www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(4): 1229-1233

www.thepharmajournal.com
Received: 13-02-2023
Accepted: 16-03-2023

K Mithun
Ph.D. Scholar (APFE)
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

S Patel
Professor (APFE)
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

D Khokhar
Assistant Professor (Biochemistry)
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
K Mithun
Ph.D. Scholar (APFE)
Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Studies on effect of different process parameters on cellulose extraction from spent lemongrass

K Mithun, S Patel and D Khokhar

Abstract
This article reports the cellulose extraction protocol and the yield determination from spent lemongrass. Spent lemongrass, an industrial by-product is a rich source of cellulose. The present study was carried out to investigate the effect of alkaline treatment processing parameters like sodium hydroxide concentration, solution temperature and immersion time on spent lemongrass cellulose yield. Box behnken design was employed and response surface methodology was adopted for statistical modelling and optimization of NaOH solution concentration, solution temperature, and immersion time with an aim to extract maximum cellulose by eliminating lignin, hemicellulose and extractives. The optimum process conditions were 2.05 w% NaOH, 80 °C temperature, and 2.08 h of extraction time with a cellulose yield of 28.08%.

Keywords: Sodium hydroxide solution, solution temperature, immersion time, cellulose yield

Introduction
Cellulose is the most abundant polysaccharide on the earth, which is a complex carbohydrate with carbon, hydrogen and oxygen atoms. Cellulose production annually is reported to be 10^5-10^{12} tonnes, respectively (Kumari *et al.*, 2019; Khan *et al.*, 2020; Lamo *et al.*, 2022) [5, 4, 6]. It is the dominant structural component of primary cell wall of all the plants. Cellulose is tasteless, odorless biodegradable material which is hydrophilic in nature, whereas, it is insoluble in water and most organic solvents. Cellulose is treated with concentrated mineral acids at high temperature to break down into glucose units.

Plant materials are primarily composed of cellulose, lignin and hemicellulose. Cellulose is a 3-D linear molecular structure with both crystalline and amorphous regions. Hemicellulose is mainly amorphous with several crystalline regions. Degree of polymerization is the basic difference between cellulose (102-104) and hemicellulose (<200). Lignin is amorphous heterogeneous 3-D nonlinear polymer which binds cellulosic components together (Bahl *et al.*, 2014). Cellulose is made up of 1,4 β-glucopyranose units, hemicellulose comprises xylose, lactose, arabinose and mannose sub units and lignin consists mainly guaiacyl, syringyl and hydroxy phenyl units (Pierson *et al.*, 2013) [12].

Cellulose, is composed of linear chains of (1→4)-linked-β-D-glucose monomers with no coils or branches. Chemical modification of cellulose is inevitable in development of plastics. Natural cellulose is cellulose I, with structures I_α and I_β. Cellulose produced by bacteria and algae is enriched in I_α while cellulose of higher plants consists mainly of I_β. Cellulose in regenerated cellulose fibers is cellulose II. The conversion of cellulose I to cellulose II is irreversible, suggesting that cellulose I is metastable whereas cellulose II is stable. With various chemical treatments it is possible to produce the structures cellulose III and cellulose IV (Eo *et al.,* 2016) [2].

Cellulose based natural fibers has diversified sources of biomass in the global village. They come in an array of forms, depending on the source, e.g., wheat, hemp, paddy, coir, jute, ramie, softwood, flax, aromatic spent wastes, kapok, cotton, hardwood, and bamboo. Plant fibers played a significant role in traditional applications for the construction of ropes, sails, and also paper.

Cellulose isolation from agricultural wastes/byproducts were reported by different researchers following varied methods wherein common sequences of steps were employed in all methods to isolate cellulose. The sequence of steps involved in isolation process were washing of raw material to remove dust and debris adhered to it, drying in an oven at ambient conditions, crushing in grinder to make powder, sieving to produce desired particle size, defatting, alkaline pretreatment, neutralization with distilled water, drying, acid treatment, neutralization using distilled water, drying to get the required cellulose. Cellulose is generally extracted from plants and its parts *i.e*., softwood, hardwood, bark *etc.*

Need has arisen for humans to extract cellulose from agro industrial wastes and that too from straw waste which will have high economic value if properly processed. In the very recent years the cellulose extraction has shifted from plant based to by-product based like rice straw, wheat straw, lemongrass straw, citronella straw, vetiver straw, little millet straw, oat straw, rye straw, kodo millet straw, finger millet straw, foxtail millet straw, brown top millet straw, proso millet straw *etc*., which are of non-wood plant based and many of these were of non-wood and non-food based cellulose sources. Researchers have reported cellulose isolation preparation from rice straw (Nasri-Nasrabadi *et al.*, 2014) [11], wheat straw (Miao *et al.*, 2020) [8], chilli leftover (Nagalakshmaiah *et al.*, 2016) [10], poplar wood (Fan *et al.*, 2013) [3], pineapple crown leaves (Prado and Spinace 2019) [14], sugarcane straw (Lu *et al.*, 2022) [7], banana pseudostem (Zope *et al.*, 2022) [16], lemongrass (Kumari *et al.*, 2019) [5], respectively.

Various researchers have reported the cellulose extraction process protocols from different food, fodder and its byproducts. The cellulose extraction process includes pretreatment, alkaline treatment, acid hydrolysis and bleaching treatments. An attempt is being made effect of alkaline treatment process parameters on the cellulose yield from lemongrass spent in the above mentioned sequence the current study.

Materials and Methods

Raw material

Lemongrass spent straw (*Cymbopogan flexuous*) (Krishna variety) was procured from Department of Plant Physiology, Agricultural Bio-chemistry, Medicinal and Aromatic Plants, IGKV, Raipur. The initial moisture content of fresh lemongrass was 63% (wb). The moisture content of spent lemongrass after oil extraction, sun dried for 7 days was determined to be 10% (db). The stored spent was washed with warm water rigorously for 3 - 4 times to remove the dirt and dust adhered on it, followed by air drying at 50°C for 24 h.

Chemicals used

Sodium hydroxide (NaOH) pellets, hydrochloric acid (HCl) solution, and hydrogen peroxide (H_2O_2) were procured from local supplier. All the chemicals are Lobe Chemie Pvt. Ltd (Mumbai) made.

Cellulose isolation procedure

Pretreatment and acid hydrolysis

Desired quantity of spent lemongrass (4-5 cm) was allowed to swell in 17.5 w% of sodium hydroxide solution for 2 h. The swollen straws were washed with distilled water and air dried at 50 °C for 20 h. and ground/crushed into powder using Food Processor 2663 grinder (Asha, India) and was sieved using a gyratory sieve shaker (Macro scientific, India) to get spent powder. The spent lemongrass powder passed through 100 mesh size (BSS 100) was used hereafter for further experimental purpose. The ground spent was hydrolyzed with 2 M HCl solution (maintaining acid to pulp ratio as 25 mL/g) at 80±5 °C for 2 h at 600 rpm (Fig. 1). The solution was cooled to room temperature, filtered using filter paper, washed with distilled water until the filtrate attains pH 7. The retentive was dried at 50 °C for 30 h.

Alkaline treatment and bleaching

Box behnken design (Table 1) was employed with three independent variables i.e., sodium hydroxide solution concentration, solution temperature and immersion time with one output i.e., cellulose yield. The three independent variables were taken at three levels each as sodium chloride concentration of 1, 2, 3 w%, solution temperature of 70, 80, 90 °C and immersion time of 1, 2, 3 h, respectively. The range of variables was fixed based on literature review and preliminary experimentation.

Table 1: Coded values and corresponding actual values used to denote independent process parameters used in Box Menken design

Independent process parameters	Data levels			
	Coded values	**X_1**	**X_2**	**X_3**
		-1	**0**	**1**
A. NaOH concentration (w %)	Actual values	1	2	3
B. Solution temperature (°C)		70	80	90
C. Immersion time (h)		1	2	3

The spent was treated with known quantities of NaOH at desired solution temperatures for desired time (Table 1) at 600 rpm to eliminate the soluble lignin, residual hemicelluloses and extractive, followed by washing with de-ionized water and drying at 50°C for 20 h. The neutral filtrate was treated with 20% w/v H_2O_2 solution at 50 °C for 1 h to eliminate insoluble lignin present, followed by washing with distilled water and air dried at 50 °C for 22 h. Cellulose was extracted using the procedure reported by Nasri-Nasrabadi *et al.* (2014) [11] with few modifications.

Cellulose yield

Cellulose yield after the removal of hemicellulose, lignin and fats was calculated by the equation given in Eqn 1.

$$\text{Cellulose yield} = \frac{\text{Finalweight of the sample (g)}}{\text{Initial weight of the sample (g)}} \times 100 \quad \text{.... (1)}$$

Statistical analysis and optimization

Experimentation was carried out according to the Box-Behnken design of 15 experimental sets in which three are the central points. The response surface data obtained from the experiments were statistically analyzed by Design Expert software 13.0.5.0 (Statease, Minneapolis, USA). Analysis of variance (ANOVA) and coefficient of regression were determined for the quadratic model fitted to the experimental data (Eqn. 2).

To study the effect of process parameters on responses (dependent variables), a second order polynomial regression equation was employed to describe the relationship.

$$Y = \beta_0 \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{2} \sum_{i=2}^{3} \beta_{ij} X_i X_j + \sum_{i=1}^{3} \beta_{ii} X_i^2 \quad \text{.... (2)}$$

Where, Y = response, βo, βi, βii, and βij are regression coefficient for intercept, linear, quadratic, and interactive terms, respectively. Xi and X_j are independent variables, respectively.

The process parameters were optimized to yield maximum amount of cellulose.

Results and Discussions

Cellulose yield

The cellulose yield ranged from 18.12% to 32.21%, respectively. The maximum cellulose yield was obtained with sodium hydroxide concentration of 2w%, solution temperature of 80°C and immersion time of 2 h, respectively. The minimum cellulose yield was obtained with sodium hydroxide concentration of 1w%, solution temperature of 70 °C and immersion time of 2 h, respectively. Table 2 shows the cellulose yield obtained at each experimental run. Response surface plots for cellulose yield by varying the concentration of process parameters were shown in Fig 1-3, respectively.

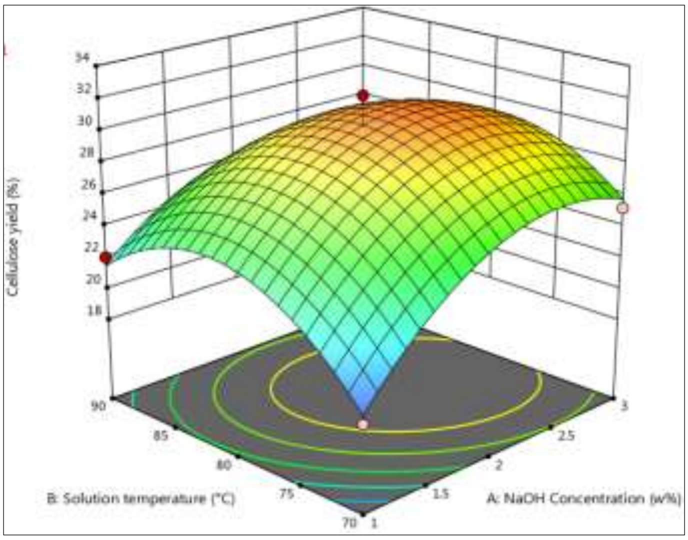

Fig 1: 3D Surface plot for cellulose yield by varying concentrations of NaOH concentration and solution temperature

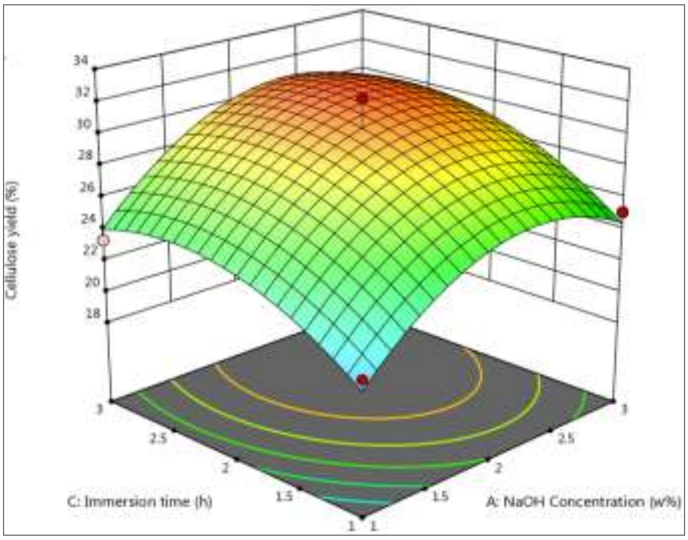

Fig 2: 3D Surface plot for cellulose yield by varying concentrations of NaOH concentration and immersion time

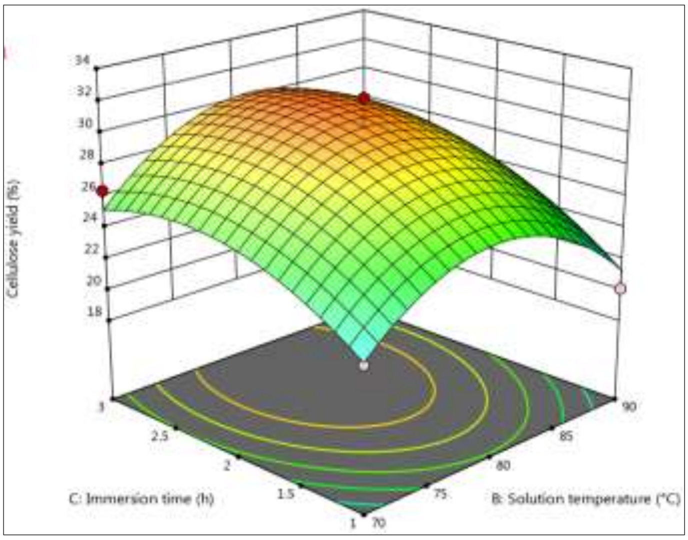

Fig 3: 3D Surface plot for cellulose yield by varying concentrations of solution temperature and immersion time

Table 2: Cellulose yield obtained from lemongrass spent at different processing conditions

Run	NaOH concentration (w %)	Solution temperature (°C)	Immersion time (h)	Cellulose yield (%)
1	2	90	1	20.09
2	1	70	2	18.12
3	1	90	2	22.05
4	2	80	2	28.84
5	2	80	2	30.11
6	2	80	2	32.21
7	1	80	3	23.34
8	2	90	3	26.78
9	1	80	1	20.92
10	3	90	2	24.46
11	2	70	1	21.64
12	3	80	1	25.14
13	3	80	3	28.67
14	2	70	3	26.42
15	3	70	2	25.21

ANOVA for cellulose yield was tabulated in Table 3. A second order model was obtained to fit the experimental data with a R^2 value of 0.94, Predicted R^2 of 0.49, Adjusted R^2 of 0.84, Adequate precision ration of 9.27, respectively. From the analysis of the second order model coefficients (Table 3), it was observed that the NaOH concentration and immersion time showed positive significant effect on the cellulose yield (p<0.05) with no effect of their interaction.

Table 3: Analysis of variance (ANOVA) for cellulose yield

Source	Sum of Squares	DF	Mean Square	F-value	p-value	
Model	210.63	9	23.4	9.76	0.0109	S
X_1-NaOH concentration	45.36	1	45.36	18.92	0.0074	S
X_2-Solution temperature	0.495	1	0.495	0.2064	0.6686	NS
X_3-Immersion time	37.93	1	37.93	15.82	0.0106	S
X_1X_2	5.48	1	5.48	2.28	0.1912	NS
X_1X_3	0.308	1	0.308	0.1285	0.7347	NS
X_2X_3	0.912	1	0.912	0.3803	0.5644	NS
X_1^2	47.08	1	47.08	19.63	0.0068	S
X_2^2	70.06	1	70.06	29.22	0.0029	S
X_3^2	19.5	1	19.5	8.13	0.0357	S
Residual	11.99	5	2.4			
Lack of Fit	6.2	3	2.07	0.713	0.6285	NS
Pure Error	5.79	2	2.9			
Cor Total	222.62	14				
R^2	0.94					
Adjusted R^2	084					
Predicted R^2	0.49					
CV (%)	6.21					
APR	9.27					

S - Significant, NS - Non-significant

The ANOVA table depicts that the model is significant and lack of fit is not significant which a good indication of model fit. The sodium hydroxide concentration and immersion time showed significant effect on the cellulose yield. The interaction terms had no significant effect on the cellulose yield, whereas the quadratic terms showed significant effect (p<0.05) on cellulose yield, respectively. The quadratic terms showed negative significant effect on the cellulose yield.

The multiple regression equation developed for cellulose yield in terms of coded factors (Eqn 3) is given below.

$$\text{Cellulose yield (\%)} = 30.39 + 2.38\, X_1 + 0.24\, X_2 + 2.18\, X_3 - 1.17\, X_1X_2 + 0.27\, X_1X_3 + 0.47\, X_2X_3 - 3.57\, X_1^2 - 4.36\, X_2^2 - 2.30\, X_3^2 \quad \ldots (3)$$

The multiple regression equation developed for cellulose yield in terms of actual factors (EQN 4) is given below.

$$\text{Cellulose yield (\%)} = -292.94 + 25.46\, A + 7.13\, B + 6.99\, C - 0.11\, AB + 0.27\, AC + 0.04\, BC - 3.57\, A^2 - 0.04\, B^2 - 2.29\, C^2 \quad \ldots (4)$$

Similar results reported by Sayakulu and Soloi (2021) [15], Lamo *et al.* (2022) [6] and Pinto *et al.* (2022) [13], respectively. Lamo *et al.* (2022) [6] indeed reported that yield of cellulose increased with the immersion time predominantly. Alkali has a solubilizing effect on hemicellulose and lignin, and mechanical stirring further increases the rate of reaction. Mohamad and Jai (2022) [9] reported that increase in the NaOH concentration increased the dissolution of lignin, hence the removal of lignin increased proportionally increased the cellulose yield significantly. The whole process protocol was successful in eliminating lignin, hemicellulose and extractives.

Process optimization and model validation

The process was numerically optimized with an aim to maximum cellulose yield. The constraints applied for optimization of extraction process are tabulated in Table 4.

Table 4: Goals and importance for optimization of process parameters for desirable values of product responses

Parameter	Goal	Importance
NaOH concentration (w %)	In range	
Solution temperature (°C)	In range	
Immersion time (h)	In range	
Cellulose yield (%)	Maximum possible	+++++

Optimum cellulose yield (28.08%) was obtained at processing conditions of 2.05 w% NaOH concentration, 80°C solution temperature, and 2.08 h with a desirability value of 0.927. The optimized result was validated by performing eight replications at the optimum processing condition and was statistically tested for its significance. The experimental result is in close agreement with the predicted optimized result wherein the above solution is validated. The predicted value of cellulose yield is 28.08% and the experimental cellulose yield is 27.23±3.42%, respectively.

Conclusions

The alkaline pretreatment and acid hydrolysis, alkaline treatment (optimized conditions) (current study) and subsequent bleaching successfully eliminated the hemicelluloses, lignin and waxes present in the sample to obtain the maximum cellulose yield. The process conditions i.e., sodium hydroxide concentration, solution temperature and immersion time were optimized so as to maximize the cellulose yield. From the current study, the optimum extraction condition of 2.05 w% of NaOH concentration, 80 °C solution temperatures, and 2.08 h immersion time resulted in a maximum cellulose yield of 28.08%. The process conditions (NaOH concentration and immersion time) showed significant effect on cellulose yield. The extracted cellulose from lemongrass spent has potential applications in paper and biodegradable packaging applications.

Acknowledgement

The authors duly acknowledge Dr. Ravindra Soni, Assistant Professor (Agricultural Microbiology), Dr. R.R. Saxena, Professor (Statistics) and Dr. Girish Chandel, Vice-Chancellor and Ex-Head (PMBB) of Indira Gandhi Krishi Vishwavidyalaya, Raipur for extending all necessary support (laboratory/intellectual) to conduct the investigation.

References

1. Bahl K, Miyoshi T, Jana SC. Hybrid fillers of lignin and carbon black for lowering of viscoelastic loss in rubber compounds. Polymer. 2014;55(16):3825-3835.
2. Eo MY, Fan H, Cho YJ, Kim SM, Lee SK. Cellulose membrane as a biomaterial: from hydrolysis to depolymerization with electron beam. Biomaterials Research. 2016;20(1):1-13.
3. Fan G, Wang M, Liao C, Fang T, Li J, Zhou R. Isolation of cellulose from rice straw and its conversion into cellulose acetate catalyzed by phosphotungstic acid. Carbohydrate Polymers. 2013;94(1):71-76.
4. Khan MN, Rehman N, Sharif A, Ahmed E, Farooqi ZH, Din MI. Environmentally benign extraction of cellulose from dunchi fiber for Nano cellulose fabrication. International Journal of Biological Macromolecules. 2020;153:72-78.
5. Kumari P, Pathak G, Gupta R, Sharma D, Meena A. Cellulose Nano fibers from lignocellulose biomass of lemongrass using enzymatic hydrolysis: characterization and cytotoxicity assessment. DARU Journal of Pharmaceutical Sciences. 2019;27(2):683-693.
6. Lamo C, Bargale PC, Gangil S, Chakraborty S, Tripathi MK, Kotwaliwale N, *et al*. High crystalline cellulose extracted from chickpea husk using alkali treatment. Biomass Conversion and Bio refinery; c2022. p. 1-9.
7. Lu S, Ma T, Hu X, Zhao J, Liao X, Song Y, *et al*. Facile extraction and characterization of cellulose Nano crystals from agricultural waste sugarcane straw. Journal of the Science of Food and Agriculture. 2022;102(1):312-321.
8. Miao X, Lin J, Bian F. Utilization of discarded crop straw to produce cellulose Nano fibrils and their assemblies. Journal of Bio resources and Bio products, 2020;5(1):26-36.
9. Mohamad NAN, Jai J. Response surface methodology for optimization of cellulose extraction from banana stem using NaOH-EDTA for pulp and papermaking. Heliyon, 2022;8:e09114.
10. Nagalakshmaiah M, Mortha G, Dufresne A. Structural investigation of cellulose Nano crystals extracted from chili leftover and their reinforcement in car flex-IR rubber latex. Carbohydrate Polymers. 2016;136:945-954.
11. Nasri-Nasrabadi B, Behzad T, Bagheri R. Extraction and characterization of rice straw cellulose nanofibers by an optimized chemo mechanical method. Journal of Applied Polymer Science. 2014;131(7):40063-40070.
12. Pierson Y, Bobbink F, Yan N. Alcohol mediated liquefaction of lignocellulosic materials: A mini review. Chemical Engineering and Process Technology. 2013;1(2):1014.
13. Pinto E, Aggrey WN, Boakye P, Amenuvor G, Sokama-Neuyam YA, Fokuo MK, *et al*. Cellulose processing from biomass and its derivatization into carboxy methylcellulose: A review. Scientific African. 2022;15:e01078.
14. Prado KS, Spinacé MA. Isolation and characterization of cellulose nanocrystals from pineapple crown waste and their potential uses. International Journal of Biological Macromolecules. 2019;122:410-416.
15. Sayakulu NF, Soloi S. The Effect of sodium hydroxide (NaOH) concentration on oil palm empty fruit bunch (OPEFB) cellulose yield. In Journal of Physics: Conference Series. 2022;2314(1):012017.
16. Zope G, Goswami A, Kulkarni S. Isolation and characterization of cellulose Nano crystals produced by acid hydrolysis from banana pseudostem. Bio Nano Science. 2022;12(2):463-471.