

## Article

# Enhancing Mesophilic Anaerobic Digestion of Waste-Activated Sludge through Heat Pretreatment and Kinetic Modeling

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**Abstract:** Sewage sludge is a useful raw material for the production of renewable energy due to its stable annual output. In this study, the enhancement of mesophilic anaerobic digestion of sewage sludge through heat pretreatment at 95 °C for 30 min was tested in an anaerobic moving bed biofilm reactor (hAMBBR). The sludge retention time was set at 20, 15, 10, and 5 days during 300 days of operation and compared to a traditional anaerobic continuous stirred tank reactor (AnCSTR) without pretreatment. Results of this research indicate that the digestion ratio of volatile soluble solids in the hAMBBR process could be improved by 50%, and the average conversion ratio of methane could be increased by 45%. When the sludge retention time (SRT) was shortened to 5 days, the methane production approached twice that of the contrast reactor. The expanded anaerobic digestion model, including activated sludge models, was utilized for operation simulation. The effect of sludge retention time (SRT) shortening on volatile suspended solids (VSS) digestibility and methane production was well reproduced with simulations. The research conclusion reveals the impact of pretreatment and reactor types on anaerobic digestion and provides the scientific basis for improving methane production and process efficiency in anaerobic digestion.

**Keywords:** sewage sludge; anaerobic digestion; heat pretreatment; kinetic model



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## 1. Introduction

Treatment and disposal of waste-activated sludge (WAS), which is continuously being produced on an annual basis, became a major environmental problem [1]. It is reported that the European Union's output of dried sewage sludge surged by more than 50%, from 6.5 MT in 1992 to 10.9 MT in 2015 [2,3]. The United States has an average annual dried sewage sludge production of 17.8 MT in the last decade [4]. In the Asia-Pacific region, the generation of dried sewage sludge in Australia is about 0.35 MT per year [5], and it is reported that over 3 million MT of sewage sludge is produced annually in Malaysia [6]. The dried sewage sludge levels in China reached 11.75 MT in 2019 [7]. It is expected that the production of sewage sludge will continue to increase in the future [8]. Therefore, it is of great significance to properly dispose of and deal with sewage sludge. Since sewage sludge contains many organic and inorganic compounds that can be toxic and harmful, finding a sustainable treatment method is challenging [9]. Research shows that turning sewage sludge into useful products or energy is still a noticeable research trend [10,11].

The current methods used in sewage sludge volume reduction and energy recovery include anaerobic digestion, incineration, pyrolysis, gasification, and others [11,12]. Anaerobic digestion (AD) is a proven sewage sludge treatment technology, and its benefits have been widely recognized [1]. For example, it can greatly reduce the amount of sludge and produce methane, an energy source [13,14]. At the same time, anaerobic digestion liquid can be used as a fertilizer [15]. Therefore, this technology has been well-applied in many countries [16]. For example, in September 2016, the Japanese Cabinet promulgated a revised version of the “Biomass Utilization Promotion Basic Plan”, which advocates sewage sludge as energy because it is a kind of biomass that is stably produced by sewage treatment plants throughout the year and meets the requirements of renewable energy [17].

ASMs and ADM1 models developed by the International Water Association (IWA) are classical dynamic models that are widely used for activated sludge-based wastewater treatment process characterization, design, optimization, and control [18,19]. The solid components of WAS are mainly composed of ordinary heterotrophic biomasses ( $X_{\text{OHO}}$ ) [20], inert particulate organic matter ( $X_{\text{I}}$ ), and unbiodegradable particulates ( $X_{\text{U}}$ ) [21]. Among these,  $X_{\text{OHO}}$  could be transformed into biodegradable particulates ( $X_{\text{S}}$ ) for biogas anaerobic fermentation after inactivation. Previous studies have shown that the structure and performance of the anaerobic bioreactor and the pretreatment of raw materials are important factors in determining the effect of anaerobic fermentation [22–26]. The selection of biofilm carrier materials is necessary to maintain microbial biomass [27]. In previous work, a mobile bed biofilm reactor was used with a sponge as the carrier to simulate the process using a computational fluid dynamics model [28].

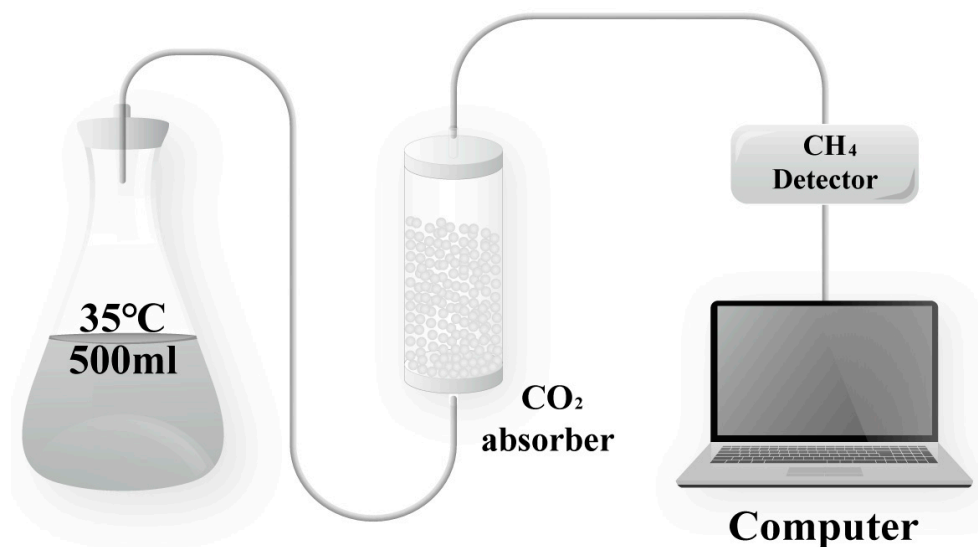
To speed up the digestion process, increase energy output, and reduce sludge volume, sewage sludge pretreatment is often used to increase the rate and volume of biogas production [29]. According to Phothilangka [30], heat pretreatment can destroy the cell wall and release a protein for biodegradation. This not only accelerates the hydrolysis rate of digestion but also improves the dewatering capacity of sludge. Some studies have shown that pretreatment using physical, chemical, and biological methods can promote enzymatic hydrolysis of biomass [31]. However, compared with other pretreatment methods, thermal pretreatment at low temperatures ( $<110\text{ }^{\circ}\text{C}$ ) is more cost-effective for two-stage anaerobic digestion [32]. On the other hand, the concentration of  $X_{\text{OHO}}$  in activated sludge has a certain correlation with seasonal change, which may affect methane production in the anaerobic process using WAS as input material. In this study, MBBR combined with thermal pretreatment was used to explore the decomposition conditions of sewage sludge; the lab-scale anaerobic process of heated WAS was improved. The biodegradable component in WAS was calculated, and the experimental data were simulated using the constructed model based on the activated sludge model. The effect of sludge retention time (SRT) shortening on volatile suspended solids (VSS) digestibility and methane production was well reproduced using simulations. The research results here provide a scientific basis for resource recycling and sustainable development.

## 2. Materials and Methods

### 2.1. Anaerobic Batch Tests

A reactor with a volume of 500 mL was filled with nitrogen and plugged with silicone rubber to prevent air from entering it. The waste-activated sludge (from the Kogasaki sewage treatment plant in Kitakyushu city) and anaerobic digestion sludge (ADS) (from the Hiagari sewage treatment plant in Kitakyushu city) were collected and concentrated regularly. Then, the Chemical Oxygen Demand (COD) concentrations of the two kinds of sludge were measured. Considering the COD concentration value, the ratio of waste-activated sludge and digestion sludge was  $F/M = 0$  or  $0.5$  under the anaerobic condition at  $35\text{ }^{\circ}\text{C}$ , and the biogas production rate was measured with a wet gas meter (Shinagawa Corporation, Japan). When  $F/M$  was  $0$ , the waste-activated sludge (WAS) was not added, but the ordinary heterotrophic organisms ( $X_{\text{OHO}}$ ) contained in the seed sludge were digested to produce biogas. The COD concentrations of the solid component of the raw sludge were

determined as the total organic matter ( $X_T$ ). Figure 1 shows the experimental device used in this study.



**Figure 1.** A schematical representation of an anaerobic batch test device.

## 2.2. Continuous Anaerobic Digestion Experiment

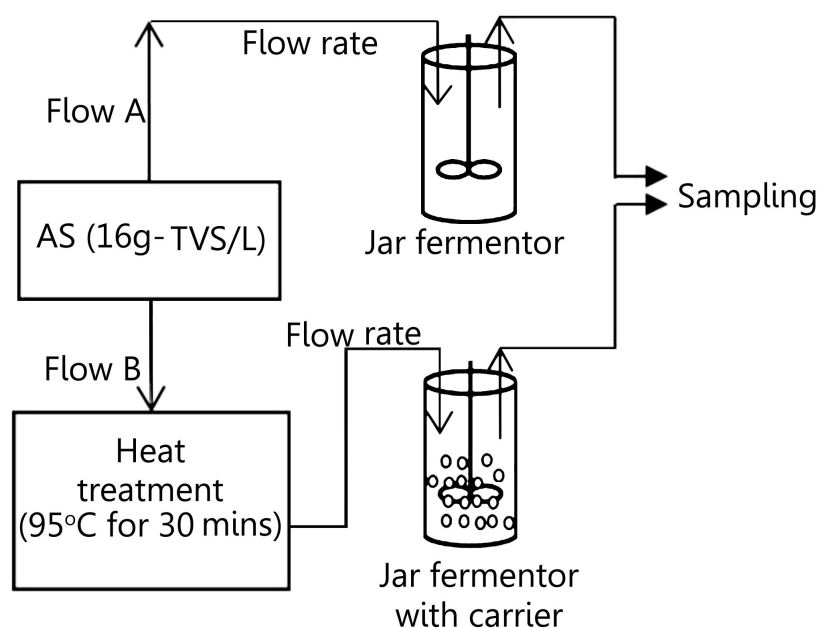
### 2.2.1. Heat Pretreatment

The collected waste-activated sludge was centrifuged and concentrated to about 16 g-TVS/L 0.5–3 times a week from the Kogasaki sewage treatment plant in Kitakyushu City and then heated with Induction Heating (IH) heating equipment. When the water temperature was heated to about 95 °C, the temperature was kept constant for 30 min. After heating, the sludge was first cooled at room temperature, then cooled at 4 °C, and finally stored in a refrigerator. The non-heated waste sludge was stored together with the heat-treated group after concentration as the sludge source for continuous experiments.

### 2.2.2. Anaerobic Long-Term Operation

As shown in Figure 2, two jar-type fermenters with an effective capacity of 3.5 L were used. One of the fermenters was a traditional anaerobic continuously stirred tank reactor (AnCSTR) with a non-heated waste-activated sludge control system, and the other one was a heated waste-activated sludge (heat pretreatment system) fermenter. The heat pretreatment system was used to promote biodegradation. The apparent volume of a hollow cylindrical carrier used in heat pretreatment anaerobic moving bed biofilm reactor (hAMBBR) was 15 mm × 15 mm × 1 mm thickness with a 30% additional amount. The anaerobic digestion sludge (about 8 g-VSS/L) extracted from the Hiagari sewage treatment plant in Kitakyushu city was used as a seed sludge, and the additional amount of waste-activated sludge was gradually changed for the continuous experiment.

The continuous anaerobic experiment was conducted at 35 °C under SRTs of 20, 15, 10, and 5 days for 300 days. The additional amount of the carrier was 30% of the total capacity of the reactor in flow B.



**Figure 2.** A schematical representation of a continuous anaerobic treatment using heated and unheated activated sludge.

### 2.3. Modeling and Simulation

The biomass reaction map for the anaerobic digestion of waste-activated sludge is shown in Figure 3. The stoichiometric coefficients of the reaction system are summarized in Table 1, and the corresponding yield coefficients are defined in the nomenclature. The expanded anaerobic digestion model (ADM1) [33], including the activated sludge model (ASMs) [34], was utilized in the simulation operation using the GPS-X simulation software. To calculate the concentration of  $X_{OHO}$  in WAS and inherent decay ( $b_{OHO}$ ) for simulation, batch tests were carried out with different F/M (waste-activated sludge/seed sludge) ratios at 0 and 0.5, respectively, and the utilized functional expressions are shown in Equations (1) and (2). Considering the death and regeneration, the relationship between  $b_{OHO}$  and decay rate in ASM1 ( $b_{OHO}$ , ASM1) is shown in Equation (3).

**Table 1.** Stoichiometric and component matrix for the model of the anaerobic digestion process.

Component →	$X_{APO}$	$X_{MPO}$	$X_{OHO}$	$S_{MPO}$	$X_S$	$X_U$	$S_{CH_4}$	Reaction Rate( $d^{-1}$ )
Process ↓								
r1 Hydrolysis	$Y_{APO}$			$1 - Y_{APO}$	$-1$			$\frac{4 \cdot X_S \cdot (X_{APO} / (X_{APO} + X_{OHO}))}{(0.035 \cdot X_{APO} + X_S \cdot (X_{APO} / (X_{APO} + X_{OHO})))} \cdot X_{APO}$
r2 Growth of methanogens		$Y_{MPO}$		$-1$			$1 - Y_{MPO}$	$0.055 \cdot S_{MPO} / (30 + S_{MPO}) \cdot X_{MPO}$
r3 Decay of heterotrophic aerobes			$-1$		$1 - f_U$	$f_U$		$b_{OHO} \cdot X_{OHO}$
r4 Decay of methanogens		$-1$			$1 - f_U$	$f_U$		$b_{MPO} \cdot X_{MPO}$
r5 Decay of acidogens	$-1$				$1 - f_U$	$f_U$		$b_{APO} \cdot X_{APO}$
r6 $CH_4$ transfer to gas phase							$-1$	

Parameters:  $f_U = 0.08$ ,  $Y_{APO} = 0.08$ ,  $Y_{MPO} = 0.04$ .

$$MPR_{(F/M=0)} = (1 - f_u) \cdot (1 - Y_{APO}) \cdot (1 - Y_{MPO}) \cdot b_{OHO} \cdot X_{OHO} - ADS \cdot e^{-b_{OHO}t} \quad (1)$$

$$MPR_{(F/M=0.5)} = (1 - f_u) \cdot (1 - Y_{APO}) \cdot (1 - Y_{MPO}) \cdot b_{OHO} \cdot (X_{OHO} - ADS + X_{OHO} - WAS) \cdot e^{-b_{OHO}t} \quad (2)$$

$$b_{OHO,ASM1} = b_{OHO} / [(1 - Y_{OHO}) \cdot (1 - f_u)] \quad (3)$$

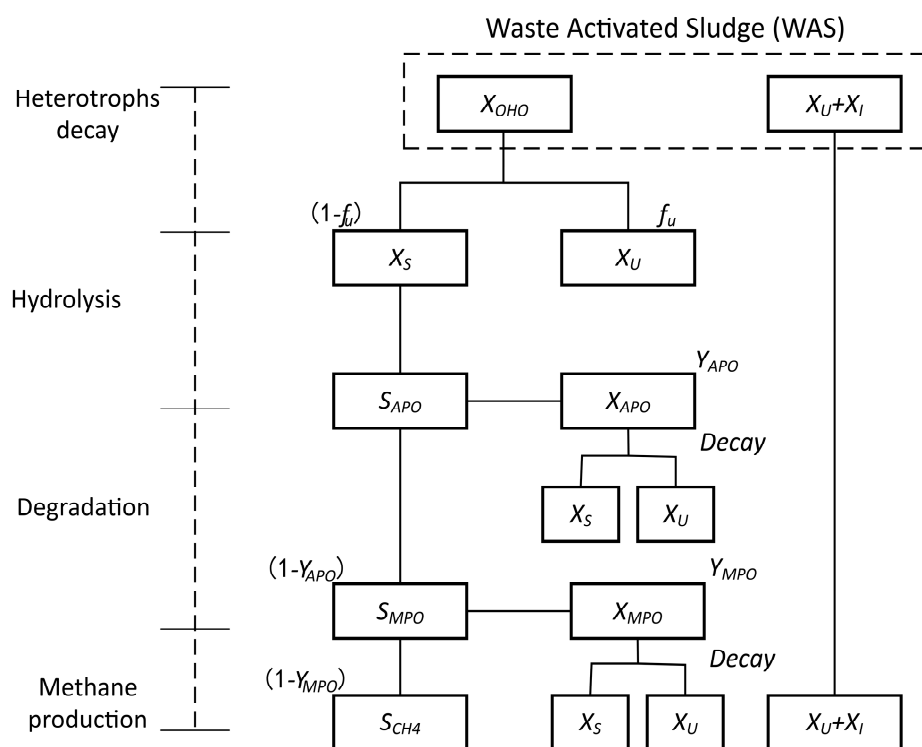


Figure 3. WAS COD biodegradation map in an anaerobic methane fermentation process.

### 3. Results

#### 3.1. Batch Tests for Kinetics

In the anaerobic methane fermentation process shown in Figure 3, the expanded ADM1, including ASMs based on the WAS COD biodegradation map, was utilized for long-term operation simulation. In order to verify the kinetics values for the simulation, several sets of batch tests were performed.

Methane production was affected by biodegradable components in WAS, and the methane production rates at different F/M ratios at 35 °C are shown in Figure 4. When F/M = 0.5, the methane production is higher than that for F/M = 0 due to the simultaneous presence of the biodegradable component  $X_{OHO}$  in WAS and seed sludge. Using Equations (1) and (2), the  $b_{OHO}$  and  $X_{OHO}$  can be calculated by finding the methane production slope of the two sets of batch tests, which were found to be of the same value, as shown in Figure 4.

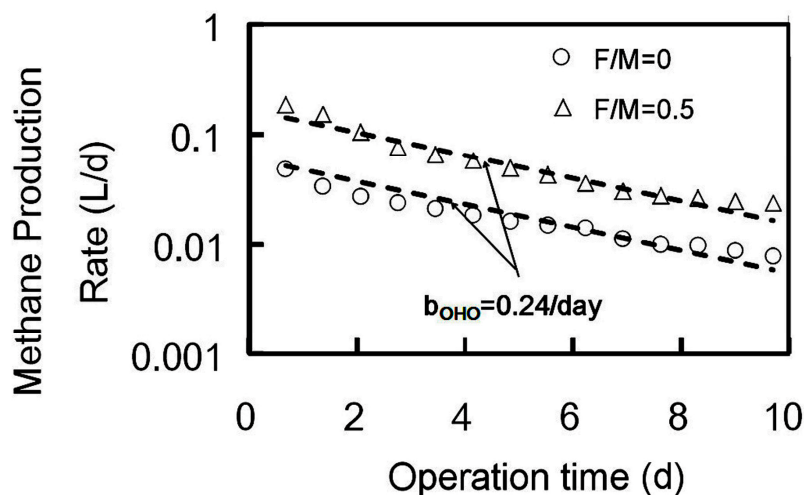


Figure 4. Methane production rate with different F/M ratios.

All simulations were performed on a platform of GPS-X™ v8.0 (Hydromantis Environmental Software Solutions, Inc., Hamilton, ON, Canada). The calculated  $b_{\text{OHO}}$  and  $X_{\text{OHO}}/X_T$  values during the long-term operation were analyzed and are shown in Figures 5 and 6, respectively. For the convenience of observation, the dynamic analog input value of  $b_{\text{OHO}}$  was fixed at a constant value of  $0.25 \text{ d}^{-1}$  ( $b_{\text{OHO, ASM1}} = 0.73 \text{ d}^{-1}$ ) during the batch experiments, and the  $X_{\text{OHO}}/X_T$  ratio (shown in Figure 6) was the result of software simulation. The corresponding kinetics values were obtained from the simulation accordingly.

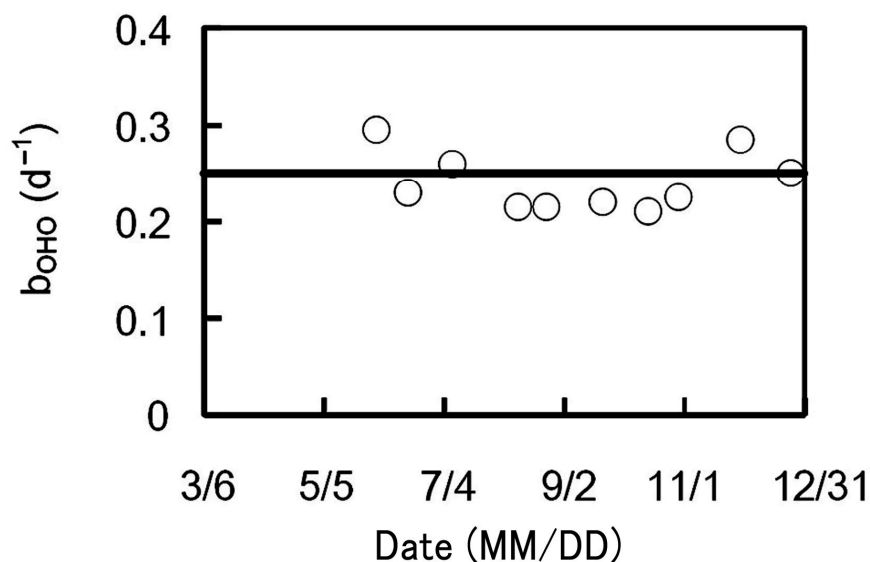


Figure 5. Calculated inherent decay in different seasons.

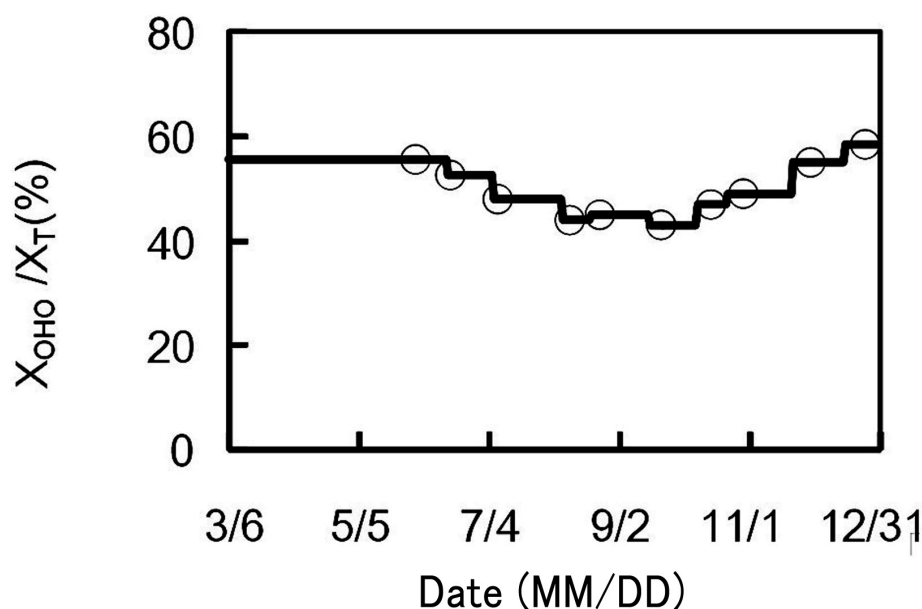


Figure 6. Calculated  $X_{\text{OHO}}/X_T$  ratio in different seasons.

As shown in Figure 5, the batch test results for  $b_{\text{OHO}}$  varied from 0.2 to 0.3. On the other hand, Figure 6 shows that the proportion of  $X_{\text{OHO}}$  during system operation was about 50%. It is clear that from July to October, and due to the high water temperature, the self-digestion rate of the activated sludge treatment system was accelerated, which in turn gradually decreased the proportion of  $X_{\text{OHO}}$  in the collected activated sludge. Among the solid



organic matter in the WAS, the majority of it consisted of the unbiodegradable particulate ( $X_U$ ), produced by the extinction of  $X_{OHO}$ , and the inert particulate organic matter ( $X_I$ ) contained in the water. The  $X_{OHO}$  is the substrate that is utilized in methane production during the anaerobic digestion process. Therefore, the amount of biogas production largely depends on the  $X_{OHO}$  proportions present in the activated sludge that changes every year.

### 3.2. Continuous Anaerobic Digestion of WAS

#### 3.2.1. COD Digestion Performance

The COD balance was calculated using Equation (4):

$$\text{COD input} = \text{COD digested} + \text{COD undigested}. \quad (4)$$

According to the input COD and residual COD after the anaerobic process, the COD digestion amount of AnCSTR and hAMBBR were calculated. As shown in Figure 7, the increase in the input COD amount caused a significant variation in the COD digestion rate, and the hAMBBR system shows higher values than the AnCSTR system.

The amount of biogas produced during anaerobic digestion depends largely on the proportion of biodegradable components in the activated sludge that changes annually. If biomass in  $X_{OHO}$  is decomposed into  $X_s$  and  $X_u$  in advance through some pretreatment, then they will be easily converted into the substrate of the acid-producing bacteria. Since  $X_{OHO}$  lives to prevent the hydrolysis of other microorganisms existing in the anaerobic digestion tank, and considering that death is the rate-limiting step of hydrolysis, it is sufficient to kill the microorganisms through simple pretreatments. This study shows that the microorganisms propagated in the activated sludge process can die rapidly under high-temperature conditions. Therefore, adding the heat-pretreated, waste-activated sludge to the mesophilic anaerobic digester can prevent direct heating of the anaerobic digester, thus reducing energy consumption and enhancing biogas recovery.

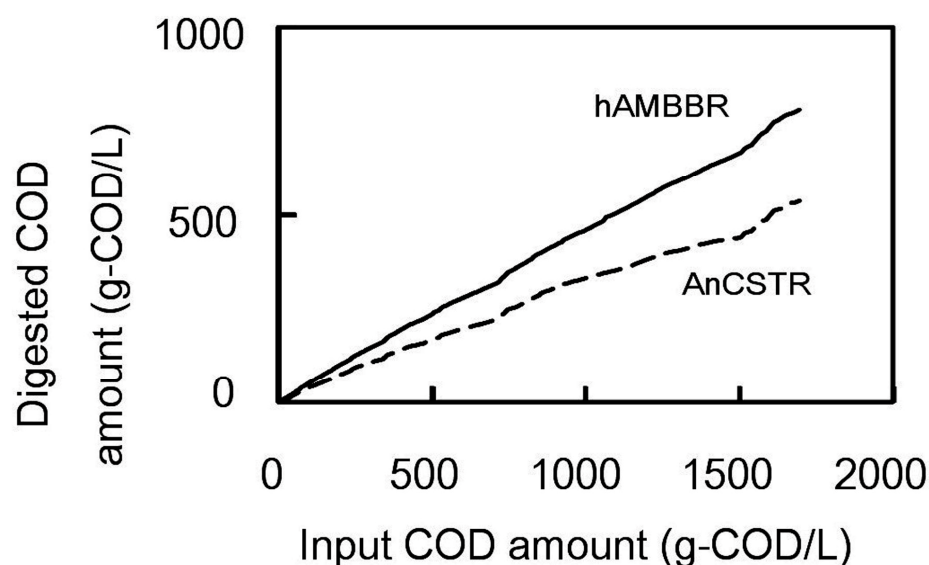
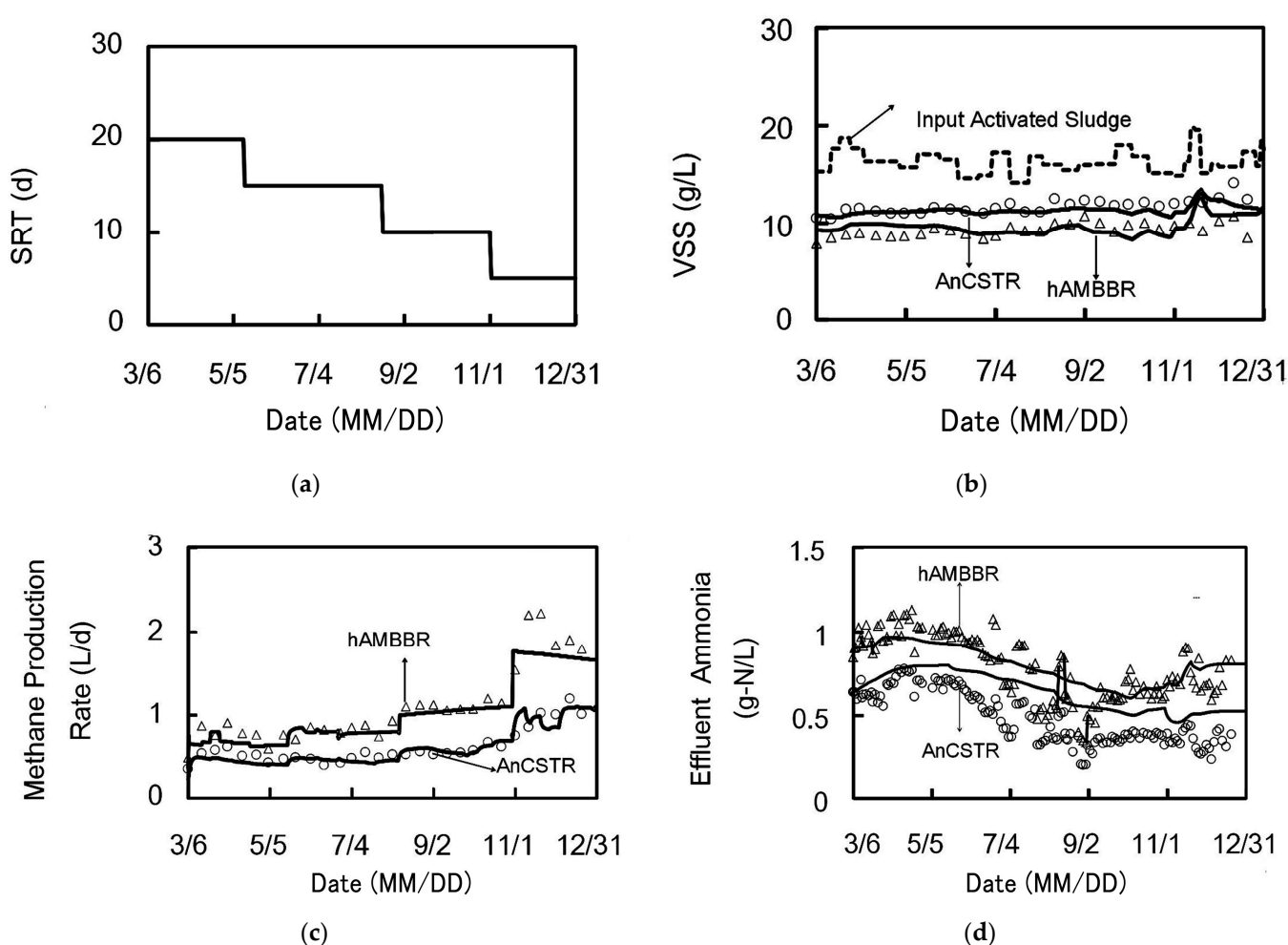


Figure 7. Digestion efficiency in hAMBBR and AnCSTR.

#### 3.2.2. VSS Digestion Performance

The SRT in the long-term operation was controlled by changing the WAS addition, as shown in Figure 8a. Figure 8b shows that the VSS concentration of the hAMBBR system was always lower than that of the AnCSTR system. When SRT = 20 d, the VSS concentration of the hAMBBR system was maintained at about 8 g-VSS/L, while that of the AnCSTR system was about 10 g-VSS/L. This behavior was also observed for SRT = 15 d and SRT = 10 d, and the VSS concentration in the hAMBBR system was significantly reduced when heated.

The digestion rate of VSS during the continuous experiment can be calculated according to the cumulative value of VSS input and the cumulative value of VSS residuals. The calculation results are shown in Table 2. When SRT = 20 d, the VSS digestibility of the AnCSTR system was about 36.3%, and that of the hAMBBR system was about 50.4%. When the SRT was shortened to 15 days, the VSS digestibility of the AnCSTR was about 31.1%, and that of the hAMBBR was about 45.5%. When the SRT was shortened to 10 days, the VSS digestibility of the AnCSTR system decreased to about 25.3%, and that of the hAMBBR system decreased to about 37.8%. Consequently, when SRT = 5 d, the VSS digestibility of the AnCSTR system was about 26.8%, and that of the hAMBBR system was about 43.3%, which is slightly higher than that at 10 days. Although there is some difference between SRT and digestion efficiency, the AnCSTR's average digestion efficiency was around 30%, while the hAMBBR's was around 44% during 300 days of operation, which is 1.5 times that of the AnCSTR system. It can be seen that the heat pretreatment promoted the biodegradation efficiency and the rate of the WAS's anaerobic fermentation process.



**Figure 8.** Simulation results of long-term anaerobic fermentation in AnCSTR and hAMBBR systems. (a) Change in sludge retention time (SRT); (b) variation in volatile suspended solids (VSS); (c) variation in methane production rates (MPR); (d) variation in ammonia in effluent.

### 3.2.3. Variation in Methane and Ammonia Production

As shown in Figure 8a,c, with the reduction of SRT and the increase in the organic loading rate (OLR), the methane production rates (MPRs) of AnCSTR and hAMBBR gradually increased. However, the MPRs in hAMBBR were significantly higher than those of the AnCSTR system. On the other hand, due to the changes in SRT, the MPRs of the heat treatment system substantially changed compared to the AnCSTR, which is shown in the



following. When SRT changed from 20 d to 10 d, the MPRs of the two reactors showed little difference, and the change range was between 0.5 L/d and 0.7 L/d. When SRT was shortened to 5 d, the difference in methane output between hAMBBR and AnCSTR suddenly increased to 1 L/d due to the increase in WAS input, whereas the MPRs of hAMBBR appeared to be around twice that of AnCSTR.

The ammonia concentrations in the two systems were also compared, as shown in Figure 8d. The gradual reduction of SRT led to a gradual reduction in the formation of ammonia. Since ammonia was produced in the process of  $X_{\text{OHO}}$  degradation, the ammonia content in hAMBBR was higher than that in AnCSTR, which also indicates that the thermal pretreatment promoted the biodegradation process.

### 3.2.4. Promoting Effect of Heat Pretreatment on Methane Fermentation

As shown in Figure 3, the transformation of biodegradable COD can be divided into four stages: heterotrophic decay, hydrolysis, degradation, and methanogenesis. Several related processes have been covered in the literature [19,35]. In the anaerobic digester, the ordinary heterotrophic organism ( $X_{\text{OHO}}$ ) is decomposed into biodegradable particulate ( $X_{\text{S}}$ ) and unbiodegradable particulate ( $X_{\text{U}}$ ) through self-digestion. The biodegradable solids are hydrolyzed in the reactor and converted into the substrate ( $S_{\text{APO}}$ ) of acidogens ( $X_{\text{APO}}$ ). According to acetogenesis, part of  $S_{\text{APO}}$  is assimilated into biomass, and the rest is decomposed into the substrate of methanogen ( $S_{\text{MPO}}$ ). In addition, the acidogens and methanogens are also decomposed into  $X_{\text{S}}$  and  $X_{\text{U}}$  through self-digestion.

However, the living ordinary heterotrophic organisms can prevent the hydrolysis of other microorganisms in the anaerobic digester, which should be easily converted into the substrate of acidogens if they are decomposed into  $X_{\text{S}}$  and  $X_{\text{U}}$  through pretreatment. Considering all the treatments, it is only necessary to make microorganisms die. The search data show that thermolysis pretreatment is the most widely used technology in sludge hydrolysis [36,37], and the microorganisms prolife rating in the activated sludge will be rapidly inactivated under high-temperature conditions. Therefore, it is possible to heat the waste-activated sludge to facilitate its digestion.

**Table 2.** VSS digestion rate in AnCSTR system and hAMBBR system.

	SRT:20 d (3/6~5/18)	SRT:15 d (5/19~8/6)	SRT:10 d (8/7~10/29)	SRT:5 d (10/30~12/6)
AnCSTR	36.3%	31.1%	25.3%	26.8%
hAMBBR	50.4%	45.5%	37.8%	43.3%

## 4. Discussion

Anaerobic digestion refers to a digestion technique in which organic matter is decomposed into  $\text{CH}_4$ ,  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ , and  $\text{H}_2\text{S}$  by facultative bacteria and anaerobic bacteria under anaerobic conditions [19]. Anaerobic digestion is widely used in biogas engineering technologies such as sewage, livestock and poultry manure, and urban organic waste treatment, which is conducive to the development of a circular economy, environmental protection, reduction of greenhouse gas emissions, and the production of renewable energy [38]. The influencing factors of anaerobic digestion mainly include material properties, digestion temperature, Carbon-to-Nitrogen ratio (C/N), pH, organic loading rate (OLR), etc. [39,40]. In the process of the anaerobic digestion of sludge, it may be possible to release organic matter and promote the hydrolysis rate of sludge through certain pretreatment techniques, such as destroying cell structures, thereby improving the anaerobic digestion performance of sludge and continuously enhancing the economy and availability of sludge anaerobic digestion technology [41,42].

Methane production is one of the key criteria to evaluate the effectiveness of anaerobic fermentation. As shown in Figure 8c, the average methane production rate of the waste sludge after thermal pretreatment increased by 45% compared to the control group. Cano et al. reported similar experimental results wherein the specific methane production rate of

sludge increased from 184 mL CH<sub>4</sub>/gVSSin to 278 mL CH<sub>4</sub>/gVSSin thermal hydrolysis [43]. In contrast, the concentration of VSS in hAMBBR has been consistently lower than in the control group, with a difference of about 44%, see Figure 8b. The above conclusions are consistent with the research results of El-Mashad et al. [44] and Zhang et al. [45] Accordingly, it was concluded that the conversion of thermally pretreated sludge to methane was more obvious than that of the control group.

After thermal pretreatment, the methane production rate of sludge increased significantly, indicating that its residence time in sludge was shortened and methane production rate was increased, as shown in Figure 8a,c. Obviously, the methane production rate of fresh sludge remained within 1 L/d, while that of the heat-pretreated sludge increased to 1 L/d on the 160th day. Subsequently, the rate gradually increased to the level of 2 L/d on the 240th day until the end of the experiment. At this stage, the sludge retention time (SRT) was reduced to 5 days. According to the research of Mottet et al. and Prorot et al., the rapid methanogenic release in the early stage of hydrolyzed sludge fermentation is directly related to the release of intracellular organic matter, and the extracellular enzyme adsorption area increased by the rupture of sludge flocs increased the reaction rate [46,47].

Based on the dynamic simulation software GPS-X 8.0 of a commercial sewage treatment plant, the anaerobic fermentation process of waste sludge was simulated by extending ASMs and an ADM1 model. The batch experimental data suggest that the extended anaerobic digestion model developed can reasonably reproduce the ammonia nitrogen concentration, VSS concentration, and methane production rate in the 300-day anaerobic fermentation process. This proves that the extended model based on ASMs and ADM1 can accurately express the reaction process of anaerobic fermentation of waste sludge. Except for the effect of reactor and thermal pretreatment on X<sub>OH</sub>O degradation and methane production, the mechanism of other influencing factors such as pH value, ionic strength, and C/N ratio on the process has not been discussed. How to improve resource and energy conversion efficiency and system stability is an important task in the field of anaerobic digestion technology, and it is also a bottleneck in the promotion and application of anaerobic digestion technology [48,49]. In the future, the mechanism of various influencing factors on anaerobic fermentation of waste sludge will be further developed, and a more mature model will be built to achieve the goal of high-speed anaerobic fermentation.

## 5. Conclusions

In this study, the extended ADM1 including the ASMs model was used to explore the anaerobic digestion process of waste-activated sludge (WAS) at a moderate temperature. The parameters obtained through batch experiments were used as the input values, and the effect of the continuous operation was simulated with GPS-X software. The results can be summarized as follows:

Firstly, when the waste-activated sludge was heated up to 95 °C for 30 min, the digestion rate of VSS in the hAMBBR system increased to about 44%, which is 1.5 times the rate in the contrast reactor. Even though SRT was shortened from 20 days to 5 days, its digestibility did not show great modifications.

Secondly, the methane production of hAMBBR increased with the reduction of SRT, and the average conversion rate of methane could be increased by up to 45%. When the SRT was shortened to 5 days, the methane production was twice that of the control reactor.

Thirdly, the simulation results showed that the extended model developed from the batch experimental data could well reproduce the effect of SRT shortening on digestibility and methane production in the experiment lasting 300 days.

Finally, the concentration of X<sub>OH</sub>O in the activated sludge showed a certain correlation with seasonal changes, which may affect methane production in the anaerobic process using WAS as the input material.

**Author Contributions:** Conceptualization, H.Y. and M.T.; methodology, B.L.; software, R.G.; writing—original draft preparation, J.W.; writing—review and editing, Y.L.; visualization, B.X.; supervision, B.L.; project

administration, R.Z.; funding acquisition, F.C. All authors have read and agreed to the published version of the manuscript.

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**Informed Consent Statement:** Informed consent was obtained from all subjects involved in the study.

**Data Availability Statement:** The authors confirm that the data supporting the findings of this study are available within the article.

**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

## Nomenclature

$b_{\text{OHO}}$	The specific decay rate of an ordinary heterotrophic organism ( $\text{d}^{-1}$ )
$b_{\text{OHO}}, \text{ASM1}$	The specific decay rate in activated sludge model No.1 ( $\text{d}^{-1}$ )
$e$	A natural constant value
$F/M$	sludge loading ( $\text{kg (BOD)}/[\text{kg (MLSS)} \cdot \text{d}]$ )
$f_u$	A fraction of biomass leading to particulate organic inert product ( $\text{g-COD}/\text{g-COD}$ )
$S_{\text{APO}}$	The substrate for Acidogens ( $\text{mg-COD}/\text{L}$ )
$S_{\text{MPO}}$	The substrate for Methanogen ( $\text{mg-COD}/\text{L}$ )
$S_{\text{CH}_4}$	Methane ( $\text{mg-COD}/\text{L}$ )
$\text{SRT}$	Sludge retention time (d)
$t$	Time (d)
$T$	Temperature ( $^{\circ}\text{C}$ )
$\text{TVS}$	Total volatile solids ( $\text{g}/\text{L}$ )
$V$	Reaction volume (L)
$\text{VSS}$	Volatile suspended solids ( $\text{g}/\text{L}$ )
$X_{\text{APO}}$	Acidogens ( $\text{mg-COD}/\text{L}$ )
$X_{\text{OHO}}$	Ordinary heterotrophic organism ( $\text{mg-COD}/\text{L}$ )
$X_{\text{MPO}}$	Methanogen ( $\text{mg-COD}/\text{L}$ )
$X_{\text{OHO-WAS}}$	Ordinary heterotrophic organisms in WAS ( $\text{mg-COD}/\text{L}$ )
$X_{\text{OHO-ADS}}$	Ordinary heterotrophic organisms in ADS ( $\text{mg-COD}/\text{L}$ )
$X_{\text{I}}$	Inert particulate organic matter ( $\text{mg-COD}/\text{L}$ )
$X_{\text{S}}$	Biodegradable particulate ( $\text{mg-COD}/\text{L}$ )
$X_{\text{T}}$	Total organic matter ( $\text{mg-COD}/\text{L}$ )
$X_{\text{U}}$	Unbiodegradable particulate ( $\text{mg-COD}/\text{L}$ )
$Y_{\text{APO}}$	The yield of $X_{\text{APO}}$ ( $\text{mg-COD}/\text{mg-COD}$ )
$Y_{\text{MPO}}$	The yield of $X_{\text{MPO}}$ ( $\text{mg-COD}/\text{mg-COD}$ )

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