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Assessing straw digestate as feedstock for bioethanol production

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ABSTRACT

Anaerobically digested agricultural waste, such as straw digestate, still consists of residual lignin and carbohydrates that may be utilised as substrate for sugars production and alcoholic fermentation. Chemical pretreatment combined with enzymatic hydrolysis was investigated as an alternative valorisation route for digestate. Acid pretreatment along with enzymatic hydrolysis was found to yield low sugars recoveries (2–39%), casting doubt on its suitability for ethanol production. In contrary, alkaline pretreatment and enzymatic hydrolysis is a better approach with elevated saccharification yields reaching up to 72%. Ethanol fermentation of alkaline pretreated digestate presented yields up to 65% consuming all the available glucose, implying that no inhibitory factors are present. Conclusively, according to the experimental results the perspective of a new integrated system is enforced. This system combines ethanol production with anaerobic digestion simultaneously producing energy in the form of ethanol and methane and improving the overall energy balance.

1. Introduction

Large amounts of residual biomass remain untreated on the harvesting fields raising concerns about their accumulation impact on soils but also their potential exploitation towards bioenergy purposes [1]. From the annual quantity of 956 MT of dry matter agricultural biomass produced in the European union in the years 2006–2015, about 442 MT constitute secondary crop products, referred as residue production [2,3]. This fraction is mainly composed of food processing waste, crop waste, and dry biomass from leaves. Even though essential for maintaining the ecosystem services, improper disposal can result in environmental pollution. Most of these residues are untreated and disposed of by burning, dumping and landfilling while smaller quantities are used for animal bedding and feed purposes, fertilizer applications or are exploited for the production of bio-energy [2,4].

On the other hand, in Europe, the number of anaerobic digestion (AD) biogas plants in 2017 reached 17783 (+11556 units since 2009) with a total installed electric capacity IEC of 10532 MW [5]. In general, there is a trend towards installations with greater capacities, dedicated to the treatment of agricultural residues, plant matter and manure. More than 6000 MW of electricity were produced from anaerobic digestion of agricultural residues [5]. Apart from biogas, which is the main product of AD and is currently used for heat and electricity production, digestate is also produced. It represents the fibrous digested substrate which is removed from the AD reactor [6]. 80–90% of the feedstock introduced into AD ends up as digestate [7]. Digestate is a highly valuable coproduct which is currently used as soil amendment or animal bedding [8]. Its physicochemical composition presents high fluctuations and is regulated by the feed material and the AD operational conditions [6,8,9].

The increasing number of biogas plant installations has an impact on the increase of the digestate produced. It is approximated that an average biogas plant in Europe treating renewable raw materials with a mean capacity of 500 kW produces about 10,000 tons of digestate per year [10].

Thus, an effort to valorize this stream in several ways has recently been reported [11–13]. Exploitation of digestate as a potential biofuel source is still in an early research stage given its "recalcitrant" lignocellulosic structure [14]. Few recent studies underline the potential of anaerobically treated wastes (AD fiber) as substrate for bioethanol due to its remaining hydrocarbon components [15–19].

Pretreatment of lignocellulosic materials is an essential step to

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degrade and change the structure of complex biomass [20]. This way, cellulose becomes more exposed and the cellulolytic enzymes can more easily penetrate its structure and convert it to monomer sugars [21,22]. Diverse pretreatment methods have been developed such as the alkali and acid pretreatment [23,24]. Dilute alkali and acid pretreatment has been verified by previous studies as an effective way to pretreat lignocellulosic biomass for biorefining, with lower fermentation time achieving increased accessibility area and improved efficiencies [15,25].

This study examined the potential of valorising digestate towards ethanol via enzymatic saccharification of the remaining fibres. The ultimate objective of this work was to showcase digestate as a bioenergy source and add value to the anaerobic digestion. Therefore, the potential of straw digestate as a feedstock for bioethanol production was assessed by the application of two chemical pretreatment methods (dilute acid and alkaline). Additionally, cellulase saccharification and ethanolic fermentation were studied.

2. Materials and methods

2.1. Fiber samples

The solid digestate resulting from the liquid - solid separation from a pilot scale anaerobic reactor (CSTR) that treated wheat straw in the premises of NTUA was used as anaerobically digested fiber. The digester operated at 35 °C and an HRT (hydraulic retention time) of 20 days. Wheat straw was obtained from Aspropyrgos province, Greece. It mainly composed of 45.1% hemicellulose, 33.8% cellulose, 16.4% lignin (15.4% Klason lignin and 1.0% acid-soluble lignin) and 4.7% ash.

2.2. Chemical pretreatment

The AD fiber sample was pre-treated in an autoclave (ISOLAB Laborgerate GmbH Autoclave) with/without dilute NaOH or H_2SO_4 at 120 °C. For each chemical pretreatment three different concentrations were examined; 2, 3 and 4% for NaOH and 1, 2 and 3% for H_2SO_4. Three retention times were studied; 60, 75 and 90 min. Pretreated mixture solutions were collected, left to room temperature and followingly centrifugated. The solid fraction was characterized in terms of solids content, lignin, hemicellulose and cellulose, whereas the liquid fraction in terms of sugars, phenolic compounds and volatile fatty acids.

2.3. Enzymatic saccharification

Enzymatic hydrolysis of pre-treated solid samples was executed at 50 °C in a shaking bath at 150 rpm by adding the cellulolytic formulation, Cellic CTec2 (Novozymes, Denmark) for 72 h. Cellulase was added at a loading according to the samples cellulosic content. Buffer solutions were used so as to set the pH around 5.0, which is the optimum pH for the enzyme activity. After 72 h, the hydrolysates were collected and centrifugated and each fraction was characterized.

2.4. Ethanol fermentation

Hydrolysates were prepared for ethanol fermentation trials by adding 1.5% Saccharomyces cerevisiae. Saccharomyces cerevisiae strain specializes mainly on glucose metabolization due to its glycolytic pathway. Such yeasts are generally employed in biorefineries so as to ferment mono-saccharides produced during cellulose hydrolysis [22]. The fermentation was conducted in 250 mL autoclavable bottles of 100 mL working volume. They were incubated at 32 °C and 50 rpm for 1 day. After 24 h, ethanol and glucose were determined in the liquid phases.

2.5. Analytical methods

Characterisation of the raw material, pretreated, hydrolysed and fermented samples was conducted according to Sluiter et al. [26]. Sugars were quantified by the marketable kit Biosis S.A. (Athens, Greece) according to the GOD/PAP method. Total organic content, TOC was determined by a Shimadzu TOC-V Total Organic Analyzer. Phenols were determined by the marketable kit Spectroquant Phenol Test 100856 and VFAs by the Spectroquant Volatile Organic Acids Test 1018909 both by Merck KGaA Mellipore, Germany. Ethanol determination was conducted through redox titration in order to assess the ethanol content in liquid phase [27]. X-ray diffractometry was applied in order to investigate the crystallinity of the samples' structure, both raw and untreated. A Bruker (Bruker-AXS, Karlsruhe, Germany) D8-Advance X-ray diffractometer was used applying the conditions of Kontogianni et al. [28].

2.6. Statistical experimental design

The principal objective of the experimental procedure was to assess in quantitative terms the effect of the main process parameters on the saccharification efficiency SG and ethanol yield Y_E (optimization parameters). SG was calculated as the glucose produced in relation to the maximal theoretical glucose produced if all the carbohydrates were converted to ethanol (Equation (1)):

$$SG = \frac{C_{glu}}{C_{cel}.f}.100(\%) \quad (1)$$

where SG is the saccharification yield,

C_{glu} is the concentration of C6 sugar after enzymatic saccharification,
C_{cel} is the initial cellulose concentration and
f is a correction parameter aiming to address the addition of water to one unit of C6 sugar during enzymatic saccharification, that is: $f = \frac{180}{162} = 1.11$) [29].

The theoretical ethanol yield was estimated from Equation (2):

$$\%\ \text{Theoretical ethanol yield}\ Y_E = \frac{[EtOH]}{0.51*[Glucose]}*100 \quad (2)$$

where [Glucose] is the initial glucose concentrations and [EtOH] is the final ethanolic concentrations.

The parameters which mostly affect the efficiency of the pretreatment scheme are chemicals' concentration, autoclave retention time and enzyme loading during enzymatic hydrolysis (controlling parameters). A 2^3 factorial experiment was carried out to assess the influence of the controlling parameters on the optimization parameters. Generally, through a 2^n factorial experiment, proper linear models are constructed interrelating the 'n' controlling parameters to the optimization parameter [30]. Table 1 presents the controlling parameters along with their levels. The experimental area of the factorial design was pre-determined through preliminary experimentation.

In each 2^3 factorial design, 8 experiments were conducted in triplicate as presented in the following Table (Table 2). In addition, four experiments in the centre of the factorial design were carried out for statistical reasons. From the experimental data collected, mathematical models were set and their adequacy was evaluated using the Fisher criterion.

Table 1
The levels of the controlling parameters at the factorial design (alkaline pretreatment or acidic pretreatment prior to enzymatic hydrolysis).

Controlling Parameter	Variation Intervals		
	Low level (-)	High Level (+)	Center (0)
Time autoclave, t_{auto} (h)	1	1.5	1.25
NaOH (%) / H_2SO_4 (%)	2 / 1	4 / 3	3 / 2
CellicCTec2, C_{enz} (μL/ g cellulose)	100	400	250

Table 2
Experimental runs of alkaline (B1–B9) and acid (A1-A9) pretreatments followed by enzymatic hydrolysis according to the factorial design.

Alkaline Pretreatment				Acid Pretreatment			
No experiment	Time autoclave (h)	NaOH (%)	CellicCTec2 (μL/g)	No experiment	Time autoclave (h)	H_2SO_4 (%)	CellicCTec2 (μL/g)
B1	1	2	100	A1	1	1	100
B2	1.5	4	100	A2	1.5	3	100
B3	1	4	100	A3	1	3	100
B4	1.5	2	100	A4	1.5	1	100
B5	1	2	400	A5	1	1	400
B6	1.5	4	400	A6	1.5	3	400
B7	1	4	400	A7	1	3	400
B8	1.5	2	400	A8	1.5	1	400
B9	1.25	3	250	A9	1.25	2	250

3. Results and discussion

3.1. Digestate characterisation

The first step in the overall pretreatment and hydrolysis procedure was the characterisation of the raw digestate used for the experimental trials (Table 3). The outcomes were used to assess the degradation degree attained by the pretreatment and saccharification processes.

The digested fibre had a cellulose content of nearly 20% indicating that its potential as a substrate suitable for bioethanol production. Nevertheless, it also presented a high content of insoluble lignin that usually constitutes the major barrier for hydrolysis [31] and thus valorisation of cellulosic content.

3.2. Effect of pretreatment

3.2.1. NaOH alkaline pretreatment

Table 4 presents the degradation efficiencies for dilute alkaline pretreatment of straw digestate.

Table 3
Raw digestate composition.

	%w/w dry base
Total Solids	9.63 ± 1.25
Water Soluble Solids	12.69 ± 0.94
Volatile Solids	74.38 ± 2.61
Cellulose	18.68 ± 4.42
Soluble lignin	1.53 ± 0.11
Insoluble Lignin	23.67 ± 1.26
Hemicellulose	16.06 ± 3.33

From the data presented in Table 4, it is obvious that in most cases the dilute alkali pretreatment led to a considerably decreased lignin content, while it triggered just a minor change in cellulose content. The hemicellulose degradation efficiencies ranged from 3.45 to 74.36%. Higher NaOH concentrations caused higher lignin and hemicellulose degradation.

In the liquid phase, the following degradation products were identified:

- Glucose 1.11–4.78 mg/g digestate
- Volatile Fatty acids 56.95–84.17 mg/g digestate
- Phenolic compounds 2.50–4.61 mg/g digestate.

The concentration of glucose identified was low given the low degradation degree of cellulose. VFAs and phenolic compounds concentrations varied according to the degradation degree of the structural polysaccharides of lignin and hemicellulose, explaining their occurrence in the hydrolysates examined. The greatest lignin degradation (77.14%) was attained with pretreatment in autoclave with 4% NaOH for 90 min at 120 °C, while the lowest lignin degradation (8.23%) was achieved through pretreatment in autoclave with 2% NaOH for 60 min at 120 °C, implying that the harshest the pretreatment conditions, the more efficient the lignin degradation.

The results presented above are in compliance with other related studies. MacLellan et al. [16] studied the alkaline pretreatment (2% NaOH, 130 °C, 2 h) of digestates from mixtures of corn strover and swine manure. High lignin degradation efficiencies over 80% were observed while the cellulose content remained

Table 4
Degradation of the total solids, lignin and structural polysaccharides of the solid fraction of straw digestate after NaOH pretreatment.

Autoclave time (h)	NaOH (%)	%TS hydrolysis			%cellulose degradation			%AIL degradation			%ASL degradation			%hemicellulose degradation		
1	2	30.67	±	2.18	1.76	±	3.68	8.23	±	3.75	62.28	±	2.11	3.45	±	5.92
1.5	4	23.59	±	2.17	2.20	±	6.83	77.14	±	4.39	75.82	±	1.27	74.36	±	1.59
1.25	3	24.01	±	0.89	5.48	±	9.04	74.27	±	3.48	68.62	±	4.16	43.78	±	16.62
1	4	29.02	±	4.76	1.64	±	7.04	76.40	±	4.77	75.16	±	2.41	44.07	±	13.20
1.5	3	19.84	±	3.33	4.14	±	1.73	71.28	±	2.75	65.15	±	1.42	44.71	±	2.42

almost intact, in full accordance with the experimental results of the present study.

Teater et al. [15] applied alkaline pretreatment on dairy manure digestate examining its potential in ethanol production. The results revealed a considerable degradation of lignin and hemicellulose with lower impact on cellulose. An increase of NaOH concentration resulted in greater lignin degradation. Optimal conditions of 3 h, 130 °C and 2% NaOH achieved the highest lignin degradation of almost 51%. Nevertheless, substantial degradations of cellulose (20–36%) and hemicellulose (32–44%) were also observed. The variation of these figures compared to our study may be attributed to the dissimilar nature and composition of the digestate feedstock. Yue et al. [18] examined the pretreatment of dairy cow faeces digestate under alkaline conditions (NaOH 0.5 M, 1 and 2% w/w and autoclave for 1, 2 and 3 h at 120 °C and 130 °C) and also verified that under alkaline conditions, lignin and hemicellulose may be partially removed and that an increased cellulose content may be received.

3.2.2. H_2SO_4 acidic pretreatment

Table 5 presents the degradation efficiencies for dilute acid pretreatment of straw digestate.

From the data presented in Table 5 it is obvious that the dilute acidic pretreatment didn't affect significantly insoluble lignin, which was the target compound. The cellulose and hemicellulose degradation efficiencies ranged from 15.32 to 43.75% and from 46.47 to 66.85% respectively. It was observed that the acidic pretreatment affected cellulose to a high degree, effect which was not noticed in the case of alkaline pretreatment.

In the liquid phase, the following degradation products were identified:

- Volatile Fatty acids 9.92–25.89 mg/g digestate
- Phenolic compounds 0.3–0.54 mg/g digestate.

The concentration of VFAs and phenolic compounds varied according to the degradation degree of hemicellulose and lignin.

The greatest lignin degradation (9.73%) was attained with pretreatment in autoclave with 1% H_2SO_4 for 60 min at 120 °C. The lowest lignin degradation (0.69%) was achieved through pretreatment in autoclave with 1% H_2SO_4 for 90 min at 120 °C. The effect of acidic pretreatment on insoluble lignin content was generally significantly lower than in alkaline pretreatment.

Yue et al. [18] examined dairy cow faeces under pretreatment with H_2SO_4 1–4% for 2 and 3 h at 120 °C and 130 °C. Acidic pretreatment under optimal conditions (H_2SO_4 3% 2 h at 130 °C) proved more effective on hemicellulose removal whereas lignin was mainly unaffected, as it was also observed in this study.

Regardless of the substrate, it has been established in literature that dilute acid pretreatment at high temperatures is an efficient scheme for the hydrolysis of hemicellulose, since it usually leads to the disruption of cellulose's crystalline structure and consequently to the increase of enzyme saccharification yield [32,33] To this end, Vancov et al. [17] studied cattle manure under acid pretreatment (H_2SO_4 2.5%, 90 min, 121 °C) reporting a degradation of hemicellulose up to 74%. Similarly to this study, inhibitory compounds such as carboxylic acids and phenols as by-products of lignin degradation were also identified.

3.3. Enzymatic saccharification of pretreated digestate

3.3.1. Enzymatic saccharification of alkaline pretreated digestate

The saccharification of alkaline pretreated digestate by cellulases addition was investigated and the glucose release expressed as saccharification yield along with the achieved carbohydrates degradation are presented in Fig. 1.

The alkaline pretreatment and hydrolysis achieved very high degradation degrees of cellulose and hemicellulose that ranged from 72.24% to 100% and from 65.09% to 99.55% respectively followed by subsequent high sugar releases (32–72%). Cellulose degradation and saccharification efficiency followed the same pattern, revealing that CellicCtec2 successfully hydrolysed the cellulose to sugar monomers readily available for ethanol fermentation. The best results were attained when digestate was pretreated in autoclave with 2% NaOH for 90 min at 120 °C and enzymatically hydrolysed with 400 μL CellicCTec2/g cellulose. Under these conditions the greatest saccharification yield achieved was 71.50%.

Similarly, Teater et al. [15] achieved overall glucose conversion up to 68.2% under alkaline pretreatment conditions of 2% NaOH and autoclave for 3 h at 130 °C. Yue et al. [19] examining animal manure digestate, under alkaline conditions achieved glucose conversion rate of 73%. Wang et al. [34] applied pretreatment of ozone combined with aqueous ammonia on rice straw digestate. Even though the chemical pretreatment scheme did not affect the lignin content, the enzymatic hydrolysis of pretreated samples presented 53–70% glucose recoveries.

Table 5
Degradation of the total solids, lignin and structural polysaccharides of the solid fraction of straw digestate after H_2SO_4 pretreatment.

Autoclave time (h)	H_2SO_4 (%)	%TS hydrolysis			%cellulose degradation			%AIL degradation			%ASL degradation			%hemicellulose degradation		
1	1	20.37	±	0.22	23.79	±	6.25	9.73	±	0.49	11.11	±	1.97	46.47	±	3.45
1.5	3	32.02	±	2.32	15.32	±	14.04	5.38	±	0.38	40.68	±	1.81	66.85	±	9.56
1.25	2	25.12	±	1.15	19.85	±	5.32	7.52	±	0.29	29.98	±	2.01	59.17	±	5.21
1	3	20.88	±	0.89	35.58	±	7.29	5.61	±	5.12	28.83	±	3.87	65.62	±	1.45
1.5	1	13.18	±	0.87	43.75	±	16.57	0.69	±	1.21	6.64	±	3.63	49.57	±	8.85

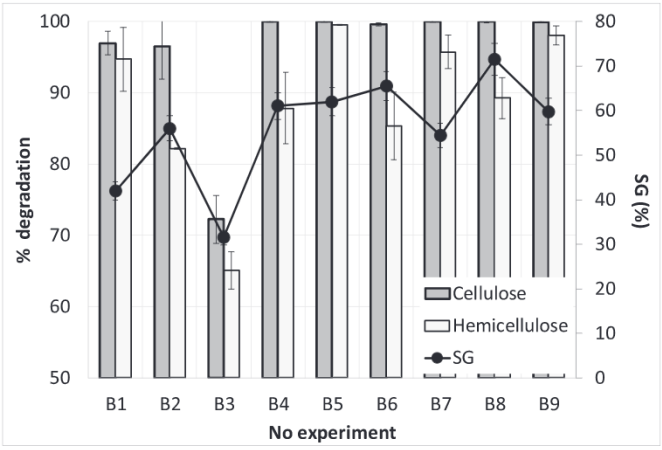

Fig. 1. Cellulose and hemicellulose degradation efficiencies and saccharification yield of straw digestate after alkaline pretreatment and enzymatic hydrolysis for experimental runs B1–B9.

3.3.2. Enzymatic saccharification of acid pretreated digestate

The saccharification of acid pretreated digestate was investigated by cellulase addition and the glucose release along with the achieved carbohydrates degradation are presented in Fig. 2.

The acidic pretreatment followed by enzymatic hydrolysis achieved lower cellulose and hemicellulose degradations that ranged from 1% to 45.64% and from 0% to 60.67% respectively. As shown in Fig. 2, the cellulose degradation and saccharification yields followed the same pattern but with lower efficiencies. The best results were attained when digestate was pretreated in autoclave with 3% H_2SO_4 for 90 min at 120 °C and enzymatically hydrolysed with 400 μL CellicCTec2/g cellulose. Under these conditions the greatest saccharification yield achieved was 38.60% which corresponds approximately to the half of the maximum achieved by the alkaline pretreatment. It was observed that cellulose degradation varied significantly. An increase in acid concentration led to increased saccharification yields.

Yue et al. [19] examining animal manure digestate, under acidic pretreatment (1% NaOH, 132 h) and hydrolysis (accelerase: 52 FPU/g, 72 h, 50 °C) achieved glucose conversion rate up to 22%.

In an overall assessment, dilute alkaline pretreatment presented better delignification results, higher glucose conversion rates and more increased efficiencies than dilute acidic pretreatment as was also verified by relevant studies [18,19].

3.4. XRD analysis

X- Ray diffractometry was employed in order to assess the structural modification that pretreatment and enzymatic hydrolysis caused to selected samples. Given cellulose's crystalline structure, the chemical pretreatment was expected to improve the diffusion of enzyme via the amorphous cellulose and to enhance the enzyme's access to the substrate. The crystallinity index, CrI, is a critical parameter directly linked to the enzymatic digestibility of the substrate representing the crystalline nature of the whole biomass [35]. It is greatly influenced by the complex structure of lignocellulose where hemicellulose and lignin are the amorphous constituents, whereas cellulose is the crystalline one. So, it is a way to unveil indirectly the amorphous content of digestate such as cellulose domains, lignin and hemicellulose.

The CrI was estimated according to Segal method [36] by use of the following equation [35–37]:

$$CrI\ (\%) = (I_{200} - I_{am})/I_{200} * 100\%$$

where: I is the height of corresponding peak.

Among the experimental runs for alkaline and acidic pretreatment, the samples that achieved the greatest efficiencies in lignin degradation and saccharification were chosen and reproduced. Given the lower efficiencies of acidic pretreatment, only alkaline sample cases were studied. Three different types of solids were set in comparison and XRD analysis:

- raw dry digestate
- dry digestate after optimal alkaline pretreatment that achieved the greatest lignin degradation (1 h autoclave, 4%NaOH, 76.40% lignin degradation)
- dry digestate after optimal alkaline pretreatment and enzymatic hydrolysis, that achieved the highest saccharification yield (1.5 h autoclaving time, 2% NaOH and 400 μL CellicCTec2/g cellulose, SG = 71.18%).

The XRD patterns and the respective crystallinity indices of raw, pretreated and enzymatic hydrolysed digestate are shown in Fig. 3. The patterns present a wide peak around 22°, for both the raw digestate and the pretreated sample scheme but with different intensity. In the case of enzymatically hydrolysed digestate, the peak is almost undetected. Previous studies verify that in XRD

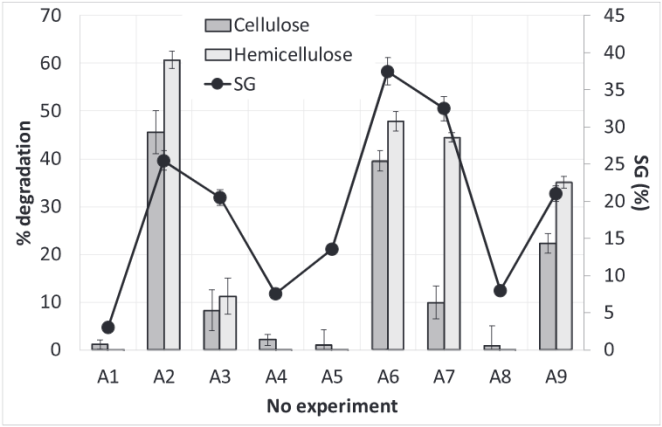

Fig. 2. Carbohydrates degradation efficiencies and saccharification yield of straw digestate after acidic pretreatment and enzymatic hydrolysis for experimental runs A1-A9.

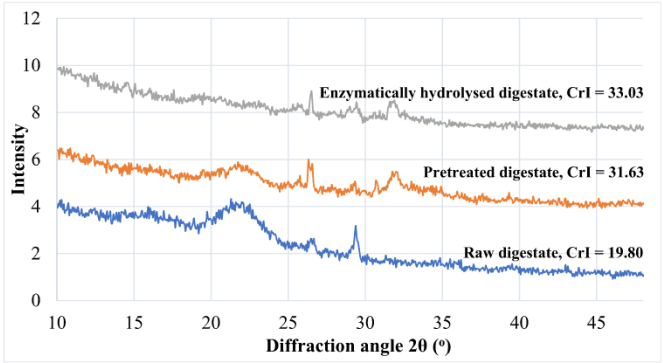

Fig. 3. XRD patterns for (a) raw dry digestate, (b) dry digestate after alkaline pretreatment that achieved the greatest lignin degradation (1 h autoclave, 4%NaOH, 76.40% lignin degradation) and (c) dry digestate after alkaline pretreatment and enzymatic hydrolysis, that achieved the highest saccharification yield (1.5 h autoclaving time, 2% NaOH and 400 μL CellicCTec2/g cellulose, SG = 71.18%).

analysis crystalline cellulose is detected around 22° [38,39]. Raw digestate presents the highest peak intensity due to the pure crystalline cellulose that remains unaffected. The cellulose peak corresponding to the pretreated digestate sampleappears with less intensity due to the degradation of the amorphous hemicellulose and lignin. A slight effect on crystalline cellulose is also observed. Crystalline cellulose is not detected on the enzymatically hydrolysed digestate sample imprinting the effective diffusion and hydrolysis occurred from the cellulolytic enzyme CellicCTec2.

The degradation of hemicellulose and lignin.was the principal result of the pretreatment schemes applied. After the pretreatment, the crystallinity of digestate was significantly high. Given that hemicellulose and lignin were degraded during pretreatment, crystalline cellulose remained partly unaffected and thus more exposed inside the overall biomass volume, a fact that could lead to elevated cellulose conversion in the stage of enzymatic hydrolysis. The CrI of the enzymatically hydrolysed digestate is lower, explaining the enzymatic diffusion in the lignocellulosic biomass and the effective crystalline cellulose hydrolysis that occurred.

Studies verify that raw lignocellulosic materials present a lower crystallinity index than pretreated samples [28,40,41]. After enzymatic hydrolysis, the CrI is reduced by 10% confirming the effect of enzymatic hydrolysis on the cellulosic fibres, degrading them to their monomer sugars.

3.5. Ethanol fermentation

Ethanol fermentation was conducted as the last step of the overall evaluation process of the digestate as suitable feedstock for ethanol production. NaOH pretreatment presented in all cases much better performance on saccharification yields than acidic pretreatment. Thus, only alkaline pretreated hydrolysates were studied as far as their potential on ethanol production.

The ethanol fermentation potential of glucose that resulted from the pretreated straw digestate was evaluated using S. Cerevisiae (baker's yeast). All experimental runs of alkaline pretreatment B1–B9 were subjected to fermentation. The fermentation profiles regarding glucose and ethanol concentrations for each experimental run are presented in Fig. 4.

Table 6 summarizes the main features of fermentation experiments in terms of final ethanol concentration, total maximum ethanolic volumetric productivity and ethanol yield. The maximum ethanolic volumetric productivity (g L^{-1} h^{-1}) is estimated from Dp/Dt where Dp is the increase in ethanol concentration over a time span Dt.

In the experimental range considered, the highest ethanol yield achieved was 65% in the experimental point 1.5 h autoclaving time, 2% NaOH and 400 μL CellicCTec2/g cellulose.

In general, slightly higher ethanol yields were observed in literature. Yue et al. [19] examining AD animal manure received after fermentation ethanol yields up to 72%. Wang et al. [34] studying rice straw digestate observed yields up to 75.2% in ethanol. According to Yue et al. [18], the ethanol efficiency ranged between 73.3 and 75.3% for alkaline pretreated and enzymatically hydrolysed digestate. Teater et al. [15] also achieved high ethanol yields up to 80.3%, comparable to the respective efficiencies of agricultural substrates.

3.6. Effect of operational parameters on saccharification and ethanol production

A factorial design was applied as a useful technique to investigate the main process variables and their interaction effects (autoclave time, chemicals concentration and enzyme dosage) on the process output in terms of saccharification (SG) and ethanol (Y_E) yields.

Based on the outcomes of the factorial experiment for acidic pretreatment and statistical processing [30], a linear model was determined, correlating the saccharification yield with the system's statistically important parameters:

$$SG_{H2SO4} = -9.44 + 10.41^{*}C_{H2SO4} + 0.03^{*}C_{enz} \quad (3)$$

This model proved to be adequate after the application of the Fisher test.

The critical parameters influencing the saccharification yield were H_2SO_4 concentration within the range of 1–3% and the enzyme dosage whereas autoclave time and the interactions among two or more parameters were negligible. The plus sign (+) in Equation (3) suggests that an increment in the H_2SO_4 concentration and/or enzyme loading leads to a higher saccharification yield.

The same analytical procedure was adopted for the alkaline pretreatment and the following model derived that proved to be adequate after the application of the Fisher test:

$$SG_{NaOH} = 2.52 + 32.04^{*}\, t_{autoclave} + 0.05^{*}\, C_{enz} \quad (4)$$

Accordingly, the parameters that were proven to influence the saccharification yield were the autoclave time and the enzyme dosage. It was shown through statistical analysis that the NaOH concentration within the range of 2–4%, as well as the interactions among two or more parameters were statistically insignificant. The plus sign (+) in Equation (4) illustrates that by increasing the autoclaving time and/or enzyme loading, a higher saccharification yield will be achieved and thus a more attractive feedstock for ethanol production will be produced.

Additionally, aiming to investigate the effect of the examined process variables on the overall process output in terms of ethanol yield, the following linear model was estimated:

$$Y_E = 5.45 + 27.18^{*}\, t_{auto} + 0.045^{*}\, C_{enz} \quad (5)$$

This proved to be adequate after the application of the Fisher test.

Autoclave time and enzyme loading remained the statistically important parameters even after ethanol fermentation as was the case for saccharification.

4. Conclusions

Conclusively, dilute alkaline pretreatment prior to enzymatic hydrolysis proved more effective than acid pretreatment reaching a saccharification yield up to 71.50% (NaOH 2%, 90 min 120 °C, 400 μL CellicCtec2/g of cellulose). This may be attributed to the high lignin degradation (up to 77%) and the structural changes of biomass verified by the XRD analysis during alkaline pretreatment that enhanced the enzyme diffusion. On the other hand, acidic pretreatment proved destructive to cellulose fibres leading to a fractional cellulose degradation before enzymatic hydrolysis. This resulted in lower sugar release and accordingly lower saccharification yields. The ethanolic fermentation of alkaline pretreated hydrolysates was efficient in all cases reaching an ethanol yield of 65%. Statistical processing of the experimental data according to the factorial design led to adequate linear models which revealed that autoclaving time and enzyme concentration are the statistically important parameters that regulate the ethanol production process from straw digestate. Thus, it was proved that digestate may stand as a potential substrate for 2nd 18generation ethanol production since it is technically feasible and with promising results.

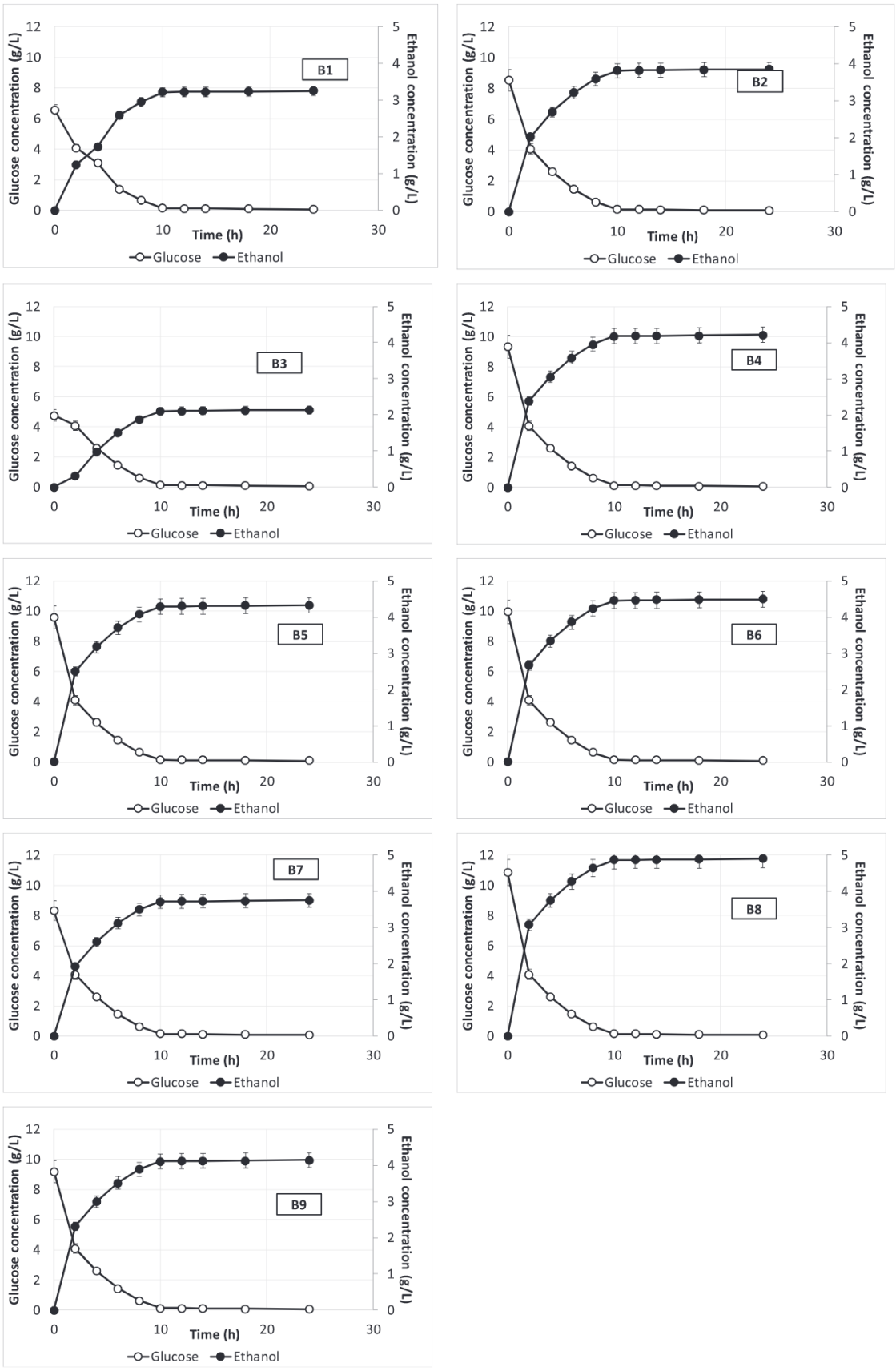

Fig. 4. Fermentation profiles of S. cerevisiae in straw digestate hydrolysates after NaOH pretreatment and enzymatic hydrolysis with CellicCTec2. Experimental runs B1–B9. Data represent mean values of two experiments.

Table 6
Results of ethanolic fermentation of alkaline and enzymatically pretreated straw digestate.

No	Ethanol (g/L)	Ethanol productivity (g/L/h)	Y_E (%)
B1	3.249	0.339	42
B2	3.848	0.281	51
B3	2.134	0.341	29
B4	4.213	0.317	55
B5	4.326	0.275	56
B6	4.491	0.269	60
B7	3.750	0.326	50
B8	4.897	0.329	65
B9	4.144	0.180	54

The growing number of AD plants and the subsequent higher amounts of digestate produced set as a priority the need for further research on valorisation routes of digestate as substrate. The bioethanol valorisation route, technically verified in this study, seems as an appealing solution. Nevertheless, the research on the field is still on bench scale. The scale-up of the processes involved is not such a trivial process and requires a systematic approach. In the framework of optimization and adjustment of the technique to industrial environment and aiming to integrate it to biorefinery networks or existing AD plants, pilot scale implementations should be promoted in order to better simulate the process and evaluate it from an economic, energetic and environmental point of view.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRediT authorship contribution statement

Vasileia Stoumpou: Investigation, Writing - review & editing. **Jelica Novakovic:** Methodology, Investigation. **Nikoleta Kontogianni:** Investigation. **Elli Maria Barampouti:** Conceptualization, Methodology, Visualization, Investigation, Data curation, Validation, Writing - original draft, Writing - review & editing. **Sofia Mai:** Conceptualization, Methodology, Visualization, Investigation, Data curation, Validation, Writing - original draft, Writing - review & editing. **Kostantinos Moustakas:** Conceptualization. **Dimitris Malamis:** Conceptualization, Methodology. **Maria Loizidou:** Supervision.

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