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 the sequential process for valorization of the organic fraction of municipal solid waste, which constitutes the mesophilic acidogenic fermentation and anaerobic digestion (AD) were studied. The results indicate that in the fermentation step, hydraulic retention time (HRT) of 4.5 days could yield higher volatile fatty acid (VFA) content of 30.5 (g-SCOD/L) as opposed to HRT of 3 days with 27.66 (g-SCOD/L). AD of residual solids out of fermentation step yielded specific methane production of 0.3 ($\frac{\mathrm{CH_4-m3}}{\mathrm{kg-VS}}$). Additionally, the effect of addition of biochar AD was investigated to conceive if it could improve the process kinetics and methane content. The results showed that feedstock/inoculum ratio of 0.3 (volatile basis) coupled with thermal and fermentative pre-treatment improved the kinetics of AD process substantially, whereas biochar addition could only improve the maximum obtainable methane content by 8 percent (76%, v/v). Furthermore, root means squared error (RMSE) obtained from the calibration in the kinetics study, showed that the first order rate model is more precise in describing the AD with biochar (6.7 vs 8.6), while the modified Gompertz is fitted better to data without biochar (9.31 vs 8.33). The economic analysis for a scale-up version with 70,000 person equivalent indicates that the proposed process could reach profit much sooner, almost 3 years in comparison to a single AD with 11 years of non-profitability. The recovery percentage of volatile solids in terms of mass was around 80%.

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

Introduction

The European Union (EU) generated about 55 million tons of organic waste per year in 2010, which mainly came from houses (40%), food waste sectors (15%) as well as business divisions (5%) [1]. Considering current Italian legislation, which forbids landfilling organic waste but treats them through biological and thermal processes like anaerobic digestion (AD), composting, and incineration with high disposal costs for secondary waste flux (75-125 €/ton) [2]. Under the pressure of such an exhaustible natural exploitation and an increasing amount of organic waste, the European Commission approved the circular economy action plan to promote sustainable recovery methods, that reduce the secondary waste flux. Approaches, 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 that respect, biological processes such as 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 this context, a sequential 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].

The laboratory study was inevitable and a prerequisite step before any scale-up AD of OFMSW. Because, it can take two main metabolic routes depending on operational conditions, namely methanogenesis or acidogenic fermentation, which may yield a stream with a low VFA/SCOD ratio or fewer appetite VFAs (such as lactic acids and alcohols), and eventually negatively impact bioproducts upstream process in terms of technical and economic feasibility[11]. So, in

our study, we are searching for a process with a desirable VFA-rich stream from fermentation and methane from methanogenesis [12], which ultimately could make the process more profitable as VFAs typically serve as platform chemicals for polyhydroxyalkanoates (PHA) synthesis process [13]–[16]. These biopolymers could be later recovered through biological processes to close the material life cycle.

Unfortunately, AD of bio-waste is highly limited by the hydrolysis step, which could be relieved by various methods such as pre-treatment, optimized (FS)/inoculum ratio, and carbonaceous material addition such as biochar [17]–[19]. 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[20].

In our article, OFMSW was separated and collected from the municipality of Treviso in northern Italy, and then 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 were used in a bio-methanation potential test (BMP) to see if the proposed methods could improve the process. The two models of first order (FO) and Modified Gompertz (MG) models were calibrated to identify the most precise one, and to 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 pre-treatment, fermentation succeeded by anaerobic

digestion. This procedure allows us to also obtain VFAs with higher added value than a single biomethane gained from single-step AD, which was currently deployed in Treviso WWTP [21].

Experimental (Material and Methods)

The overall schematic of the present study was illustrated in Figure 1. Firstly, the OFMSW was obtained and separated by a door-to-door collection system in Treviso municipality. Then after, it was transferred to the WWTP and was diluted to reach a weight ratio of 3%-4%. Next, 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 fermenter operating at conditions given in Table 1. The already existing fermentative bacteria 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 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 215 ml) during 25 days in the thermostatic bath. A total number of 8 bottles were used to observe the effect of biochar addition on biogas volume and content as well as the reaction kinetics. The experiments for each condition, namely only inoculum and either with or without biochar, were implemented in two bottles. The gas was sampled using syringe for gas characterization by gas chromatography, and then its volume was measured, and were averaged.

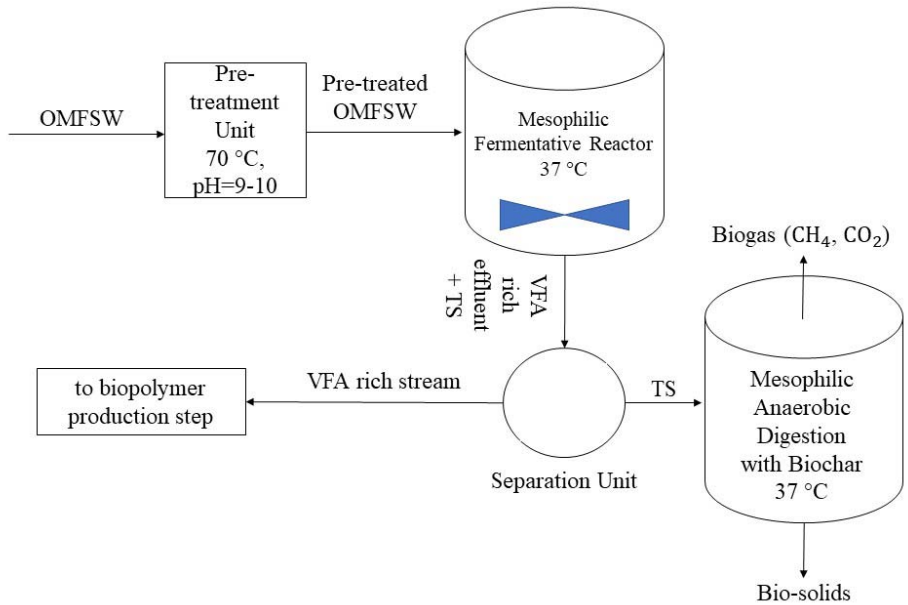


Figure 1. Schematic overview of the multi-step process for biogas and VFAs production from the OFMSW

Parameter	Mesophilic
Temperature	37 ° C
OLR 1 (kg-VS/$m^3.d$)	6.89
OLR 2 (kg-VS/$m^3.d$)	10.33
HRT 1 (days)	4.5
HRT 2 (days)	3

Table 1 The operational conditions of mesophilic fermenter

Anaerobic Pilot Unites:

The anaerobic fermentation reactor 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 was operated at a pH range of 5.95-7.55, which was controlled with soda addition in the pre-treatment unit. The mixture was blended 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 mesophilic fermenter was sampled frequently, and centrifuged to obtain the supernatant for parameters' measurements (pH, SCOD: soluble chemical oxygen demand, VFA, $\mathrm{N-NH_4^+}$, $\mathrm{P\text{-}PO_4^{3-}}$), and the tiny small fraction of residual solid part was used in solid characterization like COD, P, and Total Kjeldahl Nitrogen (TKN), and the rest was kept in the freezer to apply the BMP test later.

In BMP, the biochar was added to the bottles 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. After sampling, the mixture was manually mixed before setting back to the thermal bath. The inoculum for the BMP test was collected from 2300 $\mathrm{m^3}$ 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/$\mathrm{m^3.d}$) in the treatment plant. The inoculum was added to feedstock (residual solid from previous fermentation) based on a weight ratio of 0.3 (FS g-VS/IN g-VS). The TS and VS content (including inoculum and feedstock) in the bottles were 133 and 17.6 (g/kg) respectively. For further calculation and kinetic study, the gas volumes were first subtracted from the blank to correct for the endogenous methane production from the inoculum. The test was terminated after 25 days when the cumulative biogas production reached

89% of the final projected cumulative value. The pH, and other parameters like alkalinity, VFA, ammonia, and phosphate weren't monitored. Nevertheless, the risk of pH drop wasn't significant as the biochar addition could provide a buffer capacity and adsorption of inhibitory compounds in the solution [22], 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 [23].

Characterization of OFMSW and Carbonaceous Material:

The feedstock was characterized for different parameters with various frequencies throughout the experiment. The total and volatile solids (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 had been known that the feedstock chemical properties were quite stable.

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. The biochar was grinded into microparticles and was kept under dried condition in room temperature before adding to reactor.

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 [24]. The quantification of the VFA and gas content were 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 were 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) to see the effect of operational conditions mainly HRTs on VFA production.

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 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 values [25]. Subsequently, specific methane production (SMP) and methane production rate (MPR) were quantified. Next, two kinetic models

(first-order reaction rate and modified Gompertz model) were calibrated to biomethane data (utilizing the least-squared method) to reveal how fast the hydrolysis of the volatile solid as well the reaction proceeded (represented by K, lag phase, and maximum methane production rate).

In our study, mass and energy balance analysis were done for two scenarios: 1. A scaled-up system with mesophilic fermentation succeeded by mesophilic AD based on the optimized performance parameters 2. A single-stage mesophilic AD process as currently deployed in Treviso WWTP. All the parameters were adopted for reactors treating OFMSW collected from the municipality with an average size of 70000 person equivalent (PE). Reference parameters and boundary conditions are given in Table 2. The price of electricity was assumed to be 130 €/MWh. 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 annual net benefit of biorefinery products.

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 Processes	º C	37
Water Temperature	º C	15
Air Temperature	º C	20
Ground Temperature	º C	25
Heat Transfer Coefficient		
Outer Concrete Reactor Wall	W/(m2. º C)	0.7
Inner Concrete Reactor Wall	W/(m2. º C)	1.2
Floor	W/(m2. º C)	2.85

Table 2 Reference parameters and boundary conditions for energy balance

Results and Discussion

Bio-waste Composition and Characteristics:

The feedstock 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 3. 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 [26]. The chemical parameters of the solution phase for 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 characteristic of municipal organic solid waste [27]. Moreover, there was a slight level of VFA at 3.5 g-SCOD/L before fermentation, 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 3. Main physical-chemical features of unfermented and un-pretreated feedstock

Acidogenic Fermentation:

The fermentation step aimed at generating a stable VFA-rich stream to be used in PHA synthesizing biological process. If the secondary usage of the effluent from this step is PHA synthesizing, two vital aspects should be assured: 1. High VFA/SCOD as a desirable feature for efficient PHA synthesis step 2. Stable VFA distribution during the whole process. Indeed, even-numbered C-atoms VFA (3-hydroxybutyrate precursors; 3HBp) and the odd-numbered C-atoms VFA (3-hydroxyvalerate precursors; 3HVp) affect monomers synthesis, and consequently the PHA composition [28]. Moreover, 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 [29], [30]

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 range (6-7.5) [31], 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 one-way ANOVA statistical analysis (95% confidence interval, p-value =0.99, alternative: greater), HRT of 4.5 days yielded an average 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 similar statistical analysis 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 (Δg-VFA/g-VS_0,) for both HRTs, which is in the range of the values reported by other studies [27]. 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 4, the main physical and chemical characteristics of the effluent and solid cake (SC) from the reactor were displayed for two HRTs. The boxplots for SCOD and VFA/SCOD ratio were represented in Figure 4 and Figure 5, respectively. This result confirmed that applying HRT of 4.5 days in a scale-up fermenter treating OFMSW can yield higher VFA content, while it doesn't impact adversely the VFA/SCOD.

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
TKN (g-N/kg-TS)	8.39	8.39

Table 4. Main physical-chemical features of effluent from mesophilic fermentation and unfermented feedstock

Performance parameters for two HRTs were given in Table 5. 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 (Δg-sCOD/g-VS_0), which was a bit higher than the value, 0.2 (Δg-sCOD/g-VS_0), for HRT of 3 days [27]. 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-$\mathbf{VS_0}$)	0.24 ± 0.09	0.2 ± 0.05	0.27 [32]
$\mathbf{Y_{VFA}}$ (ΔG-VFA/G-$\mathbf{VS_0}$)	0.52 ± 0.10	0.50 ± 0.05	0.43 - 0.56[13], [33], [34]
RATE ($\frac{\mathbf{mg-SCOD_{VFA}}}{\mathbf{g-VS.d}}$)	181 ± 31.56	259 ± 25.8	-
AMMONIA RELEASE (%)	46 ± 0.14	39 ± 0.13	-
PHOSPHATE RELEASE (%)	12 ±0.09	8 ± 0.04	-

Table 5. Performance parameters of the two different operational conditions used in mesophilic 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 was 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 was the feedstock composition rather than operational procedures. As the feedstock was mainly OFMSW, the highest fraction of the VFA was butyric and acetic acid, which confirms the results observed in similar studies [23] [27]. 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).

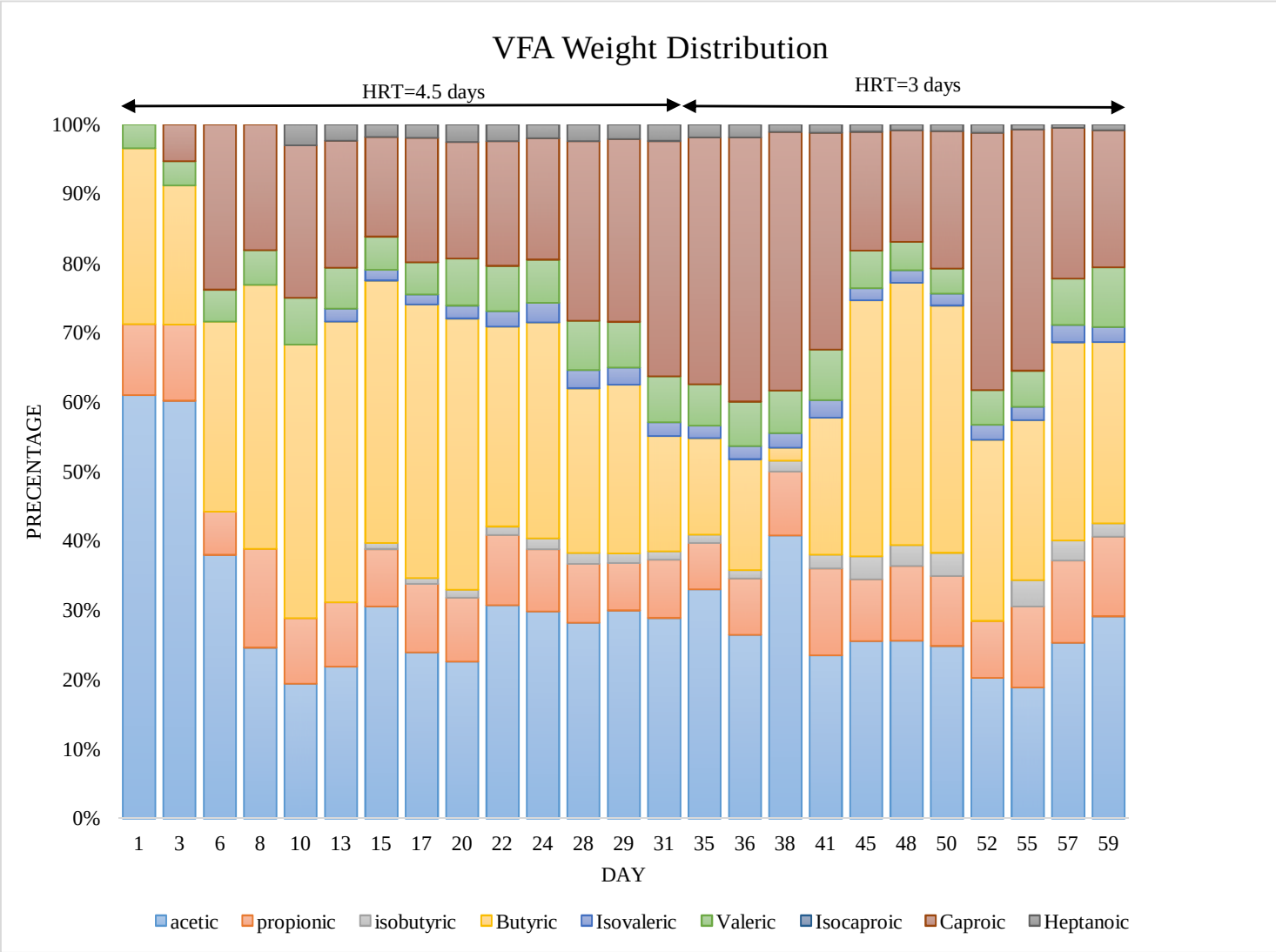


Figure 2. VFA evolution in the fermenter

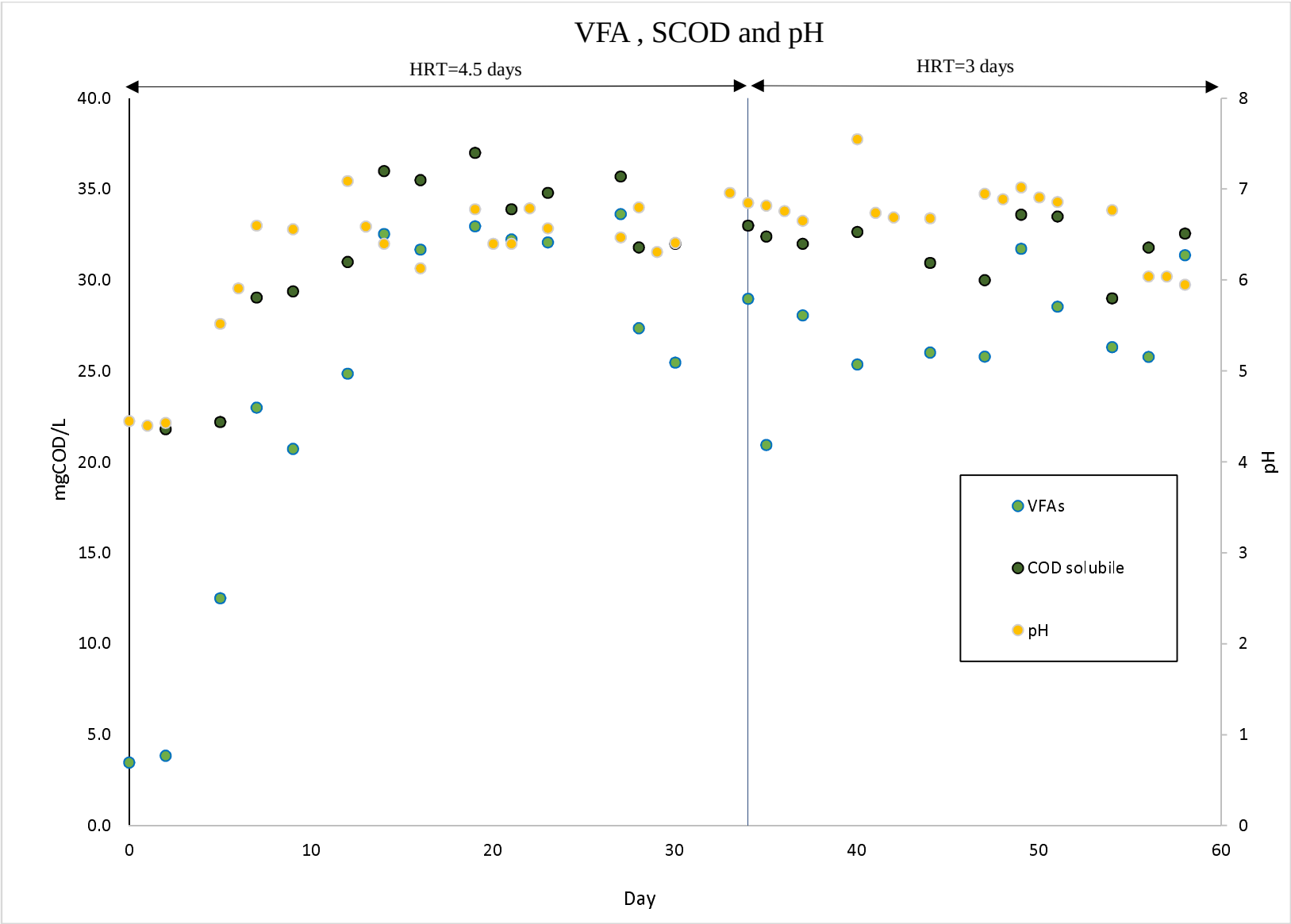


Figure 3. VFA, SCOD, and pH obtained from mesophilic experiment.

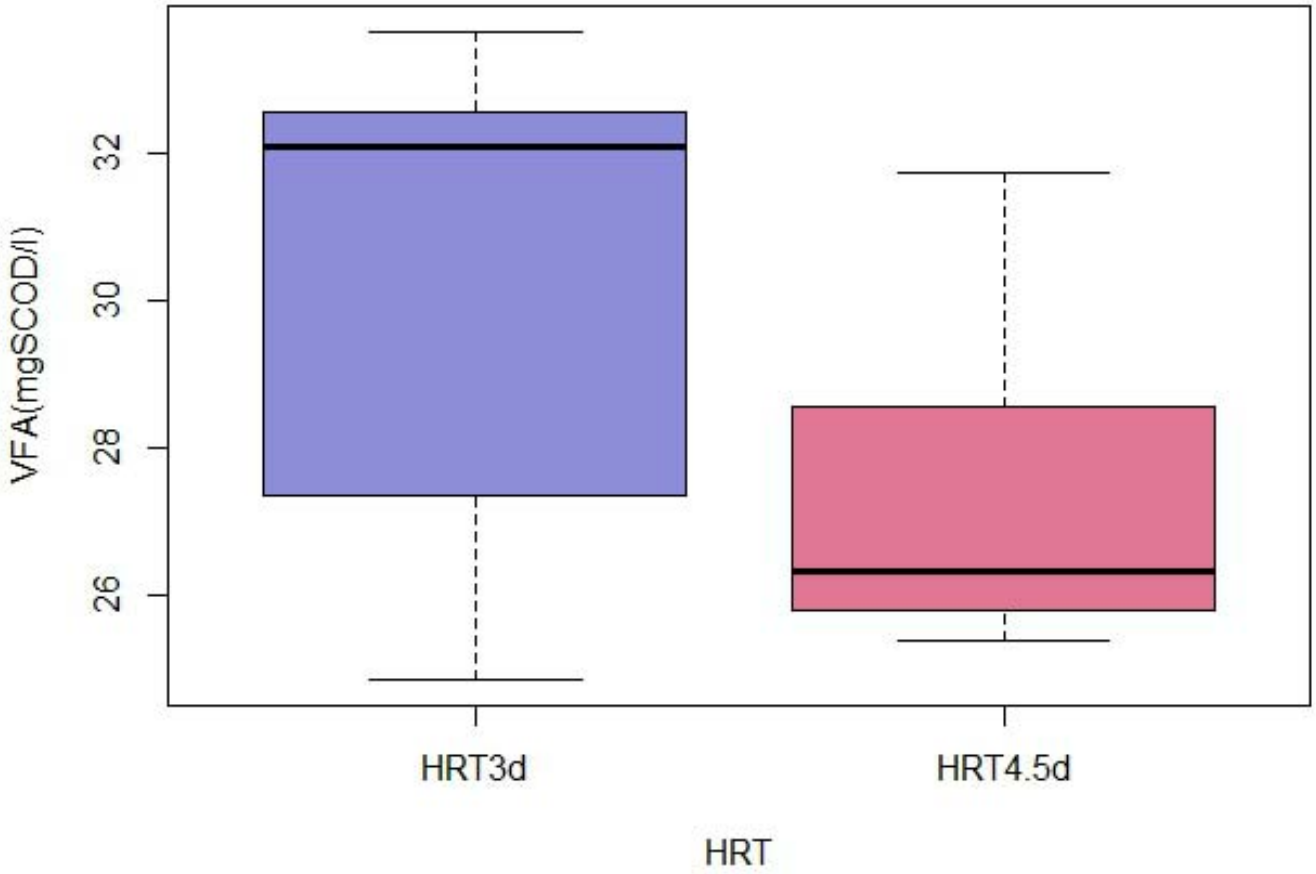


Figure 4. Boxplot of SCOD obtained in the mesophilic experiment.

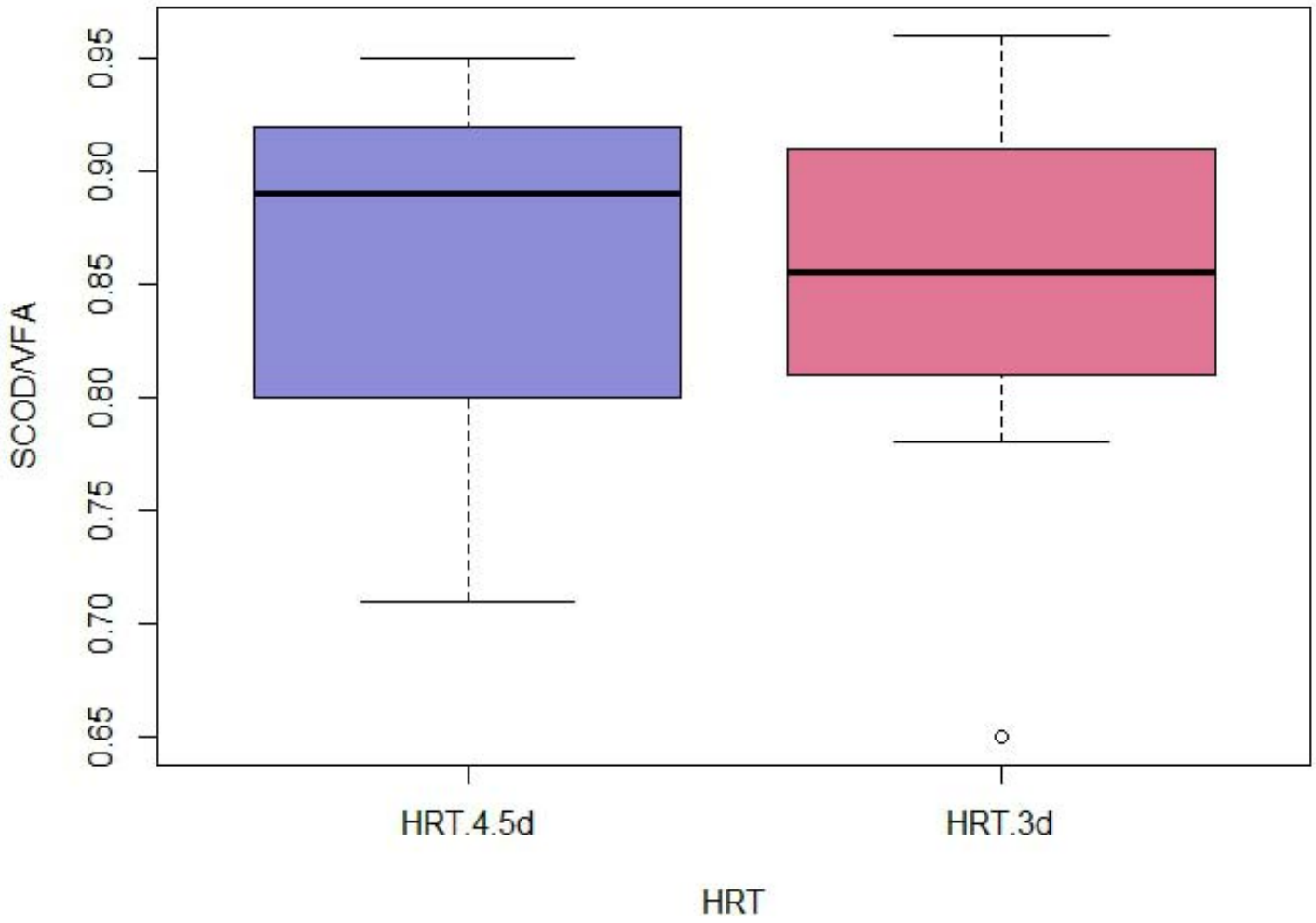


Figure 5. Boxplot of VFA/SCOD ratio obtained in the mesophilic experiment.

Anaerobic Digestion:

The residual solid part out of fermentation was further digested through a BMP test with biochar addition. The effect of biochar addition on the cumulative SMP and MPR as well as the kinetics of the process were studied. On this basis, the two models FO and MG were calibrated to the bio-methane data to identify the bottlenecks (for instance long lag phase λ and low substrate solubilization rate K) that limit digestibility and methane yield, so as perceive if our methods mitigated these inhibitory terms, which could be well represented by raise in hydrolysis rate (K), or maximum methane production rate ($\mathrm{R_m}$) and plummet in the lag phase (λ).

In this experiment, the FS/inoculum ratio of 0.3 (vs basis) was significantly higher than the quantities (1 and 0.5) reported in similar studies [21], [35]. Table *6* summarized the performance parameters of anaerobic digestion (AD) such as MPR, and SMP as well as the calibrated values and indexes for the two models in our study and the same values reported in other studies.

The MPR and SMP were 0.04-0.05 $\mathrm{CH_4\text{-}m^3_{normal}/(m^3.d)}$, and in the range of 0.24-0.3 ($\mathrm{CH_4\text{-}m^3_{normal}/kg-VS}$), with a weight average composition of 45-58 percent (v-$\mathrm{CH_4}$/v-total). While the weighted average composition of biomethane in our study was lower than the reported values in a similar study of mesophilic AD on unfermented food waste by Valentino et al. [23], the SMP value of 0.3 ($\mathrm{CH_4\text{-}m^3_{normal}/kg-VS}$), in our test stood higher than that, 0.25, reported by that study [23] on the fermented mixture of OFMSW and WAS (65%-70%, v/v). In contrast, our SMP was lower than 0.384 ± 0.006 ($\mathrm{CH_4\text{-}m^3_{normal}/kg-VS}$) in the study by Moreno et al [35], who investigated the AD on residual solids from two steps of bioethanol production and saccharification.

The comparison of hydrolysis rate 0.3 (1/day) with the value of 0.05 (1/day) in study by Karki et al. [35] (on fresh feedstock) indicates that our proposed treatment line including thermal pre-treatment, acidogenic fermentation before anaerobic digestion, and FS/inoculum ratio of 0.3 could improve the process kinetics.

Figure 7 shows the projections obtained from two calibrated models versus the observations. Although MG seems to be more precise (RMSE= 8.33 vs 9.31) in predicting the methane yield from the test without biochar, FO was more accurate, when it comes to the projection of methane yields from the tests with biochar (RMSE = almost 6 vs 10). Comparison of calibrated values in our study with those obtained in previous studies on unfermented FS revealed that addition of biochar couldn't improve the process kinetics further.

In contrary, based on observations from Figure 6 showing the fluctuation of methane content (v/v, %) in three experiments, the maximum measured 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 anaerobic digestion 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	0.4615 [27] 0.25 ± 0.01 [23] 0.384 ± 0.006 [35] 0.396 ± 0.034 [11]
K (1/day)	0.3	0.24	0.31	0.05 [21]
R_m (CH4-ml/g-VS.d)	74	41.3	70.6	54.35 [21]
λ (day)	0	0	0	4.57 [21]
RMSE (FO)	9.31	5.82	6.61	31.18 [21]
RMSE (MG)	8.33	10.53	6.67	48.6 [21]
Max CH4 content (%, v/v)	68.5	76.5	76.5	66 (63 ± 3) [23]

Table 6. SGP, MPR, Maximum methane content and calibrated indexes and values for two models in first-order reaction rates and modified Gompertz for the current study versus those in previous studies

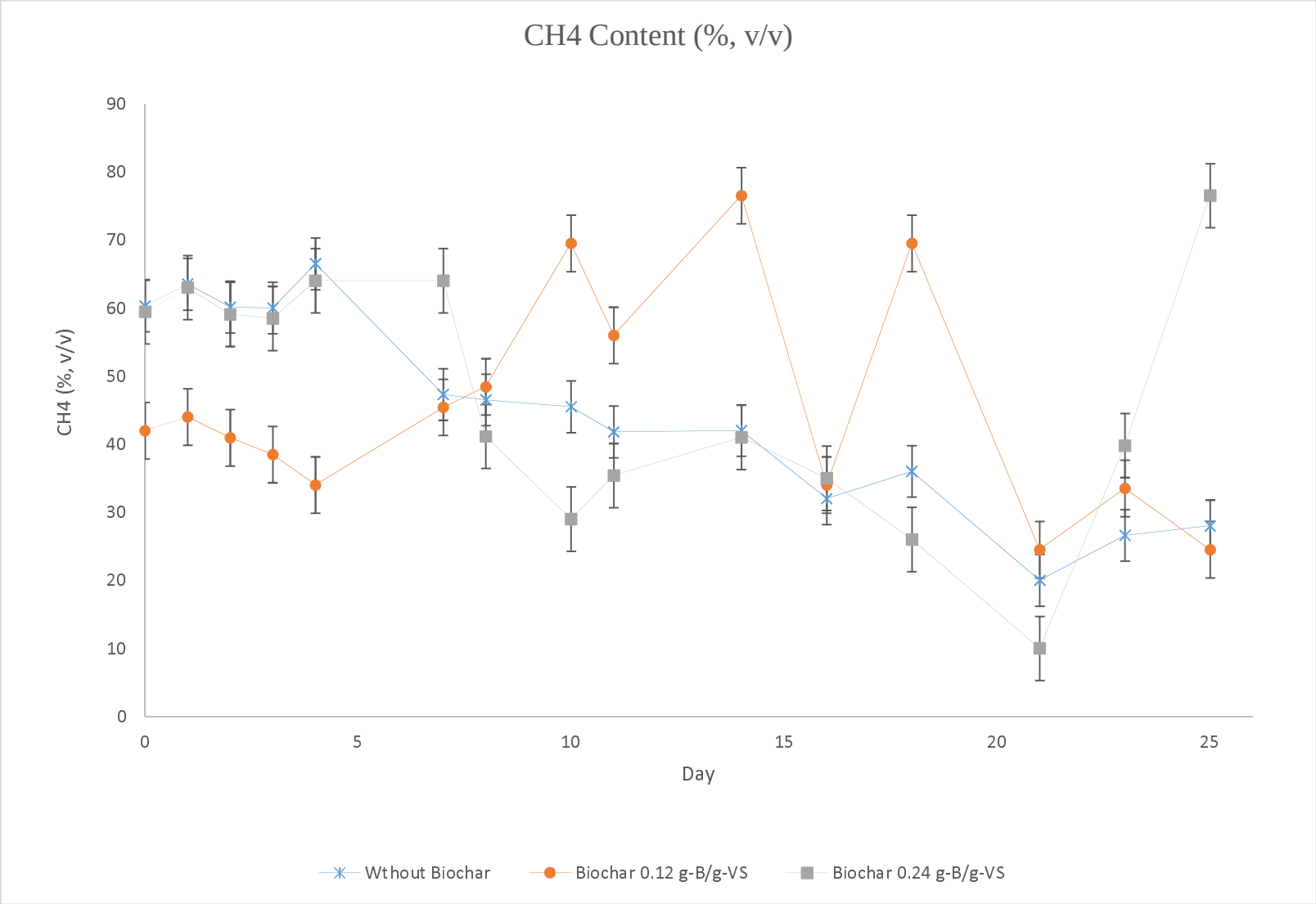
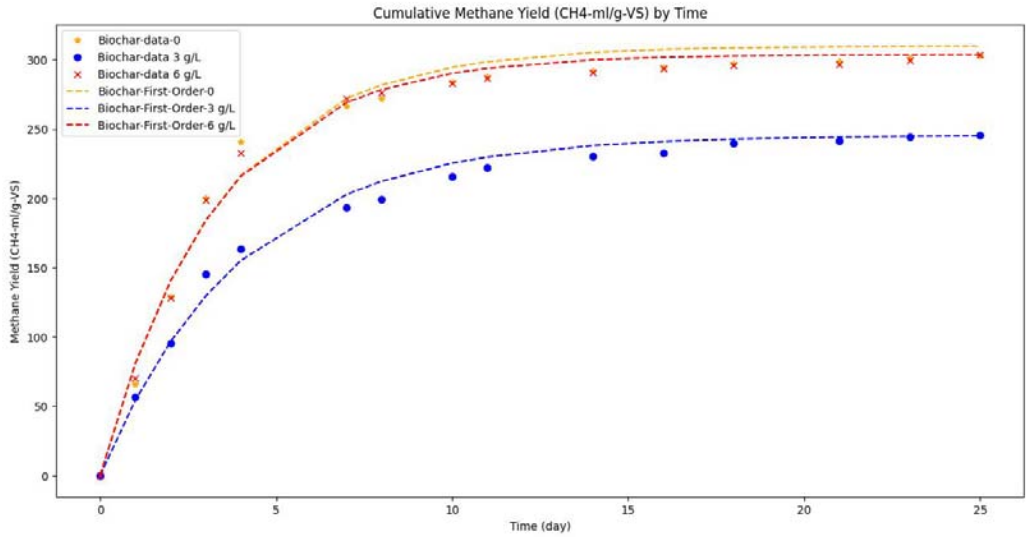


Figure 6. CH_4 content in volume basis (v/v)

(A)



(B)

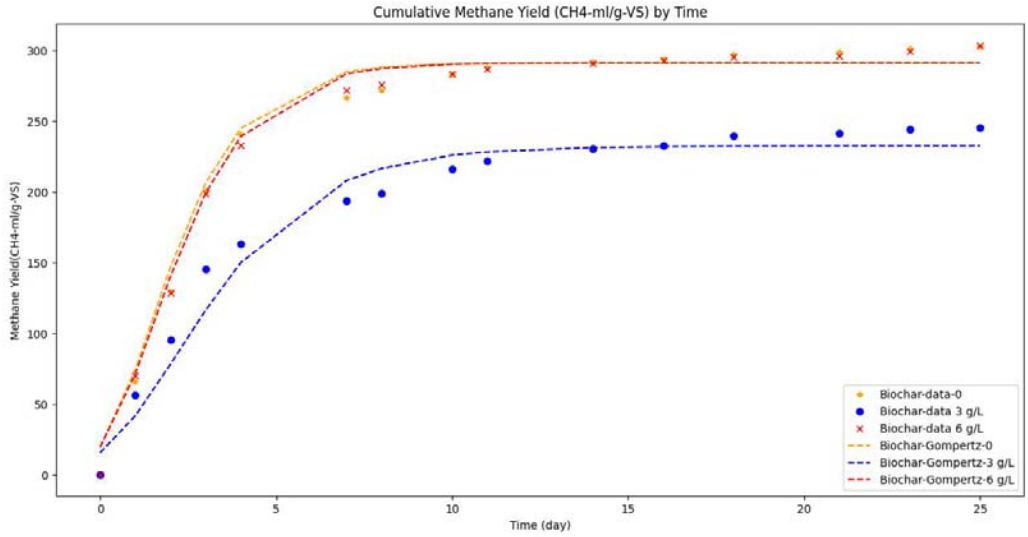


Figure 7. The projections of methane yield (CH_4-ml/g-VS) from First Order Rate (A) and Modified Gompertz models (B) vs the experimental data

Mass and Energy Balance:

The mass and energy balance were conducted for scale-up line treating OFMSW, which were collected in an imaginary municipality of 70,000 person equivalent (PE). The amount of TS production per capita was considered as 0.3 kg/PE [36]. Based on the reported values, the inlet to the line was calculated as high as 21000 (kg-TS/d). The detailed information and units of the treatment line were illustrated in supplementary documents.

In the first scenario, the pre-treated inlet after screw press with the TS removal efficiency of 80 % was 4678 (kg-TS/d) (28% dry matter), which was diluted and subsequently fed into the mesophilic fermenter operated at HRT= 4.5 days and OLR of 6.89 (kg-VS/m3.d). The yield and solubilization of the fermenter were assumed to be 0.52 ± 0.1 (g-VFA_{SCOD}/g-VS) and 0.24 (g-SCOD/g-VS). The bio-waste mixture, which was conveyed to the mesophilic fermenter, contained TS and VS levels of 4.1% and 3.1% (w/w). The gaseous flow rate out of the fermenter was assumed to be zero as HRT of 4.5 days is quite short for any gas production and adequate growth of methanogens in mesophilic condition, which was verified in batch tests. 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 113788 (kg/d), with a VFA content of 2814 (kg-SCOD/d), which could be used in the PHA synthesizing step with higher added-value products than methane [37].

The outflow from fermenter was used in separator, and the solid cake was fed into mesophilic anaerobic digester without biochar addition after diluted by water to reach TS content of 2.7% and flow rate of 113788 (kg/d). In this scenario, SGP of 0.47 (Nm^3 /kg-VS) was obtained in the whole process, considering a zero-biogas production at the fermentation stage. The mass and energy balance analyses were performed for the working volume of 608 and 2220 (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 [38].

As it was depicted in Figure 8, whilst the net amount of energy produced at the two stages process was negative, -7160 (MJ/d), the single-stage AD process generated much higher net amount of energy at 22760 (MJ/d), almost 60% of its total. Although the second scenario yields only biogas, the first scenario apart from biogas produced higher-added value biorefinery products such as VFA. As a result, the first scenario was much more profitable than the single AD process. The annual net income of the two-step recovery method was 136,850 €, almost three and half times higher than the quantity for a single AD. Consequently, as can be seen in Figure 9, the sequential recovery method started to produce benefits only after 10 years as opposed to almost 42 years of non-profitability for a single AD.

The proposed multi-step treatment line was more feasible in terms of economic profitability and technical obstacles in comparison with similar sequential valorization of OFMSW to bioethanol and biogas proposed in a study by Moreno *et al.* [35]. As it not only requires sterilization conditions imposing an additional operational cost, accounting for 40% of the total cost, but also the bioethanol concentration should be higher than 40 (g/L) to decrease the cost of subsequent distillation step.

The proposed method of separating AD and fermentation in our study was preferable than a similar study by Papa et al. [11] on single AD followed by PHA production. Indeed, the study by Papa et al. enumerated that the single-step recovery of biogas and VFAs was feasible by increasing OLR (from 6 to 3 kg VS $m^{-3}d^{-1}$) or in other words halving HRTs (from 40 to 20 days), while keeping the SMP of the reactor unaffected. Despite improvement in the biomethane production rate per reactor volume and total VFA content, the main recovery path for the volatile

solid was still gas production, accounting for more than 90% of the total conversion, in comparison with our method in which biogas and VFAs conversion owned 40% and 60% of share, respectively. It is also concluded that this single-stage AD process not only limited the VFA distribution to propionic and butyric acid, which negatively impacted the PHA synthesize step, but also having lower destruction of VS of 71% in comparison to the quantity reported in our method with about 80%.

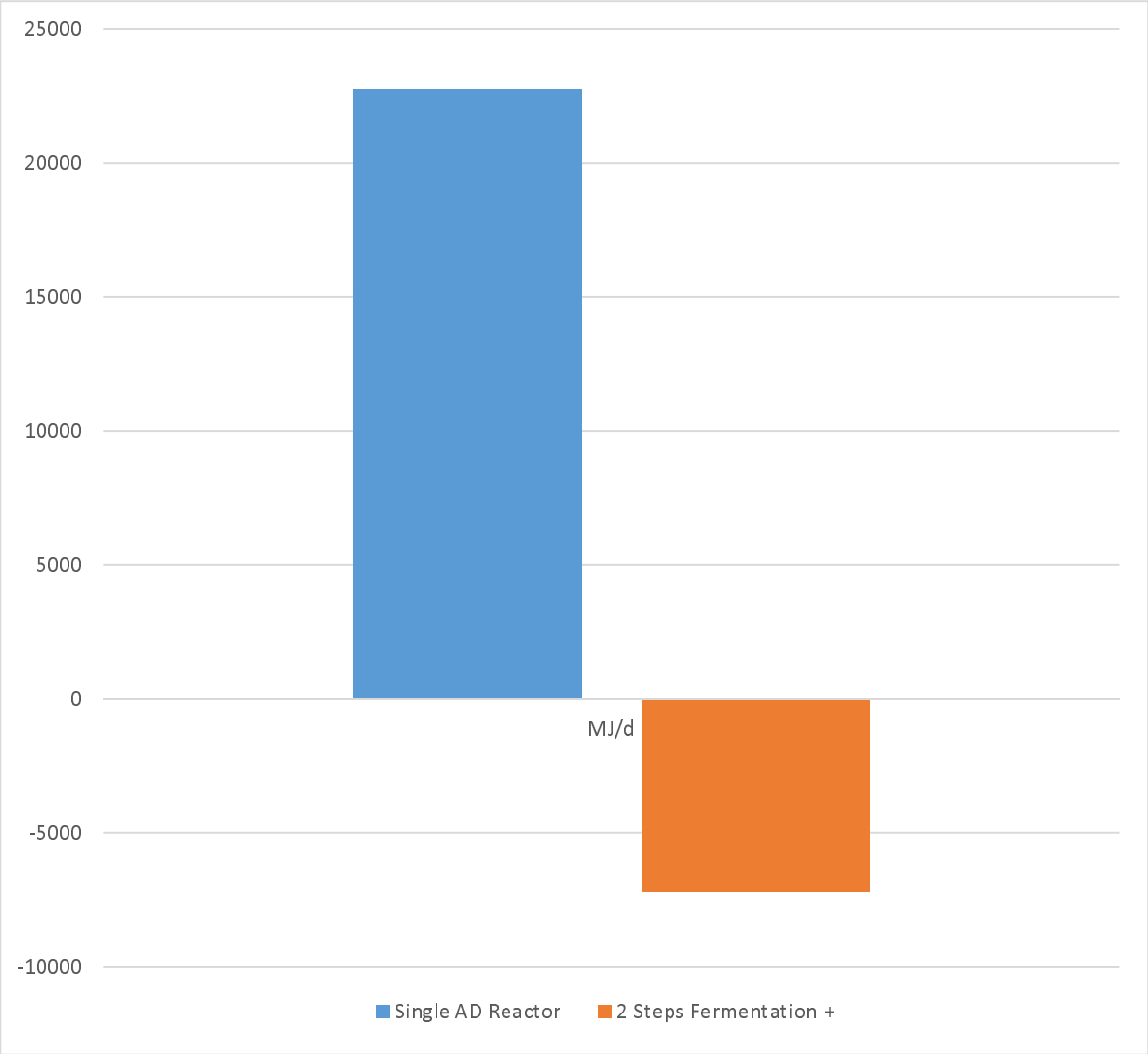
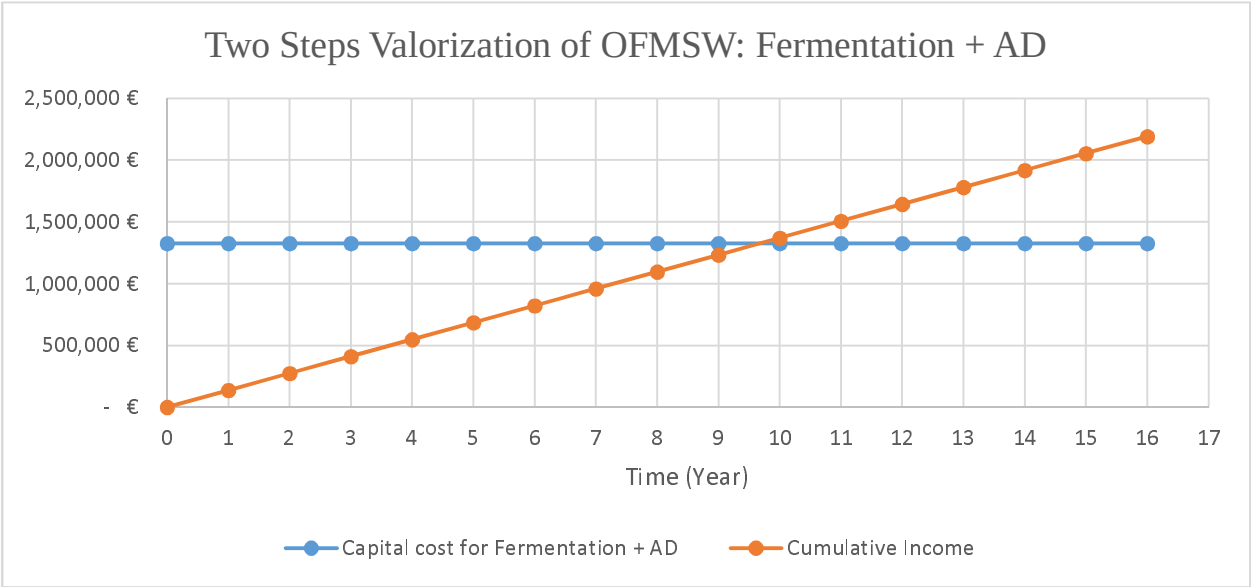


Figure 8. The Net amount of energy produced in two scenarios.



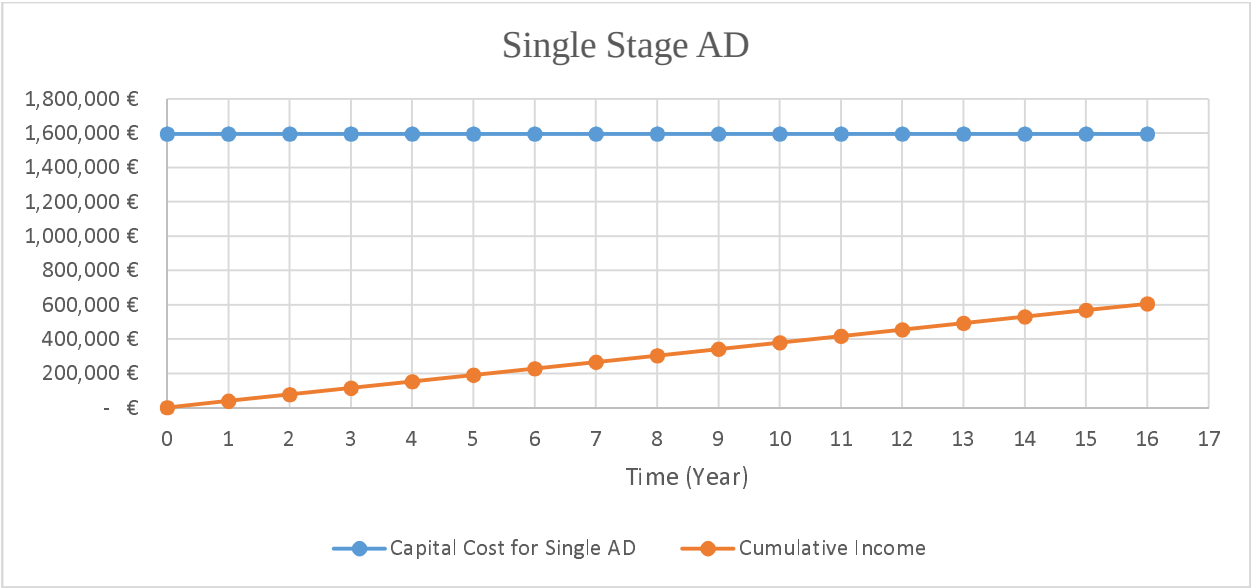


Figure 9. Capital Cost and Cumulative Yearly Income for two proposed scenarios.

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, Experimental Data of Fermentation and Anaerobic Digestion, Input to Statistical Analysis Code, Mass and Energy Balance Analysis

Acknowledgments

The authors gratefully acknowledge the Italian Ministry of University and Research (MUR) and the University of Ca ’Foscari for financial support of this research, In the Frame of Programma Operativo Nazionale Ricerca e Innovazione (PON).

References:

[1] European Commission, “Preparatory study on food waste across EU-27 for the European Commission,” 2010.

[2] L. A. Pfaltzgraff, M. De bruyn, E. C. Cooper, V. Budarin, and J. H. Clark, “Food waste biomass: a resource for high-value chemicals,” *Green Chemistry*, vol. 15, no. 2, p. 307, 2013, doi: 10.1039/c2gc36978h.

[3] D.-G. for Environment, “Circular economy action plan,” *European Commission*, 2022. https://ec.europa.eu/environment/strategy/circular-economy-action-plan_en#:~:text=The EU’s transition to a,entire life cycle of products.

[4] E. Mazur-Wierzbicka, “Towards Circular Economy—A Comparative Analysis of the Countries of the European Union,” *Resources*, vol. 10, no. 5, p. 49, May 2021, doi: 10.3390/resources10050049.

[5] A. Mattioli, G. B. Gatti, G. P. Mattuzzi, F. Cecchi, and D. Bolzonella, “Co-digestion of the organic fraction of municipal solid waste and sludge improves the energy balance of wastewater treatment plants: Rovereto case study,” *Renew Energy*, vol. 113, pp. 980–988, Dec. 2017, doi: 10.1016/j.renene.2017.06.079.

[6] V. Cabbai, N. De Bortoli, and D. Goi, “Pilot plant experience on anaerobic codigestion of source selected OFMSW and sewage sludge,” *Waste Management*, vol. 49, pp. 47–54, Mar. 2016, doi: 10.1016/j.wasman.2015.12.014.

[7] F. Girotto, L. Alibardi, and R. Cossu, “Food waste generation and industrial uses: A review,” *Waste Management*, vol. 45, pp. 32–41, Nov. 2015, doi: 10.1016/j.wasman.2015.06.008.

[8] J. Tamis, K. Lužkov, Y. Jiang, M. C. M. van Loosdrecht, and R. Kleerebezem, “Enrichment of Plasticicumulans acidivorans at pilot-scale for PHA production on industrial wastewater,” *J Biotechnol*, vol. 192, pp. 161–169, Dec. 2014, doi: 10.1016/j.jbiotec.2014.10.022.

[9] G. Pagliano, V. Ventorino, A. Panico, and O. Pepe, “Integrated systems for biopolymers and bioenergy production from organic waste and by-products: a review of microbial processes,” *Biotechnol Biofuels*, vol. 10, no. 1, p. 113, Dec. 2017, doi: 10.1186/s13068-017-0802-4.

[10] J. Sun, L. Zhang, and K.-C. Loh, “Review and perspectives of enhanced volatile fatty acids production from acidogenic fermentation of lignocellulosic biomass wastes,” *Bioresour Bioprocess*, vol. 8, no. 1, p. 68, Dec. 2021, doi: 10.1186/s40643-021-00420-3.

[11] G. Papa, T. Pepè Sciarria, A. Carrara, B. Scaglia, G. D’Imporzano, and F. Adani, “Implementing polyhydroxyalkanoates production to anaerobic digestion of organic fraction of municipal solid waste to diversify products and increase total energy recovery,” *Bioresour Technol*, vol. 318, p. 124270, Dec. 2020, doi: 10.1016/j.biortech.2020.124270.

[12] F. Micolucci, M. Gottardo, D. Bolzonella, P. Pavan, M. Majone, and F. Valentino, “Pilot-scale multi-purposes approach for volatile fatty acid production, hydrogen and methane from an automatic controlled anaerobic process,” *J Clean Prod*, vol. 277, p. 124297, Dec. 2020, doi: 10.1016/j.jclepro.2020.124297.

[13] G. Anderottola, R. Canziani, P. Foladori, M. Ragazzi, and F. Tatano, “Laboratory Scale Experimentation for RBCOD Production from OFMSW for BNR Systems: Results and Kinetics,” *Environ Technol*, vol. 21, no. 12, pp. 1413–1419, Dec. 2000, doi: 10.1080/09593332208618173.

[14] E. Stoyanova, T. Lundaa, G. Bochmann, and W. Fuchs, “Overcoming the bottlenecks of anaerobic digestion of olive mill solid waste by two-stage fermentation,” *Environ Technol*, vol. 38, no. 4, pp. 394–405, Feb. 2017, doi: 10.1080/09593330.2016.1196736.

[15] M. Sauer, D. Porro, D. Mattanovich, and P. Branduardi, “Microbial production of organic acids: expanding the markets,” *Trends Biotechnol*, vol. 26, no. 2, pp. 100–108, Feb. 2008, doi: 10.1016/j.tibtech.2007.11.006.

[16] S. Dahiya, O. Sarkar, Y. V. Swamy, and S. Venkata Mohan, “Acidogenic fermentation of food waste for volatile fatty acid production with co-generation of biohydrogen,” *Bioresour Technol*, vol. 182, pp. 103–113, Apr. 2015, doi: 10.1016/j.biortech.2015.01.007.

[17] Y. Ma, J. Gu, and Y. Liu, “Evaluation of anaerobic digestion of food waste and waste activated sludge: Soluble COD versus its chemical composition,” *Science of The Total Environment*, vol. 643, pp. 21–27, Dec. 2018, doi: 10.1016/j.scitotenv.2018.06.187.

[18] S. Montalvo, S. Vielma, R. Borja, C. Huiliñir, and L. Guerrero, “Increase in biogas production in anaerobic sludge digestion by combining aerobic hydrolysis and addition of metallic wastes,” *Renew Energy*, vol. 123, pp. 541–548, Aug. 2018, doi: 10.1016/j.renene.2018.02.004.

[19] M. Kumar *et al.*, “Critical Review on Biochar□Supported Catalysts for Pollutant Degradation and Sustainable Biorefinery,” *Adv Sustain Syst*, vol. 4, no. 10, p. 1900149, Oct. 2020, doi: 10.1002/adsu.201900149.

[20] M. Kumar *et al.*, “A critical review on biochar for enhancing biogas production from anaerobic digestion of food waste and sludge,” *J Clean Prod*, vol. 305, p. 127143, Jul. 2021, doi: 10.1016/j.jclepro.2021.127143.

[21] R. Karki, W. Chuenchart, K. C. Surendra, S. Sung, L. Raskin, and S. K. Khanal, “Anaerobic co-digestion of various organic wastes: Kinetic modeling and synergistic impact evaluation,” *Bioresour Technol*, vol. 343, p. 126063, Jan. 2022, doi: 10.1016/j.biortech.2021.126063.

[22] J. Mumme, F. Srocke, K. Heeg, and M. Werner, “Use of biochars in anaerobic digestion,” *Bioresour Technol*, vol. 164, pp. 189–197, Jul. 2014, doi: 10.1016/j.biortech.2014.05.008.

[23] F. Valentino, G. Moretto, M. Gottardo, P. Pavan, D. Bolzonella, and M. Majone, “Novel routes for urban bio-waste management: A combined acidic fermentation and anaerobic digestion process for platform chemicals and biogas production,” *J Clean Prod*, vol. 220, pp. 368–375, May 2019, doi: 10.1016/j.jclepro.2019.02.102.

[24] APHA/AWWA/WEF, *Standard Methods for the Examination of Water and Wastewater*. 2012.

[25] S. Ö. Cinar, S. Cinar, and K. Kuchta, “Machine Learning Algorithms for Temperature Management in the Anaerobic Digestion Process,” *Fermentation*, vol. 8, no. 2, p. 65, Jan. 2022, doi: 10.3390/fermentation8020065.

[26] G. Moretto, F. Valentino, P. Pavan, M. Majone, and D. Bolzonella, “Optimization of urban waste fermentation for volatile fatty acids production,” *Waste Management*, vol. 92, pp. 21–29, Jun. 2019, doi: 10.1016/j.wasman.2019.05.010.

[27] F. Valentino *et al.*, “Organic Fraction of Municipal Solid Waste Recovery by Conversion into Added-Value Polyhydroxyalkanoates and Biogas,” *ACS Sustain Chem Eng*, vol. 6, no. 12, pp. 16375–16385, Dec. 2018, doi: 10.1021/acssuschemeng.8b03454.

[28] Á. Estévez-Alonso, R. Pei, M. C. M. van Loosdrecht, R. Kleerebezem, and A. Werker, “Scaling-up microbial community-based polyhydroxyalkanoate production: status and

challenges," *Bioresour Technol*, vol. 327, p. 124790, May 2021, doi: 10.1016/j.biortech.2021.124790.

[29] F. Morgan-Sagastume *et al.*, "Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale," *Bioresour Technol*, vol. 181, pp. 78–89, Apr. 2015, doi: 10.1016/j.biortech.2015.01.046.

[30] F. Valentino, F. Morgan-Sagastume, S. Campanari, M. Villano, A. Werker, and M. Majone, "Carbon recovery from wastewater through bioconversion into biodegradable polymers," *N Biotechnol*, vol. 37, pp. 9–23, Jul. 2017, doi: 10.1016/j.nbt.2016.05.007.

[31] M. Gottardo, F. Micolucci, D. Bolzonella, H. Uellendahl, and P. Pavan, "Pilot scale fermentation coupled with anaerobic digestion of food waste - Effect of dynamic digestate recirculation," *Renew Energy*, vol. 114, pp. 455–463, Dec. 2017, doi: 10.1016/j.renene.2017.07.047.

[32] D. Bolzonella, F. Fatone, P. Pavan, and F. Cecchi, "Anaerobic Fermentation of Organic Municipal Solid Wastes for the Production of Soluble Organic Compounds," *Ind Eng Chem Res*, vol. 44, no. 10, pp. 3412–3418, May 2005, doi: 10.1021/ie048937m.

[33] S. Argelier, J.-P. Delgenes, and R. Moletta, "Design of acidogenic reactors for the anaerobic treatment of the organic fraction of solid food waste," *Bioprocess Engineering*, vol. 18, no. 4, p. 309, 1998, doi: 10.1007/s004490050448.

[34] P. Traverso, P. Pavan, D. Bolzonella, L. Innocenti, F. Cecchi, and J. Mata-Alvarez, "Acidogenic fermentation of source separated mixtures of vegetables and fruits wasted from supermarkets," *Biodegradation*, vol. 11, no. 6, pp. 407–414, 2000, doi: 10.1023/A:1011687230823.

[35] A. D. Moreno *et al.*, "Sequential bioethanol and methane production from municipal solid waste: An integrated biorefinery strategy towards cost-effectiveness," *Process Safety and Environmental Protection*, vol. 146, pp. 424–431, Feb. 2021, doi: 10.1016/j.psep.2020.09.022.

[36] L.□; E. H. P.□; T. G. Metcalf, *Wastewater engineering□: treatment, disposal, and reuse*. 2004.

[37] J. Tamis, B. M. Joosse, M. C. M. van Loosdrecht, and R. Kleerebezem, "High-rate volatile fatty acid (VFA) production by a granular sludge process at low pH," *Biotechnol Bioeng*, vol. 112, no. 11, pp. 2248–2255, Nov. 2015, doi: 10.1002/bit.25640.

[38] F. Valentino *et al.*, "Organic Fraction of Municipal Solid Waste Recovery by Conversion into Added-Value Polyhydroxyalkanoates and Biogas," *ACS Sustain Chem Eng*, vol. 6, no. 12, pp. 16375–16385, Dec. 2018, doi: 10.1021/acssuschemeng.8b03454.

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, PE: Person Equivalent, SGP: Specific Gas Production