Heliyon 10 (2024) e38773

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Case specific: Addressing co-digestion of wastewater sludge, cheese whey and cow manure: Kinetic modeling

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ABSTRACT

The study investigated the methane production efficiency in a semi-continuous laboratory experiment with periodic feeding of wastewater sludge (WWS) as primary substrate and addition of whey (CW) and cow manure (CM). The short-term behavior of a real-scale anaerobic digester with WWS and the methane production improvements with different feeding mixtures of WWS, CW and CM were addressed. Gradual addition of CW to WWS (WWS:CW:CM = 70:20:0 to 70:55:0) increased the average daily methane production to 48.6 mL CH_4/g COD/day and prevented reactor failure, but high VOA/TIC values showed that the reactors were conditionally stable evolution at an OLR of 8 g COD/L/day. Reactors that were additionally supplemented with CM (WWS:CW:CM = 70:55:10) achieved at least 12.3 % more methane than the reactors supplemented with WWS and CW alone. The highest methane production and process evolution in the reactors were achieved at OLRs between 7.5 and 8.7 g COD/L per day. After day 50, the addition of double the amount of CW further increased the methane production and VOA/TIC ratios. In this case, the OLR increased from 6.3 to 9.3 g COD/L/day. The concentration of propionic and acetic acid in all reactors increased above the recommended values and caused inhibition and instability. A strong positive Pearson correlation was found between the trace elements (Fe, Cu, Zn, Mn) detected by XRF. TE contributed to methane production, but to a lesser extent than TIC and NH4+-N. The simplified model successfully predicted methane production under a periodic feeding regime.

1. Introduction

Biogas is the main product of anaerobic digestion and consists predominantly of methane (CH_4) and carbon dioxide (CO_2), net to limited levels of hydrogen sulfide (H_2S) and water (H_2O). Anaerobic digestion provides an alternative to fossil fuels [1]. Cheese whey (CW) is a byproduct of cheese production and represents one of the high-energy products in surplus that can boost biogas production. About 9 kg of CW is simultaneously produced with 1 kg of cheese [2]. The annual production of CW is around 180–190 million tons worldwide [3]. According to the Eurostat, 55.9 million tons of CW (in liquid whey equivalent) (https://ec.europa.eu/eurostat/statistics-explained/index.php?title=Milk_and_milk_product_statistics) were produced in the EU in 2022. Despite efforts to increase human consumption of CW proteins, half of the residual liquid from cheese production is disposed of as waste [4]. When such a large

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https://doi.org/10.1016/j.heliyon.2024.e38773
Received 8 April 2024; Received in revised form 26 September 2024; Accepted 30 September 2024
Available online 1 October 2024

organic byproduct is released into the natural environment (e.g., water bodies), CW potentially poses a major ecological problem (e.g., acidification, eutrophication, impermeabilization) [2,5]. The major problems associated with the anaerobic digestion process of CW are its physicochemical characteristics: low pH and alkalinity, and nitrogen deficiency (C:N:P ~ 200:3.5:1), which can lead to inhibition of anaerobic digestion [2]. Researchers have attempted to prevent inhibition of the process by adding various chemicals (e.g., NaOH, $NaHCO_3$), which can result in large financial investments [4,6]. Instead of using different chemicals or separating the methanogenic and acidogenic phases [7] to avoid the problems of mono-digestion of CW, several researchers have proposed co-digestion of CW in combination with other wastes such as cow manure (CM) [1,8], wastewater sludge WWS [8], poultry manure [9], and food residues [10]. Co-digestion of different substrates is an alternative to mono-digestion and can improve the performance of the anaerobic digestion process as well as increase methane production [11].

In recent decades, researchers have studied anaerobic co-digestion with different combinations of substrates using different variants of the biomethane potential test (BMP test). The optimal mixtures are very important for the smooth operation of biogas plants and digesters at WWTP. Many experiments are required to determine the right combination of substrates. Biogas plant operators usually use batch tests, which sometimes do not anticipate the potential inhibitions that can occur when using some substrates [11–13]. The next step is to use laboratory or pilot scale semi/continuous testing. This can be time consuming but enables modelling on the collected data to define the correct combinations and concentrations of substrates. Hence modelling approaches are important for saving time and making the anaerobic digestion process easier to transfer from laboratory semi-continuous scale to full industrial scale. Mathematical modelling also reduces the risk of errors associated with anaerobic digestion and helps minimize the risk of imbalance and instability in the digestion process of laboratory and large-scale plants [14,15].

Further, many researchers have additionally focused on the effects of trace elements (TE) on methane production [16–21]. Trace elements are essential for microbial growth and metabolic pathways. Consequently, methane production and organic matter decomposition efficiency are determined [16]. A deficiency of TE can lead to instability and fluctuations in the anaerobic digestion process. Molybdenum (Mo), selenium (Se), and tungsten (W) play important roles in the acetogenesis phase of anaerobic digestion. Essential TE in methanogenesis are cobalt (Co), molybdenum (Mo) or tungsten (W), nickel (Ni), and manganese (Mn) [16,22]. Cobalt (Co), iron (Fe), zinc (Zn), lead (Pb), chromium (Cr), potassium (K), and copper (Cu) also play a crucial role. The concentration of TE in wastewater can vary due to various factors, such as human pollution, drainage systems, and associated industries [16]. Compared to WWS, TE concentrations in CW are very low. In the case of Zn, they are up to about 220 times lower, in the case of Fe, concentrations are about 3.5 mg/kg TS^{-1}, Mn concentrations are about 1.3 mg/kg TS^{-1}, and Mo concentrations are about 1.9 mg/kg TS^{-1} [23–25]. From this point of view, successful mono-digestion is prevented by the interplay of the lack of TE in CW, the lack of total inorganic carbon (TIC) (CO_3^{2-}), higher salinity of CW and low pH.

WWTPs generally accept an additional supply of novel substrates due to the excess quantities of organically degradable waste from the surrounding industry to ensure or improve self-sufficiency in electricity and heat supply throughout the year by improved methane production. In this regard, short-term (64 days) additions of novel substrates such as CW and CM to the anaerobic digester influent and their impact on methane yields and process parameters are case-specific. In this study, the short-term addition of CW and CM as feedstock was tested as an approach to increase the methane yield between new feedstocks and WWS at different ratios using semi-continuously upgraded 5-L AMPTS® with periodic feeding regime simulating regular weekly operation of WWTP. The periodic exchange of the digestion medium resulted in a unique dynamic pattern of system response. An integration algorithm was implemented to account for the semi-continuous mode of operation of the feed used in the bioreactors. Such discontinuities in the input conditions are difficult to model and usually cause the integration algorithm to fail. Therefore, building upon experimental data obtained in this study, a simple kinetic model was created to simulate methane production for the mixtures of WWS, CW, and CM.

2. Materials and method

2.1. Inoculum and substrates

The inocula used to start the experiments were obtained from the mesophilic anaerobic reactors of the Domžale-Kamnik wastewater treatment plant in central Slovenia (JP Centralna čistilna naprava Domžale-Kamnik d.o.o.). HRT of full-scale anaerobic digester is between 20 and 22 days. OLR varies between 3,0 ± 0,3 g VS/L.

Three different substrates were used in this study: (a) WWS from a Domžale-Kamnik WWTP in central Slovenia (JP Centralna

Table 1
Average compositions of substrates (TS – total solids, NH_4^+-N – ammonium nitrogen, COD – chemical oxygen demand) used in semi-continuous experiment.

Substrates	pH	Electrical conductivity (μS/cm)	TS (%)	COD (mg/L)	NH_4^+-N (mg/L)	NH_3 (mg/L)
inoculum	7.53 ± 0.74	3830	2.62 ± 0.22	37700		
CW – Murska Sobota[a]	4.38 ± 0.01	4400	6.54 ± 0.08	92600	224	212
CW – Ljutomer[a]	4.37 ± 0.01	2940	4.96 ± 0.09	68950	220	207
CM	7.53 ± 0.01	22500	4.12 ± 0.32	43450	3956 ± 372	3741 ± 350
Fresh CM	7.6 ± 0.01	21890	4.25 ± 0.41	67625	5805 ± 784	5490 ± 742
WWS	6.84 ± 0.01	947	2.77 ± 0.29	51200	495 ± 13	468 ± 12
Fresh WWS	7.48	3490	2.00 ± 0.19	34400	1066 ± 24	1009 ± 23

[a] CW from diary Murska Sobota and Ljutomer [28] were mixed in 1:1 ratio (volume based).

čistilna naprava Domžale-Kamnik d.o.o, https://ccn-domzale.si/index.php/sl/) [26,27] (b) CW from a diary in northeastern Slovenia (Pomurske mlekarne d.d., https://www.pomurske-mlekarne.si/en/) [28] (c) CM from a local cattle farm in central Slovenia (local farmer in Ljubljana, Bizoviška cesta, 1000 Ljubljana) The average physicochemical parameters of the inocula and substrates are listed in Table 1. A comparative analysis of the physicochemical properties of the CW used in our study with the CW used in 29 previous studies was performed (Table 2).

2.2. Experimental setup: semi-continuous experiment

The Automatic Methane Potential Test unit (AMPTS® II; Bioprocess Control ®, Sweden), upgraded to a 5-L size [11,53,54], was used for the semi-continuous experiment as previously described [11,53,54], where the methane volume is determined after the removal of acid gases such as carbon dioxide (CO_2) and hydrogen sulfide (H_2S) by passing the gas through a sodium hydroxide (NaOH) solution. This process fixes CO_2 and H_2S, allowing for an approximation of the methane (CH_4) volume based on the remaining gas volume. NaOH fixation does not account for the presence of other non-methane gases, such as hydrogen (H_2), which might be present in the biogas. These gases are not removed by NaOH and can lead to inaccuracies in methane measurement.

A set of 15 semi-continuous reactors was designed and run according to the protocol shown in Table 3. Reactors I to VII were performed in duplicate. In each reactor, 3 L of inoculum was mixed with 70 mL of WWS and the appropriate amounts of CW. Two distinct batches of CW from diary Murska Sobota and Ljutomer [28] were mixed in 1:1 ratio (volume based) and used. Reactors I received only WWS as a control. Reactors II, III, and IV received CW in addition to reactors I in different volumes: 20, 35, and 55 mL, respectively. In reactors V, VI and VII, an additional 10 mL of CM was added. Finally, in reactor VIII, 35 mL of CW and 20 mL of CM and WWS were added (Table 3). Throughout the experiment, each reactor initially received an 80 mL aliquot of water. From the experiment's inception until day 19, CM was exclusively utilized as the substrate. Subsequently, on day 20, we introduced a fresh batch of CM (collected from diary that day). Following day 47, we introduced fresh samples of WWS (collected from WWTP that day) into the reactors. Starting from day 50 onwards, the quantity of CW added to the reactors was doubled, while the volume of water added was

Table 2
Physical-chemical characteristics of cheese whey used in previous researches (n = 29). (TS – total solids, VS – volatile solids, COD – chemical oxygen demand, NH_4^+-N – ammonium nitrogen, EC – electrical conductivity).

Reference	TS (%)	VS (%)	COD (mg O_2/L)	EC (mS/cm)	NH_4^+-N (mg/L)	pH
Ghaly [6]	6.593	4.728	72220		260	4.5
			70150		78	
Malaspina et al. [29]			55434		64.31	
			68814			
Rico et al. [30]	5.51	4.78	57500		200	
Stamatelatou et al. [31]			54400	12.47		
Saddoud et al. [7]	5.93	5.61	68600			4.9
Dareioti in Kornaros [12]	7.233	6.24	93210		110	6.12
Najafpour et al. [32]	5.5	4.9	60000			5.5
						6.6
Comino et al. [33]			74400		78	4.12
Kavacik in Topaloglu [34]	5.9	4.22				6.6
Carlini et al. [35]	5.88	5.79	65000			6
Antonopoulou et al. [36]	6.77	6.27	61000			6
Kacprzak et al. [37]	6.1	5.26	66700			4.48
Gannoun et al. [38]	5.9		60000	7.6		4.46
Dareioti and Kornaros [39]	6.896	5.785	75000		100	5.69
Powell et al. [40]			71000			4.5
Shilton et al. [41]			71000			3
						4.5
Bertin et al. [42]	5.78	5.28	58500			5
Hublin in Zelić [43]	4.69	4.26	47950			3.53
Gelegenis et al. [44]	7.8	4.8	74900		60	3.5
Brown et al. [8]			72900			4.54
Maragkaki et al. [45]	6.14	4.73	6600			4.4
Fernández et al. [46]	3.31	3.01	38000		26	
Ebrahimi et al. [47]	5.5		50000			6
	6.5		70000			6.5
Ergüder et al. [48]			55250			3.44
			74500			3.92
Yang et al. [49]	6.244		71410		161	5.92
Najafpour et al. [50]	5.5	4.9	50000			5.5
	6.5		70000			6.6
Maragkaki et al. [10]	4.41	3.54	61800			3.9
Maragkaki et al. [51]	6.1	4.9	66000			5.2
Venetsaneas et al. [52]	8.69	8.09	60500			6.23
Average	6.06	5.11	62810	10.04	113.73	5.04
SD	1.08	1.10	14495	3.44	72.15	1.07

Table 3
Design of semi-continuous co-digestion experiment.

Reactor	Description	Inoculum (mL)	Sludge (WWS) (mL)	Cheese whey (CW) (mL)	Cow manure (CM) (mL)	Water (mL)	Volume replaced (mL)	WWS:CW:CM ratio[c]	HRT (d)	OLR (g COD/L) day 0–19	day 20–46[a]	day 47–49[b]	day 50–65[c]
WWS = I	Inoculum + wastewater sludge	3000	70			80	150	70:0:0	21.0	3.6	3.6	2.4	2.4
WWS + CW = II	Inoculum + Wastewater sludge + Cheese whey	3000	70	20		80	170	70:20:0	18.6	5.2	5.2	4	5.6
WWS + CW= III	Inoculum + Wastewater sludge + Cheese whey	3000	70	35		80	185	70:35:0	17.2	6.4	6.4	5.2	8
WWS + CW = IV	Inoculum + Wastewater sludge + Cheese whey	3000	70	55		80	205	70:55:0	15.6	8	8	6.8	11.2
WWS + CW + CM = V	Inoculum + Wastewater sludge + Cheese whey + Cow manure	3000	70	20	10	80	180	70:20:10	17.7	5.6	5.9	4.7	6.3
WWS + CW + CM = VI	Inoculum + Wastewater sludge + Cheese whey + Cow manure	3000	70	35	10	80	195	70:35:10	16.4	6.8	7.1	5.9	8.7
WWS + CW + CM = VII	Inoculum + Wastewater sludge + Cheese whey + Cow manure	3000	70	55	10	80	215	70:55:10	15.0	8.4	8.7	7.5	11.9
WWS + CW + CM = VIII	Inoculum + Wastewater sludge + Cheese whey + Cow manure	3000	70	35	20	80	205	70:35:20	15.6	7.3	7.7	6.5	9.3

[a] *fresh batch of CM was introduced.*
[b] *fresh batch of WWS was introduced.*
[c] *the volume of cheese whey was doubled by reducing respectively the volume of water used.*

correspondingly reduced to maintain the overall liquid volume. The reactors were kept under mesophilic conditions at 38 °C in a water bath and stirred by mechanical stirring with a duty cycle of 1 min and a 5-min regime. The experiment was run for 65 days. Every 24 h, a portion of the digestion medium was replaced with the predetermined amount of fresh feed. Reactors were not fed on weekends and holidays in accordance with WWTP operation procedure to account for the periodic feeding regime and the effect on process stability and modeling. Methane production was measured hourly. The corresponding hydraulic residence time (HRT) of reactors was calculated by dividing the total working volume of the reactor by the volume of the fresh feed replaced daily. The experiment involved recording 1566 data points over a period of 65 days for each reactor, with each data point representing the volume of methane produced within a 1-h interval (Fig. S1) as measured by AMPTS®.

2.3. Physico-chemical analysis

Total solids (TS) were determined according to APHA [55]. Electrical conductivity (EC) and pH were measured using the HQ40D portable multimeter (Hach Lange ®). Alkalinity (TIC - total inorganic carbon) and volatile organic acids (VOA) were measured using the Titralab AT1000 Series automatic titrator (Hach®) according to the manufacturer's instructions. Ammonia nitrogen (NH_4^+-N) was determined by the Nessler method. Physical and chemical analyzes were performed weekly. Chemical oxygen demand (COD) of the samples from the digestion mixture was measured in a miniaturized 96-well format assay using Agilent GC 2.5-mL vials and Teflon Septa caps with wavelength spectrometry (BIOTEK ELx808, Bio Tek Instruments, USA) (630 nm). Calibration curves of 1 g/L glucose were prepared for calculation of COD [56].

For volatile fatty acids (VFA) analyses a gas chromatograph HP 6890 Series GC System equipped with capillary column Agilent J & W GC columns DB-FFAP, 30 m × 0.530 mm × 1 μm layer of stationary phase was used. The temperature injector temperature and detector temperature (FIS - flame ionizing) were 200 ∘C and 300 ∘C, respectively. Initial oven temperature was 70 ∘C with a residence time of 1 min, then ramped at 20 ∘C min^{-1} to 120 ∘C and then 10 ∘C min^{-1} to a final oven temperature of 200 ∘C with residence time of 3 min. Carrier gas (mobile phase) was helium with flow 5 mL min−1, nitrogen flow 25 mL min^{-1}, detector gas was hydrogen at a flow rate of 30 mL min^{-1}, synthetic air at a flow rate of 400 mL min^{-1}. Ether extraction was used to prepare VFA for analyses as described before [53,54,57]. VFA analysis was performed on samples collected on days 1, 6, 13, 21, 26, 35, 42, 49, 57 and 64.

2.4. Trace elements content by X-ray fluorescence spectrometry (XRF)

Samples from the digestion mixture were air dried at 60 °C for 24h, homogenized, and 100 mg of subsamples were pressed using a pellet die and a hydraulic press. Elemental analysis was performed by X-ray fluorescence spectrometry and focused on the following elements: P, S, Cl, K, Ca, Ti, Cr, Mn, Fe, Ni, Cu, Zn, Pb, Br, Sr, and Zr. An XRF spectrometer with Rh anode (35 kV) and 5 mm beam was used to irradiate the samples. The XRF signal was detected using a silicon drift diode (SDD) (Amptek) [58]. Spectra were analyzed using LabView and quantitative analysis was performed as previously described [59]. The dark matrix (the non-responsive elements) was determined using the emission-transmission method [60]. Quality assurance for the element analysis was performed using standard reference materials: NIST SRM 1573a (tomato leaves, homogenized powder); CRM 129 (hay powder); and OU-10 (geological sample of Longmyndian greywacke, GeoPT24). Trace element content analysis was performed on samples collected on days 1, 26, 49, and 64.

2.5. Multivariate statistical analysis

Relationships between data strings of multivariate data set from the experiment (e.g., COD, VOA/TIC, pH, EC …) and methane production rates were log-transformed to determine the similarity/dissimilarity of reactors fed with mixtures of substrates, using NonMetric MultiDimensional Scaling (NM-MDS) trait space and convex-hulls in PAST software (https://www.nhm.uio.no/english/research/resources/past/) [61,62].

Statistical analyses of selected trace elements were done, using JMP ® 16.0.0 software (SAS Institute Inc., 2022). Multivariate correlations using Pearson method served as correlation of different metals and daily methane production. The confidence interval was

Table 4
Alternative models resulted from model 1 by restricting the kinetic parameters. (i. inoculum, wastewater sludge, cheese whey, cow manure).

Model	Equations	Kinetic parameters	COD fractions	Yield coefficients
Model 1	$A \xrightarrow{k_f} P$ $B \xrightarrow{k_s} C \xrightarrow{k_{int}} P$	$k_f \neq 0$ $k_s \neq 0$ $k_{int} \neq 0$	$0 < f_{easy,i} < 1\ f_{hard,i} = 1 - f_{easy,i}$	$Y_{P/A}$ $Y_{C/B}$ $Y_{P/C}$
Model 2	$A \xrightarrow{k_f} P$	$k_f \neq 0 k_s = k_{int} = 0$	$0 < f_{easy,i} \leq 1$	$Y_{P/A}$
Model 3	$A \xrightarrow{k_f} P$ $B \xrightarrow{k_s} C$	$k_f > k_s \gg k_{int} \approx 0$	$0 < f_{easy,i} < 1\ f_{hard,i} = 1 - f_{easy,i}$	$Y_{P/A}$ $Y_{C/B}$
Model 4	$A \xrightarrow{k_f} P$ $B \xrightarrow{k_s} P$	$k_f > k_{int} \gg k_s$	$0 < f_{easy,i} < 1\ f_{hard,i} = 1 - f_{easy,i}$	$Y_{P/A}$ $Y_{P/B}$
Model 5	$B \xrightarrow{k_s} C \xrightarrow{k_{int}} P$	$k_f = 0$ $k_s \neq 0$ $k_{int} \neq 0$	$0 < f_{easy,i} < 1\ f_{hard,i} = 1 - f_{easy,i}$	$Y_{C/B}$ $Y_{P/C}$

plotted at a significance level of 0.05.

2.6. Model development

A simple kinetic model, which is based on the conversion of COD to methane, was developed. The COD of each reactor originates from inoculum and different mixtures of substrates (WWS, CW and CM) as shown in Table 3. The model assumes that COD from each source (denoted by the index i) can be split in two fractions: (a) a digestible fraction ($f_{easy,\ i}$) which is converted fast and easily within the first hours to biogas and (b) the rest of COD fraction ($f_{hard,\ i} = 1\text{-}f_{easy,I}$) which is converted slowly through the production of intermediates to biogas. Fast digestible fractions of COD from all sources are combined in one entity. Similarly, poorly digestible fractions are combined in another entity. Based on these assumptions a kinetic model (Table 4) was suggested. The model consists of the following schematic reactions:

$$\underset{\text{fast digestible fraction of COD}}{A} \overset{k_{fast}}{\rightarrow} \underset{\text{methane}}{P} \tag{1}$$

$$\underset{\text{slowly digestible fraction of COD}}{B} \overset{k_{slow}}{\rightarrow} \underset{\text{intermediates}}{C} \overset{k_{inter}}{\rightarrow} \underset{\text{methane}}{P} \tag{2}$$

where A and B represent the fast and slowly digestible fractions of COD, C and P represent the intermediate compounds formed and the methane produced respectively. k_{fast}, k_{slow} and k_{int} correspond to the kinetic coefficients of the reactions. The yield coefficients for the schematic reactions (1) and (2) are denoted by the parameters $Y_{P/A}$, $Y_{C/B}$ and $Y_{P/C}$ correspondingly. $Y_{P/A}$ is expressed in mL of methane produced per mg of COD/L removed from feedstock attributed to the fast digestible fraction of COD. $Y_{P/C}$ is the yield coefficient expressed in mL of methane produced per mg of COD/L consumed from the intermediate compounds formed, while $Y_{C/B}$ is the yield coefficient in mg of COD/L of intermediates produced per mg COD of slowly digestible COD consumed. Assuming first order reaction rates the kinetic equations for reactions (1) and (2) can be written as:

$$r_A = \frac{d[A]}{dt} = -k_f[A] \tag{3}$$

$$r_B = \frac{d[B]}{dt} = -k_s[B] \tag{4}$$

$$r_C = \frac{d[C]}{dt} = k_s[B] - k_{int}[C] \tag{5}$$

$$r_P = \frac{d[P]}{dt} = Y_{P/A}\, r_A + Y_{P/C} Y_{C/B}\, r_C = Y_{P/A}\, r_A + Y_{P/B} r_C \tag{6}$$

where $Y_{P/B}$ is the combined yield of equation (2) calculated by the product $Y_{C/B}$ $Y_{P/C}$. It should be noted that [A], [B] and [C] denote the concentration of A, B and C in mg COD/L of liquid phase in the reactor. However, for methane which is released in the gas phase, [P] corresponds to the mL of methane collected per unit volume of liquid phase (V). The AMPTS® II instrument measured the volume of methane produced and collected on an hourly basis, with the value being reset to zero before the next measurement. For A, B and C their concentration are directly related with equations (3)–(5), while the recorded volumes of methane by AMTPS corresponds to the product $V\, d[P]/dt$ expressed in mL/hour calculated from equation (6). Assumption is that the rate of methane production $d[P]/\,dt$ can be reasonably approximated by the average hourly production rate $\Delta[P]/\Delta t$ recorded on hourly basis. This assumption is valid when methane production rates do not vary considerably within the time interval of 1 h.

It is significant to note that by applying different restrictions on the kinetic parameters of model 1, a set of simpler models result (model 2 to model 5 in Table 4). Model 2 assumes that part of freshly introduced COD is converted directly to methane whereas there is some recalcitrant COD which remains undigested. In model 3 the conversion of intermediates to methane is very slow and practically the reaction stops without methane production from this reaction. Model 4 assumes that slowly degradable COD is converted directly to methane in one stage without intermediate production. Finally, model 5 assumes that only part of COD is slowly degraded to methane through the production of intermediates. For the experimental protocol, see S2 – Modeling.

The model was implemented and solved in Mathematica (Wolfram Research, Inc., Mathematica, Champaign, IL, 2017). The WhenEvent option was used in NDSolve function to take into account the periodic replacement of the substrate.

3. Results and discussion

3.1. Performance of semi-continuous digesters; methane production, HRT and codigestion

Anaerobic co-digestion of WWS, CW and CM was conducted in semi-continuous experiments to quantify the effect of CW and CM addition on methane yield and production and anaerobic digestion process stability (Figs. 1 and 2). The composition of CW can exhibit variability based on several factors, including the specific cheese type, the source of milk, and the processing techniques employed in cheese production. The physical-chemical properties of CW used (mixture of CW from Murska Sobota and Ljutomer) are in accordance with those in Table 2. The measured properties of cow manure in our study are in line with literature studies (Table S1). HRT values from 15 to 21 days were achieved, which HRT are typical for anaerobic digestion units. The physical and chemical attributes of wastewater sludge and the inoculum remained within acceptable parameters and closely mirrored those of the inocula present in the online methane yield database [63].

To compare the methane production between reactors, we have analyzed daily methane production and a cumulative methane production per organic COD loading for the individual reactors. When introducing only WWS into the experimental setup (reactor I) we observed a significant decrease in average daily methane production. Initially, from 55 mL CH_4/g COD/day to 5–10 mL CH_4/g COD/day, with an average daily production of just WWS 7.5 mL CH_4/g COD/day. The methane production failure became most evident during the latter days of the semi-continuous experiment. The mono-digestion of WWS frequently encounters challenges owing to its limited organic matter content and a low C/N ratio. This renders it highly susceptible to variations in process conditions. A study by Catenacci et al. [64] underscored these difficulties, as they found methane production from WWS to be consistently meager, amounting to a mere 34 mL CH_4/g COD. Their findings shed light on the complexity of predicting methane production based solely on fundamental parameters like total and soluble COD, VS, and TS.

As an extension to methane production from WWS only, co-digestion emerges as a promising approach to augment process efficiency and performance [65]. Incorporating CM alongside CW is a common practice to potentially enhance methane yields, avoid instability, provided that the sludge possesses adequate stabilization capabilities [8,66]. In this context reactors II to VIII received different mixtures. CW, rich in COD, was introduced as a co-substrate to WWS in reactors II to IV. To challenge the stability of reactors V-VIII and to improve the methane production from CW our semi-continuous experiment, CM was added daily to reactors (Table 3).

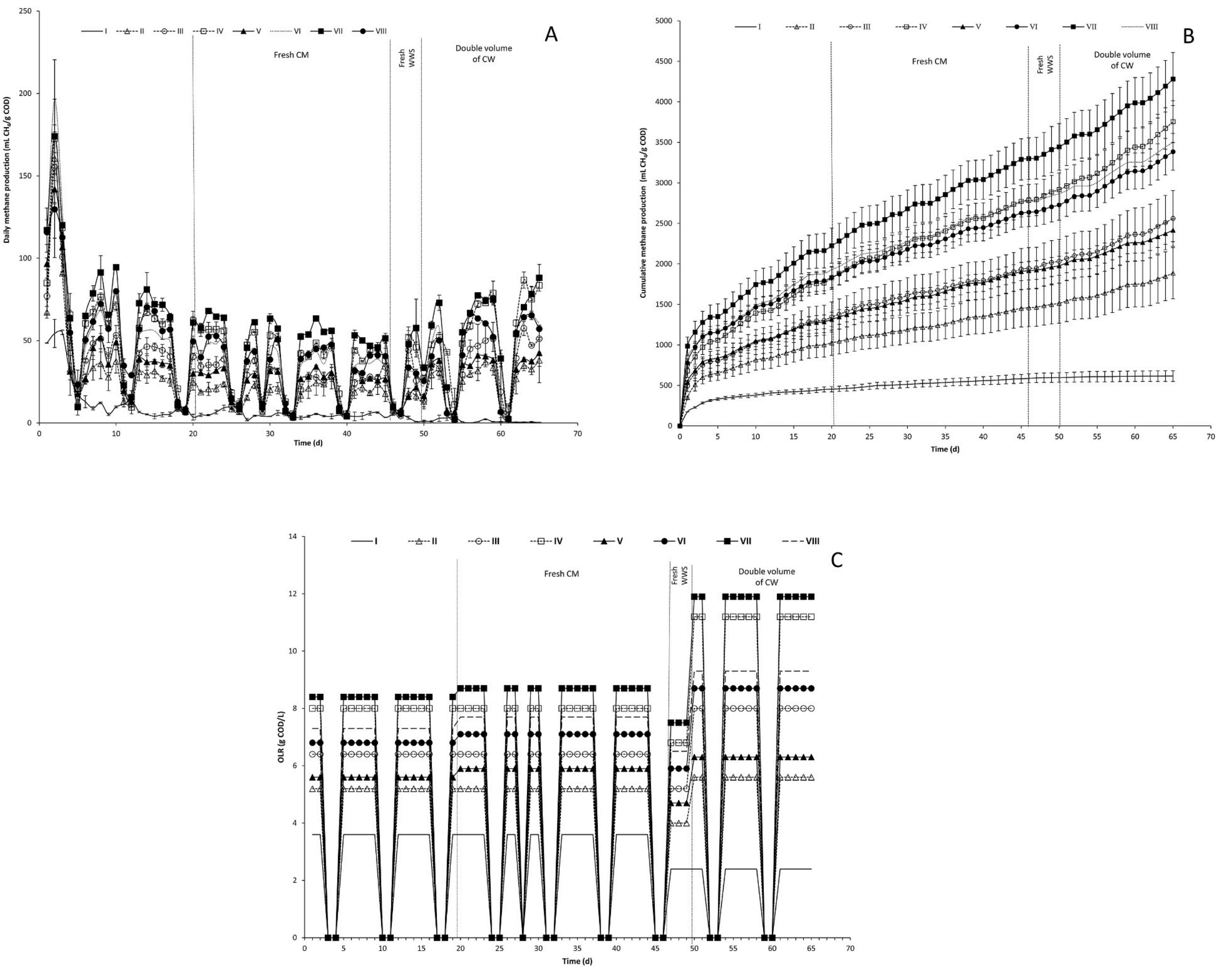

Fig. 1. Daily methane yield in semi-continuous experiment (A). Cumulative volumes of methane production (B). Organic loadings rates (OLR) during the 65 days semi-continuous co-digestion experiment (C).

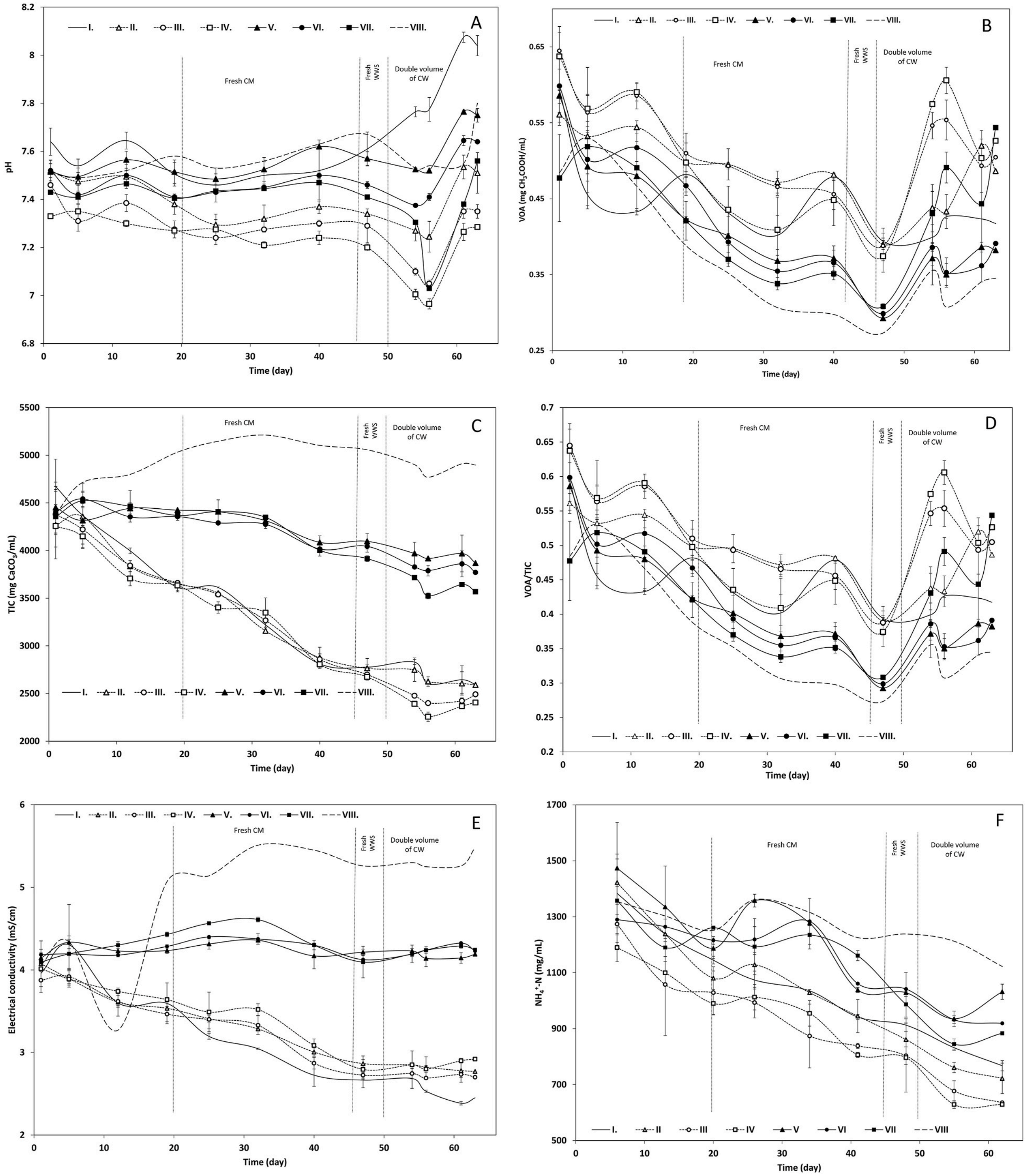

Fig. 2. Average pH (A), average volatile organic acids (VOA) (B), total inorganic carbon (TIC) (C), VOA/TIC ratio (D), electrical conductivity (EC) (E) and ammonium nitrogen (NH_4^+-N) (f) in the 65 days semi-continuous assay during. WWS (full line), co-digestion of WWS + CW (open symbols, dashed line) and WWS + CW + CM (full symbols. full line). Experiment VIII was performed without replicate and with double concentration of CM (long dashed line), (triangle – 20 mL of CW, circle – 35 mL of CW, square – 55 mL of CW).

This strategic co-digestion resulted in a substantial enhancement of methane production in reactors II, III, and IV, by 228 %, 339 %, and 546 %, respectively. Reactor IV, with the highest CW input, achieved the highest average daily methane production of 48.6 mL CH_4/g COD/day.

Furthermore, Maragkaki et al. [10] also observed improved biogas production from WWS through the addition of CW only, achieving nearly an 89 % increase. Shilton et al. [67] reported a remarkable up to 208 % enhancement in biogas production through CW supplementation, with production ranging from 246 to 356 mL/g COD, albeit without specifying methane content. In another study by Maragkaki et al. [45], the addition of 5 % CW to sewage sludge resulted in an impressive 86 % increase in biogas production, yielding 99.4 L of biogas per day at a hydraulic retention time (HRT) of 24 days.

A comparison of daily methane production per gram of COD across different mixtures during the 65-day semi-continuous experiment revealed significant differences (NP-MANOVA; $p < 0.05$). Beginning on day 50, reactors V-VIII received double the volume of CW, leading to an increase in daily methane yield, as illustrated in Fig. 1. This analysis underscored the substantial disparity in daily methane yield per gram of COD between reactors that received only WWS and those supplemented with CW and CM (NP-MANOVA; $p < 0.05$; $F = 43.58$, total sum of squares = 25.6472, within-group sum of squares = 8.14616). Comparing the average daily methane yield of reactors V-VIII to reactors II-IV in our study, there was a notable improvement of 22.9 %, 26.8 %, 8.1 %, and 2 %, respectively (see Table S2). The highest average daily methane yield, reaching 52.5 mL CH_4/g COD, was recorded in reactor VII. The best cumulative methane production per gram COD was measured for reactors VII (4280.83 mL/g COD), that was 12.3 %, 18.3 %, 20.9 % more than reactors IV, VII, VI, respectively. Other reactors produced more than 40 % less cumulative methane.

Finally, the volume of CW and periodic feeding regime did not cause failure of the process and confirmed that the co-digestion at these proportions in periodic feeding regime could be transferred to a full scale WWTP anaerobic digester. However, caution is needed in the dosing of CW, as an overabundance can result in process failure [8]. Furthermore, experiments involving mixtures of primary sludge, casein whey, and cow manure [8] resulted in operational challenges at the organic loading rate (OLR) exceeding 4.39 g COD/L/day. In contrast the reactors V-VIII in our experiments were in evolution at OLR twice as high, up to 8.7 g COD/L/day, utilizing periodic feeding regime and were in short term dosage able to sustain methane production and conditional stability regarding VOA/TIC ratios.

3.2. Process parameters during the semi-continuous anaerobic digestion of different mixtures

To quantify the effect of CW and CM addition on anaerobic digestion process stability (Fig. 2) different chemical parameters were monitored. The optimal pH range for achieving maximum methane production typically falls between 6.6 and 7.9 [68]. In our study reactor I, which exclusively received WWS, exhibited a pH increase from 7.5 to 8.1 (Fig. 2A). This rise in pH was likely attributed to the absence of CW addition, which has a notable acidifying effect, and the absence of an accumulation of short-chain fatty acids (SCFAs). In contrast, the pH values in reactors II-IV, which received higher amounts of CW, were lower, ranging from 7.2 to 7.5. Reactors V-VIII, on the other hand, had pH values ranging from 7.4 to 7.6. The introduction of CW, known for its lower pH, played a significant role in reducing the overall pH of the digestion mixture. Moreover, when the volume of CW was doubled, it led to further declines in pH, with decreases of 0.1–0.2 in reactors II, III, and IV, and 0.1 to 0.4 in reactors V, VI, VII, and VIII. This decline in pH is attributed to the slower consumption of short-chain fatty acids (SCFAs) by methanogenic Archaea, and the accelerated production of SCFAs (acetic, propionic, butyric) by anaerobic bacteria, resulting in increase of SCFA concentrations as also in the study of Prazeres et al. [2].

CM offers valuable attributes such as high TIC, essential microorganisms, TE, and vital nutrients crucial for the anaerobic digestion process [69]. For stable anaerobic digestion regarding OLR, it is generally recommended to maintain alkalinity within the range of 1000–5000 mg $CaCO_3$/L [70]. In our experiment, TIC values were initially robust, ranging from 4000 to 5000 mg $CaCO_3$/L. However, these concentrations decreased in all reactors (never below 2200 mg/l). Exception was reactor VIII, where a volume of CM maintained TIC between 4500 and 5150 mg/l, and had a notable impact on maintaining the TIC values within the reactors VIII (Fig. 2C). This stability in TIC provided adequate alkalinity, effectively averting the risk of reactor acidification. These findings suggest that CW primarily serves as the principal source of SCFAs, and the sufficient addition of CM likely contributes to the heightened methane yields observed in co-digestion experiments. Furthermore, there was a discernible surge in VOA concentrations after the 50th day of operation, coinciding with a decrease in pH during that period (Fig. 2B). Prior to this, VOA concentrations had been steadily declining. However, the increase in VOA during the last days of experiment could lead to system inhibition due to overloading. To gauge short term process evolution VOA/TIC ratio was monitored.

Ideally, VOA/TIC ratios should fall within the range of 0.3–0.4 [12,71]. Reactors supplemented with CM successfully attained the optimal VOA/TIC ratio range within 19 days of startup (see Fig. 2D). Conversely, reactors devoid of CM exhibited elevated (around 0.5) VOA/TIC ratios attributable to reduced alkalinity (TIC), potentially predisposing the system to process instability. Nevertheless, it is noteworthy that even when the VOA/TIC ratio deviated from the optimum range, no overt signs of instability, such as decreased methane yields, were observed. Notably, reactor IV demonstrated the second-highest cumulative methane production per gram COD, highlighting the pivotal role of CM as the primary alkalinity source in the digestion mixture during the co-digestion of CW, CM, and WWS (as evidenced in reactors VII). The addition of CM not only enhanced methane production but also ensured stable evolution of reactor operation at high OLR up to 7.5 g COD/L which is 35 % higher OLR compared to study of Brown et al. [8]. However, the subsequent doubling of CW volumes in reactors V to VIII (after day 50) led to increased and fluctuating VOA/TIC in reactors V-VII, predisposing the system to long-term process instability, inhibition, or even failure. Reactor VIII that received the highest volume of CM remained in the optimal ratio, which further emphases the important role of the addition of sufficient CM at even elevated OLR of 9.3 g COD/L, however additional analysis of VFA indicated inhibition at day 64. The study of Prazeres et al. [12] faced the destabilization and failure of the system when VOA/TIC ratio was 2.32 and when the HRT was shortened to 12 days.

Measurements of propionic acids in our reactors show that concentrations did not exceed 900 mg/l by day 57, which according to a study [72] may be a first indication of inhibition. In our case, acetic acid concentrations up to day 57 were below the inhibition limit of 1600 mg/l [73]. On day 64, the concentrations of both of these acids increased significantly above the recommended values in inhibition concentrations. Acetic acid reached a maximum concentration of 2050 mg/l in reactor V and propionic acid reached a maximum concentration of 7200 mg/l in reactor II. In reactor IV, the concentration of propionic acid was the lowest, 920 mg/l, while the concentration of acetic acid was 250 mg/l. Reactor IV was the least inhibited in terms of acid concentrations, but the high ratio of propionic to acetic acid (3.9) in reactor IV indicates that propionic acid is present in a relatively high proportion, which may further contribute to the inhibition. In reactors V to VII, propionic acid concentrations ranged between 1600 and 3400 mg/l. This indicates inhibition of the process. In all cases (except reactors VII and VIII), the ratio of propionic to acetic acid was greater than 1.4 at 64 days, indicating inhibition [74] and instability of the reactors.

[72–74]The fact that not all measured parameters contain valuable information was mirrored by the fact that EC values (Fig. 2E) in all reactors remained well below the inhibitory threshold of 35 mS/cm [75] and that concentrations of ammonium nitrogen (NH_4^+-N) exhibited a consistent decline in all reactors, starting from approximately 1100-1400 mg/L on day 6 and decreasing to 600–1000 mg/L by day 62. Notably, there were no significant alterations in NH_4^+-N concentrations even after doubling the volume of cheese whey (CW). Reactors with the addition of CM displayed higher NH_4^+-N concentrations, surpassing 200 mg/L (Fig. 2F), showcasing the importance of external nitrogen supplementation. Maintaining an appropriate C:N ratio, typically in the range of 20–30:1, is crucial for enhancing methane production during anaerobic co-digestion by balancing the nutrient composition. Co-digestion of dairy manure, chicken manure, and wheat straw at a C:N ratio of 27.2:1 achieved the maximum methane potential after optimization [76]. At low C:N ratios (e.g. 15–20:1), higher proportions of cheese whey beyond 50 % can lead to process instability and reduced efficiency, likely

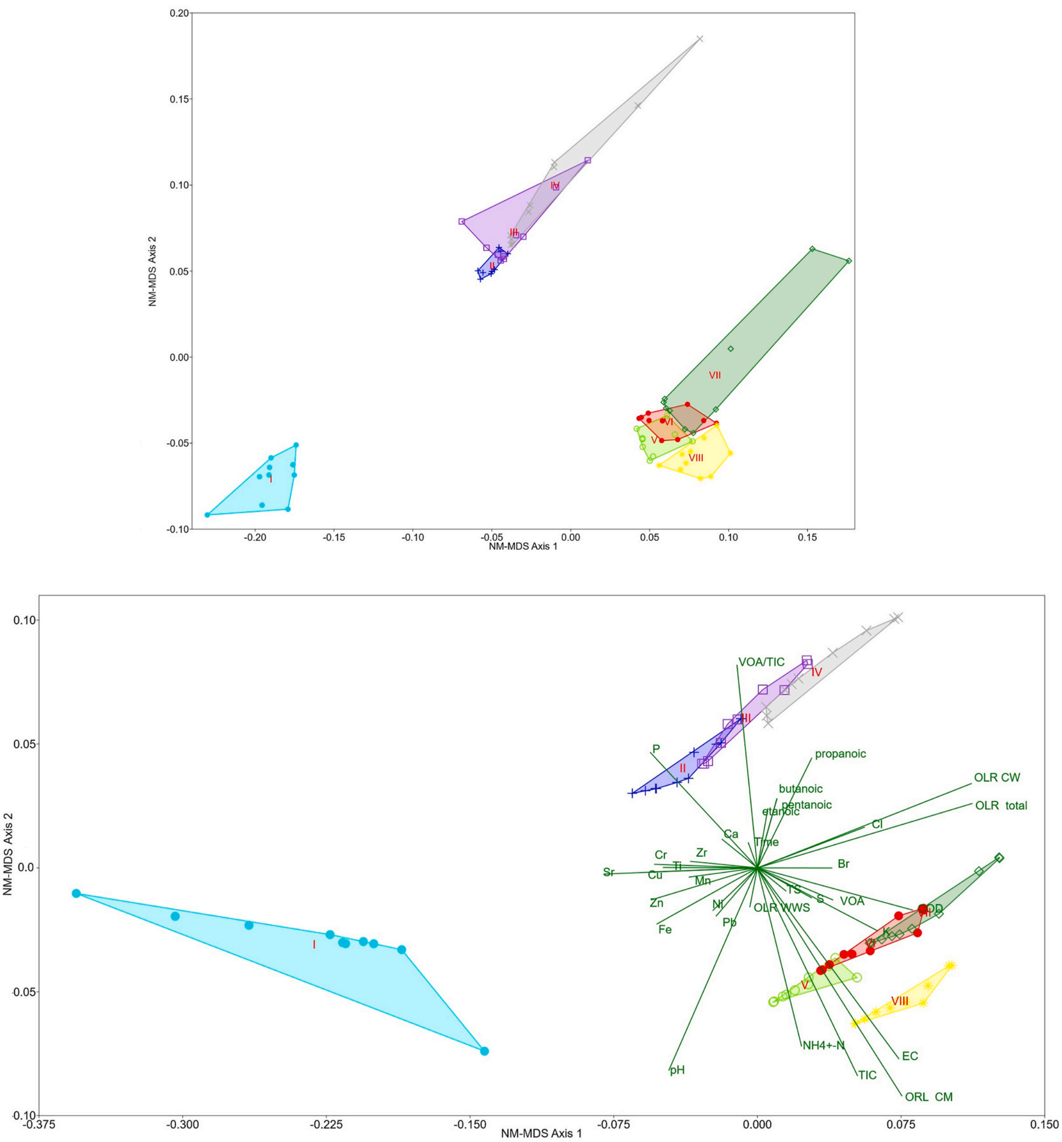

Fig. 3. (A) A Comparative Analysis of physical-chemical parameters and methane production in semi-continuous reactors, presented as overlaps in the NM-MDS Trait Space. (B) An ordination depicting the relationship between physical-chemical parameters and methane production during the 1st, 4th, 7^{th} and 9^{th} week.

due to high levels of ammonia and free ammonia that can inhibit the anaerobic digestion process and reduce methane production [77].

Changes in TS and COD are given and discussed in supplementary material (Fig. S2), next to comprehensive overview of the content of 16 different trace elements (Table S3) on four key dates: the 1st, 26th, 49th, and 64th days of the experiment. Notably, there was a consistent increase in the concentrations of potassium (K), chlorine (Cl), and bromine (Br) across all reactors in our study. However, concentrations of certain TE, such as calcium (Ca), sulfur (S), phosphorus (P), titanium (Ti), chromium (Cr), lead (Pb), and zirconium (Zr), exhibited notable fluctuations throughout the course of the experiment. In contrast, TE including iron (Fe), zinc (Zn), manganese (Mn), and copper (Cu) displayed a declining trend in the digestive mixtures of reactors II-VIII. This decline persisted despite the increased volumes of CW and CM added to the mixtures, highlighting the challenge of maintaining their concentrations in the reactors despite versatile feeding regimes, utilized in this study.

To further evaluate the distribution of these decreasing TE, normal quantile plots were generated using the Shapiro-Wilk test. It was determined that the measured data for Fe, Cu, and Pb did not conform to a normal distribution ($p < 0.05$), as indicated in Table S4.

Notably, nickel (Ni) concentrations in our case ranged from 12 to 39.2 mg/kg TS^{-1}, falling below the reported values [16]. However, concentrations of Zn, Pb, and Fe in this experiment were notably higher, ranging from 999 to 2570 mg/kg TS^{-1}, 62.4–162 mg/kg TS^{-1}, and 11700–34300 mg/kg TS^{-1}, respectively, in comparison to the values reported in the same study. Nevertheless, it's essential to note that the order of magnitude for these metal concentrations remains consistent with the range observed in prior investigations [16,78]. While metal concentration can be considered a variable influencing methanogenic activity and, subsequently, methane production rates, its impact as observed in this study was relatively modest [16,79].

A multivariate analysis examining the relationship between selected TE and daily methane production data from all reactors revealed no significant Pearson's correlation between Zn and Cu ($p = 0.29$) and daily methane production with the selected TE (see Table S5, Table S6, Fig. S3). Surprisingly, while positive correlations were observed among the TE themselves, daily methane production exhibited negative correlations with all selected TE. Among these relationships, the strongest linear Pearson's correlations ($p < 0.01$) were identified between Fe and Zn ($r = 0.91$), lead (Pb) and Zn ($r = 0.91$), Zn and Mn ($r = 0.83$), and Cu and Zn ($r = 0.85$).

Forward linear projection regression analysis was employed in our case to determine the critical time for potential process inhibition due to the depletion of TE, which would fall below recommended values in all reactors [80–82]: Fe (day 110–165), Zn (day 100–150), Mn (day 95–245), Cu (day 80–160). Consequently, it is advisable to consider supplementing the digestive mixture with TE to prolong the sustainability of the process [83].

In addition to forward projection, a NM-MDS analysis was conducted to assess the physical-chemical parameters across all reactors to unveil significant overlaps in the measured physical-chemical parameters among the various substrate mixtures. Reactors that received identical substrates exhibited overlapping convex hulls, leading to the identification of three distinct groups (Fig. 3A): Group one exclusively received WWS (reactor I); Group two comprised reactors that received both WWS and CW (reactors II-IV) and Group three encompassed reactors that received WWS, CW, and CM (reactors V-VIII). The Stress value for this analysis was calculated as 0.1498, with R^2 values of 0.7775 for the x-axis and 0.3346 for the y-axis. This distribution of reactor characteristics according to nature of substrates can be attributed to the inherent chemical composition of the substrate mixtures, resulting in divergent responses in the metabolic traits of microbial communities.

To assess the influence of physical-chemical parameters on methane production, we performed NM-MDS of these parameters in relation to methane production by week within the same dataset. The Stress value for this analysis was calculated as 0.06436, with R^2 values of 0.8701 for the x-axis and 0.06357 for the y-axis. The correlation coefficient between each environmental variable and the NM-MDS scores is presented as vectors [61]. The relative length and direction of these vectors indicate the influence of each factor. Fig. 3B illustrates that nearly all variations in the measured parameters affect methane production. Notably, factors such as NH_4^+-N and TIC play a crucial role in our case, as the NM-MDS analysis demonstrates their strong correlation. The increased concentrations of NH_4^+-N, primarily found in the cow manure OLR, lead to higher alkalinity in the reactor. Phosphorous (P) as one of the most important macronutrients for anaerobic microorganisms also played role in methane production of reactors II-IV in this study. In general, phosphorus enters anaerobic digesters primarily through the organic feedstock, such as animal manure, WWS, but also through its high abundance in milk and dairy products, including CW, where it can be found in soluble and particulate forms, depending on the specific processing and composition of the CW. Phosphates can affect the concentration and bioavailability of other cations and TE [84–88] and consequently affect the methane production.

The OLR of CW also exerted an influence on methane production, evident in reactors II-IV and reactors V-VIII, where a mixture of WWS and CW was added. Trace elements, while slightly less influential, still contribute, especially in reactors that received only WWS or CW + WWS. The Br concentration tends to be closer to reactors V-VIII.

Furthermore, elevated SCVFA factors are associated with reactors II-IV, which did not receive additional CM. Both pH and EC emerge as significant influencers of methane production, primarily in reactors V-VIII, largely due to the substantially higher salt content associated with CM compared to the other added substrates. Another noteworthy factor affecting methane production in reactors II-IV is the VOA/TIC ratio. Insufficient alkalinity within substrates can trigger acidification, resulting in a decline in pH levels. This, in turn, sets the stage for process inhibition and, in the most severe cases, potential failure of the system [12,30].

The WWTP Domžale-Kamnik produces about 2700 m_N^3 of biogas per day, of which about 62 % is methane. We recommend to supplement anaerobic reactors in the ratio WWS:CW:CM = 1.27:1:0 or WWS:CW:CM = 1.27:1:0.18, depending on the availability of CM and volume of CW. According to our results, the addition of CW to the anaerobic digester should be either short term, or with periodic dosage (as tested in this study). Further investigations are needed for the long-term performance of the process and the response to methane production and TE content. It is important to consider the practical and logistical aspects of implementing such a system, including the collection and transportation of CW to anaerobic digestion facilities, as well as the need for appropriate infrastructure and operational procedures to ensure the efficiency and stability of the biogas production process.

3.3. Modeling of methane production: mathematical simulation

The mathematical simulation involved a meticulous process of calibrating and validating model parameters using methane production data from reactor VI, which demonstrated the highest performance metrics during the calibration phase. This calibration process was geared towards minimizing the mean squared error (MAE) and ultimately yielded the most appropriate values for the model parameters (see Fig. 4 and Table 5).

One of the critical parameters determined through this process was the easily biodegradable fraction, denoted for the inoculum, WWS, CW, and CM as 0.18, 0.04, 1.00, and 0.80, respectively. This parameter signifies the degree and speed at which substrates are converted into biogas, with a scale ranging from 0 to 1. According to the model, CW emerged as the most readily and rapidly digestible substrate, followed by CM, inoculum, and sludge. Fresh CW typically comprises approximately 93–94 % water, 6.4 % total solids,

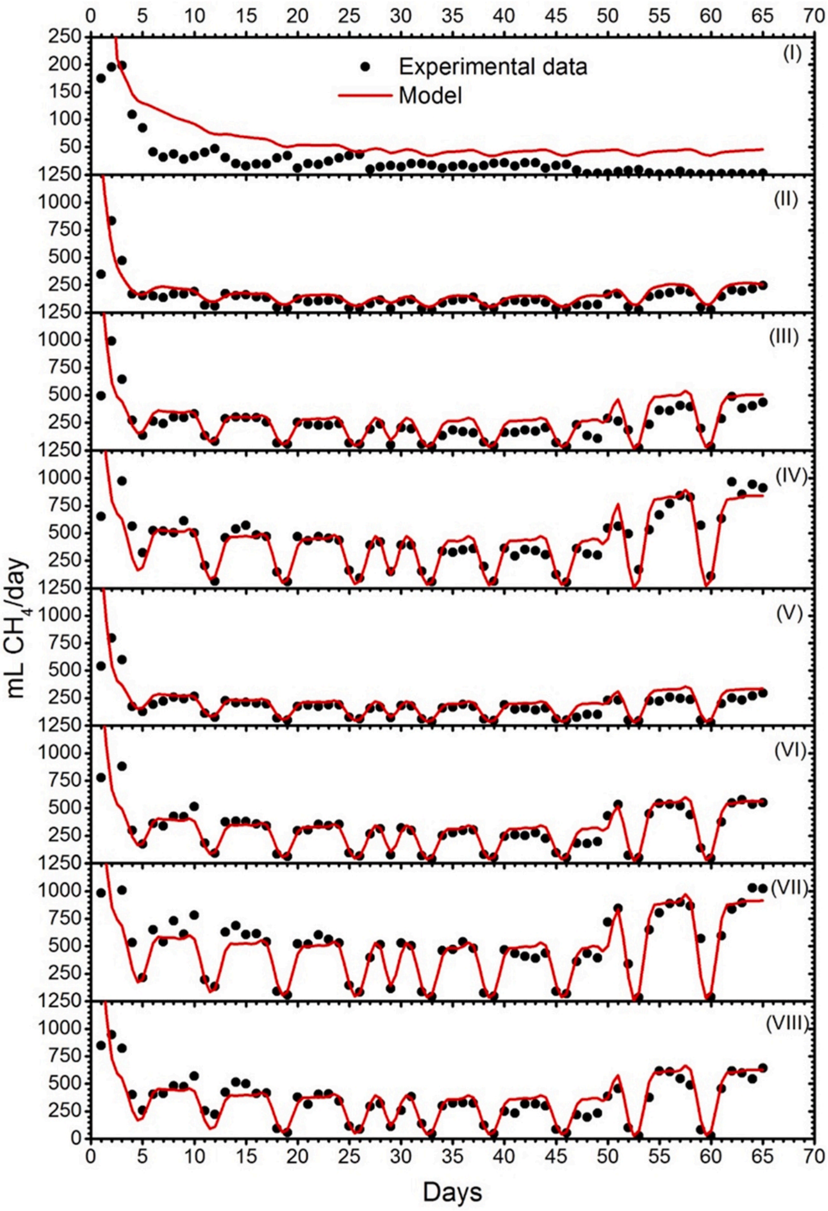

Fig. 4. The model simulation results compared to the experimental data for the eight set of reactors (I to VII)) correspond to the average daily values of reactors I to VII and (VIII) corresponds to the reactor VIII respectively. Experimental data are shown with the solid points while the model results are presented with the continuous line. The model trained on co-digestion data overestimated the single substrate (WWS) methane production by cca 58 %, showcasing the importance of the directionality of modelling for developing strategies to prevent process failures and optimize biogas production.

4.5–6% lactose, 0.55 %, 0.6–1.1 % proteins, 0.06–0.5 % fats, and 0.8–1.0 % minerals [2]. Notably, lactose, the primary source of COD in CW, undergoes swift conversion into biogas, mainly due to the absence of hydrolysis stages that often limit the digestion of complex organic substrates. Additionally, the minerals present in CW, including calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), chlorine (Cl), and sodium (Na), serve as stimulants for biogas production [89]. The relatively simple organic molecule structure, primarily disaccharides and proteins, in CW justifies the high parameter value 'f.'

In contrast, the low value of 0.04 assigned to sludge underscores its complex nature [90]. The kinetic coefficients governing fast (k_f), slow (k_s), and intermediate (k_{int}) reactions in our case were estimated at 0.066 d^{-1}, 0.004 d^{-1}, and 0.039 d^{-1}, respectively. As expected, k_f surpassed k_s, yielding a k_f/k_s ratio of 16.5. Moreover, the k_{int} value was 9.8 times higher than k_s, leading to the selection of the simplified model 4 from the options presented in Table 4. The yield coefficients $Y_{P/A}$ and $Y_{P/B}$ were estimated as 1.450 mL CH_4/(mg COD/L) and 0.413 mL CH_4/(mg COD/L), respectively. Notably, the fast and easily digestible COD fraction exhibited a methane yield 3.5 times greater than the slower and more challenging digestible COD fraction.

Unexpectedly, in very few cases data were not recorded by the data logger. Most missing data were observed in experiment I (Ia and Ib) where the hourly methane production was below the limit of detection. The results obtained from reactor I revealed a markedly low methane yield when using WWS as the sole substrate. This can be ascribed to the notably low values of 'f_{easy},' 'sludge,' and '$Y_{P/B}$.' In stark contrast, CW displayed exceptional digestibility, achieving a 100 % conversion rate into methane, a result consistent with the high '$Y_{A/B}$' values observed. CM and the inoculum each contributed 80 % and 18 %, respectively, to the fraction of the substrate that rapidly undergoes digestion.

For the purpose of model validation, we employed experimental sets I-V and VII-VIII, leveraging the same set of parameters determined from reactors VI. The validation experiments, that were entirely independent of the calibration set, with the only common factors being the source of substrates, the inoculum, and the experimental conditions, showed good correspondence between measured and simulated data (Fig. 4). In particular, experiment I can be considered as an extreme extrapolation set to validate the model performance for cases where only sludge is present in the co-digestion medium. Although the model performed poorly and over-estimated the methane production, on the other hand: (a) the model parameters were calculated by the three substrates co-digestion medium, (b) the average value of the daily production was extremely low (26.6 mL) and (c) the number of missing hourly data was the highest on this data (I) set. This affected the accuracy. The model predicted methane even when AMPTS missed recording data. The model trained on co-digestion data overestimated the single substrate (WWS) methane production by cca 58 %, showcasing the importance of the directionality of modelling for developing strategies to prevent process failures and optimize biogas production. Practical applications can benefit from the use of advanced algorithms to automate periodic feeding and maximize resource recovery efficiently.

4. Conclusions

The study conducted semi-continuous anaerobic digestion experiments to assess the co-digestion of WWS, CW, and CM for methane production. Co-digestion of CW and CM significantly enhanced methane production compared to WWS alone. Reactor VII in the semi-continuous experiment achieved the highest cumulative methane production. The study revealed the importance of substrate ratios in co-digestion scenarios. CW and CM additions had a significant impact on methane yields, especially at optimal mixing ratios. Physical-chemical parameters, such as pH, TIC, and VOA, VFA concentrations, played pivotal roles in maintaining process stability, emphasizing the need for careful monitoring and control. Fluctuations in TE concentrations, particularly iron (Fe), zinc (Zn), and copper (Cu), were observed and underscored their potential impact on long-term process sustainability. Mathematical modelling successfully simulated methane production, providing insights into substrate-specific kinetics. While the model exhibited reasonable accuracy in predicting most scenarios, further refinements are needed for extreme cases. Future research in investigating the influence of TE on anaerobic digestion across various scales and scenarios, with a focus on optimizing their concentrations for improved methane production and process stability is recommended.

Data availability statement

All relevant data are presented in the article and Supporting Information.

Ethics approval and consent to participate

Review or approval by an ethics committee was not needed for this study because no data on patients or experimental animals was used in the article. Informed consent was not required for this study because no clinical data was produced in the article.

CRediT authorship contribution statement

Blaž Stres: Writing – review & editing, Supervision, Conceptualization. **Artin Hatzikioseyian:** Writing – original draft, Validation. **Pavlina Kousi:** Writing – original draft, Visualization, Validation. **Emmanouella Remoundaki:** Writing – original draft, Visualization, Validation. **Leon Deutsch:** Writing – original draft, Investigation, Data curation. **Katarina Vogel Mikuš:** Investigation. **Gašper Rak:** Visualization, Project administration. **Sabina Kolbl Repinc:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Investigation, Conceptualization.

Table 5
Model parameters – best fit values.

Model parameter	Symbol	Value	Units
Fast digestible fraction of inoculum	$f_{easy.inoculum}$	0.18	–
Fast digestible fraction of wastewater sludge	$f_{easy\cdot\ sludge}$	0.04	–
Fast digestible fraction of cheese whey	$f_{easy\cdot\ cheese\ whey}$	1.00	–
Fast digestible fraction of cow manure	$f_{easy\cdot\ cow\ manure}$	0.80	–
Kinetic coefficient of fast reaction	k_f	0.066 (1.584)	$d^{-1}(h^{-1})$
Kinetic coefficient of slow reaction	k_s	0.004 (0.096)	$d^{-1}(h^{-1})$
Kinetic coefficient of intermediate reaction	k_{int}	0.039 (0.936)	$d^{-1}(h^{-1})$
Yield coefficient from fast digestible COD fraction to methane	$Y_{P/A}$	1.450	mL CH_4/(mg COD/L)
Yield coefficient from slowly digestible COD fraction to methane	$Y_{P/B}$	0.413	mL CH_4/(mg COD/L)

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The modeling part of this work has been financially supported by a Short-Term Scientific Mission (STSM) of Leon Deutsch at NTUA (Greece) in the frame of COST Action ES1302, European Network on Ecological Functions of Trace Metals in Anaerobic Biotechnologies. This project has been co-financed by ARIS programme P2-0180 and projects L7-4422 and J2-3056, J2-4427, J7-50152, CRP V2-2403, Horizon Europe project REMEDIES (grant agreement No. 101093964).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e38773.

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