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Optimising sewage sludge anaerobic digestion for resource recovery in wastewater treatment plants

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A B S T R A C T

Recovering resources from sludge generated during wastewater treatment is both an opportunity and a challenge. Thermophilic anaerobic digestion may optimise biogas production and digested sludge properties for further valorisation, in the framework of a circular economy. Thus, this study aimed at evaluating the effect of operational conditions, i.e. temperature, solids concentration in the sludge, and solids retention time (SRT) on the methane production, volatile fatty acids (VFA) concentration, digested sludge hygienisation, and dewaterability, during long-term anaerobic digestion. This is the first time that sludge anaerobic digestion has been evaluated for over 500 days varying control parameters for assessing concomitantly biogas, VFA, and pathogen removal outcomes with focus on resource recovery. Results showed how by shifting from mesophilic (38 °C) to thermophilic (55 °C) conditions, with a short SRT (10 days) in the reactor, the process performance was optimised. Indeed, the methane production reached a maximum of 0.4 $m^3CH_4/m^3_{reactor}\cdot d$, with a VFA concentration of 4.0 g COD/L and complete pathogen removal in the digestate, for a safe agricultural reuse. Therefore, the transition from mesophilic to thermophilic anaerobic digesters seems beneficial for the valorisation of by-products and promoting the circular economy in wastewater treatment plants.

1. Introduction

In the recent decades, academy and industry have made efforts to recover water, biofuels, biofertilizers, and other valuable bio-based products from waste streams. Likewise, there is a need to scale-up novel infrastructures and bioprocesses in municipal wastewater treatment plants (WWTP) [1,2]. In this sense, anaerobic digestion remains as the most used technique for sewage sludge stabilisation in WWTP. Through this microbiological process, organic matter is converted into biogas and digestate. Such by-products may be converted into bioenergy and biofertilizer, respectively, which are considered among the most cost-effective alternatives for closing the loop in full-scale facilities [3,4]. Moreover, intermediate compounds, namely carboxylates, produced during intermediate process stages, may also be target compounds as they can be used in a wide range of applications, for instance in chemical, textile, food, and pharmaceutical industries, as well as precursors of polyhydroxyalkanoates (PHA) for bioplastic production [5–7]. In fact, volatile fatty acids (VFA) world market demand for 2020 was evaluated in 18,500 kilotons, with an annual rate increase of 3% [8].

Although anaerobic digestion is widely used in WWTP, the process could still be optimised for resource recovery. Research and innovation have led to well-stablished technologies that enhance process performance, as sludge pre-treatments, intermediate and post-treatments [9–11]. As far as operational conditions are concerned, in most European countries, sludge digesters are still nowadays operated under mesophilic conditions (30–40 °C) by prioritizing process stability [12]. However, thermophilic digestion has long been pointed out as the most efficient in terms of organic matter removal and methane yield [13–17]. The reason for this is that the growth rates of thermophilic methanogens are higher than those of mesophilic methanogens [18]; whereas biomass yield is much lower. As a result, by accelerating the overall reaction rate it is possible to reduce the sludge retention time (SRT) and,

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consequently, the digester volume (i.e. capital and treatment cost); whilst yielding fewer amounts of biosolids to be disposed of [15,16].

Indeed, digested sludge characteristics play a role in terms of nutrients recycling in agriculture, reducing the application of chemical fertilizers in the framework of a circular bioeconomy [17]. Moreover, there is a negative correlation between the use of agrochemicals and soil sustainability [19]; since its intensive application imposes negative consequences related to environmental degradation, loss of biodiversity and decrease of long-term stability in agricultural production [20]. In this sense, the development of agroecological systems using biofertilizers coming from organic wastes may to contribute to sustainable farming. According to the US EPA Part 503, Class A and B biosolids can be applied to non-public contact sites, as agricultural land, forests, and reclamation sites (e.g. mined areas) or public contact sites, as parks and roadsides. The higher quality Class A biosolids do not show any pathogens, while complying more rigorous limits for pollutants, as heavy metals [18]. In the European Union, 40% of the sewage sludge produced is recycled in soil; yet the presence of pathogens restrains its safe agricultural reuse as biofertilizer [21]. While pathogen removal in mesophilic conditions is low or inexistent, thermophilic digestion enhances sludge hygienisation [22], which allows for direct land application as fertilizer, soil amendment, or soil conditioner in public contact sites (US EPA, Class A biosolids). Digested sludge dewaterability is another key issue in order to reduce the digestate volume and management costs, which may account for over 50% of the WWTP operating costs. From the literature, though, it is not yet clear whether thermophilic digestion improves sludge dewaterability [22,23].

On the whole, thermophilic anaerobic digestion has shown to enhance biological and chemical reaction rates, hence organic matter solubilisation and bioconversion into methane; along with pathogens and some organic micropollutants removal [16,22]. However, thermophilic digesters are prompt to instability, so the start-up and transition from mesophilic to thermophilic conditions is generally a long-term process. In lack of full-scale thermophilic inoculum, digesters may be inoculated with mesophilic digested sludge, but this implies a transition period in which mesophilic microorganisms are to be replaced by their thermophilic homologues [24].

Even though the anaerobic digestion of sewage sludge has been intensively investigated, the novelty of this study relies in its long-term performance (over 500 days), varying different operational parameters and focusing on several process outputs with the goal of recovering resources. Thus, the aim of this study is to evaluate the impact of process temperature (38, 43, 50 and 55 °C), total solids (TS) concentration in the influent (2–4%), and SRT (from 35 to 10 days) on the anaerobic digestion of sewage sludge. Process performance was monitored during the transition from mesophilic to thermophilic conditions, and focus was given to the recovery of by-products: methane production, VFA concentration, and digested sludge properties.

2. Material and methods

2.1. Sewage sludge

The sludge used in this research was collected in a municipal WWTP near Barcelona, Spain, serving a population equivalent (PE) of 130,000. Primary sludge (PS) and waste activated sludge (WAS) were thickened and mixed prior to undergoing mesophilic (38 °C) anaerobic digestion at 40 days of SRT.

The inoculum consisted of mesophilic digested sludge, while the substrate comprised a mixture of thickened PS and WAS in a volumetric ratio of 75/25%. This mixture was collected on a weekly basis and stored at a temperature of 4 °C until use. Initially, sludge with a TS concentration of approximately 2% was employed, and subsequently, the TS concentration was increased to 4%.

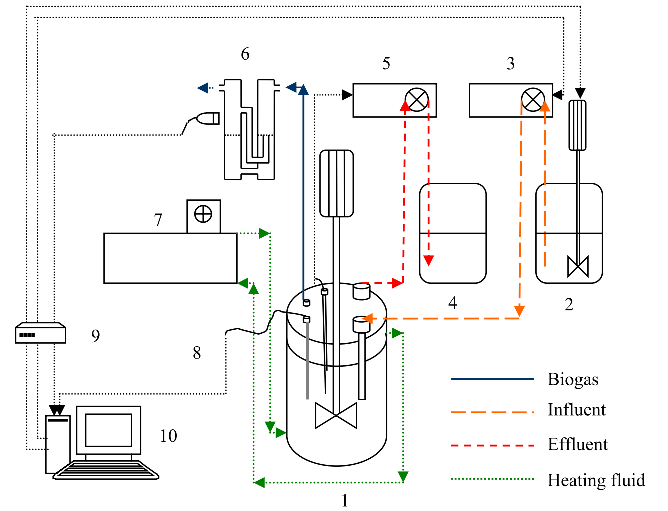

Fig. 1. Experimental set-up. (1) Continuous Stirred Tank Reactor (CSTR); (2) Sludge influent; (3) Sludge feeding pump; (4) Sludge effluent; (5) Sludge withdrawal pump; (6) Gas meter; (7) Thermostatic bath; (8) Temperature sensor; (9) Data acquisition system; (10) Computer.

Table 1
Operational conditions during long-term anaerobic sludge digestion.

Period	Days (nº)	Temperature (ºC)	SRT (days)
I	1–21	38	35
II	22–59	43	35
III	60–203	50	30
IV	204–402	55	30
V	403–439	55	25
VI	440–476	55	15
VII	477–557	55	10

2.2. Experimental set-up

The experimental set-up consisted of a laboratory-scale pilot plant comprising a continuous stirred tank reactor (CSTR) with a capacity of 5 L, which was operated in a semi-continuous mode. The sewage sludge was automatically supplied and discharged twice a day using two peristaltic pumps (Watson Marlow). The volumetric biogas production was measured through water displacement and by employing a capacitive sensor, with on-line data recording (Data Acquisition System, STEP S.L.). Temperature was monitored using a thermal sensor and maintained at the experimental range (38–55 °C) by means of a thermostatic bath (Selecta). The system set-up is shown in Fig. 1.

2.3. Experimental procedures

The anaerobic digester was operated for 18 months under the conditions outlined in Table 1. Initially, the CSTR was inoculated with 5 L of mesophilic digested sludge obtained from the full-scale WWTP. The initial operational conditions were 38 °C and 35 days of SRT, similar to the full-scale digester. After achieving stable performance, the process temperature was raised to 43, 50, and 55 °C. Once stable operation at 55 °C was achieved, the SRT was reduced to 15 and 10 days. Stable operation was identified by a fairly constant performance in terms of biogas production and volatile solids (VS) removal, as proposed in the literature [14,25]. Additionally, the TS of influent sludge was increased from 2 to 4%.

2.4. Analytical methods

The determination of TS, VS, and their soluble fractions (TDS and

Table 2
Feed and digested sludge characteristics, and operational parameters during semi-continuous anaerobic digestion of sewage sludge. Average values ± standard deviation.

Parameter	Period						
	I	II	III	IV	V	VI	VII
Operational conditions							
Temperature (°C)	38.25 ± 1.87	43.25 ± 0.32	49.38 ± 4.73	50.87 ± 1.48	55.38 ± 0.37	54.84 ± 0.47	53.09 ± 0.63
SRT (days)	37.06 ± 1.12	35.54 ± 1.20	30.29 ± 2.93	32.08 ± 4.52	30.87 ± 1.83	15.04 ± 1.40	9.97 ± 0.58
OLR (kg VS/$m^3_{reactor}$·d)	0.47 ± 0.01	0.44 ± 0.02	0.48 ± 0.06	0.75 ± 0.21	0.64 ± 0.17	2.06 ± 0.19	3.03 ± 0.33
Feed composition							
TS (g/L)	22.75	20.78 ± 0.79	21.60 ± 3.12	32.54 ± 9.74	27.23 ± 7.16	41.41 ± 1.63	39.19 ± 6.43
VS (g/L)	17.44	15.31 ± 0.53	14.42 ± 1.99	24.38 ± 7.57	20.58 ± 4.92	30.78 ± 0.72	30.39 ± 2.08
VS/TS (%)	76.66	70.70 ± 0.72	68.65 ± 3.76	72.29 ± 5.40	77.03 ± 4.27	74.72 ± 1.78	73.57 ± 1.31
Total VFA (g COD/L)	0.00	0.59 ± 0.05	1.39 ± 0.47	2.57 ± 0.63	2.23 ± 0.54	2.01 ± 0.22	2.21 ± 0.37
pH	6.65 ± 0.09	6.59 ± 0.03	6.67 ± 0.60	5.83 ± 0.22	6.12 ± 0.21	7.24 ± 0.34	6.95 ± 0.19
Effluent composition							
TS (g/L)	15.06 ± 0.48	12.14 ± 0.58	14.44 ± 1.66	16.03 ± 1.63	20.28 ± 2.07	20.98 ± 2.41	29.45 ± 1.53
VS (g/L)	9.16 ± 0.39	7.49 ± 0.29	9.00 ± 1.04	10.48 ± 1.03	13.46 ± 1.35	14.03 ± 1.13	19.65 ± 1.09
VS/TS (%)	60.82 ± 1.28	61.91 ± 0.67	62.35 ± 0.81	65.35 ± 1.43	66.17 ± 2.81	66.51 ± 4.02	66.72 ± 0.31
Total VFA (g COD/L)	0.00	0.00	1.26 ± 0.41	1.85 ± 0.38	2.06 ± 0.29	2.90 ± 0.33	3.98 ± 0.16
Acetate (g COD/L)	0.00	0.00	0.31 ± 0.19	0.23 ± 0.07	0.14 ± 0.05	0.22 ± 0.06	0.45 ± 0.06
Propionate (g COD/L)	0.00	0.00	0.47 ± 0.14	0.82 ± 0.20	1.00 ± 0.13	1.38 ± 0.16	1.79 ± 0.06
iso-Butyrate (g COD/L)	0.00	0.00	0.16 ± 0.05	0.29 ± 0.08	0.47 ± 0.03	0.58 ± 0.07	0.60 ± 0.09
n-Butyrate (g COD/L)	0.00	0.00	0.04 ± 0.02	0.00	0.00	0.00	0.00
iso-Valerate (g COD/L)	0.00	0.00	0.29 ± 0.06	0.51 ± 0.16	0.45 ± 0.03	0.71 ± 0.05	1.14 ± 0.06
n-Valerate (g COD/L)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
A/P ratio	0.00	0.00	0.98 ± 0.44	0.48 ± 0.27	0.19 ± 0.06	0.09 ± 0.10	0.22 ± 0.03
pH	7.68	8.19 ± 0.15	8.13 ± 0.15	8.20 ± 0.09	8.27 ± 0.12	8.21 ± 0.14	8.18 ± 0.06
Removal efficiency							
TS removal (%)	34.21 ± 8.76	46.59 ± 0.00	27.00 ± 14.00	48.57 ± 18.66	29.32 ± 12.96	49.39 ± 5.05	22.30 ± 18.51
VS removal (%)	35.67 ± 6.79	54.28 ± 2.09	34.66 ± 7.78	55.41 ± 2.44	35.68 ± 4.46	49.38 ± 2.95	34.08 ± 4.06
Biogas characteristics							
Biogas production (m^3/$m^3_{reactor}$ d)	0.23	0.17 ± 0.02	0.13 ± 0.06	0.31 ± 0.09	0.27 ± 0.07	0.64 ± 0.08	0.62 ± 0.06
Methane production (m^3 CH_4/$m^3_{reactor}$·d)	0.13	0.12 ± 0.01	0.08 ± 0.05	0.18 ± 0.08	0.16 ± 0.07	0.40 ± 0.03	0.40 ± 0.05
Methane yield (m^3/kgVS_{fed})	0.27	0.30 ± 0.00	0.16 ± 0.10	0.26 ± 0.15	0.24 ± 0.10	0.20 ± 0.02	0.13 ± 0.02
Methane content (%)	61.33 ± 1.13	68.56 ± 11.39	61.95 ± 5.37	65.03 ± 1.75	64.15 ± 2.81	61.90 ± 1.39	64.52 ± 3.10
Stability period							
Time (days)	1–22	44–59	78–130	145–203	319–369	442–465	522–553

VDS) was carried out following the Standard Methods [26]. For the analysis of soluble samples, centrifugation at 7000 rpm and filtration through 1.2 μm nominal pore size glass fibber filters (Albet FVC047, Spain) were required. pH and VFA, including acetic, propionic, iso-butyric, n-butyric, iso-valeric, and n-valeric acids, were analysed from the soluble samples. VFA analysis was conducted after filtration through a 0.45 μm nylon syringe filter.

VFA were quantified by gas chromatography, using a PerkinElmer AutoSystem XL Gas Chromatograph equipped with a capillary column (HP Innowax 30 m × 0.25 mm × 0.25 μm) and a flame ionization detector (FID). The carrier gas was helium (He), with a split ratio of 13 (column flow: 5 mL/min). The oven temperature was initially set at 120 °C for 1 min, followed by a constant increase to 245 °C at a rate of 10 °C/min and then maintained for 2 min. The injector and detector temperatures were set at 250 and 300 °C, respectively. The chromatograph was calibrated using dilutions of commercially available (Scharlau, Spain) VFA with concentrations ranging from 0 to 1000 mg/L of acetic, propionic, iso-butyric, n-butyric, iso-valeric, and n-valeric acids. The detection limit was 5 mg/L.

The composition of biogas was analysed with a gas chromatograph (PerkinElmer AutoSystem XL), equipped with a thermal conductivity detector (TCD). Gas samples were injected into a packed column (Hayesep 3 m 1/8 in. 100/120), and the carrier gas used was He in spitless mode (column flow: 19 mL/min). The oven temperature was maintained at 40 °C, while the injector and detector temperatures were set at 150 and 250 °C, respectively. The chromatograph was calibrated using pure samples of methane (99.9% CH_4) and carbon dioxide (99.9% CO_2). The gas volume was expressed in Standard Conditions as defined by IUPAC (273.15 K and 10^5 Pa).

Pathogen removal was assessed by analysing faecal bacteria indicators (*Escherichia coli* and *Salmonella* spp.) in fresh influent and effluent sludge samples. *E. coli* was quantified following the ISO 16649:2000 methodology, and the results were expressed as colony forming units per mL (CFU/mL). As for *Salmonella* spp., presence or absence was determined according to the NF–V08-052 methodology, with the results expressed as presence or absence per 50 mL of sample.

Sludge dewaterability was evaluated using the Capillary Suction Time (CST) test described in the Standard Methods [26]. The Triton CST filterability tester and standard filter papers (Part No. 815095) were supplied by Triton Electronics Ltd. (Essex, UK).

3. Results and discussion

3.1. Methane production

The anaerobic digestion performance under different operational conditions is shown in Table 2. The transition of temperature was conducted in three steps: from 38 to 43 °C (period I to II), from 43 to 50 °C (period II to III), and from 50 to 55 °C (period IV to V). In this way, the transition from mesophilic to thermophilic conditions was favoured by shifting the reactor temperature from the upper limit for mesophiles (43 °C) to the lower limit for thermophiles (50 °C) growth [27].

The results obtained during stable periods at 38, 43, 50 and 55 °C showed that there were little differences in methane production at long SRT (30–35 days), provided that the OLR was similar (0.13–0.20 m^3 CH_4/$m^3_{reactor}$·d) (Table 2, periods I, II, IV and V). A lower methane production was observed in period III (0.08 m^3 CH_4/$m^3_{reactor}$·d), which was associated to temperature fluctuations around 49 ± 5 °C. This is in

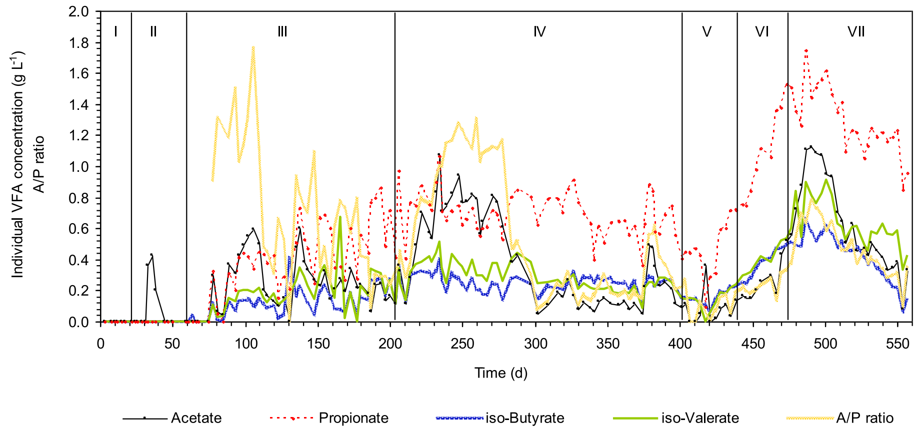

Fig. 2. Individual volatile fatty acids (VFA) concentration and acetate to propionate (A/P) ratio in digested sludge.

agreement with Palatsi et al. [28] who reported a decrease in organic matter bioconversion into methane at temperatures between 43 and 50 °C, where neither mesophiles nor thermophiles growth rates were favoured.

At 50 and 55 °C, with a SRT of 30 days (periods IV and V), the methane production was very similar (0.16–0.18 m^3 $CH_4/m^3_{reactor}$·d). Yet by decreasing the SRT to 15-10 days, while increasing the OLR to 2–3 kg $VS/m^3_{reactor}$·d, the methane production was more than doubled (0.4 m^3 $CH_4/m^3_{reactor}$·d). Some authors suggest that the benefits of thermophilic digestion, in terms of volatile solids removal and methane yield, only become evident at short SRT [29]. The time needed for full conversion of solids depends of microbial growth rates, which become 2–3 times higher for thermophilic compared to mesophilic methanogens [24]. Thus, the minimum design SRT of 15 days for mesophilic digesters [14], could be reduced to 5–8 days for thermophilic digesters. In the present study, during thermophilic operation at 55 °C, the SRT was reduced from 30 to 15 and 10 days, resulting in a stable performance even at the shortest SRT, with a methane production of 0.40 m^3 $CH_4/m^3_{reactor}$·d. It should be noticed that in CSTR, the SRT reduction comes from an increase in the flow rate, hence the OLR.

Indeed, process performance was not only improved by decreasing the SRT, but also by increasing the influent sludge solids concentration from 2 to 4% TS, hence the OLR, in the last periods (VI and VII). As indicated by Peces et al. [30], not only the hydraulic retention time (HRT), but other operational parameters are necessary to promote significant changes in predominant microbial communities and methane yield, for example the quality and quantity of input material (i.e. OLR). In this study, the highest methane production (0.40 m^3 $CH_4/m^3_{reactor}$·d) was achieved by feeding the reactor with sludge with a relatively higher concentration of solids (4% TS), decreasing the SRT to 15 and 10 days (at 55 °C) and increasing the OLR to 2–3 kg VS/m^3·d (Periods VI and VII).

To summarise, the transition to thermophilic anaerobic digestion enhanced the methane production after decreasing the SRT and increasing the OLR. This is beneficial for recovering bioenergy out of the biogas produced in WWTP. Biogas may be converted into heat and electricity in cogeneration units; or upgraded to biomethane used as biofuel in gas stations or injected into the natural gas grid. For instance, a recent study reported twice the methane yield by changing the reactor temperature from mesophilic to thermophilic (0.093 vs 0.193 m^3 CH_4/kg VS), meaning that the WWTP would become energetically self-sufficient and save 100–200 Nm^3/h of natural gas [16].

3.2. *Volatile fatty acids concentration*

The accumulation of intermediate acids and alcohols (i.e. carboxylates) produced during acidogenic and acetogenic steps of anaerobic digestion may be a drawback when the target outcome is biogas, since methanogenic activity could be inhibited. On the other hand, it may be beneficial if focus is given to VFA production for further separation of these platform chemicals used as precursors for biofuels, chemical compounds, and in several industrial processes [6,31,32]. To date, though, little is known about the factors affecting VFA accumulation and how to maximise their recovery [33].

The concentration of VFA over the whole experimental period is shown in Fig. 2. As may be observed, one of the main differences among mesophilic and thermophilic effluents referred to VFA: they were hardly detected under mesophilic operation, while they were always present under thermophilic temperatures (both at 50 and 55 °C), ranging from 1.3 to 4.0 g COD/L (Table 2). According to the literature, VFA are either not detected or found in very low concentrations in mesophilic effluents [34]. Conversely, they are generally present in thermophilic effluents, in concentrations as high as 5 g COD/L [34,35]. Not only the VFA concentration but also the VFA profile is relevant for they valorisation. For instance, in the production of PHA used for bioplastics, the monomers applied as precursors strongly influence the final properties. In this manner, short chain VFA (3–5 carbon atoms) engender a bioplastic with high crystallinity degree, fragility, and rigidity; while medium chain VFA (6–14 carbon atoms) lead to bioplastics with low crystallinity degree, softer, and with flexible properties [36].

Besides, the VFA profile may also be used as indicator of process stability. In this sense, the ratio between acetic acid and propionic acid (A/P) has been proposed to predict process failure, with a threshold of 0.5 for sewage sludge [37]. In this study, the A/P ratio was below 0.5 for all periods, but for period III, when it increased to 1.0, which is in accordance with the decrease in methane production to 0.08 m^3 $CH_4/m^3_{reactor}$·d (Table 2). As for Periods VI and VII, it may be noticed how the A/P ratio was as low as 0.09–0.22 even at a relatively high TS influent concentration (4% TS) and short SRT (10–15 days), confirming the reactor stability at 55 °C. During these periods, where the highest VFA concentration was observed, also the highest methane production (0.40 m^3 $CH_4/m^3_{reactor}$·d) was reached, indicating that methanogens were not inhibited. Other studies have reported a concomitant biogas and VFA production in single-step thermophilic reactors [38,39]. This allows for the simultaneous recovery of bioenergy from methane and VFA from the digestate. Nonetheless, for optimising the VFA production and accumulation at higher concentrations, methanogenesis needs to be inhibited to avoid the conversion of the acetogenesis end products into biogas, when the target compounds are primarily VFA [40].

In any case, thermophilic temperatures seem to be optimal for biogas and/or VFA production due to an increased hydrolysis rate of particulate organic matter. Hao and Wang [34] achieved a 10-fold increase in VFA accumulation by fermenting WAS under thermophilic conditions as compared to mesophilic conditions. These authors reported that the

Table 3
Faecal bacteria indicators in influent and effluent sludge samples.

Parameters	Influent (PS + WAS)	Effluent (digested sludge)				
Period	–	II	III and IV	V	VI	VII
Temperature (°C)	–	43	50	55	55	55
SRT (days)	–	35	30	30	15	10
E. coli (CFU/mL)	1.0×10^6	1.7×10^3	Absence	Absence	Absence	Absence
Salmonella spp. (in 50 mL)	Absence	Absence	Absence	Absence	Absence	Absence

Table 4
Digested sludge dewaterability measured as the Capillary Suction Time (CST).

Parameters	Influent (PS + WAS)	Effluent (digested sludge)		
Period	–	III and IV	V	VII
Temperature (°C)	-	50	55	55
SRT (days)	-	30	30	10
CST(s)	437	432	439	850
CST (s)/g TS/L	18	30	29	29
CST (s)/g VS/L	27	45	44	44

thermophilic reactor showed an increase in the activity of key hydrolases, raised the proportion of bacteria involved in the hydrolysis and acidification, and promoted a relative abundance of homoacetogens. Indeed, reducing the HRT from 15 to 8, 4 and 2 days in a mesophilic reactor was not enough to prompt acid accumulation and methanogens washed out, being *Methanosaeta* the predominant genus. The combination of HRT decreases with other operational parameters, as temperature increase, was pointed out to enhance VFA accumulation and methanogenic inhibition, while promoting a specific bacterial population and biochemical pathway [30]. A recent review pointed out that the main strategy for maximising VFA production is to operate reactors at high OLR and low SRT. In this case, there will be an imbalance in terms of higher VFA formation in respect to its consumption [41]. In the present study, VFA accumulation was not only promoted by increasing process temperature to thermophilic conditions, but also by decreasing the SRT from 35 to 10 days, and increasing the OLR to 3 kg VS/m^3·d.

3.3. Pathogen removal and dewaterability of digested sludge

In this study, it was clear how the anaerobic digestion temperature affected the concentration of pathogens in digested sludge, evaluated by the concentration of faecal bacteria indicators (Table 3). Compared to the concentration of *E. coli* in influent sludge (10^6 CFU/mL), a 3-log reduction was achieved by mesophilic digestion at 43 °C, while complete destruction was achieved under all thermophilic conditions assayed (50-55 °C, 10–35 days of SRT). *Salmonella* spp. was never detected. Thermophilic effluent hygienisation is widely reported in the literature, showing the potential of thermophilic anaerobic digestion in preventing the spread of pathogens in the environment upon direct land application of digestates. Indeed, a recent review highlighted how Gram-negative bacteria are sensitive to high temperature, since the fluidity and permeability of the cell membrane increases along with temperature (from 37 to 70 °C). This allows toxic chemicals in the reactor to diffuse more rapidly into the cytoplasm, and inhibits cell growth [42]. For instance, Lanko et al. [22] found a concentration of *E. coli* around 20–155 CFU/g in mesophilic digested sludge, while neither *E. coli* nor total coliforms were detected in thermophilic digested sludge. A mesophilic reactor treating sludge attained an effluent with a faecal coliform concentration of 2×10^6 CFU/g TS, which the authors appointed as Class B biosolids [43]. In a full-scale WWTP, after two-stage thermophilic digestion (55/52 °C), residual concentrations were 10^4 CFU/g for total coliforms, and 10^3 CFU/g for faecal coliforms and enterococci; while these values were 10^6, 10^5 and 10^4-10^5 after two-stage mesophilic digestion (38/35 °C) [44]. Even if pathogen removal is higher in thermophilic digesters, attention should be paid to potential re-contamination after the anaerobic digestion step.

From the point of view of plant operators, sludge dewaterability is another issue, since it affects the dose of polymer, and the final volume of digestate to be transported and disposed of, hence sludge management costs. Moreover, thermal, ultrasonic and microwave techniques have been successfully assessed for promoting sludge dewatering, however their high energy requirements render these processes uneconomical for field application [45]. Sludge dewaterability measures the capability of water entrapped into sludge flocs to be lost. In this study, it was determined by the CST in the influent and digested sludge samples (Table 4). The results obtained were similar for the influent and effluent at 50–55 °C with 30 days of SRT (430–440 s), while it was doubled upon digestion at 55 °C and 10 days of SRT (850 s). A previous study showed how an increase in sludge TS content higher than 2.6%, corresponding to an increase in OLR (up to 3 kg VS/m^3·d), deteriorated digested sludge dewaterability, which was associated with a high VFA concentration, thus high soluble organic matter concentration in the effluent [37]. Conversely, in another study evaluating digested sludge dewaterability by centrifugation, the highest dewaterability coefficient (16.1–17.4%) was achieved after thermophilic digestion as compared to mesophilic and temperature-phased anaerobic digestion (TPAD) (13.6–15.7%) [22]. So, it seems that poorer results in terms of dewaterability are obtained for thermophilic digestate using the CST, and better by centrifugation. Polymer dosage may also play a role in the latter case, which is closer to the method used in full-scale WWTP.

4. Conclusions

This study evaluated the effect of operational conditions, i.e. temperature (38-55 °C), solids concentration (2–4 %TS) in sewage sludge, and SRT (35-10 days) on the methane production, VFA concentration, digested sludge pathogen removal and dewaterability. Results showed how by shifting from mesophilic (38 °C) to thermophilic (55 °C) conditions, with a short SRT (10 days) in the reactor, the process performance was optimised. Under such conditions, the methane production reached 0.4 $m^3CH_4/m^3_{reactor}$·d, with a VFA concentration of 4.0 g COD/L, and complete pathogen removal in the digestate, for a safe agricultural reuse. Therefore, the transition from mesophilic to thermophilic digesters seems beneficial for the valorisation of by-products and promoting the circular economy in municipal WWTP.

CRediT authorship contribution statement

Ivet Ferrer: Data curation, Investigation, Methodology, Writing – original draft. **Fabiana Passos:** Writing – review & editing. **Eva Romero:** Investigation. **Felícitas Vázquez:** Conceptualization, Methodology, Supervision. **Xavier Font:** Conceptualization, Methodology, Supervision, Writing – review & editing.

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.

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References

[1] X. Wang, G. Daigger, D.-J. Lee, J. Liu, N.-Q. Ren, J. Qu, G. Liu, D. Butler, Evolving wastewater infrastructure paradigm to enhance harmony with nature, Sci. Adv. 4 (8) (2018) 1–10.
[2] S. Wainaina, M.K. Awasthi, S. Sarsaiya, H. Chen, E. Singh, A. Kumar, B. Ravindran, S.K. Awasthi, T. Liu, Y. Duan, S. Kumar, Z. Zhang, M.J. Taherzadeh, Resource recovery and circular economy from organic solid waste using aerobic and anaerobic digestion tecnologies, Bioresour. Technol. 301 (2020) 122778.
[3] P. Kehrein, M. Van Loosdrecht, P. Osseweijer, M. Garfí, J. Dewulf, J. Posada, A critical review of resource recovery from municipal wastewater treatment plants – market supply potentials, technologies and bottlenecks, Environ. Sci. J. Integr. Environ. Res.: Water Research and Technology 6 (2020) 877–910.
[4] I. Volschan Junior, R. Almeida, M.C. Cammarota, A review of sludge pretreatment methods to boost biogas production and energy sufficiency in wastewater treatment plants, J. Water Proc. Eng. 40 (2021) 101857.
[5] S. Agnihotri, D.-M. Yin, A. Mahboubi, T. Sapmaz, S. Varjani, W. Qiao, D. Y. Koseoglu-Imer, M.J. Taherzadeh, A glimpse of the world of volatile fatty acids production and application: a review, Bioengineered 13 (1) (2022) 1249–1275.
[6] V.K. Varghese, B.J. Poddar, M.P. Shah, H.J. Purohit, A.A. Khardenavis, A comprehensive review on current status and future perspectives of microbial volatile fatty acids production as platform chemicals, Sci. Total Environ. 815 (2022) 152500.
[7] X. Xu, X. Xiang, X. Dong, G. Chai, Z. Song, Y. Wang, J. Liu, Y. Han, H. Liu, Profitable wastewater treatment by directly recovering organics for volatile fatty acids production, J. Water Proc. Eng. 40 (2021) 101881.
[8] G.K. Veluswamy, K. Shah, A.S. Ball, A.J. Guwy, R.M. Dinsdale, A techno-economic case for volatile fatty acid production for increased sustainability in wastewater treatment industry, Environ. Sci. J. Integr. Environ. Res.: Water Research & Technology 7 (2021) 927–941.
[9] G. Campo, A. Cerutti, M. Zanetti, M. De Ceglia, G. Scibilia, B. Ruffino, A modelling approach for the assessment of energy recovery and impact on the water line of sludge pre-treatments, Energy 274 (2023) 127355.
[10] T. Hidaka, M. Nakamura, F. Oritate, F. Nishimura, Comparative anaerobic digestion of sewage sludge at different temperatures with and without heat pre-treatment, Chemosphere 307 (3) (2022) 135808.
[11] Z. Wang, T. Liu, H. Duan, Y. Song, X. Lu, S. Hu, Z. Yuan, D. Batstone, M. Zheng, Post-treatment options for anaerobically digested sludge: current status and future prospect, Water Res. 205 (2021) 117665.
[12] N. Bachmann, Sustainable Biogas Production in Municipal Wastewater Treatment Plants, Technical brochure International Energy Agency Published by IEA Bioenergy, 2015. ISBN 978-1-910154-22-9.
[13] H.O. Buhr, J.F. Andrews, The thermophilic anaerobic digestion process, Water Res. 11 (1977) 129–143.
[14] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Wastewater Engineering: Treatment and Reuse, fifth ed., Mc Graw-Hill Higher Education, Boston, USA, 2014.
[15] R.M. Alonso, R.S. Rio, M.P. García, Thermophilic and mesophilic temperature phase anaerobic co-digestion (TPAcD) compared with single-stage co-digestion of sewage sludge and sugar beet pulp lixiviation, Biomass Bioenergy 93 (2016) 107–115.
[16] B. Ruffino, G. Campo, A. Cerutti, G. Scibilia, E. Lorenzi, M. Zanetti, Comparative analysis between conventional and a temperature-phased anaerobic digestion system: monitoring of the process, resources transformation and energy balance, Energy Convers. Manag. 223 (2020) 113463.
[17] N. Di Costanzo, A. Cesaro, F. Di Capua, G. Esposito, Exploiting the nutrient potential of anaerobically digested sewage sludge: a review, Energies 14 (23) (2021) 8149.
[18] John Walker, A Plain English Guide to the EPA Part 503 Biosolids Rule, U.S. Environmental Protection Agency, Office of Wastewater Management, Washington, DC, 1994.
[19] I.Y.R. Odegard, E. Van der Voet, The future of food – scenarios and the effect on natural resource use in agriculture in 2050, Ecol. Econ. 97 (2014) 51–59.
[20] P. Prashar, S. Shah, Impact of fertilizers and pesticides on soil microflora in agriculture, in: Sustainable Agriculture Reviews, vol. 19, Springer, Cham, 2016, pp. 331–361.
[21] P. Wilfert, P.S. Kumar, L. Korving, G.-J. Witkamp, M.C. Van Loosdrecht, The relevance of phosphorus and iron chemistry to the recovery of phosphorus from wastewater: a review, Environ. Sci. Technol. 49 (16) (2015) 9400–9414.
[22] I. Lanko, J. Hejnic, J. Říhová-Ambrozová, I. Ferrer, P. Jenicek, Digested sludge quality in mesophilic, thermophilic and temperature-phased anaerobic digestion systems, Water 13 (2021) 2839.
[23] M. Vojtiskovà, P. Jenicek, Impact of the anaerobic digestion process configuration on the sludge dewaterability, Paliva 13 (3) (2021) 96–101.
[24] J.B. Van Lier, J. Hulsbeek, A.J. Stams, G. Lettinga, Temperature susceptibility of thermophilic methanogenic sludge: implications for reactor start-up and operation, Bioresour. Technol. 43 (1993) 227–235.
[25] H.M. El-Mashad, G. Zeeman, W.K.P. Van Loon, G.P.A. Bot, G. Lettinga, Effect of temperature and temperature fluctuations on thermophilic anaerobic digestion of cattle manure, Bioresour. Technol. 95 (2004) 191–201.
[26] APHA, Standard Methods for the Examination of Water and Wastewater, 23th edition, American Public Health Association/American Water Works Association/ Water Environment Federation, Washington DC, USA, 2017.
[27] G.D. Gebreeyessus, P. Jeníček, Thermophilic versus mesophilic anaerobic digestion of sewage sludge: a comparative review, Bioengineering 3 (2) (2016) 15.
[28] J. Palatsi, A. Gimenez-Lorang, I. Ferrer, X. Flotats, Start-up strategies of thermophilic anaerobic digestion of sewage sludge, Water Sci. Technol. 59 (9) (2009) 1777–1784.
[29] H.N. Gavala, U. Yenal, I.V. Skiadas, P. Westermann, B.K. Ahring, Mesophilic and thermophilic anaerobic digestion of primary and secondary sludge. Effect of pre-treatment at elevated temperature, Water Res. 37 (2003) 4561–4572.
[30] M. Peces, S. Astals, P.D. Jensen, W.P. Clarke, Transition of microbial communities and degradation pathways in anaerobic digestion at decreasing retention time, N. Biotech. 60 (2021) 52–61.
[31] R. Kleerebezem, B. Joosse, R. Rozendal, M.C.M. Van Loosdrecht, Anaerobic digestion without biogas? Rev. Environ. Sci. Biotechnol. 14 (2015) 787–801.
[32] A. Zhou, Z. Liu, C. Varrone, Y. Luan, W. Liu, A. Wang, X. Yue, Efficient biorefinery of waste activated sludge and vinegar residue into volatile fatty acids: effect of feedstock conditioning on performance and microbiology, Environ. Sci. J. Integr. Environ. Res.: Water research & Technology 4 (2018) 1819–1828.
[33] T. Liang, K. Elmaadawy, B. Liu, J. Hu, H. Hou, J. Yang, Anaerobic fermentation of waste activated sludge for volatile fatty acid production: recent updates of pretreatment methods and the potential effect of humic and nutrients substances, Process Saf. Environ. Protect. 145 (2021) 321–339.
[34] J. Hao, H. Wang, Volatile fatty acids productions by mesophilic and thermophilic sludge fermentation: biological responses to fermentation temperature, Bioresour. Technol. 175 (2015) 367–373.
[35] P. Zhang, Y. Chen, Q. Zhou, Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH, Water Res. 43 (15) (2009) 3735–3742.
[36] T. Wei, Q. Fang, Regulating the monomer of polyhydroxyalkanoate from mixed microbial culture: with particular emphasis on substrate composition: a review, Environ. Eng. Res. 27 (5) (2022) 210333.
[37] I. Ferrer, F. Vázquez, X. Font, Long term operation of a thermophilic anaerobic reactor: process stability and efficiency at decreasing sludge retention time, Bioresour. Technol. 101 (9) (2010) 2972–2980.
[38] M.A. De la Rubia, M. Perez, L.I. Romero, D. Sales, Effect of solids retention time (SRT) on pilot scale anaerobic thermophilic sludge digestion, Process Biochem. 41 (2006) 79–86.
[39] S. Ponsá, I. Ferrer, F. Vázquez, X. Font, Optimization of the hydrolitic-acidogenic anaerobic digestion stage (55 °C) of sewage sludge: influence of pH and solid content, Water Res. 42 (2008) 3972–3980.
[40] S. Harirchi, S. Wainaina, T. Sar, S.A. Nojoumi, M. Parchami, M. Parchami, S. Varjani, S.K. Khanal, J. Wong, M.K. Awasthi, M.J. Taherzadeh, Microbiological insights into anaerobic digestion for biogas, hydrogen or volatile fatty acids (VFAs): a review, Bioeng. 13 (3) (2022) 6521–6557.
[41] S. Wainaina, Lukitawesa, M.K. Awasthi, M.J. Taherzadeh, Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: a critical review, Bioeng. 10 (1) (2019) 437–458.
[42] M. Lin, A. Wang, L. Ren, W. Qiao, S. Wandera, R. Dong, Challenges of pathogen inactivation in animal manure through anaerobic digestion: a short review, Bioeng. 13 (1) (2022) 1149–1161.
[43] T. Forster-Carneiro, V. Riau, M. Perez, Mesophilic anaerobic digestion of sewage sludge to obtain class B biosolids: microbiological methods development, Biomass Bioenergy 34 (12) (2010) 1805–1812.
[44] J. Zábranská, M. Dohányos, P. Jeníček, J. Kutil, Thermophilic process enhancement of excess activated sludge degradability – two ways of intensification sludge treatment in Prague central wastewater treatment plant, Water Sci. Technol. 41 (9) (2000) 265–272.
[45] M. Anjum, N.H. Al-Makishah, M.A. Barakat, Wastewater sludge stabilization using pre-treatment methods, Process Saf. Environ. Protect. 102 (2016) 615–632.