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Production of bioethanol in a second generation prototype from pine wood chips

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Abstract

This paper deals with the production of bioethanol from ligno-cellulosic biomass, in particular a softwood biomass from forestry sector is tested: pine wood chip is a residual biomass obtained from coppice maintenance with a very interesting potential. Second generation bioethanol production prototype from ligno-cellulosic biomass consists of the following monitored parts: steam production system, steam explosion reactor for biomass pretreatment (temperature range 180-240 °C), enzymatic hydrolyser, fermenter and distiller. The maximum system size is around 2-3 kg input biomass each cycle.
Selected biomass are tested modifying reaction temperature and retention time of the process and optimizing severity parameter ($logR_0$ between 2.7 and 4.6). Enzymatic hydrolysis is conducted with Ctec2, cellulase complex which consists of a blend of aggressive cellulases (endocellulase and exocellulase), β-glucosidases and hemicellulase, while Saccharomyces cerevisiae yeast ("red ethanol") is used for the fermentation stage. During hydrolysis and fermentation stages intermediate collections at different time are carried out and samples analyzed in order to evaluate the progress of each phase (maximum glucose concentration obtained 18.8 mg/ml).
The results are presented in terms of raw (cellulose content around 32%) and steam exploded material composition, hydrolyzed sugars and acids content in samples, ethanol content after fermentation at different retention time. Both hydrolysis and fermentation are analyzed comparing real and theoretical efficiency. Finally, mass flows in the different selected conditions are evaluated providing a results in terms of ethanol percentage in function of raw material weight. As a result from 100 g of raw material dry basis (32 g of cellulose), 10.6 g of ethanol were obtained.

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Keywords: bioethanol, second generation biofuels, steam explosion, enzymatic hydrolysis, fermentation

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Nomenclature

WIS	water insoluble substrate
DM	dry matter
HMF	hydroxymethylfurfural
NREL	National renewable energy laboratory
HPLC	high performance liquid chromatography
AIR	acid-insoluble residue
AIL	acid-insoluble lignin
TGA	thermal-gravimetric analysis
HY	hydrolysis yield
WIS_{DM}	dry mass fraction of insoluble solids

1. Introduction

The production of bioethanol from ligno-cellulosic biomass is strategic to reach the mandatory European targets (10% replacement of fossil fuels for transport at 2020), in a sustainable technology way that avoid competition with food agriculture, allow the use of agriculture and forestry residues and reduce environmental risks which are associated to first generation biofuels [1-4]. Moreover, second generation bioethanol pathway has several promising applications in the biorefinery concept [5], from lignin processing for resin and chemicals production [6], to nanocrystalline cellulose as polymer matrix nanocomposites [7], to bioethanol reforming for power production in molten carbonate fuel cells [8].

The ligno-cellulosic biomass to bioethanol process consists of raw material pretreatment, hydrolysis, fermentation and distillation. Among physical and chemical pretreatments, necessary to remove the barriers and make cellulose more accessible to hydrolytic enzymes for conversion to glucose [9], steam explosion is the most commonly used for biomass deconstruction [10]; the physical process causes also solubilization of hemicellulosic fraction and extractives, while water insoluble substrate (WIS) is usually washed before enzymatic hydrolysis [11]. Enzymatic hydrolysis has low costs compared to acid or alkaline hydrolysis, no corrosion problem and good efficiency that can be improved using a mixture of several enzymes, in particular endoglucanase, exoglucanase and β-glucosidase. Yeasts convert sugars into ethanol, obtaining a beer (mixture of ethanol, cell mass and water); finally bioethanol is concentrated by distillation and dehydration to meet fuel specifications [12,13].

Biomass from softwood, for example pine and spruce, is a very abundant feedstock, alternative to more typical materials like arundo donax or straw [14,15], but its implementation in ethanol production is very sensitive due to the high content of lignin and the difficulties of steam explosion pretreatment for the disruption of lignin carbohydrate matrix [16-18].

The main objective of the present work is to test a specific feedstock, pine wood chip, in a second generation bioethanol prototype implemented in Biomass Research Centre laboratories in the University of Perugia [19]. The process consists of biomass pretreatment (steam explosion), solid separation from liquid, enzymatic hydrolysis and fermentation by saccharomyces cerevisiae. Steam explosion efficiency is evaluated in function of treatment time and temperature; glucose production allow to define enzymatic hydrolysis performance, while ethanol content after fermentation is the parameter for evaluating fermentation efficiency yield, overall pathway efficiency and mass flows starting from raw material dry matter (DM).

Moreover, a two steps steam explosion process has been tested to decrease process conditions (temperature in particular), as suggested in other works [20,21], but without acid treatments; the action should reduce inhibitors content produced by carbohydrate degradation, like furfurals, hydroxymethylfurfural (HMF) and acetic acid [22,23] and phenolic compounds by lignin breakdown, like vanillin, syringaldehyde, 4-hydroxybenzaldehyde and ferulic acid [24]. The objective of two steps steam explosion is to lead a pre-explosion with low temperature conditions, in order to obtain a liquid fraction with low inhibitors useful for ethanol extraction from hemicellulose, and a second steam explosion with higher temperatures, in order to maximize ethanol production from cellulose in solid fraction.

2. Materials and methods

2.1. Raw material

Pine tree wood was collected locally (Umbria region), during forestry maintenance, and after chipping stage that allowed to obtain a biomass size around 3-4 cm, finally air-dried at room temperature. The composition of the raw material was analyzed using NREL (National Renewable Energy Laboratory) method [25]. Biomass DM measured in the sample was around 85%. Hemicellulose composition was determined adding xylose, mannose, arabinose and galactose content from acid hydrolysis analysis in HPLC (High Performance Liquid Chromatography), while cellulose as glucose content. Cellulose content in raw material was 32.09% (32.09 g cellulose each 100 g biomass dry basis).

Raw material was grinded at 18 mesh, then it was extracted consecutively with water and with ethanol (two-step extraction procedure). This procedure ensured the extraction of resins, fats, wax, oils, catechol. The percentage of the extracts was referred to the dried biomass.

Cellulose and hemicellulose content of the extracted and dried solid residue was determined based on monomers content measured after a two-step acid hydrolysis procedure to fractionate the fibre. The sample was dried at 40°C for 24h to 48h to drive the sample at a final moisture content less than 10%. A first step with 72% (w/w) H_2SO_4 at 30°C for 60 min was used. In a second step, the reaction mixture was diluted to 4% (w/w) H_2SO_4 and autoclaved at 121°C for 1h. This hydrolysed liquid was then analysed for sugar content by HPLC.

The remaining acid-insoluble residue (AIR) is used to determinate acid-insoluble lignin (AIL) excluding ash content. Ash determination was performed with a extractive free biomass sample of 1-2 g by thermal-gravimetric analysis (TGA).

Percentage composition of all components presented into pine wood chip was referred to the biomass dry matter including the extractives.

After pre-treatment, the composition of solid fraction was determined as described for raw material, except that no extraction was used. Glucose concentration from enzymatic hydrolysis and ethanol concentration from fermentation were measured by HPLC. All analytical determinations were performed twice and average results are shown in table 1.

Table 1. Composition of raw material (pine wood chips).

Composition	% (dry basis)
Hemicellulose	14.22%
Cellulose	32.09%
Acetyl groups	2.78%
Ash	2.39%
Extractives	15.55%
Acid insoluble lignin (AIL)	31.15%
Other	1.82%

2.2. Steam explosion pre-treatment

Biomass was processed after air-drying (moisture content around 15%) and as received (moisture content 30-50%) in order to test a dry or wet biomass.

Biomass quantity each pre-treatment was 700-750 gr. Moreover some samples were pretreated in a two steps process, recovering WIS after the first steam explosion, charging the reactor with the collected material, and carrying out a second steam explosion.

The treatment severity was quantified by a semi-empirical parameter called severity parameter, $\log R_0$, combining treatment time and temperature according to the equation (1) [26]:

$$R_0 = t \cdot e^{[(T-100)/14.75]} \quad (1)$$

where t is the time in minutes and T the temperature in degrees Celsius.

The research campaign explored 28 pre-treatment conditions, changing severity parameter in dry and wet samples, in single and double steps steam explosions. Between the pre-treatments, 8 samples were selected for the hydrolysis and fermentation stages: main parameters of the steam-exploded samples, selected for the hydrolysis and fermentation steps, are shown in table 2.

After pre-treatment, the material was pressed in order to separate WIS from liquid fraction. WIS was washed and pressed three times to remove inhibitors and remaining hemicellulose.

Pre-treatment efficiency was described in terms of cellulosic material recovery through a sieve (pore size around 1mm) by using the following equation (2):

$$\%\ \text{Cellulose recovery} = \frac{\text{cellulose in WIS}_{\text{dm}}(\text{g})}{\text{cellulose in raw material}_{\text{dm}}(\text{g})} \quad (2)$$

Table 2. Main parameters in the steam explosion experimentations.

Sample name	Weight (gr)	Moisture (%)	Temperature (°C)	Time (min)	$LogR_0$
CP013	700	14.43	215	10	4.39
CP013W	854	44.75	215	10	4.39
CP016	700	14.43	220	10	4.53
CP016W	957	33.53	220	11	4.57
CP020W	950	32.04	170 (2nd step 220)	9 (2nd step 9)	4.50
CP028W	980	36.50	170 (2nd step 220)	30 (2nd step 9)	4.53
CP030W	980	34.15	170 (2nd step 220)	9 (2nd step 4,5)	4.21
CP037W	980	40.47	170 (2nd step 220)	30 (2nd step 4.5)	4.27

2.3. Enzymatic hydrolysis

The reaction was carried out in a bench-scale bioreactor, 6 liters capacity, equipped with a software that allow to monitor the process continuously and to maintain constant the operating conditions (pH, temperature, rotation speed).

The enzyme was provided by Novozymes, Cellic™Ctec2. In order to assess the best pretreatment conditions, WIS enzymatic hydrolysis was carried at low solids loading, 5% (g of dry solids / volume of the hydrolysis mixture). The reaction was conducted at pH 5.0, temperature 50°C, 250 rpm rotation speed at the same dosages of Cellic™ Ctec2 for 48h. Samples were collected after 0.5, 1, 2, 24 and 48 hours for glucose concentration determination. Hydrolysis yields (HY) were calculated as follows in the equation (3), considering the transformation of cellulose into glucose and cellobiose [27,28].

$$\eta_{Hy} = \frac{r_{Gg} f_G + r_{Gcb} f_{cb}}{WIS_{DM} \%_g} x\ 100 \quad (3)$$

where r_{Gg} is the molecular weight ratio of a cellulose monomer to glucose (162,16/180,18), f_G is glucose mass fraction into the slurry at the end of hydrolysis, r_{Gcb} is the molecular weight ratio of two glucan monomers to cellobiose (324.32/342.34), f_{cb} is cellobiose mass fraction, WIS_{DM} is the initial dry mass fraction of insoluble solids insert into the bioreactor, $\%_g$ is percentage of glucan in WIS_{DM}.

2.4. Fermentation

Fermentation was performed by Saccharomyces cerevisiae Red Ethanol® provided by Fermentis in dry form. After the enzymatic hydrolysis, the reactor was conducted at 5.0 pH, 32°C temperature and 150 rpm rotation speed.

A 26.9 g solution of urea (400 g/l) was addicted to bioreactor as nitrogen source. Total dry yeast (2,45 g) was rehydrated in water (24,5 g) at 30 °C for 15 min and then inoculated. The fermentation was carried out for 48h and samples were collected after 1, 3, 24 and 48h for ethanol concentration determination.

3. Results and discussion

3.1. Steam explosion

Steam explosion tests were carried out and WIS was collected evaluating the recovered fraction in percentage, as shown in fig. 1. The recovered WIS decreases if severity parameter increases, due to the solubilization of a larger quantity of material and also some losses during the recovery process; however, at the same time, the quality of biomass deconstruction should improve.

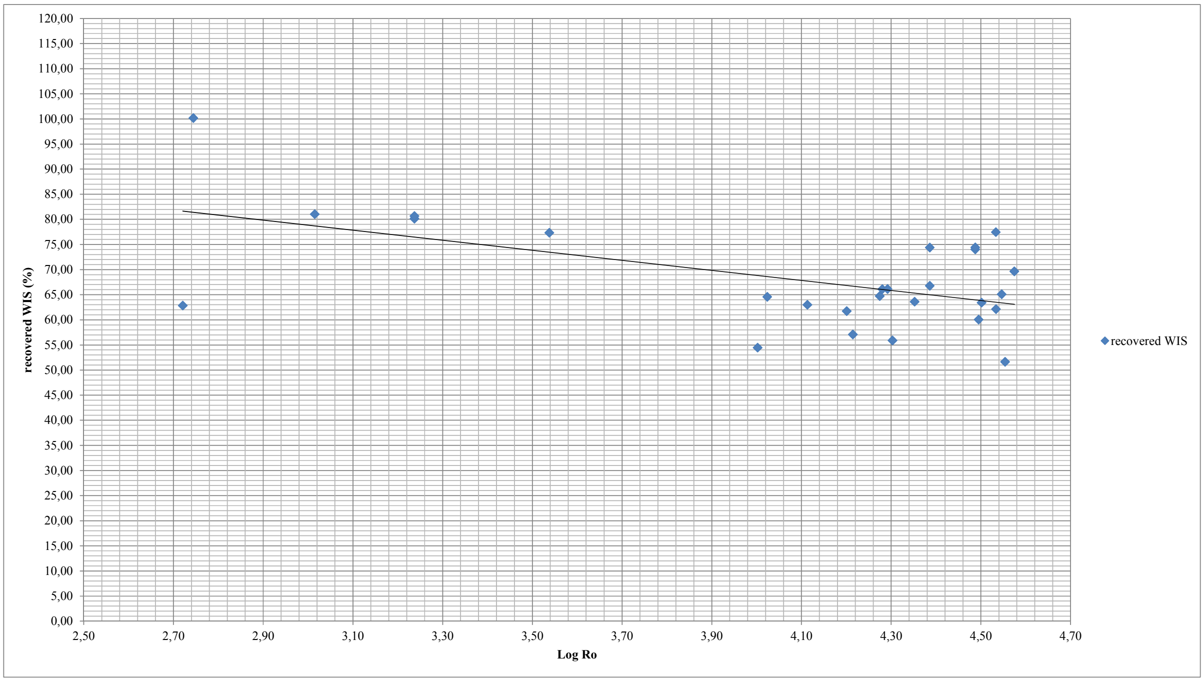

Fig. 1. WIS recovered after steam explosion pretreatment.

Steam explosions were carried out comparing dry and wet samples and comparing single-step with double-step explosion. Fig. 2 and fig. 3 show both the comparisons, considering obtained cellulose in function of severity parameter in the exploded samples.

The first comparison shows that high severity parameter values reduce cellulose content, probably cellulose degrades to other sub-products and inhibitors; low severity parameter values do not allow to deconstruct biomass and to solubilize hemicellulose and extractives.

Another interesting comparison is carried out between single-step and double-step explosion: considering the same severity parameter seems that double-step increases cellulose content, probably improving hemicellulose solubilization.

Optimal steam explosion conditions, in terms of cellulose content in the exploded sample, seems to have severity parameter between 4.2 e 4.3.

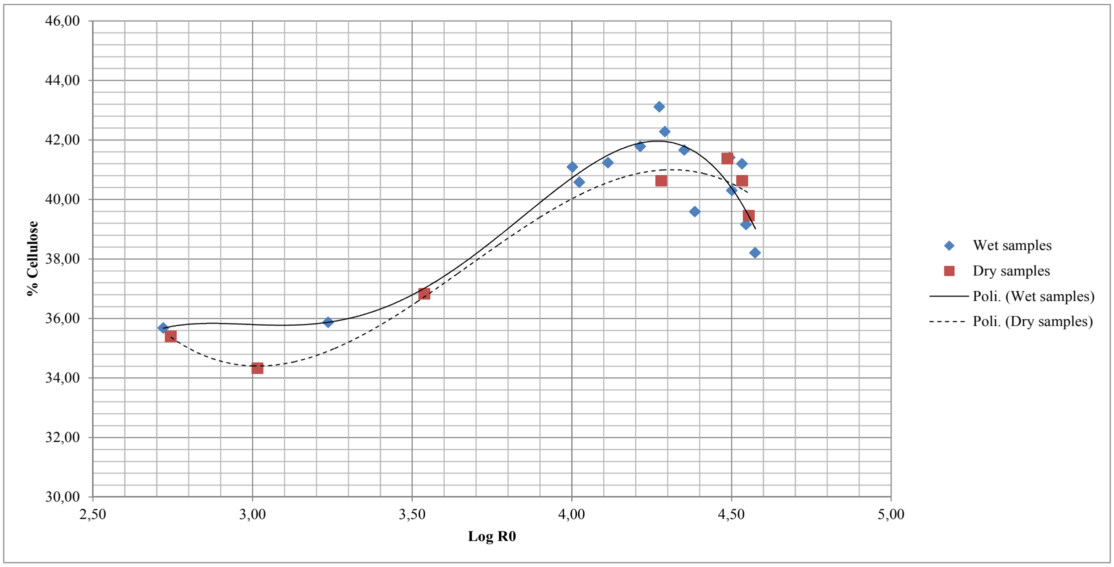

Fig. 2. Comparison between cellulose content in wet and dry exploded samples.

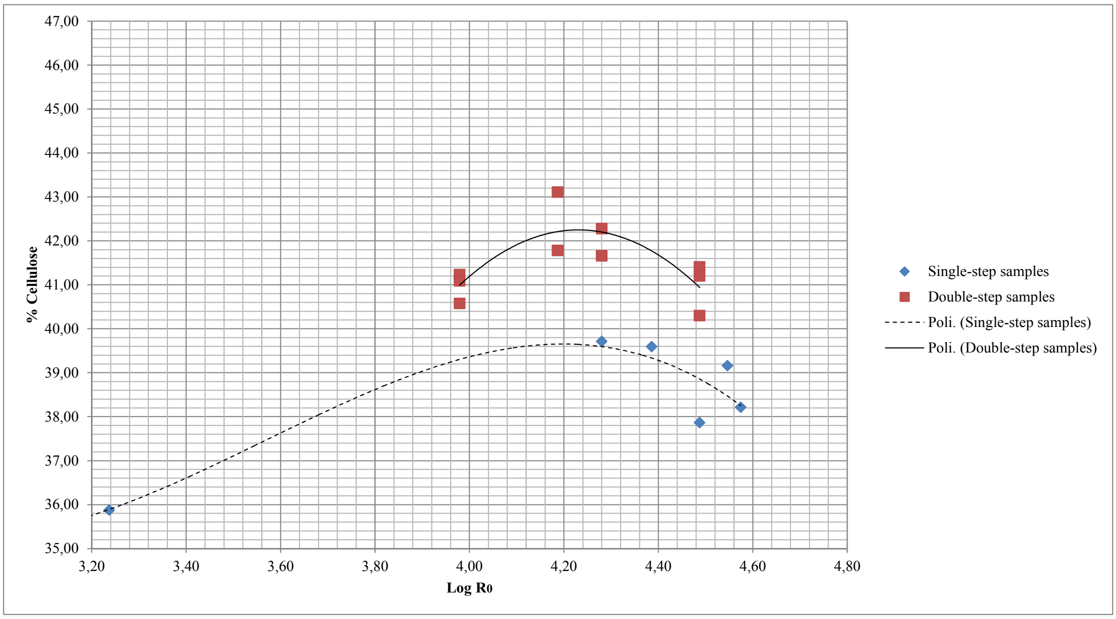

Fig. 3. Comparison between cellulose content in single- and double-step exploded samples.

After steam explosion campaign, eight samples were selected for the following stages, in the range LogR_0 4.2-4.6; table 3 shows some pretreatment results, in terms of recovered WIS and recovered cellulose in the process. Steam explosion efficiency, in percentage, is expressed as cellulose recovery; it defines recovered cellulose in WIS compared to initial cellulose charged into the reactor.

Total WIS was collected both recovering solid fraction and solid from filtered liquid. Comparing the two parameters in percentage we observe an approximately constant trend which does not influence the quantity of recovered cellulose in total, but probably influence the quality of cellulose in terms of deconstruction. Of course single-step pretreated samples present a higher cellulose recovery than double-step pretreated samples.

Considering this parameter, optimal steam explosion conditions seems to move towards higher $LogR_0$, around 4.5. Best result was obtained in CP016 sample (approximately 90% steam explosion efficiency), but both the two dry samples (CP013 and CP016) reached better results compared to wet samples.

Table 3. Steam exploded samples results.

Sample name	Charged dry matter (g)	Recovered WIS (g)	Recovered WIS (%)	Charged cellulose (g)	Recovered cellulose (g)	Recovered cellulose (%)
CP013	598.99	437.33	73.01%	192.22	163.44	85.03%
CP013W	636.64	417.56	65.59%	204.30	163.25	79.91%
CP016	598.99	429.08	71.63%	192.22	172.67	89.83%
CP016W	635.92	442.84	69.64%	204.07	167.90	82.28%
CP020W	645.62	381.60	59.11%	207.18	147,76	71.32%
CP028W	622.30	378.08	60.76%	199.70	149,52	74.87%
CP030W	645.33	344.66	53.41%	207.09	138,45	66.86%
CP037W	641.78	404.15	62.97%	205.95	164,88	80.06%

3.2. Hydrolysis

Hydrolysis tests were carried out and glucose concentration trends are shown in fig. 4, where an important amount of glucose is obtained in the first 2-4 hours (50% than overall glucose production is present after 2 hours). The higher final glucose concentration was reached in sample CP016 (18.8 mg/ml).

Probably after 48h the glucose concentration could continue to grow, suggesting to perform a 72h hydrolysis to maximize glucose production.

Fig. 5 shows hydrolysis efficiencies in function of severity parameter in pretreatment. Trend indicates an improvement of the efficiency in higher $logR_0$ samples, confirming that biomass is well deconstructed in this samples and facilitates enzyme activity. The maximum hydrolysis efficiency was obtained in sample CP016 with 82.48% yield, the same sample that reached the best pre-treatment efficiency.

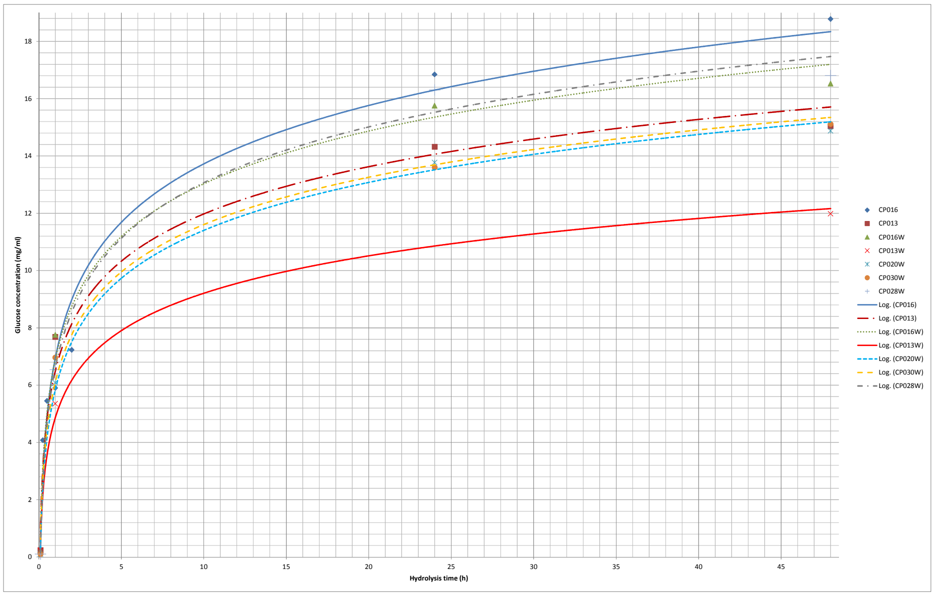

Fig. 4. Glucose concentration trends during hydrolysis processes.

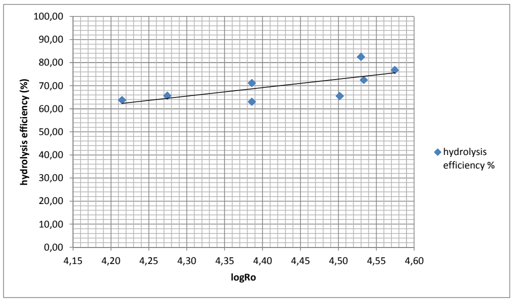

Fig. 5. Hydrolysis efficiency.

3.3. Fermentation

Fermentations were performed with Saccaromices Cerevisiae yeast for 48 h and results in function of severity parameter are shown in fig. 6.

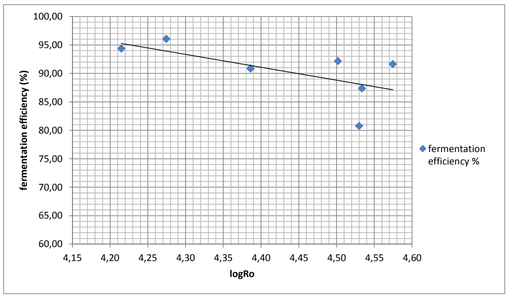

Fig. 6. Fermentation efficiency.

Fermentation efficiency, compared to theoretical efficiency, decreases with $LogR_0$, probably high severity parameter values produce more inhibitors that reduce yeast activity. CP037W reached the best performance (96.08%), while CP016 the worst (80.77%).

3.4. Overall process yields

Table 4 summarizes tests results reporting the overall process efficiency, in terms of produced ethanol each 100 g raw material dry basis.

Table 4. Overall process results.

Sample name	$LogR_0$	Overall process efficiency (g ethanol/100 g DM)
CP013 (single-step)	4.39	9.79
CP013W (single-step)	4.39	8.21
CP016 (single-step)	4.53	10.60
CP016W (single-step)	4.57	10.32
CP020W (double-step)	4.50	7.66
CP028W (double-step)	4.53	8.47
CP030W (double-step)	4.21	7.21
CP037W (double-step)	4.27	8.89

The overall process efficiency in the tested samples are in the range 7.21-10.60 g ethanol each 100 g raw material (DM). The best results was obtained in CP016, even with the worst fermentation performance, probably due to inhibitors formation during high severity parameter pre-treatment conditions. Double-step samples reached higher efficiencies with low severity parameter values (4.2-4.3) and longer treatment time in the first pretreatment step.

4. Conclusion

Pine wood chip was investigated as complementary biomass residue, from the agroforestry sector, in bioethanol production process. Experimental campaign tested samples varying severity parameter, comparing dry and wet material and evaluating a two-steps steam explosion to decrease process conditions. Considering the overall process, best performance were obtained with high $logR_0$ values between 4.5 and 4.6 (maximum yield 10.60 g ethanol/100 g raw dry material), which is a good result considering low initial cellulose content in the raw material (32%). High severity parameter values reduced both recovered WIS in pretreatment and fermentation efficiency, but reached the best hydrolysis and overall performances due to optimal biomass deconstruction. Double-step steam explosion obtained lower overall results (7.21-8.89 g ethanol/100 g raw material), but this performance can be reached with lower $logR_0$ (4.2-4.3) and could be furthermore investigated in order to reduce pre-treatment costs and minimize inhibitors formation to produce bioethanol also from hemicellulose contained in the liquid after pretreatment.

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