Evaluation on the Methane Production Potential of Wood Waste Pretreated with NaOH and Co-Digested with Pig Manure

Renfei Li 1,2, Wenbing Tan 1,2, Xinyu Zhao 1,2, Qiuling Dang 1,2, Qidao Song 3, Beidou Xi 1,2,* and Xiaohui Zhang 1,2,*

1 State Key Laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China; lirenfei1114@163.com (R.L.); wenbingtan@126.com (W.T.); zhaoxinyu1126@126.com (X.Z.); dangling819@126.com (Q.D.)
2 State Environmental Protection Key Laboratory of Simulation and Control of Groundwater Pollution, Chinese Research Academy of Environmental Sciences, Beijing 100012, China
3 Institute of Scientific and Technical Information Catas, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China; tanliu2009@126.com
* Correspondence: wbtann@126.com (B.X.); baixue215@163.com (X.Z.); Tel.: +86-10-8491-3133 (B.X.)

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Abstract: Wood waste generated during the tree felling and processing is a rich, green, and renewable lignocellulosic biomass. However, an effective method to apply wood waste in anaerobic digestion is lacking. The high carbon to nitrogen (C/N) ratio and rich lignin content of wood waste are the major limiting factors for high biogas production. NaOH pre-treatment for lignocellulosic biomass is a promising approach to weaken the adverse effect of complex crystalline cellulosic structure on biogas production in anaerobic digestion, and the synergistic integration of lignocellulosic biomass with low C/N ratio biomass in anaerobic digestion is a logical option to balance the excessive C/N ratio. Here, we assessed the improvement of methane production of wood waste in anaerobic digestion by NaOH pretreatment, co-digestion technique, and their combination. The results showed that the methane yield of the single digestion of wood waste was increased by 38.5% after NaOH pretreatment compared with the untreated wood waste. The methane production of the co-digestion of wood waste and pig manure was higher than that of the single digestion of wood waste and had non-significant difference with the single-digestion of pig manure. The methane yield of the co-digestion of wood waste pretreated with NaOH and pig manure was increased by 75.8% than that of the untreated wood waste. The findings indicated that wood waste as a sustainable biomass source has considerable potential to achieve high biogas production in anaerobic digestion.

Keywords: wood waste; biofuel; lignocellulosic biomass; NaOH pretreatment; anaerobic co-digestion

1. Introduction

Wood is a natural, renewable, and recyclable green material and bioenergy source. Under the circumstance of increasing depletion of non-renewable energy and material, getting the utmost out of wood is increasingly important. In general, almost 50% of a tree can be converted to the final products, and the rest remain as wood waste (WW) [1]. WW mainly consist of the residues from tree felling and processing, as well as discarded furniture and building materials [2]. Among these processes, sawmills account for 40–60% of the total WW generation [3,4]. The total amount of China’s WW is estimated to be 30.28 million tons in 2013 [5]. However, to date, only a minority of WW can be used for recycling and reusing, and an effective method to fully utilize these solid wastes has not been developed. Currently, the main treating options for WW are incineration for heating, thermal power
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generation, and heat recovery due to its high calorific value. However, direct incineration for energy conversion is inefficient, and large amounts of greenhouse gases and volatile organic compounds can be released during the incineration, especially in small boilers or combustion chambers that often lack emission control systems. These emissions may cause serious environmental pollution.

The biofuels produced from lignocellulosic materials (e.g., wood and agricultural crops) are a green, sustainable, renewable energy, and biofuel production has become a Chinese national strategy [6]. Anaerobic digestion offers an attractive option for the utilization of WW for biogas production [7]. Nevertheless, a high carbon-to-nitrogen (C/N) ratio and high crystalline cellulose structure make it difficult for WW to continuously and efficiently yielding biogas, which limits its large-scale application in anaerobic digestion technology [8].

Anaerobic co-digestion (AcoD) is a promising optimization technique in biogas production. This process lies in balancing the nutrients, bacterial diversity, pH, toxic compounds, and dry matter in different substrates to achieve a yield-increasing effect of CH₄ [9–11]. The methane yield of different substrates can be remarkably elevated via the AcoD technique compared with single-substrate digestion [12]. Given the ability of the intrinsic characteristics of animal manures (low C/N ratio and high NH₄–N content) to complement those of lignocellulosic biomass (high C/N ratio and rich lignocellulosic content) in digestion, mixing animal manures and lignocellulosic biomass (e.g., wood and straw) in AcoD to achieve production improvement for the biogas yield of different substrates is a common practice. For instance, Li et al. [6] recommended applying rice straw (RS) to pig manure (PM) in a (volatile solid) VS ratio of 1:1 or 1:2 can evidently increase biomethane production.

The high crystalline cellulosic structure is a substantial factor limiting the enzymatic degradation of lignocellulosic biomass during digestion [13]. To break down the linkages between lignin monomers or between lignin and polysaccharides to obtain lignocellulosic biomass, which is readily hydrolyzed, many pretreatment approaches have been established and normally categorized into three types: chemical (i.e., alkaline, acidic, and inorganic salts), physical (i.e., microwaves and liquid hot water), and biological (enzymatic and fungal). Within the pretreatment categories, NaOH pretreatment has been extensively applied to optimize biogas production of a wide range of lignocellulosic biomass [14].

Considering that the successful application of WW in anaerobic digestion to produce biomethane possibly brings considerable benefits because such feedstock is an abundant, cheap, and sustainable alternative, the utilization potentiality and optimizing strategy of WW in biogas production has to be evaluated. Although some studies have investigated the performance of biogas production from wood in anaerobic digestion [7,15,16], the information of the potential and optimizing strategy for WW to achieve high biogas production is still limited. For example, the optimizing strategy used for improving the biogas production of WW were scarce, and the comparisons of the improvements of biogas production between WW and conventional digestion substrates (e.g., lignocellulosic biomass and animal manure) under the same optimizing strategy, which can be used for more effectively assessing the optimization effect of biogas production of WW, were also lacking in previous studies. This study aimed to assess the performance and potential of methane production from WW in anaerobic digestion. The aim was achieved by (1) evaluating the disparity in biomethane productions between WW and the conventional digestion substrates (RS for lignocellulosic biomass and PM for animal manure), and (2) evaluating the improvement of biomethane production of WW under the effect of different optimizing strategies (NaOH pretreatment, AcoD technique, and their combination).

2. Results

2.1. Daily and Cumulative Methane Production from Different Substrates

Figure 1 presents the daily and cumulative methane yields with time for each digestion type. The maximum daily CH₄ yields of the single-substrate digestions of PM, WW, and RS were 14.2 (day 1), 17 (day 1), and 21.2 mL CH₄/g VS (day 2), respectively, during the 49 d of incubation (Figure 1a). After the production peak, the daily methane yield of WW was sharply decreased to approximately 2.6 mL
CH4/g VS and then remained constant (Figure 1a). Although the overall trend of daily methane yields of PM and RS dropped observably during the single-substrate digestions, some brief rise and fall during the incubation period were also observed (Figure 1a). After the maximum production peak, the daily methane yield of PM temporarily increased at days 10 to 12, 14 to 17, and 22 to 27. The daily methane yield of RS temporarily increased at days 10 to 15, 24 to 25, and 26 to 27 (Figure 1a). The mean cumulative methane yield of the single-digestion of WW was 175.81 mL CH4/g VS, which was significantly \((p < 0.05)\) lower than that of the single-digestion of PM (245.09 mL CH4/g VS) and RS (252.19 mL CH4/g VS) (Figure 1b). The average pH values of the single-digestions of WW, PS, and PM ranged from 7.5 to 8.8, 6.6 to 8.7, and 5.5 to 8.1, respectively (Figure 1c).

![Figure 1](image-url)

**Figure 1.** Daily (a) and cumulative (b) methane production and pH (c) of the single-digestions from different substrates. PM: pig manure, WW: wood waste, RS: rice straw. Different lowercase letters in the inset indicate a significant difference \((p < 0.05)\) between the methane yields of different raw materials. For the calculation of methane volume, \(P\) and \(T\) was 1 standard atmospheric pressure and \(25\ ^\circ\text{C}\), respectively.
2.2. Effects of NaOH Pretreatment on the Daily and Cumulative Methane Production from Different Substrates

After NaOH pretreatment, the maximum daily methane yields of WW and RS were increased from 17 CH₄/g VS to 19.1 mL CH₄/g VS (day 2) and from 21.2 CH₄/g VS to 22.2 mL CH₄/g VS (day 2), respectively (Figure 2a,c). Two mean production peaks were observed for the daily methane yield of WW on days 2 (19.1 mL CH₄/g VS) and 25 (6.32 mL CH₄/g VS) after NaOH pretreatment (Figure 2a). After the maximum production peak, the daily methane yield of WW temporarily increased at days 23 to 26 (Figure 2a). Three main production peaks were observed for the daily methane yield of RS on days 2 (22.2 mL CH₄/g VS), 14 (7.32 mL CH₄/g VS), and 30 (6.78 mL CH₄/g VS) after NaOH pretreatment (Figure 2a). After the maximum production peak, the daily methane yield of WW temporarily increased at days 8 to 14 and 26 to 30 (Figure 2a). Moreover, the daily methane yields of WW and RS after pretreated with NaOH were significantly ($p < 0.05$) higher than that of the untreated WW and RS, respectively (Figure 2a,c). These results showed that NaOH pretreatment could evidently increase the methane production of WW and RS in the single-substrate digestion process.

![Figure 2](image)

Figure 2. Daily (a,c) and cumulative (b,d) methane production and pH (e,f) of the single-digestions from WW and RS before and after NaOH pretreatment. PM: pig manure, WW: wood waste, RS: rice straw, WW+NaOH: WW with NaOH pretreatment, RS+NaOH: RS with NaOH pretreatment. Different lowercase letters in the inset indicate a significant difference ($p < 0.05$) between the methane yields of different raw materials.

The mean cumulative methane yield of the single digestion of WW was 243.53 mL CH₄/g VS after the NaOH pretreatment, which nearly reached the average cumulative methane yield of the single-digestion of PM (245.09 mL CH₄/g VS) (Figure 2b). When pretreated with NaOH, the mean
cumulative methane yield of RS was increased from 252.19 to 282.94 mL CH$_4$/g VS (increased by 12.2%), which was remarkably higher than the mean cumulative methane yield of PM (Figure 2d). Moreover, the mean pH values of the single-digestions of WW and RS with NaOH pretreatment ranged from 6.5 to 8.9 and 6.8 to 8.7, respectively (Figure 2e,f).

2.3. Daily and Cumulative Methane Production from Different Co-Digestion Types

Three production peaks (17.8, 6.21, and 5.87 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of WW and PM on days 1, 17, and 29 (Figure 3a). After the maximum production peak, the daily methane yield of the co-digestion of WW and PM temporarily increased at days 6 to 7, 13 to 16, and 22 to 29 (Figure 3a). Four production peaks (23, 9.4, 5.75, and 6.34 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of RS and PM on days 2, 17, 28, and 41 (Figure 3c). After the maximum production peak, the daily methane yield of the co-digestion of RS and PM temporarily increased at days 1 to 2, 11 to 17, 24 to 28, and day 37 to 41 (Figure 3c).

The average cumulative methane yield of the co-digestion of WW and PM was 234.88 mL CH$_4$/g VS, which was increased by 33.6% compared with the single-digestion of WW (Figure 4) and nearly
reached the mean cumulative methane yield of the single-digestion of PM (Figure 3b). As shown in Figure 3d, the cumulative methane yield of the co-digestion of RS and PM was remarkably ($p < 0.05$) higher than that of the single-digestions of both RS and PM. The average cumulative methane yield of the co-digestion of RS and PM was 331.12 mL CH$_4$/g VS, which was increased by 31.3% than the single-digestions of RS (Figure 4). The pH values of the co-digestion of PM and WW and the co-digestion of PM and RS ranged from 6.8 to 8.5 and 6.7 to 8.2, respectively (Figure 3e,f).

Three production peaks (20.5, 7.31, and 9.11 mL CH$_4$/g VS) were observed for the daily methane yield of WW (pretreated with NaOH) and PM on days 2, 17, and 28 (Figure 5a). Four production peaks (23.9, 9.14, 7.77, and 6.82 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of WW (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14 to 17, and 22 to 28 (Figure 5a). Four production peaks (23.9, 9.14, 7.77, and 6.82 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM on days 2, 14, 30, and 41 (Figure 5c). After the maximum production peak, the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 11 to 14, 25 to 30, and 37 to 41 (Figure 5c).

The average cumulative methane yield of the co-digestion of WW (pretreated with NaOH) and PM was 309.06 mL CH$_4$/g VS, which was increased by 75.8% compared with that of the single-digestion of WW (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14 to 17, and 22 to 28 (Figure 5a). Four production peaks (23.9, 9.14, 7.77, and 6.82 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14, 30, and 41 (Figure 5c). After the maximum production peak, the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 11 to 14, 25 to 30, and 37 to 41 (Figure 5c).

The average cumulative methane yield of the co-digestion of WW (pretreated with NaOH) and PM was 309.06 mL CH$_4$/g VS, which was increased by 75.8% compared with that of the single-digestion of WW (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14 to 17, and 22 to 28 (Figure 5a). Four production peaks (23.9, 9.14, 7.77, and 6.82 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14, 30, and 41 (Figure 5c). After the maximum production peak, the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 11 to 14, 25 to 30, and 37 to 41 (Figure 5c).

The average cumulative methane yield of the co-digestion of WW (pretreated with NaOH) and PM was 309.06 mL CH$_4$/g VS, which was increased by 75.8% compared with that of the single-digestion of WW (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14 to 17, and 22 to 28 (Figure 5a). Four production peaks (23.9, 9.14, 7.77, and 6.82 mL CH$_4$/g VS) were observed for the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 14, 30, and 41 (Figure 5c). After the maximum production peak, the daily methane yield of the co-digestion of RS (pretreated with NaOH) and PM temporarily increased at days 1 to 2, 11 to 14, 25 to 30, and 37 to 41 (Figure 5c).

The pH values of the co-digestion of PM and WW (pretreated with NaOH) and the co-digestion of PM and RS (pretreated with NaOH) ranged from 6.3 to 8.6 and 6.4 to 8.2, respectively (Figure 3e,f).
Figure 5. Daily (a,c) and cumulative (b,d) methane production and pH (e,f) of the co-digestions from WW and RS with PM after pretreatment with NaOH. WW + NaOH: WW with NaOH pretreatment, RS + NaOH: RS with NaOH pretreatment, (WW + NaOH) + PM: the AcoD of PM and WW (pretreated with NaOH), (RS + NaOH) + PM: the AcoD of PM and RS (pretreated with NaOH). Different lowercase letters in the inset indicate a significant difference ($p < 0.05$) between the methane yields of different raw materials.

3. Discussion

RS and WW belonging to lignocellulosic biomass are normally composed of three major components, namely polysaccharide cellulose and hemicellulose and the aromatic non-polysaccharide lignin. The higher the lignin content in WW, the tighter the physical structure of WW than RS (Table 1) [8]. Thus, the affinity of internal cellulose and hemicellulose of WW to hydrolase was relative weak. Maintaining the pH close to neutral (6.8–7.2) is preferable for methanogenesis, whereas the optimal pH for hydrolysis and acidogenesis is within the range of 5.5–6.5 [17,18]. At the initial stage of digestion, the accumulation of organic acids produced by hydrolysis and acidogenesis was difficult due to the low affinity of wood chips to hydrolase, thus the system pH cannot be lowered (Figure 1c). The high pH value in turn suppressed the activities of hydrolysis and acidogenesis, as well as methanogenesis of the single digestion of WW. Therefore, the methane production of RS was higher than WW during the single-substrate digestion process (Figure 1b).
Table 1. Characteristics of WW, RS, PM and inoculum.

| Parameter     | Inoculum | PM          | WW          | WW + NaOH   | RS          | RS + NaOH   |
|---------------|----------|-------------|-------------|-------------|-------------|-------------|
| TS (%)        | 18.9     | 18.1 ± 0.2  | 96.2 ± 0.6  | NA          | 92.5 ± 0.5  | NA          |
| VS (%)        | 9.8      | 9.4 ± 0.1   | 79.8 ± 0.6  | NA          | 84.4 ± 0.7  | NA          |
| Cellulose (%) | NA       | 23.6 ± 0.4  | 39.9 ± 0.3  | 41.2 ± 0.3  | 30.3 ± 1.2  | 33.7 ± 1.4  |
| Hemicellulose (%) | NA | 21.7 ± 1.2  | 36.7 ± 1.5  | 50.9 ± 1.6  | 29.2 ± 1.5  | 40.3 ± 1.8  |
| Lignin (%)    | NA       | 8.4 ± 0.1   | 13.4 ± 0.7  | 3.3 ± 0.2   | 5.4 ± 0.4   | 1.24 ± 0.2  |
| Ash (%)       | NA       | 6.9 ± 0.2   | 17.8 ± 0.3  | NA          | 15.7 ± 0.3  | NA          |
| TC (%)        | 32.2     | 26.2 ± 0.3  | 37.1 ± 0.4  | 39.1 ± 0.4  | 34.3 ± 0.5  | 36.4 ± 0.4  |
| TN (%)        | 1.4      | 1.3 ± 0.1   | 0.7 ± 0.1   | 1.4 ± 0.1   | 0.7 ± 0.1   | 1.3 ± 0.1   |
| C/N           | 23.5     | 20          | 53.5        | 27.2        | 47          | 29.1        |

Notes: NA: not analyzed; PM: pig manure, WW: wood waste, RS: rice straw. WW + NaOH: WW pretreated with NaOH, RS + NaOH: RS pretreated with NaOH.

Salehian et al. [7] found that the yield of methane produced from pine wood pretreated with 8% NaOH (100 °C for 10 min) was increased from 65 mL CH\textsubscript{4}/g VS to 178.2 mL CH\textsubscript{4}/g VS after 45 days of incubation. Similarly, Mirahmadi et al. [15] observed a 50% enhancement in methane production after the 7% NaOH pretreatment (100 °C for 2 h) of birch. Though under different conditions of alkaline pretreatment, the yield of methane in the current work of eucalyptus was also remarkably elevated from 175.81 to 243.53 mL CH\textsubscript{4}/g VS after NaOH pretreatment (Figure 2b). The complex structures of cellulose, hemicellulose, and lignin are difficult for microorganisms to degrade [19]. The daily and cumulative methane production of the single-digestions of WW and RS was evidently increased by NaOH pretreatment (Figure 2). This result was because alkali can break down the ether bonds between lignin and saponified the ester bonds between hemicellulose and lignin to weaken the internal hydrogen bonds within cellulose and hemicellulose [14], thus making cellulose and hemicelluloses accessible to hydrolytic enzymes. The NaOH pretreatment decreased the C/N ratio of WW and RS to a low level from 54.5 and 47 to 27.2 and 29.1, respectively, which was more acceptable for biodegradation over the first several days (Table 1) [20]. Notably, the improvement of methane production of the single digestion of WW (increased by 38.5%) by NaOH pretreatment was significantly higher ($p < 0.05$) than that of RS (increased by 12.2%) (Figure 4). This result might be occasioned by the following reasons. First, the NaOH pretreatment could increase the effective contact area between anaerobic microorganisms and substrates via reducing lignin content or breaking down lignin-hemicellulose complexes [14,21]. Therefore, WW, which contained higher amounts of lignin content than RS (Table 1) [8], had higher potential in increasing effective contact area when pretreated with NaOH. Second, the amounts of cellulose and hemicellulose that can be used to produce methane by methanogens were more contained in WW than in RS (Table 1). Thus, the improvement of the methane yield of WW could be visible when the effective contact area between anaerobic microorganisms and cellulose and hemicellulose was increased. Finally, after NaOH pretreatment, the pH value of the single digestion of WW was decreased to neutral, which was preferable for methanogenesis. However, the pH of the single-digestion of RS was almost unchanged (Figure 2e).

Compared with the single-substrate anaerobic digestions of PM and RS, the biomethane yield of the co-digestion of PM and RS was significantly ($p < 0.05$) increased (Figure 3). The methane yield of RS and PM co-digestion had increased by over 30% than both of the PM and RS single-digestions (Figures 3 and 4). High C/N ration and rich NH\textsubscript{4}–N content are the dominant factors limiting the methane production rates of RS and PM (Table 1), respectively [22]. Mixing PM and RS could balance the C/N ratio (Table 2) and nutrition, as well as toxic compounds generated during the digestion [23]. The AcoD with different substrates could also stimulate the synergistic effects of microorganisms for achieving improved biogas production [24].
Table 2. Experimental design.

| Treatments | Raw Material | VS Ratio | C/N Ratio |
|------------|--------------|----------|-----------|
| T1         | PM           | 20       |           |
| T2         | RS           | 47       |           |
| T3         | WW           | 53.5     |           |
| T4         | WW + NaOH    |          | 27.2      |
| T5         | RS + NaOH    |          | 29.1      |
| T6         | PM/WW        | 2:01     | 31.3      |
| T7         | PM/RS        | 2:01     | 29        |
| T8         | PM/WW(NaOH)  | 2:01     | 22.4      |
| T9         | PM/WW(NaOH)  | 2:01     | 23        |

Notes: PM/WW indicates the AcoD of PM and WW. PM/RS indicates the AcoD of PM and RS. PM/WW(NaOH) indicates the AcoD of PM and WW (pretreated with NaOH), PM/RS(NaOH) indicates the AcoD of PM and RS (pretreated with NaOH).

Co-digestion with WW had no beneficial effect on the methane production of PM (Figure 3b), which may be ascribed to the rigid and recalcitrant lignocellulosic structure of WW. On the other hand, the methane production of the co-digestion of WW and PM had nonsignificant difference with that of the single-digestion of PM (Figure 3b), suggesting WW can be utilized as a supplementary during the PM anaerobic digestion without affecting its methane production efficiency. We showed that the WW pretreated with NaOH had a satisfactory methane production performance in anaerobic digestion (Figure 2a,b). The methane production of WW treated with AcoD (pretreated with NaOH) was increased by 75.8% compared with the untreated WW. Furthermore, the growth rates of methane production of WW treated with NaOH and AcoD (pretreated with NaOH) were signally higher than the RS that under the same optimizing strategies (Figure 4). WW is widely distributed in vast rural areas and has huge reserves. Therefore, when treated with targeted approaches, WW has a considerable potential transforming from the worthless organic waste to a promising fermentation substrate.

4. Materials and Methods

4.1. Substrates and Inoculum of Anaerobic Dry Digestion

The PM and wood chips were used as the substrate of anaerobic dry digestion. The PM was obtained from the Danzhou Pig Farm (Hainan, China) and stored in a refrigerator at 4 °C before the start of the digestion experiment. The wood chips were prepared from the branches of a eucalyptus plant. The branches were crushed to obtain a diameter of less than 6 mm, soaked in biogas slurry, and acclimated for 1 week at room temperature. The inoculum was obtained from anaerobic activated sludge (Shunyi Biogas Plant, Beijing, China) and centrifuged at 10,000 r/min for 30 min. Afterward, the precipitate (inoculum) of the anaerobic activated sludge was acclimated for 1 week at room temperature, and the supernatant was used to soak the wood chips and regulate the total solid content in the anaerobic dry digestion system. The physical and chemical properties of PM, wood chips, RS, and inoculum are shown in Table 1.

4.2. Treatment Design and Incubation Experiments

A 500-mL glass vial was used as an anaerobic dry digestion reactor in this study. Nine treatments were prepared with three replicates each (Table 2). The inoculum accounted for 40% of the digestion material based on the total solid content. The digestion system was replenished with the supernatant of the centrifuged anaerobic activated sludge to 200 g of the total mass, uniformly stirred, placed in an anaerobic dry digestion reactor, and sealed with butyl rubber with an aluminum collecting gas bag. Each anaerobic dry digestion was incubated for 49 days in a constant-temperature incubator at 35 °C in the dark.
The wood chips were chemically pretreated with NaOH solution. Specifically, they were separately mixed with distilled water and NaOH solution with a mass fraction of 2% at a solid-to-liquid ratio of 1:10, uniformly stirred, treated at 90 °C for 4 h, rinsed with deionized water until neutral, and dried. The biogas slurry was extracted through a reflux operation every 12 h and injected back into the anaerobic dry digestion reactor. In this way, the biogas slurry could be evenly dispersed on the digestion substrate. The wood chips without pretreatment and no reflux operation were also used as controls.

4.3. Sample Collection and Analysis

The biogas generated during digestion was collected in a 3 L aluminum gas collecting bag. The biogas in the gas collecting bag was obtained regularly by using a 200 mL glass syringe every day to measure the biogas production with a gas flow meter (LMF-1, Cixi Instrument Co., Ltd., Shanghai, China). In addition, a gas sample was periodically collected using a 2 mL syringe for methane determinations.

Total solid (TS) and VS contents were measured in accordance with standard methods [25]. Total carbon (TC) and total nitrogen (TN) contents were determined with a high-temperature automated elemental analyzer (Vario EL cube, Langenselbold, Germany). Cellulose, hemicellulose, and lignin were observed using a semi-automatic fiber analyzer (ANKOM A200i, Longjie Instrument Equipment Co., Ltd., Shanghai, China). Methane concentrations were identified on a gas chromatograph (Agilent 6890, Santa Clara, CA, USA) equipped with a thermal conductivity detector and a flame ionization detector.

Analysis of variance (ANOVA) was performed to compare the differences in cumulative methane productions of single and co-digestions from different substrates, and the differences in the growth rates of methane production of the anaerobic digestions from WW and RS under different optimizing strategies were determined. ANOVA was conducted with SPSS 20.0 (IBM Corporation Software Group, Somers, NY, USA). Results were considered significant at \( p < 0.05 \).

5. Conclusions

In this study, NaOH pretreatment, AcoD technique, and their combination were used to test the performance and potential of the methane production of WW in anaerobic digestion. After pretreatment with NaOH was administered, the mean cumulative methane yield of the single digestion of WW increased from 175.81 mL CH\(_4\)/g VS to 243.53 mL CH\(_4\)/g VS, which was equivalent to a 38.5% increase compared with that of untreated WW. The mean methane yield of the co-digestion of WW and PM was 234.88 mL CH\(_4\)/g VS, which was higher than that of the single digestion of WW (175.81 mL CH\(_4\)/g VS) and was not significantly different from that of the single digestion of PM (245.09 mL CH\(_4\)/g VS). The mean cumulative methane yield of the co-digestion of WW pretreated with NaOH and PM was 309.06 mL CH\(_4\)/g VS, which was increased by 75.8% compared with that of the untreated WW and was higher than those of the single-digestion of WW pretreated with NaOH and the co-digestion of WW and PM. The growth rates of the methane production of WW treated with NaOH and AcoD pretreated with NaOH were considerably higher than those of the RS under the same optimizing strategies. This work could provide useful insights into the development of WW as a new sustainable and efficient alternative for biogas production.

Author Contributions: X.Z. (Xiaohui Zhang) and B.X. conceived and designed the experiments; R.L. and Q.S. performed the experiments; W.T. analyzed the data with suggestions by X.Z. (Xinyu Zhao) and Q.D.; W.T. and R.L. wrote the paper; B.X. and X.Z. (Xiaohui Zhang) proofed the paper.

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