energies

MDPI

Article

The Role of Mild Alkaline Pretreatment in the Biorefinery Upgrade of Spent Coffee Grounds

Gabriel Mota Ribeiro [1,2], Pedro L. Martins [1], Ana Cristina Oliveira [1], Florbela Carvalheiro [1], Rita Fragoso [2,*] and Luís C. Duarte [1]

[1] Unidade de Bioenergia e Biorrefinarias, LNEG—Laboratório Nacional de Energia e Geologia, Estrada do Paço do Lumiar, 22, 1649-038 Lisboa, Portugal; luis.duarte@lneg.pt (L.C.D.)
[2] LEAF—Linking Landscape, Environment, Agriculture and Food Research Center, Associated Laboratory TERRA, Instituto Superior de Agronomia, Universidade de Lisboa, Tapada da Ajuda, 1349-017 Lisboa, Portugal
* Correspondence: ritafragoso@isa.ulisboa.pt

Abstract: This work proposes a valorization route for spent coffee grounds (SCG), a widespread lignocellulosic residue, encompassing the production of: biomethane, lignin, and oligosaccharides as value-added products obtained simultaneously during a mild alkaline (NaOH) pretreatment. The studied operational variables were the reaction time (60–240 min), temperature (25–75 °C), and the NaOH concentration (0–2.5 M). The severity factor suitably describes the global process kinetics, with higher severities (log *Mo* = 5.5) yielding high product yields, 18.02% and 13.25% (on dry SCG basis) for lignin and oligosaccharides (XGMOS), respectively. Solid yield is negatively impacted by all studied variables (at the 95% confidence level). Conversely, XGMOS yield is positively influenced both by time and catalyst concentration, whereas lignin yield is only (positively) influenced by catalyst concentration. Optimal balance between product formation and potential operational costs is putatively achieved when using 0.625 M NaOH, at 50 °C for 60 min. The mild alkaline pretreated biomass (MAP-SCG) was compared to untreated SCG for biomethane production by anaerobic co-digestion with pig slurry (PS), using a ratio of biomass/PS = 1/3 (volatile solids (VS) basis). The proposed valorization route enabled the sequential production of 6.25 kg lignin, 6.36 kg oligosaccharides, and 138.05 kg biomethane per 100 kg of non-extracted SCG (and 287.60 kg pig slurry), in an integrated process that is technically feasible and promotes the circular bioeconomy.

Keywords: biogas; biomass pretreatment; circular bioeconomy; lignin-derived products; oligosaccharides

check for updates

Citation: Ribeiro, G.M.; Martins, P.L.; Oliveira, A.C.; Carvalheiro, F.; Fragoso, R.; Duarte, L.C. The Role of Mild Alkaline Pretreatment in the Biorefinery Upgrade of Spent Coffee Grounds. *Energies* **2023**, *16*, 3907. https://doi.org/10.3390/en16093907

Academic Editor: Diego Luna

Received: 31 March 2023
Revised: 27 April 2023
Accepted: 28 April 2023
Published: 5 May 2023

Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

Spent coffee grounds (SCG) are a worldwide spread lignocellulosic waste. It is estimated that up to 13 million tons of SCG are produced yearly in the world, with 24.5% being derived from domestic use and 75.5% from commercial processing [1,2].

Finding suitable solutions for the management of this waste has captured research attention, as the inappropriate disposal of this biomass creates environmental concern, e.g., the emission of greenhouse gases [2]. Furthermore, waste management guidelines in a circular bioeconomy context promote the creation of valorization routes that adopt a biorefinery approach [3]. The established biorefinery should include a cascade of integrated processes that lead to the production of several bioproducts, biofuels, and materials [4].

SCG is rich in cellulose, hemicellulose, and lignin, and also contains proteins, fats, polyphenols, and residual caffeine [5]. Therefore, the first valorization step should aim to recover the former added-value compounds to be used as food ingredients [4,6]; however, this is often not possible, mostly due to the rapid decay of this material once discarded. As such, alternative approaches must be found.

Both lignin and hemicellulose fractions could be recovered from SCG, as these fractions enable the production of several value-added products, among several options, antioxidants [7] and oligo-/monosaccharides [8], respectively, are typically the most studied. These products have potential applications in the food/feed, nutraceutical, and pharmaceutical sectors. There are several options to selectively recover hemicellulose (e.g., dilute acid hydrolysis, steam-explosion, and autohydrolysis), and/or lignin (e.g., alkaline and organosolv processes [8–12]). Unfortunately, none of the currently available process options can fulfil all requirements in terms of selectivity, efficiency, and/or economic performance, and usually a trade-off must be met for a given combination of feedstock/target product(s).

When it comes to lignin extraction, physical-chemical pretreatments are typically preferred over biological pretreatments, as the former are typically more effective, both in terms of presenting higher productivities and higher yields [13]. In fact, when the aim is to recover both the hemicellulose and lignin fractions, organosolv and mild alkaline pretreatments (MAP) are particularly useful, as both enable the simultaneous separation of these fractions from the solid feedstock and easy downstream processing for their selective recovery/purification. The mild alkaline process presents several economic advantages, mainly linked to the lower requirements regarding operational temperature and simpler equipment compared to the organosolv processes [14]. Furthermore, alkali treatments lead to the saponification of the uronic bonds between hemicelluloses and lignin, which induces swelling and increases the effective surface area of the lignocellulosic matrix, facilitating the diffusion of the hydrolytic enzymes [15,16]. The remaining (pretreated) biomass is mainly constituted by cellulose that can be upgraded by enzymatic hydrolysis followed by fermentation [17] or used directly, e.g., through anaerobic digestion (AD) for biogas production [15].

On the other hand, the need to replace fossil fuels and promote the circular bioeconomy is an opportunity to develop strategies to recover energy from waste. Several studies have proposed different technological pathways leading to the production of bioethanol [18], biodiesel [19], pyrolysis oil [20], biogas [21], or fuel pellets [22], among others [23,24]. The anaerobic digestion (AD) technology is commonly an option to valorize biomass. The AD process converts complex organic substrates into biogas, a mixture of methane (CH_4), carbon dioxide (CO_2), and other minor components such as hydrogen sulfide (H_2S), among other trace gases.

The AD process has many environmental and economic advantages [25], however, its efficiency is variable, depending, amongst other factors, on the chemical composition of the biomass. In the case of SCG, the presence of lignin increases the resistance to hydrolysis, the first stage of AD. This is due to both lignin's structure and composition [16].

In fact, a literature review by Mahmoud et al. [26] has reported that the long-term mono-digestion of SCG is linked to instability and volatile fatty acid (VFA) accumulation. Therefore, performing a pretreatment for the extraction of lignin prior to AD might enhance the production and quality of the biogas.

Another solution can be the adoption of a co-digestion regime, using SCG as a co-substrate in AD processing other waste streams (e.g., animal slurry/manure, food waste, sewage sludge, etc.), enabling a more balanced feeding and improving process performance [27,28]. Animal slurry/manure is a common main substrate at full-scale AD that shows good buffer capacity, which is relevant when using co-substrates as SCG that may lead to VFA accumulation. For example, SCG can be mixed with pig slurry to achieve a feeding with a total solids content below 15% as recommended for wet-AD systems [29].

This study aims to design a valorization route for SCG that enables the recovery of value-added compounds, namely the lignin and the hemicellulose fractions, while simultaneously making the pretreated SCG more appropriate for AD, creating a putatively more environmentally and economically viable roadmap. A mild alkaline pretreatment was studied for lignin and hemicellulose fractions' recovery, optimizing the operational conditions, such as reaction time and temperature, and the concentration of the alkaline

solution. The AD trial assessed the effect of SCG pretreatment on biogas and biomethane yields under co-digestion conditions.

2. Materials and Methods

2.1. Feedstock

Spent coffee grounds (SCG) were provided by a local cafeteria, located at the LNEG campus in Lisbon (Portugal). Upon collection, the SCG were dried at 70 °C for at least 72 h, homogenized in a defined lot, and then kept in closed plastic containers at room temperature until use.

Pig slurry (PS) was collected from a fattening/finishing farm in the Montijo region (Portugal). The sample was collected from the storage tank under stirring and was then further processed to remove coarse materials (2 mm tamisation) and stored in closed plastic containers at −20 °C until further use.

Inoculum for AD was collected from a full-scale anaerobic digester treating mixed sludge under mesophilic conditions at a wastewater treatment plant in Lisbon (Portugal). Upon collection, the inoculum was pre-incubated for 5 days at the test temperature to acclimatize it and reduce its contribution to biogas production.

2.2. Delignification and Post-Processing

SCG was treated by a mild alkaline pretreatment, using sodium hydroxide (NaOH) solution as catalyst, testing concentrations ranging from 0 to 2.5 M. The liquid/solid ratio was 10 (w/w dry basis). The treatment was carried out in polypropylene falcon tubes placed in an orbital incubator, at either 25 °C or 50 °C, or in a water bath at 75 °C, for 60, 120, or 240 min.

At the end of the reaction, the pretreated solid and the liquid alkaline liquor were separated by centrifugation at 4900× g for 15 min using a bench-top centrifuge (Ortoalresa Digicen 21 R). The pretreated solid was washed and centrifuged again, firstly with the respective NaOH solution, and then with water. After washing, the samples were dried at 70 °C for at least 72 h, then weighed, and kept in a closed plastic container at room temperature until further use.

Lignin was recovered from the alkaline liquor by acid precipitation. Briefly, equal volumes of alkaline liquor and a sulfuric acid solution with equivalent concentration (normality) were mixed at room temperature, and then an additional amount of sulfuric acid 72% (w/w) was added to reach a final excess of sulfuric acid concentration of 4% (w/w). Lignin was separated from the solution by centrifugation, under the same conditions as described above, and then washed with water and dried at 70 °C for at least 72 h, and weighed, the weight being reported as percentage of the original sample. The supernatant aliquots were stored at 4 °C until further use. The remaining liquid phase was transferred to a micro-reactor glass pressure tube and hydrolyzed in an autoclave (AJC, Portugal) at 121 °C for 60 min to hydrolyze oligosaccharides to their monomeric sugars [30].

These procedures were carried out at least in duplicate for each experimental condition. The sequence of unit operations for SCG delignification and post-processing is presented in Figure 1.

The severity of the treatment was measured using the severity factor concept for alkaline processes, as defined in [31]:

$$\log Mo = \log\left(t \times C^n \times e^{\frac{T-100}{w}}\right) \quad (1)$$

where log Mo is the severity factor, t the reaction time, C the catalyst (NaOH) concentration as measured in wt%, n is a fitted arbitrary constant with a value of 3.90 for sodium hydroxide, T the reaction temperature, and 100 is the reference temperature, both measured in °C. Finally, w is a second arbitrary constant taken to be 14.75 as usually described in the literature [31].

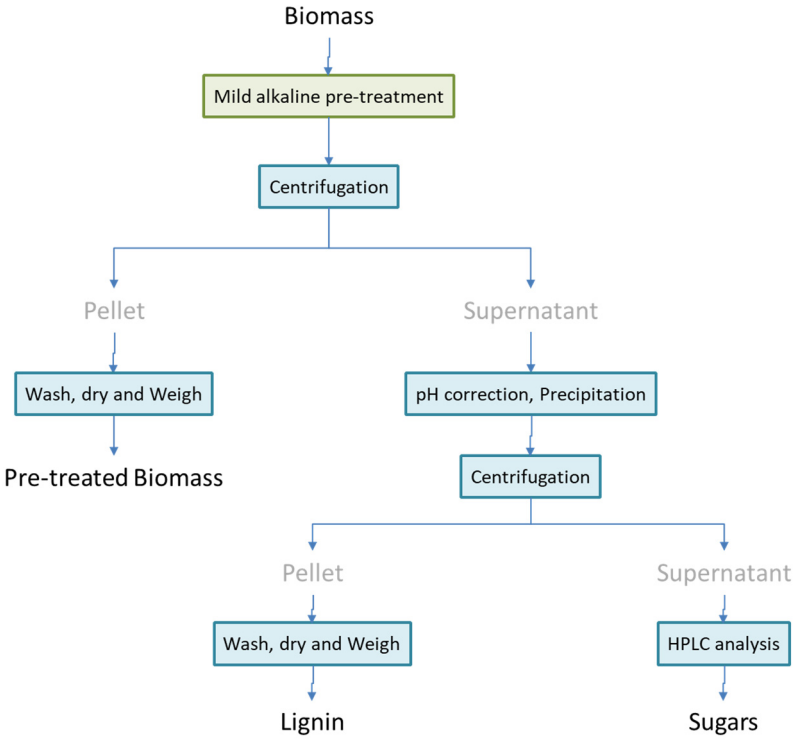


Figure 1. Sequence of unit operations for treating SCG using the proposed mild alkaline process.

2.3. Anaerobic Digestion Experiments

Anaerobic digestion trials were carried out in batch using 500 mL Schott flasks with a working volume of 350 mL kept at mesophilic conditions (37 ± 1 °C) in a water bath. As feedstock, a mixture of pig slurry as substrate and SCG (or MAP-SCG produced under the selected optimal conditions) as co-substrate was used. The ratio of SCG, or MAP-SCG, to pig slurry was 1:3 (volatile solids, VS basis). Each condition was analyzed in triplicate, using an organic load of 4.5 g VS and an inoculum:substrate ratio (I:S) of 2:1. The digesters were connected to 1 L Tedlar® bags for biogas accumulation. Daily biogas production was quantified using the water displacement method. Biogas composition was determined using a biogas sensor (Biogas 5000, Geotech by Q.E.D. Environmental Systems, Inc., Coventry, United Kingdom).

2.4. Analytical Methods

2.4.1. Quantification of Moisture and Ash Content

The moisture content was determined by oven-drying the sample at 105 °C to constant weight and the ash content was determined at 550 °C using NREL/TP-510-42622 protocol [32]. This procedure was carried out on the original SCG and the various pre-treated samples.

2.4.2. Quantification of Klason Lignin and Soluble Lignin in Biomass Samples

The determination of Klason lignin and acid-soluble lignin of the original SCG and the pretreated biomass samples was carried out by quantitative acid hydrolysis according to the NREL/TP-510-42618 protocol [33] and considering the correction for ash.

A Jasco UV-Visible spectrophotometer was used to determine the absorbance of the hydrolysis liquor aliquot, at a wavelength of 320 nm, using a value of 25 L/(g.cm) for the absorptivity and assuming a path length of 1 cm to determine the amount of acid-soluble lignin according to Beer-Lambert law [33].

2.4.3. Quantification of Carbohydrates

The hydrolysates resulting from quantitative acid hydrolysis and the liquor resulting from the mild alkaline pretreatment after the lignin precipitation and removal (pre- and post-hydrolysis) were filtered through 0.45 µm membranes and analyzed for monosaccharides, acetic acid, and potential degradation products (furfural, HMF, formic acid, acetic acid, and levulinic acid) by HPLC using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA). The chromatographic system used (Agilent, Waldbronn Germany) was equipped with RI and UV detectors (the latter set at 280 nm for furan analysis). Elution took place at 50 °C using 5 mM H_2SO_4 as eluent at a flow rate of 0.6 mL/min.

When required, quantitative acid hydrolysis samples were also analyzed using the same chromatographic system and an Aminex HPX-87P (Bio-Rad) column for elucidating some co-elutions of monosaccharides (xylose, galactose, and mannose). The samples were neutralized with barium hydroxide and passed through ion-exchange resins before analysis. Elution took place at 80 °C with H_2O as eluent at a flow rate of 0.6 mL/min.

2.4.4. High-Performance Size Exclusion Chromatography Analysis

The molecular weight distributions of the dissolved compounds in the alkaline liquor (the supernatant of the first centrifugation) were determined by high-performance size exclusion chromatography (HPSEC) analysis as described in [34] using a Toyopearl HW-55F column 10 mm mixed-B 7.5 mm i.d. column and 50 mM NaOH as eluent at 0.8 mL/min. The chromatographic analysis was carried out at room temperature using a Dionex HPLC system (Sunnyvale, CA, USA) composed of a Gina 50 Autosampler automatic injector, a GP 50 gradient pump, an LC 25 chromatography oven, and a UVD340U UV-vis detector set at 280 nm/254 nm. A column wash was carried out for 20 min between each run. The calibration relationship between retention time and molar weight was achieved using polystyrene sulfonate reference standards (PSS GmbH, Mainz, Germany). The molar masses at peak Maximum (Mw) were 148,000 Da, 63,900 Da, 29,100 Da, 9680 Da, and 1100 Da.

2.4.5. Anaerobic Digestion Analysis

Feedstocks, feeding mixtures and digestate samples were characterized according to the standard methods [35] for pH, electrical conductivity (EC), total and volatile solids (TS, VS), total and soluble chemical oxygen demand (COD_T, COD_S), and total alkalinity (TA). Total and volatile dissolved solids (TDS, VDS) were determined for feeding mixtures and digestates. Volatile fatty acids (VFA) content was determined by the titration method recommended in [36]. Inoculum was characterized by TS and VS.

2.4.6. Statistical Analysis

Statistical regression models were calculated in Microsoft Excel using the data Analysis ToolPak (Microsoft 365 Apps for enterprise). All models are calculated based on (standardized) codified values for the operational variables under study.

3. Results and Discussion

3.1. SCG Chemical Composition

The chemical composition of both SCG, and water- and ethanol-extracted SCG, were determined to identify the structural and the soluble components in this biomass. The chemical composition of the SCG used in this work is presented in Table 1.

The glucan present in SCG is entirely structural, with a value of 9.55%, similar to other reports described in the literature [37,38]. Conversely, hemicellulosic sugars' content decreases when comparing SCG with the extracted SCG sample, from 43.47% to 37.44% and 4.33% to 2.40%, respectively, for xylogalactomannan and arabinan. A similar trend can be observed for the acetyl groups, with the latter being fully removed during extraction, demonstrating that some of the compounds that are typically described as hemicellulosic components are easily removed by solvent extraction.

When looking at Klason lignin content, there is a significant reduction from SCG to extracted SCG, from 28.59% to 15.30%. As Klason lignin is not soluble, a possible explanation is that other acid-insoluble components present in the biomass may have been quantified as Klason lignin in the SCG sample, as previously reported [33,39]. Therefore, it is estimated that the real value for Klason lignin in this biomass is around 15.3 %, a similar value to the values reported in the literature [37,38].

Table 1. Composition of the total, structural, and extractable fractions of the SCG lot used in this work (given as % of dry weight).

	Structural [1]		**Extractable** [1]		**Total** [1]	
Glucan	10.22		0		9.55	
Hemicellulose	39.84		9.16		49.00	
Arabinan		2.40		1.93		4.33
Xylogalactomannan [2]		37.44		6.03		43.47
Acetyl groups		0.00		1.20		1.20
Lignin	15.77		14.96		30.73	
Klason Lignin		15.30		13.29		28.59
Soluble Lignin		0.47		1.67		2.14
Ash	0.65		1.01		1.66	
Extractives	0.00		19.70		-	
Water Extractives		0.00		7.27		-
Ethanol Extractives		0.00		12.43		-
Others (by difference)	-		-		9.06	

[1] Structural = quantification of the extracted sample; Total = quantification of the as is sample; Extractable = total-structural. [2] Xylogalactomannan was quantified by the HPLC method based on the Aminex HPX-87H column and on the use of a standard curve for xylose. Galactose, mannose, and arabinose are the only hemicellulosic sugars usually reported for this biomass, but in this work it was also possible to identify xylose (using an HPLC method based on the Aminex HPX-87P column), although in small amounts (typically less than 5% of the galactose or mannose present). However, all results presented hereafter refer to Xylogalactomannan, as it reflects better the results obtained from the utilized methods.

3.2. Mild Alkaline Pretreatment

The mild alkaline pretreatment was optimized based on its main variables: reaction time, temperature, and concentration of the NaOH solution. To verify the tendency of the solid and the lignin yields with the various conditions, a severity factor was calculated, as described in Section 2. As expected, harsher conditions of the reaction lead to lower solid yields, meaning more components have been removed from the biomass, and higher amounts of lignin recovered from the biomass (data presented in Figures S1 and S2, Supplementary Material). Figure 2 shows the relationship obtained between the solid yield and the lignin yield, where a clear correlation between the decreasing solid yield and the increasing lignin yield is presented.

This result is expected; however, the magnitude of lignin yield (reaching a maximum around 20 g/100 g biomass) is not enough to explain the observed decrease in solid yield (from 91.83% to 41.33%), meaning that the delignification is not the only factor impacting the solid yield and other components of the biomass are also being extracted during the reaction. The 7.27% of water extractives present in the biomass are also not enough to explain this difference, as even if the totality of these was removed from the biomass during the mild alkaline pretreatment, there is still a removal of 24.38% of the original biomass that is neither lignin nor water extractives, further supporting the theory that we are extracting structural components.

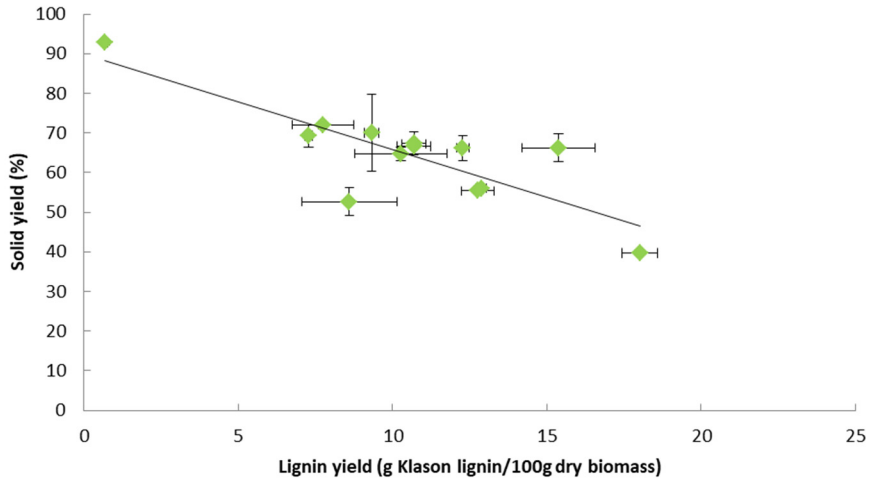


Figure 2. Relationship between the average Klason lignin yield and average solid yield obtained for the diverse tested conditions of the mild alkaline pretreatment (horizontal and vertical lines represent, respectively, each conditions' Klason lignin yield and solid yields' standard deviations).

3.2.1. Delignification of SCG

To have a better understanding of the reaction kinetics, the impact that each of the three variables studied had on the solid and lignin yield was determined. This determination allows us to identify which conditions would be the most beneficial from an industrial point of view.

Figure 3 shows the effect of the concentration of the NaOH solution used on the solid and lignin yields when the reaction time is set constant at 60 min and temperature set at 50 °C.

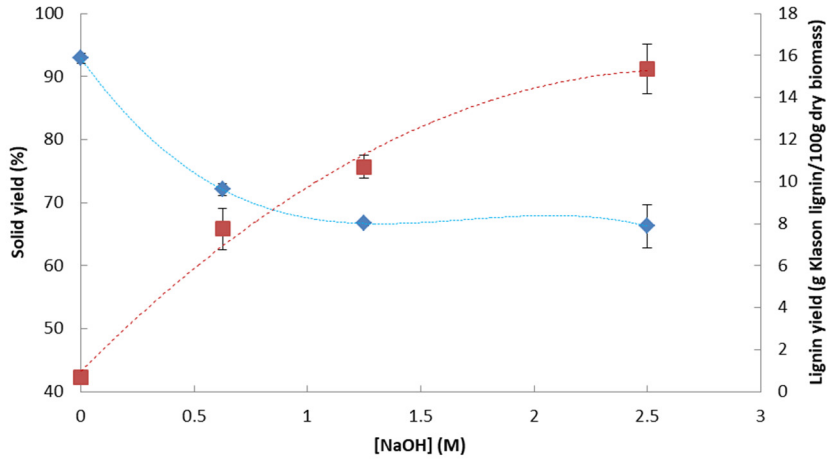


Figure 3. Effect of NaOH concentration on solid yield (♦) and Klason lignin yield (■) for the mild alkaline pretreatment carried out at 60 min and at 50 °C (lines are for eye guide only).

As expected, with the increase in the NaOH concentration there is a decrease in the solid yield and an increase in the lignin yield. Despite this, the relationship is not linear, with both the solid and lignin yields stabilizing for higher NaOH concentrations.

Figures 4 and 5 show the effects of time and temperature of the reaction on the solid and lignin yields, respectively, when the NaOH concentration is set constant at 1.25 M.

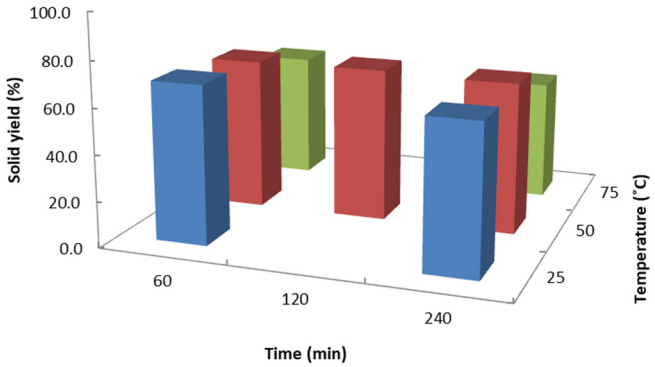


Figure 4. Effects of reaction time and temperature (blue, red, and green bars correspond to 25, 50, and 75 °C, respectively) on solid yield obtained by mild alkaline pretreatment using a catalyst (NaOH) concentration of 1.25 M.

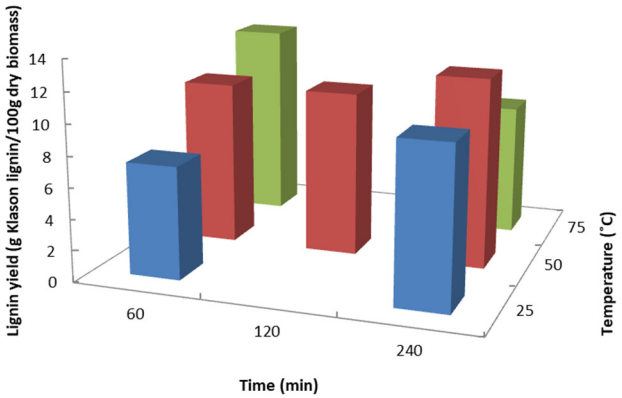


Figure 5. Effects of reaction time and temperature (blue, red, and green bars correspond to 25, 50, and 75 °C, respectively) on Klason lignin yield obtained by mild alkaline pretreatment using a catalyst (NaOH) concentration of 1.25 M.

Again, the figures show that an increase in the time, or temperature, of the reaction leads to a decrease in the solid yield and an increase in the lignin yield, although the relationship does not appear to be linear, with slight decreases in the solid yield when one of the conditions is increased.

It is interesting to note that when the reaction occurred at 75 °C for 240 min, the solid yield decreased, as expected, but the lignin yield decreased as well, in comparison with reactions performed at lower temperatures or lower times, meaning that those harsh conditions might be damaging the extracted lignin or not allowing it to precipitate, as partially described for bamboo biomass in [40].

To check if the three studied conditions were statistically relevant when it comes to the solid and lignin yields, two mathematical models were built, one for the solid yield and one for the lignin yield, using the different combinations of the conditions tested, and using the following equation:

$$Y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3, \quad (2)$$

where Y refers to either the solid or the lignin yield, β_0 is the regression coefficient at the center point, β_1 is the coefficient related to the time variable, β_2 is the coefficient related to the [NaOH] variable, and β_3 is the coefficient related to the temperature variable. Table 2 shows the results of those two models.

Table 2. Regression coefficient values for the solid and lignin yield models.

	Solid Yield		Lignin Yield	
Parameter	**Coefficients**	***p*-Value**	**Coefficients**	***p*-Value**
β_0	98.562	4.2×10^{-25}	2.231	0.086
β_1	−0.056	2.4×10^{-5}	0.001	0.789
β_2	−9.607	1.9×10^{-7}	4.965	6.6×10^{-11}
β_3	−0.261	1.7×10^{-5}	0.023	0.223

For both models, the multiple R value is higher than 0.85, meaning that the different variables are explaining the differences in the results for the solid and lignin yields. The variables are all statistically relevant when it comes to the solid yield, as all the *p*-values are considerably below 0.05. When it comes to the lignin yield, the same cannot be said, as the only variable that has a *p*-value below 0.05 is NaOH concentration, meaning that the time of the reaction and the temperature at which it occurs are not statistically relevant regarding the amount of lignin that is recovered from the reaction. A similar trend was observed for cotton stalks under similar conditions [41].

Molecular Weight of the Recovered Lignin

As a preliminary characterization, Figure 6 shows the molecular weight of the extracted lignin when the reaction occurs at 50 °C, at 60, 120, and 240 min, and with an NaOH concentration of 0.625 M, 1.25 M, and 2.5 M.

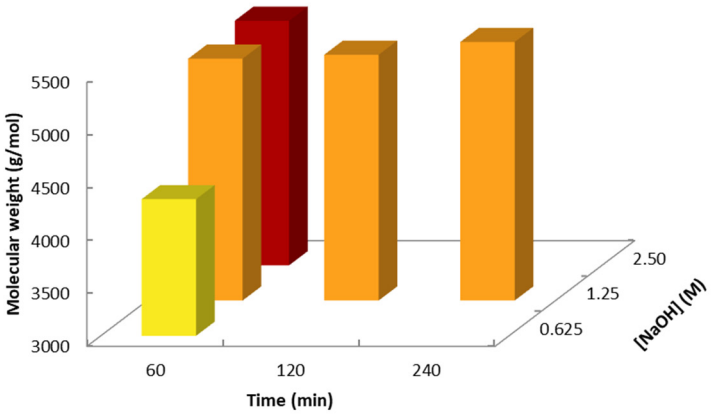


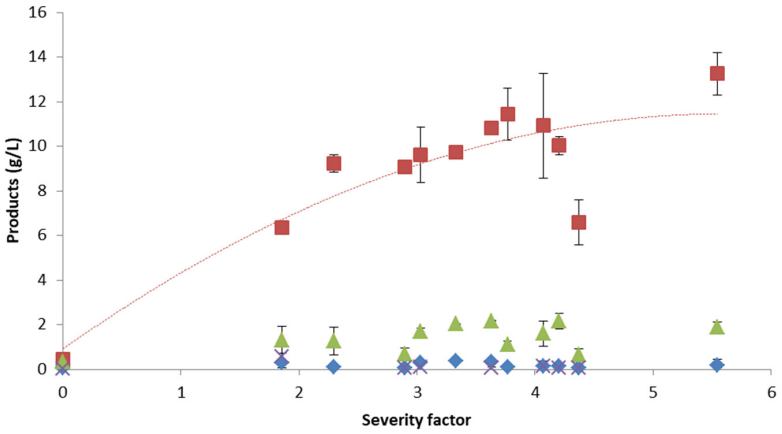
Figure 6. Molecular weight of the extracted lignin obtained by mild alkaline pretreatment at 50 °C and diverse reaction times and NaOH concentrations (yellow, orange, and red bars correspond to 0.625, 1.25, and 2.50 M, respectively).

The lignin molecules extracted are of a considerable size, with molecular weights between 4300 g/mol and 5500 g/mol, higher values than the 2400 g/mol reported by [42]. Both NaOH concentration and reaction time affect the molecular weight of the extracted lignin. The increase in time of the reaction seems to have a linear relationship with the molecular weight, but the increase in molecular weight is small, when compared with the NaOH concentration, showing again that the impact of the time of the reaction is minimal. NaOH concentration has a nonlinear relationship with the molecular weight, as the molecular weights of the extracted lignin when 1.25 M and 2.5 M were used are similar and very different from the obtained for the lower concentration tested. These results enable the classification of the extracted lignin as high molecular weight lignin [43], which can potentially be used for the production of carbon nanofibers [42].

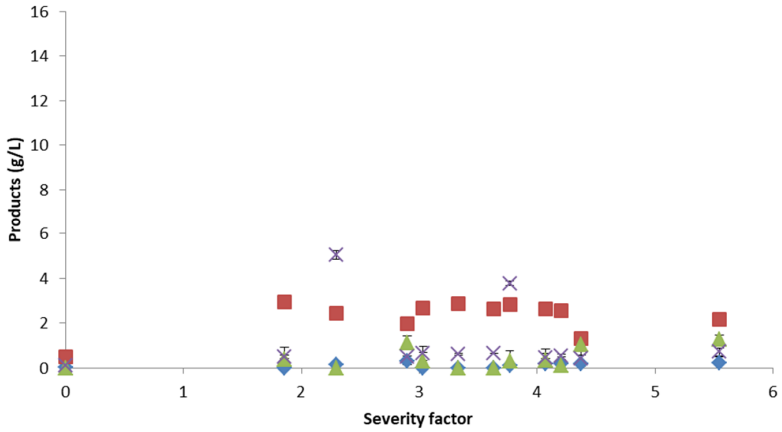
3.2.2. Oligosaccharides Production

Figure 7 presents the concentrations of glucooligosaccharides (GlcOS), xylogalactomannanooligosaccharides (XGMOS), arabinooligosaccharides (AOS), formic acid, acetic

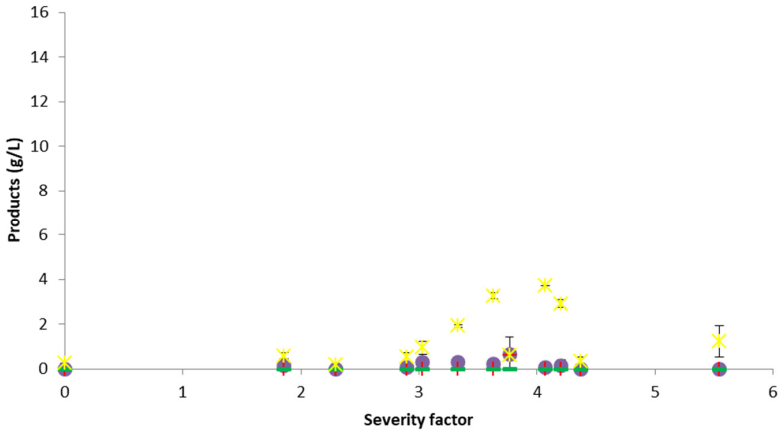
acid, levulinic acid, HMF and furfural obtained in the alkaline liquor as a function of the severity factor.



(**a**)



(**b**)



(**c**)

Figure 7. Effects of the severity factor (log *Mo*) on the composition of the alkaline liquor obtained from mild alkaline pretreatment of SCG. Panel (**a**): GlcOS (♦), XGMOS (■), AOS (▲), and AcOS (×); Panel (**b**): glucose (♦), xylose/galactose/mannose (■), arabinose (▲), and acetic acid (×); Panel (**c**): formic acid (✳), levulinic acid (●), HMF (+), and furfural (−)). Line is for eye guide only.

XGMOS seem to be the main products formed, with a maximum concentration of 13.25 g/L of XGMOS being reached, which translates to 13.25 g XGMOS/100 g SCG, and a recovery of 30.5% of the xylogalactomannan present in the biomass, whereas none of the other products exceeded concentrations of 3 g/L. There is a relationship between the [XGMOS] and the severity factor, although the relationship is not linear, as the [XGMOS] tends to stabilize when the severity factor reaches values around 5.

Previously, Branco et al. [39] reported oligosaccharides recovery values of 12.2 g/100 g biomass, with a removal of 53.2% of the original xylan present in the biomass, when using the autohydrolysis method on corn straw feedstock, and [44] reported oligosaccharides recovery values of 5.2 g/100 g biomass, which translates to a removal of 29.3% of the oligosaccharides present in the biomass, on *Annona cherimoya* seeds using the same method. This means that mild alkaline pretreatment shows to be an efficient treatment when it comes to oligosaccharides recovery when compared to autohydrolysis, the most-used method in the literature for producing oligosaccharides; the efficiencies of removal are similar, despite this method utilizing significantly lower temperatures compared to the ones reported (215 °C and 190 °C, respectively).

No other products were recovered at a significant concentration, with the highest being acetic acid at 2.17 g/L. The values for potential microbial inhibitors [45,46] such as aliphatic acids and furans are particularly low and would enable the utilization of these liquid streams to produce edible oligosaccharides, as their purification does not seem particularly challenging.

Studying the effects of the three variables (time, temperature, and NaOH concentration) on the XGMOS production in more detail, Figure 8 shows the production of XGMOS when the reaction occurred at 50 °C for 60 min, using [NaOH] of 0 M, 0.625 M, 1.25 M, and 2.5 M. Figure 9 shows the production of XGMOS when the reaction occurred using NaOH at 1.25 M, with temperatures of 25 °C, 50 °C, and 75 °C for 60, 120, and 240 min.

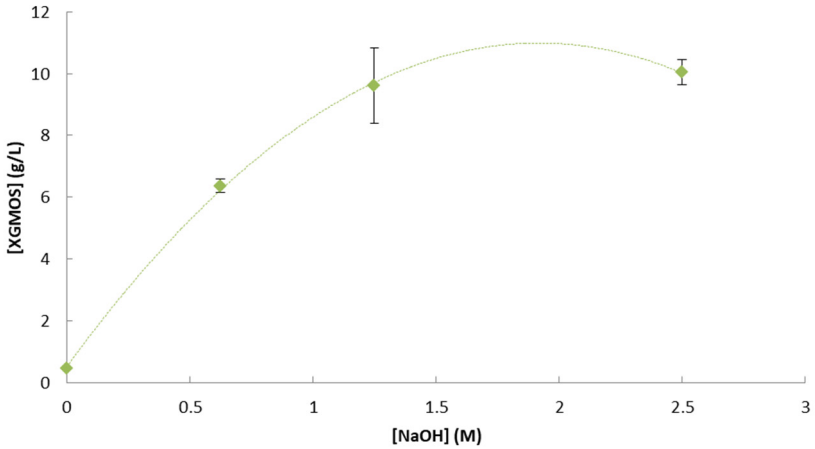


Figure 8. Effect of NaOH concentration on XGMOS titer obtained by mild alkaline pretreatment carried out at 60 min and at 50 °C (line is for eye guide only).

These results show that, for every condition, an increase in severity leads to an increase in the amount of XGMOS recovered. Despite this, time and temperature do not seem to be particularly impactful on XGMOS production compared to the impact that NaOH concentration has, in a similar trend to that observed with the lignin yield. In fact, NaOH concentration has a clear relationship with the [XGMOS], with an initial increase that seems to stabilize around the 1.25 M point.

When the reaction occurred at 75 °C for 240 min, the [XGMOS] is lower than the ones seen when reactions were performed at lower temperatures or lesser reaction time, again similarly to what happened with the lignin yield under these conditions, showing that these severe conditions may damage the products obtained, inducing oligosaccharides hydrolysis, in a similar trend to that described for bamboo biomass [40].

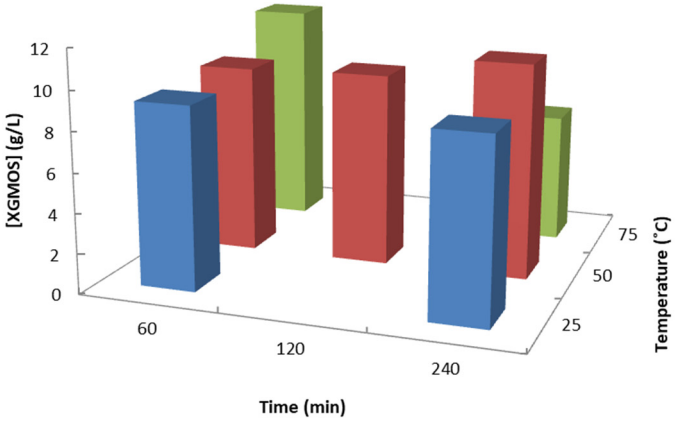


Figure 9. Effects of reaction time and temperature (blue, red, and green bars correspond to 25, 50, and 75 °C, respectively) on XGMOS titer obtained by mild alkaline pretreatment using a catalyst (NaOH) concentration of 1.25 M.

A mathematical model was built to test which of the conditions were statistically relevant regarding the production of XGMOS, as previously explained. Table 3 shows the results of the model, using the same notation described above.

Table 3. Regression coefficient values for the XGMOS yield model.

	Coefficients	***p*-Value**
β_0	8.151	2.4×10^{-24}
β_1	0.409	0.005
β_2	4.042	2.2×10^{-8}
β_3	−0.215	0.629

The multiple R value of 0.783 shows that the variables are explaining the differences in the [XGMOS]. Both time and NaOH concentration are statistically significant, with *p*-values below 0.05, unlike temperature. Despite this, the coefficient value of the NaOH concentration is around ten times larger than the time coefficient, proving that this is the most influential variable, which is also supported by previous finding for cotton stalks for similar conditions [41].

3.2.3. Chemical Composition of MAP-SCG

The most severe conditions tested (temperature = 75 °C, [NaOH] = 2.5 M and time = 240 min) led to both the highest recovered lignin yield (18.02%) and the highest [XGMOS] produced (13.25 g/L), meaning that if the goal was strictly to maximize the amounts of products recovered, these would be the optimal conditions.

However, as the main objective of this work is to find a process that is environmentally friendly and economically viable so that it can potentially be industrialized. As the time and temperature operational variables had a very limited or statistically irrelevant impact, the optimal time and temperature for this process were considered to be 60 min and 50 °C, respectively, which might have relevant impacts in economic terms, especially in the view of CAPEX due to the reduction of processing time.

Regarding NaOH concentration, this variable proved to be the most impactful on the amount of product obtained. However, that was, in part, due to the very significant increase in both the lignin yield and the [XGMOS] between extractions performed with water only ([NaOH] = 0 M) and NaOH at 0.625 M, with the amount of product obtained seemingly stabilizing when the NaOH concentration was further increased. Therefore, as NaOH is both expensive and problematic to the environment, it was decided that 0.625 M was the optimal NaOH concentration. This will putatively significantly reduce OPEX.

Table 4 shows the structural composition of MAP-SCG under the defined optimal conditions (0.625 M NaOH, 50 °C, and 60 min).

Table 4. Structural composition of the MAP-SCG obtained under the defined optimal conditions (% of dry weight).

	MAP-SCG Structural Composition	
Glucan	12.66 ± 0.09	
Hemicelulose	51.23 ± 1.09	
Arabinan		6.46 ± 0.16
Xylogalactomannan		44.77 ± 0.93
Acetyl groups		0.00 ± 0.00
Lignin	21.29 ± 0.28	
Klason lignin		20.74 ± 0.31
Soluble lignin		0.55 ± 0.04
Ash	6.58 ± 0.23	
Others (by difference)	8.24	

Compared to the SCG composition (Table 1), glucan was not significantly affected by the pretreatment, as 95.6% of the initial glucan was retained in the solid fraction, which is considered ideal for anaerobic digestion. This is expected due to the fact that cellulose is described to be considerably stable under alkaline conditions [47]. Lignin was the main targeted component in the mild alkaline pretreatment, with a decrease of around 50%. Xylogalactomannan levels decreased by around 25%, as they were the main extracted product other than lignin, and the source of the produced oligosaccharides, as described above. Arabinan presents a slight increase in comparison with the amount in the original sample, which can also be justified by the low amounts present. Again, the acetyl groups appear to be completely non-structural, as they were completely removed from the biomass. Interestingly, ash content more than doubled, meaning that, despite successive washings of the biomass with water, there was still residual NaOH present in the biomass after the end of the pretreatment, leading to an increase in inorganic compounds present in the biomass.

3.3. Anaerobic Digestion

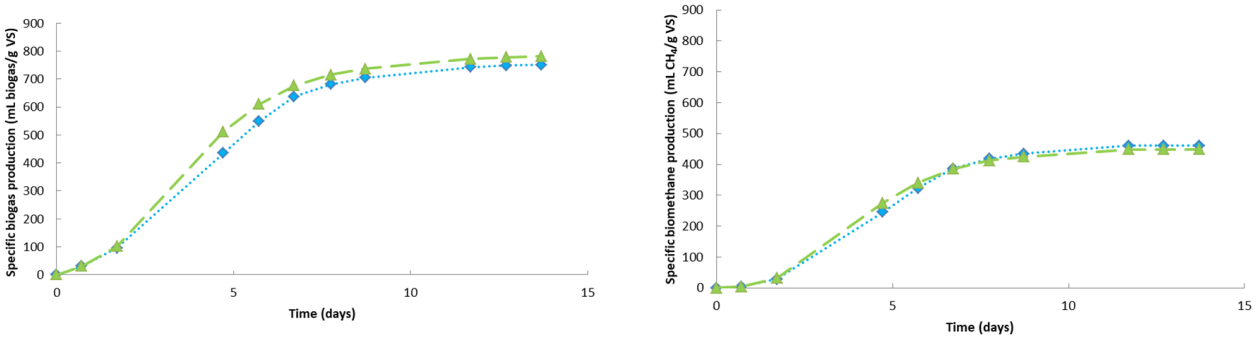
To assess the effect of the mild alkaline pretreatment on the digestibility of the SCG, an AD trial was carried out at lab-scale, using a co-digestion regime using pig slurry (PS) as substrate. The characterization of the feeding mixtures (PS + SCG and PS + MAP-SCG) used for the AD trial is presented in Table 5.

Table 5. Characterization of the feeding mixtures used in the AD trial.

Parameter	PS + SCG	PS + MAP-SCG
TS (g/L)	9.98 ± 0.16	9.69 ± 0.34
TDS (g/L)	3.13 ± 0.06	3.07 ± 0.02
VS (g/L)	7.43 ± 0.19	7.13 ± 0.40
VDS (g/L)	1.72 ± 0.01	1.65 ± 0.01
pH	6.94	6.96
EC (mS/cm)	6.69	6.57
COD_T (g/L)	11.25	11.50
COD_S (g/L)	10.75	11.25

Both feeding mixtures have a VS/TS ratio of around 74%, with PS + MAP-SCG showing a slightly lower value, which is in accordance with the higher ash content of MAP-SCG (Table 4). As can be seen from Table 5, the removal of lignin and hemicellulose during the pretreatment of SCG led to a reduction of about 4% on VS. The mild alkaline pretreatment led to a 2% increase in the $CODs/COD_T$ ratio, indicating a better availability of organic matter.

The cumulative biogas and biomethane production profiles for the co-digestion of SCG and MAP-SCG are presented in Figure 10a,b, respectively. As can be seen, the highest specific biogas production in the AD trial was achieved by the PS + MAP-SCG (782.01 mL/g SV), around 4% higher than the value obtained with PS + SCG (751.07 mL/g SV). On the other hand, Figure 10b shows that the highest specific biomethane volume was produced by PS + SCG, with a value of 460.80 mL CH_4/g SV (304.2 mLCH_4/g COD), whereas PS + MAP-SCG achieved a production of 448.93 mL/g SV (290.0 mLCH_4/g COD). These results mean that, although lignin removal increases the amount of biogas produced during the anaerobic digestion process as sugars remaining in the biomass become more available after the lignin removal, this does not translate into biomethane production because some of the sugars that were present in the initial biomass were removed during the mild alkaline pretreatment, leading to a slightly lower quality of biogas produced (61.35 g biomethane/100 g biogas for PS + SCG, 57.41 g biomethane/100 g biogas for PS + MAP-SCG). The results obtained illustrate the advantage of adopting a co-digestion regime, as the yields achieved are clearly above those for mono-digestion, which, according to a review by Gebreeyessus (2022) [4], range from 270 to 330 mL CH_4/g VS.



(**a**) (**b**)

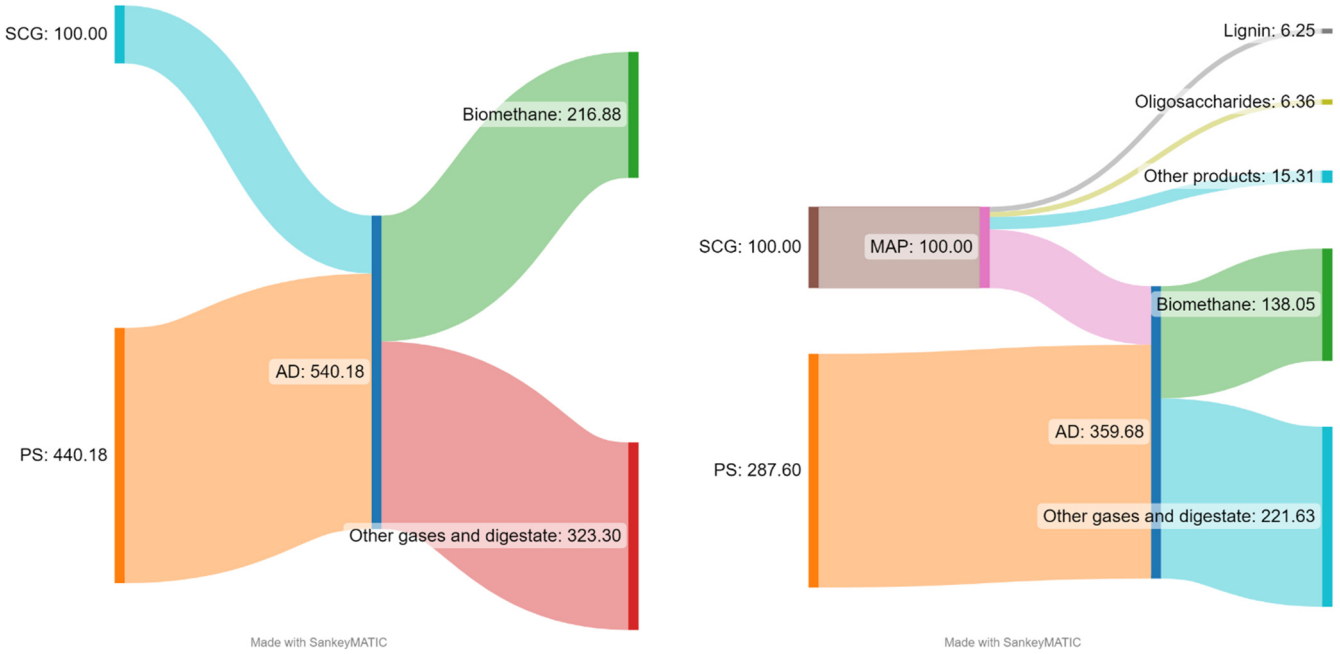
Figure 10. Cumulative biogas (**a**) and biomethane (**b**) production profiles for the co-digestion of SCG (♦) and MAP-SCG (▲) (Lines are for eye guide only).

Furthermore, the specific biomethane production values obtained in this study are higher than the results reported in the literature for co-digestion of SCG [26]. For example, Orfanoudaki et al. [28], who studied the anaerobic co-digestion of SCG and pig slurry, found a maximum methane production of 357 mL CH_4/g VS. In another study [48], a mixture of SCG and cow manure at 1:1 (VS basis) led to 225 mL CH_4/g VS.

Regarding process stability, the pH values after anaerobic co-digestion (7.06 ± 0.03 and 7.05 ± 0.02, respectively, for PS + SCG and PS + MAP-SCG) show that there was no acidification. This fact is in accordance with the VFA/TA ratios that were below 0.30, indicating a stable process [49].

3.4. Overall Mass Balance

Figure 11 shows the Sankey diagrams representing the mass balance of SCG and MAP-SCG in the defined optimal conditions, per 100 kg of biomass. The amounts of pig slurry determined were 440.18 kg and 287.60 kg, respectively, for SCG and MAP-SCG. This is because the amount of MAP-SCG entering the AD process is lower (72.08 kg) than the amount of SCG, due to the fact that the lignin and oligosaccharides had been removed from the biomass previously, and the amounts of PS entering the AD process were calculated so that the ratio of biomass to PS was kept at 1:3 (VS basis).



(**a**) (**b**)

Figure 11. Sankey diagrams for the mass balance of the overall processes of (**a**) converting SCG directly by anaerobic digestion (AD), and (**b**) the sequential utilization of mild alkaline pretreatment (MAP) and AD.

The main product obtained for both SCG and MAP-SCG is biomethane, as even if the lignin and oligosaccharides were removed from SCG, most of the biomass still goes into anaerobic digestion to produce biogas. In addition, SCG was used as a co-substrate in anaerobic digestion, and the amounts of pig slurry used were much higher than the amounts of SCG, which contributed to the large quantities of biomethane produced. However, the products obtained from the mild alkaline pretreatment present a potentially higher market value. These alternative products must be explored further to identify their true applications and market value.

4. Conclusions

An integrated valorization strategy for SCG was proposed and validated at lab-scale. This strategy successfully enabled the recovery of value-added compounds from SCG, and facilitated biofuels (biogas) production when using SCG as a co-substrate.

Mild alkaline pretreatment allowed the recovery of significant amounts of lignin and XGMOS. This was achieved under significantly milder conditions when compared to other processes such as autohydrolysis and organosolv processes, which require much higher temperatures which are typically recognized to limit their economic viability. Sodium hydroxide concentration was the operational variable that presented the stronger influence over product yields, with time and temperature having minimal impact. Furthermore, the specific biomethane production from AD after the mild alkaline pretreatment was only slightly lower when compared with non-pretreated SCG. At optimal, mild conditions it was possible to produce 138.05 kg of biomethane from 100 kg of SCG and 287.60 kg of pig slurry, retaining still a significant quantity of solid digestate that can be used as fertilizer after stabilization.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/en16093907/s1, Figure S1: Effect of the mild alkaline pretreatment on solid yield as a function of the severity factor; Figure S2: Effect of the mild alkaline pretreatment on Klason lignin recovery as a function of the severity factor.

Author Contributions: G.M.R. conducted the research and performed the analysis in collaboration with P.L.M.; G.M.R., L.C.D., A.C.O., F.C. and R.F. designed the research, performed the data interpretation and discussion, and wrote the manuscript; L.C.D. and R.F. supervised the work; L.C.D., R.F., F.C. and A.C.O. revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The work was partially supported by BRISK II (H2020 grant agreement 731101). This work was partially carried out at the Biomass and Bioenergy Research Infrastructure (BBRI-LISBOA-01-0145-FEDER-022059), supported by the Operational Programme for Competitiveness and Internationalization (PORTUGAL2020), by the Lisbon Portugal Regional Operational Programme (Lisboa 2020) and by North Portugal Regional Operational Programme (Norte2020) under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). This work was also funded by national funds through FCT—Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04129/2020 of LEAF-Linking Landscape, Environment, Agriculture and Food, Research Unit.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors thank Belina Ribeiro, Céu Penedo, and Domingos Figueiredo for their laboratory support.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Battista, F.; Zuliani, L.; Rizzioli, F.; Fusco, S.; Bolzonella, D. Biodiesel, biogas and fermentable sugars production from spent coffee grounds: A cascade biorefinery approach. *Bioresour. Technol.* **2021**, *342*, 125952. [CrossRef] [PubMed]
2. Teixeira, R.A.; Bueno, B.A.; Borges, R.M.; Bringhenti, J.R. Biochemical methane potential of spent coffee grounds via co-digestion with food waste. *Bioenerg. Res.* **2021**, 1–12. [CrossRef]
3. Stegmann, P.; Londo, M.; Junginger, M. The circular bioeconomy: Its elements and role in European bioeconomy clusters. *Resour Conserv Recycl X* **2020**, *6*, 100029. [CrossRef]
4. Gebreeyessus, G.D. Towards the sustainable and circular bioeconomy: Insights on spent coffee grounds valorization. *Sci. Total Environ.* **2022**, *833*, 155113. [CrossRef]
5. Obruca, S.; Benesova, P.; Kucera, D.; Petrik, S.; Marova, I. Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids. *New Biotechnol.* **2015**, *32*, 569–574. [CrossRef]
6. Solomakou, N.; Loukri, A.; Tsafrakidou, P.; Michaelidou, A.M.; Mourtzinos, I.; Goula, A.M. Recovery of phenolic compounds from spent coffee grounds through optimized extraction processes. *Sustain. Chem. Pharm.* **2022**, *25*, 100592. [CrossRef]
7. Ballesteros, L.F.; Ramirez, M.J.; Orrego, C.E.; Teixeira, J.A.; Mussatto, S.I. Optimization of autohydrolysis conditions to extract antioxidant phenolic compounds from spent coffee grounds. *J. Food Eng.* **2017**, *199*, 1–8. [CrossRef]
8. Ballesteros, L.F.; Teixeira, J.A.; Mussatto, S.I. Extraction of polysaccharides by autohydrolysis of spent coffee grounds and evaluation of their antioxidant activity. *Carbohydr. Polym.* **2017**, *157*, 258–266. [CrossRef] [PubMed]
9. Stahl, H.; Turek, E. Acid-hydrolysis of spent coffee grounds to produce D-mannose and D-mannitol. In Proceedings of the Fourteenth International Conference on Coffee Science, San Francisco, CA, USA, 14–19 July 1991.
10. Chiyanzy, I.; Brienzo, M.; García-Aparicio, M.; Agudelo, R.; Gorgens, J. Spent coffee ground mass solubilisation by steam explosion and enzymatic hydrolysis. *J. Chem. Technol. Biot.* **2015**, *90*, 449–458. [CrossRef]
11. Girotto, F.; Lavagnolo, M.C.; Pivato, A. Spent coffee grounds alkaline pre-treatment as biorefinery option to enhance their anaerobic digestion yield. *Waste Biomass Valor.* **2018**, *9*, 2565–2570. [CrossRef]
12. Oliva, A.; Tan, L.C.; Papirio, S.; Esposito, G.; Lens, P.N.L. Effect of methanol-organosolv pretreatment on anaerobic digestion of lignocellulosic materials. *Renew Energ.* **2021**, *169*, 1000–1012. [CrossRef]
13. Hassan, M.; Ding, W.M.; Bi, J.H.; Mehryar, E.; Talha, Z.A.A.; Huang, H.Y. Methane enhancement through oxidative cleavage and alkali solubilization pre-treatments for corn stover with anaerobic activated sludge. *Bioresour. Technol.* **2016**, *200*, 405–412. [CrossRef]
14. Padilla-Rascón, C.; Carvalheiro, F.; Duarte, L.C.; Roseiro, L.B.; Ruíz, E.; Castro, E. An integrated olive stone biorefinery based on a two-step fractionation strategy. *Ind. Crops Prod.* **2022**, *187*, 115157. [CrossRef]
15. Sambusiti, C.; Monlau, F.; Ficara, E.; Carrere, H.; Malpei, F. A comparison of different pre-treatments to increase methane production from two agricultural substrates. *Appl. Energ.* **2013**, *104*, 62–70. [CrossRef]
16. Paudel, S.R.; Banjara, S.P.; Choi, O.K.; Park, K.Y.; Kim, Y.M.; Lee, J.W. Pretreatment of agricultural biomass for anaerobic digestion: Current state and challenges. *Bioresour. Technol.* **2017**, *245*, 1194–1205. [CrossRef] [PubMed]

17. Shatalov, A.A.; Morais, A.R.C.; Duarte, L.C.; Carvalheiro, F. Selective single-stage xylan-to-xylose hydrolysis and its effect on enzymatic digestibility of energy crops giant reed and cardoon for bioethanol production. *Ind. Crops Prod.* **2017**, *95*, 104–112. [CrossRef]
18. Juárez, G.F.Y.; Pabiloa, K.B.C.; Manlangit, K.B.L.; Go, A.W. Direct dilute acid hydrolysis of spent coffee grounds: A new approach in sugar and lipid recovery. *Waste Biomass Valor.* **2018**, *9*, 235–246. [CrossRef]
19. Somnuk, K.; Eawlex, P.; Prateepchaikul, G. Optimization of coffee oil extraction from spent coffee grounds using four solvents and prototype-scale extraction using circulation process. *Agric. Nat. Resour.* **2017**, *51*, 181–189. [CrossRef]
20. Atabani, A.E.; Ali, I.; Naqvi, S.R.; Badruddin, I.A.; Aslam, M.; Mahmoud, E.; Almomani, F.; Juchelkova, D.; Atelge, M.R.; Khan, T.M.Y. A state-of-the-art review on spent coffee ground (SCG) pyrolysis for future biorefinery. *Chemosphere* **2022**, *286*, 131730. [CrossRef]
21. Vasmara, C.; Marchetti, R. Spent coffee grounds from coffee vending machines as feedstock for biogas production. *Environ. Eng. Manag. J.* **2018**, *17*, 2401–2408.
22. Limousy, L.; Jeguirim, M.; Dutournie, P.; Kraiem, N.; Lajili, M.; Said, R. Gaseous products and particulate matter emissions of biomass residential boiler fired with spent coffee grounds pellets. *Fuel* **2013**, *107*, 323–329. [CrossRef]
23. Girotto, F.; Pivato, A.; Cossu, R.; Nkeng, G.E.; Lavagnolo, M.C. The broad spectrum of possibilities for spent coffee grounds valorisation. *J. Mater. Cycles Waste* **2018**, *20*, 695–701. [CrossRef]
24. van Dam, J.E.; Harmsen, P. *Coffee Residues Utilization*; Wageningen UR-Food & Biobased Research: Wageningen, The Netherlands, 2010.
25. Chandra, R.; Takeuchi, H.; Hasegawa, T. Methane production from lignocellulosic agricultural crop wastes: A review in context to second generation of biofuel production. *Renew Sust. Energ. Rev.* **2012**, *16*, 1462–1476. [CrossRef]
26. Mahmoud, E.; Atabani, A.E.; Badruddin, I.A. Valorization of spent coffee grounds for biogas production: A circular bioeconomy approach for a biorefinery. *Fuel* **2022**, *328*, 125296. [CrossRef]
27. Kim, D.; Kim, H.; Kim, J.; Lee, C. Co-feeding spent coffee grounds in anaerobic food waste digesters: Effects of co-substrate and stabilization strategy. *Bioresour. Technol.* **2019**, *288*, 121594. [CrossRef]
28. Orfanoudaki, A.; Makridakis, G.; Maragkaki, A.; Fountoulakis, M.S.; Kallithrakas-Kontos, N.G.; Manios, T. Anaerobic co-digestion of pig manure and spent coffee grounds for enhanced biogas production. *Waste Biomass Valor.* **2020**, *11*, 4613–4620. [CrossRef]
29. Motte, J.C.; Trably, E.; Escudie, R.; Hamelin, J.; Steyer, J.P.; Bernet, N.; Delgenes, J.P.; Dumas, C. Total solids content: A key parameter of metabolic pathways in dry anaerobic digestion. *Biotechnol. Biofuels* **2013**, *6*, 164. [CrossRef] [PubMed]
30. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.J.; Sluiter, J.; Templeton, D. *Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples*; NREL/TP-510-42623; National Renewable Energy Laboratory: Golden, CO, USA, 2008.
31. Pedersen, M.; Meyer, A.S. Lignocellulose pretreatment severity—Relating pH to biomatrix opening. *New Biotechnol.* **2010**, *27*, 739–750. [CrossRef] [PubMed]
32. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.J.; Sluiter, J.; Templeton, D. *Determination of Ash in Biomass*; NREL/TP-510-42622; National Renewable Energy Laboratory: Golden, CO, USA, 2008.
33. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of Structural Carbohydrates and Lignin in Biomass*; NREL/TP-510-42618; National Renewable Energy Laboratory: Golden, CO, USA, 2012.
34. Moniz, P.; Serralheiro, C.; Matos, C.T.; Boeriu, C.G.; Frissen, A.E.; Duarte, L.C.; Roseiro, L.B.; Pereira, H.; Carvalheiro, F. Membrane separation and characterisation of lignin and its derived products obtained by a mild ethanol organosolv treatment of rice straw. *Process Biochem.* **2018**, *65*, 136–145. [CrossRef]
35. Standard Methods Committee of the American Public Health Association; American Water Works Association; Water Environment Federation. *Standard Methods for the Examination of Water and Wastewater*; American Public Health Association: Washington, DC, USA, 2017.
36. Buchauer, K. A comparison of two simple titration procedures to determine volatile fatty acids in influents to waste-water and sludge treatment processes. *Water SA* **1998**, *24*, 49–56.
37. Mussatto, S.I.; Machado, E.M.S.; Martins, S.; Teixeira, J.A. Production, composition, and application of coffee and its industrial residues. *Food Bioprocess Technol.* **2011**, *4*, 661–672. [CrossRef]
38. Ballesteros, L.F.; Teixeira, J.A.; Mussatto, S.I. Chemical, functional, and structural properties of spent coffee grounds and coffee silverskin. *Food Bioprocess Technol.* **2014**, *7*, 3493–3503. [CrossRef]
39. Branco, P.C.; Dionisio, A.M.; Torrado, I.; Carvalheiro, F.; Castilho, P.C.; Duarte, L.C. Autohydrolysis of *Annona cherimola* mill. seeds: Optimization, modeling and products characterization. *Biochem. Eng. J.* **2015**, *104*, 2–9. [CrossRef]
40. Yuan, Z.Y.; Wen, Y.B.; Kapu, N.S. Ethanol production from bamboo using mild alkaline pre-extraction followed by alkaline hydrogen peroxide pretreatment. *Bioresour. Technol.* **2018**, *247*, 242–249. [CrossRef]
41. Silverstein, R.A.; Chen, Y.; Sharma-Shivappa, R.R.; Boyette, M.D.; Osborne, J. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. *Bioresour. Technol.* **2007**, *98*, 3000–3011. [CrossRef]
42. Du, B.Y.; Zhu, H.W.; Wang, X.; Xiao, L.P.; Ma, J.L.; Chen, X.H.; Zhou, J.H.; Sun, R.C. Tuning structure of spent coffee ground lignin by temperature fractionation to improve lignin-based carbon nanofibers mechanical performance. *Int. J. Biol. Macromol.* **2021**, *174*, 254–262. [CrossRef]
43. Jin, H.Q.; Shi, H.Q.; Jia, W.C.; Sun, Y.N.; Sheng, X.R.; Guo, Y.Z.; Li, H.M.; Sun, H.D. Green solvents-based molecular weight controllable fractionation process for industrial alkali lignin at room temperature. *Int. J. Biol. Macromol.* **2022**, *207*, 531–540. [CrossRef] [PubMed]

44. Moniz, P.; Pereira, H.; Quilhó, T.; Carvalheiro, F. Characterisation and hydrothermal processing of corn straw towards the selective fractionation of hemicelluloses. *Ind. Crops Prod.* **2013**, *50*, 145–153. [CrossRef]
45. Duarte, L.C.; Carvalheiro, F.; Neves, I.; Girio, F.M. Effects of aliphatic acids, furfural, and phenolic compounds on *Debaryomyces hansenii* CCMI 941. *Appl. Biochem. Biotechnol.* **2005**, *121*, 413–425. [CrossRef]
46. Duarte, L.C.; Carvalheiro, F.; Tadeu, J.; Girio, F.M. The combined effects of acetic acid, formic acid, and hydroquinone on *Debaryomyces hansenii* physiology. *Appl. Biochem. Biotechnol.* **2006**, *130*, 461–475. [CrossRef]
47. Knill, C.J.; Kennedy, J.F. Degradation of cellulose under alkaline conditions. *Carbohydr. Polym.* **2003**, *51*, 281–300. [CrossRef]
48. Akyol, C. In search of the optimal inoculum to substrate ratio during anaerobic co-digestion of spent coffee grounds and cow manure. *Waste Manag. Res.* **2020**, *38*, 1278–1283. [CrossRef] [PubMed]
49. Bernhard, D. *Process Monitoring in Biogas Plants*; IEA Bioenergy: Vienna, Austria, 2013.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.