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Preliminary optimization of alkaline pretreatment for ethanol production from vineyard pruning

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Abstract

Vineyard pruning is a potential lignocellulosic feedstock for bioethanol production from agricultural woody residues, due to its high sugar content and ready availability in whole Europe. Ethanol production from lignocellulosic biomass requires a pretreatment step and then enzymatic hydrolysis process to release sugars for fermentation to ethanol. In this work, alkaline pretreatment with NaOH on vineyard residues was investigated on laboratory scale. The raw material was firstly characterized in order to determine cellulose, hemicellulose and lignin content, using the standard laboratory analytical procedures for biomass analysis provided by the National Renewable Energy Laboratory (NREL). Then, based on Response Surface Methodology tool (RSM), a Box-Behnken model was chosen to define a design of experiments (DoE) in terms of the three independent variables that influence the process: the NaOH concentration, the reaction time and the temperature of the pretreatment process. According to the design, 15 samples of raw material were submitted to alkaline pretreatment and subsequent enzymatic hydrolysis and the glucose yield from whole process was calculated. The statistical optimization was carried out with Minitab 17 software, in order to determine the best operative pretreatment condition by maximizing the glucose yield from enzymatic hydrolysis.
Results show that the best glucose yield was obtained at the highest sodium hydroxide concentration and temperature of the reaction; this condition allowed to achieve very significant glucose yield thanks to lignin removal performed with the pretreatment.

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Keywords: Vineyard pruning; Alkaline pretreatment; NaOH solution; Ethanol; Enzymatic hydrolysis

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1. Introduction

Lignocellulosic biomass represents a promising source for bioethanol production. The downside is the structural complexity of the material, along with the high lignin content, that hinder the digestibility of cellulose fraction during the enzymatic hydrolysis. Utilization of lignocellulosic biomass in native form results in low cellulose enzymatic digestibility yield (<20%) and involves a large consumption of enzyme [1].Therefore, a pretreatment is required and this is the limiting step.

Grapevine is one of the most common Mediterranean crop, in particular in Italy its cultivation covers about 725,000 ha, with an annual pruning amount of about 1.05 million t of dry matter [2]. After harvesting, vineyard residues are usually left in the fields and they are considered as agricultural waste. Therefore they could be a low-cost source of sugars, convertible into valuable products, such as ethanol. Although there are several studies about olive tree pruning, almond tree pruning and similar biomasses [3, 4, 5], the use of vineyard residues for ethanol production is rarely mentioned in Literature.

Alkaline pretreatment is one of the most studied pretreatment approaches, it consists in a delignification process, in which the main effect is the saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components [6].Moreover, the action of NaOH makes the lignocellulosic matrix swell, decreasing the polymerization degree and crystallinity, increasing the internal surface area, disrupting the lignin structure and finally breaking the structural linkages between lignin and carbohydrates [7, 8].The efficiency of this pretreatment process depends on several parameters, such as alkalinity level, residence time and reaction temperature. Furthermore, one advantage of this method is that usually residence time and reaction temperature are lower than other available technologies [9, 10, 11].

In order to optimize the process, response surface methodology (RSM), a multivariate statistic technique, was used. RSM allows to resolve multivariate equations starting from the data observed for an appropriate experimental design. This approach simultaneously evaluates and manages each variable of the problem to attain the best system performance[12, 13].

The aim of this work was to implement RSM statistical preliminary approach to alkaline pretreatment, in order to maximize glucose production from vineyard pruning by enzymatic hydrolysis process.

2. Materials and methods

Raw material: Vineyard pruning (Vitis vinifera) used in this study were obtained from a local farm in Umbria, Italy. Biomass was collected from Grechetto grapevine after the harvest of 2014. The raw material was air dried at room temperature to reach the equilibrium moisture content, then it was mechanically reduced to a particle size of 2 mm with a laboratory hammer mill (Retsch SM2000) for pretreatment tests. Then the grinded vineyard pruning was oven dried at 40°C to lower the moisture content below 10 wt% and stored in sealed polyethylene bags for further use.

A part of vineyard pruning sample was carried out to an additional size reduction to 0.5 mm with a centrifugal mill (Retsch ZM200) in order to analyze the chemical composition.

Alkaline NaOH pretreatment: Pretreatment experiments were carried out adding 5 g of raw material and 50 mL NaOH solution in 100 mL glass flasks, forming a slurry of biomass and alkaline solution; all the samples were then heated to determined temperature and residence time in an autoclaving system (Nuve OT 90L).

After pretreatment, the samples were cooled to room temperature and the pH value was measured; to neutralize the pretreated slurry, a small amount of H_2SO_4 (1 N) was added to reach pH equal to 7.

Then the pretreated biomass was recovered by vacuum filtration with filter paper (Filter-Lab 1249) and a Buchner funnel and then hydrolyzed.

Enzymatic digestion: Pretreated samples (5% solids loading) were then submitted to enzymatic saccharification in 250 mL Erlenmeyer flasks, added a 0.1 M citrate buffer (pH 4.6) to reach a pH 5.0.

Each flask was tightly sealed with a rubber stopper and introduced to an incubated bench-top orbital shaker (IKA KS4000 I control), and allowing to come to thermal equilibrium for 1 h at 50 °C and 100 rpm shaking. After reaching thermal equilibrium, the Cellic®CTec2 enzyme (Novozymes, USA) was added to the flasks at 0.23 g per gram of dry solid pretreated substrate for glucan digestion for 48 hours. The hydrolysates were withdrawn periodically for glucose analysis by HPLC.

Analytical procedures: the composition of the raw material and of the pretreated slurry was investigated using the technical analytical procedures provided by National Renewable Energy Laboratory [14, 15, 16]. Moisture and ash content in biomass were evaluated by termogravimetric analyzer (Leco TGA 701).

Sugar monomers were determined after acid hydrolysis by HPLC system (YoungLin 9100), equipped with Aminex HPX-87H column, a guard column and a refractive index detector; the column and detector work at 50°C at a flow rate of 0.6 mL/min with water – 0.005 M sulfuric acid as mobile phase.

Insoluble lignin content in biomass was evaluated by a thermogravimetric stage performed in a muffle furnace (Nuve MF106) at 575°C.

Aminex HPX – 87H column does not resolve the three hemicellulosic sugars xylose, mannose, and galactose. Thus, the sum of the oligomeric sugars xylan, mannan, and galactan was denoted with XMG (capitalized) while the sum of the corresponding hydrolyzed sugars, xylose, mannose, and galactose was indicated with xmg (small letters) [17].

All analytical determinations are performed in triplicate and average results are shown.

Design of experiments (DoE): a three variables Box-Behnken model was used in order to determine the optimum pretreatment parameters for maximizing the glucose production from vineyard pruning by enzymatic hydrolysis. The chosen variables are the NaOH solution concentration (C), the temperature (T) and the reaction time of the pretreatment process (RT).As preliminary evaluation, the variables were defined in three levels in the following ranges, respectively: 0.5-2.5% (w/v), 80-120 °C, 30-60 minutes.

In order to minimize the chemicals consumption and the related environmental effects, the range of NaOH solution concentration is established in a limited gap not exceeding 2.5%; the effects of temperature and time are mainly evaluated in this study.

Therefore, a total of 15 experiments with three replicates in central point (CP) were carried out in a random order in order to minimize the effects of external factors (Table 1).

Glucose yield from enzymatic hydrolysis in relation to the substrate glucan content was selected as response of RSM application,

Regression analysis and subsequently ANOVA analysis were performed with statistical software Minitab 17 (Minitab Inc.), based on full quadratic polynomial model, with a confidence level of 95% ($p = 0.05$).

3. Results and discussion

3.1. Compositional analysis of pretreated vineyard pruning and enzymatic hydrolysis results

The raw material is composed by 30.8% of glucan, 9.8% of XMG and 26.4% of lignin (dry weight basis). Based on this characterization, the composition of pretreated samples was determined and the lignin removal and glucose yield from enzymatic hydrolysis were also calculated, as reported in Table 1.

The solid recovery after pretreatment ranges between 66.3% and 83.7%, according to the rate of lignin removal. Alkaline pretreatment allows to solubilize lignin and to improve the accessibility of enzymes to cellulosic structure. The pretreatment is not very stricter in terms of alkaline solution concentration and

then the maximum lignin removal value is 32.8% of the initial content in raw material (Table 1). The lignin solubilization increases with the solution concentration and the temperature reaction; several studies performed on other agricultural residues endorse the seoccurrences [9, 18].

Table 1: Experiments performed by the Box-Behnken design (coded values of the variables in parenthesis and CP= central point), composition of pretreated vineyard pruning and calculated parameters

Run	Experimental factors			Compositional content (%)			Calculated parameters		
	NaOH concentration (% w/v)	Temperature (°C)	Residence time (min)	Glucan	XMG	Lignin	Solid recovery (%)	Lignin removal (%)	Glucose yield from EI at 48h (%)
1	1.5 (0)	80 (-1)	60 (1)	26.3	6.8	28.5	80.0	26.1	62.0
2	1.5 (0)	80 (-1)	30 (-1)	26.1	10.6	27.7	80.3	15.8	83.4
3	1.5 (0)	100 (0)	45 (0)	30.1	9.1	28.1	75.4	20.0	63.1
4	1.5 (0)	120 (1)	60 (1)	28.5	7.4	26.7	68.6	30.7	58.4
5	0.5 (-1)	100 (0)	30 (-1)	25.1	9.3	28.6	83.7	9.6	78.1
6	1.5 (0)	100 (0)	45 (0)	30.1	9.7	28.0	74.3	21.4	53.7
7	2.5 (1)	100 (0)	30 (-1)	28.3	8.3	27.8	71.2	25.3	96.9
8	1.5 (0)	120 (1)	30 (-1)	33.0	8.7	27.2	69.9	31.1	82.0
9	0.5 (-1)	120 (1)	45 (0)	29.4	10.3	29.9	80.6	8.8	54.1
10	2.5 (1)	120 (1)	45 (0)	32.1	7.2	26.8	66.3	32.8	96.2
11	2.5 (1)	100 (0)	60 (1)	31.9	11.7	27.8	71.5	27.5	82.3
12	0.5 (-1)	80 (-1)	45 (0)	30.9	10.5	27.8	82.5	13.2	72.7
13	1.5 (0)	100 (0)	45 (0)	29.7	7.5	26.9	77.8	21.1	47.3
14	0.5 (-1)	100 (0)	60 (1)	30.3	10.4	28.7	82.8	10.1	50.4
15	2.5 (1)	80 (-1)	45 (0)	31.8	9.8	25.5	77.0	25.8	56.2

The glucose yield from enzymatic hydrolysis is good related to the severity of pretreatment: the highest values of glucose are obtained at 2.5% of alkaline solution concentration and, at constant value of NaOH concentration, decreasing the time reaction. The highest cellulose digestibility is reached at operative conditions of pretreatment as 2.5% NaOH concentration, the reaction temperature of 100 °C and 30 min of residence time (run n.7).

3.2. Optimization of pretreatment for glucose production

The glucose yield from enzymatic hydrolysis was used in Response Surface Methodology tool as response to optimize the pretreatment stage.

The polynomial equation describing the glucose yield behaviour is (1):

$$\text{Glucose yield}\ (\%) = 430 - 104.4\ \text{C} - 3.33\ \text{T} - 5.65\ \text{RT} + 10.27\ \text{C}^2 + 0.01207\ \text{T}^2 + 0.0530\ \text{RT}^2 + 0.732\ \text{C T} + 0.219\ \text{C RT} - 0.0018\ \text{T RT} \quad (1)$$

The analysis of variance of the model (ANOVA) is carried out to evaluate the statistical significance of the model. The validation of the model is achieved when F value of the regression is greater than the tabulated F value; this condition is verified and the statistical significance of the model is confirmed by a P value of 0.025, lower than the confidence level (0.05).

The statistical significance observed from the ANOVA analysis is confirmed by the correlations between experimental and theoretical values, displayed in Fig. 1. The mathematical model is well fitted to experimental data with a correlation factor of 0.923.

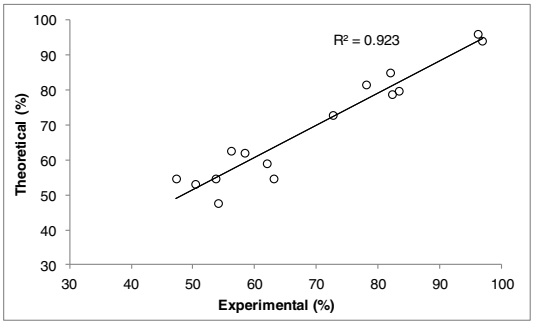

Fig. 1: Correlation between experimental and theoretical glucose yield values for vineyard pruning

The optimal condition for pretreatment to maximize the glucose yield is obtained at 2.5% NaOH concentration, performing the process at 120°C for 40 minutes; according to the initial content of glucose in the raw material, the best operative condition results in a glucose production of 202 g of glucose /kg of raw material.

4. Conclusion

A preliminary investigation on the influence of alkaline pretreatment on glucose production from vineyard pruning was performed. Three representative variables of the pretreatment were ranged in order to optimize the response as glucose yield from enzymatic hydrolysis.

The results show that mainly NaOH solution concentration and the reaction time influence the response, in particular the best solutions are obtained at high values of concentrations and low reaction time. In the investigated experimental region, the optimum glucose yield is obtained at 2.5% of NaOH concentration, 120°C of reaction temperature and 40 minutes of residence time and it is equal to 202 g of glucose/kg of raw material.

Next improvements of this study should examine a larger range of variables values, in particular the NaOH concentration that mainly influences the glucose production.

5. Copyright

References

[1] Mood, S.H.; Golfeshan, A.H.; Tabatabaei, M.; Jouzani, G.S.; Najafi, G.H.; Gholami, M.; Ardjmand, M. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. Renewable and Sustainable Energy Reviews. 27(2013), 77-93.

[2] Istat, 2011.

[3] Cara, C.; Ruiz, E.; Ballesteros, M.; Manzanares, P.; Negro, M.J.; Castro, E. Production of fuel ethanol from steam-explosion pretreated olive tree pruning. Fuel. 87(2008), 692–700.

[4] Cotana, F.; Cavalaglio, G.; Gelosia, M.; Coccia, V.; Petrozzi, A.; Nicolini, A. Effect of double-step steam explosion pretreatment in bioethanol production from softwood. Applied Biochemistry and biotechnology. 174 (2014), 156-167.

[5] Cuevas, M.; García, J.F.; Sànchez, S. Enhanced enzymatic hydrolysis of pretreated almond-tree prunings of sugar production. Carbohydrate Polymers. 99(2014), 791-799.

[6] Zheng, Y.; Pan, Z.; Zhang, R. Overview of biomass pretreatment for cellulosic ethanol production. International Journal of Agricultural and Biological Engineering. 2(2009), 51-68.

[7] Behera, S.; Arora, R.; Nandhagopal, N; Kumar, S. Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. Renewable and Sustainable Energy Reviews. 36(2014), 91-106.

[8] Camesasca, L.; Ramírez, M.B.; Guigou, M.; Ferrari, M.D.; Lareo, C. Evaluation of dilute acid and alkaline pretreatments, enzymatic hydrolysis and fermentation of napiergrass for fuel ethanol production. Biomass and Bioenergy. 74(2015), 193-201.

[9] Kim, I.; Han, J. Optimization of alkaline pretreatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass and Bioenergy. 46(2012), 210-217.

[10] McIntosh, S.; Vancov, T. Optimisation of dilute alkaline pretreatment for enzymatic saccharification of wheat straw. Biomass and Bioenergy. 35(2011), 3094-3103.

[11] Chen, Y.; Sharma-Shivappa, R.R.; Keshwani, D.; Chen, C. Potential of agricultural residues and hay for bioethanol production. Applied Biochemistry and Biotechnology. 142(2007), 276-290.

[12] Maache-Rezzoug, Z.; Pierre, G.; Nouviaire, A.; Maugard, T.; Rezzoug, S.A. Optimizing thermomechanical pretreatment conditions to enhance enzymatic hydrolysis of wheat straw by response surface methodology. Biomass and Bioenergy. 35(2011), 3129-3138.

[13] Kim, I.; Han, J.-I. Optimization of alkaline pre treatment conditions for enhancing glucose yield of rice straw by response surface methodology. Biomass and Bioenergy. 46(2012), 210-217.

[14] Sluiter, A.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton. D. Determination of Extractives in Biomass. NREL/TP-510-42619. Golden: National Renewable Energy Laboratory. 2008a.

[15] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples. NREL/TP-510-42623. Golden: National Renewable Energy Laboratory. 2008b.

[16] Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. Determination of Structural Carbohydrates and Lignin in Biomass. NREL/TP-510-42618. Golden: National Renewable Energy Laboratory. 2012.

[17] Buratti, C.; Barbanera, M.; Lascaro, E. Ethanol Production from Vineyard Pruning Residues with Steam Explosion Pretreatment. Environmental Progress&Sustainable Energy. 34 (2015), 802-809.

[18] Zhao, Y.;Wang, Y.;Zhu,J.Y. ; Ragauskas, A.; Deng, Y. Enhanced Enzymatic Hydrolysis of Spruce by Alkaline Pretreatment at Low Temperature. Biotechnology and bioengineering. 99 (2008), 1320-1328.

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