Pretreatment And Enzymatic Hydrolysis Of Miscanthus x giganteus: Influence Of Process Parameters

C. Vanderghem[1,2], Y. Brostaux[3], N. Jacquet[1,2], C. Blecker[2], M. Paquot[1]

[1]Department of Industrial Biological Chemistry
[2]Department of Food Technology
[3]Department of Applied Statistics, Computer Science and Mathematics
University of Liege, Gembloux Agro-Bio Tech, Gembloux, Belgium
cvanderghem@ulg.ac.be

Background and Objectives

Miscanthus x giganteus was delignified at atmospheric pressure by a chemical process using a mixture of formic acid/acetic acid/water[a]. The advantage of this procedures is that the acids used can be recycled by simple distillation, and the sugars and lignin in the liquid phase can be recovered in view of its valorisation.
The aim of this work was to test the ability of the process to efficiently delignify *Miscanthus* and to asses the suitability of the pulps thus obtained for enzymatic hydrolysis.
By means of Box-Behnken experimental design we investigated the effect of cooking time (1, 2 and 3 hours), formic acid/acetic acid/water volume ratio (20/60/20, 30/50/20 and 40/40/20) and temperature (80, 90 and 107°C) on the pulp yield, the residual Klason lignin content, the concentration of degradation products (furfural and hydroxymethylfurfural) in the black liquor, and the digestibility of the pulps. Using response surface methodology the optimal pretreatment process parameters were determined.

Materials and Methods

❖**Raw material:** *Miscanthus x giganteus* comes from a crop cultivated in spring 2007, harvested and air dried in spring 2009, Belgium (Tournai). The dry matter content was of 93%. The material was ground to particles around 1 mm.

❖**Pulping:** Presoaking: 50°C for 30 min in formic acid /acetic acid/water (20/60/20, 30/50/20 and 40/40/20 volume ratio). Liquid/solid ratio: 24/1.
Soaking: 80, 90 and 107°C for 1, 2 and 3 hours. Liquid/solid ratio: 24/1. Agitation: 450 rpm.
The pulps obtained were washed with water.

❖**Pulp analysis:** Pulp yield was determined gravimetrically after drying to constant weight. Lignin content was determined by the Klason lignin procedure[b].

❖**Degradation products of hexoses and pentoses :** Quantification of furfural and hydroxymethylfurfural in black liquors was performed with a Zorbax 300SB-C18 reverse phase column (Agilent) using a UV detector.

❖**Enzymatic hydrolysis:** Pulps (50 g dry matter /L) were suspended in citrate buffer (0.05 M, pH 4.8) at 50°C for 24 hours. Enzymes: Celluclast 1.5L and Novozym 188.

❖**Saccharide analysis:** High-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD). Column: PA-100.

❖**Experimental design and statistical analysis**: Optimization of process parameters was achieved using response surface methodology. To estimate the model coefficients, a three-variable modified Box-Behnken design was carried out. The corresponding matrix design is presented in table 1. Independent variables (cooking time, temperature, formic acid /acetic acid/water ratio) were standardized to the interval (1, -1). Analysis data was performed with Minitab 15 statistical software.

Results

Table 1. Experimental design and the corresponding experimental responses.

Independent variables | Dependent variables

Run order	T (°C)	t (hours)	af/aa/water	Pulp Yield	% of delignification	Furfural (ppm)	Digestibility (%)
1	90	3	40/40/20	65,4	63,2	3,85	18,61
2	107	3	40/40/20	55,4	83,57	59,44	20,89
3	107	2	20/60/20	64	60,4	9,94	10,92
4	107	3	30/50/20	54,1	86,45	54,63	29,23
5	80	2	20/60/20	94	0,87	0,06	6,94
6	107	3	20/60/20	67,1	49,94	11,55	13,89
7	107	1	30/50/20	65,1	72,46	10,34	24,17
8	90	1	20/60/20	96	0,89	0,1	10,85
9	90	2	30/50/20	80	39,32	0,51	24,78
10	90	1	40/40/20	85,1	31,28	0,34	21,36
11	80	3	30/50/20	66,6	43,34	0,4	14,18
12	90	3	20/60/20	95,9	15,22	0,34	11,99
13	107	2	40/40/20	58,2	83,49	47,02	14,22
14	80	1	30/50/20	96,7	20,93	0,09	11,62
15	80	2	40/40/20	89,7	16,71	0,22	22,33
16	107	3	20/60/20	66,8	55,13	12,27	12,84
17	107	2	40/40/20	60	71,62	11,11	16,55
18	80	2	40/40/20	93,5	12,14	0,18	19,93
19	107	3	30/50/20	52,4	86,59	49,94	25,75
20	90	1	20/60/20	92	6,08	0,1	13,33
21	107	3	40/40/20	57,4	75,64	64,31	18,24
22	90	3	40/40/20	67,7	61,9	3,36	17,67
23	80	1	30/50/20	90,8	21,52	0,11	13,68
24	107	2	20/60/20	67,6	56,35	8,97	13,06
25	90	1	40/40/20	83,4	34,66	0,39	23,57
26	90	2	30/50/20	79,7	38,26	0,53	21,98
27	80	3	30/50/20	65	46,14	0,29	19,45
28	90	3	20/60/20	84,6	24,17	0,29	11,98
29	107	1	30/50/20	65,6	72,91	10,56	34,35
30	80	2	20/60/20	92,7	4,16	0,07	10,16

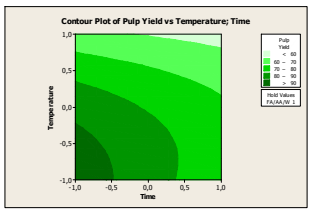
Coded units:
Temperature: 80, 90, 107°C: -1, 0, 1
Time: 1, 2, 3 hours: -1, 0, 1
fa/aa/water ratio : 20/60/20, 30/50/20, 40/40/20: -1, 0, 1

Table 2. Regression coefficients for each variable.

Factor	Pulp Yield	% of delignification	Furfural (ppm)	Digestibility (%)
T (°C)	-12,51	25,19	12,07	2,81
t (hours)	-7,57	9,05	5,19	-0
af/aa/w	-,525	13,05	5,37	3,87
T^2	-7,28	9,18	11,26	-
t^2	-	-	-	-
af/aa/w^2	6,95	-17,43	-	-7,01
T * t	4,19	-	9,40	-
T * af/aa/w	-	-	9,78	-
t * af/aa/w	-	-	-	-
R^2	90,94%	94,26%	82,11%	62,71%

The response surface regression and models were expressed without taking into account the statistically insignificant terms.

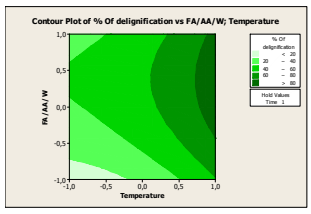
Figure 2. Effect of temperature and time on pulp yield at a fixed fa/aa/water ratio of 40/40/20.



The pulp yield mainly depends on the temperature and time and in minor extent on the fa/aa/water concentration (Table 2). Pulp yield decreases dramatically with increasing temperature and time (Fig. 2).

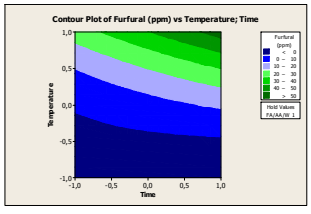
According to the surface response the maximum pulp yield (100%) could be achieved with the following conditions: Temperature: 80°C; time: 1 hour; fa/aa/water ratio: 20/60/20.

Figure 3. Effect of fa/aa/water ratio and temperature on % of delignification at a fixed time of 3 hours.



Percentage of delignification is strongly dependent on temperature and in minor extent on the fa/aa/water ratio and time (Table 2). The increase in temperature lead to an important increase in the % of delignification, reaching values of approximately 80% near 107°C when the fa/aa/water ratio is between 30/50/20 and 40/40/20(Fig. 2). The following conditions were identified to achieve the highest % of delignification (89,7%): Temperature: 107°C; time: 3 hours; fa/aa/water ratio: 35/45/20.

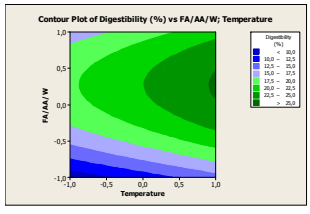
Figure 4. Effect of temperature and time on furfural production in the black liquor at a fixed fa/aa/water concentration of 40/40/20.



Furfural, the degradation product of pentoses, is significantly affected by the cooking temperature and in minor extent by time and fa/aa/w ratio (Table 2). The largest concentrations of furfural are obtained at high temperatures and times (107°C, 3h).

Hydroxymethylfurfural was detected at low concentrations (0,5 ppm).

Figure 5. Effect of fa/aa/water concentration and temperature on % of digestibility of the pulp at a fixed time of 3 hours.



Percentage of digestibility is only influenced by temperature and fa/aa/water ratio (Table2). According to response optimization, the highest digestibility in 24 hours of hydrolysis can be achieved with the following conditions: temperature: 107°C; fa/aa/water: 33/47/20.

Conclusions

❖ This chemical process using a mixture of formic acid/acetic acid /water produces a high degree of delignification of *Miscanthus* and pulps suitable for enzymatic hydrolysis.

❖ ANOVA showed that the selected models adequately fitted the experimental data with R^2 of 90.94, 94.26, 82.11 and 62,71% for the response of pulp yield, % of delignification, furfural and digestibility, respectively.

❖ The optimal parameters predicted to achieve the highest % of delignification (89,7%) were: Temperature: 107°C; time: 3 hours; fa/aa/water ratio: 35/45/20. These parameters were close to those predicted for a maximum digestibility of the pulps: temperature: 107°C; fa/aa/water: 33/47/20.

References
a. Lam, H. Q., Le Bigot Y., Delmas M., Avignon G. 2001. A new procedure for the destructuring of vegetable matter at atmospheric pressure by a catalyst/solvent system of formic acid/acetic acid. Applied to the pulping of triticale straw. *Ind. Crop. Prod. 14, 139-144.*
b. Sluiter, A. et al. 2008. Determination of structural carbohydrates and lignin in biomass. Laboratory analytical procedure. National Renewable Energy Laboratory.

Acknowledgements This study was financially supported by the Walloon Region (TECHNOSE project number 716757; LIGNOFUEL project number 716721). Ms. Virginie Byttebier is acknowledged for excellent assistance.