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Characteristics of Biogas Production and Synergistic Effect of Primary Sludge and Food Waste Co-Digestion

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Abstract

Co-digestion implementation in wastewater treatment plants enhances biogas yield, so this research investigated the optimal ratio of biodegradable waste and sewage sludge. The increase in biogas production was investigated through batch tests using basic BMP equipment, while synergistic effects were evaluated by chemical oxygen demand (COD) balance. Analyses were performed in four volume basis ratios (3/1, 1/1, 1/3, 1/0) of primary sludge and food waste with added low food waste: 3.375%, 4.675%, and 5.35%, respectively. The best proportion was found to be 1/3 with the maximum biogas production (618.7 mL/g VS added) and the organic removal of 52.8% COD elimination. The highest enhancement rate was observed among co-digs 3/1 and 1/1 (105.72 mL/g VS). A positive correlation between biogas yield and COD removal is noticed while microbial flux required an optimal pH, value of 8 significantly decreased daily production rate. COD reductions further supported the synergistic impact; specifically, an additional 7.1%, 12.8%, and 17% of COD were converted into biogas during the co-digestions 1, 2, and 3, respectively. Three mathematical models were applied to estimate the kinetic parameters and check the accuracy of the experiment. The first-order model with a hydrolysis rate of 0.23–0.27 indicated rapidly biodegradable co-/substrates, modified Gompertz confirmed immediate commencement of co-digs through zero lag phase, while the Cone model had the best fit of over 99% for all trials. Finally, the study points out that the COD method based on linear dependence can be used for developing relatively accurate model for biogas potential estimation in anaerobic digestors.

Keywords Co-digestion · Chemical oxygen demand balance · Food waste · Primary sludge · Synergy

Introduction

Municipal solid waste (MSW) generation increases throughout the years, simultaneously with the growth of cities and population, as well as consumption norms. Consequently, as its indispensable part, food waste is attributed to 8% of world emissions, which comprise the upstream food waste (FW) management, associated logistics, such as shipping and packing, and carbon productions from agricultural development and harvest. Food loss and waste would be the third largest contributor to greenhouse gas emissions if they were a nation. The average global carbon footprint of FW is around 3.3 Gt of carbon equivalent per year, the use of surface and groundwater resources is over 250 km^3, and wasted land resources are around 1.4 billion hectares [1]. There are plenty of methods that can be employed to improve FW. For management, five technologies are essentially used worldwide. During the incineration process, waste is burned in a burner, the ash is dumped, and electricity is recycled in the meantime. Anaerobic digestion (AD) is a complicated process involving numerous bacterial and methanogenic archaea combinations. The process' intended byproducts are digestate, which serves as a nutrient-rich fertilizer, and biogas, which may be used as an energy source [2]. Unlike anaerobic digestion, composting is performed under oxygenic conditions, with both processes being based on the biological degradation of organic matter. The compost's byproducts can then be used as an alternative to N, P, and K fertilizers. In landfilling, to prevent leachate from entering groundwater and surface water, FW will be placed in pits or pools and covered with an impermeable barrier. In the meantime, raw landfill gas will be collected and burned in

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units for power production [3]. Dumpsite disposal is an old way of disposing off FW, where it is dumped in a low-lying region, microbes cause the volume of the refuse to significantly diminish, and the refuse is gradually transformed into humus. Due to its higher renewable energy generation and reduced environmental effect, AD is more appropriate than other technologies.

Globally, about 1.3 billion metric tons of MSW were produced in 2010, and by 2025, the output is expected to increase to 2.2 billion metric tons a year, posing serious social, ecological, and economic problems [4] with a 2.625 trillion USD yearly cost to the world economy. Food waste exacerbates social injustice; approximately one billion people worldwide experience chronic malnutrition, and by 2050, it is anticipated that there will be a 70 to 110% rise in global food demand [5]. As published in the UNEP Food Waste Index Report 2021, 931 million tons of food (or 121 kg per person per year) were wasted in 2019. The report also finds that the average amount of household food which is wasted globally each year (74 kg per person) is remarkably similar in both low- and high-income nations, indicating that most countries can do better. According to the UN's Food and Agricultural Organization, 14% of global food production was lost through supply chain stages up to retail (but not including it) in 2019. In 2020, the entire quantity of food trash produced within the EU-27 + was predicted to be 59.9 million tons, or 116.7 kg/capita/year, with just 16% of the theoretical potential collected, reported by Bio-waste Generation in the EU (Current capture levels and future potential, 2020). The primary sources of food waste, per the European Environmental Protection Agency, are households (42%), followed by food manufacturing and processing (39%), catering facilities (14%), and wholesale and retail (5%). EU prevention initiatives implemented at households were associated with potential savings of up to 1 t CO_2-eq/t, which was reduced to a potential saving of 0.6 t CO_2-eq/t, corresponding to a 38% decrease, when accounting for macroeconomic rebound effects [6]. In addition to encouraging recycling, composting, biogas production, and reuse of materials and energy, the Waste Directive 2008/98/EC mandates a decrease in food waste at the Union level of 30% by 2025 and 50% by 2030. Such data are not available for the Republic of Serbia.

According to [7], Serbian families throw away 198,712 tons of food waste per year. The UNEP report reveals that households in Serbia generate 726,196 tons/year or 83 kg/capita/year. On average, a resident of the Republic of Serbia generates 0.87 kg of municipal waste/day or 318 kg of waste per year. Serbian hotels, restaurants, and caterers purchased 123,000 tons of raw food materials. Some 25,000 t is wasted during food preparation, which mostly refers to inedible parts. Facilities are estimated to serve 99,000 t of food, while some 15,000 t is left behind by customers. Thus, the sector produces a total of 40,000 t of FW—or almost 6 kg per capita annually. An overwhelming part of this waste—estimated at 99%—is landfilled, leaving a heavy environmental footprint. Deposited FW emits the GHG in a total amount of 28,000 t of CO_2 equivalent, which is the amount annually emitted by 6–7 thousand cars. The remaining 1% is used for the most part in composting, biogas production, and food donations. GIZ Office Belgrade estimated that in 2018, local plants exploited about 180 tons of FW. This quantity is subsequently mixed with other types of waste, like manure or garden trash, to produce roughly 22,000 m^3, a volume that may be converted into about 60 MWh of electricity. Legislation and political backing can foster a climate that encourages both consumer and company behavior changes, ensuring a venture's viability [8].

FW, which includes domestic, service, and manufacturing waste, is one of the most significant components of MSW. When we purchase more food than required, store it improperly, toss out leftovers, and overcook it, food is wasted. The composition of food waste knows to vary in terms of physical and biochemical properties in two ways: (1) geographically, following continent and collection source, and (2) seasonally, including summer and winter, holiday and working periods, and festive seasons [9]. FW has a high potential to produce renewable energy in the AD process due to its high biodegradability, concentrations of organic matter (volatile solids/total solids [VS/TS]: 0.8–0.9), and susceptibility to hydrolysis [10]. Food waste average content of TS is 27.59%, while that of VS is 25.91% [11]. As a nutritional part of foodstuffs, proteins and carbohydrates are typically thought to degrade quickly, while lipids and oils hydrolyze at slower rates. Thus, FW rich in lipids and easily degradable carbohydrates can achieve high yields, while lignocellulosic fractions (fruit and vegetable residues) have lower methane potentials. With rising willingness and ability to purchase renewables and rising waste disposal costs, interest in converting FW into bioenergy via digestion has grown [12].

Sewage is wastewater that is discharged from residential buildings as well as surrounding commercial, industrial, and governmental facilities. It includes carbon molecules like those found in human body discharge, paper, organic matter, etc. When untreated or partially treated sewage is introduced into a wastewater treatment plant's main container, the process starts. Screening and sedimentation are used to separate the solids and liquids during the initial treatment. Streams are treated further: the liquid portion using aeration, which involves oxygen addition and the use of microbes, and the solid portion, known as primary or secondary sludge, using thermal hydrolysis and AD. Anaerobic digestion, due to its simplicity and great efficiency, was historically employed as a method for removing organics rather than producing biogas [13]. Although typically constrained by poor digestion and delayed fermentation, sewage sludge also contains

significant amounts of essential microbes [14] that are beneficial for the extension of diverse groups of microorganisms involved in the process [15]. Additionally, AD plays a crucial role in treating sludge by reducing volume, enhancing stability, and getting rid of microorganisms.

Regarding the experiment, hypotheses that bio-chemical methane potential and chemical oxygen demand are proportionally related have been stated. With respect to additional biogas yield over the rated average of individuals, the synergic effect of co-digestion is noticed. Measurements in this regard were based on substrate concentrations, physical and chemical, and did not include biological testing. The mathematical description of the dependence of biogas produced in co-digestions as a function of COD masses utilized in trials reflects the novelty of the findings. Additionally, the paper proposes a model for biogas content estimation and specific biogas production of reactors based on daily COD measurements. With the aim to contribute national sustainable strategy and give an insight into the upcoming decision-making process in wastewater management policy, the objective of this paper was to exploit, test, and determine an optimal ratio on a volume basis for compatible primary sludge and food waste co-digestion in a stable manner. Furthermore, the study findings are represented in an applicable engineering way that is useful for assessments of co-digestion process performance.

Materials and Methods

Inoculum and Substrates

For batch tests, inoculum and primary sludge (PS) samples were collected from the wastewater treatment plant (WWTP) Cvetojevac/Kragujevac (7 km north-east of Kragujevac, Serbia), treating mainly municipal wastewater of approximately 175,000 population equivalents. Plant digesters treat a mixture of primary and thickened activated sludge. Feed content at this facility is processed under mesophilic conditions (approximately 37 °C) with a hydraulic retention time of about 21 days. The effluent of the digester was used as inoculum.

Food waste used for the experiment was obtained from a student dorm of the University of Kragujevac. Unfinished food from the canteen and kitchen leftovers from food preparation made up the majority of FW. Food trash was ground to obtain a representative sample after bones and non-biodegradable wastes (such as plastic bags and consumer goods) were removed. Afterwards, FW was blended until homogenized and then stored in small containers in the freezer at − 18 °C (small portions have been used as required without losing the whole sample integrity) [16]. Before preparing the feedstock for the reactors, FW was thawed at room temperature for 3 to 6 h. The features of the substrates and inoculum are displayed in Table 1 as an average of three repeats with a standard deviation.

Experimental Setup

Biochemical methane potential (BMP) experiments were applied to determine differences in biogas production between the digestion of PS and co-digestions of PS and FW. Tests were assessed in a batch assay conducted under the same state of AD regarding working order and time procedure. Benchtop experiment design consisted of an experimental unit containing two independent observational units. Samples representing individual observations were measured independently and analyzed without bulking. Each treatment has been conducted in double replicates through time. Treatments were organized in no systematic or ordered manner. The experiment was carried out over a year in the following way. The complete FW amount has been sampled as one item. Instead of being kept in a separate location, inoculum and PS were sampled on the day trials were set up. Co-/substrates were applied for feedstocks preparation just before setting up the installation. Different initial dates of the treatments were random as could, and mainly consequences of failures, breaks, and ability to access the sampling site. The experiment is randomized and replicated in a way that any unexpected variation introduced is not controlled or cannot be repeated.

The core of the observational unit was constructed of an Erlenmeyer flask (Bomex, China) and rubber stopper (Deutsch & Neumann, Germany) with two central holes. Reactors had a capacity of 5 l with a working volume of 4 l. The first hole was fitted with a gas outlet, and the other gape, sealed with a tube cap, was an outlet port used as a liquid draught tube through which content was sampled for daily analysis (Fig. 1). For daily sampling of reaction products, glass syringe (Socorex, Swiss) was used, for the pH measuring first and preparation of COD aliquots afterwards. Sampling was conducted from the same point of the reactor by placing the rubber hose through draught tube on the same height from the top of the vessel every time. Stirring and heating in the reactor were carried out with the help of a magnetic stirring system (Witeg, Germany) with a heating

Table 1 Characteristics of substrates and inoculum

	Inoculum	Primary sludge	Food waste
TS (%)	5 ± 0.02	9.65 ± 0.19	39.67 ± 0.37
VS (%)	4.28 ± 0.09	6.55 ± 0.17	34.83 ± 0.21
ρ (g/mL)	1.01	1.03	1.15
COD (g/l)	49.38 ± 1.32	70.7 ± 1.73	336.4 ± 5.64
pH	7.45 ± 0.16	6.49 ± 0.33	5.41 ± 0.13

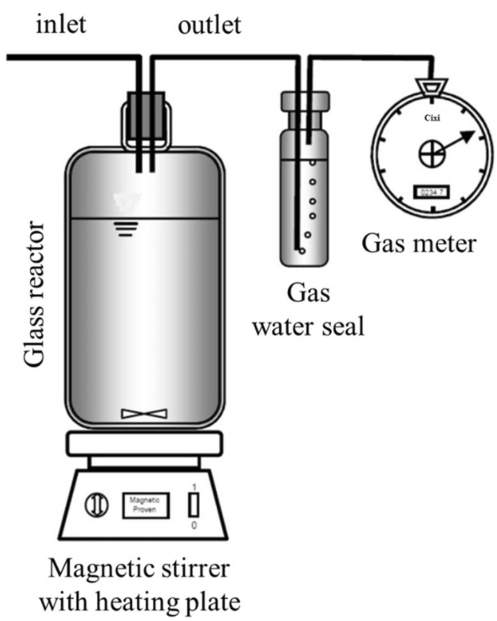


Fig. 1 Laboratory plant scheme

Table 2 Composition of feedstock used in BMP tests

V (l)	V_I	V_{PS}	V_{FW}	% (VS) FW
COD 3/1	3.458	0.407	0.135	23.56 ± 0.03
COD 1/1	3.626	0.187	0.187	30.86 ± 0.04
COD 1/3	3.715	0.071	0.214	34.35 ± 0.04
DIG	3.015	0.985	0	n/a
In	3	0	0	n/a

plate. The temperature of the content was checked twice a day by a thermal infrared camera (Flir, USA). The gas water seal was composed of a 2-l Erlenmeyer bottle, stopper, and glass tube (LMS, Germany). Biogas production was measured using a wet gas meter flow (Shanghai Cixi Instrument, China) with continuous data logging by webcam (Logitech, Europe) triggering on every hour. Afterwards, gas was collected into sampling bags (E-switch, China). All parts of the individual setting were connected by rubber hoses .

The same overall volume was added to the reactors for each experiment phase, but the PS/FW ratios were altered. Four different PS/FW proportions were tested: 3/1, 1/1, 1/3, and 1/0 (digestion). Mixtures were composed based on the volume of co-/substrate. Blank samples containing inoculum only were filled with tap water up to 4 l. Table 2 shows the reactor feeding settings for each batch assay. Based on the specific biogas yield, which is calculated as cumulative biogas produced less the inoculum contribution acquired from seed blanks over the amount of VS added before inoculation (i.e., mL biogas/g VS), each sample's biogas potential was assessed. In order to avoid limiting biomass degradation, samples were generated with an inoculum to substrate ratio of 2 (g_{VS}/g_{VS}). The total solids content was less than 7% in all prepared samples. The full-scale digestion inoculum provided basic nutrient requirements for anaerobic microorganisms, and no additional external nutrients/trace elements were added to evaluate the synergistic effects of co-digestion in providing these requirements [17]. Prior to the test, the pH values for each glass reactor ranged from 7.2 to 7.35. Flasks were flushed with Ar, then sealed to create an anaerobic environment and maintained under mesophilic conditions at 35 ± 1 °C. Hydraulic retention time (HRT) was set up at 15 days.

According to inoculum to substrate ratio (ISR), feedstocks volumes are determined as denoted (Eq. 1):

$$V = V_I + \left(V_{SS}\right) + \left(V_{FW}\right) + \left(V_W\right) \tag{1}$$

where $V, V_I, V_{SS}, V_{FW}, V_W$ are volumes of digestor content, inoculum, sludge, food waste, and water, respectively, while brackets represent optionality of usage. If the concentrations of inoculum, sludge, and food waste volatile solids are marked with $I_{VS}, S_{SS_{(VS)}}, S_{FW_{(VS)}}$, the next equation (Eq. 2) can be stated:

$$V_I \cdot I_{VS} = ISR \cdot \left(V_{SS} \cdot S_{SS_{(VS)}} + V_{FW} \cdot S_{FW_{(VS)}}\right) \tag{2}$$

When using mass contents, the equation is slightly different in form, given that $m_{SS}/\rho_{SS} = V_{SS}$, $m_{FW}/\rho_{FW} = V_{FW}$, where ρ_{SS}, ρ_{FW} are densities of sludge and food waste. Since given ratios are $\frac{V_{SS}}{V_{FW}} = \frac{1}{0}; \frac{3}{1}; \frac{1}{1}; \frac{1}{3}$, volumes can be calculated by equations (Eq. 3), respectively:

$$\begin{aligned} V_{SS} &= \frac{V \cdot I_{VS}}{ISR \cdot S_{SS_{(VS)}} + I_{VS}} \\ V_{FW} &= \frac{V \cdot I_{VS}}{ISR\left(3S_{SS_{(VS)}} + S_{FW_{(VS)}}\right) + 4I_{VS}} \\ V_{FW} &= \frac{V \cdot I_{VS}}{ISR\left(S_{SS_{(VS)}} + S_{FW_{(VS)}}\right) + 2I_{VS}} \\ V_{SS} &= \frac{V \cdot I_{VS}}{ISR\left(S_{SS_{(VS)}} + 3S_{FW_{(VS)}}\right) + 4I_{VS}} \end{aligned} \tag{3}$$

Mass-based quantitative determination of wetness at 105 °C and ignition of dried material at 550 °C were used to measure dry matter (TS) and VS. Samples were placed in crucibles and heated, then put in a furnace and burnt. The total COD and pH of the mixtures with different ratios were measured as soon as the waste streams were completely mixed with each other. During each sampling, only 5 to 7 mL were taken in order to keep the volume change negligible. The pH was recorded using a digital pH analyzer (Lutron, Taiwan). Chemical oxygen demand was analyzed by the $K_2Cr_2O_7$ closed reflux method. Diluted samples were placed in a vessel with a digestion solution and evaluated using a spectrophotometer (Lovibond,

Germany). Gnuplot 5.4 was used to create the artwork, Matlab 2016b was used for the adjustment in the models of Eqs. 4–6, and Microsoft Excel 365 for statistical analysis of ANOVA and z-test.

Kinetic Modeling

Three models were employed to match the experimental data of biogas production with the optimum solution for equations: first order regression model (Eq. 4), modified Gompertz (Eq. 5), and Cone model (Eq. 6). The first-order kinetic model was developed with the assumption that hydrolysis determines the behavior and supervises the entire process and that substrate accessibility is a limiting circumstance. The hydrolysis constant is often used to identify whether co-digestion can improve hydrolysis kinetics and subsequent biogas production rate. FW is known as a highly degradable substrate since hydrolysis and consequent alcoholic fermentation occur rapidly, while sludge is more resistant to hydrolysis. Therefore, increased anaerobic productions from biomass mixtures and higher hydrolysis rates (in the scope of fast or moderate digestibility) are anticipated when compared with mono-digestion. This model, however, does not predict conditions for maximum biological activity, lag phase, and system failures but gives valuable interpretations about hydrolysis kinetics. Differential and rearranged integral representations of first-order reaction applied on specific biogas yield are given below:

$$\begin{aligned} -\frac{dB}{dt} &= k_h B, \\ lnB &= -k_h t + lnB_0, \\ B_t &= B_0\left(1 - e^{-k_h t}\right), \end{aligned} \tag{4}$$

in which B_t is the cumulative amount of biogas per organics produced for time t (l/g COD or l/g VS), B_0 is the ultimate biogas yield (l/g), B is the specific biogas production at any given time, t is the digestion time (d), and k_h is first-order hydrolysis rate coefficient (1/d).

For a variety of dynamic biological systems, the Gompertz equation, especially its modified form, is frequently used to describe microbial cell density during methanogenic periods in terms of exponential growth rates and lag phase duration. Because of its simplicity and suitability for the batch data, it provides a realistic estimate for B_0 and has fewer uncertainties in the interpretation of results. It is commonly utilized as a model for anaerobic digestion, requires only the accumulated biogas data, and offers readily understandable parameters. In terms of this study, the lag phase period or minimum time to produce biogas was expected to be less for co-digestion than for mono-digestion, but greater than zero. The modified Gompertz model is an empirical non-linear regression model as shown in

$$B_t = B_0 exp\left\{-exp\left[\frac{R_m e}{B_0}(\lambda - 1) + 1\right]\right\} \tag{5}$$

where R_m is the maximum biogas production rate (mL/g VS/d), and λ is lag phase time (d).

Hydrolysis can also be modeled using the Contois model, as hydrolysis is considered to be a biochemical reaction facilitated by an extra-cellular enzyme produced by hydrolytic/acidogenic bacteria. Contois's equation, which is frequently used in kinetic modeling, is utilized in this research to recalculate and confirm the hydrolysis rate constants, which are predicted to be the same as or similar to those in the first-order model. The model, which links the gradually increasing hydrolytic stage of organics with degradation, is given by

$$B_t = \frac{B_0}{1 + \left(k_h t\right)^{-n}} \tag{6}$$

where n is the shape factor.

Results and Discussion

Cumulative production was normalized in two ways: (1) by specific biogas yield using the initial VS of co-/substrate (mL/g VS) and (2) by volumetric biogas production using the volumetric loading of FW and PS (mL/mL added) [18]. The overall VS concentration used in this experiment ranged from 4.84 to 5.95%. The VS showed, on average, 84% of TS content for co-digestions and 78% for mono-digestion, which indicates a high capacity for organic transformation. In the absence of VS reduction which would affect biogas production and result in notably lower yields, mass transfer from organic substance to biogas could not be carried out. Furthermore, co-digestion of FW and PS improves production compared to the sole substrate and reduces limitations of single feedstock, such as high salt concentration of FW and low VS content of PS [19]. Further on, seed sludge was analyzed for biogas potential before running the anaerobic batch experiments. The outcomes demonstrated the presence of active microorganisms in the inoculum and the ability to degrade organics in the digestion process. Digestion occurred in a steady mode for all ratios, which was partially affected by the characteristics of the inoculum already adapted to the residue type.

The average headways of specific biogas production of PS mono-digestion and volume-based co-digestion mixtures are illustrated in Fig. 2a. Batch tests indicated that adding FW increased the specific biogas yield compared to PS only, which showed the lowest average cumulative biogas production. The better digestibility of FW itself and significantly faster hydrolysis rate most likely contributed to

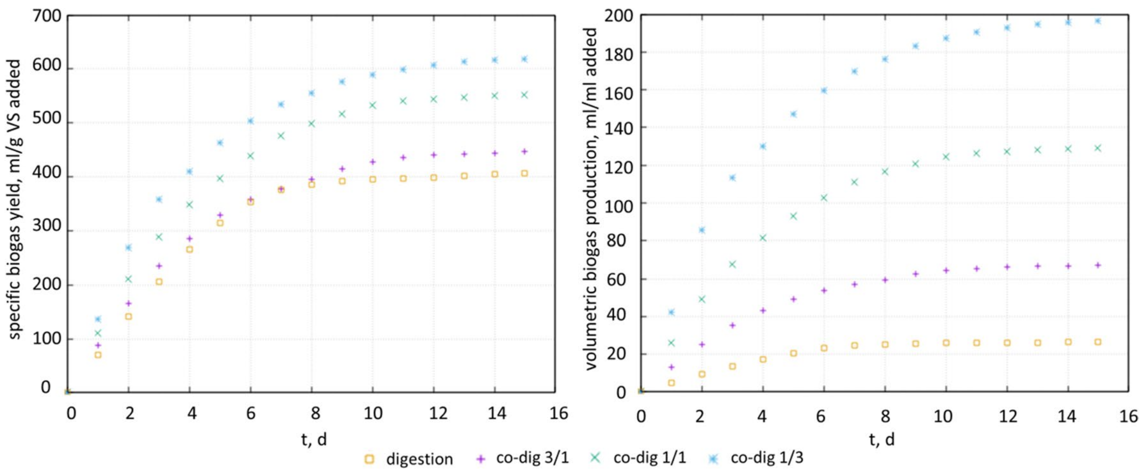


Fig. 2 Normalized cumulative production

the mixtures' increased output. After 15 days of digestion, the 3/1 trial (446.9 mL/g VS) displayed an average specific biogas yield 10% higher than PS only (407.0 mL/g VS). The average specific biogas production of 1/1 co-digestion ratio was 552.6 mL/ g VS. As shown in the figure, the optimal PS/FW mixture ratio was 1/3, with an average biogas output of 618.7 mL/g VS. In comparison to mono-digestion and 3/1 co-digestion, respectively, this implies an increase of 52% and 38%. Regarding the 1/1 PS/FW ratio, the specific biogas yield of co-dig 1/3 displayed an average growth of 12%. The highest enhancement of biogas production was observed between 3/1 and 1/1 co-digs, where 3/1 accounted for around 81% of 1/1 co-digestion, and is in accordance with VS content of the mixtures. Meanwhile, in the volumetric analysis, 1/3 (196.5 mL/mL substrate) and 1/1 co-digs (129.3 mL/mL substrate) were notably higher, approximately 52% and 92% higher than amounts of biogas produced by 1/1 and 3/1 co-digs (67.2 mL/mL substrate). The last one had an increase of around 157% compared to mono-dig (Fig. 2b). Therefore, 1 g of VS in terms of co-digestion increases biogas production from 110 up to 152% compared to PS only, and 1 mL of substrate increases biogas production within a range of 2.6–7.5 times compared to mono-dig, depending on the mixing ratio.

The increased proportion of FW in the mixture generally results in higher biogas generation. As described by [20], the biodegradability characteristics of substrates and the production of intermediate inhibitory substances will control the kinetics of the different steps of anaerobic digestion and define the biogas production curve shape. The production profiles frequently followed the exponential law. So, the production of biogas from anaerobic biomass may have grown as a result of a more suitable anaerobic environment. ANOVA analysis (Table 3) of the results obtained in this study has been conducted. The null hypothesis that there is no significant difference in biogas yield between the means of the treatments with different co-/substrates ratios is rejected. An alternative hypothesis which states that means are not the same is adopted since the *p*-value is significantly lower than 0.05. Degrees of freedom between the groups are 3 which correspond to 4 analyzed groups, and 12 within the groups matching 4 individual observations in each group. A higher value of F than F_{crit} also supports the adoption of the alternative hypothesis, since in this case, the tests have statistically different yields [21]. Furthermore, when the F is large, it suggests that the treatment effects are significant, and the differences between the groups are not due to chance.

As far as co-digestion is concerned, three mixing ratios of sewage sludge and food waste were evaluated through 16 studies [22-37]. The researchers reported the highest cumulative production under a food waste mixing ratio of 75%. In comparison with this study (619 mL/g VS), [35] reported a somewhat lower value (450 mL/g VS removed) when domestic water was used as a substrate, while [29] recorded 363 mL/g VS with yard waste as an additional co-substrate.

Table 3 ANOVA outcomes for results obtained in this study

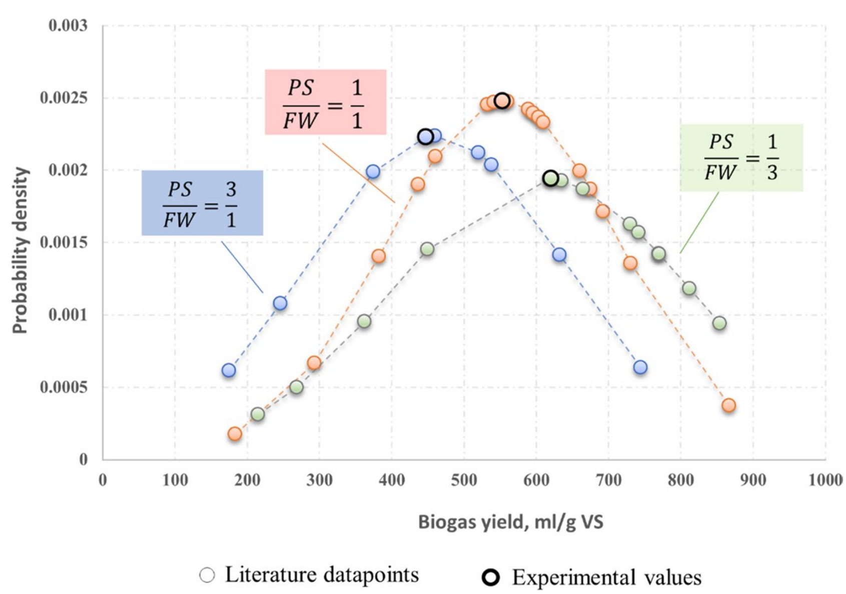
Source of variation	*SS*	*df*	*MS*	*F*	*P*-value	*F* crit
Between groups	112,744.5	3	37,581.5	67.79076	8.562E-08	3.490295
Within groups	6652.5	12	554.375			
Total	119,397	15				

According to the literature, higher yields than 0.7 l of biogas per gram of volatile solid (added) were achieved in three studies where anaerobic membrane bioreactors (AnMBR) were applied, and over 800 mL with thermophilic anaerobic membrane bioreactor implemented [26]. Meanwhile, the highest value recorded (854 mL/g VS) was from the experiment with waste-activated sludge as a substrate [28]. Furthermore, for a 1/1 ratio (co-dig 2), seven results from six studies are in 95% confidence interval (477–630 mL/g VS), to which the obtained score from this study also belongs (553 mL/g VS). The usage of biogas residue biochar showed a somewhat higher value of 675 mL/g VS [34]. On the other hand, the same study showed a minor yield of 461 mL when no biochar was used. Additionally, the bell curve of specific productions had the lowest standard deviation of 161 compared with co-digs 1 and 3, 178 and 205, respectively. As far as the 3/1 ratio is concerned (447 mL/g VS), [23] research reported a lower specific production of 246 mL/g VS, which might be explained by SARS-CoV 2 presence in the feed. In studies [37] and [22] whose results are in opposite areas of very unlikely observations (with productions of 175 and 744 mL/g VS, respectively), no additions were made. The types of FW present in the combination of residues may be the cause of differences.

Results from the experiments cited in the supplementary table and depicted in Fig. 3 correspond to a range of data against which values obtained in this study are tested under null hypothesis by *z*-test, since means and variances are known. It stated that the population mean equals a hypothesized value. In other words, measured values are probable to occur in sewage sludge and food waste co-digestion in declared ratios. The means of observations in the dataset were 461, 554, and 607 mL/g VS for ratios 3/1, 1/1, and 1/3, respectively. *P*-values of 0.82, 0.98, and 0.83 for co-digs 1, 2, and 3, respectively, indicated acceptance of the null hypothesis as true, showing a high degree of compatibility between a dataset and specific productions under the hypothetical statement. The alternative hypothesis which declared that measured values differ significantly (statistically; $p < 0.05$) from previous findings is rejected. Values obtained in this study were normally distributed but did not affect the means and variances of a literature dataset.

The average daily biogas yields and cumulative productions of PS and co-digestions are displayed in Fig. 4. In all batch trials, the biogas production began right after feeding the reactors. The peak of biogas production was seen on day 2 for co-digestion 1/3 and mono-digestion and on day 1 for 3/1 and 1/1 co-digs. During the first 4 days of the experiment, the production was significantly higher for all setups—over 62% of the entire volume of biogas. The highest cumulative production of 59.1 l achieved by co-dig 3 was nearly twice as much as that of mono digestion (32.3 l); 50% and 15% higher than co-digs 1 and 2, which amount to 39.5 l and 51.5 l of biogas, respectively. Variances between daily production rates were negligible at the ends but differed substantially at the very beginning of the process as the co-dig 2 (111 mL/g VS/d) rate was 25% higher than co-dig 1 (89 mL/g VS/d) and 20% lower than co-dig 3 (133 mL/g VS/d). When compared to co-dig 3, the daily biogas rate for mono-dig (70 mL/g VS/d) was 1.9 times lower, implying that the use of food waste as a co-substrate is strongly recommended. This effect is associated with FW as a rapidly degradable substrate since hydrolysis and consequent alcoholic fermentation rapidly transform a large amount of VS into VFA, CO_2, and H_2, while PS is more resistant to hydrolysis. Due to the typically high inoculum share in the assay, and in contrast to continuously operating systems, the substrate is only added once in a BMP test. By definition,

Fig. 3 Normal distribution of yields values



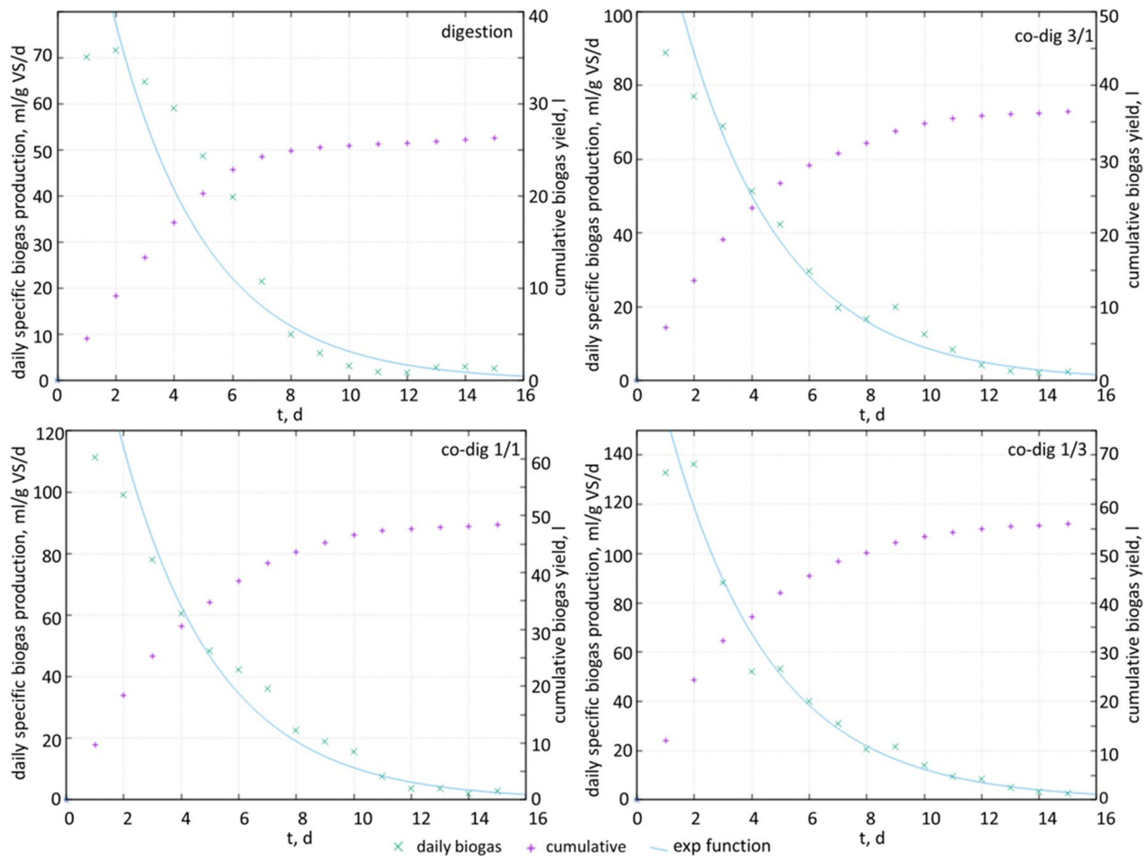


Fig. 4 Daily specific rates and cumulative production

the methane production attained in continuous tests or in large-scale plants should typically be lower than the result of a BMP test. Therefore, continuing tests are strongly advised for a more accurate estimate [38]. Within 2 weeks, the biogas production in both co-digestion and mono-digestion reactors dropped to almost zero. Figure 4 represents daily production dependencies fitting the exponential functions over 96% for co-digs and 89% for mono-dig.

The pH concentration has a big impact on the AD system since it affects how easily degradable materials dissolve and how biogas production fermentation works. It can create a suitable atmosphere for microbes since the enzymatic reactions of microorganisms depend on pH. Although most microbes prefer neutral pH settings, individual bacteria have varying optimal pH values. The somewhat acid condition of the co-/substrates pH (5.4–6.5) was adjusted by the fine pH value of the inoculum (7.45), preserving process stability and balancing overall feed pH. The pH value shown is typical of food waste. In a review study, [39] found a pH range of 4.4 to 5.8 while examining 65 food waste samples from investigations carried out between 2001 and 2014. All initial pH ranges in a study trial were acceptable, with the final readings from 7.5 to 7.8.

Co-digestion pH values did not vary with respect to mono-digestion in general. A noticeable decline on the daily production curve is observed for 1/3 co-dig. Following the onset of AD, the production rate declined from day 1 to day 4, indicating some inhibition. It might be accounted for by an abrupt rise in basicity (Fig. 5), which peaked on the fourth day of the reaction and reached a pH value of 8. Inhibition was caused by an inhibitory substance rather than the concentration of FW in the digester medium, suggesting that the FW sample itself may have been inhibitory. BMP assays can identify the acute toxicity of an inhibitor that is present in the substrate or that has been intentionally added, but they cannot identify the chronic toxicity. Apart from 1/3 co-dig, pH stayed neutral in all treatments, even without using a buffer.

Kinetic Characteristics

Mathematically, the degradation rate of every group of reactants can be characterized by a differential kinetic equation. Therefore, knowledge about biodegradation kinetics and biogas production could be helpful for specific substrate predictions. In this study, three mathematical models were

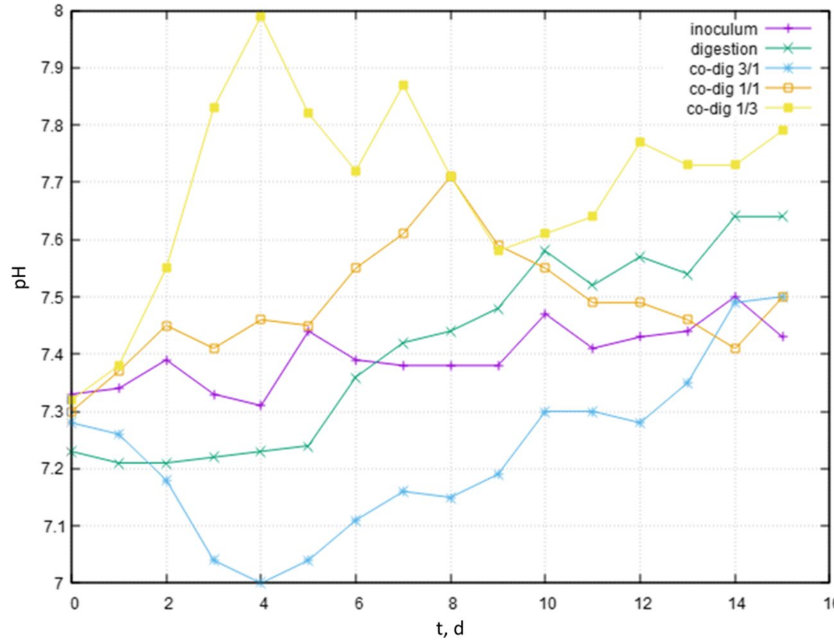


Fig. 5 Reactors pH values

applied to the results of experimental BMP tests to examine how well PS and co-digested mixture potentials could be predicted. Models were applied to simulate the production line for each trial as a function of digestion time. The experiment's eventual biogas yield (B_0) came close to matching the predictions of the first-order and Gompertz models and with a slight variance for the Cone. For digested organics, B_0 increased as VS concentration increased. The experimental cumulative biogas productions were used to check the assumption of homogeneous division around the model curve by plotting against the predicted probable values. Values that were uniformly distributed on either side of the zero (line) indicated that the models were appropriate for the current investigation. Generally, the first-order and Gompertz models fit well with experimental data with a very light exception of PS mono-digestion and co-dig 1:3, respectively, while the Cone equation fits almost perfectly. These models can explain 97.8% of BMP results. Low differences between measured and anticipated values imply that the presented models accurately predict reactor behavior.

Parameters such as biogas yield potential, maximum biogas production rate, hydrolysis rate constant, shape factor, and lag phase duration were estimated for each case and summarized in Table 4. In this regard, the first-order kinetic and Cone models are used to calculate the hydrolysis rate and the amount of biogas. A modified Gompertz model was used to calculate the minimum time to produce biogas (λ) and the growth of the biogas production rate, R_m, which was observed when the proportion of FW in co-dig mixtures increased. The before-mentioned production started after the onset of BMP assays for all co-dig reactors practically instantaneously and with less than 0.12 day for mono-digestion trial. These results are in line with the kinetic model analysis, i.e., a higher fraction of organic waste in the overall mixture volume decreases the lag phase. As a result, in actuality, co-digestion of PS with FW can boost AD efficiency by shortening the time needed for optimal biogas production.

The hydrolysis constant can be used to ascertain whether co-digestion creates advanced terms for degradation and subsequent biogas generation. The hydrolysis rate constant, k_h, varies depending on the co-/substrate type, solubility, and pH value. For instance, when canned products and kitchen waste were treated in batch co-digestion with manure, [17] calculated k_h to be 0.27 and 0.35 d^{-1}, respectively. A significantly lower rate of 0.11 d^{-1} was founded during PS co-digestion with thickened activated sludge in an equal-volume mixture [40]. Corresponding kinetic is observed in the results of this paper. The first-order kinetic model sets out a hydrolysis rate constant of 0.24 for digestion and 0.23–0.26 for co-digs, suggesting a negligible longer period to perform, while the Cone model provides some higher values for rate constants in the range of 0.3 to 0.34, showing faster and more intense hydrolysis. However, co-digestion of FW and PS had no effect on the apparent hydrolysis rate.

COD Balance

COD is a parameter that represents the extent of solubilization. The COD value indicates the amount of materials that can be chemically oxidized, which provides information on the energy content of the feedstock. Accurate COD quantity calculations for the system's entry and exit make it easier to analyze digester performance through mass fluxes. The total

Table 4 Parameters estimation from experimental data fitting with models

Models	Parameter		Units	Digestion	Co-digestions		
					3/1	1/1	1/3
First-order kinetic model	Rate constant (*k*)		1/d	0.25	0.24	0.23	0.27
	Biogas yield (Bo)	Predicted	mL/g VS added	428.17	466.58	580.00	631.96
		Measured	mL/g VS added	407.02	446.90	552.62	618.71
		Difference	%	5.20	4.40	4.95	2.14
	R-square			0.98	1.00	1.00	1.00
	rMSPE			15.16	4.88	6.56	5.37
Modified Gompertz model	Lag phase (*λ*)		d	0.12	0.00	0.00	0.00
	Max. biogas production rate (*Rm*)		mL/g VS added/d	73.23	73.95	90.78	112.03
	Biogas yield (Bo)	Predicted	mL/g VS added	404.43	439.45	546.72	601.66
		Measured	mL/g VS added	407.02	446.90	552.62	618.71
		Difference	%	0.64	1.67	1.07	2.76
	R-square			1.00	0.99	0.99	0.98
	rMSPE			3.34	9.94	12.16	19.96
Cone model	Rate constant (*k*)		1/d	0.34	0.31	0.30	0.34
	Shape factor (*n*)			1.86	1.41	1.39	1.33
	Biogas yield (Bo)	Predicted	mL/g VS added	433.06	507.48	634.32	697.50
		Measured	mL/g VS added	407.02	446.90	552.62	618.71
		Difference	%	6.40	13.56	14.78	12.73
	R-square			0.99	1.00	1.00	1.00
	rMSPE			9.41	4.55	7.86	5.10

COD concentration determined after the addition of co-/substrate in the reactor and inoculation is final for the feed and initial for the process. In this study, with a seed sludge ratio of approximately 70% of total COD for all three mixtures, the COD proportion between inoculum and co-/substrate was almost constant. This note indicates that COD is significantly attributed to FW and PS in the reactor, making feedstock's co-digestion potential high. The COD removal efficiency was calculated by Eq. 7:

$$COD_{red} = \frac{\left(COD_{\text{added}} - COD_{\text{digestate}}\right)}{COD_{\text{added}}} \tag{7}$$

where the ratio between the amount of COD reduced by and added to the digester determines the COD removal rate (in percentage when multiplied by 100). COD reductions were compared to evaluate the organic removal efficiencies from different co-/substrate ratios. Therefore, total COD removal rates for examined scenarios were 38% from 61.2 ± 0.3 to 38.1 ± 0.5 g/l for co-dig 1, 47% from 63.8 ± 0.2 to 33.7 ± 0.7 g/l for co-dig 2, and 53% from 65.1 ± 0.2 to 30.5 ± 0.6 g/l for co-dig 3. The total COD removal of 53.3% by digesting a 1/3 PS/FW feed in contrast to 26.8% for reverse proportion, under mesophilic conditions and an HRT of 34 days, has been presented by [41]. The total COD removal from anaerobic co-digestion of a mixture composed of 50% cow manure and 50% kitchen waste, vegetable and fruit residues, and MSW with 20 days HRT in a batch process was 22–41% [42]. Removal efficiencies from co-digestion of SS with olive mill wastewater, crude glycerol, and cheese way ranged between 34 and 50% for organic loading rates between 0.9 and 1.5 kg VS/m^3/d [43], which is in line with results from batch experiments in this study. Furthermore, this degree of removal matches the previously reported COD reduction value of 41% found during anaerobic degradation of FW and waste-activated sludge at a ratio of 7:3 (v/v) [44]. Concerning greasy sludge and WAS, high COD reductions (over 50%) were obtained, comparable to those of other researchers and current co-digs, regardless to the substrate ratio [45]. Finally, evidence of efficient microbiological activity from methanogenic bacteria was provided by COD elimination in conjunction with gas production.

After day 8 of our experiments, COD concentration dropped along with biogas output to a stable level. However, it is worthy of mention that settled digestate still contains a high residual COD absolutely resistible to biodegradation. The synergisms in co-/substrate pairings are further supported by higher efficiencies of COD elimination. These synergies probably resulted from the improvement of the fundamental organic nutrient compositions. To explain the co-metabolic synergistic effects, in cases when the amount of biogas produced out of co-digestion feedstock exceeds the

sum of biogas yields gathered from individual components, COD balance must be taken into account.

COD balance applied to determine the scope of synergy is expressed in Eq. (8):

$$COD_{\mathrm{In}} + COD_{PS} + COD_{FW} = COD_{Gas} + COD_{Rsd} \tag{8}$$

where the entrant COD involves inoculum, primary sludge, and food waste, and the output includes biogas yield (expressed as COD) and residue.

Biogas produced and converted into COD is presented by Eq. 9 with the aim of assessing the extent of synergism.

$$COD_{Gas} = COD_{G\_\mathrm{In}} + COD_{G\_PS} + COD_{G\_FW} + COD_{G\_Syn} \tag{9}$$

Biogas is the sum consisting of inoculum, PS, and FW biogas productions from mono trials and additional biogas due to synergetic reaction, all outlined in the form of COD [46].

After 15 days, inoculum only produced 189.1 mL/g COD, which is a low residual specific yield. Seed sludge intake was turned into biogas in the range of 11%. The remaining COD of inoculum was considered to be in solid residuals. The seed sludge was given the exact biogas potential and conversion rate in the trials that followed. Biogas production from inoculum accounts for 7.6–7.8% of the output COD (Fig. 6). The mono-digestion of PS presented an ultimate specific biogas yield of 396.0 mL/g COD. Balance calculation exhibited that 33% conversion was achieved for biogas production from mono-dig of PS based on the introduced COD. AD of sludge alone leads to lower degradation rates in comparison with values obtained for co-digs. The extent of degradation for FW during anaerobic co-digestions is supposed to be full conversion, in which the ultimate specific biogas yield of 652 mL/g COD was adopted [47]. Given that no COD conversion rate can be greater than 1, it was assumed that the maximum is reached for FW, and everything above was characterized as a result of synergetic co-metabolism. Thus, synergy demonstrates itself as the difference between measured and calculated biogas productions at the full conversion of FW and partial conversion of PS plus seed sludge background [48]. The amount of biogas expected to be generated in co-dig trials can be assessed by Eq. 10:

$$V_{co-dig} = \left(m_{COD_{co-dig}} / m_{COD_{\mathrm{mono}}} \right) * V_{\mathrm{mono}} \tag{10}$$

where $m_{\mathrm{COD.co\text{-}dig}}$ is the mass of COD added in co-digestion, and $m_{\mathrm{COD.mono}}$ and V_{mono} are the mass of COD and volume of biogas used and produced in mono experiments, respectively.

Co-digestion of sludge mixture (seed and raw primary) and FW as a co-substrate reproduced the synergetic effect as supplementary biogas production was observed. Additional biogas yields were calculated to be 7.1%, 12.8%, and 17% of output COD, assuming the same partial conversions for input COD. Synergistic COD fraction almost exceeded the output COD of seed sludge for co-dig 1 and accounted for 52% and 61% of FW COD for co-digs 2 and 3, respectively. Positive combining effects (mixing easily digested FW with more resistant sewage sludge), improved availability of macro and trace nutrients, and intense co-metabolic reaction are the causes of synergy. Analogously to previous experiments, the measured biogas equivalent of 427.6 mL/g COD for co-digestions is higher than specific production extrapolated from mono assays as well as surplus synergy production rates. Therefore, it is likely that synergistic metabolism led to better yields for seed and primary sludges as well as to a greater level of degradation.

The type of dependency between COD and specific biogas yield is a linear function which is a consequence of the exponential decline of COD as a function of time, indicating zero order reaction. The change ratio of the dependent variable, COD in this case, is a constant value. The dependent and independent quantities are directly proportional. A section on the y-axis represents the initial COD value in the reactor. At the same time, the x-axis cannot be intersected since the lag phase of COD is always present and no COD content can be withdrawn entirely (Fig. 7). The function is of descending character, which indicates that biogas yield is positively correlated with simultaneous COD consumption. After time parameter exclusion, the COD dependence graph seems suitable for biogas production predictions.

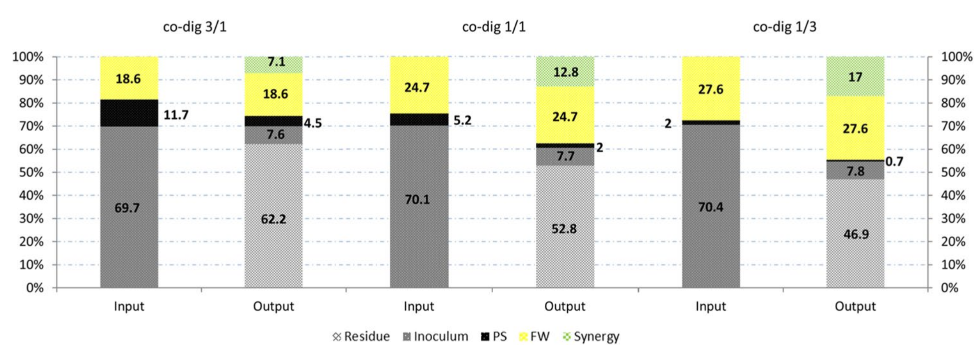


Fig. 6 Feed content and corresponding gas production expressed as COD

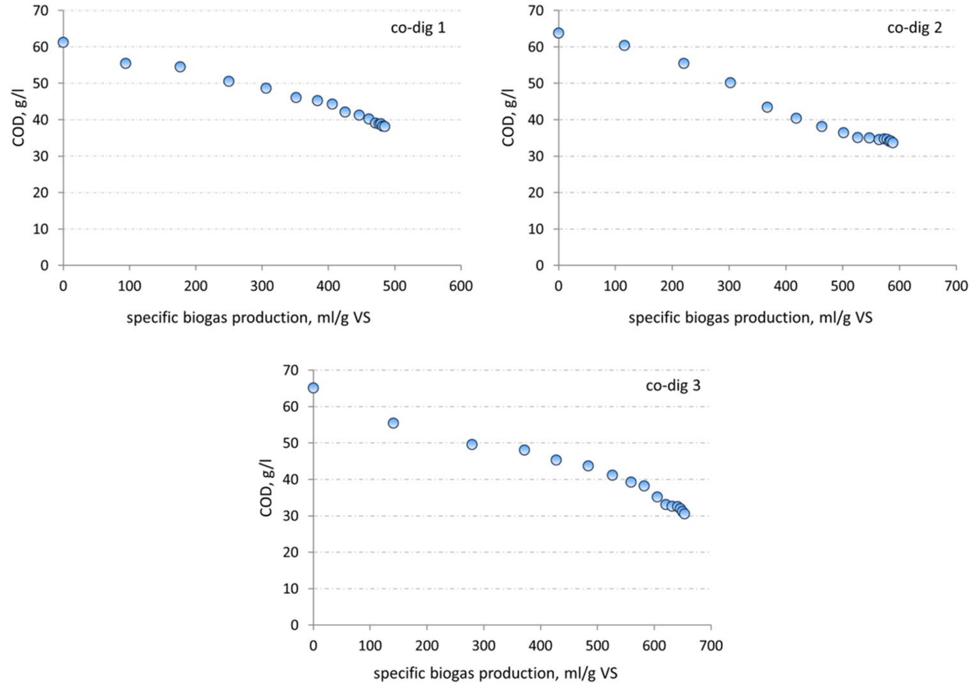


Fig. 7 COD dependence

Exponential decrement of COD was also observed with [49] and [50] in both, batch and continuous experiments, which corresponds to the phenomenon observed in this research and, consequently, to the COD dependency graph.

Conclusion

The study considered the enrichment of sewage sludge in the plant digester with the food waste used from student mess-rooms. Even though the food waste volume supplemented was relatively low, it contributed the most to total biogas generation, and that improvement can be considered as non-negligible. Additionally, co-digestion increased biogas outputs rather than reaction kinetics, while the synergistic effect was attributed mainly to the greater extent of degradation. The COD approach can be used to create a fairly accurate model for estimating biogas potential in WWTP. Standardization of COD sampling together with biological testing might be the future direction of the review.

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Author Contribution Nikola Rakic: conceptualization, methodology, investigation, writing—original draft. Vanja Sustersic: conceptualization, methodology, project administration, writing—review and editing, Dusan Gordic: supervision, resources, funding acquisition, writing—review and editing. Nebojsa Jovicic: conceptualization, supervision, writing—review and editing, Goran Boskovic: supervision, writing—review and editing. Ivan Bogdanovic: investigation, resources.

Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Competing Interests The authors declare no competing interests.

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