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Investigation on Recycling Dry Toilet Generated Blackwater by Anaerobic Digestion: From Energy Recovery to Sanitation

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Abstract: Anaerobic digestion (*AD*) has been widely adapted for blackwater treatment, however, the effect of water-conserving toilet generated blackwater on the *AD* process is still unknown. In this study, the anaerobic digestion process of dry toilet generated blackwater was investigated by means of a biomethane potential test. It was demonstrated that anaerobic digestion was inhibited and then adapted because of a high total ammonium nitrogen (TAN) level (3673.3 mg/L). The start-up period was 14.04 days and the biomethane potential of dry toilet blackwater was 402.36 $mLCH_4/gVS$ (55 days, 38 °C). Inhabitation and adaptation could be described as the increase of free ammonia nitrogen content and acetic acid concentration, followed by an enhancement of the relative abundance of acetic acid-type methanogens (from 33.53–61.52%). The main pathogen in dry toilet blackwater fermentation broth, *Pseudomonas aeruginosa*, kept multiplying in the first 8 days and then stabilized at a higher level than that of the beginning. This work showed the self-adjustment process and pathogen dynamics of dry toilet blackwater anaerobic digestion and highlights the significance of dry toilet blackwater characteristics when designing and maintaining anaerobic digestion sanitary treatment and reuse systems.

Keywords: blackwater; dry toilet; sanitation; sustainable treatment

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1. Introduction

Ecological sanitation emphasizes closing the loop of water and nutrients during wastewater management [1]. Source separation collection of blackwater (feces, urine and flushing water) and greywater (other household wastewater) is necessary for resource recovery treatment [2]. In well-designed and developed systems, blackwater can be further separately collected and treated as urine and feces, which leads to higher recovery efficiencies and better fertilizer products [3–7]. Blackwater contains high organic and nutrient levels (>50% of the organic content and 80–95% of the nutrients in domestic wastewater) [8], and the separate treatment of blackwater can maximize the possibility of resource recovery. In places with poor sanitation arrangements and facilities, dry toilets have been widely used, which can lead to a pollution of the soil and drinking water without proper treatment.

Anaerobic digestion is an energy-efficient method for blackwater treatment to recover energy and nutrients [9]. However, the stability of anaerobic digestion is susceptible to fluctuations in substrate composition (such as total solids, chemical oxygen demand, C/N ratio, and total organic carbon), accumulation of toxic substances, sludge aging and other factors [10]. The organic loading rate (OLR) of organic waste anaerobic digestion systems is normally controlled to be less than 4 g Volatile Solid/d·L [11]. Ammonia suppression is the most typical phenomenon in the anaerobic process because of nitrogen-rich materials. Ionized ammonium can directly inhibit the synthesis of methane-producing enzymes [12] and ammonia molecules can passively diffuse into cells, causing proton imbalance and potassium deficiency [13]. The biogas production process is normally inhibited when the total ammonia nitrogen (TAN) concentration exceeds 1500 mg/L and becomes toxic when TAN is $\geq$3000 mg/L. Pretreatment is usually employed to reduce ammonium concentration [14], or a support material is added to control ammonia inhibition in fermentation broths [15]. The anaerobic digestion of chicken manure was performed with high ammonia nitrogen content (TAN above 6 g/L). When average free ammonia nitrogen (FAN) concentration increased from 0.77 g/L to 0.86 g/L, the biogas yield dropped from 0.39 to 0.27 L/gVS [16]. BMP (biomethane potential) tests are generally considered to be an assessing method for an anaerobic digestion substrate. The BMP procedure involves adding small amounts of selected inoculum and substrate into serum bottles, creating anaerobic conditions and measuring biogas production over time. A cumulative biogas production curve is then obtained and the BMP value is typically expressed as a function of added volatile solids (VS) (mL·CH4/g·VS) [17]. However, the results of BMP tests can be influenced by inoculum characteristics (origin, concentration and activity), experimental conditions (gas measurement system, operational parameters, chemical parameters and inoculum to substrate ratio) [18], particle size and the nutrient media of the substrate [19].

To the best of our knowledge, a large amount of the domestic wastewater anaerobic digestion studies has focused on treatment performance and removal efficiency and mainly employed simulated blackwater or diluted blackwater from flush toilets. These types of blackwater normally have low chemical oxygen demand (COD) and TAN concentrations. Adhikari used a septic tank-upflow anaerobic sludge blanket (UASB) reactor to treat high concentration domestic sewage. The COD reached 1200 mg/L and the average removal efficiency of the COD was 88% [20]. Cunha added calcium for the recovery of phosphate granules (CaP granules) and methane from vacuum collected blackwater (BW) using an upflow anaerobic sludge blanket (UASB) reactor. The overall removal of COD was stable at above 80% [21]. In another study, a sludge blanket anaerobic baffled reactor was applied to treat blackwater. The temperature in the buffer tank ranged from 10–15 °C in wintertime and from 18–21 °C in summertime. On average, more than 78% of the influent load of the COD at a hydraulic retention time (HRT) of 3 days was removed [22].

When proposing and developing sustainable wastewater treatment systems, recovery of energy and nutrients has become the direction for future research [23]. However, minimal attention has been given to the effects of blackwater characteristics on methanogenic process stability and efficiency. The blackwater generated from dry toilets poses a challenge to decentralized treatment systems due to its high organic and ammonia load. Recently, researchers have begun to focus on the effect of blackwater generated from water-conserving toilets in anaerobic digestion. Studies of different blackwaters have suggested that differing toilet types would affect energy recovery potential due to ammonia inhibition in the methanogenesis phase [24]. Granulated activated carbon was added into the anaerobic digestion of blackwater to decrease the inhibition of TAN (2800 $\pm$ 200 mg/L) and adjust the process; biochemical methane potential and COD removal increased from 34% to 53% and 37% to 56.7%, respectively [25]. The authors investigated blackwater produced by different types of toilets, including dry, vacuum and water saving flushing toilets, and revealed remarkable differences in their properties. TAN in various blackwaters ranged from 107–3673 mg/L, and the highest TAN in concentrated blackwater was at a toxic level. The inhibition behavior during high concentration blackwater anaerobic processes remains unknown.

Considering the reuse of blackwater, pathogens can be another problem. Human feces contain a large number of pathogenic bacteria, such as *Aeromonas* spp., *Escherichia coli*, etc. (from WHO guidelines). Aerobic composting has been proved to be useful for eliminating pathogens and parasites, and reducing phytotoxicity (cress seed germination index $\geq$80%) [26,27]. The biomethane potential (BMP) test of dry toilet generated blackwater was investigated in this study, and biogas production and methane content were measured and calculated. Volatile fatty acid (VFA), ammonia nitrogen, total nitrogen and microbial diversity during the start-up period were analyzed to obtain insights into the process. The results can serve as a theoretical basis for dry toilet blackwater anaerobic digestion process design and operation.

2. Material and Methods

2.1. Blackwater

Blackwater was sampled from one household dry toilet in a rural village of Shunyi District, Beijing, used by 5 healthy adults for 1 weekday. A total of 5 L of blackwater were collected in a plastic sealed sample drum, transported to the laboratory and placed in a refrigerator at 4 °C for storage. For subsequent experiments, samples were stirred evenly and passed through a sieve with a pore size of 5 mm for the removal of impurities, such as paper, seeds and insects.

The inoculum was anaerobic granular sludge, which was obtained from an alcohol biogas digestion plant in a UASB reactor (total solids (TS) = 101.04 g/L, volatile solids (VS):TS = 67.42%, pH = 7.31).

The characteristics of the blackwater matrix are presented in Table 1.

Table 1. The characteristics of blackwater samples.

	COD (mg/L)	TAN (mg/L)	TN (mg/L)	TS (g/L)	VS (g/L)
Dry Toilet Blackwater	21,900	3673.3	5050	47.94	35.48

2.2. Biomethane Potential Test Set-Up

In the BMP tests, the inoculating container was a 500 mL jar and the biogas was collected using a double valve air bag. The fermentation jars were immersed in a water bath, the temperature was controlled at 38 °C ± 1 °C, the total substrate volume was 300 mL and the inoculum to substrate ratio was 1:1. All the BMP tests were conducted in triplicate.

The schematic of the BMP test device is shown in Figure 1. The inoculum-to-substrate ratio was selected based on preliminary tests where 3 different ratios (1:1, 2:1 and 4:1) were used. The biomethane potential of dry toilet wastewater with I/S ratio = 1:1 showed great advantages compared with other groups. The sludge was inoculated and the BMP experimental device was sealed. The biogas produced was collected using a gas bag and gas production was measured every 2–4 days. A fermentation broth sample was obtained once a day during the start-up period. Then, 5 mL of the fermentation broth was obtained from the sampling hose with a syringe, placed in a sealed cryotube and stored at 4 °C.

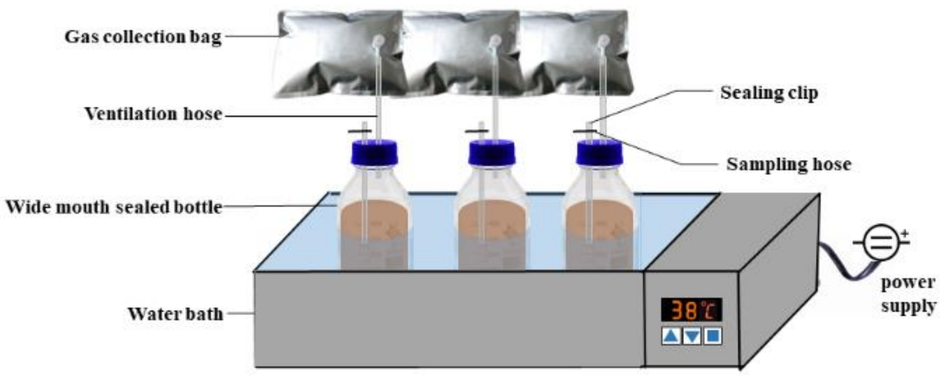

Figure 1. A schematic diagram of the biomethane potential (BMP) test device.

The fermentation broth samples were taken every day in the first 14 days for further chemical and microbial parameter tests to explore the concentrated blackwater start-up period.

2.3. Physical and Chemical Analyses

COD, NH_3-N, TN and TP were measured through the reactor digestion method (HACH method 8000). A HACH DR 2000 colorimeter and HACH DRVT00 reactor were used. pH was measured with a HACH 2100q portable water analyzer. Free ammonia concentrations were calculated from the total ammonia nitrogen (TAN) concentration, pH and temperature were calculated using Equation (1) [28]:

$$\text{FAN} = \text{TAN} \times \left[1 + \frac{10^{-pH}}{10^{-(0.09018 + \frac{2729.92}{T(K)})}}\right]^{-1} \tag{1}$$

TS and total volatile solids (TVS) were determined by the EPA method [29]. The biogas produced in the blackwater BMP test was analyzed using a portable gas analyzer (Geotech-Biogas check). The concentration of short-chain volatile fatty acids was determined by gas chromatography, and sample processing and chromatographic conditions were performed in accordance with the methods of Liu [30].

2.4. Microbial Diversity Analysis

Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China) was commissioned to conduct microbial diversity tests. The primers used were 338F-806R and mLfF-MLrR. PCR reactions were conducted using the following program: 3 min of denaturation at 95 °C, 27 cycles for 30 s at 95 °C, annealing for 30 s at 55 °C, elongation for 45 s at 72 °C, and a final extension for 10 min at 72 °C. The purified amplicons were pooled in equimolar and paired-end sequenced on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) in accordance with the standard protocols by Majorbio Bio-Pharm Technology Co. Ltd. (Shanghai, China).

2.5. Data Analysis

The versatile model of methane cumulative yield over time in anaerobic digestion was a modified Gompertz Equation [31]. The Cone Equation is commonly used for methanogenesis [32,33]. The modified Gompertz Equation and the Cone Equation were used in the analysis of the kinetics of anaerobic digestion methanogenesis.

(1) Modified Gompertz Equation

$$Y(t) = Y_m \times exp\left\{-exp\left[\frac{R_m \cdot e}{Y_m}(\lambda - t) + 1\right]\right\} \tag{2}$$

$Y(t)$—methane production rate at time, mL·CH_4/g·VS; Y_m—theoretical maximum methanogenic rate, mL·CH_4/g·VS; R_m—maximum daily methane production rate, mL·CH_4/g·VS·d; e—constant, 2.71828; λ—lag-phase duration, d; t—time, d.

(2) Cone Equation

$$Y(t) = \frac{Y_m}{1 + (k \times t)^{-n}} \tag{3}$$

$Y(t)$—methane production rate at time—mL·CH_4/g·VS; Y_m—theoretical maximum methanogenic rate, mL·CH_4/g·VS; k-rate constant, d^{-1}; t—time, d; n—shape factor, dimensionless.

The kinetic model fitting analysis and calculation of the cumulative production of methane over time were performed using ORIGIN software. Data from microbial diversity tests were analyzed using the Majorbio Cloud Platform.

3. Results and Discussion

3.1. BMP Test Results

Methane production in the blackwater BMP tests is shown in Figure 2. The methane production of the dry toilet blackwater was slow in the first 25 days. Cumulative methane production was only 30.90 mL·CH_4/g·VS on the 25th day. However, after this period, the methane production showed a dramatic increase from 68.90 mL·CH_4/g·VS on the 28th day to 402.36 mL·CH_4/g·VS on the 55th day. The proportion of methane in the biogas gradually grew and reached a peak of 79.75% on the 33rd day and then eventually stabilized at around 77%. The results show that there was a start-up period in dry toilet blackwater anaerobic digestion.

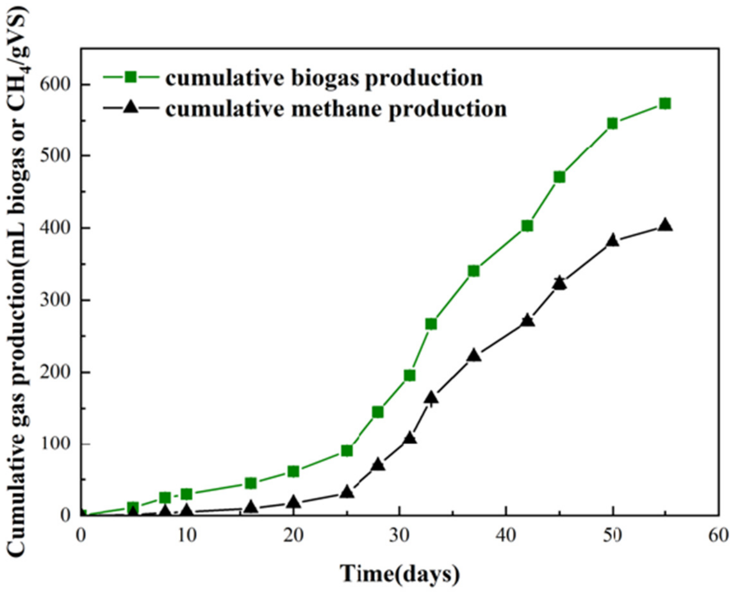

Figure 2. Cumulative biogas and methane production for BMP tests of dry toilet blackwater.

The average methane production potential of dry toilet generated blackwater was 402.36 mL·CH_4/g·VS. The biochemical oxygen demand (BOD) over chemical oxygen demand (COD) ratio of blackwater was reported to be 48–71% in the studies treating relatively low-strength blackwater [34]. Vacuum toilet blackwater with a biodegradability of 46–60% was also reported in the literature, [35] and showed that blackwater was a suitable substrate for anaerobic digestion. Compared with similar biomasses, the dry toilet blackwater showed excellent performance on biodegradability and biomethane potential. When it comes to excreta produced by other mammals, the biomethane potential of blackwater is higher than that of chicken manure, sheep manure, cow dung and pig manure under similar operating conditions, indicating that the content of long-chain organic molecules such as cellulose in human feces is relatively low (Table 2). The total ammonia in dry toilet blackwater is less than that of chicken manure (8000–12,000 mg/L), so the inhibition was not as strong as in the chicken manure anaerobic fermentation process. Moreover, the maximum methane content in biogas produced by dry toilet blackwater anaerobic fermentation was higher than that of similar biomass, indicating that the anaerobic fermentation process has a higher degradation efficiency of organic matter in blackwater. Therefore, although the concentrated blackwater did not inhibit the methanogenesis potential during anaerobic digestion, it did need a long time to start up. Because this will lead to an increase in residence time and reactor volume when applied in plants, improvement measures for the dry toilet blackwater AD process still need to be considered.

Table 2. Biomethane production potential of different biomass.

Fermentation Substrate	Operating Condition	Methanogenic Potential (mL·CH4/g·VS)	Maximum Methane Concentration (%)	Maximum Methane Production Rate (mL·CH4/g·VS·d)
pig manure [36]	35 °C 65 d	161	68	22
dairy manure [37]	35 °C, 30 d	302	64	22
food waste [37]	35 °C, 30 d	353	54	57
chicken manure [38]	35 °C, 40 d	231	60	25
pig manure [37]	55 °C, 40 d	337	65	72
municipal sewage sludge [39]	35 °C, 30 d	384	68	59
sheep manure [40]	35 °C, 60 d	273	62	–
duck manure [33]	35 °C, 60 d	441	65	–
rabbit manure e [33]	35 °C, 60 d	210	–	–
paper sludge [41]	30 °C, 30 d	269	–	39
dry toilet blackwater	38 °C, 50 d	416	78	24

The fitting results of the modified Gompertz Equation and the Cone Equation are presented in Figure 3 and Table 3. The theoretical biomethane potentials of blackwater from dry toilets was 464.46 mL·CH_4/g·VS according to the modified Gompertz Equation. The result fitted by Cone Equation was 459.14 CH_4/gVS. Neither equation could perfectly match the actual biomethane potential of the dry toilet blackwater and had differences of 10.37% and 11.64%, respectively, with the fitting results.

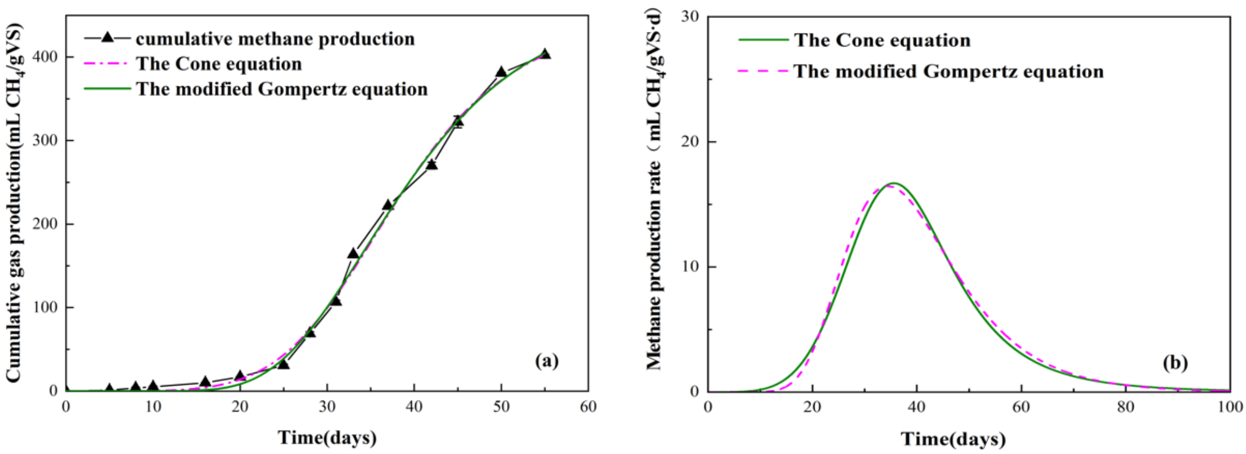

Figure 3. The cumulative methane production fitting curves (**a**) and the calculated methane production rate simulation curves (**b**) for different kinetic models.

Table 3. Kinetic analysis of two models of blackwater anaerobic digestion.

	Substrate	Y_m mL·CH_4/g·VS	R_m mL·CH_4/g·VS·d	λ d	R^2
Modified Gompertz Equation	Dry Toilet	464.46	16.46	24.04	0.9960
	Substrate	Y_m mL·CH_4/g·VS	k d^{-1}	n	R^2
Cone Equation	Dry Toilet	459.14	0.0262	5.36	0.9956

Figure 3 shows the maximum methane production rates of the dry toilet blackwater anaerobic digestion process were 16.46 mL·CH_4/g·VS·d (the modified Gompertz Equation) and 16.69 mL·CH_4/g·VS·d (the Cone Equation). Both occurred on the 35th day. The lag period λ (the duration of the start-up period) for dry toilet blackwater by the modified

Gompertz Equation was 24.04 days. In the Cone Equation, the magnitude of the *k* value can reflect the speed of the hydrolysis step. A high *k* value generally indicates that the substrate is easy to hydrolyze. It was seen that untreated dry toilet blackwater had a *k* of 0.0262 per day. Therefore, large particles of organic matter could be difficult to hydrolyze rapidly in concentrated blackwater. El-Mashad [32] used the Cone Equation to fit the methanogenic conditions of spirulina, switchgrass and a mixture of the two, and the *k* values obtained were between 0.09–0.10 per day. Li et al. [42] simulated the methanogenesis of corn stover during medium-temperature dry fermentation with the Cone Equation and obtained that untreated corn stover has a *k* value of 0.03 per day. The *k* value of the corn stover pretreated with NaOH was 0.28/d. Zhang et al. obtained the *k* values of pig, cow, chicken and rabbit manure at medium-temperature anaerobic digestion, which ranged from 0.03–0.18 per day. The *k* value gradually decreased as the organic load increased [43]. In this study, the *k* values of the dry toilet blackwater were lower than those in other research whereas the organic loads were at the same level. Thus, the hydrolysis speed of anaerobic digestion was affected when dry toilet blackwater was used as a substrate.

3.2. Ammonia Inhibition Results

3.2.1. TAN, TN, FAN, VFAs Variation

TAN, total nitrogen and free ammonia variations during the dry toilet blackwater anaerobic digestion start-up period are presented in Figure 4. On the 5th day, TAN dropped significantly from an initial 3673 mg/L to 2240 mg/L, a decrease of 35.33%. At the same time, total nitrogen (TN) also decreased 26.09%. Nevertheless, the concentration of free ammonia in the first five days was nearly unchanged, indicating that the reduced part of TAN was ammonia. Thereafter, the concentration of TAN gradually increased from the 6th day and the concentration of free ammonia in the dry toilet blackwater increased from an initial 82.3 mg/L to 189.4 mg/L on day 14. On the basis of the above-mentioned phenomenon, the passive diffusion of hydrophobic ammonia molecules into cells might lead to a decline in ammonia nitrogen and total nitrogen during dry toilet blackwater anaerobic digestion. Therefore, proton imbalance and potassium ion loss would occur, finally resulting in ammonia inhibition [28].

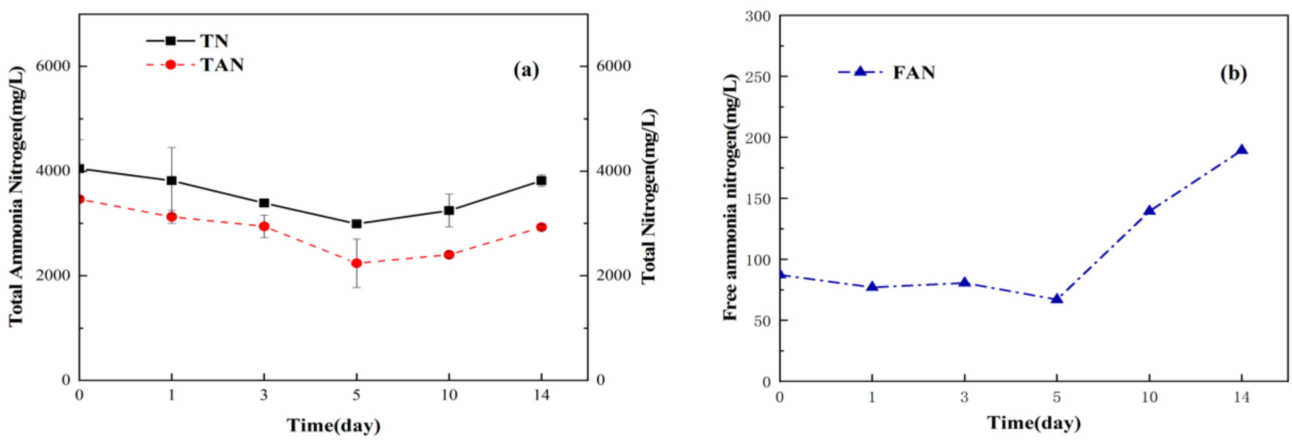

Figure 4. The course of total ammonia nitrogen and total nitrogen change (**a**) and free ammonia release (**b**) in dry toilet blackwater anaerobic digestion.

VFA content in the start-up period is shown in Figure 5. The concentration of various VFAs in the dry toilet blackwater increased slowly in the early stage, then the peak of total VFA reached 454.2 mmol on the 7th day. The concentration of acetic acid accounted for 54.3% of the VFAs. The concentration of various acids began to decrease on the 8th day. In the beginning, the substrate hydrolyzed rapidly because there was sufficient organic matter. Meanwhile, the acetic acid-type methanogen was susceptible to ammonia inhibition [44] and organic acids (especially acetic acid) gradually accumulated, thereby further suppressing the methanogenesis step. The highest concentration of VFA was 454.2 mmol. Abbassi-Guendouz et al. also found a similar phenomenon in their study on chicken manure anaerobic digestion. The VFA of the fermentation broth accumulated to 29–36 g/L in the case of the high solid content of anaerobic digestion [45].

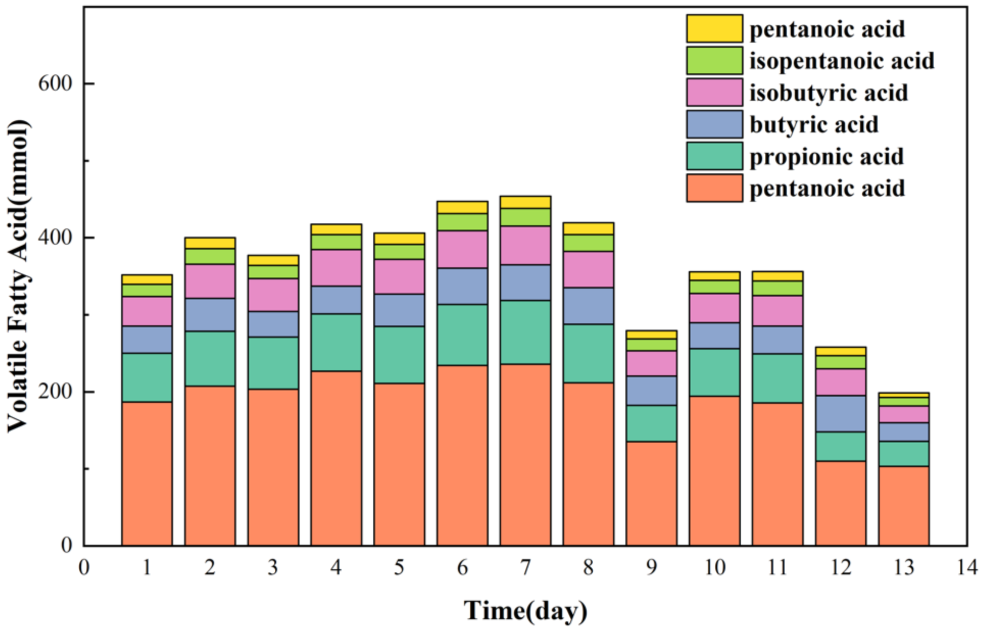

Figure 5. Fluctuation in VFA concentration during dry toilet blackwater anaerobic digestion.

3.2.2. Microbial Diversity Change

The relative abundance of the sample methanogens was analyzed (Figure 6). The most recent studies of blackwater anaerobic digestion have suggested that the predominant methanogens in the anaerobic digestion of blackwater without flushing water are *Methanosarcina* (74.9%) and *Methanoculleus* (24.3%) [46]. The results of the present study showed that *Methanosaeta* of the acetic acid-type methanogens was relatively high (30.48–63.83%) in the three scenarios. It could produce methane via the acetoclastic and the hydrogenotrophic methanogenesis pathways and was sensitive to specific inhibitors via acetoclastic methanogenesis. *Methanosaeta* was the dominant bacteria in each sample. The relative abundance of *Methanosaeta* could reach 60.22–63.83% in the initial phase of the start-up period and decreased with time in this study. This finding is consistent with the change of VFA in anaerobic digestion. During the first 8 days, the abundance of *Methanosaeta* increased due to the accumulation of acetic acid during the start-up period. After a large amount of *Methanosaeta* converted acetic acid to methane, the acetic acid concentration gradually decreased and the relative abundance of *Methanosaeta* returned to 30.48–37.45%, which was similar to the initial relative abundance of 33.53%.

Above all, the pathway for concentrated blackwater anaerobic digestion ammonia inhibition process can be summarized in Figure 7. The hydrophobic ammonia molecules derived from protein and urea can enter the cell by passive diffusion and become ammonium ions. Conversely, a proton imbalance might occur, thereby inhibiting the synthesis of methane-production enzymes and the activity of *Methanosaeta* (the acetic acid-type methanogens). However, concentrated blackwater contained plenty of organic matter. Consequently, the substrate of the hydrolysis step was sufficient, VFAs were continuously formed and this led to the accumulation of VFAs (especially acetic acid) in the early stage of fermentation. Therefore, the methanogenesis step was inhibited, while pH decreased and H_2 partial pressure increased. After the proliferation of *Methanosaeta*, the anaerobic flora gradually adapted to the dry toilet blackwater, and the ammonia nitrogen in the cells was gradually released. The consumption of organic matter led to the deceleration of the hydrolysis step. Meanwhile, methanogenesis increased, thereby relieving the inhibition caused by the accumulation of VFAs. After the above process, the start-up period of the dry toilet blackwater anaerobic digestion ended.

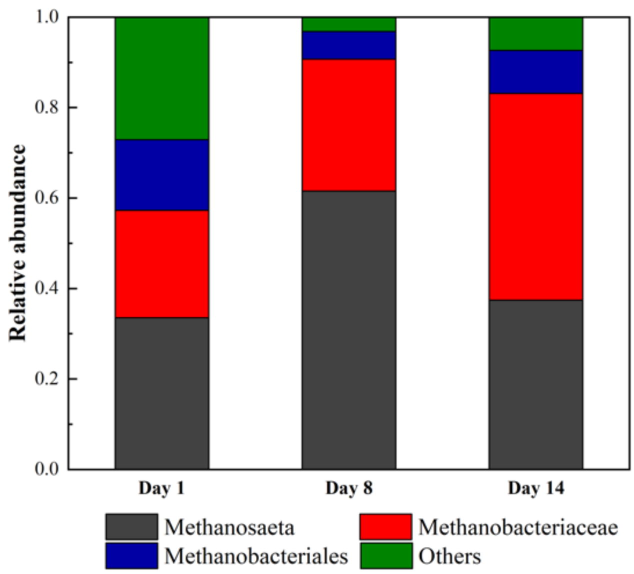

Figure 6. Relative abundance of the top three methanogens in dry toilet generated blackwater anaerobic digestion during the start-up period.

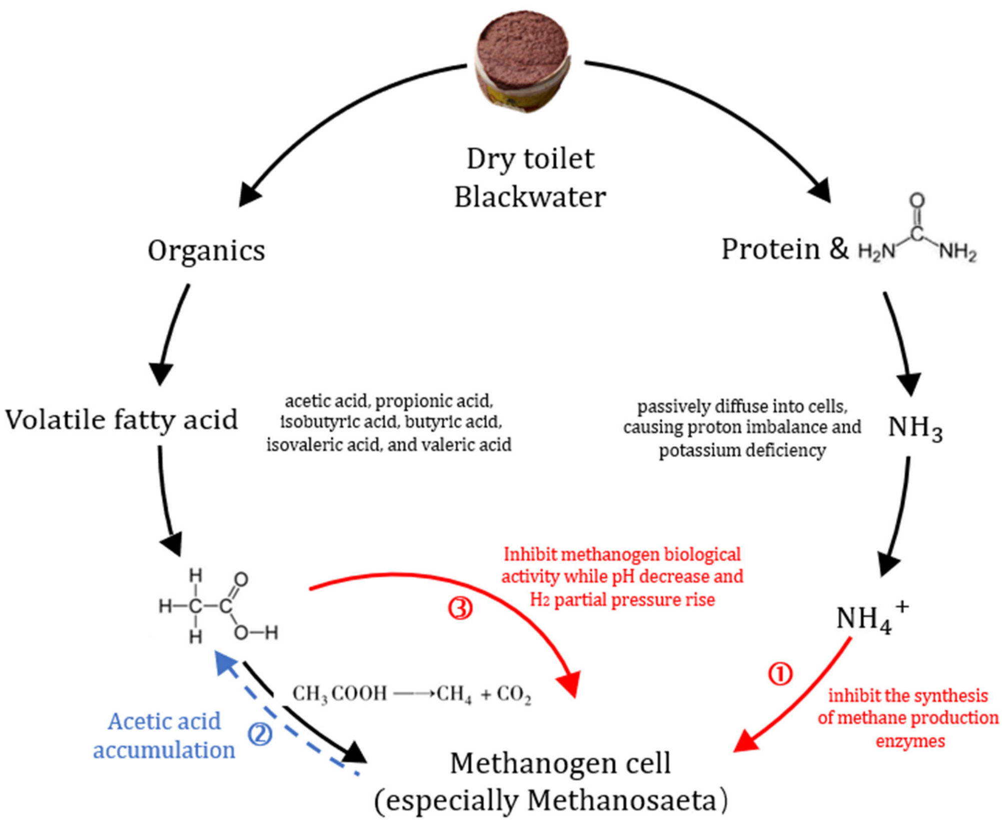

Figure 7. Concentrated blackwater ammonia inhibition principle for anaerobic digestion inhibition and self-adjustment path map.

3.3. Elimination of Pathogens during AD Process

Blackwater usually contains high concentrations of microbial contamination, including fecal bacteria (e.g., *E. coli, enterococci*), enteric pathogens (e.g., *Salmonella typhimurium, Cryptosporidium* spp., *Giardia* spp.) and opportunistic pathogens (e.g., *Pseudomonas aeruginosa*) [47–49]. Enteric infections can be transmitted by those pathogens, leading to diseases such as enteritis, typhoid/paratyphoid fever, salmonellosis and cholera. Exposure to such pathogens during blackwater reuse, for example, when irrigating plants with a fermentation broth, may contribute to disease transmission [26]. Chlorine disinfection and UV irradiation has been widely applied for reducing pathogens. The anaerobic digestion process has also proven to be effective for abating the concentration of pathogenic bacteria, having the capability to reduce pathogenic bacteria in blackwater from an initial 10^{24} CFU per 100 mL to 10^3 CFU per 100 mL after anaerobic and chemical treatments [25].

To further explore the impacts of the anaerobic digestion process in terms of sanitation, a microbial analysis for pathogens was performed (Table 4). The PCR results of total bacterial DNA concentration showed a significant increase from 28,398 copies/20 µL on the 1st day to 73,613 copies/20 µL. Proportionally, the top four pathogenic bacteria in dry toilet blackwater were *Pseudomonas*, *Bacteroides*, *Tissierella* and *Anaerosalibacter*. Among them, *Pseudomonas aeruginosa* was the main pathogen in dry toilet blackwater, accounting for 9.64–72.91% of total pathogens. The concentrations of *Pseudomonas* on the 1st, 8th and 14th days were 2738, 17,968, 16,180 copies/20 µL, respectively. Therefore, untreated dry toilet blackwater has a high risk of infection, including malignant external otitis, endophthalmitis, endocarditis, meningitis, pneumonia and septicemia [50]. The proportion and concentration of pathogenic bacteria kept growing during the start-up period of anaerobic digestion and then the content of pathogens decreased due to anaerobic acidogenic bacterial propagation, while the concentration of pathogens gradually stabilized. The pathogenic bacterial result changes indicated that in the early period of anaerobic digestion *Pseudomonas*-based pathogens would continue to multiply and pose a safety challenge for the reuse of digested blackwater. Therefore, subsequent disinfection of the fermentation broth may be still required for dry toilet blackwater treatment. Composting could be an effective disinfection method that may be feasible in remote locations. The survival fraction of *Pseudomonas aeruginosa*, the main pathogen in dry toilet blackwater, was less than 10^{-6} after a 30 min heat-treatment at 50 °C [51].

Table 4. Relative abundance and concentration of Pseudomonas.

Time (Day)	1st	8th	14th
Relative abundance	5.47	72.02	13.88
Concentration (copies/20 µL)	2738	17,968	16,180

4. Conclusions

It was revealed that dry toilet generated blackwater was more biodegradable than similar biomass and had a biomethane potential of 402.36 mLCH_4/gVS and a methane content of 78%. High initial TAN (3464.33 mg/L) in dry toilet blackwater fermentation broth needed a lag period (7–8 days) for start-up, and the abundance of *Methanosaeta* increased with free ammonia release and VFA accumulation for adaptation. Nevertheless, the concentration of *Pseudomonas* kept growing in the lag period, then gradually stabilized, while the proportion of pathogenic bacteria showed a trend of significantly falling after rising. The existence of a lag period will lead to an increase in reactor volume and construction costs. According to the above self-adjusting mechanism of microorganisms, adding acetic acid-type methanogens or reducing the ammonia content in the dry toilet blackwater through a pretreatment process will contribute to solve this problem. Meanwhile, this work has proved that ammonia inhibition during the dry toilet blackwater AD process will not affect the efficiency of methane production, which allows household anaerobic treatment systems to be applied in remote and decentralized areas. When considering anaerobic digestion for dry toilet generated blackwater, wastewater properties, such as ammonia concentration, that may affect the stability of treatment systems should be carefully considered and a further disinfection process after anaerobic digestion is indispensable for blackwater reuse.

Author Contributions: S.Z. conceived of, designed and performed the experiments and also wrote the paper; X.Z. analyzed the data and reviewed the paper; Z.L. gave critical commentary and revisions; X.W. contributed analysis tools; L.Y. also performed the experiments and collected the data. All authors have read and agreed to the published version of the manuscript.

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