Opportunities and challenges: experimental and kinetic analysis of anaerobic co-digestion of food waste and rendering industry streams for biogas production

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Abstract

Large amounts of food waste and sewage sludge exert a hazardous environmental impact in several countries. Producing biogas and digestate from food and industrial waste is one of the solutions for waste management, stabilization of sludge, resource and energy recovery and reductions in the amount of waste. However, biogas production from such substrates has challenges in degradation efficiency, inhibitory effects and other challenges, and thus co-digestion and pretreatment techniques could be applied to enhance biogas production. The aim of this study is to explore the effects of co-digestion of food waste, meat and bone meal and rendering wastewater sludge. First, thermal pretreatment was performed (35°C, 5 days) by adding the rendering-industry streams to food waste in the amounts of 0, 5, 10 and 15% on a total solid basis, and further anaerobic digestion (40.5°C, ca. 40 days) was then performed. Both

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experimental and kinetic analysis were conducted, and the major factors regarding opportunities and challenges in the two-stage process are discussed. Results have shown that both co-substrates from rendering industry decreased the biogas yield of food waste. When 5% of them was added to food waste, meat and bone meal decreased biogas production by 12%, and wastewater sludge decreased it by 23%. Both co-substrates, on the other side, increased the rate of reaction of food waste digestion when applying different common kinetic models.

Highlights

- Rendering streams were studied as co-substrates to food waste for biogas production
- Experimental study of thermal pretreatment and anaerobic digestion was performed
- Food waste could cause inhibition of the anaerobic digestion process
- Kinetic parameters were estimated for anaerobic digestion of selected mixtures
- Rendering streams decrease biogas production, while increase the rate of reaction

Keywords

Food Waste; Rendering Industry Streams; Thermal Pretreatment; Anaerobic Co-Digestion; Biogas Production; Experimental Research; Kinetic Analysis

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Abbreviations

AE Agroproteinka Energija; AD Anaerobic Digestion; EU European Union; FW Food Waste; IN Inoculum; LCFAs Long Chain Fatty Acids; MBM Meat and Bone Meal; SCOD Soluble Chemical Oxygen Demand; TS Total Solids; VFAs Volatile Fatty Acids; VS Volatile Solids; WWS Wastewater Sludge

Nomenclature

COD	Chemical Oxygen Demand (g/L)
k	Reaction Rate Constant (d^{-1})
m	Mass (g, kg)
N	Number of Data Points
n	Shape Factor ($-$)
NH_4-N	Ammonium Nitrogen (g/L)
pH	Power of Hydrogen ($-$)
R	Biogas Production Rate (Nm^3/(kg TS·d))
RMSE	Root Mean Square Error (Nm^3/kg TS)
S	Biogas Yield (Nm^3/kg TS)
t	Time (d)
TIC	Total Inorganic Carbon (g $CaCO_3$/L)
V	Volume of Gas (Nm^3)
VFAs	Volatile Fatty Acids (g CH_3COOH/L)

Subscripts

exp	Experimental
i	Data Point
m	Maximum
mod	Model

Greek Symbols

λ	Lag phase (d)

1   Introduction

A circular economy and a "closing the loop" approach have been recognised in EU energy policy as very important factors in the security of energy supply and the threat to climate change [1]. Implementation of a multi-waste management concept has shown that it is possible to treat various waste streams at the same facility [2], where anaerobic digestion technology has shown the highest applicability level of all energy recovery methods.

The latest European directive on the promotion of the use of energy from renewable sources (REDII) positions future biogas production towards the utilisation of more sustainable feedstocks, such as lignocellulosic materials and the biomass fraction of waste and residues [3]. The rationale behind the decision to consider only such substrates in anaerobic digestion (AD) has been related to the unfavourable side effects of currently used substrates, which are mainly cultivated crops, such as maize [4]. Among the side effects are increasing food prices [5] and the environmental footprint of the biogas industry because of crop production [6].

Among all bio-waste types in the EU28, food waste (FW) is the most interesting on account of worldwide trends towards landfill reduction [7] and increasing separate collection of waste that can be further used in the production of renewable heat and electricity [8], or renewable gas [9]. The European Commission has estimated that around $88 \cdot 10^6$ t/y of FW are generated in the EU28, which is equal to ca. 173 kg/y of FW per person [10].

Energy recovered from FW (food waste and loss) can significantly contribute to better energy self-sufficiency and to a reduction in fossil energy consumption [11]. There are many conversions pathways for FW to produce energy and value-added materials [12]. Compared with disposal methods such as landfill, incineration and composting, AD is a promising technology for FW management, since FW is a wet organic material. Co-digestion with animal manure and sewage sludge are practical options for AD of FW for biogas production [13]. AD is a conversion process from which versatile uses of products, methane and digestate, are

possible, in the industrial, domestic and transport sectors. Emphasis in this work will be placed on the experimental analysis of two-stage AD, its kinetics and on the limitations of the AD process relating to FW.

The paper is structured as follows. First, the comprehensive review is presented and focuses on the presentation of FW resources, pretreatment and degradation of FW, co-digestion of FW and the common co-substrates in the two-stage AD, and on an overview of the use of animal and rendering by-products in AD (Section 1.1). Further, in Section 1.2 limiting factors in AD of FW are presented, focusing on the inhibition affecting biogas production, caused by the compounds like ammonia, long chain fatty acids (LCFAs), volatile fatty acids (VFAs) and metal elements. The last Section of the review presents commonly applied kinetic models and estimated kinetic parameters in AD of FW.

The second part of the paper is devoted to testing the scientific contribution and objectives of this research through an experimental and modelling study of the two-stage AD of FW, where in the first stage rendering industry streams were thermally pretreated and added as co-substrates for AD (second stage). Finally, four of the common kinetic models were applied to perform kinetic analysis of biogas yield, and to estimate kinetic parameters of the mixtures for AD.

1.1 Resource and energy recovery from food waste

In general, there are two types of FW; the first is edible FW that is generated during food consumption by consumers and could be reduced or avoided, while the second is inedible FW which cannot be avoided, such as peels, bones, stalks and skin [14]. FW represents a significant part of food consumption; around one third of all food intended for human consumption becomes FW [15]. FW is generated mostly in homes, restaurants and in food services (schools, hospitals, old people's homes etc.), but also in distribution and in food stores,

and along the production supply chain [16]. FW that is collected is mostly processed in biogas plants, followed by processing in composting plants [17].

The complex origins of raw FW result in a wide and heterogenous composition, where the main component of raw FW is water, ca. 70-90%. The dry matter basis is ca. 5-50% lipids, ca. 0-20% starch and ca. 2-20% proteins [18]. The high share of water in FW makes it unsuitable for incineration, gasification or pyrolysis, while at the same time, it is highly applicable in a wet AD process [19]. Utilising FW for wet AD has shown high potential in reducing environmental burdens: for instance 6,600 t of FW can be substituted for 9,900 t of maize silage, resulting in a carbon footprint reduction of 42% [20].

However, the approach to AD of FW is slightly different compared to AD of energy crops and animal manure [4], which is usually performed as a single-stage process [21]. In comparison to maize silage and cattle manure, FW has characteristics that vary considerably, depending on the source of the FW. Multi-stage AD of FW offers higher stability [22] and more efficient conversion of biomass to biogas [23]. The first stage of AD, also known as hydrolysis, is usually the rate-limiting step in the case of FW that defines the overall biogas production rate [24]. To achieve more efficient hydrolysis and avoid low conversion rates, pretreatment methods are introduced [25]. After pretreatment, the second stage includes degradation of organic material under anaerobic conditions where biogas is produced.

1.1.1   Pretreatment of food waste

Prior to the pretreatment, raw FW is ground or milled into smaller particles to improve the carbon accessibility [25]. There are several applicable pretreatment methods for FW, such as thermal, mechanical, thermo-chemical, chemical and biological pretreatment [26]. Thermal hydrolysis has been recognised as the most efficient and least complex method of hydrolysing macromolecules in FW for easier degradation [27].

1.1.2 Degradation of organic material under anaerobic conditions

The second stage includes anaerobic degradation of organic material. AD has been recognised as an economic and effective option to reduce FW landfill, groundwater pollution and emission of toxic gases [28]. In addition, AD of FW is considered a recycling method [29] that additionally contributes to its promotion in the framework of sustainable development and circular economy.

Two-stage AD could enhance conversion and thus increase the yield of biogas. A study on anaerobic degradation of kitchen FW showed an increase in biogas production of ca. 30-40% when thermal pretreatment was performed using temperatures of 90-120°C with durations of 70 and 50 min [30]. Synthetic FW (a mixture of fruits/vegetables, pasta/rice, bread/baked goods, meat and fish) yielded negative effects in terms of biogas production when thermal pretreatment was performed at temperatures higher than 120°C and a pretreatment time longer than 4 h [31].

On the other hand, thermal pretreatment of canteen FW and waste activated sludge at temperatures higher than 200°C resulted in reduced biogas production [32]. At higher temperatures, complex polymer compounds are formed which inhibit the second stage (AD process) [31]. Based on a literature review, thermal pretreatment of FW is recommended at lower temperatures and prolonged duration, since increasing temperature does not significantly increase biodegradation but rather decreases the potential for biogas production by forming inhibitory intermediate compounds.

1.1.3 Anaerobic digestion of food waste and co-substrates

Several studies have been performed regarding anaerobic co-digestion of FW and co-substrates. It was found that, especially in batch processes, the substrate to inoculum ratio has

a significant impact on the process performance of FW digestion. In the case of batch AD of FW, the substrate to inoculum ratio has varied as follows 1:1 [4], 1.4:1 [33] and 3.0:1 [34].

Some examples of co-digestion studies of FW are as follows: Canteen FW and parthenium weed were studied for biogas production using microwave irradiation and steam pretreatment on a laboratory scale [35], where by adding pretreated parthenium weed to FW, pH control was improved as compared to untreated weed. Canteen FW in co-digestion with rice straw showed an approximately 70% higher biogas yield compared to mono-digestion of FW [36]. Thermally pretreated canteen FW and waste activated sludge were studied for biogas production, where the results showed that 24 h pretreatment using fungal mash resulted in a 6% increase in soluble chemical oxygen demand (SCOD), and the SCOD removal during biogas production was estimated to be between 70 and 90% [37].

The co-digestion of pretreated FW and yard waste gave a biogas yield of 431 NmL/g VS, while untreated FW and yard waste had a biogas yield of 335 NmL/g VS [38]. Adding sewage sludge and yard waste to cafeteria FW showed synergistic effects in terms of biogas production compared to mono-digestion of FW [39]. Co-digestion of FW composed of bread, rice, spaghetti, vegetables, fruits and meat gave a 1.4-fold higher methane yield compared to sludge mono-digestion. Adding organic FW to sludge increases the organic content in the mixture and improves the digestibility of the mixture [40]. Anaerobic co-digestion of restaurant FW and sewage sludge showed that, when adding 10% of sludge to FW, biogas production is stable [41].

Based on the literature review, it was found that substrates in second-generation biogas production like FW, various sludge types and other waste types are highly heterogenous, and thus process behaviour is highly unpredictable. Therefore, it is important to investigate limiting factors and obstacles to their use in AD.

1.1.4   Animal and rendering industry streams in anaerobic digestion

The rendering industry processes animal by-products into more valuable materials with the goal of preventing land and water pollution caused by irresponsible handling of those by-products [42]. Animal by-products are classified in three categories [42]:

- Category 1 – materials with the highest risk for public health, animals, or the environment (animals affected by diseases, e.g. mad cow disease) that cannot be utilised in AD in any kind of form [43];

- Category 2 – animal by-products that can be recovered in biogas plants approved by national rules (manure or digestive tract content) and

- Category 3 − animal by-products that could be used for human consumption; however, for commercial reasons, they are not intended for human consumption. Such products are fully suitable to be recovered in biogas plants.

After processing animal by-products, the main rendering industry streams are wastewater sludge (WWS), meat and bone meal (MBM) and grease trap sludge, which is mostly used in biodiesel production [44].

In the meat and rendering industry, the AD process acquires a high potential for renewable energy production and reduction of greenhouse gas emissions [45]. However, relatively high financial investment and the inappropriateness of some streams like fat, oil and grease for biogas production have been recognised as major concerns in installing AD technology in a rendering plant [45].

Meat processing by-products have shown high yields in biogas production when co-digested with pig manure and process water from a rendering facility [46]. To keep such an AD process stable, a limit has been set on a maximum 10% of MBM share in a mixture. MBM has proven to have high TS content of approximately 98.5% and a relatively low carbon to nitrogen ratio (C/N) of 4.19 [47], which could significantly contribute to ammonia inhibition during AD

[48]. Therefore, MBM can be added to lignocellulosic substrates with a very high C/N ratio, like wheat straw and pine wood [49], to achieve the optimum C/N value for biodegradation. WWS from meat waste processing showed both ammonia and VFAs inhibition in AD when loaded higher than 3.8 g COD/g VS [50]. At lower loadings, inhibition was not detected, and the biogas yield of WWS achieved values of 0.53-0.55 Nm^3/kg VS. From the literature review, it was found that rendering industry streams can only be utilised for AD if they meet safety regulations to be utilised in biogas production and if they are added to a base substrate in smaller portions to maintain stability during the process.

1.2   Limiting factors in anaerobic digestion of food waste

Inhibition has been identified as the main obstacle to using FW as a substrate for AD [51]. Owing to the complex nature of FW, there are many inhibitory compounds that can affect AD and the biogas production rate. Some of the most frequent inhibitory compounds are presented and analysed in the subsections below.

1.2.1   Ammonia

One of the most important inhibitory compounds related to AD of FW is the excess nitrogen (protein) content in FW [52]. To quantify the share of nitrogen in a substrate and to estimate its potential for ammonia inhibition, the C/N ratio is usually used. A wide range of C/N values for FW is reported, between 16.5 and 46.8 [53]. Usually, an optimum C/N ratio for biodegradation is between 27 and 32 [52], which makes FW as a substrate for AD highly unpredictable in terms of whether or not it will lead to ammonia inhibition during the process.

When considering ammonia inhibition, it is important to distinguish two different concentrations of nitrogen that are usually used. The first is total ammonia nitrogen (TAN), which refers to both nitrogen from ammonia (NH_3-N) and ammonium (NH_4-N) that is present in the liquid phase during the process. Free ammonia refers to the concentration of unionised

ammonia (NH_3) in the same liquid phase. Free ammonia is a toxic form of ammonia that causes ammonia inhibition during AD. The threshold concentration for ammonia inhibition depends on the type of substrate and inoculum [53], and on AD conditions like temperature and pH [54]. When quantifying the ammonia inhibition in AD of FW, the threshold is usually expressed in terms of NH_4-N concentration, since it is easily measurable. Several reports have shown that a wide range of threshold concentration for ammonia inhibition exists, between 2 and 6 g/L [53,55].

1.2.2 Long Chain Fatty Acids (LCFAs)

During the hydrolysis of FW, lipids are degraded into long chain fatty acids (LCFAs) [56], of which palmitic, stearic and oleic acids are reported to be the most common [57]. The theoretical methane potential of lipids is higher than that of carbohydrate and proteins, between approximately 850 and 1,050 NL/kg VS [58]. However, lipids are usually not suitable for mono-digestion, owing to LCFAs inhibition, which is caused by an accumulation of LCFAs due to the slow lag phase of acidogenesis [58]. An excess of LCFAs results in physical adsorption on the cell membrane of microorganisms and in stagnation of the molecular transfer [51]. Since FW is usually rich in lipids (ca. 5 g/L [51]), LCFAs inhibition can be expected. The reported threshold for LCFAs inhibition during AD is 0.5-1.5 g COD/L [59]. If there is no LCFAs inhibition in the acidogenesis stage, then volatile fatty acids (VFAs) are being produced.

1.2.3 Volatile Fatty Acids (VFAs)

As the concentration of VFAs increases during acidogenesis [60], the pH value in the system drops and can cause inhibition in acetolactic methanogenesis. Such a phenomenon has been reported for AD of kitchen FW, where the pH was reported in the range of 2.3-5.1 [61]. The optimum pH range for the acetogenesis of FW is estimated between 6.8 and 7.6 [62]. A study on AD of canteen FW showed that propionic acid is the most responsible VFA for causing

inhibition [63]. Moreover, the same research showed that manual adjustment of pH by adding chemicals could not reverse VFA inhibition but could only delay the process failure.

On the other hand, if the concentration of VFAs in the system is too low, it indicates that acidogenesis was inhibited and no acids were produced [64]. Such an observation was determined using the VFAs/SCOD ratio, which showed that strong acid conditions (pH=4.0) favour inhibition of acidogenesis and delay conversion of soluble acids to acetic acid that is further converted to methane [64]. Therefore, to avoid inhibition caused by VFA in two-stage anaerobic digestion, it is important to adjust the pH value to be in a range of 6.4-7.8 [62]. An experimental study on co-digestion of FW and pig manure revealed that the threshold inhibition concentration of VFAs ranged between 16.5 and 18.0 g/L [65].

1.2.4 Metal elements

Metal elements are essential for a stable and efficient AD process [51]. In general, there are two groups of metals important for AD: light and heavy metals. Iron, nickel, selenium and cobalt have proven to be the most important heavy metals responsible for the stable AD of FW. Their excess can cause disruption in the function and structure of enzymes that lead to inhibition [66]. However, inhibition caused by heavy metals is not usually a concern in AD of FW, since they are usually not sufficiently present in FW [51].

In contrast, light metals like sodium (Na), potassium (K) and calcium (Ca) are more present in FW and could be the cause of salt inhibition [51]. The threshold for sodium inhibition in AD of kitchen FW was between 8 g Na/L [67] and 12 g Na/L [68]. In the case of a calcium presence in AD of FW, the threshold was set at a value of 7 g Ca/L, while the optimum concentration of calcium was reported at between 0.15 and 0.30 g Ca/L [69]. The threshold for potassium inhibition is estimated at approximately 7.5 g K/L [70].

In laboratory conditions, salt inhibition can be detected by measuring electrical conductivity [68]. To avoid salt stress during AD and inhibition in biogas production, the

overall conductivity should be maintained below 30 mS/cm, which has been estimated as the general threshold value [71].

A summary of limiting factors that impact the anaerobic digestion of food waste through an inhibition is presented in Table 1.

Table 1. A summary of limiting factors in anaerobic digestion of food waste

Limiting factor	Inhibition threshold
Ammonia	2-6 g NH_4-N/L
LCFAs	0.5-1.5 g COD/L
VFAs	16.5-18.0 g CH_3COOH/L
Salts	30 mS/cm

1.3 Kinetic modelling of anaerobic digestion of food waste

Kinetic analysis and estimation of the kinetic parameters of AD are important in predicting the behaviour of an anaerobic system and in optimising biogas production [72]. Results of the kinetic analysis quantify the impact of changing process variables like pH, total solids, added co-substrate and others on the rate of biogas production and biogas yield [73]. The most common kinetic models for AD of organic biomass are ADM1, Modified Gompertz, Monod [74], the First-order model and the Cone model [75].

Estimated kinetic parameters for AD of FW performed in a batch mode [75] yielded a value of the first-order kinetic parameter equal to 0.099 d^{-1}, while the Modified Gompertz kinetic parameter was equal to 0.126 d^{-1}. Changing the FW composition and finding its impact on the value of kinetic parameters constituted an attractive method in studying FW capacity for AD [76]. It was established that using an exponential model (First-order model) resulted in a wide range of rate constant values for VS reduction, between 0.55 and 3.63 d^{-1}.

Application of more complex models like the original Anaerobic Digestion Model No. 1 (ADM1) [77] or modified ADM1 [78] to simulate AD of FW showed well-predicted methane production. In addition, these complex models can identify which processes within AD have the most effect on biogas production and are the possible cause of inhibition.

It is important to emphasise, however, that as the complexity of the applied model increases, there are certain limitations, such as the necessity for more data regarding the substrate and the process and more time to successfully apply the model.

1.4   Scientific contribution of the research

Based on the detailed literature review, there is no reported research on AD of FW using rendering industry streams as co-substrates. This study presents a comprehensive experimental and modelling study of the two-stage AD of FW on a laboratory scale when rendering industry streams are added as co-substrates during thermal pretreatment in portions up to 15% on a TS basis. The "closing the loop" approach between the biogas plant and the rendering plant via integrated waste management will be evaluated with the following objectives:

(i)   to assess the impact of rendering plant industry streams, MBM and WWS, on the efficiency of thermally-pretreated FW collected from the biogas plant handling FW as a base substrate;

(ii)   to determine the yield of biogas and to evaluate the stability and efficiency of AD of selected FW, MBM and WWS mixtures by monitoring several important process variables over time, and

(iii)   to estimate kinetic parameters for AD of selected mixtures.

2   Materials and methods

In this section, an overview of applied materials and methods is presented. First, the substrate sampling in the biogas and rendering plant is described, followed by description of

chemical analyses and the laboratory set-up. The last part describes the kinetic modelling of the AD process.

2.1 Substrate and inoculum sampling

The substrates were collected from two companies located near the city of Zagreb, Croatia. FW and inoculum were sourced from the Agroproteinka Energija biogas plant, and MBM and WWS from waste of categories 2 and 3 were collected from the Agroproteinka rendering plant. The inoculum was sampled in an anaerobic digester of the biogas plant and had slightly less than 5% of TS. WWS is sampled at the rendering plant after a decanter centrifuge for dewatering in the wastewater treatment facility [79]. Two sets of experiments were carried out. For the first set of experiments, FW (FW1), the co-substrate MBM and the inoculum (IN1), were sampled on February 15, 2019, while for the second set of experiments, FW (FW2), the co-substrate WWS and the inoculum (IN2) were sampled on April 15, 2019.

2.2 Chemical analysis

First, TS content of substrates and inoculum were determined in an oven (Universal Oven UN 30) at 105°C. Around 30 g of raw substrate was placed in a ceramic crucible and dried in an oven until constant weight. TS content was determined in three parallels.

During the two-stage process (pretreatment and AD), an analysis of the gas and liquid phases was conducted, using the standard equipment found in biogas plants. Gas phase composition was analysed by an OPTIMA 7 biogas analyser, and the following gases were measured: methane (CH_4), carbon dioxide (CO_2) and hydrogen sulphide (H_2S). For the liquid phase, several analyses were performed. COD was determined by Hach LCK cuvette tests and LT 200 Series COD reactor for digestion. Temperature was set at 148°C and time was set to 120 min. The concentration of NH_4-N was also analysed by Hach LCK cuvette tests. A DR 3900 spectrophotometer with RFID technology was used to measure concentrations of COD

and NH_4-N. pH was measured by a Hach HQ440d pH-meter. The concentration of VFAs and total inorganic carbon (TIC) was measured by a TitraLab AT1000 Series Potentiometric Titrator. Before each use, the instruments were calibrated according to the declared procedure. Analysis of both the gas and liquid phases were conducted in triplicate, and the average values are presented.

2.3 Experimental set-up

Two experiments were conducted, where first the substrates (FW and MBM and FW and WWS) were hydrolysed at 35°C for 5 days, and further AD was performed on pretreated substrates to possibly replicate biogas production in Agroproteinka Energija (AE) company.

2.3.1 Thermal pretreatment

In the first stage, MBM and WWS were added to FW in the amounts of 0, 5, 10 and 15% on a TS basis. The reason for adding co-substrates in such small fractions as compared to some previous reports [33,34] is to maintain process stability. The mixtures were prepared in triplicate with a total mass of 60 g TS added to a container of 1.0 L volume. After adding the mixtures to the containers and sealing them with parafilm, the containers were placed in a heated bath. Thermal pretreatment of mixtures was conducted for 5 days at a temperature of 35.0°C, which was maintained by a SC 100 immersion circulator (Thermo Scientific™). Substrates for the second stage (AD) were then selected based on measured changes to the variables in the liquid phase.

2.3.2 Anaerobic digestion

In the second stage, AD of pretreated mixtures was performed. Two mixtures were selected for AD: a control mixture (with no added co-substrate, thus only FW) and a mixture with the overall best parameters according to the selection criteria as analysed after thermal

pretreatment. AD was performed in 500 mL volume filter flasks, which were placed in a heated bath where the temperature was set and maintained at 40.5°C.

In total, 20 g of TS was added to each reactor. For all the batch assays, the ratio between substrate (mixture after thermal pretreatment) and inoculum was set to 1:1 on a TS basis. At the start of the process, the pH value in the reactor was set to 7.8 (as in an anaerobic digester of AE company) using an NaOH solution (pH=13). Finally, demi-water was added so that 5% of the TS content in the reactor (also, as in an anaerobic digester of AE company) was achieved.

Biogas production was measured according to the DIN 38414-8 standard method [80] using a graduated 400 mL eudiometer in 5 mL increments, water as a confining liquid and a levelling bottle of 1.0 L. To subtract biogas production in substrate assays, the blank assay containing only inoculum was set. Figure 1 presents the schematics for batch-mode two-stage AD composed of a) thermal pretreatment and b) anaerobic digestion.

Biogas yield (S, expressed in Nm^3/kg TS) of each mixture is calculated using the following equation [81]:

$$S = \frac{V(\text{biogas})}{m(\text{substrate})} \tag{1}$$

where V(biogas) is the cumulative biogas production given at 101,325 Pa and 0°C (also called normalized volume of biogas, Nm^3 [82]) and m(substrate) is the mass of the studied mixture expressed in kg TS.

a) Thermal pretreatment at 35.0°C
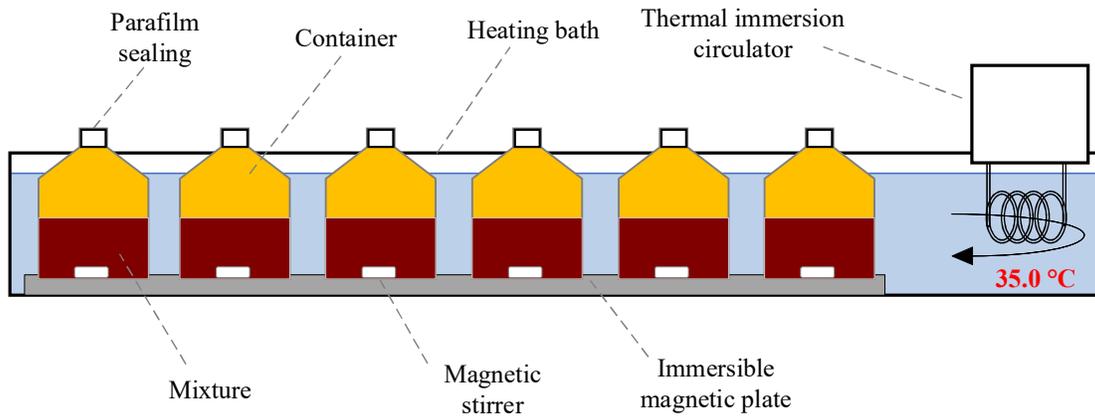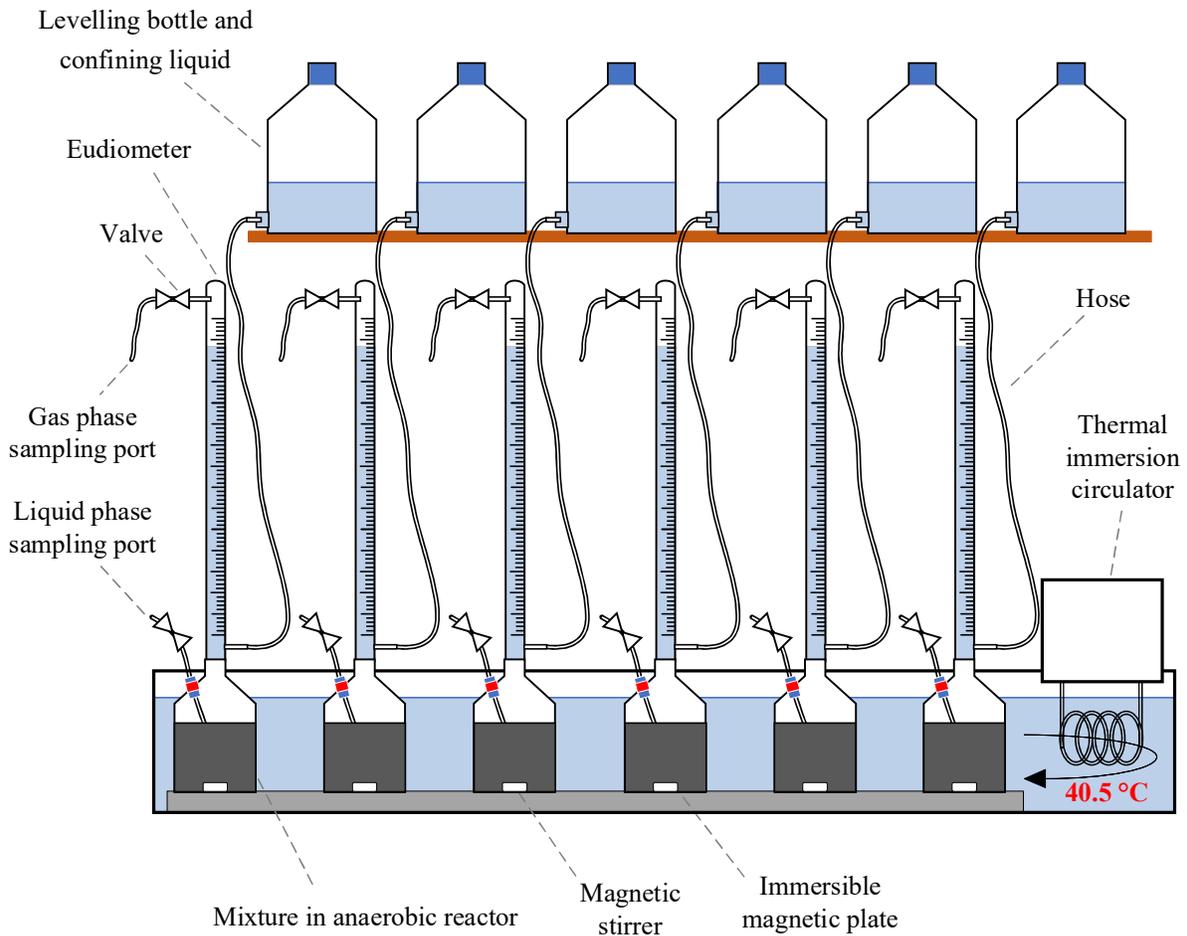

b) Biogas production at 40.5°C

Figure 1 Schematic diagram for batch-mode a) thermal pretreatment and b) biogas production

2.4 Kinetic modelling

Kinetic study of AD from FW and rendering industry streams was carried out, using four different models. Cumulative biogas production during batch AD [83] was being estimated, using the models as presented in Table 2.

Table 2. Kinetic models for AD

Model	Mathematical definition	
First-order [84]	$S(t) = S \cdot \left(1 - \exp\left(-k \cdot t\right)\right)$	(2)
Monod [85]	$S(t) = S \cdot \left(\dfrac{k \cdot t}{1 + k \cdot t}\right)$	(3)
Modified Gompertz [86]	$S(t) = S \cdot \exp\left(-\exp\left(\dfrac{R_{\mathrm{m}}}{S} \cdot \exp\left(\lambda - t\right) + 1\right)\right)$	(4)
Cone [75]	$S(t) = \dfrac{S}{1 + \left(k \cdot t\right)^{-n}}$	(5)

$S(t)$ is the time reported biogas yield [Nm^3/kg TS]; k is the first-order reaction constant (First-order model) or rate constant (Monod and Cone model) [d^{-1}]; R_{m} is the maximum biogas production rate [Nm^3/(kg TS·d)], λ is the lag time [d] and n is the dimensionless shape factor.

Root-mean square error (*RMSE*) was used to indicate the quality of the model's fit to experimental data, which was calculated using the following equation [38]:

$$RMSE = \sqrt{\dfrac{\sum\limits_{i=1}^{N} \left(S_{\mathrm{exp,i}} - S_{\mathrm{mod,i}}\right)^2}{N}} \tag{6}$$

where $S_{\mathrm{exp,i}}$ is the average biogas yield obtained in the experiment, $S_{\mathrm{mod,i}}$ is the biogas yield obtained by the model, and N is a number of measurements (data points). To find the optimum value of kinetic parameters (k, R_{m}, λ, n) using these models, the values of kinetic parameters were such to achieve the highest match of the model with the experimental data, and thus the lowest value of *RMSE*.

3   Results and discussion

In this section, the results of experimental and kinetic analysis are presented. The authors would like to note that data on the chemical composition and physical properties of substrates are confidential, while the results of the research, such as TS content, parameters measured before and after pretreatment and during AD are shown in the following.

3.1   Total solid content of substrates and inoculum

Table 3 shows the TS content of the substrates and inoculum that were used for two-stage hydrolysis and AD.

Table 3. TS content of substrate and inoculum samples

Substrate/Inoculum	Total solid content [%]
FW1	19.58 ± 2.23
FW2	19.98 ± 0.31
MBM	99.30 ± 0.52
WWS	12.60 ± 0.03
IN1	4.44 ± 0.01
IN2	4.53 ± 0.01

FW has a TS content of approximately 20%, which is in the range of values found in the literature: 7.6-39.5%. [37–39,87–89]. The wide span of TS in FW is mainly due to FW composition. In an AE biogas plant, the TS content of 5% is achieved by adding water or some waste liquid stream such as spoiled milk, juice, waste soup from restaurants, or any liquid waste available for use.

MBM showed the share of TS to be almost 100%, while WWS had a much lower TS content, ca. 13%. The moisture content in MBM is usually around 5% [90] or even below 2% [47], as in this study. Such a high TS content makes MBM highly suitable for incineration as a

supplement to or replacement for coal [91]. MBM is typically incinerated when it fails to meet the standards for use as animal feed (waste category 1) [42].

WWS shows TS content to be in a range, as reported previously, between 10.8-16.9% [37,39,87]. The inoculum has a TS content slightly less than 5%, which is in the range of the TS content in biogas plants [92], and is a relatively common value for digestion of FW [93].

3.2 Thermal pretreatment of food waste and rendering industry streams

Thermal pretreatment of FW and rendering industry streams, MBM and WWS, was evaluated by monitoring the change in pH, NH_4-N and COD. Values of parameters measured before and after pretreatment are shown in Figure 2 left, for the first experiment (co-substrate MBM, inoculum IN1, sampled on February 15, 2019) and in Figure 2 right, for the second experiment (co-substrate WWS, inoculum IN2, sampled on April 15, 2019). The coloured bar in Figure 2 represents the average value of the variable for the given mixture, while range bars delimit the actual range of values measured in the experiments [94].

The results in Fig. 2 a) show that both FW (FW1 and FW2, collected at different times) show a similar range of pH during the pretreatment, between 3.40 and 3.50. According to the literature, the reported pH range of FW is very wide, between 3.7 and 6.1 [38,64,87,95]. Adding MBM to FW1 slightly increases the pH, from about 3.5 (0% MBM) to about 3.9 (15% MBM). Such a trend was anticipated, since MBM is the product of alkaline hydrolysis where NaOH is used to dissolve animal industry streams in rendering plants [96]. On the other hand, WWS showed no significant change in pH. The results also show that after pretreatment, the pH values remain similar to those before pretreatment in all the cases analysed.

Figure 2 b) show the impact of adding rendering industry streams to FW in terms of NH_4-N concentration. The FW2 sample (right) had a greater share of nitrogen-rich material than the FW1 sample (left). Values are slightly higher compared to previously reported values, which are about 0.203 g/L [89]. With more MBM, and especially WWS, in the substrate, NH_4-N

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concentration increased, since both animal industry streams are rich in proteins [97] that hydrolyse during the pretreatment and increase NH$_4$-N concentration.
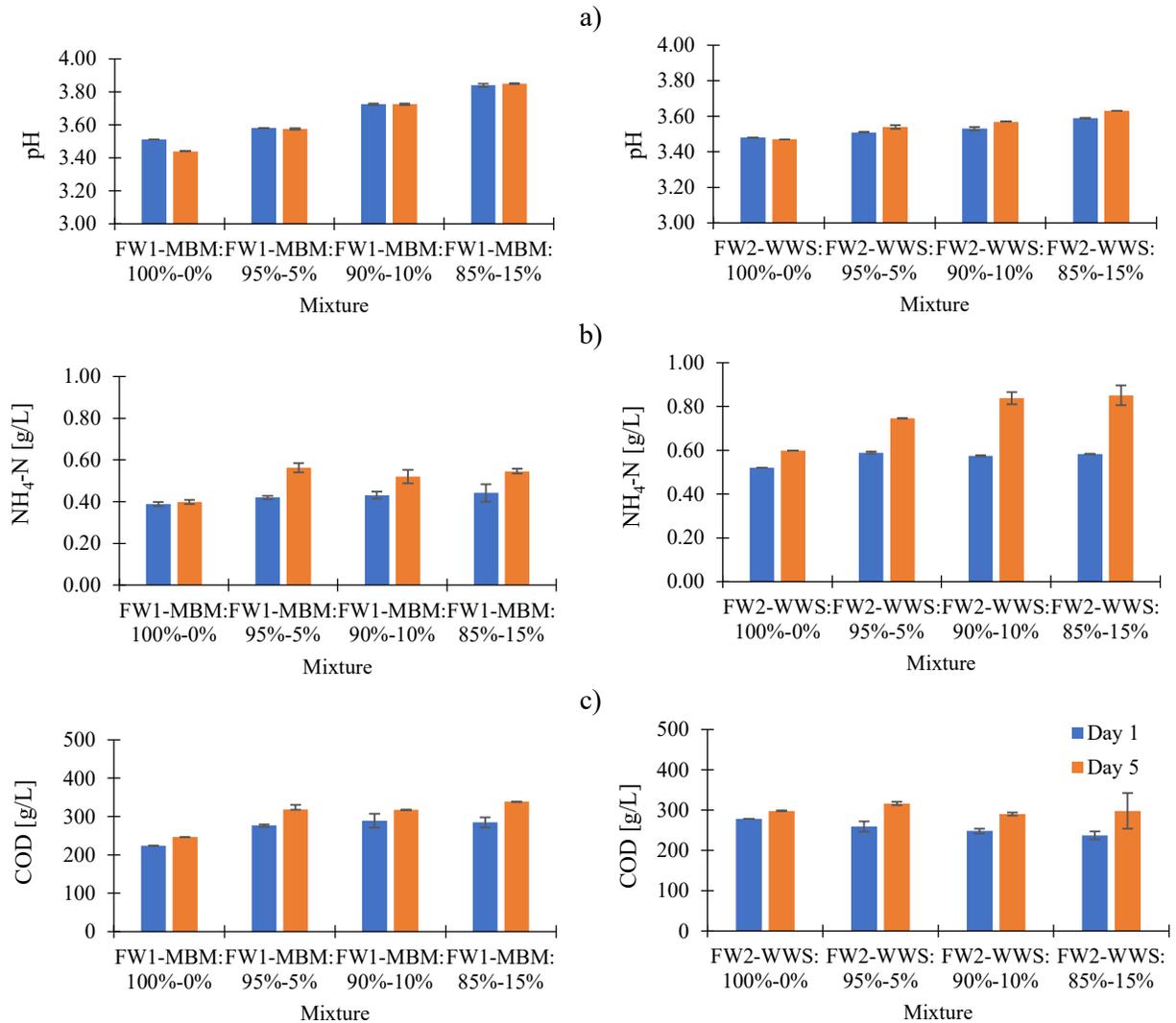

Figure 2 Change in a) pH, b) NH$_4$-N and c) COD during pretreatment of FW1 and MBM

(left) and FW2 and WWS (right)

COD values for the samples are shown in Figure 2 c). FW2 has a slightly higher COD value (298 g/L) compared to FW1 (224 g/L). Results of the research are in line with results obtained for cafeteria FW with a pH of 4.2 ± 0.3, where COD was 197 ± 42 g/L [98].

As a result of the pretreatment, COD increased by 7 - 26%, more in the case of FW2-WWS. When adding MBM to FW1, an increasing trend of COD occurs, while in the case of

FW2-WWS, a decreasing trend is obtained, which is expected, since WWS is a low-organic material [99].

Based on these results, selection criteria were determined to decide which samples to select to reveal their impact in terms of AD. The mixture with the highest relative increase in COD and the lowest relative increase in NH_4-N concentration during pretreatment was selected. The first indicator stands for the higher amount of degradable organic matter, which in theory corresponds to higher biogas yield. The second criterion is related to prevention of ammonia inhibition during AD. Based on the chosen criteria, mixtures FW1-MBM: 95%-5% and FW2-WWS: 95%-5% were selected for the second AD stage.

3.3 Anaerobic digestion of food waste and rendering industry streams

In the second stage, AD of FW1, FW1-MBM (95%-5%), FW2 and FW2-WWS (95%-5%) were carried out. During the process, analyses were performed for both gas and liquid phases. In the gas phase, biogas yield and composition were measured, while in the liquid phase, pH, VFAs, TIC, NH_4-N, COD and electrical conductivity were analysed.

3.3.1 Gas phase

Figure 3 a) - d) shows the results of variables for the gas phase of AD for thermally pretreated mixtures of FW and rendering industry streams.

For the AD of FW, the reported biogas yield is 0.27-0.64 Nm^3/kg VS [100] that amounts to 0.24-0.58 Nm^3/kg TS, using an average VS/TS ratio of 0.90 [100]. In this research, the following biogas yields were obtained: for FW1 – 0.566 Nm^3/kg TS, for FW1-MBM – 0.499 Nm^3/kg TS, for FW2 – 0.252 Nm^3/kg TS and 0.195 Nm^3/kg TS for FW2-WWS. Such a wide range of values is a result of the FW heterogeneity (taken at two different times).
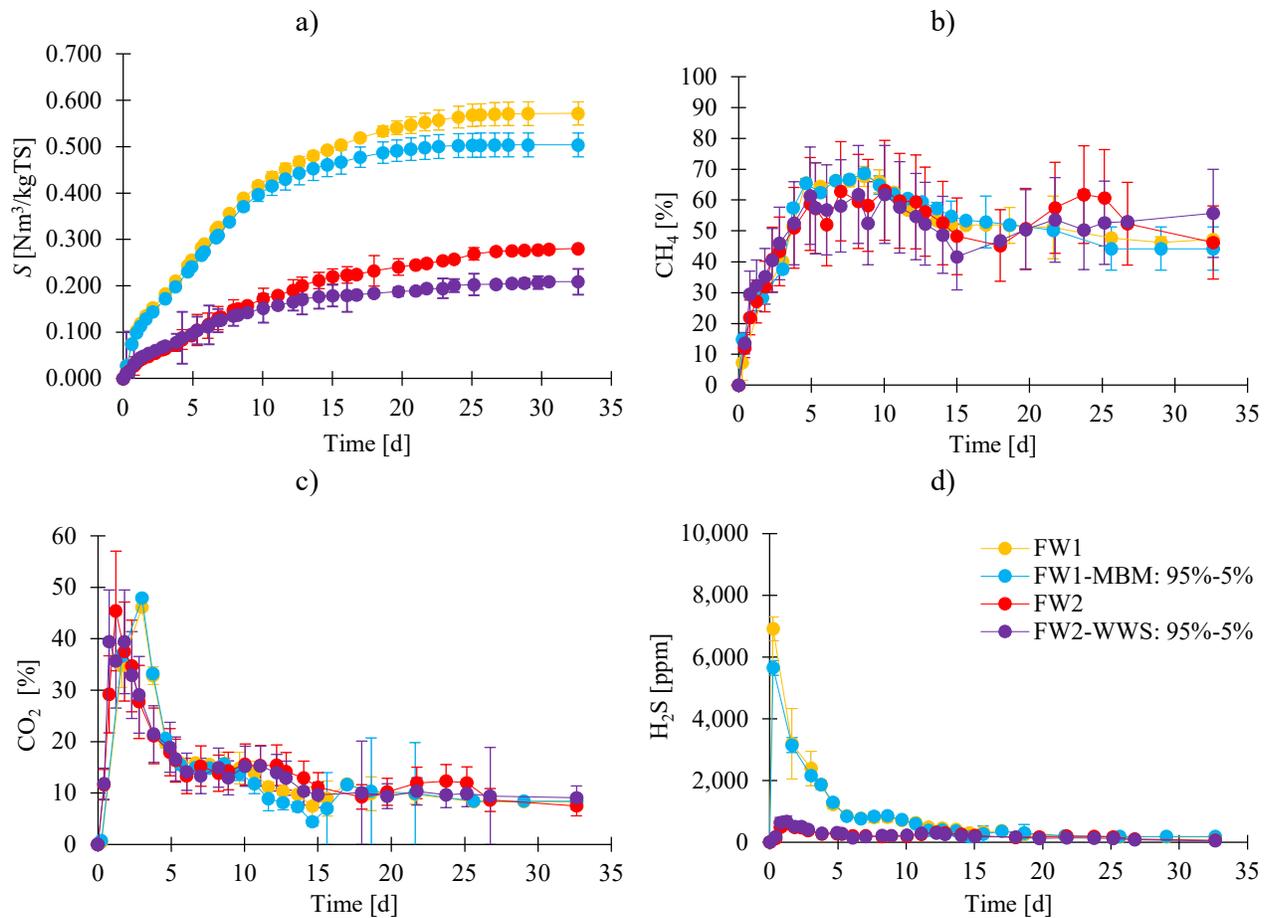
Figure 3 Variables in a gas phase, a) biogas yield, and concentrations of b) CH_4, c) CO_2 and

d) H_2S during AD

In this research, it is estimated that sample FW2 is not very different from FW1, since the material that biogas plant receives usually comes from the same sources, and analysis of the liquid phase (see Figure 4) contributes to that statement. Based on the shape of the biogas yield profiles [101] shown in Figure 3a), it can be concluded that inhibition in AD of FW2 occurred, resulting in about 2.25-fold lower biogas yield compared to FW1. More detailed discussion of the causes of inhibition in the process will be provided in the following subsection on analysis of the liquid phase.

This research showed that both rendering industry streams have decreased biogas yield of FW when added in portions of 5% on a TS basis. It has been stated that FW contains fungi and yeast that enhance its biodegradability during AD [102]. MBM and WWS are sterile

industry streams of alkaline hydrolysis, and when added to FW in AD, they could possibly decrease the size of the bacterial community of fungi and yeast in FW, which is reflected in a slightly lower biogas yield.

According to the previous report, in the steady state period, the biogas produced from FW reported CH_4 concentrations to be approximately between 53% and 59%, while the CO_2 concentration in biogas was in the range of around 40-47% [88]. In this study, similar concentrations of the main biogas components in the steady state period (after day 20) was observed. By comparing CH_4 and CO_2 profiles in Figures 3b) and 3c), it can be observed that the FW2 and FW2-WWS mixtures showed slightly lower CH_4 and slightly higher CO_2 content in biogas before stabilizing (days 5-20).

The profiles of H_2S concentration in biogas during the AD showed that the FW1 sample had a much higher content of sulphur-rich materials than the FW2 sample. The highest reported H_2S concentration in the experiments was obtained one day from the start of the process and reached approximately 7,000 ppm. According to the literature, raw biogas can have up to 10,000 ppm of H_2S [103]. In both cases, rendering industry streams reduced H_2S generation during AD of FW, which could be a promising topic for further exploration in the future, since high H_2S concentration during combustion produces high amounts of SO_2, which affects biogas engines on account of corrosion [104].

It is also important to note that for both batch experiments, as biogas was produced, it displaced the air which was trapped in the reactor headspace at the start of the process and decreased the share of oxygen in the gas phase. By displacing oxygen and other gases by biogas, anaerobic conditions in reactors were achieved and maintained. Other contaminants such as nitrogen, water vapour and oxygen can be present in raw biogas in amounts up to 15, 3 and 5% [103]. In this research, the maximum oxygen content in produced biogas was 5%, while concentrations of nitrogen and water vapours were not measured.

3.3.2 Liquid phase

Figure 4 a) - f) shows the results of variables for the liquid phase of AD of thermally pretreated mixtures of FW and rendering industry streams.
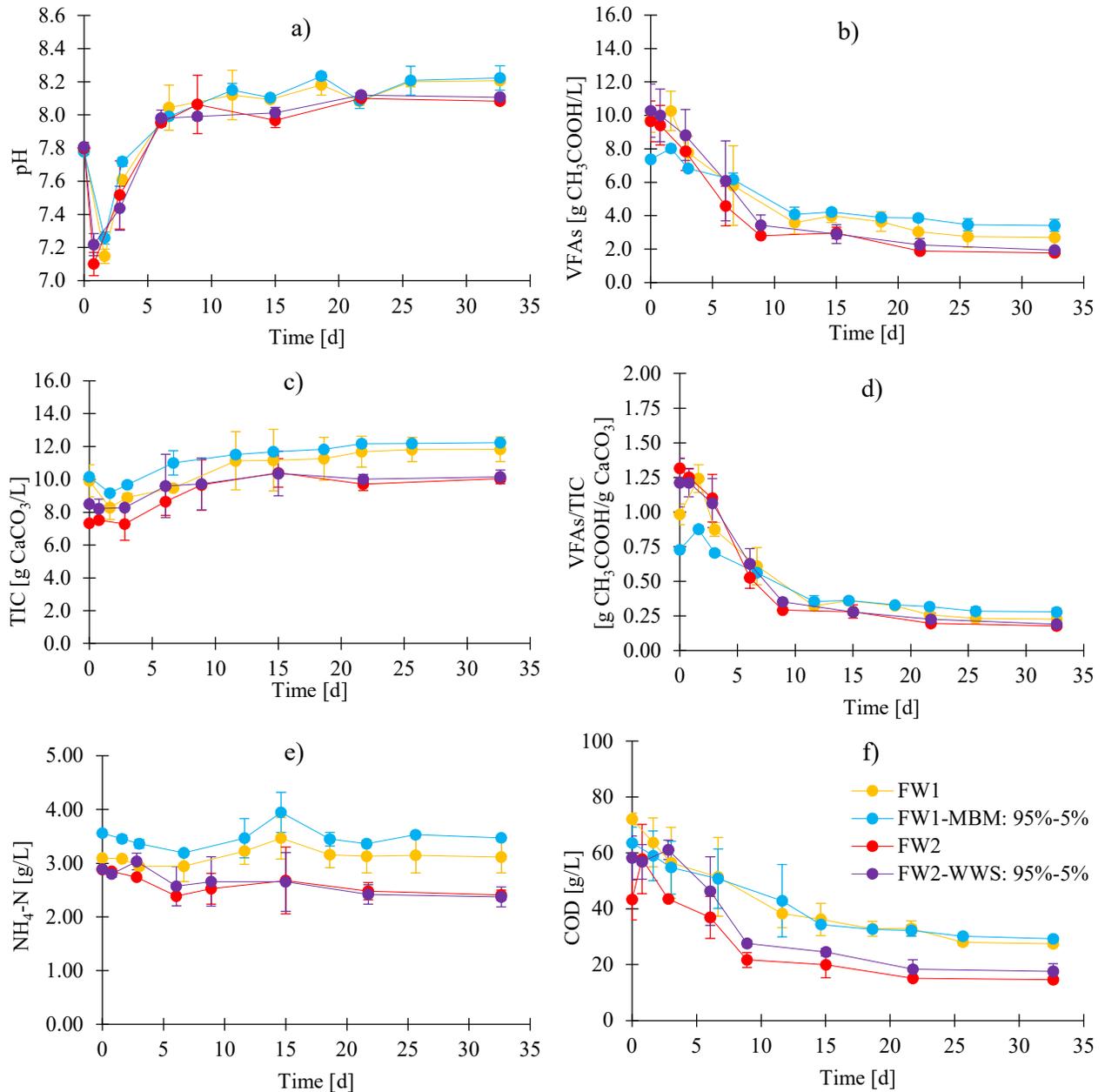

Figure 4 The change in variables in a liquid phase, a) pH, b) VFA, c) TIC, d) VFA/TIC, e) NH_4-N and f) COD during AD of selected mixtures

The profile of pH values determined in this research follows the theoretical pathway. During the first days of the process, pH value drops because of acidogenesis and acetogenesis,

while it subsequently increases as VFAs are consumed to produce CH_4 and CO_2 [105]. As mentioned before, MBM is a product of alkaline hydrolysis of animal industry streams [96], and when added as a co-substrate to FW, it slightly increases pH (see Figure 4a). FW2 showed a similar range of pH values to that of FW1. As with MBM, WWS slightly increased the pH of FW. According to some previous studies, the pH values for AD of FW ranged between 6.0 and 8.5 [35,106]. The pH values obtained in this research showed that there was no indication of inhibition in biogas production caused by poor pH control for FW2 and WWS [107].

Figure 4b) shows the reported profile of VFAs during AD of selected FW mixtures. VFAs are generated during the acidogenesis stage, which causes the drop in pH as shown in Figure 4a). For the AD of FW, it was reported that the concentration of VFAs ranged between ca. 10.0 to 11.0 g/L, while pH ranged between 7.5 and 9.0 [108]. However, another study showed that the maximum value of VFA concentration during AD of FW was even below 5.0 g/L, while pH was above 8.0 [89]. During the entire process, FW1 achieved a VFA conversion of 81.8%, while FW1-MBM achieved a VFA conversion of 57.5%. Adding MBM to FW causes lower generation of VFAs, which was reflected in lower levels of conversion to biogas and consequently lower biogas yield, as shown in Figure 3a). The VFA conversion was 81.6% for FW2, and 81.2% when WWS was added to FW2. Results show that VFAs in all mixtures under analysis were converted successfully, which is an indication of non-inhibited acidogenesis and acetogenesis steps. Based on that, it can be concluded that inhibition of biogas production for FW2 and FW2-WWS cannot be caused by LCFA or VFA accumulation [109].

The profile of TIC in these mixtures shown in Figure 4c) follows similar trends as the pH profile shown in Figure 4a), since the TIC value represents the buffering capacity of the mixture (ability to change pH by adding acids or alkaline) [93]. The range of TIC values during AD of FW was reported to be between 8.0 and 9.5 g $CaCO_3$/L [110]. Results of this research have proven to be in a slightly broader range: for FW, between 8.272 ± 0.715 g $CaCO_3$/L and 11.835

± 0.933 g $CaCO_3$/L, while for FW2, the range was between 7.285 ± 1.006 g $CaCO_3$/L and 10.396 ± 1.613 g $CaCO_3$/L. In both cases, the addition of MBM and WWS yielded slightly increased TIC values.

Usually, a high VFAs/TIC ratio (<0.4) is an indicator that the AD reactor is overfed by substrate and that the process is unstable [106]. Such an interpretation is valuable only if AD is studied in continuous operation. In this research, a batch AD was performed, which showed that the VFAs/TIC ratio can go above 1.0 with the process remaining stable. Adding MBM to FW1 decreased the VFAs/TIC ratio, since MBM showed a negative effect in term of VFA production. On the other hand, WWS did not significantly affect the VFAs/TIC ratio for FW2. In the case of batch AD of food-processing industrial waste, the VFAs/TIC ratio at the start of the process was approximately 0.70; after 6 days it increased to around 2.3 and later dropped, reaching the final value of ca. 0.25 after 30 days from the start of the process [111].

Ammonia inhibition of biogas production using FW is a relatively common inhibition type in AD, caused by protein-rich material present in FW [112]. It has been determined that, in the case of AD of FW, there is a wide range in the NH_4-N inhibition threshold concentration, between 2 and 6 g/L [55]. As expected, adding MBM to FW1 increased the release of ammonia during AD, similar to what was observed during the pretreatment stage. However, these higher concentrations of NH_4-N when MBM was added to FW did not affect the stability of AD, since the biogas production was not inhibited, as shown in Figure 3a). It can be seen in Figure 4e) that the highest NH_4-N concentration is achieved when adding MBM to FW1. Among the reasons for stable behaviour (despite a comparably high NH_4-N concentration) is adaptation of the microbial community in a digester over time to operation at higher NH_4-N concentrations (compared to others) without causing a failure in the process [113]. The FW2 and FW2-WWS mixtures had much lower concentrations of NH_4-N than FW1, from which we can conclude that ammonia inhibition cannot be the reason why FW2 gave such a reduced biogas production.

Figure 4f) shows the change in COD of these mixtures during the AD. According to the literature, FW shows a wide range of COD values at the start of the process, between 69.92 and 181.05 g/L [55,89,114]. The efficiency of COD removal during AD was approximately the following: 61.9% for FW1, 53.9% for FW1-MBM, 74.7% for FW2, and 71.2% for FW2-WWS. In the literature, it has been reported that COD removal efficiency of two-stage AD of dining hall FW was 78.7% [115], while the COD removal efficiency during AD of canteen FW was slightly lower, between 51 and 62% [109].

Based on the results presented in Figure 4, there is no indicative measure in the liquid phase of what caused the inhibition in AD of the FW2 and FW2 mixtures with WWS, since those samples showed almost identical parameter values as FW1 and FW1-MBM.

Finally, to further explore the possible cause of inhibition, electrical conductivity was measured at the end of the process, which could show possible salt inhibition [67]. The explanation of salt-inhibition mechanisms is that a high presence of sodium ions during AD reduces the conversion of acetate to products (inhibition of methanogenesis) and reduces the potential to produce biogas [116]. In this study, it was noticed that the measured biogas composition (Figure 3b) showed lower methane and higher CO_2 concentrations in the biogas for FW2 and its mixture with WWS. Since the last stage of AD, methanogenesis is related to conversion of acetate and CO_2 to methane, methanogenesis of FW2 is shown to be relatively inefficient.

Measurements of electrical conductivity gave the following results, for FW1 8.99 ± 0.54 mS/cm, for FW1-MBM 9.00 ± 0.39 mS/cm, for FW2 9.96 ± 0.63 mS/cm and for FW2-WWS 9.60 ± 0.44 mS/cm. Results indicate that higher conductivity (higher concentrations of salts [117]) is obtained for FW2. However, the values are still way below the general threshold for salt inhibition of 30 mS/cm [71]. It is possible that a slightly higher concentration of salts in

FW2 resulted in the lower biogas yield, but it is highly improbable to expect that an approximately 10% higher electrical conductivity resulted in 2.25-fold lower biogas yield.

After experimental analysis of two-stage AD of FW and rendering industry streams, a high-level of heterogeneity for FW was confirmed. Analysis revealed that most process variables display the usual behaviour; however, despite that, the AD process was inhibited for a certain FW sample. Rendering industry streams showed antagonistic effects in terms of biogas production when added to FW. It was also noted that their addition to FW slightly improved stability, since a narrower range of reported values was obtained between studied parallels.

3.4 Kinetic parameters of AD

The kinetic parameters of AD for the mixtures were further estimated. Results of the applied kinetic models with the lowest *RMSE* are shown in Figure 5, while Table 4 displays the calculated kinetic parameters.

The best fit of a model to the experimental data for all these mixtures was obtained by the First-order kinetic model, where the estimated reaction rate constant for FW1 was 0.135 d^{-1} and for FW2, 0.097 d^{-1}. As expected, the rate constant for FW2 is lower (by 28%) compared to FW1, owing to the occurrence of inhibition. These results are in line with previous reports. The first-order reaction rate constant for AD of FW has shown a wide range of values, between 0.027 d^{-1} and 0.49 d^{-1} [72,75,118–120].

In this study, Monod kinetics proved to be the least applicable among the models studied, because of the highest *RMSE* values. Application of the Modified Gompertz model in AD of thermally pretreated FW gave a lag phase (λ) equal to 0 d, which was also reported in some previous studies [72,120,121]. Kinetic analysis using the Cone model showed that FW has a shape factor equal to $n=1.6$, and a reaction rate constant between 0.145 and 0.200 d^{-1}. A previous report on the application of a Cone model in AD of FW gave a similar shape factor (1.3) and rate constant (0.126 d^{-1}) [75].

Previous studies have shown that adding co-substrates like seaweed [119], waste cardboard [122], distillery grains [123], pig manure [65] and certain types of wastewater biosolids [124] to FW decreases the value of kinetic parameters. On the other hand, co-substrates like sewage sludge [125], rice straw [36] and dairy manure [126] increase the reaction rate of AD when added to FW.
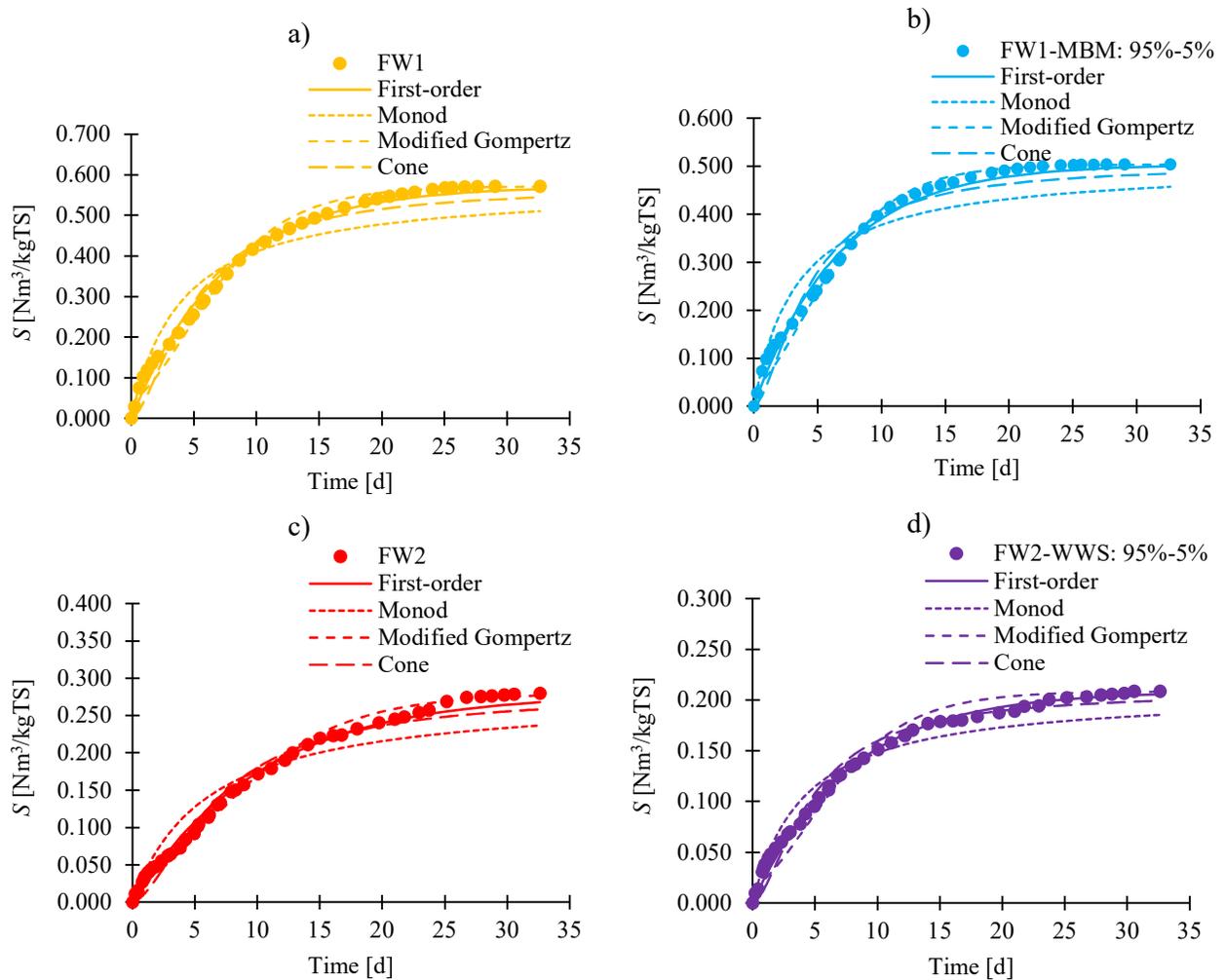

Figure 5   Kinetic analysis of biogas yield for a) FW1, b) FW1-MBM, c) FW2 and d) FW2-WWS

Table 4. Estimated kinetic parameters for AD of selected mixtures

Model	Parameters	Mixtures			
		FW1	FW1-MBM 95%-5%	FW2	FW2-WWS 95%-5%
First-order	k [d^{-1}]	0.135	0.150	0.097	0.131
	$RMSE$ [Nm^3/kg TS]	0.0150	0.0153	0.0079	0.0052
Monod	k [d^{-1}]	0.255	0.300	0.168	0.245
	$RMSE$ [Nm^3/kg TS]	0.0512	0.0476	0.0259	0.0146
Modified Gompertz	R_m [Nm^3/(kg TS·d)]	0.0845	0.0950	0.0623	0.0850
	λ [d]	0	0	0	0
	$RMSE$ [Nm^3/kg TS]	0.0218	0.0171	0.0090	0.0112
Cone	k [d^{-1}]	0.200	0.230	0.145	0.210
	n [$-$]	1.6	1.6	1.6	1.6
	$RMSE$ [Nm^3/kg TS]	0.0305	0.0288	0.0136	0.0106

4 Conclusion

This study has investigated the thermal pretreatment of rendering industry streams, MBM and WWS with FW obtained from a biogas plant, and further biogas production potential has been explored. Thermal pretreatment of these mixtures at a temperature of 35°C for a 5-day duration showed no impact on the pH, while concentrations of both COD and NH4-N increased. AD of both samples containing MBM or WWS causes antagonistic effects in terms of biogas production when added to FW. Adding 5% MBM to FW1 decreased biogas production by 12%, while adding 5% WWS to FW2 decreased biogas production by 23%.

This research has also shown that there is a relatively high probability of inhibition during AD of FW, on account of the variety and complexity of FW. In addition, it was found that a certain inhibition could occur that could not be detected using the standard equipment applied in biogas plants.

Based on kinetic analysis, rendering industry streams showed an increase in the reaction rate of AD from FW, determined by means of the different kinetic models used in this study.

In this research, First-order kinetics showed the highest match between the experimental and model data, where the reaction rate constant increased from 0.135 d^{-1} to 0.150 d^{-1} when MBM was added in 5% share to FW, and from 0.097 d^{-1} to 0.131 d^{-1} when WWS was added to FW.

The experimental approach has also shown that process variables, such as pH, LCFAs, VFAs, VFAs/TIC ratio, NH_4-N and electrical conductivity behave as usual, although the AD process might be inhibited. The research has proved that utilisation of waste and residue materials to produce advanced biofuels, such as biogas, is more complex and requires higher level analysis, compared to the use of common substrates to produce biogas, e.g. cultivated energy crops.

Future research should focus on analysing more detailed causes of inhibition during AD of FW and on exploring how to prevent such inhibitions.

Acknowledgements

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