kitchen waste as feedstock for bioethanol and biogas production in a biorefinery setup. Mater Today Proc. 2023;106:144–152. https://doi.org/10.1016/j.matpr.2023.08.379.
7. Jain S, Kumar S. Advances and challenges in pretreatment technologies for bioethanol production: A comprehensive review. Sustain Chem Clim Action. 2024;5:100053. https://doi.org/10.1016/j.scca.2024.100053.

8. Anukam A, Berghel J. Biomass pretreatment and characterization: A review. In: IntechOpen eBooks. 2020. https://doi.org/10.5772/intechopen.93607.
9. Jeong S, Lee J. Optimization of pretreatment condition for ethanol production from oxalic acid pretreated biomass by response surface methodology. Ind Crops Prod. 2016;79:1–6. https://doi.org/10.1016/j.indcrop.2015.10.036.
10. Canam T, Town J, Iroba K, Tabil L, Dumonceaux T. Pretreatment of lignocellulosic biomass using microorganisms: Approaches, advantages, and limitations. In: InTech eBooks. 2013. https://doi.org/10.5772/55088.
11. Chen J, Ma X, Liang M, Guo Z, Cai Y, Zhu C, Wang Z, Wang S, Xu J, Ying H. Physical–chemical–biological pretreatment for biomass degradation and industrial applications: A review. Waste. 2024;2(4):451–473. https://doi.org/10.3390/waste2040024.
12. Chittibabu S, Saseetharan MK, Kalaivani MR, Rajesh MP. Optimization of microwave-assisted alkali pretreatment and enzymatic hydrolysis of banana pseudostem. Energy Sources A Recovery Util Environ Eff. 2014;36(24):2691–2698. https://doi.org/10.1080/15567036.2011.574193.
13. Namboonlue S, Ngowsakul K, Nakarat K, Kongsinkaew C, Subjalearndee N, Uttayopas P, Charoenrat T, Laemthong T. Predictive reducing sugar release from lignocellulosic biomass using sequential acid pretreatment and enzymatic hydrolysis by harnessing a machine learning approach. Comput Struct Biotechnol J. 2025;27:4246–4256. https://doi.org/10.1016/j.csbj.2025.09.027.
14. Zheng Z, Guo X, Zhu K, Peng W, Zhou H. Artificial neural network–genetic algorithm to optimize wheat germ fermentation condition: Application to the production of two anti-

tumor benzoquinones. Food Chem. 2017;227:264–270. https://doi.org/10.1016/j.foodchem.2017.01.077.

15. Seidl PR, Goulart AK. Application of computational methods for pretreatment processes of different biomass feedstocks. Curr Opin Green Sustain Chem. 2020;26:100366. https://doi.org/10.1016/j.cogsc.2020.100366.
16. Ghani ZA, Yusoff MS, Zaman NQ, Zamri MFMA, Andas J. Optimization of preparation conditions for activated carbon from banana pseudo-stem using response surface methodology on removal of color and COD from landfill leachate. Waste Manag. 2017;62:177–187. https://doi.org/10.1016/j.wasman.2017.02.026.
17. Martín C, Dixit P, Momayez F, Jönsson LJ. Hydrothermal pretreatment of lignocellulosic feedstocks to facilitate biochemical conversion. Front Bioeng Biotechnol. 2022;10:846592. https://doi.org/10.3389/fbioe.2022.846592.
18. Sathish T, Prakasham RS. Enrichment of glutaminase production by Bacillus subtilis RSP-GLU in submerged cultivation based on neural network–genetic algorithm approach. J Chem Technol Biotechnol. 2010;85(1):50–58. https://doi.org/10.1002/jctb.2267.
19. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal Chem. 1959;31(3):426–428. https://doi.org/10.1021/ac60147a030.
20. Lu F, Wang C, Chen M, Yue F, Ralph J. A facile spectroscopic method for measuring lignin content in lignocellulosic biomass. Green Chem. 2021;23(14):5106–5112. https://doi.org/10.1039/D1GC01507A.
21. M. Zappalà, B. Fallico, E. Arena, and A. Verzera, “Methods for the Determination of HMF in Honey: A Comparison,” Food Control16 (2004): 273–277, https://doi.org/10.1016/j.foodcont.2004.03.006.

22. H. Sihotang, “Synthesis 2-((Furan-2-yl) methylene)-1- phenilhydrazone From Furfural Results of Corn Isolation and Its Utilization as Corrosion Inhibitor in Zinc (Zn) Metal,” Journal of Chemical Natural Resources 3 (2022): 35–42, https://doi.org/10.32734/jcnar.v3i1.9335
23. Gilman J, Walls L, Bandiera L, Menolascina F. Statistical design of experiments for synthetic biology. ACS Synth Biol. 2021;10(1):1–18. https://doi.org/10.1021/acssynbio.0c00385.
24. Das S, Negi S. Enhanced production of alkane hydroxylase from Penicillium chrysogenum SNP5 (MTCC13144) through feed-forward neural network and genetic algorithm. AMB Express. 2022;12(1):28. https://doi.org/10.1186/s13568-022-01366-1.
25. Parkhey P, Ram AK, Diwan B, Eswari JS, Gupta P. Artificial neural network and response surface methodology: A comparative analysis for optimizing rice straw pretreatment and saccharification. Prep Biochem Biotechnol. 2020;50(8):768–780. https://doi.org/10.1080/10826068.2020.1737816.
26. Xu J, Li C, Dai L, Xu C, Zhong Y, Yu F, et al. Biomass fractionation and lignin fractionation towards lignin valorization. ChemSusChem. 2020;13(17):4284–4295. https://doi.org/10.1002/cssc.202001491.
27. Kaithvar P, Singh H, Negi S. Integrated chemical and enzymatic pretreatment of Tinospora cordifolia aerial root for cellulose enrichment and nanofiber fabrication. Starch. 2026;78(7). https://doi.org/10.1002/star.70260.
28. Liu F, Li J, Yu H, Li Y, Wang Y, Gao H, et al. Optimizing two green-like biomass pretreatments for maximum bioethanol production using banana pseudostem by effectively

enhancing cellulose depolymerization and accessibility. Sustain Energy Fuels. 2021;5(13):3467–3478. https://doi.org/10.1039/D1SE00613D.

29. Moodley P, Rorke DC, Gueguim Kana EB. Development of artificial neural network tools for predicting sugar yields from inorganic salt-based pretreatment of lignocellulosic biomass. Bioresour Technol. 2019;273:682–686. https://doi.org/10.1016/j.biortech.2018.11.034.
30. Luo H, Gao L, Liu Z, Shi Y, Xie F, Bilal M, et al. Prediction of phenolic compounds and glucose content from dilute inorganic acid pretreatment of lignocellulosic biomass using artificial neural network modeling. Bioresour Bioprocess. 2021;8(1):134. https://doi.org/10.1186/s40643-021-00488-x.
31. Pappu SMJ, Gummadi SN. Artificial neural network and regression coupled genetic algorithm to optimize parameters for enhanced xylitol production by Debaryomyces nepalensis in bioreactor. Biochem Eng J. 2017;120:136–145. https://doi.org/10.1016/j.bej.2017.01.010.
32. Shi C, Yang H, Chen T, Guo L, Leng X, Deng P, et al. Artificial neural network–genetic algorithm-based optimization of aerobic composting process parameters of Ganoderma lucidum residue. Bioresour Technol. 2022;357:127248. https://doi.org/10.1016/j.biortech.2022.127248.
33. Haldar D, Shabbirahmed AM, Mahanty B. Multivariate regression and artificial neural network modelling of sugar yields from acid pretreatment and enzymatic hydrolysis of lignocellulosic biomass. Bioresour Technol. 2023;370:128519. https://doi.org/10.1016/j.biortech.2022.128519.

34. Triveni Interchem Pvt. Ltd. Lignin powder (10000 g/mol) – industrial grade [Internet]. TradeIndia; [cited 2026 Oct]. Listed price: ₹83/kg; minimum order quantity: 25 kg. Available from: https://www.tradeindia.com/products/lignin-powder-10000-g-mol-c12887175.html
35. Tokyo Chemical Industry (India) Pvt. Ltd. Xylan from corn core (CAS No. 9014-63-5), product number X0078 [Internet]. [cited 2026 Oct]. Listed price: ₹6200 per 25 g. Available from: https://www.tcichemicals.com/IN/en/p/X0078

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table3Supplementary.docx