CHEMICAL ENGINEERING TRANSACTIONS
VOL. 49, 2016
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Marco Bravi, Tajalli Keshavarz

ISBN 978-88-95608-40-2; **ISSN** 2283-9216
DOI: 10.3303/CET1649038

Response Surface Optimization of Dilute Sulfuric Acid Pretreatment of Switchgrass (*Panicum virgatum* L.) for Fermentable Sugars Production

Ana I. Paniagua-García*[a], Rebeca Díez-Antolínez[a], María Hijosa-Valsero[a], Marta E. Sánchez[b], Mónica Coca[c]

[a]Centre of Biofuels and Bioproducts. Instituto Tecnológico Agrario de Castilla y León (ITACyL), Villarejo de Órbigo, E-24358, León, Spain
[b]Chemical and Environmental Bioprocess Engineering Group. Natural Resources Institute (IRENA), Universidad de León, Avenida de Portugal 42, E-24071, León, Spain
[c]Department of Chemical Engineering and Environmental Technology, Universidad de Valladolid, C/Doctor Mergelina s/n, E-47011, Valladolid, Spain
pangaran@itacyl.es

Pretreatment of lignocellulosic materials to disrupt their recalcitrant structures is a crucial step on second generation biofuel production. In this study, response surface methodology (RSM) was used to investigate the effects of dilute sulfuric acid hydrolysis conditions on switchgrass (*Panicum virgatum* L.). A central composite rotatable design (CCRD) was applied to assess the effect of acid concentration (0.16 – 1.84% (w/w)), solid load (4.9 – 30.1% (w/w)) and hydrolysis time (9.6 – 110.5 min), on glucose, xylose and total sugars (glucose, xylose and arabinose) recovery yields and total inhibitors generated (acetic acid, levulinic acid, 5-hydroxymethylfurfural and furfural) after acid hydrolysis. Afterwards, enzymatic hydrolysis of the solid phase was performed as a second treatment step. Experimental data were fitted to a second order polynomial model to find the optimum acid hydrolysis conditions by multiple regression analysis. The results show a strong dependence of total sugars recovery and total fermentation inhibitors on acid concentration, and a weaker dependence on solid load and acid hydrolysis time. The optimized hydrolysis conditions, predicted by the polynomial model, were 1.72% (w/w) of sulfuric acid concentration and 112.0 min of acid hydrolysis time, for a fixed value of 10.0% (w/w) of solid load, with a total sugars yield of 78.8% for acid hydrolysis combined with a subsequent enzymatic hydrolysis step.

1. Introduction

The transport sector worldwide depends almost entirely on fuels derived from petroleum. A strategy for reducing the rate of fossil fuel consumption is to increase the use of renewable biofuels. Lignocellulosic materials (e.g., corn stover, switchgrass, wheat straw, forest wastes, etc.) enable the production of second generation biofuels, since these raw materials are not employed as human or animal food. In addition, lignocellulosic biomass is abundant, cheap and renewable and consists of approximately 75% polysaccharides (Bayer et al., 2007). Switchgrass (*Panicum virgatum* L.) is a perennial warm-season grass with high productivity, rapid growth, low water and nutritional requirements and a high tolerance to heat, cold and drought (McLaughlin et al., 1999). This vegetal species can be used for the production of biofuels, and most research studies have been focused on ethanol generation (Keshwani and Cheng, 2009).
The conversion of lignocellulosic materials to bioalcohols consists of three main steps: pretreatment, enzymatic hydrolysis and fermentation. The objective of the pretreatment is to alter the structure of the lignocellulosic matrix to increase cellulose digestibility. For efficient conversion of lignocellulosic biomass into fermentable sugars, the choice of pretreatment is extremely important (Diaz et al., 2013; Karapatsia et al., 2014). Each pretreatment has certain disadvantages, especially regarding the generation of compounds that can potentially act as inhibitors of enzymatic hydrolysis and fermentation. Acid based hydrolysis is considered

Please cite this article as: Paniagua A.I., Diez-Antolinez R., Hijosa-Valsero M., Sanchez M.E., Coca M., 2016, Response surface optimization of dilute sulfuric acid pretreatment of switchgrass (panicum virgatum l.) for fermentable sugars production, Chemical Engineering Transactions, 49, 223-228 DOI: 10.3303/CET1649038

to be an economical viable pretreatment process (Chandra et al., 2007). Dilute acid pretreatment targets the hemicellulose fraction liberating pentose sugars while redistributing lignin and disrupting the crystalline structure of cellulose fibrils. Cellulose hydrolysis, based on the selective degradation of cell-wall components by specific enzymes (Zuorro et al., 2015), is thus enhanced by the resulting increase in porosity and overall surface area of the treated material (Jeoh et al., 2007).
Response surface methodology (RSM) has been used successfully in many fields, including biomass pretreatments (Avci et al., 2013). In this paper, pretreatment of switchgrass with dilute sulfuric acid was conducted, using RSM with a central composite rotatable design (CCRD). The objective was to find the optimal conditions for the main hydrolysis parameters: acid concentration, biomass load and hydrolysis time, leading to maximum recovery of sugars and minimum generation of inhibitors in order not to affect the further stages of enzymatic hydrolysis and acetone-butanol-ethanol (ABE) fermentation with *Clostridium* sp.

2. Material and methods

2.1 Raw material

Switchgrass (*P. virgatum*), *Alamo* variety, was used to conduct the experimental runs. Switchgrass was grown in a test plot of ITACyL (Agrarian Technologic Institute of Castile and Leon) located in Valladolid (Spain). The material was produced and harvested at the beginning of November 2013. Feedstock was dried in an oven at 45ºC for 48 h, ground in a SM100 Comfort rotary mill (Retsch GmbH, Haan, Germany) and passed through a 1.0 mm screen (Ruangmee and Sangwichien, 2013). Milled material was stored at room temperature in airtight containers until being used.

2.2 Acid hydrolysis pretreatment

Dilute sulfuric acid at three different acid concentrations: 0.5, 1.0 and 1.5% (w/w) was used to treat 10 g of milled switchgrass at three different solid loads: 10, 17.5 and 25% (w/w). Pretreatment was performed in an autoclave at 121ºC (103 kPa) during three different times: 30, 60 and 90 min. The ranges and levels of the independent variables were determined according to preliminary studies. Different combinations of acid concentration, solid load and acid hydrolysis time were planned by an experimental design according to the RSM design.
After the autoclave pretreatment, the samples were cooled and the solid residue was recovered by vacuum filtration and washed with distilled water until it reached neutral pH, using a Buchner funnel with cellulose filters (Model 1238, Filter Lab, Barcelona, Spain). The liquid of the acid hydrolysis and the first aliquot of wash water were collected, separately, to measure the recovered volumes and analyze fermentable sugars (glucose, xylose and arabinose) and inhibitors (acetic acid, levulinic acid, 5-hydroxymethylfurfural (5-HMF) and furfural) concentrations. Pretreated solids were dried in an oven at 45°C during 48 h. Thereafter, moisture content was determined before storing in a plastic container at room temperature to be further used as substrate for the enzymatic saccharification.

2.3 Severity factor

The severity factor (SF) allows the comparison of different experimental conditions of dilute sulfuric acid pretreatment by combining the variables time, temperature and acid concentration according to Eq (1), (Lee et al., 2013):

$$SF = \log\{t * exp[(T_H - 100)/\ 14.75]\} - pH \quad (1)$$

where t is the acid hydrolysis time (min), T_H is the acid hydrolysis temperature (°C) and pH is the acidity of the dilute sulfuric acid solution used for the pretreatment.

2.4 Enzymatic hydrolysis

Pretreated solids were used as a substrate to study the effect of dilute sulfuric acid pretreatment in the subsequent hydrolysis of sugars by enzymes. The cellulose hydrolyzing enzymes Novozyme 50013 (cellulose complex), with an activity of 70 filter paper units (FPU)/g, and Novozyme 50010 (β-glucosidase), with an activity of 250 cellobiose units (CBU)/g, were used in all experiments. Enzymes were kindly provided by Novozymes A/S (Bagsvaerd, Denmark).
Enzymatic hydrolysis tests were carried out in 100 mL capped Erlenmeyer flasks containing 1 g of pretreated switchgrass (dry basis) and 33 mL of 50 mM citrate buffer (pH 4.8) together with 70 µL β-glucosidase enzyme (22 CBU/g pretreated biomass) and 300 µL cellulase complex enzyme (25 FPU/g pretreated biomass). Samples were incubated at 50°C and 150 rpm in an Infors HT Minitron orbital shaker (Infors AG, Bottmingen, Switzerland) during 48 h. After that, the samples were cooled at room temperature, filtered and washed with 20 mL distilled water using a Buchner funnel with cellulose filters. The liquid of the enzymatic hydrolysis and the wash water were taken separately and used for sugar analysis. Enzymatic hydrolysis was evaluated by

the ratio of total sugars mass (glucose, xylose and arabinose) released by unit of dry biomass (mg total sugars/g dry biomass).

2.5 Analytical methods

Moisture, ash and protein contents of the biomass were determined according to the National Renewable Energy Laboratory procedures (NREL, 2008). Structural carbohydrates including glucan, xylan and arabinan, as well as acid insoluble lignin (AIL) and acid soluble lignin (ASL) were examined using a two-stage sulfuric acid hydrolysis procedure (NREL, 2008).

The concentrations of released sugars (C6 sugars reported as glucose and C5 sugars reported as a total of xylose and arabinose), organic acids (acetic acid and levulinic acid), furfural and 5-HMF were determined according to NREL (2008). These compounds were quantified in liquid hydrolyzates on an Agilent 1200 HPLC equipment (Agilent Technologies, Santa Clara, CA, USA) with a 300 x 7.8 mm i.d. cation exchange column Aminex HPX-87H (Biorad, Hercules, CA, USA) and a Refractive Index Detector (RID, G1362A, Agilent Technologies). The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.6 mL/min and 60 °C. The injection volume was 20 µL. All samples were filtered through 0.22 µm filters prior to analysis.

2.6 Experimental design

A CCRD with three variables was used to determine the optimum combination of acid concentration, biomass load and hydrolysis time for maximizing the sugar recovery while minimizing the inhibitor concentrations. The experimental design was planned using Minitab 16 software (Minitab, State College, Pennsylvania, USA), which resulted in 19 trials. The experiments were performed with different combinations of the independent variables that include: 8 trials for factorial design, 6 trials for axial points (two for each variable) and 5 trials for replication of the central point. Each variable was studied at three levels with two axial points as can be seen in Table 1. The analysis of variance (ANOVA) of the results, the fitting models and the optimum determination were performed with Minitab 16 software.

Table 1: Experimental design showing actual (and coded) values for independent variables; severity factors (SF) and observed and predicted values for responses. Notes*: Obs: observed; Pred: predicted.*

Trial	Independent variables			SF	Glucose, mg/g		Xylose, mg/g		Tot Sug, mg/g		Tot Inh, mg/g	
	Ac conc, %	Sol load, %	t, min		Obs	Pred	Obs	Pred	Obs	Pred	Obs	Pred
1	1.5(+1)	25(+1)	30(-1)	1.58	145	148	191	198	364	376	26	28
2	1(0)	17.5(0)	60(0)	1.71	150	144	211	211	385	382	26	27
3	0.5(-1)	10(-1)	30(-1)	1.10	118	111	91	93	233	226	15	14
4	1(0)	17.5(0)	60(0)	1.71	146	144	224	211	397	382	29	27
5	0.5(-1)	10(-1)	90(+1)	1.58	139	136	192	185	361	348	25	23
6	1(0)	17.5(0)	9.6(-1.68)	0.91	122	123	104	118	253	268	21	20
7	1(0)	17.5(0)	60(0)	1.71	142	144	215	211	385	382	30	27
8	1(0)	17.5(0)	110.5(+1.68)	1.97	164	163	225	211	420	406	30	30
9	1(0)	30.1(+1.68)	60(0)	1.71	136	132	148	145	307	300	22	20
10	1.5(+1)	10(-1)	30(-1)	1.58	156	159	233	216	421	410	30	31
11	1.5(+1)	10(-1)	90(+1)	2.06	176	178	245	262	462	480	36	37
12	1.84(+1.68)	17.5(0)	60(0)	1.97	175	166	243	240	456	441	36	34
13	1(0)	17.5(0)	60(0)	1.71	143	144	206	211	378	382	26	27
14	1(0)	17.5(0)	60(0)	1.71	140	144	198	211	363	382	27	27
15	1.5(+1)	25(+1)	90(+1)	2.06	164	171	219	217	412	419	30	31
16	0.16(-1.68)	17.5(0)	60(0)	0.91	63	72	29	32	102	117	3	4
17	0.5(-1)	25(+1)	90(+1)	1.58	112	108	76	92	206	218	12	12
18	1(0)	4.9(-1.68)	60(0)	1.71	161	165	234	238	429	437	31	32
19	0.5(-1)	25(+1)	30(-1)	1.10	81	79	44	28	141	123	6	6

3. Results and discussion

3.1 Characterization of switchgrass

The compositional analysis of the untreated switchgrass (% dry basis) was: 33.0±0.4 glucan, 21.4±0.4 xylan, 2.4±0.4 arabinan, 20.3±1.2 acid insoluble lignin, 4.6±0.2 acid soluble lignin and 7.0±0.4 ash. The glucan, xylan, arabinan, lignin and ash contents of switchgrass were in agreement with values reported by other authors (Hu and Wen, 2008; Monti et al., 2008). The total protein in the feedstock was 1.6±0.1%. It is well known that there is a strong correlation between protein and the degree of cell wall lignification. Low protein content leads to high lignin contents (Hatfield et al., 1994).

3.2 Effect of acid hydrolysis conditions on sugar recovery and inhibitor generation

After performing the dilute sulfuric acid pretreatment, a significant amount of fermentable sugars was found in the hydrolysates, especially xylose. After pretreatment, recovered solids were subjected to subsequent enzymatic hydrolysis that leads to glucose release. Total sugars recovery and total inhibitors generation were calculated as the sum of the compounds recovered in the liquid of dilute acid pretreatment and in the enzymatic hydrolysates. Glucose, xylose, total sugars [sum of glucose, xylose and arabinose] and total potential inhibitors [sum of acetic acid (the main inhibitor produced), levulinic acid, 5-HMF and furfural] were calculated on the basis of grams of dry switchgrass. The results are presented in Table 1, which relates the severity factor (SF) and independent variables of dilute acid pretreatment with the observed responses. It was observed that the highest values of SF produced the highest concentrations of hydrolyzed sugars and inhibitors. It was verified that the removal of hemicelluloses (higher xylose recovery) enhanced the digestibility of cellulose (higher glucose recovery), as already observed by Chandra et al. (2007).

3.3 Statistical analysis

An ANOVA was performed to analyze the experimental data given in Table 1 and evaluate the effects of variables and their possible interactions. Coefficients of the model were evaluated by regression analysis and tested for their significance. ANOVA fittings of quadratic models for all responses are shown in Table 2.

Table 2: ANOVA table for the adjusted model of different responses.

Response	Source	Sum of squares	DF	Mean square	F Value	p-value	R^2 / Adj R^2
Glucose	Model	15128.3	9	1680.9	35.17	0.000	0.97/0.95
	Lack of fit	373.1	5	74.6	5.24	0.067	
	Residual	430.1	9	47.8			
Xylose	Model	87098.5	9	9677.6	43.85	0.000	0.98/0.96
	Lack of fit	1617.8	5	323.6	3.51	0.124	
	Residual	1986.5	9	220.7			
Total sugars	Model	196532.3	9	21837.1	67.29	0.000	0.99/0.97
	Lack of fit	2311.2	5	461.9	3.03	0.152	
	Residual	2920.7	9	324.9			
Total inhibitors	Model	1512.7	9	168.1	47.72	0.000	0.98/0.96
	Lack of fit	19.9	5	4.0	1.41	0.396	
	Residual	31.7	9	3.5			

The significance shown by R^2 and adjusted R^2 values indicates that variation in the responses was attributed to the three independent variables at more than 95% confidence level. The large F values and the corresponding small p-values also confirm that the models are significant. The goodness of the fit for the models is therefore assumed.

A three-variable quadratic polynomial regression model to predict glucose (Y_G), xylose (Y_X), total sugars (Y_{TS}) and total inhibitors (Y_{TI}) as a function of the three independent parameters: acid concentration (%, A), solid load (%, B) and hydrolysis time (h, C) was developed. The models in terms of non-coded variables are given in Eq (2), (3), (4) and (5):

$$Y_G = 52.07 + 128.21A - 1.28B + 0.39C - 36.37A^2 \quad (2)$$

$$Y_X = -72.71 + 326.52A - 3.66B + 2.96C - 101.40A^2 - 0.02C^2 \quad (3)$$

$$Y_{TS} = 75.72 + 395.86A - 10.10B + 3.41C - 142.31A^2 - 0.02C^2 + 4.65AB \quad (4)$$

$$Y_{TI} = -0.82 + 40.95A - 0.47B + 0.10C - 11.64A^2 \quad (5)$$

These equations predict all the responses considerably well with high R^2 and low p-values (Table 2). The importance of the independent variables and their effects can also be explained by the magnitude and sign of the coefficients. As observed from Eqs (2)-(5), acid concentration and hydrolysis time had a positive influence, whereas solid load had a negative influence on sugar recoveries and inhibitor generation. The magnitudes of the acid concentration linear terms were considerably higher than those of hydrolysis time and solid load. These observations are in agreement with previous works with sorghum bagasse that pointed out that the main effect was given by the acid concentration followed by hydrolysis time (Heredia-Olea et al., 2012). However, the quadratic terms of acid concentration negatively contributed to all responses. In addition,

quadratic terms of hydrolysis time negatively contributed to xylose and total sugars recoveries, and were not significant (p-values < 0.05) to glucose and total inhibitors. The interactions between independent variables were only significant for the positive interaction of acid concentration and solid load on total sugars. The predicted results obtained from quadratic models are shown in Table 1.

3.4 Optimization and response surface plots

The ANOVA analysis of optimal conditions was mathematically performed based on glucose, xylose, total sugars and total inhibitors. The objective was to maximize fermentable sugar recoveries while keeping the total inhibitors below 40 mg/g to avoid inhibition of *Clostridium* sp. The optimum values for the independent variables were found to be: 0.96% (w/w) acid concentration, 4.9% (w/w) solid load and 110.5 min hydrolysis time. The predicted and observed responses at optimal conditions are shown in Table 3.

Table 3: Predicted and observed responses at optimal conditions for the three-independent-variable model.

Responses	Pred (mg/g)	$Yield_{pred}$ (%)	Obs (mg/g)	$Yield_{obs}$ (%)	Deviation (%)
Glucose	178.7	48.7	197.0	53.7	10.2
Xylose	254.9	104.9	253.4	104.2	-0.6
Total sugars	476.4	74.8	485.8	76.3	2.0
Total inhibitors	35.8		36.6		2.2

The optimal values for the solid load and hydrolysis time correspond to the axial points of the experiment. Therefore, another complementary CCRD was used to design an experiment taking into account only acid concentration and hydrolysis time (the most significant variables), with wider ranges of values to know if the optimal values were beyond the axial points, and optimize the same responses for switchgrass. For this experiment, the solid load was fixed at 10% (w/w) for all trials (Avci et al., 2013; Ruangmee and Sangwichien, 2013). The experiment resulted in 13 trials (4 trials for factorial design, 4 trials for axial points and 5 trials for replication of the central point). Acid concentration was studied at three levels (0.75, 1.88 and 3.00%) with two axial points (0.28 and 3.47%) and hydrolysis time at three levels (30, 90 and 150 min) with two axial points (5.2 and 174.9 min). A two-variable quadratic polynomial regression model was developed, which was estimated as significant with an ANOVA. Figure 1 shows the contour plots associated to the response surface. The contour plots seem to confirm that there were significant interactions between acid concentration and hydrolysis time on glucose, xylose and total sugars recoveries and total inhibitors generation. Figure 1 shows that acid concentration had a more significant influence on glucose, xylose, total sugars recoveries and total inhibitors generation than the hydrolysis time because there were more response line levels located in the predefined range of acid concentration. The final optimal conditions for glucose, xylose, total sugars recoveries and total inhibitors generation were: acid concentration of 1.72% (w/w) and hydrolysis time of 112.0 min. The responses for these optimal conditions are shown in Table 4.

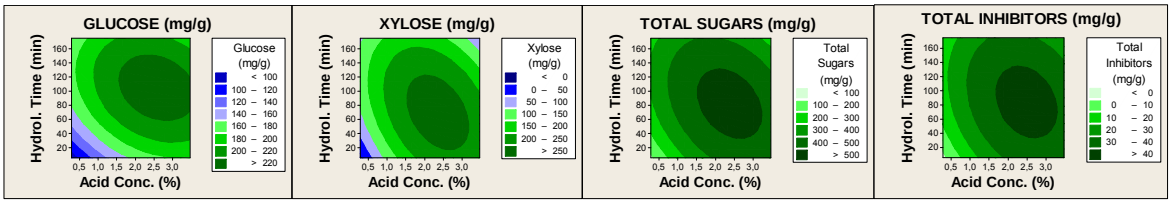

Figure 1: 2-D contour plots between coupled acid concentration (%) and hydrolysis time (min) for glucose (mg/g), xylose (mg/g), total sugars (mg/g) and total inhibitors (mg/g).

Table 4: Predicted and observed responses at optimal conditions for the two-independent-variable model.

Responses	Pred (mg/g)	$Yield_{pred}$ (%)	Obs (mg/g)	$Yield_{obs}$ (%)	Deviation (%)
Glucose	222.9	60.8	196.8	53.7	-11.7
Xylose	245.0	100.8	266.0	109.4	8.6
Total sugars	502.1	78.8	502.2	78.8	0.0
Total inhibitors	39.4		42.9		8.9

Sugar yields observed for the optimal conditions for two experiments (three independent variables and two independent variables) were similar, and there were no significant differences between them ($p < 0.05$).

4. Conclusions

The RSM is an effective method to optimize the operation parameters (acid concentration, solid load and hydrolysis time) of dilute sulfuric acid pretreatment of switchgrass to predict the maximum glucose, xylose and total fermentable sugars yields and a concentration of total inhibitors that does not affect the subsequent ABE fermentation by *Clostridium* sp. Optimal conditions were achieved with 1.72% (w/w) acid concentration, 112.0 min hydrolysis time and 10.0% (w/w) solid load. Under these conditions, a maximum glucose release of 196.8 mg/g (53.7% yield), a xylose release of 265.99 mg/g (109.4% yield) and total sugar release of 502.2 mg/g (78.8% yield), with a generation of total inhibitors of 42.9 mg/g, were obtained.

Acknowledgments

Authors are grateful to ITACyL and European Regional Development founds (FEDER) (POCTEP Project Nº 0450_AGRI_SOS_E_6) for financial support. MH-V is supported by a postdoctoral contract (DOC-INIA, grant number DOC 2013-010) funded by the Spanish Agricultural and Agrifood Research Institute (INIA) and the European Social Fund. Authors thank R. Antón and N. del Castillo for their technical help.

References

Avci A., Saha B.C., Dien B.S., Kennedy G.J., Cotta M.A., 2013, Response surface optimization of corn stover pretreatment using dilute phosphoric acid for enzymatic hydrolysis and ethanol production, Bioresource Technology, 130, 603-612.

Bayer E.A., Lamed R., Himmel M.E., 2007, The potential of cellulases and cellulosomes for cellulosic waste management, Current Opinion in Biotechnology, 18, 237-245.

Chandra R., Bura R., Mabee W., Berlin A., Pan X., Saddler J., 2007, Substrate pretreatment: the key to effective enzymatic hydrolysis of lignocellulosics, Biofuels, Ed. Olssen L., Springer, Heidelberg, 67-93.

Diaz A., Le Toullec J., Blandino A., de Ory I., Caro I., 2013, Pretreatment of rice hulls with alkaline peroxide to enhance enzyme hydrolysis for ethanol production, Chemical Engineering Transactions, 32, 949-954, DOI: 10.3303/CET1332159.

Hatfield R.D., Jung H.J.G., Ralph J., Buxton D.R., Weimer P.J., 1994, A comparison of the insoluble residues produced by the Klason lignin and acid detergent lignin procedures, Journal of the Science of Food and Agriculture, 65, 51–58.

Heredia-Olea E., Pérez-Carrillo E., Serna-Saldívar O., 2012, Effects of different acid hydrolyses on the conversion of sweet sorghum bagasse into C5 and C6 sugars and yeast inhibitors using response surface methodology, Bioresource Technology, 119, 216-223.

Hu Z., Wen Z., 2008, Enhancing enzymatic digestibility of switchgrass by microwave assisted alkali pretreatment, Biochemical Engineering Journal 38 (3), 369-378.

Jeoh T., Ishizawa C., Davis M., Himmel M., Adney W., Johnson D., 2007, Cellulase digestibility of pretreated biomass is limited by cellulose accessibility, Biotechnology Bioengineering, 98, 112-122.

Karapatsia A., Penloglou G., Pappas I., Kiparissides C., 2014, Bioethanol production via the fermentation of *Phalaris aquatica* L. hydrolysate, Chemical Engineering Transactions, 37, 289-294, DOI: 10.3303/CET1437049.

Keshwani D.R., Cheng J.J., 2009, Switchgrass for bioethanol and other value-added applications: A review, Bioresource Technology, 100, 1515-1523.

Lee J.Y., Ryu H.J., Oh K.K., 2013, Acid catalyzed hydrothermal severity on the fractionation of agricultural residues for xylose rich hydrolyzates, Bioresource Technology, 132, 84-90.

McLaughlin S.B., Bouton J., Bransby D., Conger B.V., Ocumpaugh W.R., Parrish D.J., Taliaferro C., Vogel K.P., Wullschleger S.D., 1999, Developing switchgrass as a bioenergy crop. Perspectives on New Crops and New Uses, Ed. Janick J., ASHS Press, Alexandria VA, 282-299.

Monti A., Di Virgilio N., Venturi G., 2008, Mineral composition and ash content of six major energy crops, Biomass and Bioenergy, 32, 216-223.

NREL (National Renewable Energy Laboratory, USA), 2008, Standard Procedures for Biomass Compositional Analysis < www.nrel.gov/biomass/analytical_procedures.html> accessed 28.10.2015

Ruangmee A., Sangwichien C., 2013, Response surface optimization of enzymatic hydrolysis of narrow-leaf cattail for bioethanol production, Energy Conversion and Management, 72, 381-388.

Zuorro A., Lavecchia R., Maffei G., Marra F., Miglietta S., Petrangeli A., Familiari G., Valente T., 2015, Enhanced lipid extraction from unbroken microalgal cells using enzymes, Chemical Engineering Transactions, 43, 211-216, DOI: 10.3303/CET1543036.