Importance of storage time in mesophilic anaerobic digestion of food waste

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ARTICLE INFO
Article history:
Received 16 August 2015
Revised 23 October 2015
Accepted 2 November 2015
Available online 16 January 2016

Keywords:
Food waste
Bio-pretreatment
Storage
Fermentation
Biochemical methane potential
Hydrolysates

ABSTRACT
Storage was used as a pretreatment to enhance the methanization performance of mesophilic anaerobic digestion of food waste. Food wastes were separately stored for 0, 1, 2, 3, 4, 5, 7, and 12 days, and then fed into a methanogenic reactor for a biochemical methane potential (BMP) test lasting up to 60 days. Relative to the methane production of food waste stored for 0–1 day (285–308 mL/g-added volatile solids (VSadded)), that after 2–4 days and after 5–12 days of storage increased to 418–530 and 618–696 mL/g-VSadded, respectively. The efficiency of hydrolysis and acidification of pre-stored food waste in the methanization reactors increased with storage time. The characteristics of stored waste suggest that methane production was not correlated with the total hydrolysis efficiency of organics in pre-stored food waste but was positively correlated with the storage time and acidification level of the waste. From the results, we recommend 5–7 days of storage of food waste in anaerobic digestion treatment plants.

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Introduction

Production of urban food waste on a global scale is predicted to increase by 44% from 2005 to 2025 (Adhikari et al., 2006). Because of its high moisture content, as well as organic- and nutrient-rich composition, food waste is considered as a valuable biomass resource for biomethane recovery using anaerobic digestion (AD) (Kiran et al., 2014; Komemoto et al., 2009; Wang et al., 2015b). AD is a widely used but complicated technology for the treatment of food waste, in which organic matter is converted to methane and carbon dioxide under an oxygen-free environment (Jiang et al., 2013). AD of food waste can be generally divided into two steps, i.e., fermentation and methanization.

Hydrolysates, which are fermentation products, significantly affect the performance of methanization reactors. Therefore, optimum environmental and operational parameters influencing acid-phase digestion of food waste, including pH (Jiang et al., 2013; Lim et al., 2008; Wang et al., 2014), temperature (Jiang et al., 2013; Komemoto et al., 2009; Lim et al., 2008; Vanwonterghem et al., 2015), hydraulic retention time (HRT) (Lim et al., 2008; Wang et al., 2015a), inoculum-to-substrate ratio (Forster-Carneiro et al., 2008; Xu et al., 2012), and organic loading rate (Jiang et al., 2013; Lim et al., 2008), have been intensively investigated. Some studies have focused on the physical or chemical pretreatment of food waste in order to enhance the hydrolysis or to alter the properties of food waste and thus to facilitate subsequent methanization through
processes such as microwave treatment (Marin et al., 2010; Shahriari et al., 2013), ultrasonication (Cho et al., 2013; Elbeshbishy and Nakha 2011), mechanical grinding (Izumi et al., 2010), alkali treatment (Lin et al., 2013), thermal treatment (Li and Li and Jin, 2015; Wang et al., 2006), ozonation (Ariunbaatar et al., 2014), and enzyme treatment (Kim et al., 2006).

Nevertheless, the aforementioned pretreatment methods are costly and decrease the efficiency of extracting energy from food waste. By contrast, simple storage can be used as an alternative pretreatment step. Xu et al. (2011) reported that anaerobic storage of dewatered sludge improves its biodegradability, increasing the soluble organic acid content from 90 to 2400 mg/L and increasing the soluble organic carbon content from 220 to 1650 mg/L. However, there are few studies on the effect of storage on food waste digestion. Although food storage in many industrial practices lasts from 0 h to tens of days, operators only consider transportation or loading requirements. Compared with sludge and other solid waste, food waste has low microbial load because of sanitation and cooking processes. There are studies focusing on the fermentation time of food waste in a fermentation reactor using an inoculum. For example, Wang et al. (2015a) used retention times of 1, 3, 5 days for an acidogenic reactor for food waste using mesophilic two-phase AD. They used a 1:1 inoculum-to-substrate ratio on total solids (TS) basis. The highest acidification effect was achieved with 5 days of fermentation. Lim et al. (2008) studied the effect of different HRT (4, 8, 12 days) on the production of volatile fatty acids (VFAs) in food waste inoculated with digested sludge and found that total VFAs produced by 8 and 12 days of fermentation were much more than those formed after 4 days. However, to the best knowledge of the authors, there are no studies on the storage pretreatment of unincinerated food waste to obtain optimal hydrolysates for subsequent methane production.

Therefore, the objective of this work was to investigate the effects of storage time on the properties of the hydrolysates from food waste and on the performance of the subsequent methanization. A further aim was to determine the optimal storage time for AD treatment plants for food waste.

1. Methods and materials

1.1. Substrates and inoculant

Food waste used in the study was collected from the canteen of Tongji University, Shanghai, China. It consisted of cooked rice, vegetables, fish, eggs, etc. After bones and inert materials were removed, the waste was homogenized in a shredding machine. Anaerobic sludge obtained from an up-flow anaerobic digester of a paper mill was used as inoculant. The food waste and sludge had a TS content of 24.1 wt.% and 11.8 wt.%, respectively, and volatile solid (VS) content of 88.2% dry weight (dw) and 60.8% dw, respectively.

1.2. Experimental set-up

The prepared waste was first fed into fermentation reactors and incubated in an oscillating cultivation box (SPX-250-Z-S, Yuejin, China) at 35 ± 2°C for 0, 1, 2, 3, 4, 5, 7, and 12 days, respectively. Polystyrene centrifuge tubes of 50 mL without lids served as the storage reactors, covered by Parafilm (WS54956, Bemis, USA) which was pricked to enable the escape of biogas produced during fermentation, so as to simulate the anoxic conditions of storage. Four parallel experiments were performed for each fermentation retention time. In one parallel experiment, the waste was immediately stored at 4°C in a refrigerator for property characterization. In the other three, waste was used as feed stock for a methanization reactor and stored at −20°C before use.

Methanization of the pre-fermented waste was carried out in an automatic methane potential test system (AMPTS II, Bioprocess Ltd., Sweden) under mesophilic conditions (35 ± 1°C) maintained by a thermostatic water-bath incubator. Serum bottles (1 L) were hermetically sealed with rubber stoppers having two metal tubes to enable separate sampling of liquids and gas flow. The biogas produced first flowed into a bottle with 3 mol/L NaOH to absorb CO₂ and the remaining gas volume was measured by the principle of water-displacement and buoyancy. Tests were carried out in triplicate. Two blank reactors were used to measure the quantity of methane produced by the inoculum. The reported methane production of food waste was after deducting the background value of the inoculated sludge. The flow chart of the storage and methanization experiments is shown in Fig. 1. Mechanical stirring was neither employed in storage nor in AMPTS operation, but the liquid in methanization reactors was homogenized by hand-shaking before sampling.

The recipe for methanization included food waste that had been subjected to various times of fermentation, inoculant, nutrient solutions, and distilled water. The mixture had a TS content of 105 g/L and a VS content of 72 g/L. The inoculant-to-substrate ratio on a VS basis was 2:1. The preparation of nutrient solutions was based on documented methods (ISO, 1998). The initial pH was adjusted to 6.8−7.2 by using 1 mol/L HCl and 1 mol/L NaOH solutions. Reactors were purged from the bottom with nitrogen gas for 5 min. Liquid samples were collected every 2 days within the first 24 days of digestion.

1.3. Analytical methods

The pH, TS, VS, VFAs, and total organic carbon (TOC) were measured to evaluate the characteristics of hydrolysates at various fermentation times. The pH, TOC, and VFAs of each liquid sample in the methanogenic reactor were analyzed to study the degradation of organic matter in the reactor. Methane production was automatically recorded by the AMPTS system.

The TS and VS content were analyzed by using standard methods (APHA et al., 2012). The pH and VFA content were measured with a pH meter (6230M, Jenco, USA) and high-performance liquid chromatography system (LC-20AD, Shimadzu, Japan) respectively. TOC values were obtained from the difference between total carbon (TC) and inorganic carbon, both of which were analyzed with a TOC analyzer (TOC-VCPN, Shimadzu, Japan). The total nitrogen (TN) value can also be obtained from the TOC analyzer. Prior to the analysis of VFAs
and TOC, the liquid samples were centrifuged at 21,200 × g for 10 min to remove particles.

2. Results and discussion

2.1. Effects of storage time on the physicochemical characteristics of hydrolysates

2.1.1. Efficiency of hydrolysis

As shown in Table 1, there was no significant difference in TS (23.26 wt.%–26.66 wt.%), VS (87.42% dw–91.68% dw), dissolved organic carbon (DOC) (3624–3998 mg/L) or dissolved nitrogen (DN) (117–144 mg/L) of samples except for F12. The efficiency of hydrolysis of organics in samples F0–F7 based on the ratio between DOC of hydrolysate and total carbon in the initial food waste was 26.0%–29.1%. The efficiency generally increased with fermentation time, except for F7, and the efficiency of protein hydrolysis, calculated by the ratio between DN of the hydrolysate and total nitrogen in the initial food waste, was found to be less than 15% for F0–F7.

F12, however, had a lower VS content (88.5%) and a higher DN concentration (184 mg/L). As listed in Table 1, the hydrolysis of proteins was not improved in the first 7 days of storage, but significantly increased in 12 days. The efficiency of hydrolysis of proteins and organics for F12 was found to be 23.9% and 31.5%, respectively; therefore, F12 had more readily biodegradable products for methane production.

2.1.2. Acidification efficiency

Acetic acid was only detected in F1, F2 and F12 and accounted for 12%, 7.2% and 10.4% of the total acid concentration, respectively. Lactic acid was the dominant acid in all fermentation tanks, increasing steadily in the first five days, from 286.5 mg-C/L for F1 to 873.8 mg-C/L for F5. However, after another few days, it increased slowly to 1022.6 mg-C/L for F12. No acid was observed in the methanization reactor of fresh food waste (F0).

The acidification efficiency was calculated from the ratio of carbon content contained in VFAs and lactate to the DOC, and it showed the same trend as total acid concentration (Fig. 2). The acidification rate increased from 0 (F0) to 29.44% (F12). In particular, it increased quickly from 0 to 8.83% in the first two days and then increased slowly to 21.87% for F5 and to 29.44% for F12. The low acidification rate indicates that >70% of the dissolved organic matter was not transformed to acids. Poor acidification and low hydrolysis performance may have resulted from the absence of inoculums and the limited population of microorganisms in the food waste. Lactic acid comprised the largest proportion of dissolved organic acids in all hydrolysates but F0, being several times higher in concentration compared with VFAs. This result is consistent with the data from Table 1.

### Table 1 – Characteristics of inoculum and food waste subjected to different days of storage.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>F0</th>
<th>F1</th>
<th>F2</th>
<th>F3</th>
<th>F4</th>
<th>F5</th>
<th>F7</th>
<th>F12</th>
<th>Sludge</th>
</tr>
</thead>
<tbody>
<tr>
<td>TS (%)</td>
<td>24.13 ± 1.04</td>
<td>23.26 ± 0.16</td>
<td>24.67 ± 1.13</td>
<td>23.87 ± 0.19</td>
<td>24.77 ± 0.92</td>
<td>26.66 ± 1.14</td>
<td>24.83 ± 0.24</td>
<td>28.15 ± 1.25</td>
<td>11.8</td>
</tr>
<tr>
<td>VS (%)</td>
<td>88.22 ± 3.78</td>
<td>91.32 ± 0.24</td>
<td>87.42 ± 2.55</td>
<td>90.76 ± 0.14</td>
<td>90.90 ± 2.17</td>
<td>91.68 ± 4.83</td>
<td>91.04 ± 0.18</td>
<td>88.45 ± 0.55</td>
<td>60.8</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>3793 ± 82</td>
<td>3689 ± 171</td>
<td>3681 ± 65</td>
<td>3839 ± 22</td>
<td>3976 ± 139</td>
<td>3998 ± 157</td>
<td>3624 ± 170</td>
<td>3879 ± 98</td>
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<tr>
<td>DN (mg/L)</td>
<td>139 ± 1</td>
<td>117 ± 4</td>
<td>125 ± 6</td>
<td>124 ± 2</td>
<td>138 ± 11</td>
<td>144 ± 5</td>
<td>137 ± 13</td>
<td>184 ± 11</td>
<td></td>
</tr>
<tr>
<td>Hydrolysis efficiency (%)</td>
<td>27.1</td>
<td>27.2</td>
<td>26.0</td>
<td>27.6</td>
<td>29.1</td>
<td>29.0</td>
<td>26.1</td>
<td>31.5</td>
<td></td>
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<tr>
<td>Hydrolysis efficiency of proteins (%)</td>
<td>14.3 ± 0.0</td>
<td>12.5 ± 0.5</td>
<td>12.8 ± 0.7</td>
<td>13.0 ± 0.2</td>
<td>13.4 ± 1.0</td>
<td>14.4 ± 0.5</td>
<td>13.2 ± 1.2</td>
<td>23.9 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>VFAs (mg-C/L)</td>
<td>0</td>
<td>39.1 ± 0.9</td>
<td>39.6 ± 2.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>119.2 ± 2.3</td>
<td>–</td>
</tr>
<tr>
<td>Lactate (mg-C/L)</td>
<td>0</td>
<td>286.5 ± 10.5</td>
<td>507.4 ± 3.5</td>
<td>617.7 ± 18.4</td>
<td>675.9 ± 8.2</td>
<td>873.8 ± 10.4</td>
<td>721.6 ± 28.7</td>
<td>1022.6 ± 13.2</td>
<td>–</td>
</tr>
<tr>
<td>VFAs + Lactate (mg-C/L)</td>
<td>0</td>
<td>325.6 ± 10.8</td>
<td>547.0 ± 4.6</td>
<td>617.7 ± 18.4</td>
<td>675.9 ± 8.2</td>
<td>873.8 ± 10.4</td>
<td>721.6 ± 28.7</td>
<td>1141.8 ± 15.4</td>
<td>–</td>
</tr>
<tr>
<td>Acidification efficiency (%)</td>
<td>0</td>
<td>8.83 ± 0.16</td>
<td>14.87 ± 0.31</td>
<td>16.09 ± 0.43</td>
<td>17.00 ± 0.45</td>
<td>21.87 ± 0.70</td>
<td>19.92 ± 0.14</td>
<td>29.44 ± 0.49</td>
<td>–</td>
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<tr>
<td>pH</td>
<td>6.33 ± 0.07</td>
<td>4.43 ± 0.10</td>
<td>4.23 ± 0.06</td>
<td>3.92 ± 0.01</td>
<td>3.91 ± 0.01</td>
<td>4.1 ± 0.00</td>
<td>3.72 ± 0.01</td>
<td>3.92 ± 0.08</td>
<td>–</td>
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</tbody>
</table>

Note: the data is presented in mean value and standard deviation. F0, F1, F2, F3, F4, F5, F7 and F12 refer to food waste obtained from 0, 1, 2, 3, 4, 5, 7 and 12 days of storage, respectively. TS: total solid; VS: volatile solid; DOC: dissolved organic carbon; DN: dissolved nitrogen; VFAs: volatile fatty acids.
with the observation of Wang et al. (2014) that lactic acid is the main product when food waste is fermented at pH 4.0.

Contrary to the acid concentration and acidification efficiency, pH decreased with the prolonging of storage (Fig. 2). F0 had a pH of 6.3, which decreased to 4.4 (F1) after one day of storage. F7 even had a low pH of 3.7. Although much more acid was detected in the fermentation reactors, F12 and F5 had pH values higher than that of F7, suggesting a stronger buffering capacity due to the higher DN concentration. F5 and F12 also showed higher efficiency in hydrolysis and acidification compared with F7, probably because of the inhibition caused by the low pH of F7.

2.2. Biomethane production from hydrolysates obtained with various lengths of storage

Biochemical methane potentials obtained in 21 days (i.e., BMP21) and in 60 days (i.e., BMP60) for batch AD were used to evaluate the material’s biodegradability (German, 2001; Thygesen et al., 2014). Fig. 3 shows the cumulative methane production of the hydrolysates at preset fermentation periods. The BMP21 values for food waste pre-stored for 0, 1, 2, 3, 4, 5, 7, and 12 days were 311 ± 5, 336 ± 22, 442 ± 143, 385 ± 30, 397 ± 91, 484 ± 15, 571 ± 24, and 479 ± 137 mL/g-VSadded, respectively. The BMP60 values were 285 ± 8, 308 ± 31, 530 ± 197, 466 ± 106, 418 ± 119, 618 ± 12, 696 ± 43, and 639 ± 174 mL/g-VSadded, respectively. Therefore, the methane production rate and final methane yield were significantly affected by the input of pre-hydrolyzed substrates. The methane yields of F0–F1 were only in the range of 285–308 mL/g-VSadded, whereas those of F2–F4 and F5–F12 were 418–530 mL/g-VSadded and 618–696 mL/g-VSadded, respectively, and with a more narrow data deviation for F5 and F7, indicating improved process stability.

2.3. Behavior of VFAs, DOC, and DN in a methanogenic reactor

The pre-hydrolyzed food waste was further hydrolyzed, acidified, and transformed to methane with the help of inoculum. The DOC and VFAs in all methanization reactors decreased significantly in the first 6 days, because VFAs were readily converted into methane by methanogenic bacteria. Except for F0 and F1, DOC and VFAs started to increase on day 8, reaching 3358 and 1383 mg-C/L, respectively, in F12; and except for F5, the food wastes treated with longer storage time generally maintained higher concentration in 24 days overall (Fig. 4a, b), indicating that storage as the pretreatment made the transition from organic matter to dissolved organic carbon and further to VFAs much easier. In methanogenic reactors, the accumulation of DOC and VFAs indicates higher rates of hydrolysis and acidification compared with the methanogenesis rate. F0 and F1, however, had the lowest DOC (930–1343 mg-C/L) and VFA content (32–195 mg-C/L) and increased little within 24 days of methanization, implying inefficiency in organic matter degradation.

The acidification rates of F0 and F1 were within 0–25% during the 24 days. The rates for F2–F12, however, increased from the 8th day on, reaching 21%–48% on the 24th day. Therefore, methanogenic reactors fed with hydrolysates that underwent longer storage times led to greater conversion of DOC into VFAs and hydrolysis of more organic matter, as discussed above. Considering the methane production data in Fig. 3, we can deduce that longer storage time can enhance the methane yield and production efficiency by improving the hydrolysis and acidification of food waste.

DN in anaerobic reactors, which indicates the hydrolysis of proteins, increased from 831–1665 mg-N/L (2nd day) to 1983–2525 mg-N/L (24th day) (Fig. 4c). As the difference between various lengths of storage is not significant, the protein degradation was independent of the hydrolysate characteristics.

3. Discussion

3.1. Relationship between hydrolysate characteristics and methane yield

As indicated by the bivariate analysis (Table 2), BMP21 and BMP60 are not correlated with the efficiency of hydrolysis of
organic matter, but are positively correlated with the acidification efficiency during storage \((p < 0.05; p < 0.01)\) and storage time \((p < 0.05)\). Lactic acid mainly contributed to the acidification efficiency, which increased steadily from 0 \((F_0)\) to 29.4\% \((F_{12})\) with storage time. Accordingly, the methane production increased considerably from \(F_0\) to \(F_{12}\) in 21 and 60 days.

Although the lactic acid concentration increased with storage time, the total hydrolysis efficiency of organic matters was not correlated with storage time, whereas the efficiency of protein hydrolysis increased with storage time. The total hydrolysis efficiency varied slightly in 0–7 days and increased sharply from day 7 (26.1\%) to day 12 (31.5\%). It was not correlated with BMP21 and BMP60, both of which increased with increasing storage time. Therefore, the underlying reason is that except for proteins, considerable amounts of organic matters were not converted to DOC, but their structures were modified during storage. As a result, the solid materials were more readily biodegraded and transformed into acids, although the efficiency of total hydrolysis based on the DOC content appeared to be similar. This led to improved methane yield, as demonstrated by the improved efficiency of hydrolysis and acidification during methanization (Fig. 3).

### 3.2. Optimal storage time for the digestion of food waste

The obtained results demonstrate that longer storage time can improve acidification efficiency and can provide better substrate for methanization, and, therefore, lead to significantly increased methane production. After 0 or 1 day of storage, the methane yield of food waste in 60 days was 285–308 mL/g-VS\(_{\text{added}}\), increasing within 2–4 days to 418–530 mL/g-VS\(_{\text{added}}\) and further to >600 mL/g-VS\(_{\text{added}}\) in 5 and 7 days with more stable performance. One of the reactors even produced 763 mL/g-VS\(_{\text{added}}\) in 12 days. But with the prolonging of storage, the improvement in either acidification efficiency or methane production did not increase as quickly as in the first 5–7 days. Longer storage time
also requires more storage space and reduces the treatment capacity. Considering that only 5 or 7 days of storage can result in high efficiency for substrate acidification (20%–22%) and thus greatly improve methane yield, we suggest 5–7 days of storage for fermentation followed by methanization in engineering practice.

**Table 2 – Correlations between characteristics of hydrolysates and methane yield.**

<table>
<thead>
<tr>
<th></th>
<th>BMP21 Pearson Correlation</th>
<th>BMP60 Pearson Correlation</th>
<th>Hydrolysis efficiency during storage</th>
<th>Hydrolysis efficiency of proteins during storage</th>
<th>Lactate Acidification efficiency during storage</th>
<th>Storage time</th>
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<tr>
<td>BMP21</td>
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BMP21 and BMP60: Biochemical methane potentials obtained in 21 days and 60 days respectively.

**Correlation is significant at the 0.01 level (2-tailed);**

* Correlation is significant at the 0.05 level (2-tailed).
3.3. Comparison with two-stage anaerobic digestion technology

Two-stage digestion refers to the combination of a fermentation reactor and methanogenic reactor. In the literature, two-stage digestion is usually reported to have a methanogenesis efficiency of 7%–23% (Shen et al., 2013; Liu et al., 2006; Lim et al., 2013) higher than one-stage digestion, while some studies found that the methane production in two-stage systems might be comparable or even inferior to single stage systems due to lower bacteria diversity and negative effects of the syntrophic association between acidogens/acetogens and methanogens (Schievano et al., 2012; Merlino et al., 2013).

Compared to two-stage digestion, storage treatment together with methane fermentation can already increase methane production by 40% in 21 and by 100% in 60 days as demonstrated in this study, and has great advantages in terms of economy and manipulation. Storage is a routine unit in plants, which is simple and does not need special maintenance, while the fermentation reactor in two-stage digestion, known for its complexity, requires the construction of a fixed reactor, mixing equipment, larger space, maintenance, and inoculum, etc. The storage method reduces the infrastructure cost and maintenance cost, so a significant cost reduction benefit from replacing the fermentation reactor by a storage tank can then be anticipated. Meanwhile, the storage treatment is feasible since it takes place in a simple storage tank and there is no need for inoculation or addition of any other conditioner, reducing the material cost and requiring much less manipulation. Our results partly explain why one-stage digestion is more popular in practice, since storage + one-stage digestion can just reach the efficiency of two-stage digestion and is much simpler for management.

In addition, only Xu et al. (2011) mentioned anaerobic storage as a pretreatment for enhanced biodegradability of dewatered sewage sludge. Some researchers used VFAs, which can be produced during storage of food waste, as an acid-pretreatment method for enhancing the methanogenesis of cellulolic materials and achieved greater solubilization, thus facilitating the subsequent methane production (Trzcinski and Stuckey, 2015; He et al., 2008). Food waste used in this experiment contains carbohydrates, proteins, fats and etc. As can be seen, the DOC and DN concentration in the methanization reactor varies for food waste that has gone through different lengths of storage, implying that the degradation of carbohydrates and proteins as well as fats is affected by the storage length. Therefore, it might be concluded that the storage method can be applied to biomass of various compositions, but the behavior in individual cases needs further investigation.

4. Conclusions

Pretreatment by storage was found to greatly affect the rate and yield of methane production of food waste in mesophilic anaerobic digestion. Acidification efficiency in fermentation as well as efficiency of further hydrolysis and acidification during subsequent methanization increased with increasing storage time, but the improvement was less efficient after 5–7 days. Relative to the methane production of food wastes subjected to 0–1 day of storage, that achieved with food waste subjected to 5–12 days of storage increased by >40% in 21 days and doubled in 60 days on average. For economy, 5–7 days of storage of food waste prior to being processed in anaerobic digesters is recommended.

Acknowledgments

This work was supported by the National Basic Research Program (973) of China (No. 2012CB719801), the National Natural Science Foundation of China (Nos. 51378375, 51178327; 21177096), the Fundamental Research Funds for Central Universities (No. 0400219272), and the Collaborative Innovation Center for Regional Environmental Quality.

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