# Valorization of Organic Fraction of Municipal Solid Waste Through Production of Volatile Fatty Acids (VFAs) and Biogas

Hojjat Borhany*†

†Affiliation: Department of Environmental Sciences Informatics and Statistics, "Ca Foscari" University of Venice, Via Torino 155, Mestre-Venice, Veneto, 30170, Italy

*Corresponding Author: hoj.borhany@gmail.com

## Abstract

The economic and technical feasibility of a multiple-step recovery process from the organic fraction of municipal solid waste, which constitutes the acidogenic fermentation and anaerobic digestion (AD), was studied and optimized concerning HRT and inoculum/feedstock (FS) ratio. It was also investigated if the addition of biochar to AD could improve the process kinetics and methane content. The results indicate that HRT of 4.5 days could yield higher VFA content (30.5 g-SCOD/L) as opposed to HRT of 3 days (27.66 g-SCOD/L). Moreover, it was seen that inoculum/FS of 3.3 (volatile solid basis) could increase the specific methane yield (0.3 $\frac{\mathrm{CH_4-m3}}{\mathrm{kg-VS.d}}$) and improve it kinetically, whereas biochar addition could only improve the maximum obtainable methane content (76%, v/v). Additionally, root means squared error (RMSE), obtained from the calibration of a kinetics study, showed that the first order rate is more precise in describing the biochar AD (6.7 vs 8.6), whilst the modified Gompertz is fitted better to data without biochar (9.31 vs 8.33). The economic analysis for a scaled-up version with 70,000 PE indicates that the proposed two-step process could reach profit much sooner, almost 3.5 years in comparison to a single AD with 11 years of non-profitability.

**Keywords:** Acidogenesis Fermentation, Anaerobic Digestion (AD), Kinetics Study, Biochar, First Order Rate (FO), Modified Gompertz (MG), Mass and Energy Balance

## Introduction:

In the European Union (EU), about 55 million tons of organic waste was produced annually, which mainly came from houses (40%), food waste sectors (15%) as well as business divisions (5%) [1]. Considering current national legislation, which forbids landfilling organic waste but treats them through biological and thermal processes like anaerobic digestion (AD), composting, and incineration with high disposal cost (75-125 €/ton) [2]. Under the pressure of such an exhaustible natural exploitation and an increasing amount of organic waste, the policymakers in the European Commission gave the circular economy action plan the green light to promote sustainable recovery methods, that reduce the secondary waste flux. These approaches, which are typically recommended in the context of circular economy, assume a "take-use-reuse" viewpoint that wants to close the circuit of cycles extended product life, and treat the wastes as precious recyclable materials [3][4]. In this context, biological processes such as acidogenic fermentation and AD have been deployed widely in many EU states to gain either platform chemicals such as volatile fatty acids (VFAs) or biogas from organic wastes produced in urban areas[5]– [8].

In that respect, a multiple-step treatment route to obtain various bioproducts such as VFAs and bio-hydrogen, with higher added-value markets than single bio-methane, at distinct steps was aimed in the recent studies in the hope of either redesigning the existing single-step plant or integrating them into bio-refinery platforms [9], [10].

Because AD can take two main metabolic routes: methanogenesis or acidogenic fermentation depending on operational conditions and avoiding VFA stream with a low VFA/SCOD ratio or fewer appetite VFAs (lactic acids and alcohols), which could negatively impact bioproducts upstream process alongside technical and economic feasibility of the whole process, the laboratory study was inevitable and a prerequisite step before going to the up-scaled industrial version. So

here, we are searching for a process with a desirable VFA-rich stream from fermentation and methane from methanogenesis [11], which ultimately could make the process more profitable as VFAs typically serve as platform chemicals for polyhydroxyalkanoates (PHA) synthesis process[12]–[14]. These biopolymers could be later recovered through biological processes to close the material life cycle.

Nonetheless, AD of bio-waste is highly limited by the hydrolysis step, which could be relieved by various methods such as pre-treatment, optimized inoculum/FS ratio, and carbonaceous material addition such as biochar [15]–[17]. The latter method was recently realized with several advantages to the process, such as improving the process stability, accelerating the process rate, buffering potency and alkalinity, inhibitors adsorption, enriched microbial functionality, and electron transfer mechanism, which ultimately could improve $CH_4$ generation by fostering hydrolysis, acetogenesis, and methanogenesis[18].

In this study, OFMSW, which was separated and collected from the municipality of Treviso in northern Italy, was firstly pre-treated (70 °C, pH=9-10) for 24 hours before feeding to the semi-continuous stirred tank reactor (SCSTR) for mesophilic fermentation under varied organic loading rates (OLRs) and HRTs. The residual solids out of this step, which was recalcitrant for further biodegradation, were used in a bio-methanation test (BMP) to see if the proposed methods could improve the process. The calibration of two models, first order (FO) and Modified Gompertz (MG) models were conducted to identify the most precise model and see how proposed procedures affect the kinetics of the process.

Therefore, here, we search for an efficient, beneficial, and environmentally friendly multiple-step approach for organic waste valorization, which is omnipresent in the urban area, and their economic value not attained yet, through fermentation succeeded by boosted anaerobic digestion.

This procedure allows us to obtain a wide range of biochemical products such as VFA with higher added value than a single biomethane obtained from single-step AD, which was currently deployed in Treviso WWTP [19].

## Experimental (Material and Methods).

In Figure 1, you could see the overall schematic of the treatment line used in this study. Firstly; the OFMSW was obtained and separated by a door-to-door collection system in Treviso municipality and then after, it was transferred to the WWTP and was diluted to reach a weight ratio of 3%-4%. Then, the prepared feedstock was pre-treated at pH = 9-10 at the temperature of 70˚C for 24 hours before it was fed manually into the 5L mesophilic reactor operating at OLRs (6.89-10.33 kg-VS/$m^3$. d) and HRTs of 4.5 and 3 days. The fermentative bacteria, already existing in the substrate, can convert the organic matter into VFAs, hence, no anaerobic inoculum was used. The tests were characterized in terms of VFA yield, VFA concentration, SCOD, ammonia, and phosphate release. The output from the mesophilic fermentation reactor was centrifuged and the solid part was separated for further mesophilic BMP test. This batch test was done with the addition of biochar in two diverse dosages (0.12-0.24 g-Biochar/g-VS) in bottles of 250 ml (working volume 216 ml) during 25 days in the thermostatic bath. A total number of 8 bottles were used in this experiment to observe the effect of biochar addition on biogas volume and content alongside the reaction kinetic. The first two bottles contain only inoculum. The 3$^{rd}$ and 4$^{th}$ contain feedstock and the 5$^{th}$ and 6$^{th}$ contain feedstock + 3 g/L biochar and the 7$^{th}$ and 8$^{th}$ contain feedstock + 6 g/L biochar.

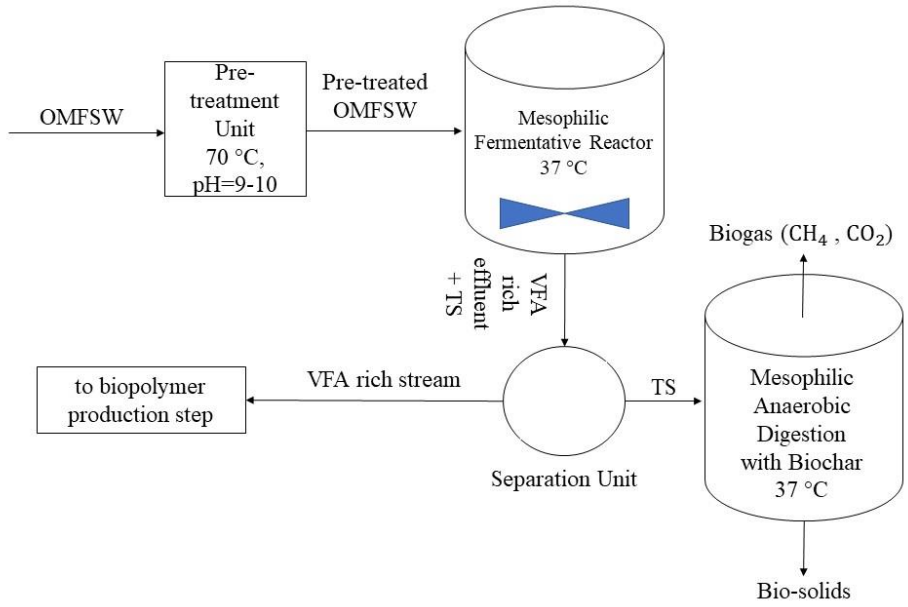



Figure 1. Schematic overview of the lab scale multi-step process for biogas and VFA production from the OFMSW

## Anaerobic Pilot Unites.

The anaerobic fermentation reactor used in this study was a type of semi-continuous stirred tank reactor (SCSTR) with an operational volume of 4.5 L, which was fed and emptied manually and regularly according to HRTs. The mesophilic fermenter in this study operated at a pH range from 5.95-7.55 (controlled with soda addition in the pre-treatment unit) with separate operational conditions of HRT of 4.5 and 3 days with relative OLRs of 6.89 – 10.33 (kg-VS/$m^3$.d). The mixture was mixed mechanically and the whole system was kept in the oven to hold the temperature constant at a level of 37°C. The output from the fermenter was centrifuged and the supernatant was separated to be used in relevant parameters measurements (SCOD, VFA, $N-NH_4^+$, $P\text{-}PO_4^{3-}$) and the solid part was collected to apply the BMP test. The biochar was added to the bottles (250 ml, 216 ml working volume) to reach two different biochar concentrations of 3 and 6 (g/L), and they were kept in the thermostatic bath to maintain the temperature at the level of

37 °C. The gas volume was measured by syringe and the mixture was manually mixed before setting back to the thermal bath. The inoculum BMP test was collected from 2300 $m^3$ CSTR anaerobic digester treating thickened wasted activated sludge (WAS) and squeezed OFMSW mixture under the mesophilic condition at an OLR of 1.8-2.0 (kg-VS/$m^3$. d), in the treatment plant, and was acclimated for two weeks under mesophilic conditions before the application. The acclimated inoculum was added to residual solid from previous mesophilic fermentation based on a weight ratio of 3.3 (g-VS/g-VS,), which was much higher than the similar studies [19]. The TS and VS concentration (including inoculum and feedstock) in the bottles were 221 and 3.9 (g/kg) respectively. Each series of the anaerobic digestion condition, meaning that blank or only inoculum, without biochar (control test) and two various biochar concentrations, were implemented in two bottles and after gas characterization using gas chromatography, biogas volume was measured. For further calculation and kinetic study, the gas volumes were first subtracted from the blank to correct for the background methane potential of the inoculum. The BMP test was terminated after 25 days when the cumulative biogas production reached 89% of the final projected cumulative value.

## Characterization of OFMSW and Carbonaceous Material.

The feedstock was characterized for different parameters with various frequencies throughout the experiment. The total and volatile solids content (TS/VS) measurements were done several times during the experiment immediately under freshly arrived feedstock. Other parameters like COD, TKN, P, and VFA were once measured on fresh feedstock as it was known that the feedstock chemical properties were quite stable. In general, the feedstock contains 40 ± 3.5 g/kg of TS and 31 ± 3.28 g/kg of VS, meaning that it contains more than 70% of the volatile fraction, which approves its highly biologically treatable characteristics.

The carbonaceous material used in this study was biochar, which is produced by the local supplier and its main chemical features were reported in Table 1 in supplementary documents.

## Analytical methods.

The reactor output was sampled and characterized three times a week for SCOD and VFA characterization, and two times a week for P-$PO_4^{3-}$, $NH_4$-N, while its pH was measured daily (unless Saturday and Sunday). The characterization of the solid part in the effluent was done once a month for Total Kjeldahl Nitrogen (TKN), P, and COD (mg-COD/kg-TS). The TS and VS characterization were adopted in 105 º C and 550 º C ovens for 24 hours. Except for VFA, all the remaining analyses were carried out following the Standard Methods [20]. The quantification of the VFA and gas content was conducted using AGILENT 6890N gas chromatograph, which was described in detail in the supplementary document.

## Calculations and Mass Balance.

The parameters characterizing the performance of the mesophilic fermentation were calculated in the days, when the data are available, and the process reached the pseudo-steady-state condition. The state of stability of the process was evaluated based on the VFA content and pH. The main parameters and their formula were reported in the supplementary documents. The exploratory data analysis and statistical description of VFA data were performed by the open-source program R (The R Foundation for Statistical Computing, version 3.5.0).

The COD solubilization, VFA yield, and rate were calculated based on their final concentration (mg/L), and initial unfermented feedstock characterizations, especially the values for $SCOD_0$(mg/L) ,$VFA_{(0)}$ (g/L), and $VS_{(0)}$ (g/kg). The release for ammonia and phosphate were determined based on their final concentrations (mg/L) and concerning the nitrogen and phosphor content available in the unfermented solid part, reported for TKN (g/kg), P (g/kg) and $TS_{(0)}$ (g/kg). In this study, the weight distribution of VFA was also conducted, because the fermented effluent potentially could be utilized in the MMC-PHA production process. Indeed, the high VFA/SCOD is required in an efficient PHA synthesize step and VFA distribution like even numbered C-atoms VFA (3-hydroxybutyrate precursors; 3HBp) and the odd-numbered C-atoms VFA (3-hydroxyvalerate precursors; 3HVp) affects monomers synthesis and consequently the PHA composition [21].

In BMP, the biogas volumes were measured daily, and with much less frequency, its content was characterized. To fill those values where gas characterization wasn't measured (day= 0, 2, 3, 7, 8, 11, 23), K-Nearest Neighbors Algorithm (KNN) imputation (number of neighbors=4, weights=distance) was employed to impute the missed values [22]. After having all the gas characterization data, specific methane production (SMP) and methane production rate (MPR)

were quantified for the final cumulative bio-methane volume. Consequently, two kinetic models (first-order reaction rate and modified Gompertz model) were calibrated to biomethane data (utilizing the least-squared method), and the results revealed how fast the hydrolysis of the volatile solid happen as well as how fast the reaction proceeded (lag phase and maximum methane production rate).

In our study, mass and energy balance analysis were conducted for two scenarios: either a single-stage mesophilic AD process, which was similarly deployed in Treviso WWTP, or a scaled-up system with mesophilic fermentation succeeded by mesophilic AD based on the obtained performance parameters. The purpose of comparing these two scenarios was the identification the optimal one in terms of surplus thermal energy and electricity production as well as the net benefit of biorefinery products. All the parameters were adopted for reactors treating OFMSW collected from the municipality with an average size of 70000 (PE). Reference parameters and boundary conditions are given in Table 1. In the energy analysis, the price of electricity was assumed to be 130 €/MWh.

| Parameter | Unite | Value |
|---|---|---|
| **Biogas** | | |
| Low Heat Value Biogas | MJ/N.m3 | 23.02 |
| **Combined Heat and Power (CHP)** | -- | 0.5 |
| Thermal Energy yield | -- | 0.4 |
| Electrical Energy yield | | |
| **Boundary Conditions** | | |
| Operative Temperature Anaerobic | º C | 37 |
| Processes | º C | 15 |
| Water Temperature | º C | 20 |
| Air Temperature | º C | 25 |
| Ground Temperature | | |
| **Heat Transfer Coefficient** | W/(m2. º C) | 0.7 |
| Outer Concrete Reactor Wall | | |
| Inner Concrete Reactor Wall | W/(m2. º C) | 1.2 |
| Floor | W/(m2. º C) | 2.85 |

Table 1 Reference parameters and boundary conditions for energy balance

## Results and Discussion

### Bio-waste Composition and Characteristics:

The feedstock used in this study was quite stable in terms of its physical and chemical characteristics throughout the experiment. The main parameters monitored on the feedstock were shown in Table 2. It has a TS content of 40 ± 3.5 (g/kg) and VS content of 31 ± 3.28 (g/kg), meaning that 70.2% ± 2.73% of the OFMSW constitutes the biodegradable part, which could support the fermentation process. The chemical composition of the solid part was 12.9 (g-N/kg-TS), 4 (g-P/kg-TS), and 565 (g-COD/kg-TS), which is in the range of the values reported for typical OFMSW in Italy [23]. The chemical parameters of the solution phase for mesophilic feedstock were 325 mg/L of N-$NH_4^+$, 14 mg/L of P-$PO_4^{3-}$ and 25.8 g-SCOD/L. The feedstock COD: N: P ratio was 100/2.2/0.7, meaning that nutrients such as phosphor and nitrogen were limiting substrates in fermentation, which is the typical characteristics of municipal solid waste [24]. Moreover, there was a slight level of VFA at 3.5 g-SCOD/L as a result of the already presence of fermentative bacteria.

| Parameters | Values |
|---|---|
| TS (g/kg) | 41 ± 3.15 |
| VS (g/L) | 29 ± 3.28 |
| TKN (gN/kg-TS) | 12.9 |
| P (gP/kg-TS) | 4 |
| COD (gCOD/kg-TS) | 565.0 |
| COD:N:P | 100:2.2:0.7 |
| CODsol (g/L) | 25.8 |
| N-$NH_4^+$(mg/L) | 325 |
| P-$PO_4^{3-}$ (mg/L) | 14 |
| VFA (g-SCOD/L) | 3.5 |
| VS/TS (g/g) | 70.2% ± 2.73% |

Table 2. Main physical-chemical features of unfermented and un-pretreated feedstock

## Acidogenic Fermentation

The fermentation step aimed at generating a VFA-rich stream to be used in PHA synthesizing biological process. However, this stream could also be consumed by bacteria in the secondary wastewater treatment step, denitrification [25], which requires easily biodegradable carbon sources to support their metabolism. If the secondary usage of the effluent from this step is PHA synthesizing, two vital aspects should be assured: 1. High VFA/SCOD 2. Stable VFA distribution during the whole process. Indeed, the high VFA/SCOD ratio guarantees high efficiency in biomass selection in PHA producing step. On the other hand, the stability in the VFA spectrum means a predictable and reproducible PHA monomer production and consequently stability in the physical and mechanical features of synthesized polymers[26], [27]. The stability of the fermenter in terms of pH variation shows that despite a sudden fall in pH during the startup phase of the reactor, it was controlled and stood at the optimal value window (6-7.5) [28], which was mainly attributed to the addition of soda (40% NaOH-g/H2O-g) to the fresh feedstock during the pre-treatment, which brought the initial pH as high as 9-10. According to Figure 3, the process stability was achieved after 14 days, which was roughly 3 times HRTs (4.5 days).

Based on the statistical analysis using R programming (95% confidence interval, p-value =0.99, alternative: greater), HRT of 4.5 days yielded a VFA concentration of 30.3 ± 4.4 (g-SCOD/L), which was higher than 27.6 ± 2.4 (g-SCOD/L) for HRT of 3 days. Furthermore, the statistical analysis using R showed that HRT of 4.5 days yielded a similar VFA/SCOD ratio (roughly 0.84) as opposed to HRT of 3 days (confidence interval 95%, p-value = 0.56), which corresponds to a VFA yield of 0.52 ($\Delta$g-VFA/g-$VS_0$, ) for both HRTs, which is in the range of the values reported by other studies [24]. While two HRTs exhibited quite similar stable processes in terms of variation in pH (standard deviation of 0.25 vs 0.26 in steady state condition), in terms of VFA concentration variation HRT of 3 days was more stable than HRT of 4.5 days (standard deviation of 1.43 vs 2.64 in steady state condition). In Table 3, the main physical and chemical characteristics of the effluent and solid cake (SC) from the reactor were displayed for the effluent from the fermenter for two HRTs. The boxplots for SCOD and VFA/SCOD ratio were represented in Figure 4 and Figure 5, respectively.

| Parameters | Mesophilic Fermented HRT:4.5 days | Mesophilic Fermented HRT:3 days |
|---|---|---|
| TS (g/kg) | 43 ± 5.15 | 41.5 ± 0.71 |
| VS (g/kg) | 23.6 ± 2.7 | 25.5 ± 2.12 |
| COD (g-SCOD /kg TS) | 202 | 213 |
| SCOD (g-COD/L) | 33.3 ± 2.64 | 32.1 ± 1.43 |
| $N\text{-}NH_4^+$ (mg/L) | 563 ± 74.8 | 526 ± 66.6 |
| $P\text{-}PO_4^{3-}$ (mg/L) | 32 ±14.24 | 26.6 ± 6.24 |
| VFA (g-SCOD/L) | 30.3 ± 4.4 | 27.6 ± 2.4 |
| P (g-P/kg-TS) | 3.45 | 3.45 |
| TKN (g-N/kg-TS) | 8.39 | 8.39 |

Table 3. Main physical-chemical features of effluent from mesophilic fermentation and unfermented feedstock

Performance parameters for two HRTs were given in Table 4. As can be seen, the HRT of 4.5 days solubilized more solids, yielded higher COD solubilization, and released more nutrients (nitrogen and phosphorous) than the HRT of 3 days. COD solubilization for HRT 4.5 days was at the level of 0.24 ($\Delta$g-sCOD/g-$VS_0$), which was a bit higher than the value, 0.2 ($\Delta$g-sCOD/g-$VS_0$), for HRT of 3 days [24]. Similarly, ammonia and phosphate releases were 46% and 12% for HRT of 4.5 days as opposed to the lower values of 39% and 8% for HRT of 3 days.

| Parameter | Mesophilic HRT: 4.5 days, OLR: 6.89 (kg-VS/$m^3$.d) | Mesophilic HRT: 3 days OLR: 10.33 (kg-VS/$m^3$.d) | Previous Similar Studies |
|---|---|---|---|
| pH | 6.6 ± 0.25 | 6.9 ± 0.26 | 5.2-5.6 |
| Solubilization (Δg-sCOD/g-$VS_0$) | 0.24 ± 0.09 | 0.2 ± 0.05 | 0.27 [29] |
| $Y_{VFA}$ (Δg-VFA/g-$VS_0$) | 0.52 ± 0.10 | 0.50 ± 0.05 | 0.43 - 0.56[25], [30], [31] |
| Rate ($\frac{mg-SCOD_{VFA}}{g-VS.d}$) | 181 ± 31.56 | 259 ± 25.8 | - |
| Ammonia Release (%) | 46 ± 0.14 | 39 ± 0.13 | - |
| Phosphate Release (%) | 12 ±0.09 | 8 ± 0.04 | - |

Table 4. Performance parameters of the two different operational conditions used in fermentation.

The distribution of the VFA in terms of equivalent g-SCOD weight ratio for two HRTs was enumerated in Figure *2*. As can be seen, the VFA distribution is quite stable during the operation of the reactor regardless of deployed operational conditions, and the main fractions were butyric acid (26%-31%, std: 0.14%), acetic acid (27%, std: 0.07%), caproic acid (22%-26%, std: 0.1%) and propionic acid (9.2%-9.9%, std: 0.02%). This finding is in accordance with other studies that reported the main factor in determining the VFA distribution is the feedstock composition rather than operational procedures. In this experiment, as the feedstock was mainly OFMSW, the highest fraction of the VFA is butyric acid as was observed in similar studies [32] [24]. The VFA spectrum is of importance because the VFA stream with higher dominance of even numbers of carbon atoms means higher 3-hydroxybutyrate (HB) monomer synthesis compared to the 3-hydroxyvalerate

(HV), which is correlated with the net prevalence of odd numbers of carbon atom acids (propionic, valeric, and isovaleric acid).

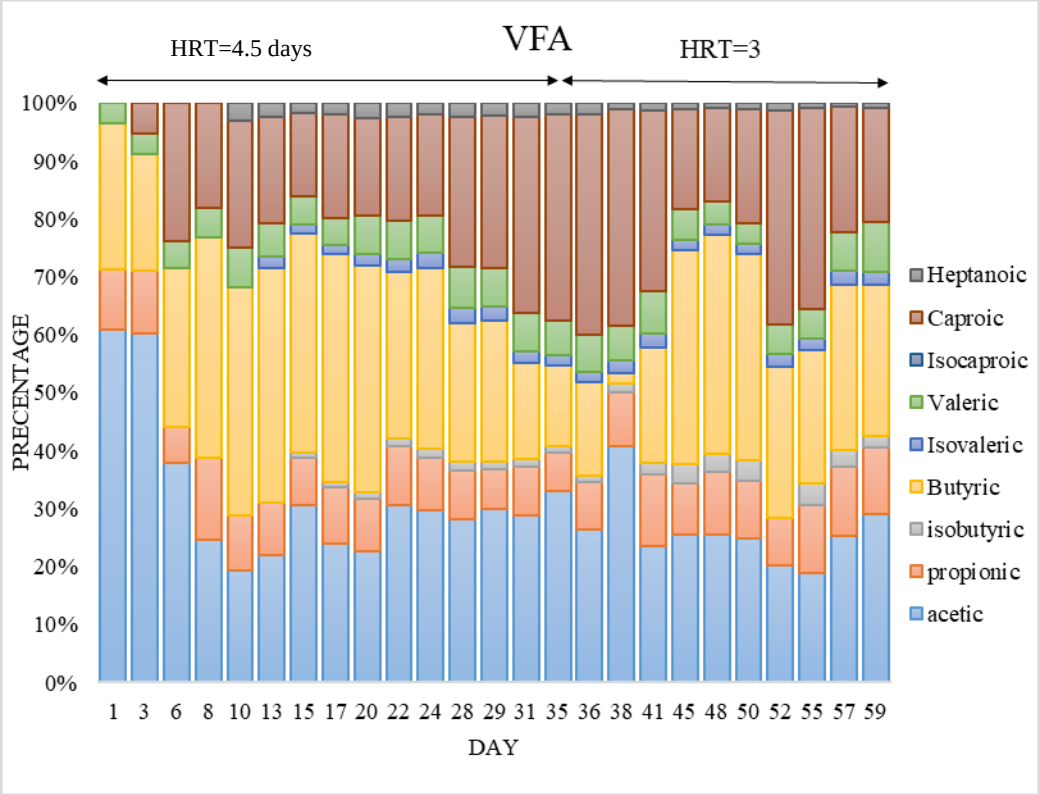



Figure 2. VFA evolution in the fermenter

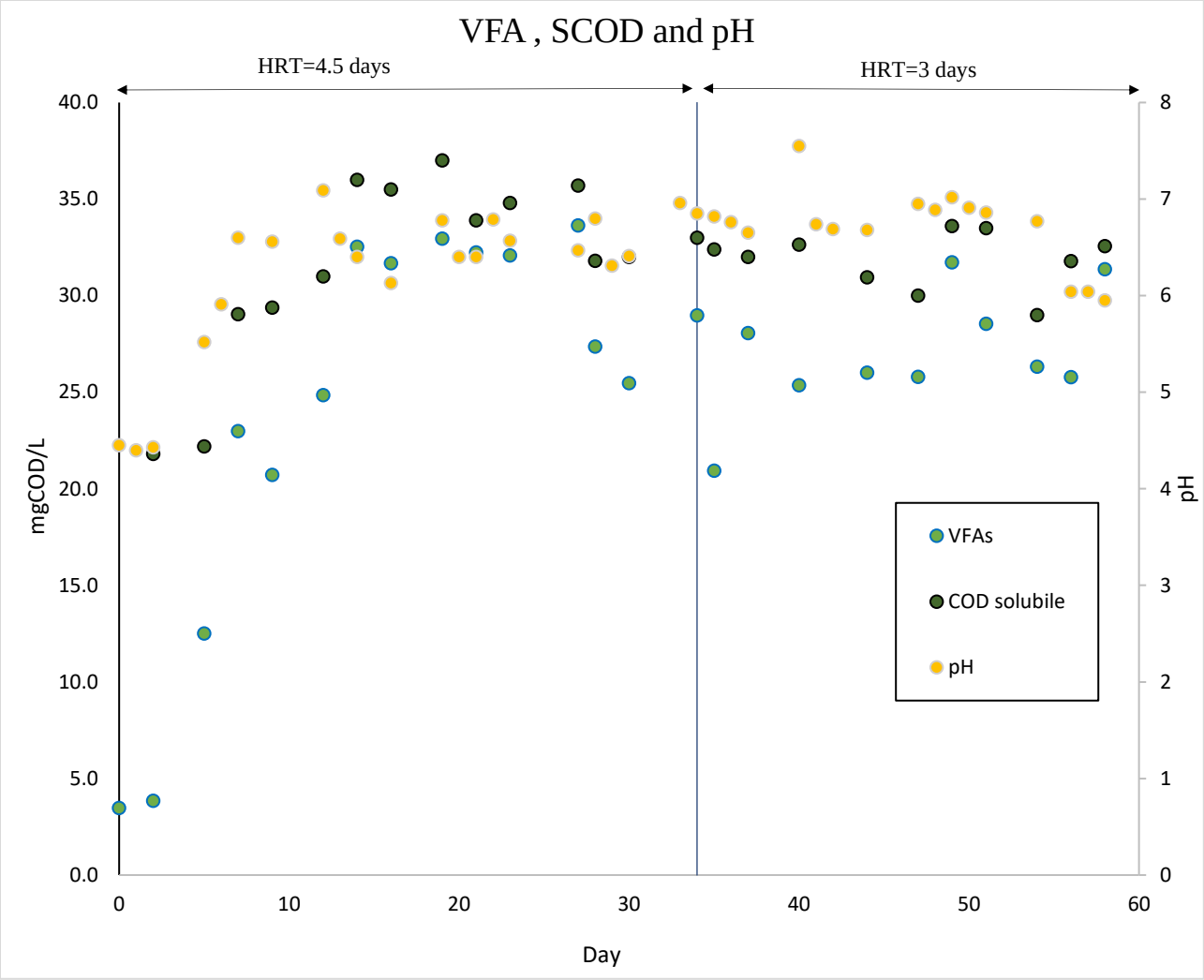



Figure 3. VFA, SCOD, and pH obtained from mesophilic experiment

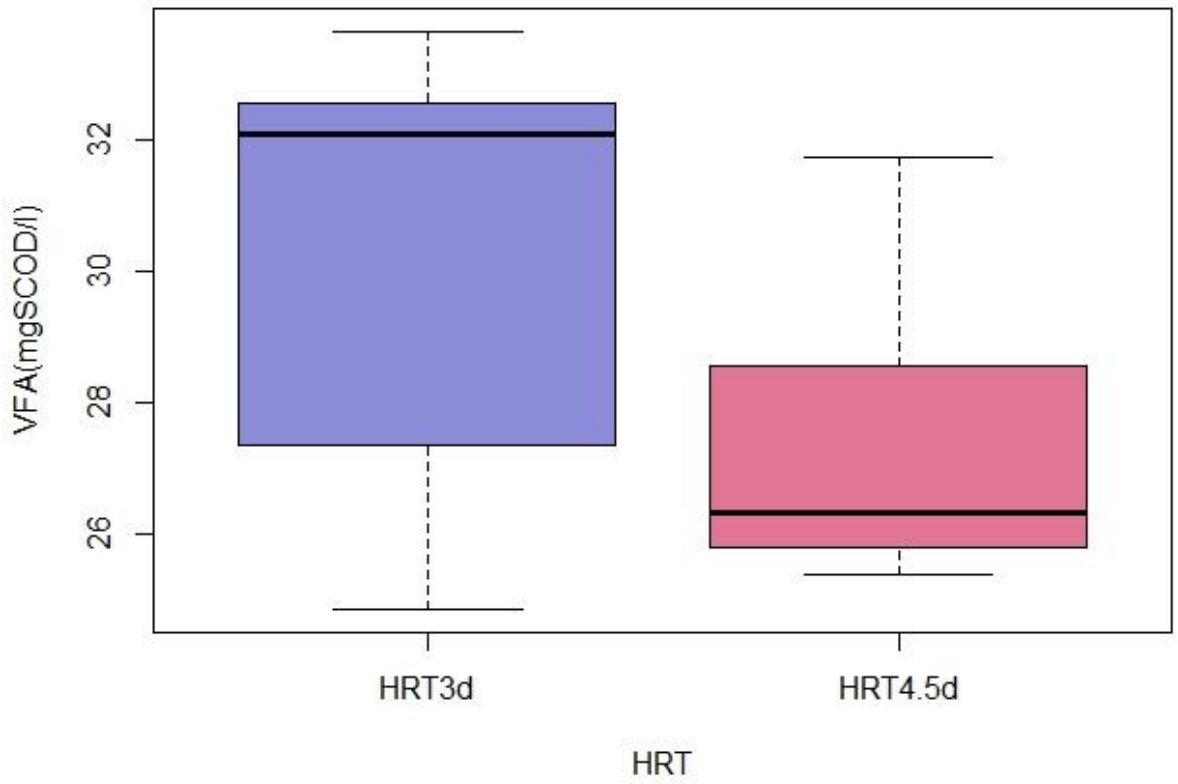



Figure 4. Boxplot of SCOD obtained in the mesophilic experiment

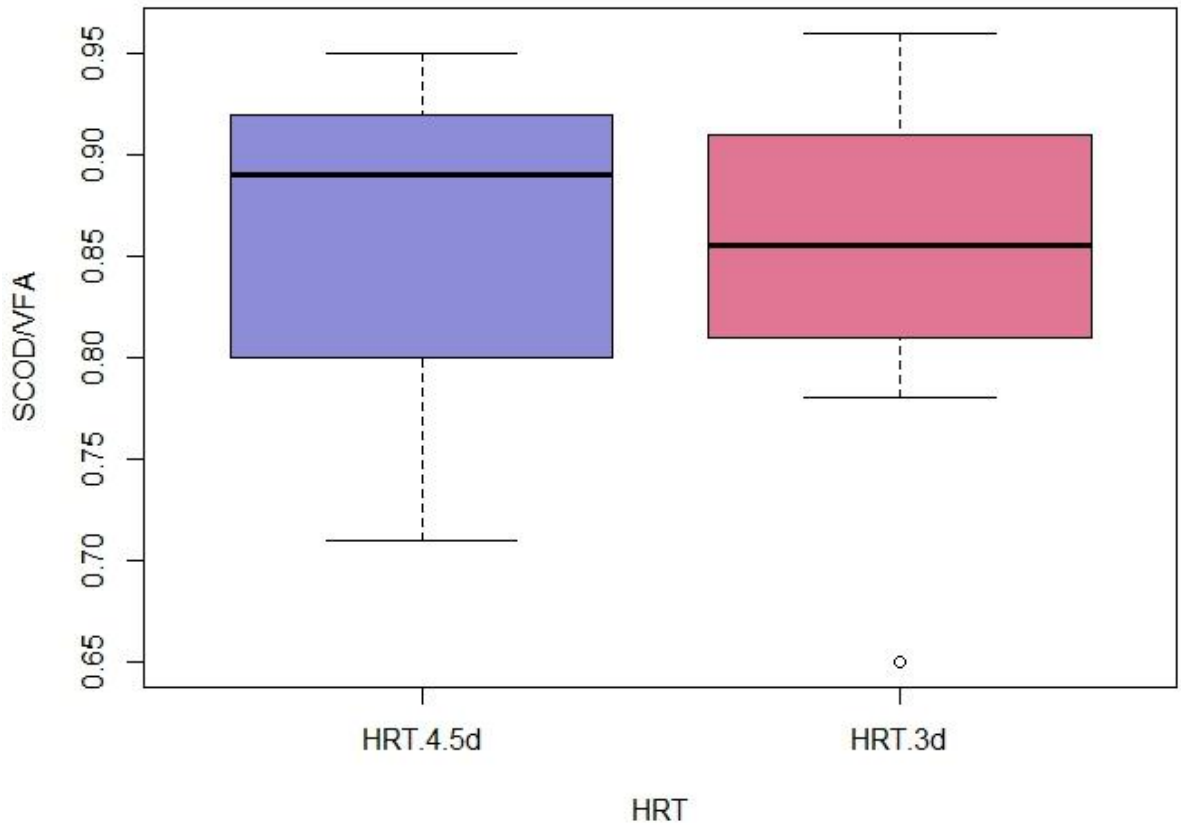



Figure 5. Boxplot of VFA/SCOD ratio obtained in the mesophilic experiment

**Anaerobic Digestion:**

The effluent from mesophilic fermentation was centrifuged and filtered, and the solid part was further digested through a BMP test with biochar addition and optimized inoculum/FS ratio. In this section, the effect of these two terms on the cumulative SMP and MPR alongside the kinetics of the process was studied. In this context, two models (first-order rate, modified Gompertz model) were calibrated to the bio-methane data to help us to identify the bottlenecks that limit digestibility and methane yield, for instance, long lag phase and low substrate solubilization rate (K). And to perceive if our methods mitigated these inhibitory terms and so raised the kinetics of the reaction by surging hydrolysis rate (K), or maximum methane production rate $R_m$ and shortening the lag phase ($\lambda$).

In this experiment, while the TS content of the feedstock to the reactor in terms of weight ratio was 22.1%, its VS content stood at 3.9 %. This huge dissimilarity is mainly attributed to not only low VS content in both inoculum and feedstock as a result of biodegradation of the putrescible part in the anaerobic fermentation step but also high inoculum/FS ratio (almost 3.3, which was much higher than the quantity, 1, in a similar study [19] ) and was found based on experience to optimize the bio-methane production from the residual fermented solid. The solid part of the feedstock contains 207.5 g-COD/g-TS and nitrogen as high as TKN= 7.7 (g-N/g-VS), and a phosphor content of 3.45 (g-P/kg-TS). This means that COD: N:P ratios in the feedstock were 100:2.85:1.66, which makes it suitable for anaerobic digestion. Moreover, in this study, the biochar dosage was in the range of zero to 0.24 g-biochar/g-VS, which was increased in two equal steps. As the process was done in batch condition, the pH, and other parameters like alkalinity, VFA, ammonia, and phosphate weren't monitored and only the gas volume was quantified and characterized by GC. Nevertheless, the risk of pH drop wasn't significant as the biochar addition

could provide a buffer capacity to the solution [33], and the considerable part of readily biodegradable COD of the feedstock was already converted to VFA in the previous step, and as result, the process was easily controlled even in transient condition when the risk of methanogenic inhibition was high [32].

Table 5 summarized the performance parameters of anaerobic digestion (AD) such as MPR, and SMP alongside the calibrated values for the two models. The MPR in this study was 0.04-0.05 $CH_4$-$m^3_{normal}/(m^3.d)$ and SMP, in the range of 0.24-0.3 ($CH_4$-$m^3_{normal}/kg-VS$), with an average composition of (v-$CH_4$/v-total) 45%, which was much lower than the reported values in a similar study of mesophilic AD on unfermented food waste [19]. In contrast, SMP values, of 0.3 ($CH_4$-$m^3_{normal}/kg-VS$), in our test stood higher than that, 0.25, reported by a study conducted by Valentino et al [32] on the fermented mixture of OFMSW and WAS (65%-70%, v/v). Nevertheless, from the data presented in Table 5, it can be understood that while the optimized inoculum/FS ratio boosted methane production significantly, the addition of biochar couldn't improve it further.

Moreover, the effect of biochar addition and inoculum/FS ratio on biogas production kinetics was studied by calibration of the parameters of two models (first-order rate, modified Gompertz model) to daily cumulative methane yield. Figure 7 shows the projections obtained from these models versus the observations. Although the modified Gompertz model seems to be more precise (RMSE= 8.33 vs 9.31) in predicting the methane yield from the test without biochar, the first-order rate model was more accurate, when it comes to the projection of methane yields from the tests with biochar (RMSE = almost 6 vs 10). Similarly, from a comparison of calibrated values in our study versus those obtained in previous studies on unfermented FS, it cannot be inferred that the

biochar addition could improve the process kinetics further, but the optimized inoculum/FS ratio did.

Figure *6* shows the fluctuation of methane content (v/v, %) in three experiments, which were obtained from the measurements and data imputation. As can be seen, the maximum content (v/v, %) of the methane in the two sets of tests with biochar addition was around 76%, which was roughly 8% higher than the value for the test without biochar.

The results of this study indicate that the residual solids out of the fermentation, which seems to be not appropriate for further VFA production to be used in PHA biological synthesizing, could be used in boosted anaerobic digestion by biochar addition and optimized inoculum/FS ratio to gain the most possible amount of valuable bioenergy.

| Parameters | Without biochar | Biochar (0.12 g-biochar/g-$VS_{added}$) | Biochar (0.24 g-biochar/g-$VS_{added}$) | Previous studies |
|---|---|---|---|---|
| SMP (($CH_4$-$m^3_{normal}/kg-VS$) | 0.303 | 0.245 | 0.30 | 0.40 ± 0.02 [24]<br>0.25 ± 0.01 [32]<br>(mesophilic fermented feedstock) |
| K (1/day) | 0.3 | 0.24 | 0.31 | 0.05 [19] |
| $R_m$ (CH4-ml/g-VS.d) | 74 | 41.3 | 70.6 | 54.35 [19] |
| $\lambda$ (day) | 0 | 0 | 0 | 4.57 [19] |
| RMSE (FO) | 9.31 | 5.82 | 6.61 | 31.18 [19] |
| RMSE (MG) | 8.33 | 10.53 | 6.67 | 48.6 [19] |
| Max CH4 content (v/v) | 68.5 | 76.5 | 76.5 | 66 [32] |

Table 5. SGP and Calibrated values for two models in first-order reaction rates and modified Gompertz for the current study versus those in previous studies

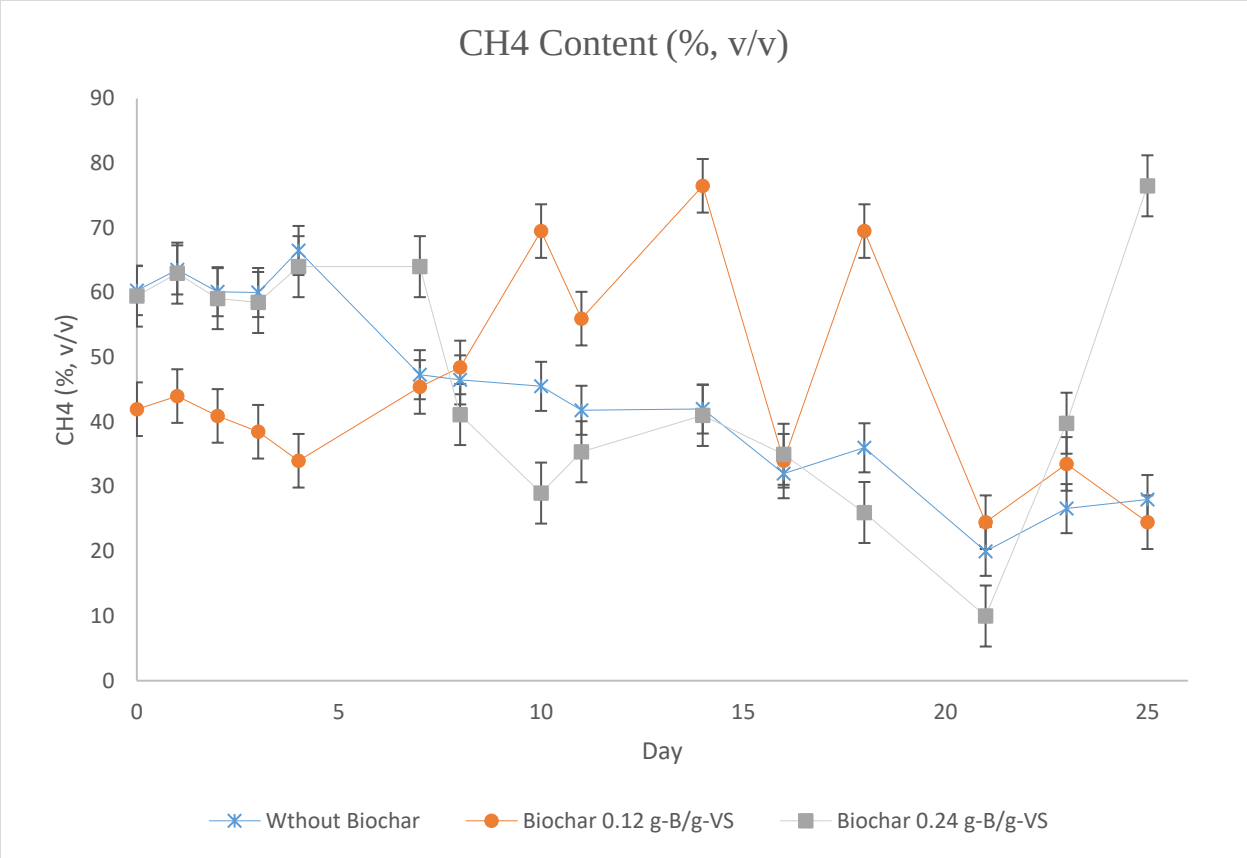
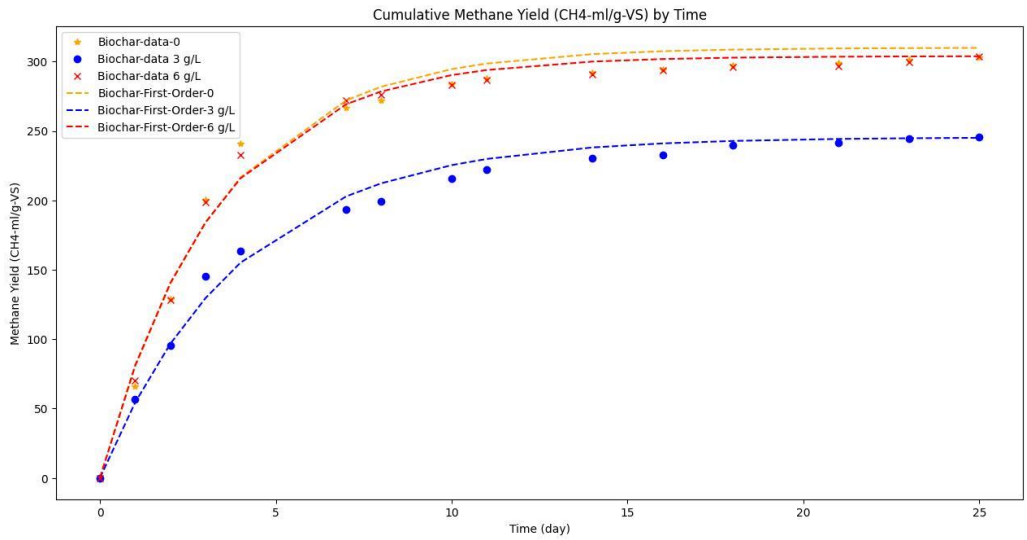



Figure 6. $CH_4$ content in volume basis (v/v)

(A)




(B)

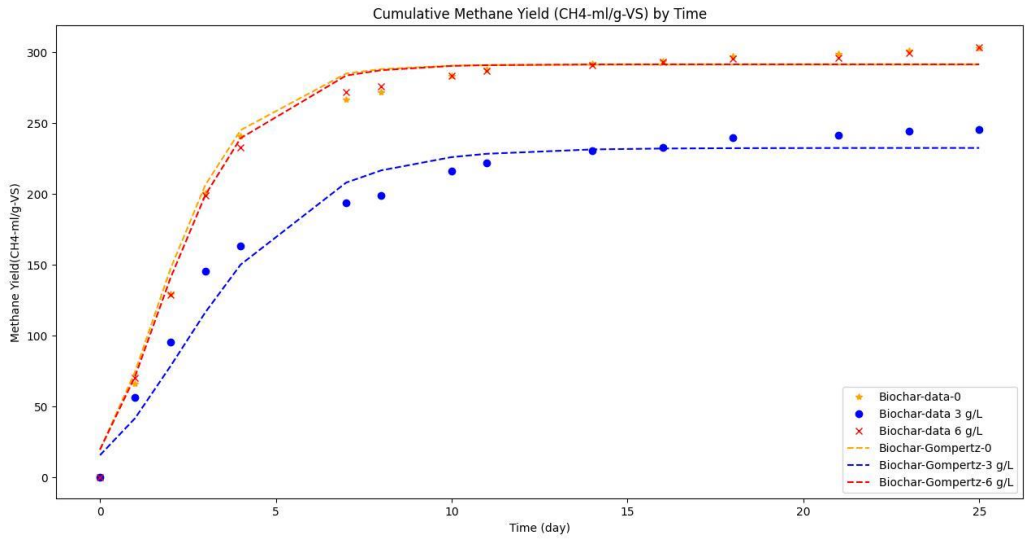



Figure 7. The projections of methane yield ($CH_4$-ml/g-VS) from First Order Rate (A) and Modified Gompertz models (B) vs the experimental data

**Mass and Energy Balance:**

The mass and energy balance was conducted for an industrial up-scaled industrial fermenter and digester treating OFMSW obtained from an imaginary municipality of 70,000 PE. The amount of TS production per capita was considered as 0.3 kg/PE [34]. Based on the reported values, it can be concluded that the inlet is as high as 21000 kg-TS/d. The detailed line was represented in the Excel file available in supplementary documents.

In the first scenario, the pre-treated inlet gained after screw press with the efficiency of 80 % for TS was 4678 kg-TS/d (15% dry matter), which was subsequently fed into the mesophilic fermentor operated at HRT= 4.5 days and OLR of 10 (kg-VS/m3.d). The yield of the fermenter was assumed to be 0.52 ± 0.1 (g-$VFA_{SCOD}$/g-VS). The bio-waste mixture, which was conveyed to the mesophilic fermenter, contained TS and VS levels of 5% and 4.5% (w/w). The gaseous flow rate out of the fermenter was 29 ($CH_4 - m^3$/d) with a purity of 5%, which was corresponding to 982 (kg-VS/d), meaning that almost 21% of the inlet VS was converted to gas at this step. The outlet out of this step was used in the separator to gain solid cake (SC) overflow and liquid phase. The liquid stream out of the fermenter had a volumetric flow rate of 75056 (kg/d), almost 80% of the input, with a VFA content of 2177 (kg-SCOD/d), which could be used in the PHA synthesizing step with higher added-value products than methane [35].

The SC overflow was diluted with water to reach the flow rate of 3320 (kg-TS/d) before feeding to the AD process without biochar addition. In this scenario, SGP of 0.414 ($Nm^3$ /kg-VS.d) was obtained in the whole process, considering a small biogas production at the fermentation stage (SGP of 0.14 ($Nm^3$ /kg-VS.d). The mass and energy balance analyses were performed for the working volume of 419 and 1736 $m^3$ of the fermenter and anaerobic digester, respectively. It was assumed that the gained biogas was consumed in combined heat and power (CHP) units with an

overall efficiency of 0.5 [37]. As it was depicted in Figure 8, whilst the net amount of energy produced at the two stages process stood at 27% of total energy, 10608 (MJ/d), the single-stage AD process produced a higher amount of net energy, almost 62% of the total energy, at 27818 (MJ/d), without any other higher added-value biorefinery products such as VFA. The second scenario, however, allows the production of VFA, which makes it much more profitable than the single AD process. The net income of the latter recovery method was three times higher than the quantity for a single AD. Consequently, as can be seen in Figure *9*, the two steps process method starts to produce benefits only after 3.5 years as opposed to almost 11 years for a single AD.

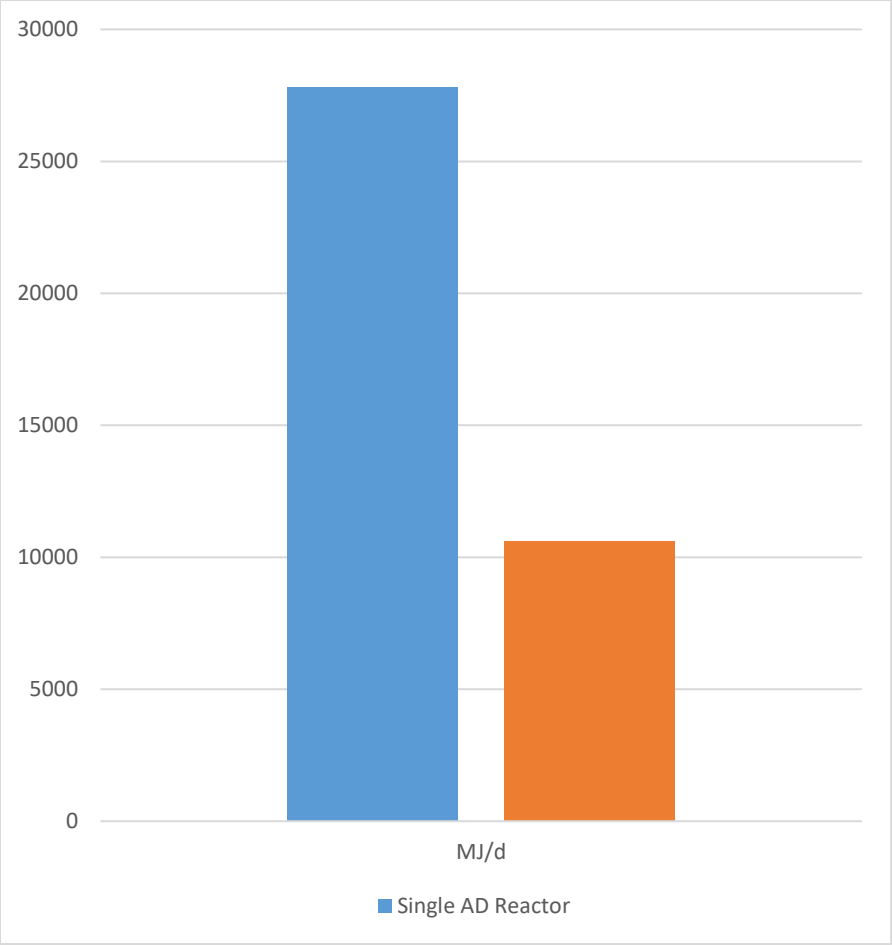
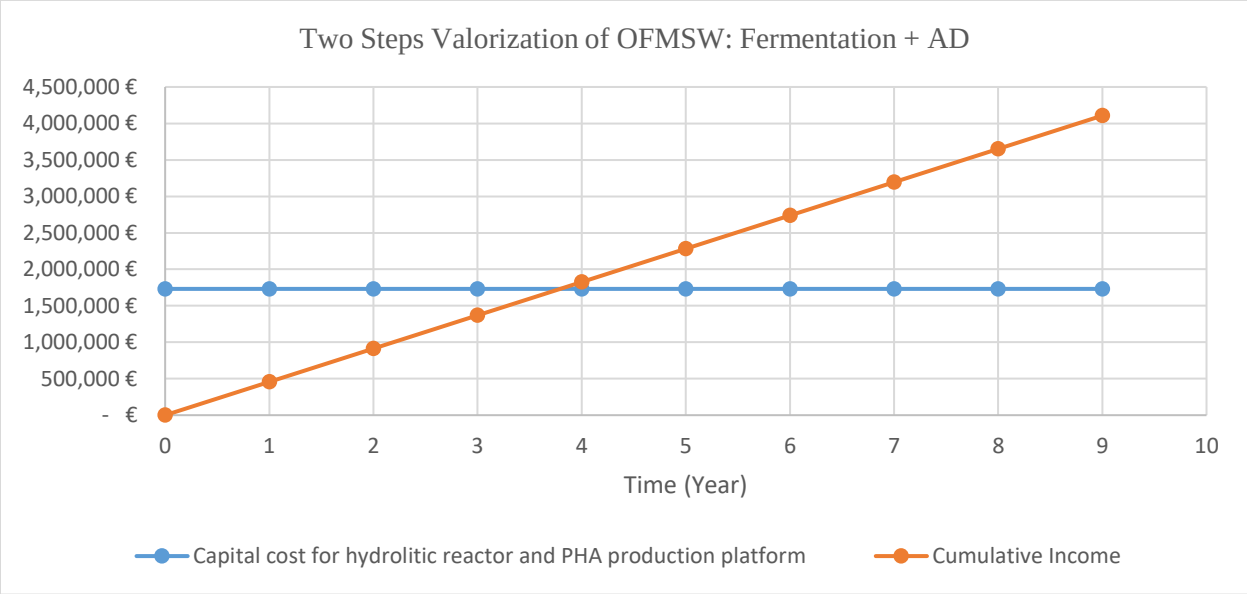



Figure 8. The Net amount of Energy produced in two scenarios



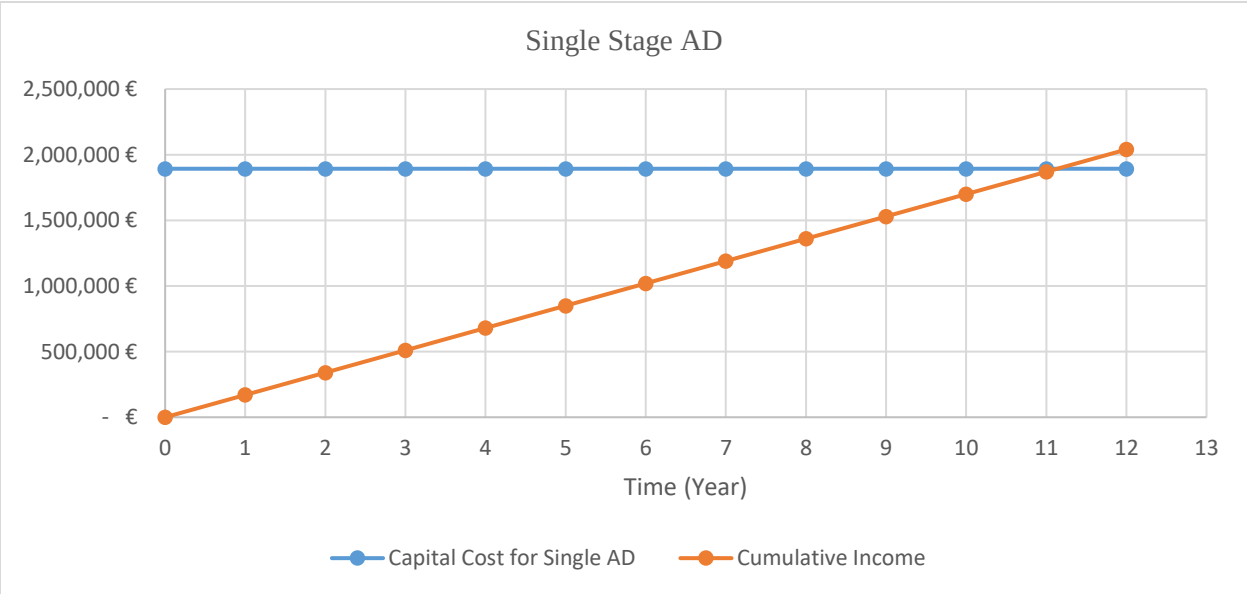




Figure 9. Capital Cost and Cumulative Yearly Income for two proposed scenarios

## Associated Content

Carbonaceous Characteristics, Gas Chromatography Procedure for Gas and VFA Characterization, Equations for Performance Parameters and Kinetics Study, KNN Imputation Code, Statistical Analysis code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## Abbreviations

VFA: Volatile Fatty Acid, HRT: Hydraulic Retention Time, OLR: Organic Loading Rate, OFMSW: Organic Fraction of Municipal Solid Waste, EU: European Union, PHA: Polyhydroxyalkanoates, TS: Total Solids, VS: Volatile Solids, N: Total Nitrogen, P: Total Phosphor, AD: Anaerobic Digestion, SCSTR: Semi-Continuous Completely Stirred Tank Reactor, COD: Chemical Oxygen Demand, TKN: Total Kjeldahl Nitrogen, BMP: Bio-methanation Test, 3HBp: 3-hydroxybutyrate precursors, 3HVp: 3-hydroxyvalerate precursors, KNN: K-Nearest Neighbors Algorithm, SMP: Specific Methane Production, MPR: Methane Production Rate, SBR: Sequencing Batch Reactor, std: standard deviation, RMSE: Root Means Squared Error, FO: First Order, MG: Modified Gompertz, FS: Feedstock, SCOD: Soluble Chemical Oxygen Demand, SC: Solid Cake