

Article

Enhancing Bioenergy Yields from Sequential Bioethanol and Biomethane Production by Means of Solid–Liquid Separation of the Substrates

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Abstract: The production of second-generation ethanol using lignocellulosic feedstock is crucial in order to be able to meet the increasing fuel demands by the transportation sector. However, the technology still needs to overcome several bottlenecks before feasible commercialization can be realized. These include, for example, the development of cost-effective and environmentally friendly pretreatment strategies and valorization of the sidestream that is obtained following ethanol distillation. This work uses two chemical-free pretreatment methods—nitrogen explosive decompression (NED) and synthetic flue gas explosive decompression—to investigate the potential of a bioethanol production sidestream in terms of further anaerobic digestion. For this purpose, samples from different stages of the bioethanol production process (pretreatment, hydrolysis, and fermentation) and the bioethanol sidestream went through a separation process (involving solid–liquid separation), following which a biomethane potential (BMP) assay was carried out. The results show that both factors being studied in this article (involving the pretreatment method and the separation process) served to influence methane yields. Liquid fractions that were obtained during the process with NED gave rise to methane yields that were 8% to 12% higher than when synthetic flue gas was used; fermented and distillation sidestream gave rise to the highest methane yields (0.53 and 0.58 mol CH₄/100 g respectively). The methane yields from the liquid fractions were between 60–88% lower than those that were obtained from solid fractions. Samples from the bioethanol sidestream (solid fraction) that were pretreated with NED had the highest methane yield (1.7 mol CH₄/100 g). A solid–liquid separation step can be a promising strategy when it comes to improving the energy output from lignocellulosic biomass and the management of the ethanol distillation sidestream.

Keywords: anaerobic digestion; bioethanol; biofuel; lignocellulose; sidestreams; zero-waste

1. Introduction

Bioethanol is one the most successful alternatives in terms of the replacement of fossil fuels in the transport sector. Its commercial-scale production is centred on the use of starchy and sugary food-grade feedstock and, therefore, it is regarded as a first-generation ethanol. However, regardless of whether the food-versus-fuel debate still holds true, it has been recognized that first-generation ethanol plants cannot be fully responsible for fulfilling the increasingly demanding requirements of a fossil-free

energy sector [1]. Therefore, the search is on for alternative sources of energy using non-edible biomass as a feedstock. Among the different forms of technology and the various processes that are currently available, the use of lignocellulosic biomass as a feedstock for the production of second-generation biofuels has been reported as a promising replacement for fossil fuels, due mainly to its lower costs, higher levels of accessibility, and its sustainability [2].

Lignocellulosic biomass is composed of three major polymers: cellulose (40–60%); hemicellulose (20–40%); and lignin (10–25%) [3]. Due to its recalcitrant and complex structure, the conversion of cellulosic biomass into ethanol requires four sequential steps: pretreatment, hydrolysis, fermentation, and distillation. The pretreatment step needs to be added in order to open up the structure and ensure proper access to its polymers [4]. Pretreated material is further converted into sugar monomers using acids or enzymes (this being the hydrolysis step). In the next step (fermentation), yeast or bacteria are used to convert the sugars into ethanol. After the fermentation process has been completed, the broth is distilled so that the ethanol can be recovered.

From the four steps previously reported, great attention has been paid to the pretreatment method, since it is fundamental for a successful deconstruction of the lignocellulosic structure. It has been discussed in the literature that the pretreatment stage is responsible for 30% of the overall process costs. This fact highlights the need to develop more energy- and environmentally friendly pretreatment alternatives [5]. Recently, a pretreatment strategy centered on explosive decompression using nitrogen or synthetic flue gas was developed. Since chemicals are not used in these methods, their utilization could potentially be an attractive solution for pretreatment of lignocelluloses [3,6,7]. The nitrogen explosive decompression makes use of nitrogen molecules being smaller than water molecules, meaning they are easily able to enter the cells of the feedstock. In the flue gas explosive decompression, the gas is introduced into the reactor using a modified injection tube with a circular form at the bottom, which allows the gas to diffuse better into the reaction broth. Due to the composition of the flue gas (80% nitrogen and 20% carbon dioxide) and the characteristics of the modified injection tube, carbon dioxide molecules will be dissolved in water and penetrate more easily into the biomass. This will lead to the production of carbonic acid and to the start of an acid-catalyzed autohydrolysis process. As a result, the hemicellulose will be easily dissolved, making biomass more accessible for the anaerobic microorganisms and enhancing biomethane production [7–9]. Raud et al. [10] investigated the efficiency of nitrogen and flue gas explosive decompression pretreatment methods in bioethanol production. The authors concluded that ethanol yields and fermentation efficiencies of samples pretreated with flue gas were lower than samples pretreated with nitrogen and further research needed to be done with these pretreatment methods in order to determine which inhibitory compounds are formed in different production steps and how to remove them in order to improve the overall bioethanol yields of the process. Due to the characteristics of both pretreatment methods and the research gaps that were found in the literature, further studies on the performance of these pretreatment methods are of interest.

Lignocellulosic materials have been a matter of intense research in terms of uncovering the production of second-generation ethanol. For this purpose, pilot plants and demo plants have been constructed around the world, but feasible commercial production of second generation ethanol is still cumbersome. This is related to the intrinsic complexity of the process involving, for instance, an energy-demanding pretreatment to open up the recalcitrant structure, costly enzymes, and the low use of the biomass since hemicellulose and lignin fractions are generally not converted into ethanol. Additionally, various lignocellulose-based byproducts are left unused during substrate processing and, therefore, represent an environmental problem [1,3].

Anaerobic digestion has been reported as a promising handling option for the range of products that originate from second-generation ethanol plants. In addition, it can be used as a strategy to offset the costs involved in the production of ethanol plants, to improve the energy efficiency of the production chain, and to reduce that chain's environmental impacts [3]. Bioethanol production followed by biogas production has been reported in the available literature as a promising solution when it comes to enhancing the energy balance of the bioethanol production chain [11]. Elsayed et al. [12]

studied the potential of sequential bioethanol and biogas production. The authors showed that these strategies improve the energy output that is produced by the biomass. Calicioglu and Brennan [13] utilized duckweed as a feedstock so that they could investigate the energetic potential of sequential bioethanol and biomethane production. The results showed that duckweed has more potential for use as biomethane than it does for bioethanol production and that samples that had been through the sequential process had an energy gain of 70.4%.

Nevertheless, there is a continuous search for strategies that aim to improve the efficiency of second-generation bioethanol production. Solid–liquid separation has also been reported as a solution to improve the overall biogas yields. Cestonaro do Amaral et al. [14] investigated the potential of solid separation in various types of manure. The authors concluded that, due to the composition of the samples after the separation process, this is an interesting option in terms of improving biogas and biomethane yields. Dos Anjos et al. [15] analyzed the influence of solid–liquid separation in the substrates for further biogas production. It was reported that, in the separation process, the digesters have a faster stabilization time, higher biogas and biomethane yields, and better retention times. Kokko et al. also studied the benefit of the solid–liquid separation strategy. The authors separated the solid and liquid fraction of sedimented fiber samples and concluded that both fractions (solid and liquid) are promising sources of feedstock for the anaerobic digestion process. These studies suggest that, irrespective of where the separation process takes place (such as in sludge or substrate), the composition of the supernatant and the solid fraction will be distinct and both can be promising when it comes to biogas/biomethane production.

This study aims to enhance the bioenergy yields from combined bioethanol and biomethane production from barley straw-based second-generation ethanol by means of the solid–liquid separation strategy. For this purpose, two pretreatment methods were applied (explosive decompression using nitrogen and synthetic flue gas) and a particular focus was given to their impact on the sidestream origin as well as to samples from different stages of the bioethanol production process (pretreatment, hydrolysis, and fermentation).

2. Materials and Methods

2.1. Bioethanol Production

2.1.1. Biomass and Pretreatment

To study the improvement of biomethane yield from the bioethanol production sidestream by means of solid–liquid separation, barley straw (*Hordeum vulgare*) was used as substrate. The feedstock was grown in a field belonging to the Estonian University of Life Sciences (which is located near Tartu, Estonia) and was harvested in 2013. The straw was dried to a water content of <10%, and then was ground down and sieved to small-size fractions (1–3 mm) using the Cutting Mill SM 100 comfort (from Retsch GmbH).

The biomass was pretreated using two different pretreatment methods, these being nitrogen explosive decompression (NED) and synthetic flue gas explosive decompression (which was composed of 20% carbon dioxide and 80% nitrogen). For this end, 100 g of biomass was introduced into the 2 L non-stirred pretreatment vessel (Series number 4600, from the Parr instrument company, USA) and mixed with 800 g of distilled water. The samples were heated from 23 °C up to 150 °C at a pressure of 30 bar. Once the samples had reached a temperature of 150 °C, the heater was switch off and a retention time of one minute was applied. Then, the pretreatment vessel was cooled down naturally (to near 80 °C) in a process that lasted for approximately one hour. After that, a process of rapid decompression was applied to release the pressure from the vessel [7].

2.1.2. Hydrolysis, Fermentation, and Distillation

The enzymatic hydrolysis was carried out in 1000 mL Erlenmeyer shake flasks (with a working volume of 1000 mL), using 30 FPU g⁻¹ cellulose of the commercial cellulase mixture, Accellerase1500 (purchased from DuPont de Nemours). The samples were placed in the shaker incubator at 200 rpm and 50 °C for a period of twenty-four hours [3].

The hydrolysates were moved to 1000 mL florence flasks. In order to convert the glucose into ethanol, 2.5 g of the yeast *Saccharomyces cerevisiae* (Turbo yeast T3) was added to the samples. This yeast is commonly available in brewery shops and this batch was stored in the refrigerator until used. The fermentation process was carried out under anaerobic conditions, across a period of seven days at room temperature.

Following the conclusion of the fermentation stage, the samples were distilled in a Buchi R-210 Rotavapor System from BÜCHI Labortechnik (Switzerland) at 175 mbar and a bath temperature of 60 °C. The remaining material from the distillation process (the bioethanol sidestream) was further utilized in the biomethane potential (BMP) test.

2.2. Biomethane Potential

The BMP test that was utilized in this study is based on the procedures that have been described by Owen et al. [16]. For this end, samples from different fractions (solid and liquid) and stages (pretreatment, hydrolysis, and the fermentation stage, plus the bioethanol sidestream) were used as a substrate.

The BMP test was carried out in 575 mL plasma bottles with a total volume (substrate and inoculum combined) of 200 mL and an inoculum-to-substrate ratio (I/S ratio) of 0.25. The inoculum being utilized in the experiments was collected from the wastewater treatment plant in Tartu. Prior to use, it was incubated at a temperature of 36 °C for a period of four days to reduce the nonspecific gas production. The experiments were carried out in triplicate during a period of 41 days, and the gas composition was evaluated daily during the first two weeks, every other day in weeks three and four, and once a week in weeks five and six. Biogas production was determined by measuring the pressure growth in the test bottles. To ensure this, a pressure meter (a BMP-Testsystem WAL) was used to register the pressure before and after the GC analysis. The methane content was measured chromatographically using a gas chromatograph (CP-4900 Micro-GC, Varian Inc). After that, the initial, final, and cumulative amounts of methane were calculated based on Equations (1)–(3):

$$[\text{CH}_4 \text{ I}] = MF \frac{P_I V_{HS}}{R (273.15 + T)} \quad (1)$$

$$[\text{CH}_4 \text{ F}] = MF \frac{P_F V_{HS}}{R (273.15 + T)} \quad (2)$$

$$[\text{CH}_4 \text{ C}]_t = ([\text{CH}_4 \text{ I}]_t - [\text{CH}_4 \text{ F}]_{t-1}) + [\text{CH}_4 \text{ C}]_{t-1} \quad (3)$$

where P_I (Pa) is the headspace pressure that was registered before the GC analysis was carried out, V_{HS} (m³) is the volume of the bottle's headspace, MF is the methane percentage measured by GC, R is the ideal gas constant (8.314 Jmol⁻¹K⁻¹), T is the incubation temperature (°C), P_F (Pa) is the headspace pressure that was registered after the GC analysis, $[\text{CH}_4 \text{ I}]_t$ (mol CH₄) is the initial amount of methane in the bottle's headspace in the current period of time, $[\text{CH}_4 \text{ F}]_{t-1}$ (mol CH₄) is the final quantity of methane in the bottle's headspace in the previous period of time, and $[\text{CH}_4 \text{ C}]_{t-1}$ (mol CH₄) is the cumulative methane that was produced in the previous period of time.

The amount of methane produced by the actual substrate (pretreated, hydrolyzed, fermented, and sidestream) was calculated taking into the account that 100 g of raw biomass was originally added into the system. The results are presented in mol CH₄ per 100 g of raw biomass. The extended version of the biomethane potential methodology is available in our previous publications [7,17].

2.3. Chemical Analysis

The composition of the straw (cellulose, hemicellulose, and lignin) was determined in an ANKOM 2000 analyzer according to the standardized neutral detergent fiber method, which was proposed by Van Soest et al. [18], and the official methods of analysis that are used by the Association of Official Analytical Chemists (AOAC) [19]. The moisture content was measured in a Kern MLS-50-3D moisture analyzer (from Kern & Sohn GmbH, Balingen - Germany).

The solid and liquid fractions were collected after the pretreatment, hydrolysis, fermentation, and distillation steps by means of a separation process. The broth from the various stages of bioethanol production was separated in a centrifuge (Thermo Scientific Heraeus megacentrifuge, Waltham, USA) at 10,000 rpm for twenty minutes until the solid and liquid fraction were totally separated.

The liquid fractions that were collected after the pretreatment, hydrolysis, fermentation, and distillation steps were quantified in terms of ethanol, glycerol, acetic acid, glucose, xylose, arabinose, cellobiose, galactose, and mannose by means of the use of HPLC equipment (Prominence-i LC-2030C 3D Plus, Shimadzu, Japan) coupled with a RID detector at 60 °C (20A, Shimadzu, Japan). Sugar concentrations were measured using the column Rezex RPM-monosaccharide Pb²⁺ (Phenomenex, Torrance, USA) at 85 °C and Milli-Q water at 0.6 mL/min as the mobile phase, while the other molecules were quantified using the column HPX-87H (Biorad, Hercules, USA) at 50 °C and 5 mM of sulphuric acid at the same flow rate as in the mobile phase.

2.4. Statistics

The results were analyzed using the GraphPad Prism 5 software, using descriptive statistics, and Shapiro–Wilk and Kolmogorov–Smirnov’s normality tests. The Kruskal–Wallis test was used to study the differences between the variables and this was followed by Dunn’s correction for multiple comparisons. The error bars in the figures represent two standard deviations. The results were regarded as significantly different when the *p*-value was <0.05 (with confidence intervals of 95%).

3. Results

3.1. Chemical Composition

The chemical composition of samples (involving total solids and volatile solids) from the different stages and fractions of the bioethanol production process are presented in Table 1. As can be seen from the table, the ‘total solids’ (TS) content of untreated barley straw was at 931 g/kg and the volatile solids (VS) content was at 963 g/kgTS. For samples from the liquid fraction of bioethanol production that were pretreated with NED, the TS content varied between 18.0 g/kg (pretreated) and 34.2 g/kg (hydrolyzed). In samples that were pretreated with flue gas, the TS content followed the same trend. It was lower in the pretreated material (17.1 g/kg) and higher in the hydrolysis stage (37.8 g/kg). As for those samples that came from the solid fraction that had been pretreated with NED, the hydrolyzed material had the highest TS content (139 g/kg), followed by bioethanol sidestream (128 g/kg), fermented samples (123 g/kg), and pretreated barley straw (118 g/kg). When considering those samples that were pretreated with flue gas, the TS results can be seen as being within the range of 113 g/kg (bioethanol sidestream) and 144 g/kg (hydrolyzed).

The liquid fraction of both pretreatments, plus hydrolysis, fermentation, and distillation were further analyzed in terms of sugars, ethanol, glycerol, and acetic acid concentrations (Table 2). Cellobiose, galactose, and mannose were not detected in any of the analyzed samples and there were high levels of variability in the replicates; in general their concentrations were lower than 0.7 g/L. Arabinose concentrations varied between 0.3–0.8 g/L in all analyzed samples. Both pretreatments resulted in a similar profile of sugars, glycerol, and acetic acid. The yeast was able to uptake at least 98% of the present glucose and the ethanol yield was higher in the hydrolysate, which came from the biomass that had undergone the NED pretreatment (0.49 g ethanol/g glucose), when compared with the hydrolysate from the flue gas pretreatment at 0.29 g ethanol/g glucose).

Table 1. Mean values for total solids and volatile solids of samples from different fractions and steps of bioethanol production, pretreated with nitrogen explosive decomposition (NED) and flue gas (\pm represents the standard deviation).

Fraction	Pretreatment	Variable	TS	VS
			g/kg	g/kgTS
-	-	Untreated	931 \pm 0	963 \pm 0
Liquid Fraction	NED	Pretreated	18.0 \pm 0.07	997 \pm 0
		Hydrolyzed	34.2 \pm 0.09 ^a	997 \pm 0
		Fermented	20.1 \pm 0.8	997 \pm 0 ^b
		Sidestream	23.4 \pm 0.7 ^a	997 \pm 0 ^b
	Flue Gas	Pretreated	17.1 \pm 0.1	997 \pm 0
		Hydrolyzed	37.8 \pm 0.1 ^a	997 \pm 0 ^b
		Fermented	19.7 \pm 0.4	997 \pm 0
		Sidestream	19.7 \pm 0.7	997 \pm 0
Solid Fraction	NED	Pretreated	118 \pm 0	996 \pm 0 ^b
		Hydrolyzed	139 \pm 2	995 \pm 0 ^b
		Fermented	123 \pm 1	995 \pm 0
		Sidestream	128 \pm 6	995 \pm 0
	Flue Gas	Pretreated	125 \pm 4	995 \pm 1 ^b
		Hydrolyzed	144 \pm 2	995 \pm 0
		Fermented	116 \pm 3 ^a	995 \pm 0 ^b
		Sidestream	113 \pm 3 ^a	996 \pm 0 ^b

^{a,b} The superscript letters indicate no significant differences ($p < 0.05$) between averaged values.

Table 2. Concentrations of glucose, xylose, glycerol, acetic acid, and ethanol (g/L) in samples from different stages of bioethanol production that has been pretreated with NED and flue gas.

		Glucose (g/L)	Xylose (g/L)	Glycerol (g/L)	Acetic Acid (g/L)	Ethanol (g/L)
NED	Pretreated	0.48 \pm 0.02	0.6 \pm 0.4	<0.25 ^a	1.53 \pm 0.03	-
	Hydrolyzed	13.7 \pm 0.8	4.06 \pm 0.18	<0.25 ^a	1.81 \pm 0.01	1.15 \pm 0.05
	Fermented	0.25 \pm 0.09	3.6 \pm 0.4	0.7 \pm 0.1	2.2 \pm 0.2	6.3 \pm 0.0
	Sidestream	0.8 \pm 0.3	3.8 \pm 0.6	0.71 \pm 0.06	2.6 \pm 0.3	8.4 \pm 0.7
Flue Gas	Pretreated	0.64 \pm 0.14	<0.25 ^a	<0.25 ^a	1.25 \pm 0.07	-
	Hydrolyzed	15.1 \pm 1.7	4.1 \pm 0.3	<0.25 ^a	1.62 \pm 0.00	0.60 \pm 0.00
	Fermented	<0.25 ^a	2.1 \pm 0.2	0.53 \pm 0.08	1.7 \pm 0.3	4.4 \pm 1.3
	Sidestream	0.39 \pm 0.04	4.1 \pm 0.6	0.79 \pm 0.06	2.3 \pm 0.3	7.3 \pm 0.7

^a Below the detection level of 0.25 g/L.

3.2. Methane Recovery from Solid and Liquid Fractions of the Bioethanol Production Process

Figure 1 represents the biomethane potential of samples taken from the liquid fraction of different stages of the bioethanol production process (pretreated, hydrolyzed, fermented, and bioethanol sidestream).

As is presented in Figure 1 and Figure 3, at the end of the anaerobic digestion process for samples taken from the liquid fraction that were pretreated with NED, the pretreatment stage had the lowest methane yield (0.19 mol CH₄/100 g), while fermented samples and the bioethanol sidestream had the highest yields (0.53 mol CH₄/100 g and 0.58 mol CH₄/100 g, respectively). Samples from the liquid fraction that were pretreated with flue gas followed the same trend. The methane yields varied between 0.17 mol CH₄/100 g (in the pretreatment stage) and 0.52 mol CH₄/100 g (the bioethanol sidestream). The differences between the methane yields of the solid and liquid fractions are statistically significant (Table 3).

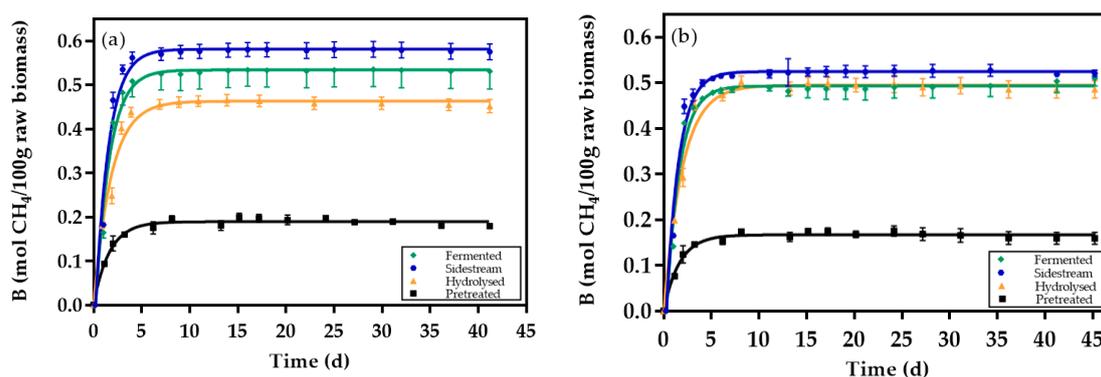


Figure 1. Experimental results and the respective fitting curves of samples from the liquid fraction of pretreated, hydrolyzed, fermented material, and the bioethanol sidestream. (a) The liquid fraction of samples that have been pretreated with NED; (b) the liquid fraction of samples that had been pretreated with flue gas. Error bars show two standard deviations.

Table 3. Statistically significant results between the parameters being studied in the methane recovery analysis.

Fraction	Pretreatment	Variable	B_{\max} mol CH ₄ /100 g
Untreated	-	-	0.91 ± 0.02 ^{a,g,h}
Liquid Fraction	NED	Pretreated	0.19 ± 0.00 ^e
		Hydrolyzed	0.46 ± 0.00 ^{e,f}
		Fermented	0.53 ± 0.01 ^g
		Sidestream	0.58 ± 0.01 ^{g,h}
	Flue Gas	Pretreated	0.17 ± 0.00 ^e
		Hydrolyzed	0.49 ± 0.01 ^{f,g,l}
		Fermented	0.49 ± 0.01 ^{f,l,m}
		Sidestream	0.52 ± 0.01 ^{g,h,l,m}
Solid Fraction	NED	Pretreated	1.3 ± 0.03 ^a
		Hydrolyzed	1.5 ± 0.02 ^b
		Fermented	1.6 ± 0.02 ^{b,c}
		Sidestream	1.7 ± 0.02 ^{b,c,d}
	Flue Gas	Pretreated	1.4 ± 0.04 ^{b,i}
		Hydrolyzed	1.6 ± 0.07 ^{b,d,j}
		Fermented	1.5 ± 0.02 ^{a,b,c,i,j,k}
		Sidestream	1.3 ± 0.03 ^{a,i,k}

^{a,b} The superscript letters indicate no significant differences ($p < 0.05$) between averaged values.

Figures 2 and 3 show the biomethane potential of samples from the solid fraction of different stages of the bioethanol production process (pretreated, hydrolyzed, fermented, and bioethanol sidestream). For samples from the solid fraction that were pretreated with NED, the methane yields varied between 1.3 mol CH₄/100 g (pretreated) and 1.7 mol CH₄/100 g (bioethanol sidestream). For samples pretreated with flue gas, the maximum methane yield was 1.6 mol CH₄/100 g (hydrolyzed), followed by 1.5 mol CH₄/100 g (fermented sampled), 1.4 mol CH₄/100 g (pretreated), and 1.3 mol CH₄/100 g (bioethanol sidestream).

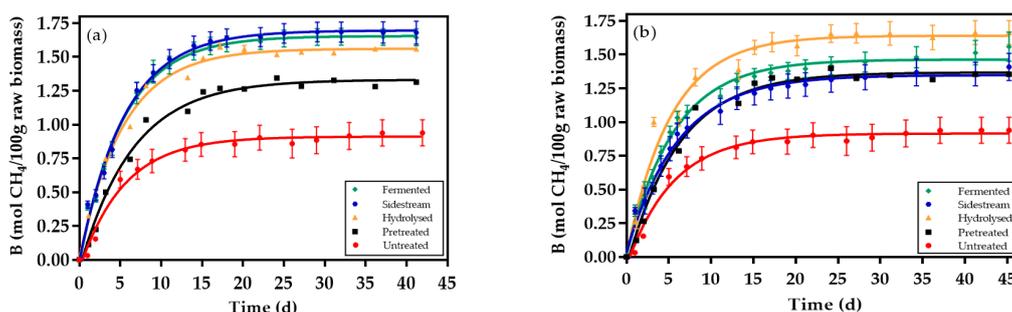


Figure 2. Experimental results and respective fitting curves for samples from the solid fraction of pretreated, hydrolyzed, fermented material, and the bioethanol sidestream. (a) Solid fraction for samples that were pretreated with NED; (b) solid fraction for samples that were pretreated with flue gas. Error bars show two standard deviations.

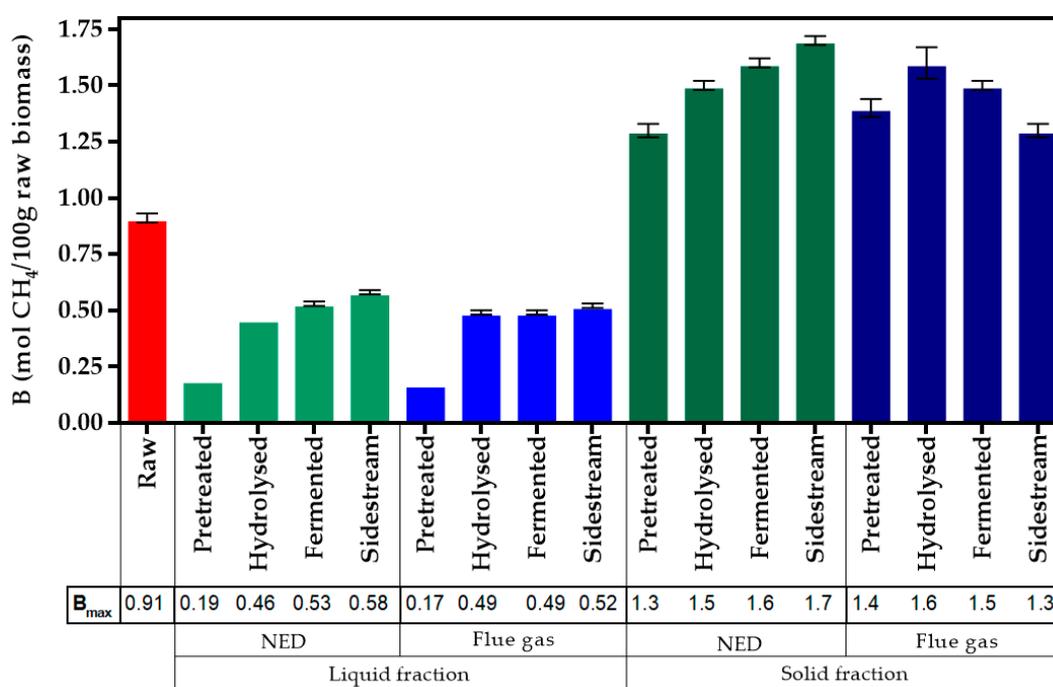


Figure 3. Maximum methane yield (B_{max}) for the fitting curves of samples taken from the liquid and solid fraction of pretreated, hydrolyzed, fermented material, and the bioethanol sidestream, pretreated with NED and flue gas. Error bars show two standard deviations.

3.3. Kinetics Rate and Digestion Time

Figure 4 illustrates the kinetic rate constant and correlation coefficient for samples from the liquid and solid fraction that were pretreated with NED and flue gas. For samples from the liquid fraction that were pretreated with NED, the kinetic rate varied between 0.55 d^{-1} (hydrolyzed) and 0.72 d^{-1} (bioethanol sidestream). For samples that were pretreated with flue gas, the highest kinetic rate was reported in the bioethanol sidestream (0.76 d^{-1}), followed by that for fermented (0.73 d^{-1}), pretreated (0.60 d^{-1}), and hydrolyzed material (0.52 d^{-1}). For samples from the solid fraction that were pretreated with NED, the kinetic rate varied between 0.16 d^{-1} (pretreated) and 0.18 d^{-1} (hydrolyzed material and bioethanol sidestream). For samples that were pretreated with flue gas, the hydrolyzed material had the highest rate constant (0.19 d^{-1}), followed by that for fermented samples (0.18 d^{-1}), pretreated barley straw, and bioethanol sidestream (0.16 d^{-1}). The correlation coefficients for all of the fitting curves varied between 0.9778 and 0.9945.

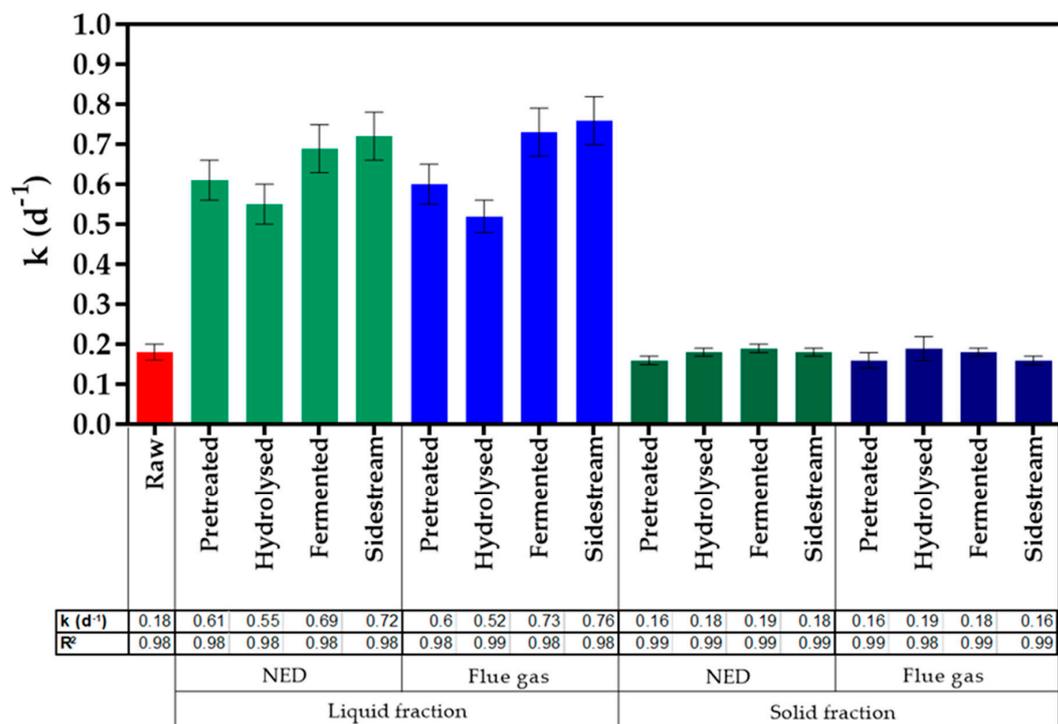


Figure 4. Kinetic rate constant (k) and correlation coefficient (R^2) for the fitting curves of samples from the liquid and solid fraction of pretreated, hydrolyzed, fermented material, and the bioethanol sidestream, pretreated with NED and flue gas. Error bars show two standard deviations.

Table 4 illustrates the time needed for the micro-organisms to achieve 85% B_{max} and 95% B_{max} . For samples from the liquid fraction that were pretreated with NED and flue gas, the bioethanol sidestream had the shortest digestion time (3.5 to 3.6 days). This achieved 95% of the methane potential around one day before the hydrolyzed samples and about half a day before the pretreated material. Samples from the solid fraction of pretreated material had the longest digestion time (18.6 to 18.9 days), followed by the bioethanol sidestream (16.6 to 18.1 days), fermented material (16.1 to 16.7 days), and hydrolyzed samples (15.6 to 16.1 days).

Table 4. Digestion time (85% B_{max} and 95% B_{max}) for samples from the liquid and solid fraction of pretreated, hydrolyzed, fermented material, and the bioethanol sidestream, pretreated with NED and flue gas.

Fraction	Pretreatment	Variable	85% B_{max}		95% B_{max}	
			mol CH ₄ /100 g	Days	mol CH ₄ /100 g	Days
Untreated	-	-	0.88	14.0	0.98	21.7
Liquid Fraction	NED	Pretreated	0.16	2.6	0.18	4.1
		Hydrolyzed	0.39	2.8	0.44	4.5
		Fermented	0.45	2.4	0.51	3.8
		Sidestream	0.49	2.4	0.55	3.6
	Flue Gas	Pretreated	0.14	2.6	0.16	4.1
		Hydrolyzed	0.42	3.0	0.47	4.8
		Fermented	0.42	2.4	0.47	3.6
		Sidestream	0.45	2.3	0.50	3.5
Solid Fraction	NED	Pretreated	1.1	12.5	1.3	18.9
		Hydrolyzed	1.3	10.2	1.5	16.1
		Fermented	1.4	10.2	1.6	16.1
		Sidestream	1.4	10.5	1.6	16.6
	Flue Gas	Pretreated	1.2	12.0	1.3	18.6
		Hydrolyzed	1.4	9.9	1.6	15.6
		Fermented	1.2	10.6	1.4	16.7
		Sidestream	1.1	11.5	1.3	18.1

3.4. The Ratio of Methane in the Produced Biogas

The ratios of methane in the biogas that has been produced for samples from the liquid and solid fractions that were pretreated with NED and flue gas are represented in Figure 5. For the liquid fractions of samples that were pretreated with NED, the highest methane ratios were achieved on day four, from fermented samples ($\text{CH}_4\text{:Biogas} = 0.74$), followed by hydrolyzed material ($\text{CH}_4\text{:Biogas} = 0.74$; $t = 9$), pretreated samples ($\text{CH}_4\text{:Biogas} = 0.69$; $t = 7$), and bioethanol sidestream ($\text{CH}_4\text{:Biogas} = 0.67$; $t = 2$). At the end of the anaerobic digestion process, hydrolyzed material and samples from the fermentation stage had the highest methane ratio ($\text{CH}_4\text{:Biogas} \approx 0.74$), followed by pretreated samples and the bioethanol sidestream ($\text{CH}_4\text{:Biogas} \approx 0.66$). For samples that were pretreated with flue gas, material from the fermentation stage and bioethanol sidestream had the highest methane ratio ($\text{CH}_4\text{:Biogas} = 0.75$; $t = 2$), followed by pretreated samples ($\text{CH}_4\text{:Biogas} = 0.69$; $t = 5$), and hydrolyzed material ($\text{CH}_4\text{:Biogas} = 0.58$; $t = 5$). At the end of the anaerobic digestion process, the biomethane production proved to have a very distinct performance. Fermented material had the highest methane ratio ($\text{CH}_4\text{:Biogas} = 0.73$), followed by bioethanol sidestream ($\text{CH}_4\text{:Biogas} = 0.69$), pretreated barley straw ($\text{CH}_4\text{:Biogas} = 0.62$), and hydrolyzed material ($\text{CH}_4\text{:Biogas} = 0.55$). Statistically significant differences were found between the ratio of $\text{CH}_4\text{:Biogas}$ of the different samples that had been pretreated with NED and flue gas.

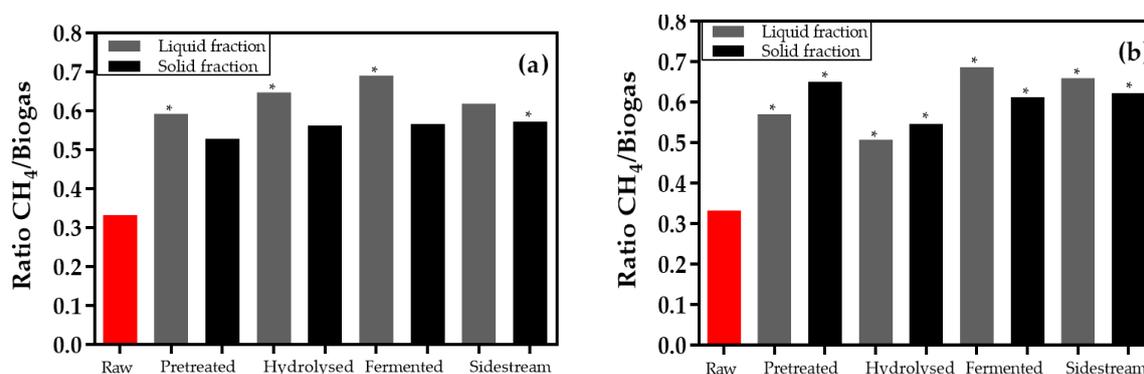


Figure 5. The ratio of the methane volume in the biogas volume for samples taken from the solid and liquid fraction of bioethanol production (a) pretreated with NED; (b) pretreated with the flue gas (the asterisks on top of the bars indicate significant differences ($p < 0.05$)).

For those solid fractions of samples that had been pretreated with NED, the highest methane ratio was reported in samples that came from the bioethanol sidestream ($\text{CH}_4\text{:Biogas} = 0.73$; $t = 1$), followed by fermented material ($\text{CH}_4\text{:Biogas} = 0.71$; $t = 1$), hydrolyzed samples ($\text{CH}_4\text{:Biogas} = 0.66$; $t = 7$), and pretreated barley straw ($\text{CH}_4\text{:Biogas} = 0.63$; $t = 7$). At the end of the anaerobic digestion process, hydrolyzed samples had the highest methane ratio ($\text{CH}_4\text{:Biogas} = 0.62$), followed by the bioethanol sidestream ($\text{CH}_4\text{:Biogas} = 0.61$), fermented material ($\text{CH}_4\text{:Biogas} = 0.60$), and pretreated samples ($\text{CH}_4\text{:Biogas} = 0.59$). Statistically significant differences were found between the methane ratios of pretreated and hydrolyzed samples from the solid fraction (those that had been pretreated with NED) ($p < 0.05$). For samples that had been pretreated with flue gas, the maximum methane ratio was achieved in samples that came from the bioethanol sidestream ($\text{CH}_4\text{:Biogas} = 0.78$; $t = 2$), followed by fermented material ($\text{CH}_4\text{:Biogas} = 0.73$; $t = 1$), hydrolyzed samples ($\text{CH}_4\text{:Biogas} = 0.67$; $t = 5$), and pretreated barley straw ($\text{CH}_4\text{:Biogas} = 0.65$; $t = 5$). At the end of the process the methane ratio varied between $\text{CH}_4\text{:Biogas} = 0.59$ (pretreated material) and $\text{CH}_4\text{:Biogas} = 0.65$ (fermented samples). Statistically significant differences were found between the ratios for $\text{CH}_4\text{:Biogas}$ in the different samples that had been pretreated with flue gas.

3.5. Mass Balances

The mass balances of samples pretreated at 175 °C and 30 bar with nitrogen and flue gas explosive decompression were performed in previous publications [8,10]. In these studies, samples from the solid and liquid fractions were separated after the hydrolysis and fermentation. The results showed that there was a biomass loss during the pretreatment step. From the initial 100 g of initial biomass added into the pressure vessel, only 87.2–92.6 of dry biomass was obtained after the pretreatment. The results of the fiber analysis carried out in these studies revealed that the hemicellulose content decreased from 32.9 g to 6.4 g (for samples pretreated with NED) and from 32.9 to 6.3–7.0 (for samples pretreated with flue gas). The cellulose loss was between 11–21%, while the lignin content increased from 5.4 g to 7.8–11.9 g. After the hydrolysis step, the samples went through a separation process and the cellulose content was about 17.1–17.8 g. The glucose yields reported after the hydrolysis step were similar for both pretreatment methods (15.2 g for samples pretreated with NED and 15.8–16.0 for samples pretreated with flue gas). The ethanol yields reported from the liquid fraction after the fermentation stage were between 5.6–9.4 g (for samples pretreated with flue gas) and 9 g for samples pretreated with NED.

4. Discussion

4.1. Chemical Composition

Concerning the TS composition, in both fractions (solid and liquid), the samples taken from the hydrolysis step had the highest levels of TS content, probably due to the added enzyme which works as a catalyst, improving hydrolysis efficiency and leading to higher TS contents [20]. In almost all situations, samples from the pretreatment stage had the lowest TS values. This may be due to the pretreatment method that partially decomposed the dry matter. The difference in the total solids content of untreated barley straw (nine to ten times more) and the remaining samples is mainly due to the fact that untreated material was air dry barley straw, while the other samples were diluted (100 g/L) in distilled water for the pretreatment process [7]. Statistically significant differences were found between the TS content of different variables. When comparing the TS content of samples from the solid fraction that had been pretreated with NED (using solid separation) with our previous research (without any solid–liquid separation), the results were 35% to 53% higher, while for samples that had been pretreated with flue gas the figures were improved by 34% to 55%. An improvement in the TS content means that there is more substrate available to be converted into methane, thereby improving the overall biogas and biomethane yields [3,7].

Regarding the VS content, the lowest value was reported in untreated material (963 g/kgTS), while the highest value was found in samples from the liquid fraction, where it remained the same (997 g/kgTS) for samples that had been pretreated with both NED and flue gas in all stages of the process. The VS content of samples from the solid fraction varied between 995 g/kgTS and 996 g/kgTS. Unlike the TS results, the VS content was similar both with and without solid–liquid separation. Research has shown that high VS content is desired in terms of the anaerobic digestion process [21]. This is an indicator of the biodegradability of the substrate and of its potential to produce methane (per gram of volatile solids of the substrate). A higher VS content leads to higher volumetric and specific biogas and biomethane productions [22].

The high variability in the concentrations of cellobiose, galactose, and mannose is probably due to the complexity of the materials in addition to the low concentration of the compounds, resulting in the poor resolution of the peaks in the chromatograms. The lack of temperature control during fermentation may have influenced the yields, as well as the pretreatment decompression levels. A slow opening of the pressure valve may not totally disrupt the plant cell wall, which leads to a low digestibility of the biomass and, therefore, lower ethanol yields. The distillation process concentrated the ethanol that was present rather than promoting evaporation, so possibly higher temperatures and/or longer times

should be employed. However, as the VS content did not change, the ethanol present should not have a significant impact on the data that has been obtained in further experiments.

4.2. Methane Recovery From Solid and Liquid Fractions of Bioethanol Production Process

Overall, the methane yields of samples that were pretreated with NED were 8–12% higher (statistically significant) (pretreated, fermented, and bioethanol sidestream) than that of samples that were pretreated with flue gas. Only samples from the hydrolysis step (which had been pretreated with NED) had methane yields that were lower than for samples that were pretreated with flue gas (being 6% lower). It has been reported in previous studies that the flue gas pretreatment method is more effective in the hydrolysis step than is the NED pretreatment method (being 6% higher) [3,7]. Nevertheless, when compared with samples from the solid fraction, the liquid fraction had low biomethane yields (60–88% lower), mainly due to the separation process. In that separation process, the liquid fraction will be composed mainly of hemicellulose, while the solid fraction will be composed mainly of cellulose and lignin. The composition of the solid fraction is particularly important when it refers to biomethane production. Research has shown that high amounts of lignin (>100 g/kg volatile solids) decrease methane yields, mainly because it limits the biomass degradation [23]. On the other hand, high cellulose content tends to improve bioethanol and biomethane yields [5].

The differences between the methane yields of samples that were pretreated with NED and flue gas and between the solid and liquid fraction were statistically significant ($p < 0.05$). When comparing the results that had been obtained in this study with our previous research (without solid–liquid separation), the maximum biomethane yields were highly improved in terms of samples from different stages of bioethanol production when pretreated with NED. The biomethane potential of the bioethanol sidestream was 45% greater than that of samples that were without the solid–liquid separation, followed by hydrolyzed material (33%), samples from the fermentation stage (32%), and pretreated barley straw alone (17%). The methane yields for samples that had been pretreated with flue gas were also improved by 44% in pretreated material, 33% in samples from the hydrolysis step, 15% in the fermentation stage, and 8% in bioethanol sidestream [3,7].

Drosg et al. [24] studied the potential of stillage fractions for the anaerobic digestion process. The authors concluded that the anaerobic digestion of thin stillage (the liquid fraction) can provide an additional energy recovery level of 41%, while wet cake (the solid fraction) has an energy recovery level of 57%. Town et al. [25] also investigated the biochemical methane potential of wheat-based stillage. The results showed that the stillage as a whole produced 96% of the methane that was expected of it, while thin stillage produced 125% of the methane predicted, and the wet cake produced 95% of the methane estimated. This shows that solid–liquid separation is a suitable method when it comes to enhancing methane production from the bioethanol sidestream. It can also be considered as an additional pretreatment after each step of the bioethanol production process (pretreatment, hydrolysis, fermentation, and distillation), since it enhances solids removal and improves the efficiency of the anaerobic digestion process. The separation of the solid and liquid fractions is a promising solution in terms of enhancing the energy output from the biomass and an effective strategy in terms of the large quantity of bioethanol sidestream that is generated following the distillation process.

4.3. Kinetics Rate and Digestion Time

In both pretreatment methods, the kinetic rate constant tends to increase when another step is added to the process—except for the hydrolysis step. This is mainly because, in the pretreatment stage, there was a small quantity of sugars available and a high number of micro-organisms were present, meaning that the sugars were consumed quickly. On the other hand, the hydrolysis step had more sugars available (than in the pretreatment stage), but had a limited number of micro-organisms at the beginning of the process. Therefore the reaction took more time to start because the micro-organisms first needed to grow and adapt to the environment and only then, just after the conclusion of that first stage, will the sugars be consumed. As a result, the overall kinetic rate of the process will be

slower. In real scale applications, this may represent an added cost since additional strategies may need to be implemented to keep the process efficient and overcome this rate-limiting step. Some of the strategies that can be utilized to decrease retention time in the hydrolysis step include a search for alternative pretreatment methods that will biodegrade the substrates easily; the utilization of substrates with a high solid content; increasing the organic load of the system; or carrying out the experiments in expensive high-rate bioreactors to achieve better performance levels. Research has shown that the utilization of high-rate systems means that the intermediate products that are generated in the process can actually be handled, without any risk of its collapse, leading to better hydrolysis rates [26]. In real scale applications this may represent an added cost, since additional strategies may need to be implemented to keep the process efficient and overcome this rate-limiting step. Some of the strategies that can be utilized to decrease the retention time in the hydrolysis step include searching for alternative pretreatment methods that will biodegrade the substrates easily; utilization of substrates with a high solid content; increasing the organic load of the system; or carrying out the experiments in expensive high-rate bioreactors to achieve better performances. Research has shown that high-rate systems are capable of handling the intermediate products generated in the process, without the risk of its collapse, leading to better hydrolysis rates [26]. The correlation coefficients that have been obtained in this study indicate that the equations being utilized to generate the fitting curves successfully express the variability of the experimental results.

When comparing the results that have been obtained in this study with our previous research results (for samples from the solid fraction), the speed of the substrate biodegradation was slower with solid–liquid separation than it was without solid–liquid separation. For samples that were pretreated with NED, the kinetic rate constant was 11–31% lower, while for samples that had been pretreated with flue gas the figure was 10–30% slower [3,7]. However, the overall biomethane production was between 8–45% higher, mainly due to the composition of the samples. These differences in the kinetic rate and in the biomethane yields can be explained by the separation process. With the solid–liquid separation, the samples from the liquid fraction had smaller amounts of total solids and had mainly hemicellulose in their composition, which is easily biodegradable. This means that the liquid fraction is less recalcitrant than the solid fraction and the micro-organisms will require less time to acclimatize to the environment. Therefore, they will be able to access the substrates faster and the time required to start anaerobic activity will be shorter, leading to better kinetic rates. Also, as the majority of the sugars are available in the solid fraction, the methane production in the liquid fraction will be smaller. On the other hand, with the separation process the solid fraction will have higher organic loads and a higher sugar content than will the liquid fraction, since the cellulose and lignin are aggregated mainly in the solid fraction. As lignin is highly recalcitrant, samples from the solid fraction will have a lower digestibility, making the rate of the reaction slower [5]. However, as the samples from the solid fraction have a high content of cellulose, once the bacteria start the degradation process there is more substrate available than in samples that are without solid–liquid separation, increasing the overall volume of biogas and biomethane being produced. When regarding biomethane production, these results show that the liquid fraction from bioethanol sidestream samples achieve the highest methane yield in the shortest time.

From the process point of view, this study analyzed the importance of keeping the solid and liquid fractions in one stream or separating them at different stages of the process in order to minimize energy and water usage, keep sugar and ethanol concentrations high, and avoid the generation of large quantities of sidestream at the end of the production chain. Carrying large masses of material through the production chain (such as water) can be energy intensive. It will require high levels of energy input in the distillation step and for the handling of the sidestream generated at the end of the process. If, in the pretreatment stage, a high quantity of inhibitory products are generated, it would be advantageous to remove the liquid fraction at this stage. If not, keeping both fractions of the broth (both solid and liquid) for the hydrolysis step will save water consumption. In the hydrolysis stage it is important to analyze whether it is also beneficial to carry both the solids and the liquid fraction

into the fermentation step. If the hydrolysis process is effective in the conversion of available cellulose fibers into sugar monomers then there will be no need to carry on the solids. However, if hydrolysis still continues during the fermentation stage, it can be profitable to forward the solids as well into the fermentation step. Finally, the main advantage of the separation is reported in samples that came from the sidestream. As samples from the liquid fraction have a relatively low biomethane production rate in all stages of the process, great attention should be paid to the solid fraction of the bioethanol sidestream. These samples can be used for high solid digestion, which is known to reduce water usage, enhance digestate handling, and improve biomethane yields [27,28]. Although some biomass losses can be registered (up to 10%) during the separation process [29], leading to a reduction in the bioethanol yield, the results show that the separation process is more advantageous when it comes to biomethane production (anaerobic digestion) than it is for bioethanol production (cellulases). This is mainly due to the composition of the samples.

4.4. Mass Balances

The biomass loss reported after the pretreatment was caused probably by the solubilization of the cellulose and hemicellulose, and by the partial evaporation of the moisture. As 17.1–17.8 g of unhydrolyzed cellulose were reported in the solid fraction of the enzymatic hydrolysis, further studies on samples from different stages of bioethanol production process are needed in order to increase cellulose solubilization and, therefore, improve glucose and ethanol efficiency.

5. Conclusions

This study evaluated pretreatment methods, which involved nitrogen explosive decompression and synthetic flue gas explosive decompression, in order to be able to investigate the potential offered by combined bioethanol and biomethane production. For this, samples from different fractions (solid and liquid) and stages of bioethanol production process (pretreatment, hydrolysis, and fermentation stage, and bioethanol sidestream) were used for further biomethane production. The results show that solid–liquid separation is an effective strategy for enhancing methane yields from combined bioethanol and biomethane production and improve the energy output from the biomass. For samples that, with solid–liquid separation, produce biomethane, yields from the bioethanol sidestream were 45% greater than that of samples that did not undergo the separation process. This strategy was particularly advantageous for samples from the solid fraction of bioethanol sidestream. The methane yields from samples from the liquid fraction were 60% to 88% lower than from samples from the solid fraction. Statistically significant differences were found between the methane yields from samples from the solid and liquid fraction ($p < 0.05$). Both pretreatment methods utilized in this study (flue gas and nitrogen explosive decompression) were effective in the pretreatment of the biomass. However, samples pretreated with NED had biomethane yields 8% to 12% higher than samples that were pretreated with flue gas. The results show that biomethane production from the bioethanol sidestream by means of solid–liquid separation is a promising solution in terms of enhancing the energy output from the biomass, and a promising strategy in terms of the large quantity of bioethanol production sidestream that can be generated following the distillation process. Future research will include the implementation of various separation pathways in the different stages of the bioethanol production process in order to improve the overall bioethanol and biomethane yields. The results will be further utilized in the design and implementation of a pilot-scale anaerobic digestion plant.

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