processes

MDPI

Article

Enhancing Single- and Two-Stage Anaerobic Digestion of Thickened Waste-Activated Sludge through FNA-Heat Pretreatment

Salomeh Chegini and Elsayed Elbeshbishy *

Department of Civil Engineering, Faculty of Engineering, Architecture and Science, Toronto Metropolitan University (Formerly Ryerson University), 350 Victoria Street, Toronto, ON M5B 2K3, Canada; salomeh.chegini@torontomu.ca

* Correspondence: elsayed.elbeshbishy@ryerson.ca; Tel.: +1-(416)-979-5000 (ext. 557618)

Abstract: This study aimed to investigate the effect of combined Free Nitrous Acid (FNA)-Heat (i.e., FNH) pretreatment on single- and two-stage anaerobic digestion (AD) of thickened waste-activated sludge (TWAS). Single-stage AD was conducted in batches, while two-stage AD involved acidogenic fermentation under semi-continuous flow followed by batch methanogenesis. FNH pretreatment was applied before the acidogenic stage, using 1.4 mg HNO_2-N/L FNA concentration at 25 °C, 37 °C, and 60 °C for 24 h. Among the scenarios, the most promising results were observed with two-stage AD fed with FNH-pretreated TWAS at 60 °C, showing higher COD solubilization and a reduction in volatile solids. Combined FNA-Heat pretreatment in two-stage AD yielded elevated methane production (363–415 mL CH_4/g VS added) compared to single-stage digestion. Methane yields from FNA-Heat pretreated single-stage ranged from 332 to 347 mL CH_4/g VS added, contrasting with 212 mL CH_4/g VS added for untreated TWAS. Methane generation commenced early in both untreated and pretreated samples, attributed to soluble substrate abundance.

Keywords: two-stage anaerobic digestion; thermochemical pretreatment; acidogenic fermentation; solubilization; methane production; thickened waste-activated sludge

Citation: Chegini, S.; Elbeshbishy, E. Enhancing Single- and Two-Stage Anaerobic Digestion of Thickened Waste-Activated Sludge through FNA-Heat Pretreatment. *Processes* **2024**, *12*, 345. https://doi.org/10.3390/pr12020345

Academic Editors: Farshid Torabi and Sabeti Morteza

Received: 3 October 2023
Revised: 19 January 2024
Accepted: 24 January 2024
Published: 6 February 2024

1. Introduction

In the realm of wastewater treatment, anaerobic digestion stands as a recognized technology for stabilizing municipal sludges and harnessing methane gas. Traditionally, the anaerobic digester processes a mixture of primary (PS) and thickened waste-activated sludge (TWAS). While PS is readily digestible, TWAS poses a challenge due to its slower digestion rate [1]. In the case of TWAS digestion, the initial step, hydrolysis, often becomes the bottleneck. The intricate aggregation of microorganisms and extracellular polymeric substances (EPS), alongside the bound water content, necessitates extended treatment times and leads to limited rates and yields of biogas production [2].

To tackle these challenges, various pre-treatment (PT) techniques have emerged to enhance sludge solubilization and disintegration. These techniques span mechanical, thermal, chemical, and biological processes [3], all aimed at breaking down EPS and cell membranes, thereby releasing intracellular and extracellular components that are more readily biodegradable during AD digestion. However, many of these methods prove cost-intensive due to chemical and energy demands, coupled with adverse environmental impacts [4].

Considering this backdrop, the biorefinery concept emerges as a beacon of innovation, poised to not only address the challenges intrinsic to anaerobic digestion but also to redefine the paradigm of waste-to-resource conversion. The essence of the biorefinery approach lies in its potential to extract multifaceted value from sludge's intricate composition, transforming it into a gamut of valuable outputs, including biofuels and bioproducts [5–7].

By integrating diverse PT techniques within the framework of a biorefinery, the inherent complexity of sludge can be strategically harnessed to yield an array of products, effectively minimizing waste and optimizing resource utilization. In alignment with the global thrust toward circular economies, where resource optimization and environmental consciousness hold sway, the biorefinery ethos offers a compelling pathway [7].

The incorporation of biorefinery principles not only surmounts the challenges posed by traditional anaerobic digestion but also metamorphoses them into platforms for resource efficiency, economic viability, and environmental stewardship. Through the prism of the biorefinery paradigm, sludge transitions from being a challenge to an untapped resource, embodying the core tenets of sustainable and integrated waste management practices [5,6].

A promising contender in this arena is free nitrous acid (FNA), a biocidal chemical agent that exists in equilibrium with nitrite in an acidic environment. FNA holds the potential for advancing the circular economy, as it can be generated through partial nitritration of ammonium-rich digestate liquor [8]. Prior studies have investigated FNA's effects on enhancing sludge biodegradability. Wang et al., for instance, demonstrated that FNA pre-treatment at concentrations of 0.4–2.1 mg HNO_2-N/L for 24 h increased methane production by 10–30% over a 20-day digestion of secondary sludge. FNA was found to accelerate hydrolysis and boost methane potential [9]. Yet, solely relying on FNA for anaerobic digestion does not meet the requirements for delivering class A biosolids [10]. Hence, integration with other techniques or further treatment becomes necessary. For instance, when combined with low thermal energy (FNH), FNA has demonstrated the capability to inactivate fecal coliforms [10]. Moreover, rooted in thermodynamics and the Arrhenius equation, higher temperatures have been proven to accelerate chemical reactions [10–12].

Taking this into consideration, the concept of combined FNA and thermal pre-treatment emerges, with the potential to expedite the degradation of carbohydrates, lipids, proteins, and DNA by leveraging the effects of FNA and its derivatives (e.g., NO, N_2O_3, and NO_2). This fusion could lead to even greater methane production during AD, surpassing the outcomes of sole FNA pre-treatment. Notably, the availability of heat energy in wastewater treatment plants (WWTPs) equipped with anaerobic sludge digestion and cogeneration makes this approach feasible [13]. Interestingly, while combined FNA with heat pretreatment (FNH) has not been extensively studied in the context of the AD process, its potential effectiveness suggests a straightforward implementation.

In earlier studies that employed FNA, the digestion process predominantly comprised a single-stage methanogenic reactor. However, emerging insights highlight the potential benefits of physically dividing the process into two stages (staged AD), which promises improved bacterial metabolisms, encompassing acidogenesis and methanogenesis, in conducive environments [14]. Combining FNH with staged anaerobic digestion, a process that divides AD into two stages, offers a chance to optimize bacterial metabolisms [15,16].

Against this backdrop, the present study hypothesized that the conjunction of FNH pretreatment with the staged AD process could optimize the hydrolysis/fermentation phase in the acidogenic digester, consequently elevating biomethane production. To date, no information has been available regarding the application of such a strategy to enhance fermentation and bioenergy recovery from FNH-pretreated TWAS. Hence, the central objective of this research was to evaluate the impact of combined FNH pretreatment on the enhancement of single- and two-stage AD of TWAS. It's noteworthy that this study specifically explores the integration of FNH pretreatment prior to the acidogenic stage (first stage) of the two-stage AD process.

The study comprised three distinct phases, each focusing on evaluating FNH pretreatment performance, acidogenic digester performance, and methanogenic digester performance. Throughout each phase, two systems operated in parallel: one employed FNH-pretreated sludge (PT-TWAS) as the test system, while the other used raw TWAS as the control system. The overarching aim was to comprehensively compare the effects of PT on the performance of single-stage and two-stage digestion in terms of methane production.

2. Materials and Methods

2.1. Chemicals

All reagents and chemicals were used as received without further purification and were supplied either by VWR or Sigma-Aldrich. Sodium nitrite $\geq$ 97.0%, crystals ACS (MW: 69 g/mol), were purchased from VWR, Canada, and pre-determined amounts of sodium nitrite stock solution were used for FNA pretreatment.

2.2. Substrate and Inoculum

In this study, samples of Thickened Waste-Activated Sludge (TWAS) and inoculum were obtained from the Ashbridge Bay Wastewater Treatment Plant, a prominent facility managed by the City of Toronto, Ontario, Canada. This plant, with a capacity of 818 ML/day (818,000 m^3/day), serves a population of approximately 1.6 million Toronto residents [17]. TWAS samples were collected from the outflow of the flotation tanks. Feed sludge samples were collected weekly and stored separately at 4 °C. Daily feedstock volumes were taken about two hours before feeding. Standard laboratory analyses were conducted to assess the physical, biochemical, and biodegradable characteristics of the inoculum, raw, pretreated, and digested samples.

Pretreatment experiments exclusively targeted the TWAS, while the digesters were fed with either raw TWAS or pretreated TWAS. The primary characteristics of TWAS (with standard errors from triplicate measurements) were as follows: total solids (TS) 36.2 $\pm$ 0.7 g/L, volatile solids (VS) 25.8 $\pm$ 0.5 g/L, total chemical oxygen demand (TCOD) 43.6 $\pm$ 1.6 g/L, soluble chemical oxygen demand (SCOD) 2.2 $\pm$ 0.2 g/L, and pH of 6.7 $\pm$ 0.1.

For the subsequent Biochemical Methane Potential (BMP) tests elaborated in Section 2.5, the inoculum was procured from the effluent of anaerobic digesters operating under mesophilic conditions within a temperature range of 34–38 °C. These digesters received around 6500 m^3/day of primary sludge and 1600 m^3/day of TWAS. The average Solid Retention Time (SRT) and organic loading rate were 18 days and 1.1 kg VS/m^3, respectively. The primary characteristics of the inoculum (with standard errors obtained through triplicate measurements) were TS 22.7 $\pm$ 1.5 g/L, VS 14.6 $\pm$ 0.8 g/L, TCOD 16.9 $\pm$ 1.5 g/L, SCOD 0.8 $\pm$ 0.09 g/L, and pH of 7.1 $\pm$ 0.1. Additionally, BMP testing was carried out on both raw and pretreated TWAS to assess biodegradability. The inoculum underwent degassing for seven consecutive days under mesophilic conditions before the commencement of the BMP test [14].

2.3. Pretreatment

As outlined in Table 1, the pretreatment phase involved the utilization of three reactors, each with a working volume of 2 L. The process encompassed subjecting raw TWAS samples to FNA pretreatment within continuously stirred reactors, conducted at three distinct temperatures: 25, 37, and 60 °C. The selection of FNA dosage range, pH, and temperature was based on preliminary experiment results and a thorough literature review from earlier studies [10,17,18]. These considerations align with the broader aim of promoting sustainability in waste treatment practices. The goal was to minimize energy usage (opting for lower temperatures) and reduce chemical dosage, making the process environmentally friendly and economically feasible.

Table 1. FNA-Heat Pretreatment conditions before the acidification process.

Sample ID#	~FNA (mg HNO_2-N/L)	Temperature (°C)	pH
Raw TWAS (Control)	0	24 $\pm$ 1	6.7 $\pm$ 0.1
PT-25	1.4	24 $\pm$ 1	5.5 $\pm$ 0.1
PT-37	1.4	37 $\pm$ 1	5.5 $\pm$ 0.1
PT-60	1.4	60 $\pm$ 1	5.5 $\pm$ 0.1

Each experiment spanned a duration of 24 h. Initial adjustment of the sample's pH to a target of 5.5 ± 0.1 was accomplished using a solution of 1.0 M HCl, and the pH was not actively controlled during the pretreatment phase. In the case of FNA pretreatment, meticulous preparation was undertaken for a nitrite stock solution (5 g N/L) utilizing sodium nitrite salt ($NaNO_2$). This prepared stock solution was then carefully introduced into the batch reactors to attain the desired FNA concentration of 1.4 mg HNO_2-N/L.

With due consideration to the pH and temperature conditions, the necessary volume of the stock solution was calculated employing the equations governing acid equilibrium (Equations (1) and (2)).

$$S[HNO_2 - N] = \frac{S[NO_2 - N]}{(K_a \times 10^{pH})} \quad (1)$$

$$K_a = \frac{e^{-2300}}{273 + T} \quad (2)$$

where: K_a, ionization constant; T, temperature (°C), and S, the concentration stated as nitrogen [10].

Following the pH adjustment process and the addition of the specified FNA dosage, the samples underwent thermal pretreatment with an exposure time of 24 h. This thermal pre-treatment was executed within a Grant Combined Orbital/Linear Shaking Water Bath, specifically the Model OLS200, manufactured by Grant Instruments (Royston, UK), with gentle agitation at approximately 150 rpm. The water bath's temperature was set to three distinct levels: 25 ± 1, 37 ± 1, and 60 ± 1 °C. The sludge was gently mixed at a range of 100–150 rpm to ensure uniformity throughout the retention period. As a point of comparison, control experiments were carried out without any pH adjustment or nitrite addition, and these control experiments were conducted at room temperature.

2.4. Hydrolysis/Acidification Experiment–Semi-Continuous

Four semi-continuous glass bioreactors, each with a 2 L operating volume, were employed—one for control and three for pretreated samples. Schematic diagrams illustrating the experimental setups for the acidification/fermentation process in the control and pretreated systems are shown in Figures 1 and 2 respectively.

Within the control system, raw TWAS (R-TWAS) underwent semi-continuous fermentation with a 3-day solid retention time (SRT), yielding fermenter effluent referred to as F-TWAS. In the pretreatment systems, effluents from the pretreatment units underwent subsequent semi-continuous fermentation processes.

Stirred fermenters were independently fed semi-continuously (once per day) with raw and FNA-heat pretreated TWAS. The volume of the added substrate and its ratio to the inoculum were adjusted to sustain an organic loading rate (OLR) of 1 g-TCOD/g-VSS per day. Before sealing the fermentation reactors with rubber stoppers, the reactor content was flushed with nitrogen gas for a minimum of 5 min to establish anaerobic conditions. The pH was maintained at 5.5 ± 0.1 by adjusting the feed pH using sodium hydroxide (NaOH) or hydrochloric acid (HCl). These acidification/fermentation tests took place in a mesophilic water bath set at 37 °C ± 1, with continuous stirring at 150 rpm using impellers.

Daily, 500 mL of fermented effluent was withdrawn, followed by replenishment with 500 mL of either raw or pretreated TWAS, resulting in a 3-day hydraulic retention time (HRT). A digital drive tubing pump (Masterflex L/S® Digital Drive with PTFE-Tubing Pump Head supplied from Avantor Fluid Handling, Devens, MA, USA) facilitated decanting and feeding.

The fermentation process was initially extended for 12 days (equivalent to 4 HRT) to attain a steady-state condition (where parameter variations were under 10%). This state was then sustained for an additional 12 days, resulting in a total fermentation period of 24 days. Throughout the experiment, water levels in the water bath were maintained daily due to evaporation. In the final week of fermentation, effluents from the fermenters (fermented

TWAS and fermented-pretreated TWAS) were collected daily to serve as feed substrates for the subsequent BMP test.

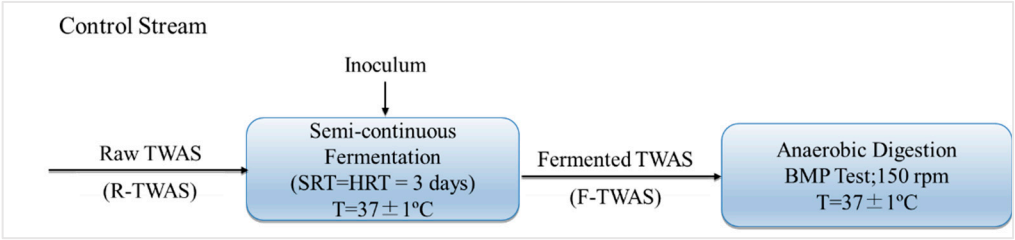

Figure 1. Experimental design of control system.

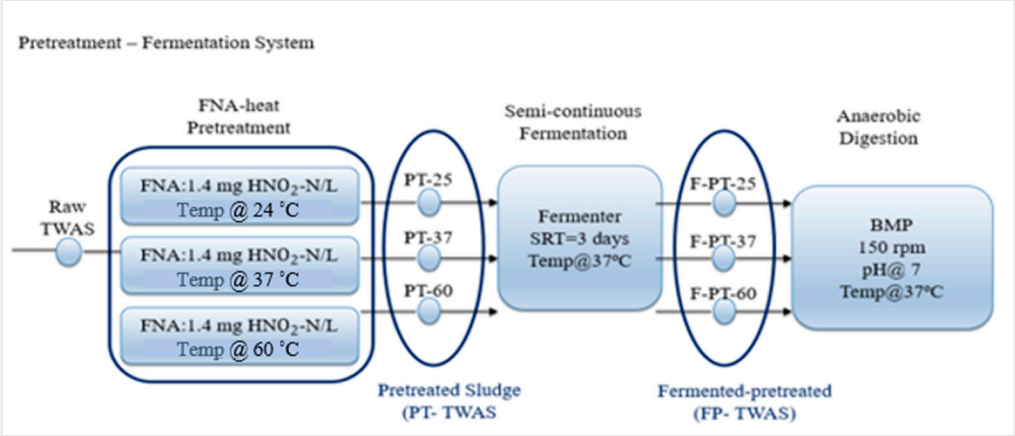

Figure 2. Experimental design of FNA-heat pretreatment and semi-continuous acidification/fermentation of control system.

2.5. BMP

Biochemical methane potential (BMP) tests represent a pivotal experimental approach for evaluating and contrasting the kinetics and methane-yielding potential of diverse substrates throughout anaerobic degradation processes. These tests were executed in batch mode, following the established protocol outlined by Angelidaki et al. [19,20]. All tests were meticulously conducted in triplicate, utilizing 150 mL of inoculum sourced from the Ashbridge WWTP. The food-to-microorganism (F/M) ratio was approximately 1 for each experimental iteration. Prior to the initiation of BMP testing, a 7-day degassing period was observed for the inoculum. Additionally, to establish an accurate baseline for methane production from the substrate, three blank digesters containing solely 150 mL of inoculum (i.e., devoid of the substrate) were established.

Inoculums are instrumental in initiating the digestion process and minimizing any lag phase, consisting of an active anaerobic culture introduced to the digesters. Ensuring anaerobic conditions, the substrate and inoculum were meticulously mixed. Subsequently, the bottles were hermetically sealed using butyl rubber stoppers and aluminum crimp caps after a nitrogen gas flush (maintained for 3 to 5 min at 5 to 10 psi). The sealed bottles were then placed within a Thermo Scientific Benchtop Orbital shaker, specifically the MaxQ model. To simulate mesophilic conditions in line with the batch reactor setup, the incubator temperature was maintained at 37 ± 1 °C. Throughout the procedure, a rotational speed of 150 rpm was sustained. The BMP experiments extended until biogas generation diminished to negligible levels, typically spanning around 44 days. Over the course of the experiments, methane production was gauged daily or every alternate day. The quantification of methane production from the sludge was ascertained by subtracting biogas production from the blank samples, and the outcome was expressed as the volume of methane generated per gram of VS added (mL CH_4/g VS added).

2.6. Analytical Methods

To execute the soluble analysis encompassing all tests except those concerning solids and total COD, the raw, pretreated, and digested TWAS samples underwent filtration utilizing a 0.45 µm VWR Vacuum Filtration System, specifically the Model 10040-462 (VWR, Radnor, PA, USA). To expedite the filtration process, samples were appropriately diluted with DDW (double-distilled water). All analyses were meticulously carried out either in duplicate or triplicate to ensure robust results.

The quantification of parameters, including total solids (TS), volatile solids (VS), total chemical oxygen demand (TCOD), and soluble chemical oxygen demand (SCOD), was executed in accordance with established standard protocols [21]. For absorbance measurements, a HACH spectrophotometer (model 3900) (HACH, Ames, IA, USA) was employed. The determination of total volatile fatty acids (VFAs) was achieved through a calorimetric approach using the TNT 872 kit, following the prescribed HACH procedure. In detail, the method involved the systematic collection of a well-mixed sample, preparation of reagents from the TNT 872 kit, and calibration of the calorimeter using known VFA standards. The actual colorimetric reaction occurred within a designated chamber, leading to a discernible color change proportional to the VFA concentration. Precise measurement of this color intensity was facilitated using the HACH spectrophotometer, ensuring robust and reliable data analysis. The overall methodology upheld stringent quality control measures, including blanks and replicates, to fortify the accuracy of results.

As for the measurement of biogas volume, a manual approach was adopted involving a 100 mL Poulten and Graf Fortuna™ (Wertheim, Germany) air-tight glass syringe. The measured biogas volume was subsequently normalized to standard pressure and temperature conditions (STP; 1 atm and 0 °C) [14].

2.7. Performance Analysis

The efficiency of suspended solids reduction (R) achieved through different samples (i.e., pretreated, fermented, and fermented pretreated) is calculated using Equation (3):

$$\text{Solids Solubilization (R \%)} = ((\text{SSi} - \text{SSt})/\text{SSi}) \times 100 \quad (3)$$

where SSi represents the suspended solids (TSS and VSS) concentration before treatment in g/L, and SSt represents the suspended solids concentration after treatment in g/L.

The extent of COD solubilization, expressed as the percentage conversion of particulate COD (PCOD) to soluble COD (SCOD), is calculated using Equation (4).

$$\text{COD solubilization (\%)} = (\text{SCOD}_f - \text{SCOD}_i)/\text{PCOD}_i \times 100 \quad (4)$$

where $SCOD_f$ and $SCOD_i$ are the SCOD concentrations of TWAS after and before pretreatment, respectively. PCODi was the initial particulate COD. The particulate COD was considered the difference between TCOD and SCOD.

The biodegradability of TWAS was quantified by comparing the total methane production to the theoretical yield from fully biodegradable organic material (as per Equation (5)).

$$\text{Biodegradability (\% BD)} = (B_0 \text{ measured}/B_0 \text{ theoretical}) \times 100 \quad (5)$$

where B_0 Measured is experimental ultimate methane production and, B_0 theoretical is the theoretical methane potential based on the initial TCOD of the substrate (398 mL/g TCOD added at 37 °C).

3. Results and Discussion

3.1. Disintegration of TWAs through FNA Pretreatment and Fermentation Process

3.1.1. Suspended Solid Reduction

This study section focuses on comparing the impact of combined FNA-heat pretreatment on the reduction of sludge-suspended solids with that of the fermentation process,

specifically when the pretreatment is applied before fermentation. Figure 3 illustrates the concentrations of suspended solids (TSS and VSS) in the raw and processed (fermented and/or pretreated) TWAS samples.

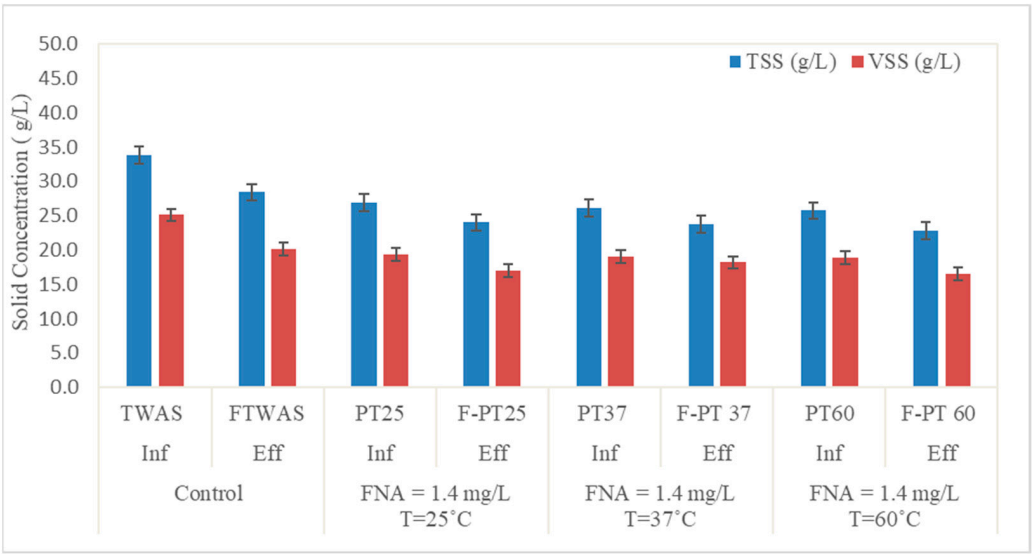

Figure 3. Concentration of the raw, pretreated, and fermented sludge; Combined FNA-heat pretreatment (error bars represent the standard errors).

The efficiency of suspended solids reduction (R) achieved through pretreatment (PT), fermentation (F), or fermented pretreated (FP) samples is further compared in Figure 4.

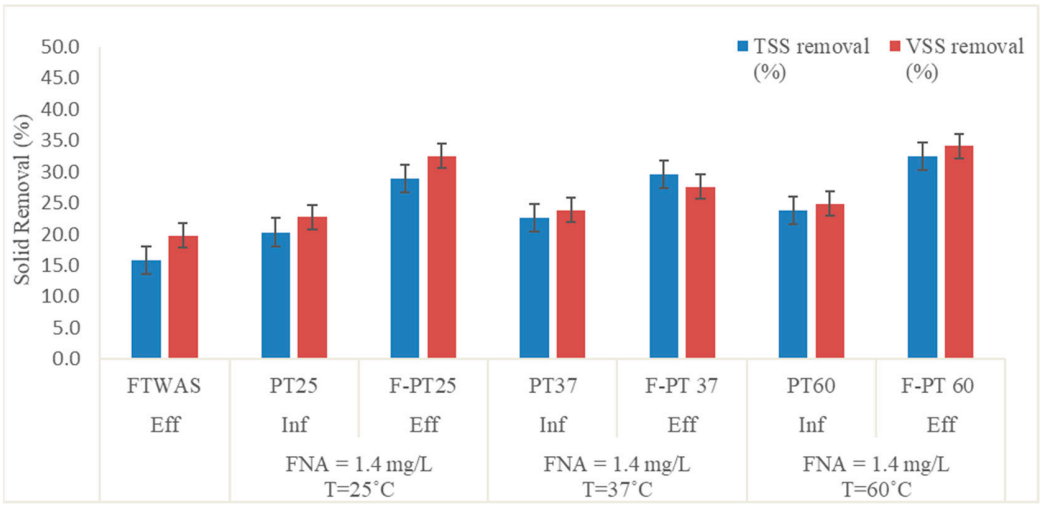

Figure 4. Percentage of suspended solids reduction via FNA-heat pretreatment, fermentation, and combined pretreatment/fermentation processes (error bars represent the standard errors).

As depicted in Figure 3, the suspended solid concentrations of all pretreated samples demonstrated a notable decrease compared to the non-treated TWAS (control). The percentage of solid reduction efficiency exhibited enhancement with increasing temperatures. Among the pretreated samples (PT-25, PT-37, and PT-60), the most significant reductions in TSS and VSS were observed in the "PT-60" condition, measuring at 25.7 ± 1.4 and 18.9 ± 0.9 g/L, respectively. This corresponds to a substantial enhancement of approximately 24% in TSS reduction efficiency and 25% in VSS reduction efficiency at PT-60. This outcome points to the effectiveness of applying FNA pretreatment at temperatures below 60 °C. This process facilitates the transformation of TWAS-suspended solids into a

soluble form by disintegrating the TWAS structure and converting larger high-molecular substances into organic matter with lower molecular weight, such as monosaccharides and amino acids [18]. This finding aligns with our observations from the initial phase of this study. The elevated solid reduction percentage achieved through combined pre-treatment reflects a heightened potential for sludge disintegration when compared to untreated sludge. These results harmonize with a previous study where pre-treating full-scale WAS at room temperature with an FNA dosage of 1.8 mg HNO_2-N/L led to a 17% increase in VS destruction [22]. Notably, our study detected even greater VSS reduction, potentially attributed to the nature of the waste and the higher temperatures applied (up to 60 °C).

According to Figures 3 and 4, it is evident that the fermentation of pretreated samples across all tested scenarios led to significantly improved efficiency in reducing suspended solids compared to the isolated application of either pretreatment alone (PT-samples) or fermentation alone (F-TWAS).

The TSS and VSS contents of TWAS decreased from 33.8 ± 2.0 g/L and 25.1 ± 1.9 g/L (R-TWAS) to 28.4 ± 3.1 g/L and 20.0 ± 1.5 g/L after undergoing the fermentation process (F-TWAS). This reflects an approximate reduction efficiency of 16% for TSS and 20% for VSS. Notably, the most substantial reduction in suspended solids was achieved through the fermentation process following FNA-pretreated samples at 60 °C (F-PT-60), resulting in remarkable reductions of 32.5% for TSS and 34.1% for VSS. As a result, the TSS and VSS content of TWAS reached their lowest levels at 22.8 ± 1.1 and 16.5 ± 0.9, respectively.

It is important to highlight that the fermentation process did not yield significant gas production. These observations support the conclusion that the reduction in TSS and VSS observed in this study primarily stemmed from the disintegration of the TWAS structure rather than mass removal during the fermentation process.

3.1.2. COD Solubilization

Enhancing the solubility of organic materials through pre-treatment before the anaerobic digestion (AD) process aims to alleviate the hydrolysis phase's rate-limiting nature [12,23]. Improved COD solubilization typically signifies enhanced pre-treatment efficacy, translating to superior anaerobic digestion performance. This elevation in COD solubilization is expected to bolster the hydrolysis rate and acidogenic efficiency within the acid phase reactors, providing readily biodegradable components [24–26].

This study segment delves into evaluating the impact of pre-treatment (PT), fermentation (F), and combined fermentation/pretreatment (F-PT) processes in terms of COD solubilization.

Based on the presented findings in Figure 5, the soluble chemical oxygen demand (SCOD) content of the untreated TWAS (RTWAS) exhibited an increase from 2.2 ± 0.1 g/L to 10.1 ± 0.2 g/L post the fermentation process (F-TWAS). However, the pretreatment phase demonstrated more efficient COD solubilization compared to the fermentation process alone. For all conditions, SCOD concentrations following FNA-Heat pretreatment surpassed those of non-pre-treated TWAS samples. These outcomes align with prior studies reporting an increase in SCOD concentration through the application of both isolated and combined FNA-based pre-treatment for TWAS [27,28].

This augmentation in SCOD arises from the release of extracellular polymeric substances (EPS) and intracellular organic matter as a result of cell membrane disruption caused by pretreatment [3,8,15].

In congruence with the findings on the reduced efficiency of suspended solids (Figure 4), the fermentation of pretreated samples across all scenarios demonstrated greater effectiveness in achieving higher COD solubilization compared to the fermentation of untreated TWAS (Figure 6).

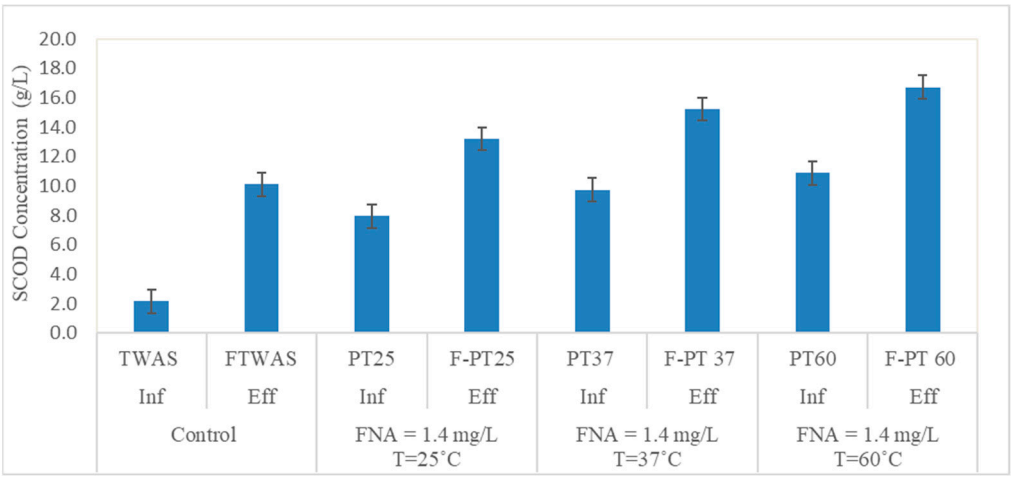

Figure 5. SCOD concentration (g/L) in the fermented, FNA-heat pretreated, and raw TWAS (error bars represent the standard errors).

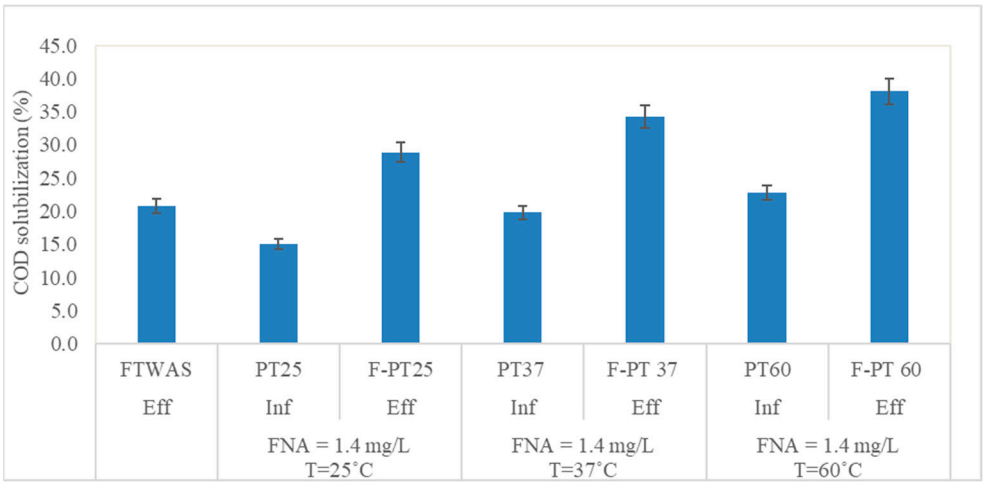

Figure 6. Degree of COD solubilization (%) in the fermented/FNA-heat pretreated samples compared to the raw TWAS (error bars represent the standard errors).

Among the range of scenarios explored, the most effective strategy for disintegrating the structure of TWAS was the employment of a fermenter supplied with PT-60 (F-PT-60).

Regarding the total chemical oxygen demand (TCOD), it was expected to remain constant before and after pretreatment [17]. The findings revealed an insignificant variation (<5%) in TCOD concentration post pretreatment. Thus, the implementation of FNA and heat pretreatment effectively preserved TCOD throughout the pretreatment procedure.

A comparison between the results of COD solubilization and those of suspended solids (TSS and VSS) reduction unveiled an intriguing trend: In specific scenarios, the extent of COD solubilization was lower than the percentage reduction in suspended solids. This observation can be attributed to the methodology employed in SCOD analysis, where samples underwent filtration using 0.45 µm syringe filters. On the contrary, suspended solid analysis involved filtering samples through 1.2 µm filter papers. Consequently, some particulate matter transitioned from a solid form to a colloidal phase within the size range of 0.45–1.2 µm, contributing to the reduction in suspended solid concentration without affecting the content of soluble substances, such as SCOD.

3.1.3. VFAs Production

Figure 7 depicts the concentrations of total Volatile Fatty Acids (VFAs) within both control and pretreatment systems during semi-continuous mesophilic fermentation. The

enhancement percentage in total VFA concentration resulting from the processes of fermentation, pretreatment, and combined fermentation/pretreatment is also illustrated in the same figure.

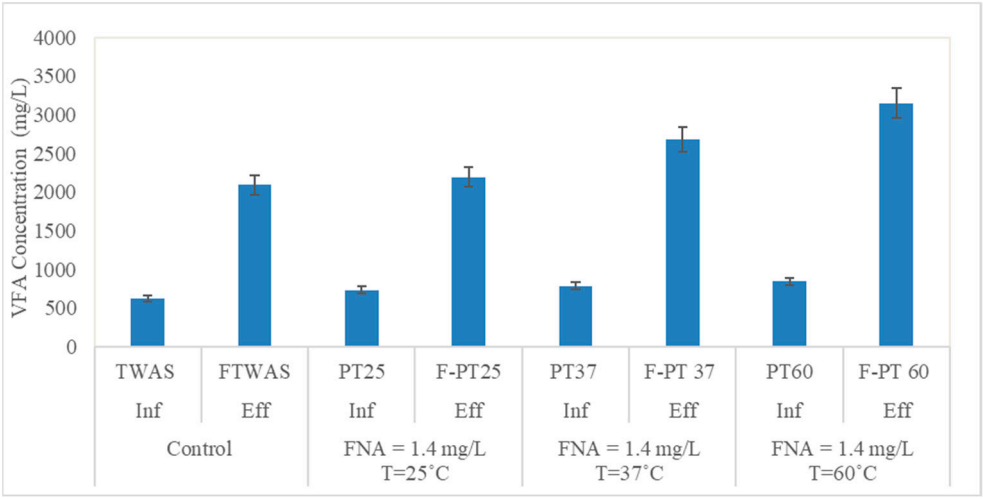

Figure 7. Total VFAs concentration (mg/L) in the fermented/FNA-Heat pretreated samples compared to the raw TWAS (error bars represent the standard errors).

The overarching trend observed in Figure 7 indicates a rise in VFA concentration following all pretreatment scenarios. As depicted, the application of FNA-Heat pretreatment led to a heightened total VFA content, elevating it from 628 ± 64 mg/L in R-TWAS to a peak of 845 ± 50 mg/L (specifically in the "PT-60" condition). This accounts for a significant 35% increase. However, it is important to note that the augmentation in VFA content after pretreatment was predominantly attributed to the acidogenic fermentation phase.

In contrast, within the control system, the fermentation process brought about an elevation in the total VFA content of raw sludge, escalating it from 628 ± 64 mg/L to 2095 ± 117 mg/L.

A similar trend was observed within the pretreatment systems, where the total VFA content initially increased to 845 ± 50 mg/L during the pretreatment phase (specifically in PT-60). Subsequently, at the conclusion of the consecutive pretreatment/fermentation process (PT + F-PT60), a substantial rise in VFA concentration was achieved, reaching 3152 ± 140 mg/L.

It is noteworthy that while the combination of FNA and heat pretreatment had a notable impact on enhancing the soluble fraction of organics and promoting sludge disintegration, its influence on the VFAs concentrations of the pretreated sludge (PT samples) remained relatively modest.

Notably, gas production was closely monitored throughout the semi-continuous fermentation process, revealing that the fermentation of both the raw and pretreated TWAS samples yielded no substantial gas generation. It is important to highlight that the initial pH of the fermenters was diligently maintained within the optimal range of 5.5 ± 0.2, as recommended for the fermentation process. Consequently, the lack of substantial hydrogen generation (less than 5%) can be primarily attributed to the comparatively low concentrations of soluble carbohydrates found in both the untreated and pretreated Thickened Waste Activated Sludge (TWAS). These soluble carbohydrates, mainly glucose, function as the favored electron donors and carbon sources for microorganisms that produce hydrogen.

3.2. Anaerobic Digestion

3.2.1. Methane Production Yield

The batch reactors were employed in conducting the biochemical methane potential experiment, aiming to assess the anaerobic biodegradability.

For comparison within a consistent framework, the cumulative methane productions from the BMP experiments were normalized per substrate unit mass of added COD (mL CH_4/g TCOD added) and per substrate unit mass of added VS (mL CH_4/g VS added). Remarkably, all treated TWAS samples, whether pretreated, fermented, or a combination thereof, exhibited greater methane production compared to the raw TWAS. Figure 8 showcases the biomethane yield per substrate unit mass of added VS (mL CH_4/g VS added) for both pretreated and/or fermented as well as the raw TWAS.

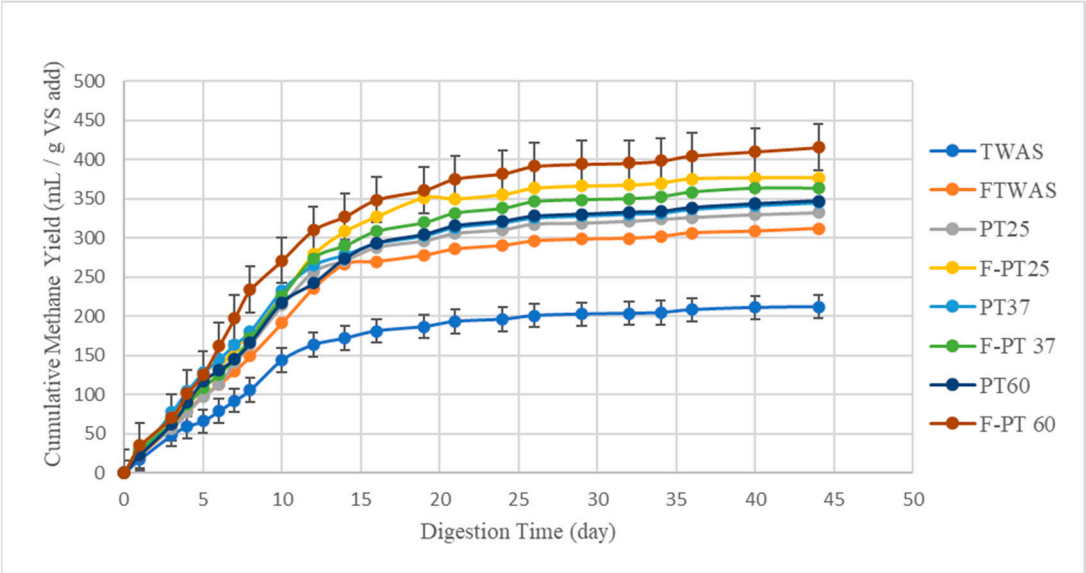

Figure 8. Cumulative methane production (mL/g VS added) comparison between FNA-Heat pretreated and/or Fermented samples and the raw TWAS. Error bars are shown only for the control and the sample with the highest cumulative methane yield (i.e., F-PT-60), with omitted bars for other samples as errors were less than 5%, enhancing figure clarity.

The observed higher methane yields in all treated samples, regardless of the specific treatment approach, align with findings from other relevant references [29]. Consistent with existing literature, this outcome emphasizes the positive influence of pretreatment in improving the hydrolysis stage of anaerobic digestion (AD) [3,30]. The enhanced methane production in treated samples is indicative of the effectiveness of pretreatment methods in facilitating the breakdown of complex organic matter, ultimately leading to improved biogas generation during the AD process. These results are in line with broader research trends supporting the beneficial impact of pretreatment on biogas production from organic substrates [17,27,29,31–35]. The results from our study align with findings from other references that highlight the efficiency of a two-stage anaerobic digestion (AD) process compared to a single-stage configuration [1,36]. Specifically, the implementation of the combined FNA-Heat pretreatment within the two-stage AD process led to significantly higher methane yields, ranging from 363 to 415 mL CH_4/g VS added. This notable increase in methane production contrasts with the methane yields achieved by the FNA-Heat pretreatments in the single-stage configuration, which varied between 332 and 347 mL CH_4/g VS added. Moreover, these yields were considerably higher than the untreated waste-activated sludge (TWAS), which yielded only 212 mL CH_4/g VS added. These consistent findings emphasize the enhanced efficiency and biogas production potential associated with the two-stage AD process, particularly when coupled with the FNA-Heat pretreatment. Furthermore, the methane production commenced promptly following the initiation of anaerobic digestion, devoid of significant lag phases, both for the untreated raw sample and the pretreated sludges. This outcome was attributed to the abundance of soluble substrates readily available for the anaerobic microorganisms [37–39].

It is noteworthy that the application of the sole FNA-pretreatment process at lower temperatures (i.e., PT-24 and PT-37) and the sole fermentation process showed lower methane yield compared to combined pretreatment-fermentation.

As depicted in Figure 8, the fermented and/or pretreated samples not only yielded higher ultimate methane production (after 44 days) in comparison to the control reactor (R-TWAS) but also consistently exhibited greater methane recovery at each specific time point. This observation substantiates the absence of any inhibitory effects stemming from the pretreatment/fermentation process during anaerobic digestion.

3.2.2. Anaerobic Biodegradability

The evaluation of the efficacy of both single-stage and two-stage AD processes was extended to assess their impact on biodegradability, as depicted in Figure 9. The biodegradability of TWAS was quantified by comparing the total methane production to the theoretical yield from fully biodegradable organic material (as per Equation (5). where B_0 Measured is experimental ultimate methane production, and B_0 theoretical is the theoretical methane potential based on the initial TCOD of the substrate (398 mL/g TCOD added at 37 °C).

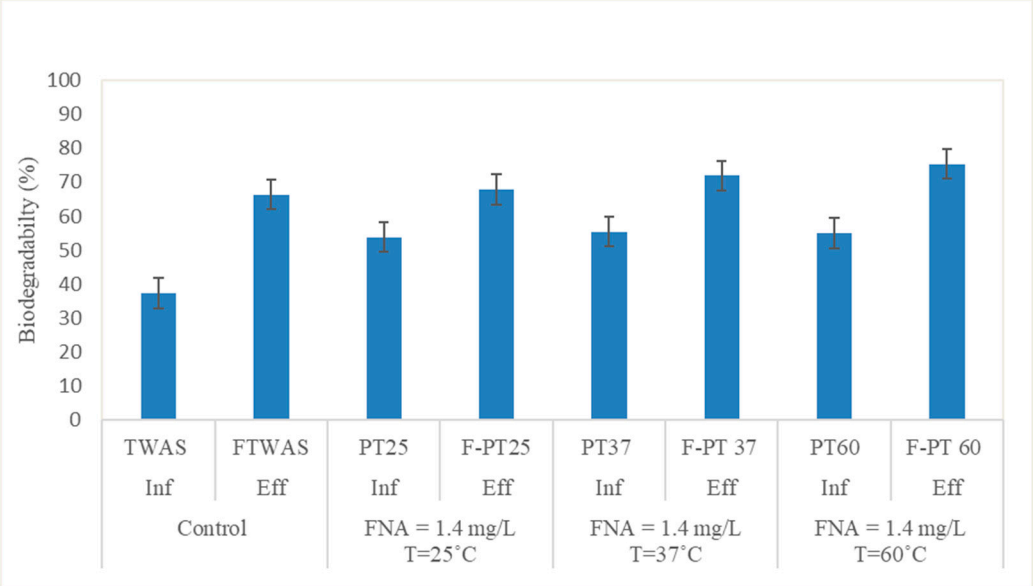

Figure 9. Biodegradability of FNA-Heat pretreated/fermented samples compared to the raw TWAS (error bars represent the standard errors).

The inherent biodegradability of the TWAS utilized in this study was approximately 37%. As illustrated in Figure 9, all treated samples exhibited enhanced biodegradability compared to the untreated TWAS. This enhancement can be attributed to the presence of readily biodegradable compounds within the treated substrates (either pretreated and/or fermented), which were introduced into the anaerobic digesters [40–42].

3.2.3. COD Mass Balance

A comprehensive COD mass balance was executed across all digesters to assess the precision of the experimental data. The mass balance was established by considering the initial and final TCOD concentrations within the digesters, with the deduction of the theoretical methane production per unit mass of TCOD. A comparison between the methane production results obtained from this study and those deduced through TCOD mass balance revealed a divergence of under 10% for all digesters. Moreover, the COD mass balance ranged from 89% to 94% across all reactors, thereby affirming the reliability of the experimental data.

3.2.4. Kinetic Analysis

For determining kinetic parameters with a reliable level of confidence, the Modified Gompertz (MG) equation (Equation (6)) was employed. In this equation, B(t) signifies the cumulative methane production at the time, P represents the ultimate biogas production parameter, Rm denotes the maximum methane production rate per day, λ stands for the length of the lag phase (day), and t signifies the duration of anaerobic digestion (day).

$$B(t) = P \times \exp\left\{-\text{expexp}\left[\frac{R_M \times e}{P}(\lambda - t) + 1\right]\right\} \quad (6)$$

The Modified Gompertz (MG) model was utilized to fit the experimental data from the BMP tests to the theoretical model. The summarized results for parameters such as P (ultimate methane production in mL), Rm (maximum methane production rate in mL/day), and Lam (lag phase time in days) are presented in Table 2.

Table 2. The kinetic study of the single and two-stage AD process using the Gompertz Model; FNA-heat.

	AD		**Gompertz Kinetic Model**			
Exp.ID#	**MMPR * (mL/d)**	**Cumulative CH_4 (mL)**	**P (mL CH_4)**	**Rm (mL CH_4/d)**	**Lam (d)**	**R^2**
Control	21 ± 1.0	228 ± 9	222	16	0.5	0.999
F-TWAS	20 ± 0.8	274 ± 11	267	20	0.9	0.999
PT-25	23 ± 1.1	278 ± 14	272	21	1.1	0.999
F-PT-25	21 ± 0.6	274 ± 9	278	20	0.9	0.999
PT-37	23 ± 0.2	286 ± 15	276	20	0.1	0.999
F-PT-37	24 ± 0.5	297 ± 23	291	21	0.9	0.999
PT-60	23 ± 0.4	302 ± 21	276	19	0.5	0.999
F-PT-60	27 ± 0.5	311 ± 24	299	23	0.7	0.999

* Maximum Methane Production Rate (mL CH_4/d).

The estimated parameters exhibit alignment with the experimental data, with the trend of ultimate methane production (P) closely mirroring the observations from the experiments. Notably, the highest predicted cumulative methane production, reaching 299 mL CH_4, was attained by the "F-PT-60" sample.

Regarding the estimated lag phase, the variations were within the range of 0.1 to 1.1 days for the different treatment scenarios. Most treated samples demonstrated a lag phase of less than 1 day. A concise lag phase offers advantages, particularly in terms of not necessitating an extended residence time, which subsequently leads to reduced reactor volume requirements and lowers the operational expenses associated with the process.

The values of Rm, representing the maximum methane production rate, spanned from 16 to 23 mL/day across different samples. This range included values for both untreated TWAS and "F-PT-60." The agreement between the experimental data and the estimated parameters was consistent, further confirming the reliability and validity of the model.

4. Conclusions

This study investigates the impact of combined FNA-heat pre-treatment on sludge-suspended solids reduction, comparing it to the fermentation process. Pretreated samples showed significant decreases in suspended solid concentrations, with the most substantial reductions observed at 60 °C. Fermentation of pretreated samples, especially after FNA pretreatment at 60 °C, demonstrated improved suspended solids reduction and enhanced soluble chemical oxygen demand (SCOD) solubilization. Total chemical oxygen demand (TCOD) remained constant after pretreatment, and total volatile fatty acids (VFAs) concentration increased due to acidogenic fermentation. Biochemical methane potential (BMP)

experiments revealed higher methane production in all treated samples, with the combined FNA-Heat pretreatment in a two-stage anaerobic digestion process yielding the highest methane yields. The study concludes that the combined FNA-heat pretreatment and fermentation effectively reduce suspended solids, enhance methane production, and improve the biodegradability of treated waste-activated sludge samples.

Author Contributions: Methodology, S.C. and E.E.; data curation, S.C. and E.E.; writing—original draft preparation, S.C.; writing—review and editing, S.C. and E.E.; supervision, E.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Data will be available upon request.

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

References

1. Sarwar, R.; Elbeshbishy, E.; Parker, W.J. Codigestion of high pressure thermal hydrolysis-treated thickened waste activated sludge with primary sludge in two-stage anaerobic digestion. *Environ. Prog. Sustain. Energy* **2018**, *37*, 425–433. [CrossRef]
2. Wang, X.; Jiang, C.; Wang, D.; Yang, Y.; Fan, L.; Xu, S.; Zhuang, X.J. Quorum sensing responses of activated sludge to free nitrous acid: Zoogloea deformation, AHL redistribution, and microbiota acclimatization. *Water Res.* **2023**, *238*, 119993. [CrossRef] [PubMed]
3. Dai, Z.; Liu, L.; Duan, H.; Li, B.; Tang, X.; Wu, X.; Liu, G.; Zhang, L.J.S. Improving sludge dewaterability by free nitrous acid and lysozyme pretreatment: Performances and mechanisms. *Sci. Total Environ.* **2023**, *855*, 158648. [CrossRef] [PubMed]
4. Biswal, B.K.; Huang, H.; Dai, J.; Chen, G.-H.; Wu, D. Impact of low-thermal pretreatment on physicochemical properties of saline waste activated sludge, hydrolysis of organics and methane yield in anaerobic digestion. *Bioresour. Technol.* **2020**, *297*, 122423.
5. Manzanares, P. The role of biorefinering research in the development of a modern bioeconomy. *Acta Innov.* **2020**, *37*, 47–56. [CrossRef]
6. Lopes, T.F.; Łukasik, R.M. Economic, social and environmental impacts attained by the use of the effluents generated within a small-scale biorefinery concept. *Acta Innov.* **2020**, *36*, 57–63. [CrossRef]
7. Ximenes, J.; Siqueira, A.; Kochańska, E.; Łukasik, R.M. Valorisation of agri-and aquaculture residues via biogas production for enhanced industrial application. *Energies* **2021**, *14*, 2519. [CrossRef]
8. Wang, C.; Wang, Y.; Chen, Z.; Wei, W.; Chen, X.; Mannina, G.; Ni, B.-J. A novel strategy for efficiently transforming waste activated sludge into medium-chain fatty acid using free nitrous acid. *Sci. Total Environ.* **2023**, *862*, 160826. [CrossRef] [PubMed]
9. Hendriks, A.; Zeeman, G. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour. Technol.* **2009**, *100*, 10–18. [CrossRef]
10. Wang, Q.; Jiang, G.; Ye, L.; Yuan, Z. Enhancing methane production from waste activated sludge using combined free nitrous acid and heat pre-treatment. *Water Res.* **2014**, *63*, 71–80. [CrossRef]
11. Pijuan, M.; Wang, Q.; Ye, L.; Yuan, Z. Improving secondary sludge biodegradability using free nitrous acid treatment. *Bioresour. Technol.* **2012**, *116*, 92–98. [CrossRef] [PubMed]
12. Wang, J.; Zhang, Z.; Ye, X.; Huang, F.; Chen, S. Performance and mechanism of free nitrous acid on the solubilization of waste activated sludge. *RSC Adv.* **2018**, *8*, 15897–15905. [CrossRef] [PubMed]
13. Zupančič, G.D.; Roš, M. Heat and energy requirements in thermophilic anaerobic sludge digestion. *Energy* **2003**, *28*, 2255–2267. [CrossRef]
14. Azizi, A.; Koupaie, E.H.; Hafez, H.; Elbeshbishy, E. Improving single-and two-stage anaerobic digestion of source separated organics by hydrothermal pretreatment. *Biochem. Eng. J.* **2019**, *148*, 77–86. [CrossRef]
15. Jiang, W.; Tao, J.; Luo, J.; Xie, W.; Zhou, X.; Cheng, B.; Guo, G.; Ngo, H.H.; Guo, W.; Cai, H.J.C. Pilot-scale two-phase anaerobic digestion of deoiled food waste and waste activated sludge: Effects of mixing ratios and functional analysis. *Chemosphere* **2023**, *329*, 138653. [CrossRef] [PubMed]
16. Wang, E.; Sun, H.; Chen, P.; Zheng, Y.; Guo, J.; Dong, R.J.B.T. Two-step anaerobic digestion of rice straw with nanobubble water. *Bioresour. Technol.* **2023**, *376*, 128928. [CrossRef] [PubMed]
17. Chegini, S.; Okoye, F.; Elbeshbishy, E. Chemical pretreatment of thickened waste activated sludge (TWAS) to improve biogas production. In *Residuals and Biosolids Conference 2020*; Water Environment Federation: Alexandria, VA, USA, 2020.
18. Zhang, T.; Wang, Q.; Ye, L.; Batstone, D.; Yuan, Z. Combined free nitrous acid and hydrogen peroxide pre-treatment of waste activated sludge enhances methane production via organic molecule breakdown. *Sci. Rep.* **2015**, *5*, 16631. [CrossRef]
19. Angelakis, A.N.; Snyder, S.A. *Wastewater Treatment and Reuse: Past, Present, and Future*; Multidisciplinary Digital Publishing Institute: Basel, Switzerland, 2015.
20. Angelidaki, I.; Alves, M.; Bolzonella, D.; Borzacconi, L.; Campos, J.; Guwy, A.; Kalyuzhnyi, S.; Jenicek, P.; Van Lier, J.B. Defining the biomethane potential (BMP) of solid organic wastes and energy crops: A proposed protocol for batch assays. *Water Sci. Technol.* **2009**, *59*, 927–934. [CrossRef]

21. Van Lier, J.B.; Mahmoud, N.; Zeeman, G. Anaerobics for Wastewater Treatment. Paper Presented at The 3rd International Meeting on Environmental Biotechnology and Engineering. 2008, pp. 415–456. Available online: https://research.wur.nl/en/publications/anaerobics-for-wastewater-treatment (accessed on 23 January 2024).
22. Wei, W.; Wang, Q.; Zhang, L.; Laloo, A.; Duan, H.; Batstone, D.J.; Yuan, Z. Free nitrous acid pre-treatment of waste activated sludge enhances volatile solids destruction and improves sludge dewaterability in continuous anaerobic digestion. *Water Res.* **2018**, *130*, 13–19. [CrossRef]
23. Bonilla, S.; Choolaei, Z.; Meyer, T.; Edwards, E.A.; Yakunin, A.F.; Allen, D.G. Evaluating the effect of enzymatic pretreatment on the anaerobic digestibility of pulp and paper biosludge. *Biotechnol. Rep.* **2018**, *17*, 77–85. [CrossRef]
24. Choi, J.-M.; Han, S.-K.; Lee, C.-Y. Enhancement of methane production in anaerobic digestion of sewage sludge by thermal hydrolysis pretreatment. *Bioresour. Technol.* **2018**, *259*, 207–213. [CrossRef]
25. Ohimain, E.I.; Izah, S.C.J.R.; Reviews, S.E. A review of biogas production from palm oil mill effluents using different configurations of bioreactors. *Renew. Sustain. Energy Rev.* **2017**, *70*, 242–253. [CrossRef]
26. Zahedi, S.; Romero-Güiza, M.; Icaran, P.; Yuan, Z.; Pijuan, M. Optimization of free nitrous acid pre-treatment on waste activated sludge. *Bioresour. Technol.* **2018**, *252*, 216–220. [CrossRef]
27. Atelge, M.; Krisa, D.; Kumar, G.; Eskicioglu, C.; Nguyen, D.D.; Chang, S.W.; Atabani, A.; Al-Muhtaseb, A.H.; Unalan, S. Biogas production from organic waste: Recent progress and perspectives. *Waste Biomass Valoriz.* **2020**, *11*, 1019–1040. [CrossRef]
28. Saha, M.; Eskicioglu, C.; Marin, J. Microwave, ultrasonic and chemo-mechanical pretreatments for enhancing methane potential of pulp mill wastewater treatment sludge. *Bioresour. Technol.* **2011**, *102*, 7815–7826. [CrossRef] [PubMed]
29. Atelge, M.; Atabani, A.; Banu, J.R.; Krisa, D.; Kaya, M.; Eskicioglu, C.; Kumar, G.; Lee, C.; Yildiz, Y.; Unalan, S.J.F. A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. *Fuel* **2020**, *270*, 117494. [CrossRef]
30. Uthirakrishnan, U.; Sharmila, V.G.; Merrylin, J.; Kumar, S.A.; Dharmadhas, J.S.; Varjani, S.; Banu, J.R. Current advances and future outlook on pretreatment techniques to enhance biosolids disintegration and anaerobic digestion: A critical review. *Chemosphere* **2022**, *288*, 132553. [CrossRef]
31. Apollo, S.; Onyango, M.S.; Ochieng, A.J.C.E.J. Integrated UV photodegradation and anaerobic digestion of textile dye for efficient biogas production using zeolite. *Chem. Eng. J.* **2014**, *245*, 241–247. [CrossRef]
32. Akgul, D.; Cella, M.A.; Eskicioglu, C. Influences of low-energy input microwave and ultrasonic pretreatments on single-stage and temperature-phased anaerobic digestion (TPAD) of municipal wastewater sludge. *Energy* **2017**, *123*, 271–282. [CrossRef]
33. Bao, H.; Yang, H.; Zhang, H.; Liu, Y.; Su, H.; Shen, M.J.E.R. Improving methane productivity of waste activated sludge by ultrasound and alkali pretreatment in microbial electrolysis cell and anaerobic digestion coupled system. *Environ. Res.* **2020**, *180*, 108863. [CrossRef]
34. Kim, J.; Yu, Y.; Lee, C. Thermo-alkaline pretreatment of waste activated sludge at low-temperatures: Effects on sludge disintegration, methane production, and methanogen community structure. *Bioresour. Technol.* **2013**, *144*, 194–201. [CrossRef] [PubMed]
35. Ma, B.; Peng, Y.; Wei, Y.; Li, B.; Bao, P.; Wang, Y. Free nitrous acid pretreatment of wasted activated sludge to exploit internal carbon source for enhanced denitrification. *Bioresour. Technol.* **2015**, *179*, 20–25. [CrossRef] [PubMed]
36. De Gioannis, G.; Muntoni, A.; Polettini, A.; Pomi, R.; Spiga, D.J.W.M. Energy recovery from one-and two-stage anaerobic digestion of food waste. *Waste Manag.* **2017**, *68*, 595–602. [CrossRef] [PubMed]
37. Duan, H.; Gao, S.; Li, X.; Ab Hamid, N.H.; Jiang, G.; Zheng, M.; Bai, X.; Bond, P.L.; Lu, X.; Chislett, M.M. Improving wastewater management using free nitrous acid (FNA). *Water Res.* **2020**, *171*, 115382. [CrossRef]
38. Zhang, L.; Duan, H.; Ye, L.; Liu, L.; Batstone, D.J.; Yuan, Z. Increasing capacity of an anaerobic sludge digester through FNA pre-treatment of thickened waste activated sludge. *Water Res.* **2019**, *149*, 406–413. [CrossRef]
39. Zhang, T. FNA-Based Pre-Treatment of Primary, Secondary and Digested Sludge. Ph.D. Thesis, The University of Queensland, St Lucia, QLD, Australia, 2016.
40. Alpaslan Kocamemi, B.; Çelik, S.; Senol, A.B.; Kurt, H.; Erken, E. Paradigm Shift in Domestic Wastewater Treatment: Toward Energy Minimization, Greenhouse Gas Emission Reduction, and Resources Recovery. In *Wastewater Management and Technologies*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 211–237.
41. Ghosh, A.; Ray, A.; Goswami, A.; Ali, O.A.; Singh, P.K.; Pattnaik, R. Recent Development and Innovations in Integrated Biogas-Wastewater Treatment. In *Biorefinery for Water and Wastewater Treatment*; Springer: Berlin/Heidelberg, Germany, 2023; pp. 271–297.
42. Zhao, Q.; Ying, H.; Liu, Y.; Wang, H.; Xu, J.; Wang, W.; Ren, J.; Meng, S.; Wang, N.; Mu, R. Towards low energy-carbon footprint: Current versus potential P recovery paths in domestic wastewater treatment plants. *J. Environ. Manag.* **2023**, *344*, 118653. [CrossRef]