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OPTIMIZATION OF MICROWAVE ASSISTED DELIGNIFICATION OF WOOD RESIDUES BY SURFACE RESPONSE METHODOLOGY

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1. Introduction

Due to effects such as global warming, environmental pollution and health hazards directly linked with the intensive exploitation of fossil fuels, there is an urgent need to find alternative energy resources. Vegetal biomass is a renewable resource with high availability worldwide. Current research is focused on developing efficient processes to transform this resource into bio-fuels and value-added fine chemicals [1, 2]. Vegetal biomass contains cellulose, hemicellulose and lignin in variable amounts. Pre-treatment plays a major role in breaking down the lignin barrier for the recovery of cellulose from lignocellulosic biomass. The effectiveness of a pre-treatment should be assessed following some criteria: minimizing energy input and the formation of inhibitors; maximizing the yield of fermentable sugars and preservation of cellulosic and hemicellulosic fractions [3]. There are several options of pre-treatment for lignocellulosic biomass and among them the most promising are: mineral acids, alkali, organosolv, wet oxidation, CO_2 and steam explosion. A considerable amount of lignocellulosic biomass is generated as waste byproduct through agricultural practices and processing. Thus, the objective of this research is to improve the yield of enzymatic hydrolysis of wood residues by removal of lignin using alkaline wash assisted by microwave heating.

2. Experimental

Wood chips residue from a furniture factory (in Bucharest) were used for the experiments. The wood residue was milled and sieved. The 0.315-0.5 mm fraction was retained for the experiments. The wood residues were subjected to successive extractions with toluene:ethanol 1:2 solvent and then with water, for 6 hours, in a Soxhlet extractor, in order to remove wax and other extractives which can act as inhibitors for delignification and enzymatic hydrolysis. The wood residue was then dried for 48h at 40^0C.

2.1 Determination of the composition of wood residue

The lignin and structural carbohydrates content of the wood residue (extracted) was determined according to NREL 42618 standard [4] by acid hydrolysis in two steps. In the first step, the wood residue is put into contact for 1 hour with 72% sulfuric acid. For the second step the solution is diluted to 4% sulfuric acid and autoclaved for 1 hour at 121^0C. Acid insoluble lignin is filtered, dried and weighted. The solution is neutralized and passed through 0,45 mm nylon filter before HPLC analysis for the determination of monosaccharides. The HPLC analysis was performed with a Jasco 2000+ System equipped with an RI detector. The monosaccharides were separated in isocratic conditions with 0,5 ml/min ultrapure water as eluent, at 80^0C, on a Carbosep Corgel 87P (7.8 x 300 mm)

Column. The results obtained for the characterization of the wood residue are presented in table 1.

Table 1. Composition of wood residue from furniture manufacturing

Chemical composition	Determination method	%
Cellulose	Glucose - HPLC	51.3
Hemicellulose	Xylose - HPLC	19.6
Lignin	Klason lignin – gravimetry after acid hydrolysis	25.5
Extractives	Toluen+Ethanol extractives - gravimetry	2.8
	Water extractives - gravimetry	0.8

2.2 Microwave alkaline treatment

The treatments of the extracted wood residue were carried out in alkaline conditions, for 30 minutes, in a pressurized microwave reactor (Synthwave-Milestone). This 800 mL reactor is able to provide severe conditions: high temperatures (up to 300^{0}C) and pressures (up to 200 bar). The treatments were performed with a volume of 250 mL solution of 0.4M NaOH, 1 mL of Fatty acid methyl esthers (FAME), variable amount of extracted wood residue, different temperatures and 7 bar Argon pressure.
Figure 1 presents a one of the diagrams in which the experimental conditions (temperature, pressure and microwave power) are monitored and registered during alkaline treatment in the Synthwave microwave reactor. In order to maintain a constant temperature of 60^{0}C inside the microwave reactor, the microwave power delivered inside the reactor was kept at 500-800W in the heating region of the profile and at 150-200W in the constant region of the temperature profile.

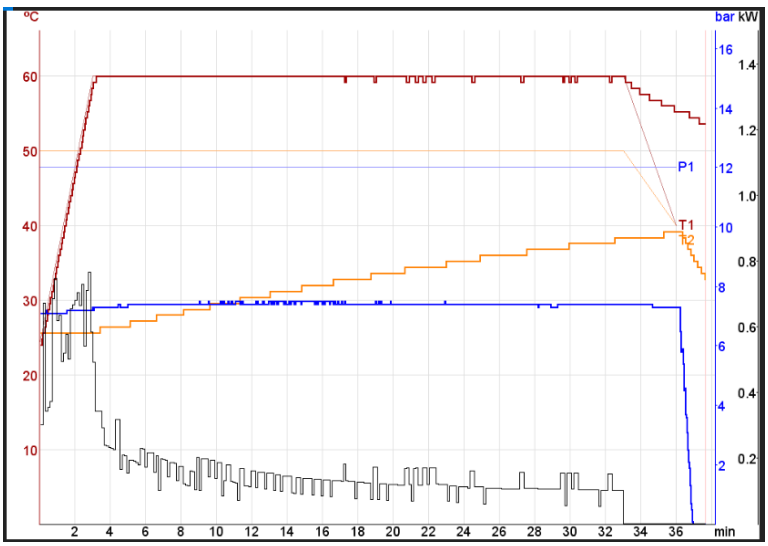

Fig. 1. Monitoring of alkaline treatment of wood residues in Synthwave microwave reactor: (Graph colour: temperature in red; pressure in blue; microwave power in black)

2.3 Lignin content

The efficiency of the alkaline wash was assessed by monitoring the concentration of lignin (determined by UV absorbance at 320 nm against a calibration curve) from the alkaline washing solution.

2.4 Determination of sugars from enzymatic hydrolysis

The solid biomass obtained after the alkaline treatment was washed with distilled water until neutral pH and subjected to enzymatic hydrolysis. Enzymatic hydrolysis was performed at 50^0C, in a shaking water bath, with 1 g of treated wood residue, 25 mL of buffer solution pH=5 (citric acid/ Na_2HPO_4) and 0.6 mL Celluclast 1.5L enzyme. Sugar concentrations were determined every 24h for three days to monitor the yield of enzymatic hydrolysis. Sugar concentration (as glucose equivalents obtained per one gram of dry mass) was determined following a modified dinitrosalicylic acid assay [5].

2.5 Central composite design – lignin and glucose equivalents by enzymatic hydrolysis

The treatment conditions were established according to the results obtained from a screening and characterization study carried out before constructing the experimental matrix for optimization (in Design Expert 11).

Table 2. The experimental matrix and the values determined for lignin and glucose equivalents from enzymatic hydrolysis of wood residue

		Factor 1	Factor 2	Response 1	Response 2	Response 3	Response 4
Std	Run	A:temperature	B: wood residue	Lignin	Glucose Equivalents-day 1 (EG1)	Glucose Equivalents-day 2 (EG2)	Glucose Equivalents-day 3 (EG3)
		^{0}C	g	mg/g wood residue	mg/g wood residue	mg/g wood residue	mg/g wood residue
10	1	60	3.75	19.3595	78.27	95.69	99.38
12	2	60	3.75	19.8549	78.11	97.84	102.25
8	3	60	5.52	15.5074	83.2	105.42	106.1
2	4	80	2.5	26.6245	82.74	99.31	104.48
11	5	60	3.75	18	80.78	94.25	100.38
5	6	32	3.75	9.9068	77.24	87.7	92.98
3	7	40	5	11.5167	73.04	87.79	93.45
6	8	88	3.75	31.9907	98.61	109.98	114.77
1	9	40	2.5	12.1358	86.04	101.2	105.05
7	10	60	1.98	22.3591	88.75	92.27	100.28
4	11	80	5	24.6122	93.63	104.39	115.51
9	12	60	3.75	17.9974	85.91	96.46	104.87

The screening allowed the careful selection of the most important factors that affect the lignin removal from wood residue: liquid to solid ratio (quantity of wood residue) and temperature. All experiments were carried out in duplicate. A Central Composite Design (CCD) model type was selected to be fitted with the experimental data for optimization. All experiments were carried out in duplicate. Table 2 contains the CCD experimental matrix with 12 experiments carried out with different settings of the independent factors (wood residue quantity and temperature) and the values determined experimentally for the two responses (lignin concentration and glucose equivalents).

3. Results and discussion

Fitting analysis of different model types to experimental values of lignin concentration indicate that the best model is linear (table 3). The correlation coefficients ($R^2_{adjusted}$ = 0.95 and $R^2_{predicted}$ = 0.91) are very close to 1 and there is a very small difference between their values.

Table 3. Fit summary for the concentration of lignin

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	< 0.0001	0.1993	0.9510	0.9170	Suggested
2FI	0.6581	0.1674	0.9463	0.8842	
Quadratic	0.4026	0.1421	0.9471	0.8255	
Cubic	0.1752	0.1674	0.9668	0.5855	Aliased

ANOVA of the linear model fitted for the concentration of lignin (table 4) confirm that the selected model is significant and the model is fitted. The factors that have significant effect on the concentration of lignin are the two independent factors considered: temperature and wood residue quantity.

Table 4. ANOVA of the linear model fitted for the concentration of lignin

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	451.39	2	225.69	107.68	< 0.0001	significant
A-temperature	432.41	1	432.41	206.31	< 0.0001	
B-wood residue	18.98	1	18.98	9.05	0.0147	
Residual	18.86	9	2.10			
Lack of Fit	16.15	6	2.69	2.98	0.1993	not significant
Pure Error	2.71	3	0.9034			
Cor Total	470.25	11				

The second response – glucose equivalents from enzymatic hydrolysis of microwave alkaline treated wood residue – was monitored for 3 consecutive days (EG1, EG2 and EG3

in table 2). The data were fitted for each individual response. In table 5 is presented the fitting for the data collected in the 3rd day of enzymatic hydrolysis. It can be observed that a two factor interaction model is suggested.

Table 5. Fit summary for the glucose equivalents determined after 3 days of enzymatic hydrolysis of microwave alkaline treated wood residue

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.0083	0.1142	0.5787	0.2789	
2FI	0.0027	0.4381	0.8563	0.7050	Suggested
Quadratic	0.3818	0.4143	0.8610	0.6358	
Cubic	0.2414	0.5684	0.8976	0.6564	Aliased

ANOVA of the model fitted for the glucose equivalents (table 6) confirm that the selected model is significant and the model is fitted. The factors that have significant effect on the concentration of lignin are the temperature and the interaction of temperature with wood residue quantity. The quantity of wood residue as single term does not have an significant effect on response as shown by its p-value which is well above significance level of 0.05.

Table 6. ANOVA of the model fitted for the glucose equivalents from enzymatic hydrolysis of microwave alkaline treated wood residue (3 days)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	477.35	3	159.12	22.86	0.0003	significant
A-temperature	341.99	1	341.99	49.12	0.0001	
B-wood residue	7.34	1	7.34	1.05	0.3347	
AB	128.03	1	128.03	18.39	0.0027	
Residual	55.69	8	6.96			
Lack of Fit	38.22	5	7.64	1.31	0.4381	not significant
Pure Error	17.47	3	5.82			
Cor Total	533.04	11				

The exploration of the experimental surfaces (figure 2) of the fitted models indicate the dominant effect of temperature as independent factor. Although this suggests that an increase of temperature would provide higher values of lignin concentration removed from the wood residue, previous experiments that were carried out showed a negative effect of very high temperatures on the performance of enzymatic hydrolysis. This can be explained by the fact that with the increase of temperature, degradation of lignin and structural carbohydrates is enhanced generating compound with inhibitory effect to the enzymes from hydrolysis [6].

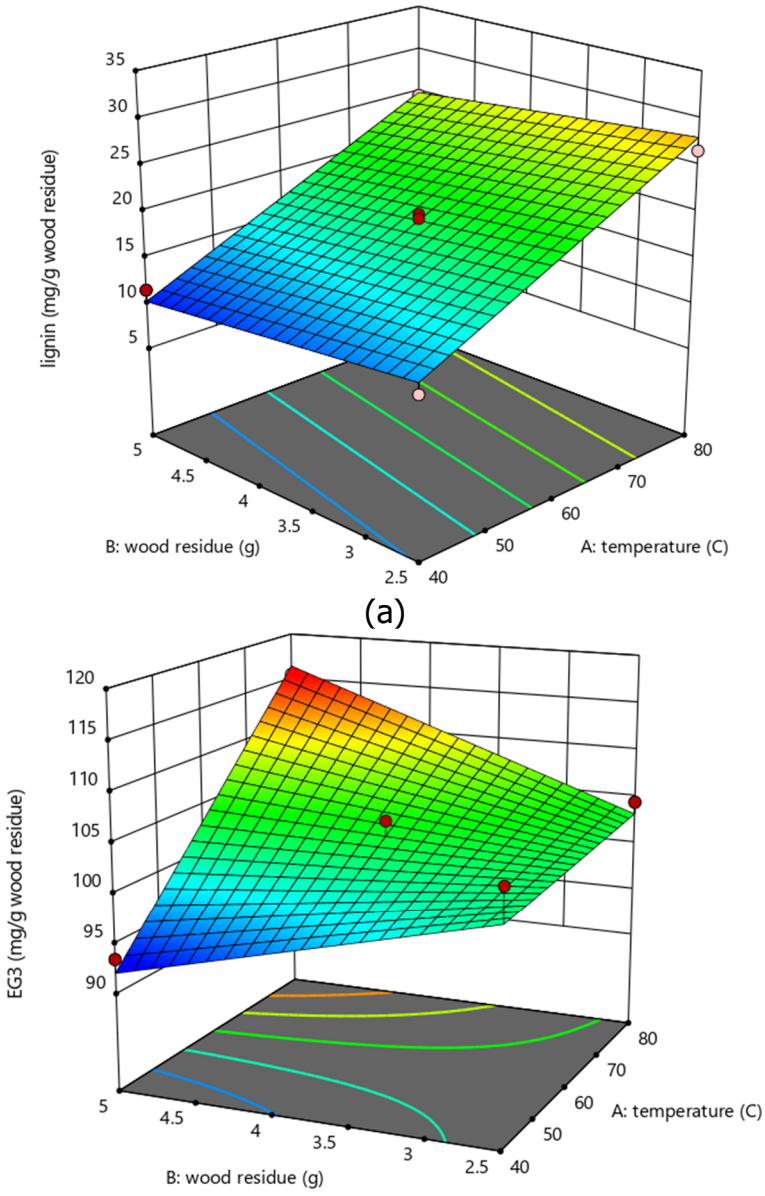

Fig. 2. 3D plot of response surfaces function of the independent factors with significant effect for: a) lignin concentration; b) glucose equivalents from enzymatic hydrolysis of microwave alkaline treated wood residue (3 days)

Optimization of experimental conditions within the experimental space was carried according to the following criteria: minimization of temperature and maximization of the solid residue quantity, lignin concentration and glucose equivalents. There are 8 solutions that satisfy the conditions imposed for the desirability function built for optimization and all

of them are found in the same region of the response surface. One of the optimal solution (22.09 mg lignin / g wood residue and 111.67 glucose equivalents / g wood residue) indicated by the model for these optimization criteria is obtained for the following coordinates: 72^{0}C and 5 g of wood residue (figure 3).

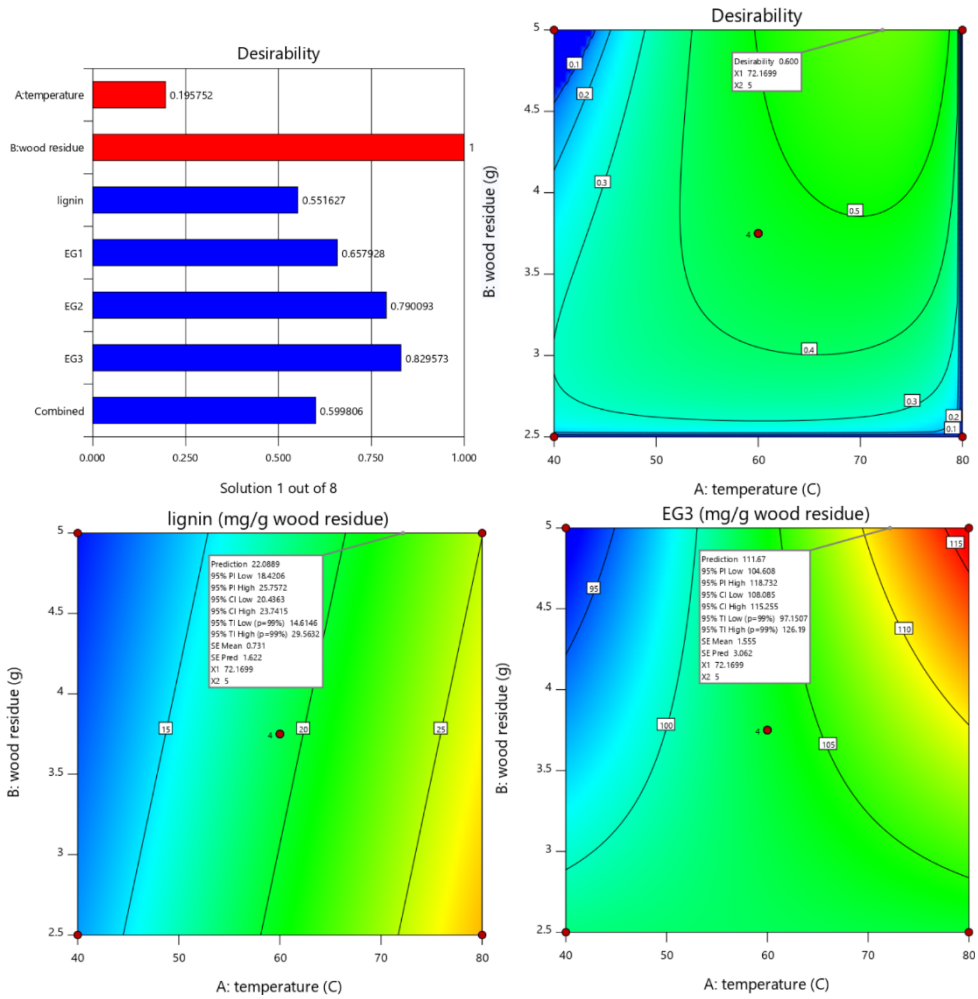

Fig. 3. Optimization (maximization) of the responses (lignin and glucose equivalents) function of the independent variables (temperature and quantity of wood residue)

4. Conclusions

This study is focused on improving the enzymatic hydrolysis of wood residue from furniture manufacturing by microwave alkaline treatment. Response surface methodology was used in order to find the best settings of the independent parameters (temperature and wood residue quantity) considered for the alkaline treatment. The optimization function (desirability) indicated a narrow range of settings for the temperature and the quantity of wood residue at which optimal performance is achieved: 72^{0}C and 5 g of wood residue.

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