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Acidogenic fermentation of food waste and sewage sludge mixture: Effect of operating parameters on process performance and safety aspects

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ABSTRACT

The production of added-value bio-products and energy from waste streams while minimizing environmental impacts is a crucial aspect within the circular economy's principles. The biorefinery can be an exit to the constant increasing of organic food waste and sewage sludge to solve the issues of waste disposal. This work deals with the production of volatile fatty acids (VFA) as added-value products from food waste and sewage sludge mixture in a pilot scale acidogenic fermentation process. Moreover, due to the lack of information about safety aspects in the literature, the explosive risk of the fermenter has been assessed by means of the quantification of lower flammability limit (LFL) of the generated flammable gases. Different temperature and feedstock's composition were tested, as well as the effect of thermal hydrolysis. Mesophilic fermentation (37 °C) on thermally hydrolysed feedstock (48 h at 72 °C) ensured stability in terms of VFA production at high concentration (30 ± 2 $gCOD_{VFA}/L$) and COD_{VFA}/COD_{SOL} ratio (0.86 ± 0.09). Such condition also showed high LFL (28.9%), corresponding to a less hazardous condition compared to the other investigated, especially the thermophilic ones where LFL changed between 18% and 26%.

1. Introduction

The approach of the circular economy is based on the reuse of the produced waste to give them a second life and thus recover bio-products with high added value (Battista et al., 2020). Organic food waste, as well as the sewage sludge produced from domestic wastewater treatment plants (WWTPs) are the two biggest sources originated from the urban scenario. Their availability is linked to human activities, and it is not an issue for practices focused on their valorisation. To recycle this waste without environmental pollution, the biorefinery represents a truly innovative approach opposed to their disposal, which allow to obtain different high added value products such as biofuel, biogas and, among others, volatile fatty acids (VFA; Ubando et al., 2020). VFA are a group of carboxylic acid that can be used as carbon sources for several applications: chemical building blocks, solvents and pharma industries, biopolymer synthesis (Owusu-Agyeman et al., 2020). Presently, at the industrial scale, VFA are produced from pure cultures that require sterile cultivation conditions and that involves high production cost and non-renewable resources (petroleum) but the large availability of renewable feedstock or renewable resources (organic food waste, wastewater sludge, agricultural waste, etc.) opens new possible routes. The most known and widely used VFA are acetic, propionic and butyric acids, which represent a whole production close to 4.000.000 t/year, with a market price of 400–800 €/ton for acetic acid, 1700–1880 for propionic, 1800–2000 for butyric, 2200–2700 for valeric and 2000–2500 €/ton for caproic acid (Jankowska et al., 2017).

Currently, biological VFA production using a mixed microbial consortium (MMC) is gaining more interest to ensure the reducing dependence on non-renewable fuels by allowing the disposal of municipal organic waste and working under non-sterile conditions

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(Esteban-Gutiérrez et al., 2018). Nowadays, VFA production from organic waste is widely discussed in the literature and authors have used a large variety of substrates coupled with mixed microbial cultures (MMC) through acidogenic fermentation process (Lukitawesa et al., 2020; Owusu-Agyeman et al., 2020; Dahiya and Mohan, 2019). The development of a whole biorefinery has been also recently discussed (Valentino et al., 2018) and the results were obtained in a multi-steps pilot scale platform in which the organic fraction of municipal solid waste has been utilised for VFA, biogas and biopolymer (e.g. polyhydroxyalkanoates, PHA) production. In this scenario, the production of VFA-derived biopolymers has been assessed given the PHA biodegradability and its properties which make this compound suitable to replace fossil-derived polymers such as polyethylene and polypropylene (Torres-Giner et al., 2018). In addition to producing VFA in the liquid phase, acidogenic fermentation of organic waste also offers the possibility to produce a solid-rich phase and a gaseous phase (Valentino et al., 2019). The solid-rich phase can be utilized in high-solids anaerobic digestion process producing biogas and fertilizers for agriculture; the produced gas is usually a mixture of H_2 and CO_2, in relative percentage that could change based on the operating parameters (among others temperature and hydraulic retention time, HRT; Micolucci et al., 2020). Apart from the attractive possibility to recover H_2, the gaseous mixture can be hazardous to workers, especially when H_2 content achieved a volumetric percentage above 35% v/v (Micolucci et al., 2020).

Several authors have studied how certain parameters affect the acidogenic fermentation of food waste, alone or in combination with wastewater sludge, to produce VFA: temperature, pH, HRT, organic loading rate (OLR) (Ali et al., 2021; Li et al., 2018; Cheah et al., 2019). These parameters can activate different metabolic pathways to produce a particular number of carbon atoms in the fatty acid chain and have an influence on the gases produced during the process. On the other hand, in the framework of the European legislation, assessment of risks to the safety and health of workers arising from the presence of hazardous chemical agents must be considered. A first attempt for the assessment of such risk has been made by applying a dedicated algorithm to a bioprocess describing the PHA production from fermented waste streams (Incocciati et al., 2020). However, this algorithm was simplified to the chemical risk only (due to the possible use of chemicals in the bioprocess) and it did not include the explosive risk coming from the gas mixture produced in the fermentation process.

In the present study, several operating parameters have been applied in the pilot scale acidogenic fermenter located in Treviso wastewater treatment plant (WWTP) and modified to investigate their effects on the production of VFA, starting from a substrate consisting of a mixture of food waste and sewage sludge. In this specific geographical context, the food waste came from the source separate collection in the Treviso province (northeast Italy). Given the lack of literature data concerning the safety aspect of this type of process, an explosive risk assessment has been carried out to identify the most dangerous conditions for workers' safety.

2. Materials and methods

2.1. Organic substrates

The substrates utilized in this work were: (a) the municipal secondary sludge (MS) coming from the static thickener, after the biological nutrient removal (BNR) process applied to the domestic WWTP of Treviso, and (b) the food waste (FW) coming from the source separate collection of more than 50 districts in the Treviso province. After collection, the FW is usually transferred into a dedicated plant for the mechanical trituration and squeezing; the squeezed fraction is co-digested with the MS in the adjacent full-scale anaerobic digester and the solid fraction is sent to composting. Both FW and MS were collected weekly and characterized in terms of total solids and volatile solids (TS and VS), soluble chemical oxygen demand (COD_{SOL}), VFA, ammonia ($N\text{-}NH_4^+$), phosphate ($P\text{-}PO_4^{3-}$), Total Kjeldahl Nitrogen (TKN) and organic phosphorus (P) (Table 1). The waste streams were utilized for this work in the pilot area located in the same full-scale plant.

Table 1
Summary of the physical-chemical features of the waste stream.

Parameter	FW (squeezed fraction)	MS (thickened)
TS (g/L)	120 ± 7	27 ± 1
VS (g/L)	108 ± 4	19 ± 1
VS/TS (%)	90 ± 2	70.0 ± 0.8
COD_{SOL} (g/L)	32 ± 3	0.51 ± 0.03
COD_{VFA} (g/L)	6 ± 1	–
Ammonia (g $N\text{-}NH_4^+$/L)	168 ± 25	437 ± 41
Phosphate (g $P\text{-}PO_4^{3-}$/L)	54 ± 6	128 ± 8
TKN (g N/kg TS)	19 ± 3	45 ± 3
P (g P/kg TS)	6 ± 1	2.0 ± 0.7

2.2. Acidogenic fermenter: operating parameters

The acidogenic fermentation was carried out in a 0.380 m^3 Continuous Stirred Tank Reactor (CSTR) equipped with a mechanical stirrer, under temperature control by using a thermostatic jacket and without pH control. The reactor was operated for more than 3 years (not continuously), and seven operating conditions were tested; the different applied parameters are summarized in Table 2. For the purpose of this study, the final VFA concentration (COD_{VFA}) was taken into account as well as the ratio between the VFA (as COD) and the COD_{SOL}. This parameter is particularly relevant in the perspective of utilizing the VFA-rich stream for PHA production, since high COD_{VFA}/COD_{SOL} ratios correspond to a high yield of PHA (gPHA/g waste) (Valentino et al., 2018). In all runs, COD_{VFA} concentrations and COD_{VFA}/COD_{SOL} ratio were continuously monitored, and the composition of the generated gas mixture was quantified. The pH was not controlled; hence, all runs exhibited slightly acidic values (between 4.5 and 5.0) in most of the operation period. A pre-hydrolysis step (72 °C, 48 h; only in the last two runs) was applied to facilitate the following fermentation process.

2.3. Analytical methods

The liquid phase of the reactor's effluent was monitored three times per week for soluble COD, pH and VFA (both concentration and distribution). The COD measurements were performed in accordance with the Standard Methods (APHA/AWWA/WEF, 2012). The VFAs were quantified by AGILENT 6890 N gas chromatograph equipped with a flame ionization detector (with T of 200 °C), a fused silica capillary column, DB-FFAP (15 m x 0.53 mm × 0.5 µm thickness of the film), and hydrogen as gas carrier. The analysis was conducted by increasing the temperature from 80 °C to 200 °C, with a rate of 10 °C/min. Each sample was centrifuged and filtered with a 0.20 µm filter porosity before analysis.

Biogas production was quantified by a flow meter (Ritter CompanyTM), while CO_2 and H_2S percentage in the biogas was determined through a portable infrared gas analyser GA2000TM (Geotechnical In- strumentsTM). Both CH_4 and H_2 were quantified by a gas chromatograph GC Agilent Technology 6890 NTM equipped with a column HP-PLOT MOLESIEVETM (30 m x 0.53 m ID x 25 µm thickness of the film), using a thermal conductivity detector (TCD) at 250 °C. The injector was maintained at a constant pressure of 70 kPa, with a temperature of 120 °C. Samples were taken using a gas-type syringe of 200 µL as volume. The analyses were conducted at a

Table 2
Operating parameters and feedstock composition of seven CSTR Run fermentation processes.

Run	HRT (days)	Window time (days)	OLR (kg VS/m^3 d)	T (°C)	Feedstock (% v/v)	Hydrolysis (°C, h)
1	5.5	0–85	11.5	55	FW(50%) -MS (50%)	–
2a	5.5	0–120	9.9	55	FW(40%) -MS (60%)	–
2b	5.5	121–180	8.3	55	FW(30%) -MS (70%)	–
2c	5.5	180–250	8.3	42	FW(30%) -MS (70%)	–
3a	5.5	0–140	8.3	37	FW(30%) -MS (70%)	–
3b	5.5	140–420	8.3	37	FW(30%) -MS (70%)	72 °C, 48 h
4	5.5	0–53	8.3	25	FW(30%) -MS (70%)	72 °C, 48 h

constant temperature of 40 °C for 8 min, by using Argon as gas carrier.

2.4. Data analysis

To better represent VFA distribution, the molar ratio between odd numbered acids and the total VFA was quantified. The substrate solubilisation was calculated by the ratio between the COD_{SOL} (as net concentration subtracted to the initial COD_{SOL}; COD_{SOLin}) and the initial VS (VS_{in}) of the feedstock:

$$\text{Solubilisation} = (\text{mg } COD_{SOL} - \text{mg } COD_{SOLin})/\text{g } VS_{in}$$

The VFA yield was quantified as the ratio between the produced VFA (as net concentration subtracted to the initial VFA; COD_{VFAin}) and the VS_{in}:

$$\text{Yield} = (\text{g } COD_{VFA} - \text{g } COD_{VFAin})/\text{g } VS_{in}$$

For those runs which the thermal hydrolysis was applied, both substrate solubilization and VFA yield were calculated considering the feedstock before the hydrolysis as starting point.

All the parameters characterizing reactor performances were calculated after steady states were achieved. Descriptive statistics and exploratory data analysis were performed using the open source program, R (The R Foundation for Statistical Computing, version 4.0.3).

2.4.1. Explosion risk assessment and determination of Lower Flammability Limit (LFL) of flammable mixture

The acidogenic fermentation is an anaerobic process, however, the oxygen presence inside the fermenter cannot be categorically excluded. A potentially explosive atmosphere is composed of air and flammable compounds or mixture. The explosion can cause serious injuries to workers and huge damages to the infrastructures, and it occurs when the three following conditions are simultaneously met: (a) oxygen presence; (b) flammable substance concentration between its lower flammability limit (LFL) and upper (UFL) flammability limit (these limits affect the flammability range); (c) presence of ignition sources (hot surfaces, sparks, etc.), which can provide the required activation energy.

In industrial plants, there are several components (compressors, valves, flanges, etc.) that can become sources of flammable gases/vapours released in case of failure. Two thresholds (LFL and UFL) define the flammability range. Below the LFL, the flammable substance/mixture concentration is too lean to burn (there is an air excess); over the UFL, the mixture is too rich in flammable substance, and it cannot be ignited because the air quantity is insufficient (Dwyer et al., 2011). The LFL and UFL calculation are fundamental for ensuring the processes' safety and preventing explosions. The flammability limits data of a single flammable compound (hydrogen, methane, etc.) can be easily found in the literature; however, the mixtures flammability data are not available, and they must be quantified by experimental tests or calculation methods. The latter are simple and extremely quick (Mendiburu et al., 2018; Zhou et al., 2020). In this study, Le Chatelier's Law (Mashuga and Crowl, 2000) has been chosen for its simplicity and fast application for the calculation of LFL of flammable mixture:

$$LFL_{mixture}(v/v\%) = \frac{100}{\sum_{i=1}^{n} \frac{x_i}{LFL_i}}$$

Where "x_i" is the volumetric percentage of a single flammable compound and "LFL_i" indicates the lower flammability limit of the single flammable compound.

The gaseous mixture LFL has been solely calculated for the following reasons: (a) LFL represents the minimum concentration of vapor/gas in air below which the flame propagation will not occur in presence of an effective ignition source; (b) alarms in process industry, triggered by gases detectors, depend on LFL. The gases' LFL (expressed in volumetric percentage), produced by acidogenic fermentation are reported in Table 3 (Lauri et al., 2021).

3. Results and discussions

3.1. CSTR acidogenic fermentation and VFA production

All runs were performed in two conditions of temperature (mesophilic and thermophilic) without any pH-control strategy. Hence, the pH of the system tended to be 4.4–5.2 as the operative range of values. According to Lee et al. (2014), for optimal yields of VFA production, it is necessary to maintain the pH above 4.0 to not affect the fermentative microorganisms' activity, independently from the operating temperature. Maximal VFA production needs to be accomplished by obtaining the highest possible value of COD_{VFA}/COD_{SOL} ratio, which is the key parameter for the evaluation of an acidification process. Fig. 1 shows the VFA production trends as a function of time for all runs. Run 1 was operated for 85 days (roughly 15 HRTs), at 55 °C with a FW content of 50% (v/v) in a mixture with 50% (v/v) of MS. The total VFA concentration was characterized by a significant fluctuation and the range of variability was quite high (between 10 and 30 gCOD/L). Thermophilic temperature favored the organic matter solubilization; therefore, the average COD_{SOL} concentration was 51 ± 7 g COD_{SOL}/L. The oscillatory trend of VFA meant process instability and, despite the high VFA concentration potentially achievable, the average COD_{VFA}/COD_{SOL} ratio was 0.49 ± 0.07, meaning that 50% of solubilized matter remained unconverted into VFA. Compared to Run 1, the following Run 2 was characterized by lower content of FW (40% in Run 2a and 30% in Run 2b-c, respectively). A very long lag phase characterized the process start-up, and despite the reduced amount of FW in the mixture, the process suffered the same instability problems and performances' fluctuation as

Table 3
LFL of potentially explosive gases in fermentation reactor.

Gas	LFL (v/v %)
H_2	4
CH_4	4.4
H_2S	3.9

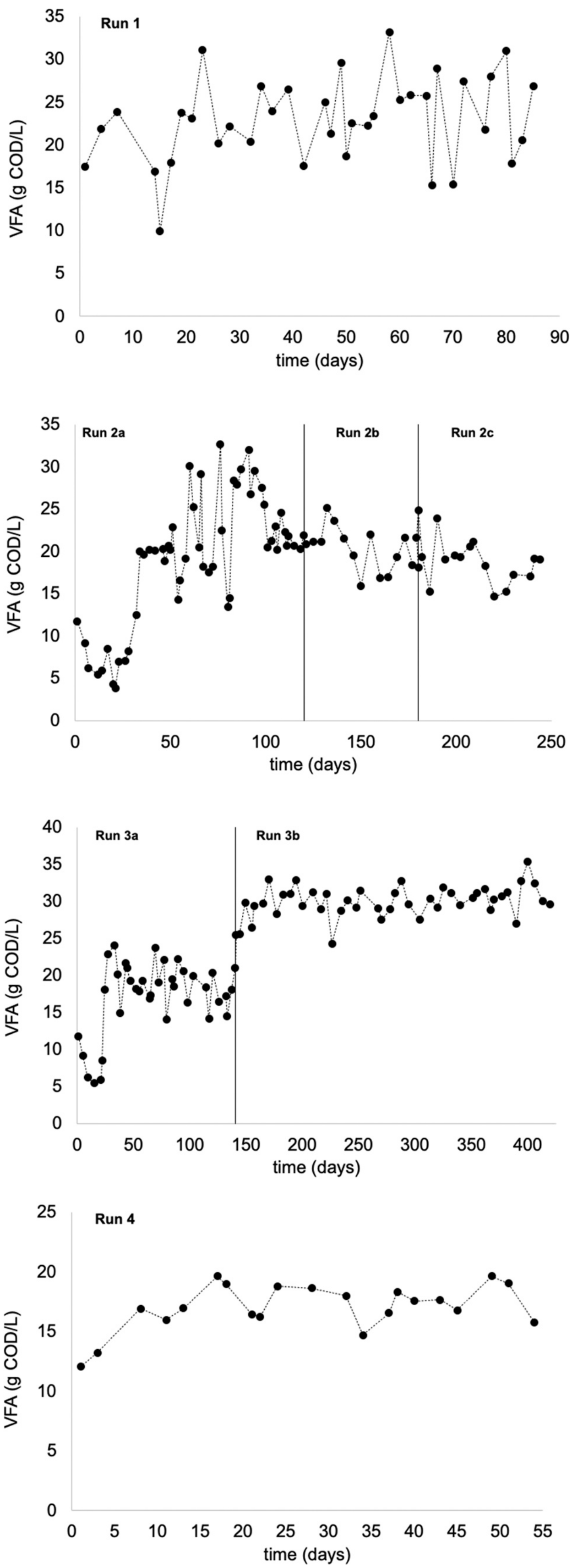

Fig. 1. Trends of produced VFA in the seven CSTR runs.

observed in Run 1. The average value of COD_{VFA}/COD_{SOL} ratio was 0.53 ± 0.06, statistically similar to the value obtained in Run 1 (ANOVA, Tukey HSD post-hoc test, $\alpha = 0.05$; Table 4). It has been underlined that thermophilic condition tends to increase the fermentation rate (Girotto et al., 2017) and, even though the FW content has been reduced to 40% w/w, the process remained unstable because of the fast fermentability of the feedstock and the frequent pH fluctuations (often below 4.5). Run 2b and 2c instead, exhibited a nearly stable VFA production ringing between 16 and 25 gCOD/L for Run 2b and between 15 and 23 gCOD/L for Run 2c, with an average VFA concentration of 20 ± 3 and 19 ± 3 $gCOD_{VFA}/L$ respectively. Compared to Run 2a, a significant increase of COD_{VFA}/COD_{SOL} ratio was detected: 0.64 ± 0.05 for Run 2b and 0.75 ± 0.04 for Run 2c. The simultaneous decrease of both FW (to 30% v/v) and temperature (to 42 °C) established the ideal condition inside the bioreactor for high stability in the acidification process; hence, a good selective pressure for the VFA-microorganism producers (Yi et al., 2014). In other words, low FW content combined to mesophilic temperature allowed to obtain good acidification yield and process stability. These results were in line with previous experiments (Morgan-Sagastume et al., 2015; Garcia-Aguirre et al., 2017), which demonstrated that mesophilic temperature was better performing than thermophilic one for VFA production on FW and MS. Run 3a and 3b were performed at a lower temperature (37 °C), maintaining the FW-MS content of 30–70% v/v; in addition, in Run 3b, the bioreactor was fed with thermally hydrolysed feedstock (72 °C, 48 h). Run 3a showed a stable VFA production after 25 days (roughly 4.5 HRTs); the average VFA concentration was equal to 20 ± 3 $gCOD_{VFA}/L$ on 26 ± 3 $gCOD_{SOL}/L$. Hence, an average COD_{VFA}/COD_{SOL} ratio of 0.73 ± 0.04 was achieved (substantially similar to Run 2c; ANOVA, Tukey HSD post-hoc test, $\alpha = 0.05$). Run 3b gave remarkable improvements in terms of acidification yield. VFA production was substantially higher than previous runs, achieving an average VFA concentration of 30 ± 2 gCOD/L coupled with the highest COD_{VFA}/COD_{SOL} ratio (0.86 ± 0.09; ANOVA, Tukey HSD post-hoc test, $\alpha = 0.05$; Table 4) observed. Hence, the separate thermal hydrolysis enhanced the solubilization of volatile solids and made them more available to the fermentative microorganisms' consortium. In accordance with these considerations, the fermentation yield was 0.62 ± 0.2 g COD_{VFA}/g VS_{in}, much higher than other yield obtained with no thermally hydrolysed feedstock. Run 4 was carried out at 25 °C, exploiting the benefits associated with the thermal hydrolysis previously discussed. Despite of the partially solubilised organic matter and the observed process stability from day 10 to the end of the run, the low temperature did not sustain the fermentative biomass as it was in the previous Run 3b: the average VFA concentration was the lowest observed (18 ± 2 gCOD/L), with a COD_{VFA}/COD_{SOL} ratio remarkably decreased compared to Run 3b (0.70 ± 0.02).

Fig. 2 summarizes the average VFA and COD_{SOL} concentrations, as well as the COD_{VFA}/COD_{SOL} ratio in all the acidogenic fermentation tests. As previously discussed, Run 3b shows optimum results and depicts the crucial roles of the thermal hydrolysis, especially dealing with feedstock containing a high volumetric fraction of secondary MS, which is a substrate not easily convertible into VFA (Morgan-Sagastume et al., 2015). In the literature, some authors positively discussed the effect of thermal pre-treatment on acidogenic fermentation for VFA production from organic waste. In Morgan-Sagastume et al. (2011), the high-pressure thermal hydrolysis (HPTH) applied on sewage sludge enabled up to 5-fold increase in VFA yield and 6-fold increase in VFA production rate. El Gnaoui et al. (2020) described the effects of thermal pre-treatment (100 °C, 30 min) on food waste for the enhancement of feedstock biodegradability in following anaerobic digestion process (measured a

Table 4
Characteristics of the fermentation effluents and fermentation performances obtained in all CSTR runs.

Parameter	Unit	CSTR Test						
		Run 1	Run 2a	Run 2b	Run 2c	Run 3a	Run 3b	Run 4
Total VFA	$gCOD_{VFA}/L$	24 ± 5	22 ± 4	20 ± 3	19 ± 3	20 ± 3	30 ± 2	18 ± 2
Soluble COD	$gCOD_{SOL}/L$	51 ± 3	46 ± 5	32 ± 2	25.0 ± 0.7	26.4 ± 0.7	35.3 ± 0.7	24.6 ± 0.3
VFA fraction	COD_{VFA}/COD_{SOL}	0.49 ± 0.07	0.53 ± 0.06	0.64 ± 0.05	0.75 ± 0.04	0.73 ± 0.04	0.86 ± 0.09	0.70 ± 0.02
pH	–	4.4 ± 0.3	4.6 ± 0.3	5.0 ± 0.2	4.9 ± 0.2	5.1 ± 0.2	5.2 ± 0.1	5.2 ± 0.1
Solubilisation	$gCOD_{SOL}/gVS_{in}$	539 ± 45	608 ± 39	477 ± 28	329 ± 21	360 ± 25	555 ± 27	319 ± 18
Yield	$gCOD_{VFA}/gVS_{in}$	0.33 ± 0.04	0.37 ± 0.03	0.41 ± 0.02	0.37 ± 0.01	0.38 ± 0.02	0.62 ± 0.02	0.34 ± 0.01

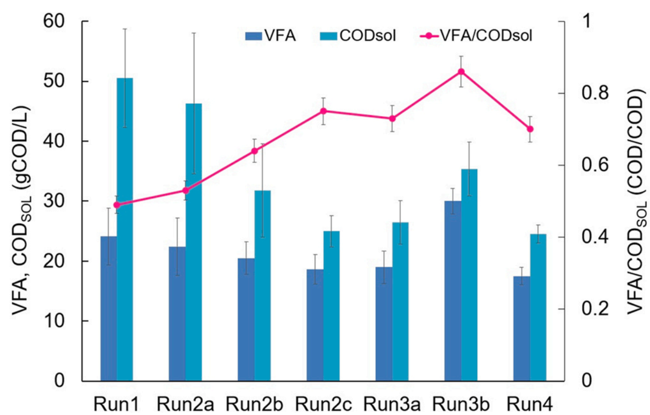

Fig. 2. Average VFA concentrations and COD_{SOL} levels achieved in the seven CSTR runs.

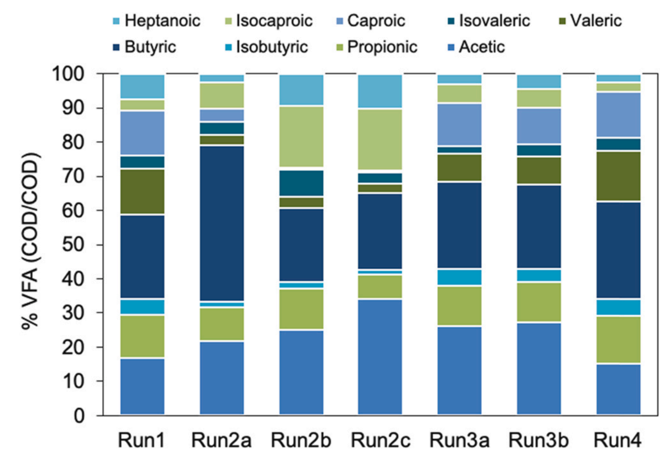

Fig. 3. Percentage of individual VFAs respect to total VFA in each run.

methane yield). Ali et al. (2021) reached high concentration of VFA (up to 60 g/L) in mesophilic batch fermentation tests, starting from selected food waste: apart from the adopted pH and inocula, the results showed the benefits of the thermal pretreatment on VFA production rates and yields. Moretto et al. (2019) demonstrated how VFA production in mesophilic and pH-controlled alkaline acidogenic fermentation (37 °C, pH 9.0) on batch and CSTR tests (at laboratory scale) can be optimized by applying thermal pre-treatment (76 °C for 72 h). In countertrend to the previous observations, the work of Vidal-Antich et al. (2021) showed that the hydrolysis pre-treatment did not enhance the fermentation yields and showed only minor kinetic improvements in sewage sludge and food waste mesophilic batch fermentation. The maximum fermentation yield was 0.48 $gCOD_{VFA}/gVS$, in tests conducted with sludge and food waste at 50% v/v each. In this past study, the thermal hydrolysis was only applied to the sewage sludge; it is expected to have comparable observations with all the cited studies (including this work) if such pre-treatment is applied to both carbon sources.

In summary, the main results obtained in the discussed seven runs are resumed in Table 4.

3.2. VFA distribution

The impact of changing operating parameters on VFA composition was also investigated. Fig. 3 shows the content (in percentage, COD basin) of each VFA compared to the total, in all runs. Among the short-chain and medium-chain VFA, acetic, propionic and butyric acids were those mostly present, with a content between 55% and 60% (and close to 80% in Run 2a). Valeric, caproic and isocaproic acid were also present in non-negligible quantities. Some literature examples show different VFA profiles in relation to the feedstock composition (Zhou et al., 2013), also reporting a wide range of process parameters such as pH, OLR, temperature, HRT, etc. (Moretto et al., 2019; Strazzera et al., 2018). In this study, all experiments have been carried out at the same pH and HRT. Hence, the differences in terms of VFA compositions were due to the feedstock composition, temperature, and thermal pre-treatment (where applied). More specifically, the decrease in food waste content (from Run 1 to Run 2a) caused a decrease in the longer-chain VFA (valeric, caproic and heptanoic acids) and an increase of short-chain VFA, especially acetic and butyric acids. The further decrease of food waste content (to 30% v/v) led to a substantial reduction of butyric acid with an increase of longer chain VFA (iso-caproic and heptanoic acid). No substantial changes were observed between Run 2b and 2c, where the T was decreased to mesophilic condition (42 °C) by maintaining similar feedstock composition. Therefore, feedstock composition played a crucial role in the VFA spectrum, while temperature did not, at least in the explored range (42–55 °C) from Run 1 to Run 2c. The further decrease of the temperature to 37 °C (Run 3a) still showed a net dominance of acetic, propionic, and butyric acids (closed to 60% COD basin); on the other hand, valeric (8.3%) and caproic (12.8%) acids increased remarkably if compared to Run 2c (2.7% and 0.5% respectively), which was conducted with the same feedstock composition and at 42 °C. The thermal hydrolysis applied before mesophilic fermentation temperature (37 °C, Run 3b) did not have any relevant effect on VFA distribution, compared to Run 3a. Previous lab-scale experiments conducted at mesophilic temperature did not show relevant change in VFA distribution when thermal pre-treatment was applied: a similar dominance of acetic and butyric acids was observed in fermented FW-MS mixture (Moretto et al., 2019). Even describing the benefits of the thermal hydrolysis on sewage sludge fermentation (2–5fold increase in VFA yield and 4–6fold increase in VFA production rate), also Morgan-Sagastume et al. (2011) found that the VFA spectrum was dependent on the type of feedstock more than hydrolysis application. Thermally hydrolysed sludge was utilised in mesophilic and thermophilic fermentation tests by Zhang et al. (2019); also in this case, the VFA spectrum was similar in the fermentation liquids coming from hydrolysed and raw sludge (dominance of acetic, butyric and valeric acids), independently from the chosen fermentation temperature. On the other hand, starting from an equally pre-treated mixture and conducting the fermentation process al low temperature (25 °C; Run 4), butyric (28.5%), valeric (14.9%) and caproic acid (13.6%) increased, at the expense of acetic acid, which decreased from 27.4% (Run 3b) to 15.3% (Run 4).

Previous researchers highlighted the importance of pH as the main parameter to drive a selective VFA production from organic waste residues in acid, neutral and alkaline environments (Garcia-Aguirre et al., 2017). In this study, the volume required for the pilot scale reactor did not allow the use of chemicals for pH control, not practical in the perspective of a full-scale application. Hence, the pH was similar in all runs; however, the effect of temperature and thermal hydrolysis on both VFA concentration and composition have been discussed.

Despite the observed differences in VFA distribution in all runs, the average molar concentration of acids with an even number of carbon atoms (C_{2+n}) was always higher than the molar fraction of acids with an odd number (C_{3+n}). Accordingly, the fermentation liquids were strongly dominated by the presence of C_{2+n}, with a ratio $[C_{3+n}/(C_{3+n} + C2_{+n})]$ in the range 0.15–0.31 mol/mol. This is a key aspect in the perspective of VFA utilization for microbial bioplastics synthesis as a tool for predicting the biopolymers composition and their potential application (Valentino et al., 2017).

It's important to highlight that the use of mixed consortium is generally associated to numerous metabolic pathways in the fermentation process, which led to a differentiation of the products and to a diversified VFA spectrum (Kumar et al., 2022). In a perspective of a process scaling up, techniques for the separation of single VFA must be considered.

3.3. Flammable gases production and calculation of LFL of gaseous flammable mixture

Besides VFA, the acidogenic fermentation of organic matter by anaerobic microorganisms generates by-products, such as flammable gas and solids residues. Apart from the inert carbon dioxide (CO_2), three flammable gases were usually found and analysed: methane, hydrogen, and hydrogen sulphide. All these gases belong to category 1 A, which is reported in regulation (CLP) n°1272/2008 EC (European Parliament and the Council of the European Union, 2008). Table 5 shows the average concentrations of gases generated in the fermenter and Fig. 4 shows the related trends (given the low or negligible content, the H_2S trends were not shown). In all runs, CO_2 had the highest concentration, which ranged between 77% and 85% v/v.

The volumetric H_2 concentration was higher in Run 1, 2a and 2b (the average is respectively equal to 18.0 ± 0.7, 14.8 ± 0.4 and 12.6 ± 0.3 respectively), where thermophilic temperature (55 °C) was applied. Higher volumetric H_2 content was also related to higher FW content, since FW is a more putrescible material compared to MS, and more rich organic matter (as volatile solids or soluble COD) (Gottardo et al., 2017). With the decrease of the temperature to 42 °C, the H_2 percentage in the gas phase decreased accordingly (10.4 ± 0.3% v/v; Run 2c). It is visible how hydrogen was steadily

Table 5
Gaseous phase composition in the acidogenic fermenter and related LFL.

Run	Gaseous phase composition (%; v/v - ppm)				LFL (%)
	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (ppm)	
1	77.5 ± 0.6	3.5 ± 0.2	18.0 ± 0.7	0.36 ± 0.05	18.5
2a	80.9 ± 0.3	3.1 ± 0.3	14.8 ± 0.4	0.4 ± 0.1	22.1
2b	84.3 ± 0.4	2.4 ± 0.3	12.6 ± 0.3	0.4 ± 0.1	26.3
2c	84.0 ± 0.4	4.8 ± 0.2	10.4 ± 0.3	0.06 ± 0.02	27.0
3a	85.1 ± 0.3	1.9 ± 0.5	12.0 ± 0.5	0.04 ± 0.02	29.0
3b	84.9 ± 0.3	3.9 ± 0.1	10.2 ± 0.3	0.04 ± 0.01	28.9
4	83.7 ± 0.5	5.6 ± 0.3	9.6 ± 0.5	0.36 ± 0.05	26.6

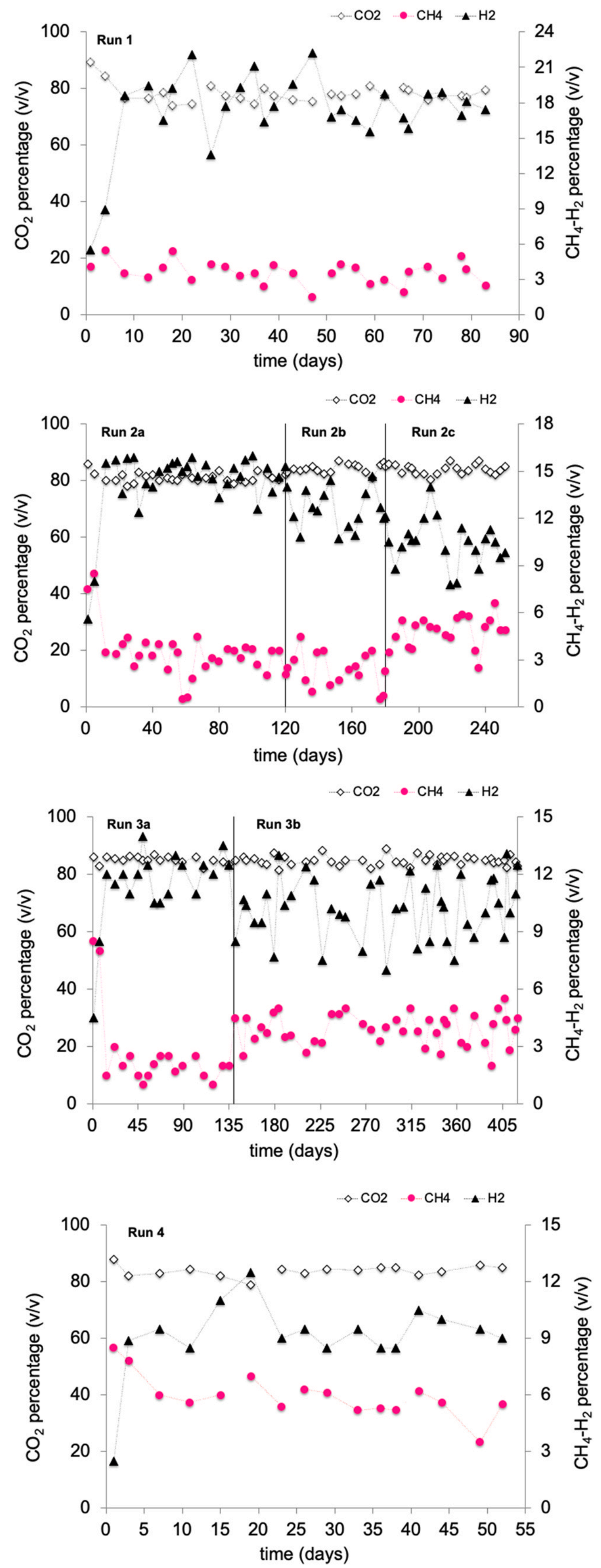

Fig. 4. Volumetric percentage of gases (CO_2, H_2, CH_4) generated per days during all the CSTR runs.

around 18% in Run 1. Such value was never achieved in the following runs, meaning that the high content of food waste gave readily biodegradable organic substrates to hydrogen-producers' microorganisms, easily activated by high temperature (55 °C; Pu et al., 2019). Lower temperature showed lower H_2 content in the gas phase (in the range 9.6–12%; runs 2c, 3a-b, and 4). On the contrary, except for Run 3a, the CH_4 amount was higher under mesophilic conditions than in thermophilic conditions. The relatively low HRT applied in all runs should not favor the methanogenic bacteria, which generally require at least 15–20 days as HRT and 7.0–8.0 as pH; the presence of CH_4, even though at a low level (1.9–5.6% v/v), could be related to the CH_4-producers' bacteria already present in feedstock (which was stored under quiescent conditions). H_2S was also detected, but at very low (0.36–0.40 ppm; Run 1, 2a-b, and 4) or negligible (0.04–0.06 ppm; Run 2c, 3a-b) content. Even though thermal hydrolysis improved the VFA production or the activity of fermentative bacteria, the flammable gases production was not particularly affected.

As required by the "Le Chatelier's law", the volumetric percentage of each flammable gas was used to calculate the LFL in each run. All lower flammability limits were always higher than those of single gases. The lowest LFL (18.5%) was obtained in Run 1, and it was mainly due to the highest percentage (18% v/v) of H_2. In runs 2a-b, also under thermophilic temperature, the food waste content was reduced and a decrease in H_2 concentration was observed; in addition, CH_4 and H_2S concentrations remained constant. Therefore, an LFL increase was observed (up to 26.3%). When the temperature was decreased to 42 °C (Run 2c), the gaseous mixture's LFL increased because of the decrease of H_2 content (10.4 ± 0.3% v/v). With the further decrease of the temperature (37 °C; Run 3a), the LFL accordingly increased to 29.0%. By applying the thermal hydrolysis (Run 3b), no substantial change in the LFL was observed (28.9%). A little decrease of LFL was observed in Run 4 (25 °C after thermal hydrolysis), attributable to the increase in CH_4 (5.6 ± 0.3).

Based on calculated LFL data, Run 1 was the most dangerous scenario due to the lowest LFL, while Run 3a-b corresponded to the safest operating conditions since a higher quantity of the flammable mixture is required for the ignition. Accordingly, the CO_2 content was higher in Run 3a-b (84.9–85.1% v/v): it is extremely important to highlight that inert gas like CO_2 helps to dilute the hazardous gas mixture (John et al., 2011) and makes it less dangerous. Therefore, high CO_2 content is recommended over the course of the acidogenic fermentation process addressed to VFA synthesis.

3.4. Recommendations for improving the safety of acidogenic fermentation reactor

The resources' recovery from organic waste in anaerobic bioreactors can generate combustible dusts and flammable gases, which could make the environment more hazardous for workers (Lauri and Pietrangeli, 2021; Lauri et al., 2021). Therefore, employers should adopt adequate safety measures aimed at preventing the workers' exposure for their protection. In the anaerobic acidogenic fermentation process, the formation of potentially explosive atmospheres is extremely unlikely because of exiguous oxygen presence; however, some recommendations should be considered to ensure a high safety level. The concentration of O_2, H_2, CH_4 and H_2S must be continuously monitored by specific sensors or probes. It is advisable to duplicate or triplicate the sensors' number to avoid interruption in the monitoring, in case of sensors' failure. With reference to flammable gases (H_2, CH_4 and H_2S), the detector must be characterized by specific technical requirements, such as high sensitivity, explosion proof, intrinsic safety, stability, and quick response. The LFL of the flammable mixture has important outcomes in terms of process safety because it is used to set the alarm thresholds. When the settled concentrations are exceeded, the gas detector triggers alarms, which cannot be arrested until an action is taken and therefore the alarms must be audible and visible.

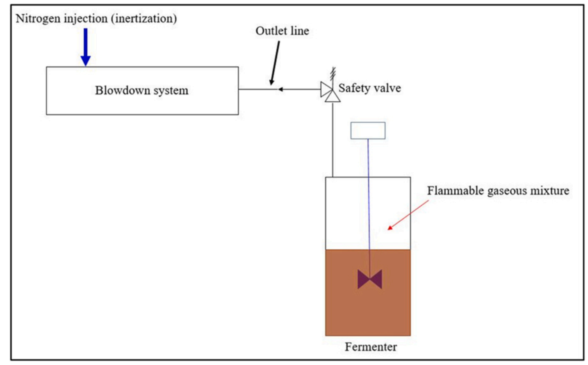

Fig. 5. Blowdown system to be installed in the acidogenic fermentation reactor.

With reference to industrial safety, LFL investigation provides fundamental and important information, because the alarms, triggered by gases detectors, depend on LFL. Indeed, in industrial processes, two alarm thresholds are generally established: (a) low-level alarm, and (b) high-level alarm.

The low-level alarm can act as a warning of a potential problem requiring investigation. On the contrary, the high-level alarm can trigger an emergency response, such as evacuation or shutdown, which has to be quickly adopted for ensuring the workers safety. The low-level alarm should be set as low as practicable, preferably no higher than 0.15 LFL, whereas the high-level alarm should be no more than 0.45 LFL.

As observed in this work, the feedstock composition strongly affected the LFL: relatively low content of food waste (30% v/v) had a positive impact on acidification performances (VFA concentration and COD_{VFA}/COD_{SOL} ratio) and safety aspects. LFL is also strongly affected by the operating temperature, which can be easily adjustable. The temperature was controlled by a thermostatic jacket and a hydraulic seal was installed for preventing dangerous overpressures. It is also noteworthy that the worst scenario in terms of safety (Run 1) was characterized by H_2 content of 18.0 ± 0.7% v/v; in case of selective H_2 generation in similar acidogenic fermentation process (usually thermophilic condition at HRT close to 3.0 days), H_2 content can increase up to 40% v/v (Gottardo et al., 2017). In practice, this can further decrease the LFL, which can act as an important tool in the evaluation of the process safety for workers. In vision for a full-scale plants implementation, it is recommended to install safety valves for avoiding overpressures inside the fermentation reactor, because these valves decrease the pressure in a shorter time compared to the hydraulic seal. To ensure the continuous fermenter operating, safety valves duplication is fundamental. As the acidogenic fermentation generates a gaseous flammable mixture, the fermenter vent must be conveyed to a blowdown tank, where it is recommended to inject nitrogen (inert gas) to dilute the atmosphere and inhibit the creation of a potentially explosive mixture. According to Directive 2014/34/EU (European Parliament and the Council of the European Union, 2014), all devices (level indicator, pressure and temperature transmitter, etc.), equipment's control, safety systems, etc., which are intended for use in Atex zones (areas characterized by the possible presence of potentially explosive atmospheres), must be certified. (Fig. 5).

4. Conclusions

The municipal organic waste represents a good feedstock for the VFA production as added-value products by acidogenic fermentation. High content of FW (above 40% v/v) showed low potential in the conversion of volatile solids into VFA and difficulties in the control of the process (low stability due to frequent pH fluctuations). On the other hand, with a FW content below 40%, the mesophilic temperature coupled to a thermal hydrolysis as pre-treatment was the best option for VFA production in CSTR acidogenic fermenter for both final concentration and acidification grade (COD_{VFA}/COD_{SOL}). In general, the VFA composition was not substantially affected by thermal pre-treatment, but it was seriously modified by the feedstock composition and the chosen temperature in the acidification process.

In a perspective of full-scale implementation, LFL can be considered an extremely useful tool for assessing explosion risk in acidogenic fermentation since the dangerousness of the process is mainly due to the flammable gases production. The operating conditions adopted in this study led to a volumetric CO_2 (inert gas) percentage always high (in the range 77–85% v/v); moreover, mesophilic temperature tended to increase the value of LFL (close to 29). Remarkably, this condition has been found in parallel to the highest VFA production (30 ± 2 g COD_{VFA}/L) and acidification yield (0.62 ± 0.02 g COD_{VFA}/g VS_{in}). Hence, it should be considered as the representative condition for a sustainable and realistic fermentation in a scaled-up version of the urban waste acidogenic fermentation process. In addition, even though anaerobic fermentation does not support the explosive risk due to the absence of O_2 in the gas phase (in theory), its concentration monitoring is highly recommended in a full-scale plant.

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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