Lignocellulosic Ethanol Production from the Recovery of Stranded Driftwood Residues

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Keywords: cellulose hydrolysis, steam explosion, bioethanol, stranded driftwood residues

Abstract:

This paper builds upon a research project funded by the Italian Ministry of Environment, and aims to recover stranded driftwood residues (SDRs), in order to transform a potential pollution and safety issue into valuable bio-resources. In particular, one of the experiments consisted of bioethanol production from lignocellulosic residues. The SDRs were gathered from the Italian coast (Abruzzo Region, Italy) after an intense storm. The biomass recalcitrance, due to its lignocellulosic structure, was reduced by a steam explosion (SE) pretreatment process. Four different pretreatment severity factors (R?) were tested (LogR? 3.65, 4.05, 4.24 and 4.64) in order to evaluate the pretreated material's accessibility to enzymatic attack and the holocellulose (cellulose plus hemicellulose) recovery. A first enzymatic hydrolysis was performed on the pretreated materials by employing a solid/liquid (S/L) ratio of 1% (w/w) and an enzyme dosage of 30% (w enzyme/w cellulose), in order to estimate the maximum enzymatically accessible cellulose content. Since the primary goal of pretreatment and hydrolysis is to convert as much cellulose as possible into monomeric glucose and recover all the holocellulose, the two pretreated materials showing these features were selected for bioethanol production process. The pretreated materials underwent a semi-simultaneous saccharification and fermentation (SSSF). The SSSF process was performed into two lab-scale bioreactors (5 L) with an S/L ratio of 15% and an enzyme dosage of 15% for five days. The efficiency of the whole bioethanol production process was assessed as ethanol overall yields (g ethanol/100 g raw material). The best overall yield was achieved by sample BS04 (8.98 g ethanol/100 g raw material).

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Abstract: This paper builds upon a research project funded by the Italian Ministry of Environment, and aims to recover stranded driftwood residues (SDRs), in order to transform a potential pollution and safety issue into valuable bio-resources. In particular, one of the experiments consisted of bioethanol production from lignocellulosic residues. The SDRs were gathered from the Italian coast (Abruzzo Region, Italy) after an intense storm. The biomass recalcitrance, due to its lignocellulosic structure, was reduced by a steam explosion (SE) pretreatment process. Four different pretreatment severity factors (R_0) were tested (Log R_0 3.65, 4.05, 4.24 and 4.64) in order to evaluate the pretreated material's accessibility to enzymatic attack and the holocellulose (cellulose plus hemicellulose) recovery. A first enzymatic hydrolysis was performed on the pretreated materials by employing a solid/liquid (S/L) ratio of 1% (w/w) and an enzyme dosage of 30% (w enzyme/w cellulose), in order to estimate the maximum enzymatically accessible cellulose content. Since the primary goal of pretreatment and hydrolysis is to convert as much cellulose as possible into monomeric glucose and recover all the holocellulose, the two pretreated materials showing these features were selected for bioethanol production process. The pretreated materials underwent a semi-simultaneous saccharification and fermentation (SSSF). The SSSF process was performed into two lab-scale bioreactors (5 L) with an S/L ratio of 15% and an enzyme dosage of 15% for five days. The efficiency of the whole bioethanol production process was assessed as ethanol overall yields (g ethanol/100 g raw material). The best overall yield was achieved by sample BS04 (8.98 g ethanol/100 g raw material).

Keywords: stranded driftwood residues; bioethanol; steam explosion; cellulose hydrolysis

1. Introduction

The accumulation of stranded driftwood residues (SDRs) is an issue that has been afflicting the Italian coastal zone for a long time and it has been particularly amplified in recent years because of increased flooding [1]. The intense precipitations originating the rapid and large increase in discharge in the channel network frequently trigger slope instabilities, such as landslides and debris flows. These colluvial processes, coupled with the fluvial dynamics, supply large volumes of both sediments and large wood trunks to the channels. The wood residues enter into the channels and they are transported downriver during high flows, accumulating in the dead water area of the stream, or are flushed directly into the sea. During a coastal storm, the SDRs can end up on coastal beaches, causing the formation of heaps whose abundance decreases as the distance from the river mouth increases [2]. SDRs are of great concern to local authorities since they cause difficulties in the important economic sector of seaside tourism. In fact, SDRs deposited along the coast are unsightly and unwelcoming to tourists or beachgoers, resulting in losses for seaside tourism [3]. For these reasons



SDRs have to be removed from the beaches with huge costs for local authorities and considerable environmental impacts, due to their landfill disposal. The Italian situation was deeply studied by Biomass Research Centre (CIRIAF-CRB) in the context of a research project alongside with the Italian Ministry of the Environment and Protection of Land and Sea. A data-gathering campaign for the period of 2010–2014 showed that every year, about 200,000 t of SDRs accumulate along the Italian shores. At present, in Italy, SDRs are classified as municipal solid waste, so each municipality, on the basis of its own management system, performs the collection and the disposal into a landfill or the on-site incineration of SDRs (about 90% and 10%, respectively). The first approach is characterized by high management cost, which is estimated to be around 120 €/ton (mean of the values provided by municipalities), and environmental issues, such as the occupation of valuable land space and air-water-soil pollution due to discharging wood degradation products into the atmosphere, earth and groundwater. The on-site incineration is obviously a low-cost management approach, but it can be very dangerous for the environment. In fact, burning SDRs can produce polychlorinated dibenzo-p-dioxins and polychlorinated dibenzo-p-furans, because of saline water absorption [4]. Since SDRs are mainly composed of lignocellulose, the production of second-generation bioethanol could be a solution for the high management cost and environmental issues related to SDRs. Second-generation bioethanol is a valuable bio-resource that could potentially allow municipalities to earn around 150–200 €/ton of SDRs, reducing the high management cost [5,6]. Lignocellulosic ethanol is an alternative to thermochemical energy production (combustion, gasification or pyrolysis) from this kind of biomass; ethanol can substitute a fraction of the gasoline in the transport sector or, after a reforming process, feed fuel cells [7-9]. Second-generation bioethanol is produced starting from lignocellulosic biomass after an appropriate pretreatment method, saccharification by cellulosic enzyme and yeast fermentation. Several pretreatment methods [10-12] have been proposed in order to alter or fractionate this type of biomass and render its main components available for saccharification and fermentation. The Steam explosion (SE) process is an eco-friendly pretreatment [13] that uses hot steam for deconstructing the lignocellulosic biomass entirety [14,15]. The SE pretreatment allows the recovery of a large fraction of sugars derived from hemicellulose (both monomeric and oligomeric) into an aqueous solution and a lignocellulosic pulp where cellulose is more easily subjected to enzymatic attack [16,17]. Three different process configurations can be employed for the production of bioethanol from pretreated biomass, such as separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF) and semi-simultaneous saccharification and fermentation (SSSF). The SHF process is divided into two steps, the first performed at the optimal conditions for the enzyme (50 $^{\circ}$ C and pH 5) and the second for the yeast (32 °C and pH 5), while in the SSF process the operational conditions are intermediate between enzymatic hydrolysis and fermentation (37 °C and pH 5). SSSF consists of a pre-hydrolysis (4–24 h) followed by SSF [18,19]. Recent studies showed variable results of SHF and SSF in terms of ethanol yield, depending on the operational conditions, microorganism and biomass employed [20–23].

On the basis of these premises, an experiment on SDRs collected from Italian coast (Abruzzo Region) for bioethanol production was carried out in order to transform a potential pollution and safety issue into valuable bio-resources, maximizing the bioethanol yield.

The SDRs were pretreated by SE at four different $LogR_0$ (3.65, 4.05, 4.24 and 4.64) in order to evaluate the pretreated material's accessibility to enzymatic attack and the holocellulose recovery. The produced water-insoluble substrates (WIS) underwent enzymatic hydrolysis with an solid/liquid (S/L) ratio of 1% and an enzyme dosage of 30% in order to estimate the maximum enzymatically accessible cellulose content. Two SSSF processes with an S/L ratio of 15% and an enzyme dosage of 15% were carried out for the samples with the highest hydrolysis yield and better compromise between hydrolysis yield and holocellulose recovery. The results are presented in terms of ethanol yield, and expressed as grams of produced ethanol on 100 grams of raw material (RM).

2. Materials and Methods

2.1. Feedstock

SDRs were gathered from the Adriatic coast in Abruzzo (Giulianova, Italy) after a storm. About 100 kg of SDRs were collected in 1000 m² area selecting pieces of different size in order to obtain a representative sample.

The 100 kg of SDRs were subjected to size-reduction (2–3 cm) by a cutting mill and then dried at 40 °C for one week. After the drying process the SDRs were quartered until a sample of 10 kg was obtained. In this method the RM is first thoroughly mixed on a hard, clean surface and then formed into a cone in the centre of the surface. The cone is divided into four even quarters which are then separated from each other. Two opposite quarters are removed and the two remaining quarters are mixed together again. This process is repeated until a sample of the required size is obtained.

The quartered SDRs (13.30% moisture content) were characterized according to the National Renewable Energy Laboratory (NREL) [24] analytical methods for biomass in order to evaluate its composition. The same SDRs were used for the SE tests.

2.2. Biomass Pretreatment

SE pretreatment of SDRs were performed in order to deconstruct the lignocellulosic biomass. The SE pretreatment plant was described in a previous work by the same authors [15]. The efficiency of the pretreatment is affected by time and temperature according to Equation (1):

$$R_0 = t \times e^{(T-100)/14.75} \tag{1}$$

where *t* is time (min) and *T* temperature ($^{\circ}$ C).

The pretreatment range was chosen according to literature data [25], where lignocellulosic biomass was usually pretreated by SE at temperatures between 170 and 220 °C and residence time between 5 and 15 min. The maximum temperature value employed in the experiments was 220 °C in order to avoid cellulose degradation which starts at 230 °C [26] while the time was kept within 15 min in order to deconstruct the biomass in a reasonable time, considering a potential industrial optimization. Temperatures below 170 °C would not deconstruct the biomass in an efficient way reducing the saccharification yield [17]. In this work four different LogR₀ (3.65, 4.05, 4.24 and 4.64) were used to pretreat SDRs. The pretreatment conditions are summarized in Table 1.

Sample ID	T (°C)	T (min)	LogR ₀
BS01	190	10	3.65
BS02	190	25	4.05
BS03	210	10	4.24
BS04	210	25	4.64

Table 1. Steam explosion (SE) conditions.

For each LogR₀ values, two consecutive explosions were executed using 500 g of RM for each explosion. After the SE a WIS rich in cellulose and lignin and a pretreatment liquor (PL) rich in pentose sugars were obtained. The WIS was washed with water with a S/L ratio equal to 1:10 at 50 °C for 30 min. The two obtained WIS for each LogR₀ were gathered and an aliquot of WIS was selected through quartering sub-sampling method and analyzed according to NREL analytical methods for biomass [27]. The PL was analyzed according to NREL analytical methods for biomass [28].

2.3. Enzymatic Hydrolysis

The four WIS obtained after the SE process underwent enzymatic hydrolysis for 96 h. The tests were performed in 50 ml flask with a S/L ratio of 1% in an orbital shaker (300 rpm) under controlled

conditions (T = 50 °C and pH = 5). Each enzymatic hydrolysis was performed twice using citrate buffer for pH maintenance and sodium azide as antibiotic as suggested by NREL procedure [29]. The enzyme (NS-22192) provided by Novozyme, was employed at a dosage of 30% (w enzyme/w cellulose).

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

The hydrolysis yields at each time (4, 24, 48, 72 and 96 h) (Hy_t %) were calculated as following Equation (2):

$$[Hy_t\% = \frac{r_{G_c} \times f_G}{WIS_l \times C_{\%}} \times 10^4]$$
⁽²⁾

where r_{Gc} is the molecular weight ratio of 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.

2.4. SSSF (Semi-Simultaneous Saccharification and Fermentation)

The two samples that showed the highest hydrolysis yields were chosen to undergo the SSSF process with a S/L ratio of 15%.

The SSSF process consisted on a 4 h pre-saccharification step of pure enzymatic hydrolysis (T = 50 °C and pH = 5) followed by a 92 h step of SSF (T = 37 °C and pH = 5). The whole process was conducted in a Biostat[®] A-Plus-Sartorius reactors with an integrated control system of pH, temperature and stirring. The enzyme NS-22192, was employed at a dosage of 15%. A suggested dosage (1.41 g) of dry yeast (Ethanol Red[®]) provided by Fermentis company was employed for all trials. Since this kind of yeast is not naturally able to ferment C5 sugars, the PL was not used for ethanol production in the experimentation.

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

The ethanol production for each sample during SSSF was calculated as gram of produced ethanol on grams of cellulose in input as reported in Equation (3):

$$[EtOHy\% = \frac{EtOH}{WIS_l \times C_{\%} \times 0.5661} \times 10^4]$$
(3)

where 0.5661 is referred to the maximum theoretical ethanol yield obtainable from cellulose, and it is derived from the product between the cellulose to glucose (1.11) and glucose to ethanol (0.51) maximum theoretical yields.

The overall ethanol yield (*OY*) displays the ratio between the produced ethanol (g) and 100 grams of RM which went through the whole process (SE plus SSSF) as shown in Equation (4).

$$[OY = \frac{EtOH}{WIS_{I}} \frac{WIS_{r}}{RM} \times 100]$$
(4)

where the WIS_r is the total water-insoluble substrate recovered after the SE pretreatment (g). WIS_r/RM (g) represents the SE pretreatment yield.

The relative overall ethanol yield $(OY_{\%})$ was calculated as follows in Equation (5):

$$[OY_{\%} = \frac{OY}{C_{RM\%} \times 0.5661} \times 100]$$
(5)

where $C_{RM\%}$ is the cellulose content in RM. All the variables regarding solid materials in the calculations are considered on dry basis.

3. Results and Discussion

The starting RM showed the following composition (Table 2).

Table 2. Raw material (RM) percentage composition and the relative standard deviations (S.D.). The cellulose and hemicellulose percentages are expressed as their monomeric sugars.

Component	Cellulose %		Hemice	ellulose %		Acetyl %	Lignin %	Extractives %	Ash %	Tot. %	Other %
Monomer	Glucose	Xylose	Galactos	e Mannose	Arabinose						
RM	31.43	12.63	0.75	1.03	0.50	4.27	27.77	6.19	4.86	85.30	14.70
S.D.	0.59		(0.23		0.04	1.53	0.15	0.35	1.70	

As shown in Table 2, the cellulose content of RM was about 31%, a little lower than other lignocellulosic biomasses (e.g., cardoon or Phragmites australis) [16,19]. This lower value of cellulose could be related to a partial decomposition of organic matter that occurs when biomass is exposed for a long time to weathering and the action of natural micro-organisms. The hemicellulose is composed mainly by xylose (>85%), a pentose sugar that cannot be fermented by Saccharomyces cerevisiae.

Moreover, high SD values indicated that the RM was rather nonhomogeneous because of its origin.

The four WIS obtained after the SE treatment were characterized in order to evaluate their cellulose and hemicellulose content. Table 3 shows the pretreatment conditions, the WIS recovered after the SE and their content in cellulose, hemicellulose and lignin.

Table 3. Water-insoluble substrates recovery (WIS_r) and composition: hemicelluloses (H); cellulose (C); lignin (L).

Sample	LogR ₀	WISr	%H	%C	%L
BS01	3.65	78.34%	12.71%	39.52%	36.83%
BS02	4.05	77.06%	5.80%	44.30%	44.02%
BS03	4.24	73.63%	2.82%	44.73%	44.00%
BS04	4.64	69.09%	0.82%	46.81%	47.07%

The WIS_r after the pretreatment showed a variation in mass, decreasing at an increased severity factor, since hemicellulose is easily degraded by hot steam. This determined an enrichment in the cellulose, which was not even degraded by harsher conditions. As demonstrated by other studies [14,30], SE does not affect the lignin fraction, causing an enrichment in its percentage into the WIS.

Since cellulose was not degraded by the pretreatment, the PL after the SE was only analyzed in terms of total solubilized sugars from the hemicellulose. This value was used for determining the hemicellulose dissolved into the liquid. The percentages of hemicellulose recovered into the PL, the WIS, and its total recovery after the SE pretreatment are shown in Table 4.

Table 4. Hemicellulose recovery (Hr) in pretreatment liquor (PL) and WIS.

Sample	LogR ₀	%H _r PL	%H _r WIS	%Total H _r
BS01	3.65	17.86	66.79	84.65
BS02	4.05	14.79	29.95	44.76
BS03	4.24	11.80	13.94	25.74
BS04	4.64	5.27	3.82	9.09

The data in Table 4 show that the percentage of hemicellulose turning into WIS considerably dropped at an increasing severity factor. The $^{\circ}$ H_r PL showed percentages equal to 17.86% for LogR₀ 3.65 and at the same severity factor the amount of hemicellulose present in the WIS was 66.79%. At higher severity factors the hemicellulose recovered into the WIS started decreasing, but at the same

time the %H_r PL showed the same trend, suggesting its conversion into inhibitors such as furfural, 5-HMF, levulinic acid and formic acid [31]. Furthermore, after the SE pretreatment, it is desirable to recover as much hemicellulose as possible and at the same time deconstruct the lignocellulosic matrix. This condition might be verified by samples BS02 and BS03 that presented an amount of %Total H_r equal to 44.76% and 25.74%, respectively. The enzymatic hydrolysis (S/L ratio of 1%) was performed twice and an aliquot of each sample was filtered and analyzed at 24, 48, 72, and 96 h by the HPLC system. This S/L ratio was chosen in order to determine the maximum enzymatically accessible cellulose. In order to describe the enzymatic hydrolysis, the conversion of cellulose into glucose with respect to time was plotted in Figure 1.



Figure 1. Glucose concentration trend during enzymatic hydrolysis at solid/liquid (S/L) ratio = 1%.

The H_y % increased for increasing LogR₀ values; in fact, the higher the pretreatment severities were, the more cellulose was hydrolyzed into glucose, showing a similar trend at every LogR₀. In all the samples the H_y % decreased after 24 h due to the simultaneous effect of the substrate decreasing and the product inhibition of the glucose [32]. Especially for sample BS04, a rapid growth was observed in the first 24 h of the process (\approx 95% of total hydrolyzed cellulose) while only \approx 5% was hydrolyzed between 24 and 96 h. This was probably due to a higher accessible fraction of cellulose, resulting in a faster hydrolysis process. Moreover, the results in Figure 1 showed that a pre-hydrolysis step of 4 h for the SSSF process could release an appropriate amount of glucose for starting the yeast fermentation [33] when a S/L ratio of 15% is employed.

The final values of the enzymatic hydrolysis yield were calculated at 96 h and according to the results obtained in Table 5, the best result in terms of H_{y96} % was achieved by sample BS04 and was equal to 83.17%.

Table 5. Enzymatic hydrolysis results after 96 h at S/L ratio = 1% and enzyme dosage = 30%.

Sample	LogR ₀	$H_{Y96}\%$	Glucose(g)
BS01	3.65	21.10%	0.46
BS02	4.05	44.07%	1.08
BS03	4.24	51.18%	1.33
BS04	4.64	83.17%	2.16

The lowest result was obtained by sample BS01 which showed an H_y % of 21.10%, while samples BS02 and BS03 showed a similar result, around 50%. In Table 5, we reported the grams of glucose

produced for each sample at the described working conditions. As shown, these values increased with the pretreatment severity. Moreover, Table 5 gives the idea that SDRs are very recalcitrant, since they need to be pretreated at $LogR_0$ values above 4.24 for achieving an H_y % higher than 50%. These $LogR_0$ values are very high and atypical for lignocellulosic biomass [34] and they are probably due to the nature of SDRs where the weathering could cause a cellulose collapse, making it less accessible to the enzyme's action.

According to the data shown in Tables 4 and 5, the samples BS02 and BS04 were chosen to undergo the SSSF process with a S/L ratio of 15% and an enzyme dosage of 15%. The former presented the best compromise between %Total H_r and H_v % while the latter presented the best H_v %.

The SSSF process consisted of a 4 h step of pure enzymatic pre-hydrolysis and a 92 h step where the conditions were intermediate for the hydrolysis and fermentation. The advantage is that the fermentation removes glucose from the solution which regulates the product inhibition of the enzymatic activity, especially for beta-glucosidase [35]. In Figure 2 the data obtained from the SSSF (sample from BS02 and BS04), relative to both glucose consumption and ethanol production, were plotted as a function of time.



Figure 2. Ethanol production and glucose consumption in BS02 (red) and BS04 (blue) at S/L ratio = 15% and enzyme dosage = 15% by semi-simultaneous saccharification and fermentation (SSSF) process.

Despite the glucose concentration after 24 h being close to zero, the ethanol concentration persisted to grow, meaning that the enzyme's action on cellulose was working efficiently between 24 h and 96 h.

In fact, despite the intermediate temperature values for the enzymes, the removal of the glucose from the solution by yeast fermentation indicated a decrease of the product inhibition. A simultaneous ethanol production resulted in the total consumption of accessible cellulose after 72 h of the SSSF process for sample BS02 and 96 h for sample BS04. In any case, the ethanol production of BS04 was always higher than that of BS02 for all samplings analyzed. Since the initial glucose concentration (4 h) was similar for the two samples, the best result of BS04 was related to its enhanced cellulose digestibility, due to a more efficient pretreatment.

Table 6 reports the yields of samples BS02 and BS04 at 96 h in terms of grams of ethanol on 100 grams of cellulose loaded into the bioreactor (EtOH_y%) and 100 grams of RM (OY). The last value, OY%, states the percentage of maximum theoretical ethanol production achieved from SDRs by the whole production process.

Since samples BS02 and BS04 had a similar content of cellulose (Table 3), the higher the ethanol production (mg/mL) was, the higher the EtOH_y% was. In addition, even though the WIS_r of BS04 was about 8% lower than that of BS02 (Table 3), the OY% of BS04 (50.48%) was about 8% higher than that of BS02 (42.28%). The reason for this result is again due to the enhanced cellulose digestibility of

BS04. Regarding the hemicellulose, also supposing its total recovery and employment by hemicellulase enzymes and genetically modified Saccharomyces cerevisiae or other yeast able to ferment C5 sugars, the OY% of sample BS02 would still be similar to sample BS04. In fact, the total hemicellulose recovered after the SE of sample BS02 was equal to 44.76% (Tables 3 and 4), which is only 2/2.5 g of ethanol on 100 g RM, hypothesizing the same efficiency obtained with the cellulose conversion. This scenario would require a real increase in complexity and cost of the whole production process, so for SDRs it is more convenient only to optimize the SE pretreatment for cellulose conversion into ethanol.

The best OY% displayed in Table 6 is lower than the ones achieved from other lignocellulosic biomass pretreated at lower $LogR_0$, with the same S/L ratio and similar enzyme loadings [36].

Sample	EtOHy%	ΟΥ	ΟΥ%
BS2	22.0361	7.5227	42.28
BS4	27.7724	8.9818	50.48

Table 6. Ethanol production results at S/L ratio = 15% by SSSF process.

The OY% obtained at the high $LogR_0$ employed for the pretreatment is related to the recalcitrant nature of SDRs, which is probably due to the origin of these residues. In order to support this thesis, further studies on SDRs could be performed to investigate the fine structure of cellulose, hemicellulose and lignin. However this OY% could permit municipalities to earn around 50–100 \notin /ton of SDRs [5,6], reducing the high management cost related to the disposal of SDRs into landfill.

4. Conclusions

This work proved the suitability of SDRs as a substrate for the production of second-generation ethanol. The best overall yield was achieved by sample BS04 (8.98 g ethanol/100 g RM) after undergoing SE pretreatment at $LogR_0$ 4.64, enzymatic hydrolysis (0.15 g enzyme/g cellulose) with a S/L ratio of 15% and yeast fermentation by Saccharomyces cerevisiae.

In particular, the experiment showed that a good OY can be achieved only by pretreating the SDRs at a high $LogR_0$ and so giving up the hemicellulose recovery and employment. This aspect could be related to the origin of SDRs, which could have produced a collapse of the whole lignocellulosic matrix, causing difficulties in ethanol production. Other experiments will have to carried out for understanding more thoroughly the lignocellulosic matrix of SDRs.

Since SDRs are classified as waste, the production of 90 grams of ethanol on 1 kg of RM could be considered an efficient way to valorize these lignocellulosic residues, allowing municipalities to reduce the costs linked to SDR management. However, future feasibility research should be performed for demonstrating the economical advantage of SDR conversion into bioethanol, taking into account the cost of collecting, aggregating and transporting of this biomass. In addition, since the great variability of SDRs is related to the variability of biomasses that belong to a specific geographical area, a future work could be carried out for statistically validating the magnitude of the SDR sampling method proposed here.

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Author Contributions: Gianluca Cavalaglio coordinated the design of the experiments, supervised the laboratory experimentation and wrote the article. Andrea Nicolini and Anna Laura Pisello designed and supervised the experiments. Franco Cotana supervised the research activities and the article preparation. Mattia Gelosia supervised the laboratory experimentation and analyzed the data. Silvia D'Antonio performed the laboratory experimentations.

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