Article

Anaerobic Biodegradation of Wheat Straw Lignin: The Influence of Wet Explosion Pretreatment

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Abstract: Large amounts of lignin residue is expected in the future when biorefineries for producing biofuels and bio-products will increase in numbers. It is, therefore, valuable to find solutions for using this resource for the sustained production of useful bioenergy or bio-products. Anaerobic digestion could potentially be an option for converting the biorefinery lignin into a valuable energy product. However, lignin is recalcitrant to biodegradation under anaerobic conditions unless the structure is modified. Wet oxidation followed by steam explosion (wet explosion) was previously found to make significant changes to the lignin structure allowing for biodegradation under anaerobic conditions. In this study, we examine the effect of wet explosion pretreatment for anaerobic digestion of wheat straw lignin under mesophilic (37 °C) conditions. Besides the biorefinery lignin produced from wheat straw, untreated lignin was further tested as feed material for anaerobic digestion. Our results showed that wet exploded lignin pretreated with 2% NaOH showed the highest lignin degradation (41.8%) as well as the highest methane potential of 157.3±9.9 ml/g VS. The untreated lignin with no pretreatment showed the lowest methane yield of 65.8±4.8 and only 3.5% of the lignin was degraded. Overall, increased severity of the pretreatment was found to enhance anaerobic degradation of lignin.

Keywords: lignocellulosic materials; anaerobic digestion; wet explosion pretreatment; lignin; cellulose; hemicellulose

1. Introduction

Energy security, as well as greenhouse gas emissions, are some of the greatest global challenges of the 21st century. Moreover, solar, wind, and hydropower are facing challenges of variations in supply as well as storage [1]. Biomass-based bio-refineries are, therefore, emerging steadily over the world as a way to supply security and stability into the renewable energy system. Biomass-based bioenergy production is advantageous over other processes as it can be integrated into the existing energy infrastructure and energy supply chain. Lignocellulosic biomass including wood waste, crop residues, and energy crops can be used for producing different types of biofuels, such as bio-oil, biogas and bioethanol, to improve energy security with concomitant reduction of the dependency on the conventional fossil fuels [2–4].

Currently, the lignocellulosic bio refineries are mainly utilizing the carbohydrate fraction of biomass leaving the lignin fraction behind as a waste material. The paper industries are further only utilizing the cellulose fraction of the biomass for paper production while producing steam or wood pellets from the lignin or discharging this fraction as
a waste [5]. With paper consumption and the number of biomass-based biorefineries on the rise, lignin waste production is increasing. About 79 billion liters of second-generation biofuel is planned for being produced in US during 2022. This will not only be helpful in overcoming the consumption of conventional fossil fuels but will also produce a huge amount of lignin waste (62 million tons) [6]. Therefore, the full use of the whole biomass to generate biofuel will not only improve economics of the process but will also make the process more sustainable [7].

Promising feedstocks for biofuel production are wheat and rice straw, grasses, forestry residues, corn stover, saw dust, cotton stocks, olive pruning, olive stones, and wood chips [5,8]. Wheat straw has major potential because of its abundancy and the fact that it is relatively easy to convert into fermentable sugars [9]. During the processing of wheat, roughly 1.3-1.4 kg of wheat straw is produced from 1 kg of grain [10]. The total annual production of wheat is about 681.92 million tons [11]. Wheat contains ca. 15% lignin, 35–45% cellulose, and 20–30% hemicellulose [12]. Due to its higher carbohydrate content, it is currently being employed for bioethanol production in the agro-industries while the lignin is not being utilized to its potential. The main aim of this paper is to develop a cost effective and simple process to convert this fraction to value-added products in order to boost up the economics of the bioethanol industry.

Currently, different chemical processes are being used for lignin degradation at an industrial scale. Since these chemicals are costly, it affects the overall viability of these processes [13]. Due to the troubled economics associated with chemical lignin conversion, biological processes are receiving attention. In nature, the degradation of lignin is done by bacteria and fungi [14], where fungi are considered to be the most efficient microorganisms for lignin degradation [15]. A drawback of fungal processes is their need for specific temperatures and pH (temperature in the mesophilic range and pH in the acidic range) [16]. Bacteria on the other hand have the potential of being active even under extreme environmental conditions [17].

Most bacterial lignin degradation was studied using aerobic bacteria with the rationale that lignin is recalcitrant under anaerobic conditions. However, a few studies have shown some potential of anaerobic bacteria to degrade lignin under natural conditions [18–20]. Interestingly, some recent laboratory scale studies have shown significant conversion of lignin into biogas after oxidative pretreatment of the material [21,22]. Oxidative pretreatment, therefore, seems to be a promising way to enhance anaerobic digestion of lignin.

Several types of pretreatments were tested for enhancing anaerobic digestion of lignocellulosic materials, including ethanolic ammonia pretreatment [23], hydrothermal pretreatment [24], alkali pretreatment [25,26], acidic pretreatment [27], enzymatic pretreatment [28], and ammonium bicarbonate pretreatment [29]. Even these pretreatments have shown significantly increased biogas production due to lignin solubility during pretreatment. These processes are troubled by the addition of costly chemicals, which further can affect the final use and disposal of the digestate after the AD process [30]. Moreover, these pretreatments did not increase the availability of remaining lignin to the AD microorganisms during the AD process.

The wet explosion process is composed of a wet oxidation process combined with a steam explosion process [31–33]. The wet explosion pretreatment was found effective for enhancing biogas yield from different lignocellulosic materials [31]. This process needs the addition of oxygen or air as the only additive and is, therefore, environmentally friendly. The process further produces negligible amounts of inhibitory compounds during the pretreatment. These processes are troubled by the addition of costly chemicals, which further can affect the final use and disposal of the digestate after the AD process [30]. Moreover, these pretreatments did not increase the availability of remaining lignin to the AD microorganisms during the AD process.

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the degree of methoxylation of the lignin fraction in biomass materials, making it accessible to anaerobic digestion [22]. In this study, we examine the use of wet explosion pretreatment of wheat straw lignin in combination with the addition of NaOH to examine the potential effect on lignin conversion during pretreatment [26]. Anaerobic digestion of lignocellulosic materials is facing the challenge of the low degree of lignin conversion. Hence, this study can significantly contribute to finding ways of overcoming this challenge.

2. Materials and Methods

2.1. Materials

The wheat straw and clarified manure used in the study were collected from 5D farms Pasco USA. The wheat straw samples were air dried at ambient conditions for 15 days to remove moisture and then ground to 10 mm particle size before wet explosion pretreatment. The clarified manure is manure water collected from a screw press installed at 5D farms to remove manure fibers for use as bedding at the farm. The clarified manure was stored at 4 °C after collection to mitigate any changes.

2.2. Wet Explosion Pretreatment and Enzymatic Hydrolysis

The pretreatment of the ground wheat straw was done in a 100 L wet explosion pilot reactor. The pretreated material was subjected to enzymatic hydrolysis to remove the carbohydrate fraction as sugars. Enzymatic hydrolysis of the pretreated samples was performed over 90 hours at pH 5 and 50°C in an 8 L Applikon reactor by the addition of cellulolytic enzymes: Cellic®CTec2:27.8 mg protein/g glucan and Cellic®HTec2;10% of CTec2 (Novozymes, USA). The resulting lignin fraction ligninW1 was recovered by water washing of the final residue followed by solid–liquid separation to ensure that all sugars were removed. Lignin W1 was further pretreated at 220 °C for 10 minutes under different concentrations (0, 1, and 2%) of NaOH to produce lignin W2, lignin W3 and lignin W4. All the pretreatment conditions for the lignin samples are shown in Table 1.

| Lignin Type | Temperature (°C) | Time (min) | Oxygen (%) | NaOH (%) | D.M (%) |
|-------------|------------------|------------|------------|----------|---------|
| Lignin W1   | 190              | 25         | 7.5        | 0        | 25      |
| Lignin W2   | 220              | 10         | 4          | 0        | 15      |
| Lignin W3   | 220              | 10         | 4          | 1        | 15      |
| Lignin W4   | 220              | 10         | 4          | 2        | 15      |

2.3. Semi-Continuous Anaerobic Digestion

The pretreated and untreated lignin samples were anaerobically digested in five 2 L semi-continuous anaerobic digestion systems using clarified manure as inoculum. The feedstock was prepared by mixing 65% of the pretreated lignin with 35% of clarified manure. The anaerobic digestion was carried out in five glass bioreactors under mesophilic conditions (37 °C). The AD experiment was conducted using a hydraulic retention time of 15 days and 60 ml of the feed material was fed to each of the reactors on a daily basis at a fixed time. The methane production from all the reactors was also measured on a daily basis at a fixed time using the water displacement method. As well as the wheat straw lignin, a similar bioreactor was further started using untreated lignin as a feed material to compare the performance of the biorefinery lignin, which still contains some cellulose in addition to lignin with a pure lignin stream.

2.4. TS/VS and Compositional Analysis

The TS and VS of the undigested and digested samples were measured by gravimetric analysis [32].
For compositional analysis, the pretreated and untreated lignin feed along with the effluent samples were dried at 37 °C for 48 h in an oven to attain a moisture content of below 10% as per the NREL standards [34,35]. A total of 0.3 g of the thoroughly dried samples were solubilized in 3 mL; 72% (weight basis) sulfuric acid at 30 °C in water bath. The samples were then autoclaved at 121 °C for 1 h to ensure dilute acid hydrolysis (4% weight basis) [34,36]. Two-step hydrolysis was performed on feed and effluent samples for measuring lignin and the total carbohydrate content of all the samples. A UV spectrophotometer (Jenway 6405 UV/Visible, NJ, USA) was used to measure the acid-soluble lignin contents of the samples at a wavelength of 320 nm. The carbohydrate contents of the lignin samples were measured using an ultimate 3000 HPLC system. Carbohydrate and lignin contents of the samples were measured in triplicates.

2.5. Gas Analysis

The biogas composition was analyzed on a daily basis using a mass spectrophotometer Universal Gas Analyzer, UGA series (Stanford Research Systems, Sunnyvale, CA, USA). The methane contents of biogas were measured by taking a sample in a syringe then the composition was examined by connecting the syringe with the gas analyzer.

2.6. pH and VFA Analysis

The stability of all the reactors was monitored by measurements of pH and the concentration of volatile fatty acid (VFA). An Oakton pH-510 pH analyzer was used for pH analysis and VFA concentration was analyzed by taking 2 mL of the effluent sample after centrifuging for 15 min at 10,000 rpm. The supernatant of the centrifuged sample was collected using a 3 mL syringe and subsequently diluting the sample 2 times using 4 mM of sulfuric acid. The diluted sample was then filtered and VFA concentration was determined by using an UltiMate® 3000 HPLC system (Dionex, Sunnyvale, CA, USA) with an Aminex® 87 H column 250 × 4.6 mm (Bio-Rad, Hercules, CA, USA) and a Shodex RI-101 refractive index detector as previously described [37].

3. Results and Discussion

3.1. Compositional Analysis

The composition of feed and effluent samples was analyzed after the completion of every hydraulic retention time of bioreactor operations and the average results of all retention times are presented in Table 2. It is evident from the table that a major fraction of the organic matter in all lignin feed stock samples are composed of lignin. The highest lignin content was found in untreated lignin, i.e., 97.1%. It was found that 93.5% of the total lignin in the untreated lignin was composed of acid insoluble lignin and only 3.6% of the total lignin was composed of acid soluble lignin. Carbohydrate contents in the untreated lignin was found to be lowest in comparison to the other feed stocks, i.e., 2.9%. The lignin contents in the samples after both pretreatments, i.e., lignin W1, lignin W2, lignin W3, and lignin W4 were found to be 66.4, 67.9, 68.8, and 70.8%, respectively, where the acid insoluble lignin contents were found to be 93.8, 93.5, 93.3, and 93.2% of the total lignin in lignin W1, lignin W2, lignin W3, and lignin W4, respectively. It was also observed that the acid soluble lignin increased with increased concentrations of NaOH during pretreatment. The highest acid soluble lignin of 6.71% of the total lignin was found in lignin W4 and the lowest acid soluble lignin was found in untreated lignin with 4.2% of the total lignin. The acid insoluble lignin is the lignin having a molecular weight higher than 850 kD, which needs pretreatment before it can be degraded by anaerobic bacteria [7]. Carbohydrate contents of lignin in lignin W1, lignin W2, lignin W3, and lignin W4 was found to be 33.6, 32.1, 31.2, and 38.7, respectively.

The lignin to carbohydrates ratio of all the feed materials, i.e., untreated lignin, lignin W1, lignin W2, lignin W3, and lignin W4 was found to be 33.4, 1.9, 2.1, 2.2, and 2.4, respectively. The highest lignin to carbohydrates ratio was observed in untreated lignin with a
value of 33.4 indicating that the lignin content in untreated lignin was far higher than in pretreated wheat straw. The lignin to carbohydrates ratio of the effluents show that wet exploded lignin with the highest decrease is lignin W4 with 2% NaOH added during the pretreatment. This indicate that lignin W4 had the highest consumption of lignin into biogas, while the high lignin content in untreated lignin indicates that there was consumption of lignin into biogas in this sample. The addition of 2% NaOH during the pretreatment is expected to decrease the molecular weight of the lignin fragments, which allows the AD process to get access to the lignin in accordance with the study conducted by Rodriguez et al. [38].

| Feed Stock | Sample Type | Carbohydrates (% of VS) | Acid Soluble Lignin (% of VS) | Acid Insoluble Lignin (% of VS) | Lignin: Carbohydrates Ratio |
|------------|-------------|--------------------------|-----------------------------|-----------------------------|-----------------------------|
| Raw Lignin | Feed        | 2.9 ± 0.7                | 3.6 ± 0.15                  | 93.5 ± 1.1                  | 33.4 ± 2.1                  |
|            | Effluent    | 1 ± 0.4                  | 3.1 ± 0.11                  | 95.9 ± 1.2                  | 99 ± 1.7                    |
| Lignin W1  | Feed        | 33.6 ± 0.8               | 4.1 ± 0.13                  | 62.3 ± 0.8                  | 1.9 ± 0.2                   |
|            | Effluent    | 31.2 ± 0.2               | 3.4 ± 0.2                   | 65.4 ± 0.6                  | 2.2 ± 0.1                   |
| Lignin W2  | Feed        | 32.1 ± 0.6               | 4.4 ± 0.1                   | 63.5 ± 1                   | 2.1 ± 0.09                  |
|            | Effluent    | 34.4 ± 0.3               | 3.4 ± 0.1                   | 62.2 ± 0.7                  | 1.9 ± 0.1                   |
| Lignin W3  | Feed        | 31.2 ± 0.5               | 4.6 ± 0.12                  | 64.2 ± 0.8                  | 2.2 ± 0.4                   |
|            | Effluent    | 34.7 ± 0.1               | 3.3 ± 0.2                   | 62 ± 1.2                    | 1.9 ± 0.3                   |
| Lignin W4  | Feed        | 29.2 ± 0.6               | 4.8 ± 0.3                   | 66 ± 0.6                    | 2.4 ± 0.5                   |
|            | Effluent    | 38.7 ± 0.4               | 3.2 ± 0.1                   | 58.1 ± 0.4                  | 1.6 ± 0.2                   |

3.2. Methane Potential of the Feed Tocks

The methane production from the different samples is shown in Figure 1. The average methane yield for the first 30 days for untreated lignin, lignin W1, lignin W2, lignin W3 and lignin W4 was found to be 45.4 ± 20.2, 59.5 ± 30.9, 65.6 ± 35.2, 76.8 ± 41.4 and 87.9 ± 52 mL/gVS, respectively. After 30 days, methane yield stabilized in all bioreactors. Average methane yield during the 31st to 62nd days for untreated lignin, lignin W1, lignin W2, lignin W3 and lignin W4 was found to be 65.8 ± 4.8, 94.7 ± 5.6, 122.9 ± 6.2, 137.4 ± 7.9 and 157.3 ± 9.9 mL/gVS, respectively. Overall, the lignin which underwent wet explosion pretreatment yielded a higher methane yield in comparison to the untreated lignin. Lignin W1, lignin W2, lignin W3, and lignin W4 yielded 43.7, 86.7, 108.6, and 138.8% extra methane in comparison to the untreated lignin. The secondary wet exploded lignin further produced higher methane production as compared to the primary wet exploded lignin. The secondary wet exploded lignin W2, lignin W3, and lignin W4 produced 29.8, 45.1, and 66.3% extra methane in comparison to the one with only the primary wet explosion pretreatment (lignin W1). The reason that the wet explosion pretreatment yielded higher quantities of methane in comparison to the untreated lignin can be explained by the fact that parts of the lignin and the hemicellulose fractions of the biomass samples solubilized during the pretreatment making these parts accessible to anaerobic bacteria [7]. Moreover, the feed samples pretreated by the addition of alkali during the secondary wet explosion yielded more methane in comparison to the ones pretreated without a base addition. Lignin W4 and W5 yielded 11.7 and 27.9% extra methane in comparison to the lignin W2 without the addition of base during the pretreatment. Lignin W5 pretreated with the addition of 2% NaOH resulted in the production of 14.7% extra methane in comparison to lignin W4 with a lower concentration of 1% NaOH.

The results of this study are comparable with Khan and Ahring, (2020) [21], but the overall methane yield from the lignin under mesophilic conditions is 28% lower compared to using thermophilic conditions. Raw lignin has further not been studied previously for conversion into methane and it is obvious that the higher lignin and lower carbohydrate
content compared to biorefinery lignin had a significant negative influence on the methane yield.

Figure 1. Methane yield from all feed stocks.

3.3. Stability of the AD Process

The stability of the AD process was monitored continuously throughout the whole study by measurements of pH and VFA as shown in Table 3. As shown, the concentration of VFAs is significantly higher in pretreated lignin samples in comparison to untreated lignin. Moreover, it is also evident that the concentration ofVFAs in secondary wet exploded lignin samples are higher compared to the primary wet exploded lignin samples. This can be explained by the fact that during wet explosion pretreatment of lignocellulosic materials, lignin is depolymerized with simultaneous degradation of the side chains of the acetyl groups of hemicellulose [39]. The VFA concentration was reduced in all the effluent samples compared to the feed material due to the consumption by anaerobic bacteria and archaea during AD. Table 3 shows the VFA concentration of the samples during the highest/lowest methane yield of the bioreactors after stabilization. The untreated lignin produced the smallest amount of methane after 26 days of incubation, i.e., 62 ml/g VS. The VFA analysis of the samples showed considerably higher concentration of VFA and lower pH, i.e., 6.95 of the samples on that day. On the other hand, the VFA concentration was found to be the lowest on the day when the reactor yielded the highest amount of methane, i.e., on the 56th day of the experiment where the pH was found to be 7.67. Similarly, lignin W1, W2, W3, and W4 produced the highest methane yield on the 51st, 50th, 38th, and 46th day of the experiment where the VFA concentration was found to be lowest in all reactors. The pH of these reactors remained above 7, even during the lowest methane yield. The pH of the reactors was found to be higher on the day of the highest methane yield compared to the day when the reactors produced the lowest methane after stabilization. The pH of lignin W1, W2, W3, and W4 was found to be 7.63, 7.68, 7.7, and 7.7, respectively, on the day when they yielded the highest methane.
Table 3. VFA concentration and pH of the feed and effluent samples.

| Feed Stock | Sample Type       | Acetic Acid (g/L) | Propionic Acid (g/L) | Isobutyric Acid (g/L) | Butyric Acid (g/L) | Isovaleric Acid (g/L) | Valeric Acid (g/L) | Isocaproic Acid (g/L) | Hexanoic Acid (g/L) | Heptanoic Acid (g/L) | Formic Acid (g/L) | pH   |
|------------|-------------------|-------------------|----------------------|----------------------|-------------------|----------------------|-------------------|----------------------|---------------------|---------------------|-------------------|------|
| Raw Lignin | Feed              | 0.3               | 0.4                  | 0                    | 0.2               | 0                    | 0.1               | 0                    | 0                   | 0                   | 0                 | 7.59 |
|            | (Effluent)$_{\text{max CH}_4}$ | 0.1               | 0.2                  | 0                    | 0.1               | 0                    | 0                 | 0                    | 0                   | 0                   | 0                 | 7.67 |
|            | (Effluent)$_{\text{min CH}_4}$ | 0.2               | 0.3                  | 0                    | 0.1               | 0                    | 0                 | 0.1                  | 0                   | 0                   | 0                 | 6.95 |
| Lignin W1  | Feed              | 2.5               | 2                    | 0.1                  | 1.7               | 0                    | 0                 | 0.2                  | 0                   | 0                   | 0                 | 7.55 |
|            | (Effluent)$_{\text{max CH}_4}$ | 0.7               | 0.4                  | 0                    | 0.5               | 0                    | 0                 | 0                    | 0                   | 0                   | 0                 | 7.63 |
|            | (Effluent)$_{\text{min CH}_4}$ | 0.9               | 0.7                  | 0.1                  | 0.8               | 0                    | 0.1               | 0                    | 0.1                 | 0                   | 0                 | 7.41 |
| Lignin W2  | Feed              | 3.6               | 2.2                  | 0.2                  | 2                 | 0                    | 0                 | 0.3                  | 0                   | 0                   | 0                 | 7.44 |
|            | (Effluent)$_{\text{max CH}_4}$ | 0.9               | 0.3                  | 0                    | 0.7               | 0                    | 0                 | 0                    | 0                   | 0                   | 0                 | 7.68 |
|            | (Effluent)$_{\text{min CH}_4}$ | 1.2               | 0.9                  | 0.1                  | 1                 | 0                    | 0.1               | 0                    | 0.2                 | 0.1                 | 0                 | 7.46 |
| Lignin W3  | Feed              | 3.8               | 2.7                  | 0.5                  | 2.3               | 0                    | 0                 | 0                    | 0.4                 | 0                   | 0                 | 7.41 |
|            | (Effluent)$_{\text{max CH}_4}$ | 0.7               | 0.6                  | 0                    | 0.8               | 0                    | 0                 | 0                    | 0.1                 | 0                   | 0                 | 7.7  |
|            | (Effluent)$_{\text{min CH}_4}$ | 1                  | 1.2                  | 0.3                  | 1                 | 0                    | 0                 | 0.1                  | 0                   | 0                   | 0                 | 7.5  |
| Lignin W4  | Feed              | 4.3               | 2.8                  | 0.3                  | 2.5               | 0                    | 0                 | 0                    | 0.3                 | 0                   | 0                 | 7.35 |
|            | (Effluent)$_{\text{max CH}_4}$ | 0.8               | 0.5                  | 0                    | 0.3               | 0                    | 0                 | 0                    | 0                   | 0                   | 0                 | 7.7  |
|            | (Effluent)$_{\text{min CH}_4}$ | 1.2               | 1                    | 0.2                  | 1.2               | 0                    | 0.1               | 0                    | 0.1                 | 0                   | 0                 | 7.3  |
3.4. Biodegradability of Substrate

The composition of undigested and digested lignin samples was analyzed to understand what fractions of the lignin samples were consumed by anaerobic bacteria during the anaerobic digestion process. The VS destruction of all the different feed materials during AD are shown in Figure 2. The VS analysis of the effluent showed that the VS reduction in the untreated lignin, lignin W1, W2, W3, and W4 was 9.1%, 18.4%, 23.9%, 29%, and 36.9%, respectively. The results clearly revealed that VS reduction increased after pretreatment and the increase was dependent on the severity of the pretreatment. The highest volatile solids degradation was observed in lignin W4 with 36.9% degradation followed by lignin W3, W2, W1, and untreated lignin. The wet exploded samples showed higher volatile solids degradation in comparison to untreated sample. The secondary wet exploded lignin with 2% NaOH showed the highest VS reduction as far higher than the primary wet exploded lignin W1 and the secondary wet exploded lignin W2 and W3. Lignin W4 with 2% NaOH showed 21.2, 35.3, 50, and 75.3% more VS degradation compared to lignin W3, W2, W1 and untreated lignin, respectively. Previous work has shown that wet explosion further increased the methoxylation of lignin leading to enhanced volatile solids destruction [21]. In this study, we demonstrate that the addition of NaOH further depolymerized lignin into smaller fragments, which again increased the degradation of lignin [38]. Moreover, the wet explosion pretreatment improved the methoxylation of lignin, which makes it easy to degrade under anaerobic conditions [40].

![Figure 2. Volatile solids contents of the feed and effluent sample.](image)

3.5. Biogas Composition

The methane content of the biogas from the bioreactors are shown in Figure 3. The average methane percentage after stabilization of untreated lignin, lignin W1, lignin W2, lignin W3, and lignin W4 was found to be 55.9 ± 0.8, 55.1 ± 0.8, 54.6 ± 1, 54.2 ± 1, and 53.8 ± 1, respectively. The methane contents in lignin W4 was found to be the lowest as compared to the other samples, which follows the fact that this sample has the highest carbon conversion, and that lignin produces more carbon dioxide relative to methane.
4. Conclusions

Our study showed that the lignin produced by the biorefinery industry, such as the cellulosic ethanol industry, can be used as a feedstock for anaerobic digestion after wet explosion pretreatment to produce sugars for bioethanol production. The wet explosion pretreatment can increase the biodegradability of lignin and by adding alkali as part of the process, more lignin can be converted to methane. The highest alkali concentration tested was (2%) (sample W4), and a total of 41.8% of the lignin was degraded during anaerobic digestion. This resulted in 138.8% more methane compared to the untreated lignin.

Author Contributions: M.U.K.: Conceptualization, formal analysis, investigation, data curation, writing—original draft; B.K.A. Conceptualization, investigation, supervision, reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by CAHNRS App A program under the project with the title: An Integrated process for Increased Bioenergy production and Nutrient Recovery during AD. PG 00017118.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data collected for this study will be made available upon request from the corresponding authors.

Acknowledgments: We thank Philip Teller for performing the pretreatment of the lignin samples.

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

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