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Article

Sustainable Ethanol Production from Common Reed (*Phragmites australis*) through Simultaneuos Saccharification and Fermentation

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Abstract: *Phragmites australis* (common reed) is a perennial grass that grows in wetlands or near inland waterways. Due to its fast-growing properties and low requirement in nutrients and water, this arboreal variety is recognized as a promising source of renewable energy although it is one of the least characterized energy crops. In this experiment, the optimization of the bioethanol production process from *Phragmites australis* was carried out. Raw material was first characterized according to the standard procedure (NREL) to evaluate its composition in terms of cellulose, hemicellulose, and lignin content. Common reed was pretreated by steam explosion process at three different severity factor (R_0) values. The pretreatment was performed in order to reduce biomass recalcitrance and to make cellulose more accessible to enzymatic attack. After the pretreatment, a water insoluble substrate (WIS) rich in cellulose and lignin and a liquid fraction rich in pentose sugars (xylose and arabinose) and inhibitors were collected and analyzed. The simultaneous saccharification and fermentation (SSF) of the WIS was performed at three different solid loadings (SL) 10%, 15%, 20% (*w*/*w*). The same enzyme dosage, equal to 20% (g enzyme/g cellulose), was used for all the WIS loadings. The efficiency of the whole process was evaluated in terms of ethanol overall yield (g ethanol/100 g raw material). The maximum ethanol overall yields achieved were 16.56 and 15.80 g ethanol/100 g RM dry basis for sample AP10 and sample AP4.4, respectively. The yields were reached

working at lower solid loading (10%) and at the intermediate $LogR_0$ value for the former and at intermediate solid loading (15%) and high $LogR_0$ value for the latter, respectively.

Keywords: steam explosion; Ethanol; simultaneous saccharification and fermentation; *Phragmites australis*

1. Introduction

The supply of sustainable energy, in order to reduce the dependence on fossil sources and to address climate change, is becoming a global priority [1–3]. Biomass is a suitable and renewable primary energy resource that can provide a substantial contribution to meet future energy demand in a sustainable way. The usage of biomass can provide heat, electricity, chemicals, as well as alternative transportation fuels such as bioethanol or biodiesel. In particular, a great attention has been dedicated to the production of second generation bioethanol. The use of lignocellulosic feedstock such as organic wastes, forestry residues, high-yielding woody and grass energy crops for ethanol production, significantly decreases potential pressure on land use [4] and reduces greenhouse gas emissions when compared to first-generation biofuels [5].

Lake environments offer a good availability of lignocellulosic biomass such as common reed (*Phragmites australis*), which is a perennial grass that grows in wetlands or near inland waterways. This plant has numerous traditional applications, being used for local handicrafts such as roofs, baskets, and beach umbrellas [6].

Due to its vigorous growth and difficulty of eradication, in some areas of the world, common reed is typically regarded as an invasive weed. Nevertheless, recently, for its fast growing properties, *Phragmites australis* is becoming to be regarded as a promising source of renewable energy. Thanks to recent advancements in conversion technologies, common reed's processing residues can be used as a feedstock for the production of cellulosic ethanol, giving an added value to rural economy [7].

The whole cellulosic ethanol production process consists on a pretreatment of lignocellulosic biomass followed by enzymatic hydrolysis and fermentation of the released sugars. The pretreatment step is necessary to reduce biomass recalcitrance and make cellulose more accessible to enzymatic attack. Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials [8]. In this process the biomass is treated with saturated steam at high pressure and high temperature for few minutes before the material is exposed to atmospheric pressure. After the explosion it is possible to recover a solid matrix rich in cellulose and lignin while hemicellulose is broken down into free pentose sugars that are collected into the pretreatment liquor. The drawback of steam explosion pretreatment is that high severity conditions brought to cellulose degradation as well as the generation of compounds that may be inhibitory to the microorganisms used in downstream processes [9].

Hydrolysis of polymeric sugars and their fermentation into ethanol can be performed in two separate steps (SHF process), or alternatively the two steps can be merged together at intermediate conditions in one process known as simultaneous saccharification and fermentation (SSF). There are pros and cons associated with both of these processes [10,11].

It has been demonstrated that the SSF process allows minimization of the accumulation of inhibitory hydrolysis products as well as contamination risk because of the presence of ethanol [12]. Furthermore the process integration of hydrolysis and fermentation in one reactor reduces the overall capital cost. The disadvantage of the SSF process is that enzymes and yeasts cannot work at their best operative conditions e.g., with respect to temperature and pH. In fact, *Saccharomyces* strains and fungal cellulases (most commonly used for the hydrolysis step) require an operating temperature of 35 °C and 50 °C respectively. Working with lower temperatures in SSF could reduce the hydrolysis rate because of the increased processing times [13].

In this experiment, the production of second generation bioethanol from *Phragmites australis* residues was performed. The biomass was pretreated through steam explosion at three different severity factor values. SSF process was carried out in order to quantify the ethanol yields assessing the contribution to the process of both the pretreatment severity and the solid loading (SL).

The pretreatment efficiency was also evaluated in terms of hemicellulose recovery from the liquid fraction. This is important because a large number of biochemicals and biomaterials can be produced from hemicellulose bioconversion. Hexose and pentose sugars can be employed for the production of lactic acid, xylitol, and other value-adding compounds [14]. Furfural from xylose dehydration, has many applications in oil refining, plastics, pharmaceutical, and agrochemical industries [15]. Therefore the improvement of the hemicellulose recovery efficiency is a challenge that can maximize biorefinery integrated process economics.

2. Materials and Methods

2.1. Feedstock

Phragmites australis residues were kindly provided from a local handicraft firm (Zoppitelli Company), which collected the common reed from Lake Trasimeno in central Italy. The moisture content was equal to 5.95%. The common reed was first chipped down to a final size of 1–2 cm and it was then stored in the dark. The raw material was characterized according to the National Renewable Energy Laboratory (NREL, Golden, CO, USA) analytical methods for biomass in order to evaluate its composition [16].

2.2. Biomass Pretreatment

Steam explosion pretreatment of common reed was performed in order to separate the three main components of the biomass: cellulose, hemicellulose, and lignin. The steam explosion pretreatment plant was described in a previous work by the same author [17]. The pretreatment conditions are described by the severity factor $LogR_0$ [18] according to Equation (1):

$$R_0 = te^{\left[{}^{(T-100)}/_{14.75}\right]} \quad (1)$$

where t is the time (s) and T is the temperature (°C).

In this study, three different $LogR_0$ (3.6, 4.0, and 4.4) were used to pretreat *Phragmites australis*. For every $LogR_0$ value six consecutive explosions were executed using 500 g of dry biomass for each explosion. After the steam explosion, a Water Insoluble Substrate (WIS) rich in cellulose and lignin

and a pretreatment liquor rich in pentose sugars were separated by a sieve (pore size around 1 mm). The WIS was washed with water with a solid/liquid ratio equal to 1:10 at 50 °C for 30 min. The whole material was then pressed and the recovered liquid was mixed with the pretreatment liquor in order to obtain only one liquid fraction. The liquid fraction was analyzed according to National Renewable Energy Laboratory (NREL, Golden, CO, USA) analytical methods for biomass [19].

Meanwhile, the WIS was carefully blended and then a small fraction of WIS was selected through quartering sub-sampling method and analyzed according to the National Renewable Energy Laboratory (NREL, Golden, CO, USA) analytical methods for biomass [9].

Cellulose and hemicellulose recovery after steam explosion in both solid and liquid fractions were calculated according to Equations (2) and (3):

$$X_{rs\%} = \frac{X}{X_{RM\%} \times RM} \times 10^4 \quad (2)$$

where X_{rs} is cellulose or hemicellulose fraction in WIS ($C_{rs\%}$ and $H_{rs\%}$, respectively).

$$X_{rl\%} = \frac{X}{X_{RM\%} \times RM} \times 10^4 \text{ zr} \quad (3)$$

where X_{rl} is cellulose or hemicellulose fraction in liquid fraction (C_{rl}% H_{rl}% respectively).

2.3. SSF (Simultaneous Saccharification and Fermentation)

The SSF process consisted on a 24 h pre-saccharification step of pure enzymatic hydrolysis (T = 50 °C and pH = 5) followed by a 72 h step of SSF (T = 37 °C and pH = 5). The whole process was conducted in Biostat® A-Plus-Sartorius reactors (Goettingen, Germany) with an integrated control system of pH, temperature and stirring. The enzyme, ™Ctec2 (Bagsvaerd, Denmark) provided by Novozyme, was employed at a dosage of 20% (g enzyme/g cellulose). A suggested dosage (1.41 g) of dry yeast (Ethanol Red® (Marcq-en-Baroeul, France) provided by Leaf Technologies company was employed for all trials. Since this kind of yeast is not naturally able to ferment C5 sugars, the pretreatment liquor wasn't used for ethanol production in the experiment.

Glucose and ethanol concentrations from SSF were determined by High Performance Liquid Cromatography HPLC (Dionex Ultimate 3000—Thermo Scientific (Sunnyvale, CA, USA) equipped with a Biorad Aminex HPX-87H column and an RI detector.

The hydrolysis yield (Hy_{24}) at the end of the pre-saccharification step (24 h) was calculated according to Equation (4):

$$Hy_{24}\% = \frac{r_{Gc} \times f_G}{WIS_l \times C_{\%}} \times 10^4 \quad (4)$$

where r_{Gc} is the molecular weight ratio of a cellulose monomer to glucose (162.16/180.18), f_G is the glucose mass fraction (g) into the slurry at the end of the hydrolysis, WIS_l is the water insoluble substrate loaded into the bioreactor (g) and C% is the cellulose percentage found in the WIS characterization.

The final hydrolysis yield (Hy_{96}%) at the end of the SSF process (96 h) was calculated according to Equation (5):

$$Hy_{96}\% = \frac{C_f - C_i}{C_i} \tag{5}$$

where C_i is the initial (24 h) cellulose content (g) in WIS_l, while C_f is the final cellulose content in $WIS_{l(f)}$ at the end of the process (after 96 h). The cellulose content was calculated as follow:

$$C_i = C\% \times WIS_l \times 100 \tag{6}$$

$$C_f = C\% \times WIS_{l(f)} \times 100 \tag{7}$$

The fermentation yield was calculated as follows in Equation (8):

$$Fy\% = \frac{EtOH}{(C_f - C_i) \times 1.11} / 0.51 \times 100 \tag{8}$$

where *EtOH* (g) is the produced ethanol during the SSF process; 1.11 and 0.51 are respectively cellulose to glucose and glucose to ethanol maximum theoretical yields.

The overall ethanol yield (*OY*) displays the ratio between the produced ethanol (g) and 100 g of RM which went through the whole process (SE plus SSF) as shown in Equation (9).

$$OY = \frac{EtOH}{WIS_l} \times \frac{WIS_t}{RM} \times 100 \tag{9}$$

where the WIS_t is the total water insoluble substrate recovered after the steam explosion pretreatment (g). WIS_t/RM (g) represents the steam explosion pretreatment yield.

The relative overall ethanol yield ($OY_{\%}$) was calculated as follows in Equation (10):

$$OY_{\%} = \frac{OY}{C_{RM\%} \times 0.5661} \times 100 \tag{10}$$

All the variables regarding solid materials in the calculations are considered on dry basis.

2.4. Experimental Setup

Figure 1 summarizes the experimental setup employed in this work. During all the experimentations the enzyme dosage value was fixed while the $LogR_0$ and the solid loading values were considered as variables. For each severity factor three different SSF trials (AP3.6, AP4.0 and AP4.4) at 15% (*w*/*w*) of SL were performed. For a fixed $LogR_0$ value (4.0) three different SSF trials (AP10, AP15 and AP20) at different SL values (10%, 15%, 20% *w*/*w*) were carried out.

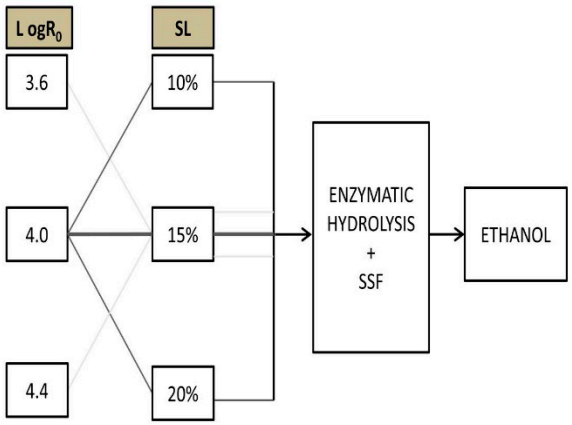

Figure 1. Experimental setup.

At the intermediate conditions ($LogR_0$ = 4.0 and SL = 15% *w*/*w*) a single SSF process was carried out. The results obtained at this condition were employed to evaluate both solid loading and $LogR_0$ effects. The samples were called AP15 and AP4.0 when referring to solid loading and $LogR_0$ effects respectively.

3. Results and Discussion

3.1. Phragmites australis Characterization

The raw material composition is shown in Table 1.

Table 1. Raw material composition.

	H%	C%	Ac%	L%	E%	A%	Total%	Other%
Phragmites australis	20.51	38.13	3.92	23.02	6.90	4.25	96.72	3.28
Standard deviation	0.62	0.36	0.16	0.92	0.23	0.04	1.20	1.20

H = hemicellulose, C = cellulose, Ac = acetyls, L = lignin, E = extractives, A = ash.

3.2. Steam Explosion

Steam explosion pretreatment at three different $LogR_0$ values (3.6, 4.0 and 4.4) was performed. Table 2 reports the WIS characterization for each severity factor value.

As expected, at the lower $LogR_0$ value, the WIS contained less cellulose (48.96%) as well as a higher hemicellulose fraction (12.50%). This finding demonstrated that the pretreatment at lower $LogR_0$ values was relatively ineffective, since only about half of the hemicellulose and acetyl groups were removed compared to the RM composition (Table 1).

Table 2. Steam exploded material characterization.

Log R_0	H%	C%	Ac%	L%	A%	Total%	Other
3.6	12.50 ± 0.05	48.96 ± 0.27	2.19 ± 0.06	28.87 ± 0.80	3.34 ± 0.15	95.86 ± 0.82	4.14 ± 0.82
4.0	4.01 ± 0.9	54.61 ± 0.28	0.42 ± 0.42	33.99 ± 0.51	3.19 ± 0.21	96.22 ± 0.67	3.78 ± 0.67
4.4	1.20 ± 0.10	52.07 ± 0.53	0.39 ± 0.01	38.63 ± 0.29	4.21 ± 0.15	96.49 ± 0.79	3.51 ± 0.79

H = hemicellulose, C = cellulose, Ac = acetyls, L = lignin, A = ash.

At a $LogR_0$ value of 4.4, the cellulose fraction started to decrease since cellulose was probably degraded by severe pretreatment conditions. The highest enrichment in cellulose (54.61%) was obtained at intermediate severity conditions. This value also ensured a hemicellulose and acetyl groups residue in WIS equal to 4.01% and 0.42%, respectively. These values were similar to those showed by $LogR_0$ 4.4 (Table 2). This data were satisfying in terms of pretreatment efficiency so it was unnecessary to work at more severe conditions.

Since lignin was not removed by the steam explosion pretreatment [20], its percentage showed an increasing trend along with the removal of other components. At the intermediate $LogR_0$ value, the WIS showed an intermediate lignin enrichment corresponding to 33.99%.

The liquid fraction analysis was performed in order to evaluate hemicellulose recovery for the potential utilization of its derivative as biobased building-blocks.

The C_{rl}% and H_{rl}% in terms of their monomeric and oligomeric form at different severity conditions are shown in Figures 2 and 3.

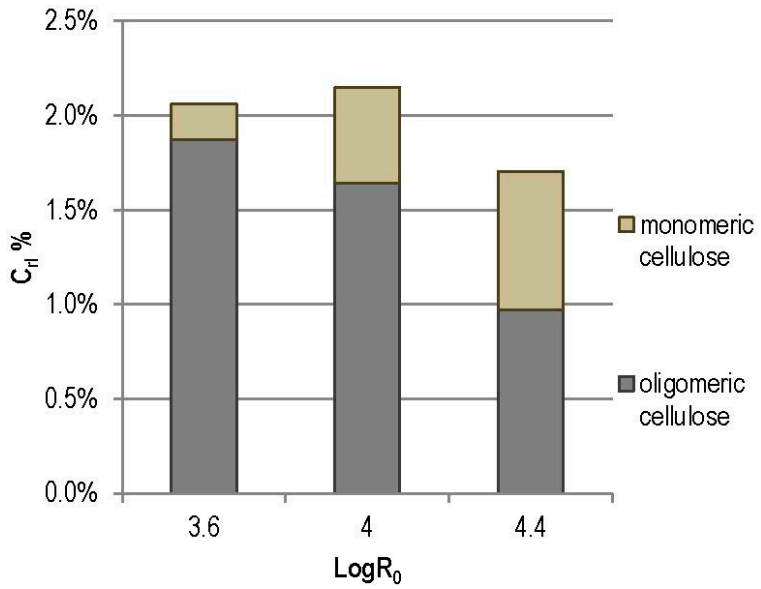

Figure 2. Monomeric and oligomeric cellulose in the liquid fraction after steam explosion.

Figure 2 shows that the C_{rl}% was very low (*i.e.*, <2.2%) at every $LogR_0$. At growing severity conditions monomeric/oligomeric cellulose ratio increased. The total cellulose recovery (C_{rl}%) reached a maximum value for LogR0 4.0 and a minimum value for LogR0 4.4. The decrease in C_{rl}% for $LogR_0$ of 4.4 was probably due to glucose conversion into inhibitors [21].

A similar trend was observed for the hemicellulose fraction (Figure 3) where a consistent H_{rl}% reduction, ranging from 37.6% to 2.5%, was obtained with increasing $LogR_0$. The significant decrease of the hemicellulose recovery was due to the formation of inhibitory products such as acetic acid, furfural, 5-HMF (Figure 4) [22].

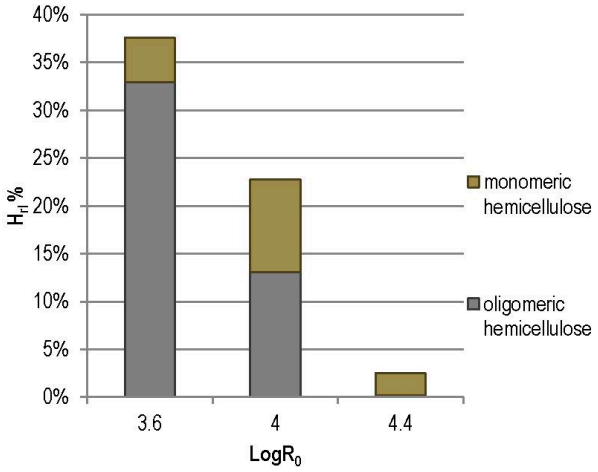

Figure 3. Monomeric and oligomeric hemicellulose in the liquid fraction after steam explosion.

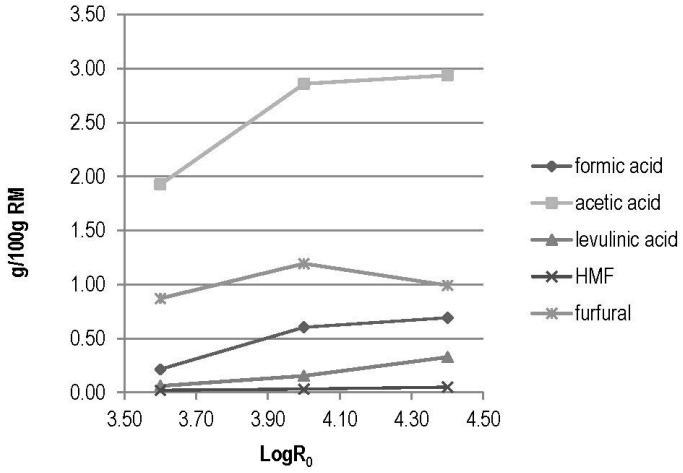

Figure 4. Inhibitors concentration in liquid fraction after steam explosion.

At the highest severity condition, the H_{rl}% as well as the oligomeric sugars were almost completely absent. The low H_{rl}% could be explained by the high acetyls groups content in *Phragmites australis* biomass that were converted into acetic acid during pretreatment. The acetic acid generates hydronium ions that lead to the acidification of the liquor, catalyzing the hemicellulose degradation [13].

The modest H_{rl}% at every $LogR_0$ value, especially above 4.0, suggested that steam explosion was not the most suitable pretreatment for the complete fractionation and recovery of *Phragmites australis* components, in particular hemicellulose. Hence, it would be interesting to explore other pretreatment processes or to employ a double-step steam explosion. The double-step steam explosion could allow a good hemicellulose recovery in the first step and a good biomass deconstruction in the second step, as just tested for other biomass such as pinewood chips [23].

Inhibitors concentrations (g/100 g RM) are displayed in Figure 4. As expected, inhibitors concentrations increased along with the pretreatment severity. As mentioned above 5-HMF, furfural and acetic acid came from cellulose and hemicellulose degradation. 5-HMF breakdown brought to formic and levulinic acid formation. The formic acid was also formed from furfural degradation at severe pretreatment conditions [15]. The furfural concentration drop displayed in Figure 4 can be

explained, along with the formic acid formation, also by its evaporation, since furfural is highly volatile [24].

Considering the low H_{rl}%, C_{rl}% (especially for monomeric component) and the potential inhibition of the hemicellulose degradation products [25], in this experiment the liquid fraction was not used in the fermentation phase. The use of liquid fraction would lead to an acetic acid concentration in the bioreactor approximately between 4 g/L and 7 g/L for all the samples. This acetic acid concentration alone could strongly inhibit the efficiency of Ethanol Red® yeast (Marcq-en-Baroeul, France) [26].

3.3. SSF

3.3.1. Effect of Solid Loading

A SSF of the samples pretreated at a $LogR_0$ of 4.0 at three different solid loadings (10%, 15%, 20% *w*/*w*) was performed. A fixed dosage of enzyme (20%) was employed. The enzymatic hydrolysis yields (Hy_{24}% and Hy_{96}%), the fermentation yield (Fy%), and the overall ethanol yield (OY) for each sample are shown in Table 3.

Table 3. SSF performances at different WIS.

WIS	$LogR_0$	SL% (*w*/*w*)	Hy_{24}%	Hy_{96}%	ΔHy% [a]	Fy%	OY
AP10	4	10	60.27	84.22	21.84	87.70	16.35
AP15	4	15	49.63	70.80	21.17	88.24	12.92
AP20	4	20	42.25	58.33	16.08	89.41	11.54

[a] $\Delta Hy\% = Hy_{96}\% - Hy_{24}\%$.

Both Hy_{96}% and OY showed a decreasing trend while the Fy% showed similar values for each sample. As displayed in Table 3, AP10 sample reached higher Hy_{96}% (84.22%) probably because of the modest glucose inhibition. On the contrary, at high solid loadings, the reaction mixture was subjected to a higher stress due to mixing and mass transfer limitation [27] as well as the presumable glucose inhibition [28]. It was notable that the maximum glucose concentration value (g/L) after 24 h of pre-hydrolysis step for the sample AP15 and AP20 was about 67.0 g/L, despite the larger solid loading of the latter. This suggests that perhaps at this glucose concentration the enzyme is almost entirely inhibited by its product.

The ΔHy% was very similar for AP10 and AP15 but lower for AP20. This was maybe caused by the higher lignin content at this solid loading that inhibited the cellulases activity [29].

The ethanol, glucose and glycerol concentrations (g/L) reached from the samples at the three different solid loadings are displayed in Figure 5. As expected, AP10 reached lower ethanol production values while AP20 reached higher values because a larger quantity of substrate was processed at the same time.

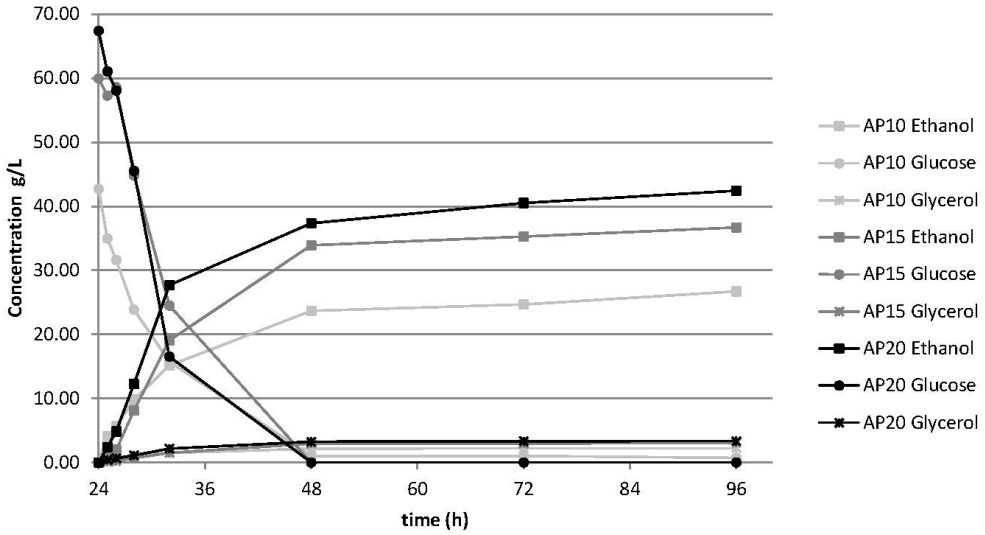

Figure 5. Ethanol, Glucose and Glycerol concentrations during SSF at different SL.

3.3.2. Effect of $LogR_0$

A second SSF trial was performed, working with a fixed solid loading value (15% *w*/*w*) and at three different $LogR_0$ values (3.6, 4.0, 4.4), in order to test the severity factor contribution to ethanol yield. The enzymatic hydrolysis yields (Hy_{24}% and Hy_{96}%), the fermentation yield (Fy%) and the overall ethanol yield (OY) for each sample are shown in Table 4.

Table 4. SSF results at different $LogR_0$.

WIS	$LogR_0$	SL% (*w*/*w*)	C_r%	Hy_{24}%	Hy_{96}%	ΔHy% [a]	Fy%	OY
AP3.6	3.6	15	98.17	38.88	38.88	-	98.39	8.64
AP4.0	4.0	15	96.08	49.63	70.80	21.17	88.41	12.92
AP4.4	4.4	15	90.77	62.38	91.78	29.40	82.55	15.80

[a] $\Delta Hy\% = Hy_{96}\% - Hy_{24}\%$.

As shown, the cellulose conversion after 24 h (Hy_{24}%) and 96 h (Hy_{96}%) increased for increasing severity factor values. This was probably due to the biomass pore size enlargement [30] as well as available surface area [31], that gave a boost to cellulose accessibility for enzyme [32]. This was also confirmed by the AP3.6 sample, where the hydrolysis stopped just after 24 h (ΔHy% = 0), indicating that all the accessible cellulose was probably converted. Furthermore, the data in Table 4 shows that the cellulose recovery (C_{rs}%) dropped at increasing severity condition confirming the cellulose mass loss at high $LogR_0$. Despite the lowest cellulose recovering value (C_r = 90.77%), AP4.4 sample achieved the highest OY (15.80 g/100 g RM). The higher the hydrolysis yield was, the higher ethanol concentration was achieved (Figure 6). In these trials the Fy% values showed a dropping trend, and AP4.4 obtained the lowest yield (82.55%). This was probably due to the higher content of phenolic compounds, derived from lignin degradation during steam explosion pretreatment, that could inhibit the fermentation phase [14,33]. Moreover, HPLC data showed a significant HMF and furfural concentration into the hydrolysate liquid, especially at higher severity factors, which could contribute

to reduce the fermentation efficiency. The presence of inhibitors in the WIS could be due to an unsuccessful washing step of biomass after steam explosion pretreatment.

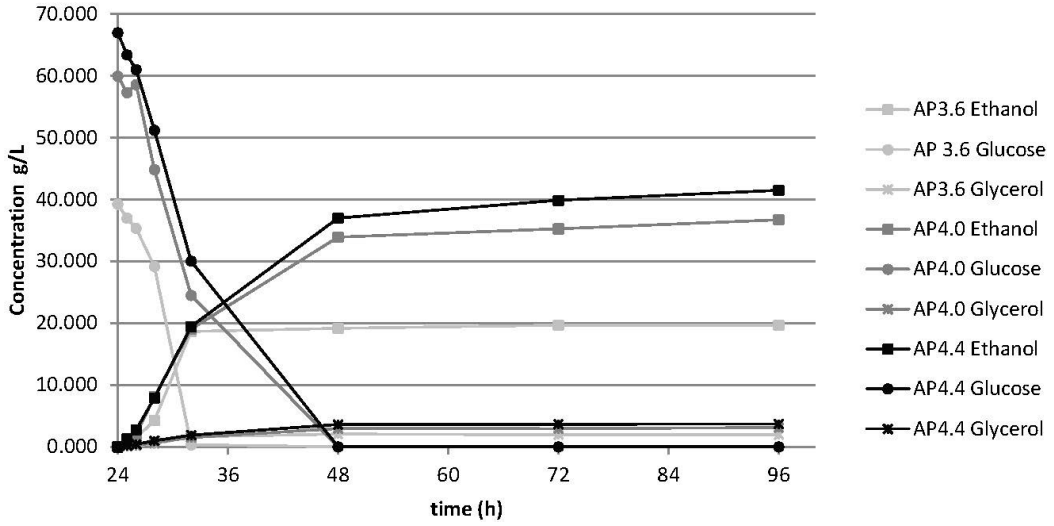

Figure 6. Ethanol, Glucose, and Glycerol concentrations during SSF at different $LogR_0$.

3.3.3. Relative Overall Ethanol Yields and Ethanol Concentrations

Converted cellulose (Hy_{96}%) and relative overall ethanol yield ($OY_{\%}$) values of each sample are reported in Figure 7.

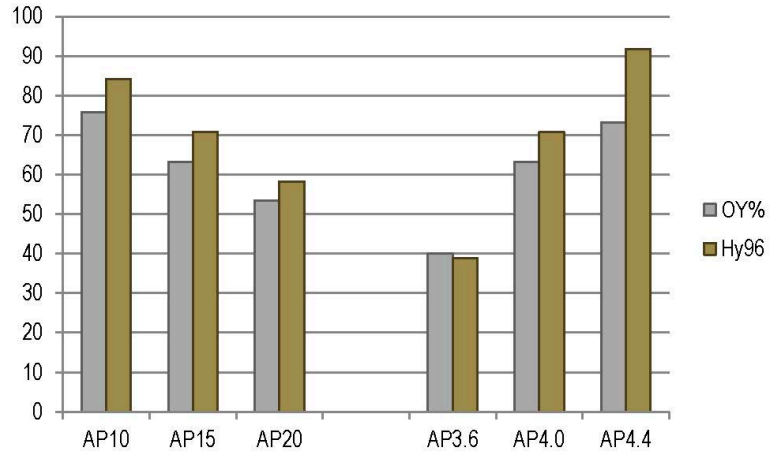

Figure 7. Converted cellulose and ethanol overall yields results.

As expected, maximum $OY_{\%}$ as well as Hy_{96}% was achieved working with low solid loading and at high $LogR_0$ values. The best samples in term of $OY_{\%}$ were AP10 (75.75%) and AP4.4 (73.21%) while the worst were and AP3.6 (38.88%) followed by AP20 (53.49%). The best $OY_{\%}$ values were found to be very similar, this is probably due to the enhanced cellulose accessibility which balanced cellulose mass loss (AP4.4) and reduced mass transport limitation and glucose inhibition (AP10).

The final ethanol concentration (% *w*/*w*) was calculated in order to evaluate the distillation suitability as separation technology (Figure 8). It is known that the energy requirement at low alcohol

concentrations (<3.5% *w*/*w*) is considerably larger compared to high concentrations [34]. Indeed, under this threshold limit the distillation is considered energetically-economically inconvenient.

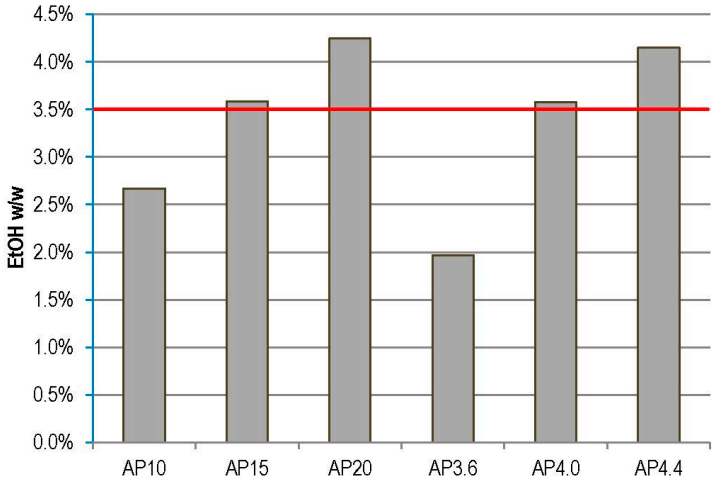

Figure 8. Ethanol concentrations in the different test.

As displayed, the only two samples that extensively exceeded the 3.5% value were AP20 and AP4.4. Despite the sample AP10 reached the highest $OY_{\%}$, the final ethanol concentration (2.67% *w*/*w*) was below the 3.5% threshold limit. On the other hand AP4.4 showed an OY% slightly lower but a good final ethanol concentration (4.15% *w*/*w*). The sample AP20 achieved the best final ethanol concentration (4.24% *w*/*w*), but the next to last OY%.

These results suggest that a good compromise between $OY_{\%}$ and ethanol concentration should be at Log R_0 between 4.0 and 4.4 and solid loading between 15% and 20% at the cost of producing more inhibitors. Further research could be aimed at investigating these ranges.

4. Conclusions

In this experiment, *Phragmites australis* was tested as a feedstock for the production of lignocellulosic ethanol. Common reed confirmed to be a promising biomass for its interesting cellulose content (38.1%) and for its low recalcitrance. A good biomass deconstruction and consequent overall yields were reached with intermediate pretreatment conditions. The hemicellulose recovery in the liquid fraction was very low at every pretreatment condition, especially for $LogR_0$ values greater than 4.0.

Phragmites australis showed overall relatives yields similar to those found in literature [3], for both high and low solid loadings ($OY_{\%} \approx 73\%$). High rates of cellulose hydrolysis and overall ethanol yields were reached by working with both low solid loading and medium $LogR_0$ (sample AP10), and medium solid loading and high $LogR_0$ (sample AP4.4). The sample AP4.4 and AP10 produced 15.80 and 16.56 g ethanol/100 g RM dry basis, respectively. The worst results in terms of overall yield were achieved by samples AP3.6 and AP20. The final ethanol concentration (% *w*/*w*) was calculated in order to evaluate the convenience of ethanol recovery by the distillation process. Good final ethanol concentrations were achieved by the samples AP4.4 (4.15%) and AP20 (4.24%), which exceeded the distillation threshold limit (3.5%).

Moreover, the trials showed that it was not possible to optimize the overall yield and the hemicellulose fraction recovery at the same time for *Phragmites australis* pretreated by steam explosion. On the contrary, the steam explosion process was effective for the production of cellulosic ethanol and in order to maximize the ethanol overall yield as well as final ethanol concentration, biomass should be pretreated at high $LogR_0$ values (≥4.0) and high solid loadings (≥15%).

As future development of this research it would be interesting to carry out further experiments using solid loadings within the range of 15%–20% and pretreating the biomass above $LogR_0$ 4.0, taking into account the possibility to create a statistical model with more variables and levels for the severity factor, the solid loading, and the enzyme dosage.

Author Contributions

Franco Cotana coordinated all the activities; Gianluca Cavalaglio designed the research and revised the manuscript. Anna Laura Pisello collaborated in writing the paper and revising the manuscript. Mattia Gelosia collaborated in designing the research, performed the tests and analyzed the data. Enrico Pompili performed the research and analyzed the data David Ingles performed the research, analyzed the data and wrote the paper. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Salata, F.; de Lieto Vollaro, A.; de Lieto Vollaro, R.; Mancieri, L. Method for energy optimization with reliability analysis of a trigeneration and teleheating system on urban scale: A case study. *Energy Build*. **2015**, *86*, 118–136.
2. De Lieto Vollaro, R.; Guattari, C.; Evangelisti, L.; Battista, G.; Carnielo, E.; Gori, P. Building energy performance analysis: A case study. *Energy Build*. **2015**, *87*, 87–94.
3. De Lieto Vollaro, R.; Evangelisti, L.; Carnielo, E.; Battista, G.; Gori, P.; Guattari, C.; Fanchiotti, A. An integrated approach for an historical buildings energy analysis in a smart cities perspective. *Energy Procedia* **2014**, *45*, 372–378.
4. Salata, F.; de Lieto Vollaro, A.; de Lieto Vollaro, R. A model for the evaluation of heat loss from underground cables in non-uniform soil to optimize the system design. *Therm. Sci*. **2015**, *19*, 461–474.
5. Naik, S.N.; Goud, V.V.; Rout, P.K.; Dalai, A.K. Production of first and second generation biofuels: A comprehensive review. *Renew. Sustain. Energy Rev*. **2010**, *14*, 578–597.
6. Sathitsuksanoh, N.; Zhu, Z.; Templeton, N.; Rollin, J.A.; Harvey, S.P.; Zhang, Y.-H.P. Saccharification of a Potential Bioenergy Crop., Phragmites australis (Common Reed), by Lignocellulose Fractionation Followed by Enzymatic Hydrolysis at Decreased Cellulase Loadings. *Ind. Eng. Chem. Res*. **2009**, *48*, 6441–6447.

7. Szijarto, N.; Kádár, Z.; Varga, E.; Thomsen, A.B.; Costa-Ferreira, M.; Réczey, K. Pretreatment of Reed by Wet Oxidation and Subsequent Utilization of the Pretreated Fibers for Ethanol Production. *Appl. Biochem. Biotechnol*. **2009**, *155*, 386–396.
8. Sun, Y.; Cheng, J. Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour. Technol*. **2002**, *83*, 1–11.
9. Kumar, P.; Barrett, D.M.; Delwiche, M.J.; Stroeve, P. Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Ind. Eng. Chem. Res*. **2009**, *48*, 3713–3729.
10. Cotana, F.; Cavalaglio, G.; Gelosia, M.; Coccia, V.; Petrozzi, A.; Ingles, D.; Pompili, E. A comparison between SHF and SSSF processes from cardoon for ethanol production. *Ind. Crop. Prod*. **2015**, *69*, 424–432.
11. Rana, V.; Eckard, A.D.; Ahring, B.K. Comparison of SHF and SSF of wet exploded corn stover and loblolly pine using in-house enzymes produced from T. reesei RUT C30 and A. saccharolyticus. *SpringerPlus* **2014**, doi:10.1186/2193-1801-3-516.
12. Philippidis, G.P.; Smith, T.K.; Wyman, C.E. Study of the enzymatic hydrolysis of cellulose for production of fuel ethanol by the simultaneous saccharification and fermentation process. *Biotechnol. Bioeng*. **1993**, *41*, 846–853.
13. Kádár, Z.; Szengyel, Z.; Réczey, K. Simultaneous saccharification and fermentation (SSF) of industrial wastes for the production of ethanol. *Ind. Crop. Prod*. **2004**, *20*, 103–110.
14. Yoon, K.Y.; Woodams, E.E.; Hang, Y.D. Enzymatic production of pentoses from the hemicellulose fraction of corn residues. *LWT Food Sci. Technol*. **2006**, *39*, 388–392.
15. Mamman, A.S.; Lee, J.-M.; Kim, Y.-C.; Hwang, I.T.; Park, N.-J.; Hwang, Y.K.; Chang, J.-S.; Hwang, J.-S. Furfural: Hemicellulose/xylosederived biochemical. *Biofuels Bioprod. Biorefin*. **2008**, *2*, 438–454.
16. Sluiter, J.; Sluiter, A. Summative Mass Closure. Technical Report NREL/TP-510-48087-revised July 2011. Available online: http://www.nrel.gov/docs/gen/fy11/48087.pdf (accessed on 25 August 2015).
17. Cotana, F.; Cavalaglio, G.; Gelosia, M.; Nicolini, A.; Coccia, V.; Petrozzi, A. Production of Bioethanol in a Second Generation Prototype from Pine Wood Chips. *Energy Procedia* **2014**, *45*, 42–51.
18. Overend, R.P.; Chornet, E.; Gascoigne, J. Fractionation of lignocellulosics by steam-aqueous pretreatments [and discussion]. *Philos. Trans. Royal Soc. Lond.* **1987**, *321*, 523–536.
19. Sluiter, A. *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples*; National Renewable Energy Laboratory: Golden, CO, USA, 2006.
20. Ruiz, H.A.; Rodríguez-Jasso, R.M.; Fernandes, B.D.; Vicente, A.A.; Teixeira, J.A. Hydrothermal processing, as an alternative for upgrading agriculture residues and marine biomass according to the biorefinery concept: A review. *Renew. Sustain. Energy Rev*. **2013**, *21*, 35–51.
21. Palmqvist, E.; Hahn-Hägerdal, B. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. *Bioresour. Technol*. **2000**, *74*, 25–33.
22. Almeida, J.R.M.; Modig, T.; Petersson, A.; Hähn-Hägerdal, B.; Lidén, G.; Gorwa-Grauslund, M.F. Increased tolerance and conversion of inhibitors in lignocellulosic hydrolysates by Saccharomyces cerevisiae. *J. Chem. Technol. Biotechnol*. **2007**, *82*, 340–349.

23. Cotana, F.; Cavalaglio, G.; Gelosia, M.; Coccia, V.; Petrozzi, A.; Nicolini, A. Effect of Double-Step Steam Explosion Pretreatment in Bioethanol Production from Softwood. *Appl. Biochem. Biotechnol*. **2014**, *174*, 156–167.
24. Horn, S.J.; Nguyen, Q.D.; Westereng, B.; Nilsen, P.J.; Eijsink, V.G.H. Screening of steam explosion conditions for glucose production from non-impregnated wheat straw. *Biomass Bioenergy* **2011**, *35*, 4879–4886.
25. Kim, S.-K.; Park, D.H.; Song, S.H.; Wee, Y.J.; Jeong, G.T. Effect of fermentation inhibitors in the presence and absence of activated charcoal on the growth of Saccharomyces cerevisiae. *Bioprocess Biosyst. Eng*. **2013**, *36*, 659–666.
26. Demeke, M.M.; Dietz, H.; Li, Y.; Foulquié-Moreno, M.R.; Mutturi, S.; Deprez, S.; Den Abt, T.; Bonini, B.M.; Liden, G.; Dumortier, F.; *et al*. Development of a D-xylose fermenting and inhibitor tolerant industrial Saccharomyces cerevisiae strain with high performance in lignocellulose hydrolysates using metabolic and evolutionary engineering. *Biotechnol. Biofuels* **2013**, doi:10.1186/1754-6834-6-89.
27. Spindler, D.; Wyman, C.E.; Mohagheghi, A.; Grohmann, K. Thermotolerant yeast for simultaneous saccharification and fermentation of cellulose to ethanol. *Appl. Biochem. Biotechnol*. **1988**, *17*, 279–293.
28. Andrić, P.; Meyer, A.S.; Jensen, P.A.; Dam-Johansen, K. Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis: I. Significance and mechanism of cellobiose and glucose inhibition on cellulolytic enzymes. *Biotechnol. Adv*. **2010**, *28*, 308–324.
29. Várnai, A.; Siika-aho, M.; Viikari, L. Restriction of the enzymatic hydrolysis of steam-pretreated spruce by lignin and hemicellulose. *Enzym. Microb. Technol*. **2010**, *46*, 185–193.
30. Grethlein, H.E. The Effect of Pore Size Distribution on the Rate of Enzymatic Hydrolysis of Cellulosic Substrates. *Nat. Biotechnol*. **1985**, *3*, 155–160.
31. Fan, L.T.; Gharpuray, M.M.; Lee, Y.H. *Cellulose Hydrolysis*; Springer-Verlag: Berlin, Germany, 1987.
32. Hendriks, A.; Zeeman, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol*. **2009**, *100*, 10–18.
33. Ando, S.; Arai, I.; Kiyoto, K.; Hanai, S. Identification of aromatic monomers in steam-exploded poplar and their influences on ethanol fermentation by Saccharomyces cerevisiae. *J. Fermentation Technol*. **1986**, *64*, 567–570.
34. Vane, L.M. Separation technologies for the recovery and dehydration of alcohols from fermentation broths. *Biofuels Bioprod. Biorefin*. **2008**, *2*, 553–588.