Acidogenic Fermentation of Urban Organic Waste: Effect of Operating Parameters on Process Performance and Safety

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Biorefinery represents an innovative approach for waste management, where products at the end of their service life are seen as valuable resources for the production of high added value bio-products or bio-fuels. In this context, acidogenic fermentation is gaining scientific and commercial interest, since it allows to improve waste/wastewater treatability as well as additional recovery of volatile fatty acids (VFA), the building blocks for the chemical industry or precursors of reduced chemicals in conventional organic chemistry. This work illustrates the results of a study aimed at highlighting the effects of different inputs (feedstock composition and pre-treatment; temperature) on process performance and safety. The fermentation process was applied on a mixture of food waste and sewage sludge (FWs-SS) within the contest of Treviso municipality (northeast Italy). The VFA production and relative ratio respect to soluble chemical oxygen demand (COD_{SOL}) were evaluated, as well as the variation of lower flammability limit (LFL) of flammable gaseous mixture, produced during the acidogenic fermentation. Thermal pre-treatment (72°C, 48 h) enhanced the solubilization of the organic matter, which was converted into VFA in batch mode under mesophilic conditions (37°C). The VFA level increased up to 30 ± 3 g COD/L, with high COD_{VFA}/COD_{SOL} ratio (0.86 ± 0.05). This condition was also characterized by the lowest level in volumetric percentage of flammable gases such as H_2, CH_4 and H_2S. Variation of LFL of gaseous mixture as function of fermenter operating parameters has been investigated by Le Chatelier's Law. The high concentration of CO_2 (greater than 80% v/v) increased the LFL and therefore the flammable mixture was considered poorly hazardous. In practice, the variation of fermenter operating parameters to the final optimized protocol caused a continuous LFL growth, up to 29.3%, corresponding to a safer operation of fermentation reactor.

1. Introduction

The biorefinery concept, as the key to the circular economy approach, contributes to environmental sustainability by reducing the consumption of raw materials and decreasing amount of waste. Consequently, research on the use of renewable resources for the industrial processes has been intensive in the last years: product and process innovation in industrial manufacturing is the goal and an essential element of competition between companies, as it will improve their business performance (Nghiem et al., 2017). In the context of anaerobic treatment for urban waste valorisation, there are some other valuable by-products which can compete with methane and have a market for itself, such as volatile fatty acids (VFA). Carboxylic acids are building blocks for the chemical industry (Sauer et al., 2008), as precursors of reduced chemicals and derivatives (esters, ketones, aldehydes, alcohols and alkanes) in conventional organic chemistry (Dahiya et al., 2015), or constitute a valuable resource for biodegradable polymer production (Valentino et al., 2017).Within biorefinery context, the VFA production is carried out through fermentation processes, which may involve the use of single anaerobic bacterial strains or Mixed Microbial Cultures (MMC). Although MMC may lead to lower yields in terms of VFAs, they have several advantages, since non-sterile conditions are needed,

and risk of contamination is decreased (Valentino et al., 2017). Process parameters and/or operating conditions such as temperature, pH, hydraulic retention time (HRT) and organic loading rate (OLR) may affect the fermentative activities of the MMC community in terms of VFA yield and distribution. Moreover, the production of other fermentation by-products, such as longer chain fatty acids, other carboxylic acids, alcohols and biohydrogen can be also driven by the chosen operating parameters/conditions in the fermentation process (Moretto et al., 2019). An update review focused the attention on the VFA production from food wastes (FWs), optimal candidates for the biorefinery processes as one of the most abundant organic substrates annually produced around the world (Strazzera et al., 2018). The authors evidenced that FWs present high organic matter content (100–150 g COD/L) and nitrogen and phosphorous concentrations (2-15 g/L and 0.5-1.0 g/L, respectively) which make them adequate for VFA. The analysis of the most promising pre-treatments indicates that 0.5–3% HCl and H_2SO_4 additions and thermal pre-treatment (140–170°C) increase the organic matter's solubilisation. The best operative conditions are a slightly controlled pH (6.0–7.0), short HRT (1–7 days), thermophilic temperatures and OLR of about 10 gTS/L d (Total Solids). For some of the applications requiring VFA, the composition of fermentation products is a critical aspect when proposing the valorisation of organic waste streams into VFA (Valentino et al., 2017). With particular emphasis on the urban scenario, FW and sewage sludge (SS) are the two major carbon sources which could be easily converted into VFA (Valentino et al., 2019). This study is aimed at highlighting the effects of some process parameters on fermentation performance and safety aspects. The influence of these parameters is emphasized in terms of VFA production and variation of lower flammability limit (LFL) of flammable gaseous mixture, produced during the process. It has been demonstrated that VFA production is strictly connected with fermentation performance, whereas LFL has outcomes on process safety suggesting that the success of an industrial application is strongly dependent on performance and safety level improvement.

2. Materials and Methods

The typical MMC technology utilized for biopolymer (polyhydroxyalkanoates; PHA) production is based on three main stages (Moretto et al., 2020). Firstly, the acidogenic fermentation of the organic substrate for the production of volatile fatty acids (VFA), which are the preferred substrate of PHA-producing microorganisms; then, two aerobic steps are necessary for a) the selection/enrichment of biomass (MMC) able to synthetize PHA (PHA-accumulators) and b) the final accumulation of PHA inside the cellular walls up to 50-60% on cell dry weight. In both aerobic stages, the C-source is the VFA-rich stream obtained in the first acidogenic fermentation step.

2.1 Acidogenic fermentation: operating parameters

The role of acidogenic fermentation is the carbon conversion into VFA (as Chemical Oxygen Demand; COD_{VFA}), and their accumulation in the liquid phase. For this purpose, it was considered fundamental to play on the COD_{VFA}/COD_{SOL} ratios which express the conversion rate of the organic substrate in preponderance towards the VFA (Valentino et al. 2017). The anaerobic fermentation was carried out in a 380 L Continuous Stirred Tank Reactor (CSTR) equipped with a mechanical stirrer, with a hydraulic retention time (HRT) of 5 days, organic loading rate (OLR) of 10-17 kg VS/m^3 d (Volatile Solids), and pH 4.5-5.5. The temperature (T) was controlled by means of a thermostatic jacket. Five operating conditions (Run 1-5) were performed as summarized in Table 1. The COD_{VFA} concentration, COD_{VFA}/COD_{SOL} ratio and the composition of the gas mixture were monitored and quantified. The volumetric FWs-SS percentages were changed from Run 1 to following runs; the T was progressively decreased from Run 1-2 (55°C) to Run 3 (42°C) and Run 4-5 (37°C). Thermal pre-treatment was only applied in Run 5, before mesophilic fermentation. In each run, the process was considered sufficiently stable and under steady state after consistent VFA production was reached. No inoculum was used at the beginning of the process, since the FWs already contained fermentative consortia. COD, VFA as well as gaseous phase were analysed as reported in Micolucci et al. (2016).

Table 1: Feedstock composition and operating parameters of the five CSTR fermentation process

Run	Window time (d)	Feedstock	T (°C)	Thermal pre-treatment	OLR (kg VS/m^3 d)
1	0 - 120	FWs 40-45%; SS 55-60%	55	-	15 - 17
2	121 - 180	FWs 30-35%; SS 65-70%	55	-	10 - 13
3	181 - 250	FWs 30-35%; SS 65-70%	42	-	10 - 13
4	0 - 140	FWs 30-35%; SS 65-70%	37	-	10 - 13
5	141 - 420	FWs 30-35%; SS 65-70%	37	72°C - 48 h	10 - 13

2.2 Explosive risk assessment and safety aspects

The analysis has been focused to assess the creation of potentially explosive atmospheres in the CSTR. Explosion risk assessment is composed by specific steps (Molino et al., 2012), from the identification of hazardous properties of substances (e.g. flammability range) and the emission sources of flammable compounds to the identification of measures aimed at ensuring explosion safety. A potentially explosive atmosphere is generated by a mixture of air and flammable compounds (vapors, gases or dusts), which, after ignition, allows self-sustaining flames propagation. Hence, an explosive atmosphere represents a real problem if the three following conditions contemporaneously occur: a) presence of flammable compounds, having a concentration (in air) between lower flammability limit (LFL) and upper flammability limit (UFL); b) air presence; c) ignition sources presence (sparks, hot surfaces, etc.).

Even though the FWs-SS fermentation can produce a flammable gaseous mixture, it is important to highlight that the CSTR fermenter is an anaerobic reactor and therefore the formation of explosive atmospheres is extremely improbable because of oxygen absence. However, in order to guarantee a high safety level an oxygen monitoring needs to be continuously carried out. In the industrial plants exist several components (valves, flanges, pumps, compressors, etc.), which can become potential sources of release of flammable compounds in case of failure (Lauri et al., 2018). These releases could generate hazardous zones due to the presence of potentially explosive atmospheres. In accordance with Atex Directive 99/92/EC, the employer is obliged to classify the workplaces, where potentially explosive atmospheres could occur. Hence, the LFL is a crucial parameter for improving the safety level.

2.3 Calculation of the Lower Flammability Limit (LFL)

Substances/mixtures flammability domain represents the concentrations range, in which a flammable mixture or compound can fire or explode in presence of an active ignition source. Two thresholds delimit such range: lower flammability limit (LFL) and upper flammability limit (UFL).In order to prevent fires and explosions a detailed knowledge of flammability range is required. Flammable compounds (hydrogen, methane, ethanol, etc.) are generally provided with safety data sheets, but, in case of flammable mixtures, the flammability data often are scarce or unavailable. Such limits can be estimate by experimental tests, which usually consume time and money and sometimes are impossible because of emergent requirements. In literature, in order to assess mixtures flammability limits some predictive models are available. In this study, the chosen model is the Le Chatelier's Law, which is widely used for its simplicity, fast application and effectiveness in estimating the flammability limits of gaseous mixtures (Mashuga and Crowl, 2000):

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

Where:

- x_i is the volumetric percentage of flammable substance;
- LFL_i is the lower flammability limit (expressed in volumetric percentage) of the single flammable compound.

The attention has been only focused on lower flammability limit for the following reasons: when flammable compounds concentrations are lower than LFL, the process is safer, because LFL represents the minimum concentration of vapor/gas in air below which the flame propagation will not occur in presence of an ignition source; on the other alarms triggered by gases detectors, solely depend on lower flammability limit.

3. Results and discussion

3.1 Acidogenic fermentation and VFA production

The first three runs were continuously conducted. The FWs content in the mixture was 40-45% v/v (Run 1) and it was maintained for 120 days (24 HRTs). The higher OLR (due to the higher FWs content) caused significant fluctuation of VFA concentration and lower conversion yield of COD_{SOL} into VFA. VFA concentration and COD_{SOL} were 23 ± 6 g COD_{VFA}/L and 45 ± 8 g COD_{SOL}/L), with a COD_{VFA}/COD_{SOL} ratio of 0.53 ± 0.11, meaning that a significant fraction of solubilized COD (or VS) was not converted into VFA. By decreasing the FWs content (Run 2), a more stable VFA production was observed, even with lower average value 21 ± 3 g COD_{VFA}/L; COD and VS conversion into VFA led to a higher COD_{VFA}/COD_{SOL} ratio of 0.64 ± 0.07 compared to Run 1. In Run 3, despite the lowest VFA level (19 ± 1 g COD_{VFA}/L), higher COD_{VFA}/COD_{SOL} ratio of 0.75 ± 0.06 was observed. The temperature of 42°C (lower kinetics compared to Run 1-2) and the synergistic effect of higher SS content (compared to Run 1) led to higher process stability in terms of performances fluctuations (VFA concentration and COD solubilization). The following two Runs 4-5 were conducted by maintaining lower FWs content (30-35% v/v) and applying mesophilic temperature of 37°C. In Run 5, a thermal feedstock pre-treatment was performed in a separated tank. In both runs, the process showed stable performances and the

pH maintained itself between 5.0 and 5.5, due to the biological sludge buffering capacity (Cabbai et al., 2016). Run 4 showed a stable VFA production, between 18 and 21 g COD_{VFA}/L. The COD_{VFA}/COD_{SOL} ratio values fluctuated from a minimum of 0.65 up to 0.79. In practice, no substantial differences were observed in the acidification performances between Run 3 and Run 4. On the contrary, Run 5 was characterized by the highest VFA production and COD_{VFA}/COD_{SOL} ratio compared to all the previous runs. The average VFA concentration and COD_{VFA}/COD_{SOL} ratio were equal to 30 ± 3 g COD_{VFA}/L and 0.85 ± 0.05 respectively.

From the VFA-rich stream utilization perspective, Run 5 showed optimum results and it proved the crucial role played by the thermal pre-treatment stage before mesophilic fermentation. Recent studies (Moretto et al., 2019) have demonstrated similar outcomes in a bench-scale fermentation test on similar urban waste mixture: high COD and VS solubilization following by remarkable acidification can be achieved by applying thermal pre-treatment and alkaline fermentation (pH 9).

3.2 Flammable gases classification and calculation of LFL of gaseous flammable mixture

Acidogenic fermentation of FWs-SS feedstock produced a flammable gaseous mixture, which is composed by hydrogen (H_2), methane (CH_4), hydrogen sulfide (H_2S) and carbon dioxide (CO_2). Apart from CO_2 (inert gas), flammable gases (H_2, CH_4 and H_2S) belong to category 1A reported in regulation (CLP) n°1272/2008 EC, and they have a LFL expressed in volumetric percentage (4.0%, 4.4% and 3.9% respectively for H_2, CH_4 and H_2S). Indeed, the most hazardous substances/mixtures are characterized by low LFL and large flammability range. An effective improvement of safety level has been achieved by optimizing the fermentation process conditions (in terms of VFA production). In fact, variation of LFL of gaseous mixture as function of fermenter operating parameters (Runs 1-5) has been investigated by Le Chatelier's Law.

The following Figure 1 shows the gaseous mixture composition in the five performed CSTR runs. The H_2 production seemed to be negatively affected by the mesophilic temperature and lower FWs content in the mixture in accordance with a previous study (Micolucci et al., 2016); similar trend was observed also for H_2S. Both gases exhibited the highest content (%, v/v) in Run 1, with higher FWs content and T: 14.7 ± 0.4 % and 0.5 ± 0.02 % for H_2 and H_2S respectively. Run 5 exhibited more variability in the percentage for both gases but the lower average content (10.2 ± 0.8 H_2 % and 0.04 ± 0.01 H_2S %) contributed to the improvement of the safety level. On the contrary, CH_4 level seemed to be more abundant under mesophilic T, in the fermentation trials conducted with lower amount of FWs in the feedstock: 4.8 ± 0.5 % and 3.9 ± 0.4 % were the average CH_4 content (v/v) in Run 3 and Run 5. The trend of CH_4 percentage is in contrast to H_2 and H_2S trends and they differently affected the safety level of the fermentation process.
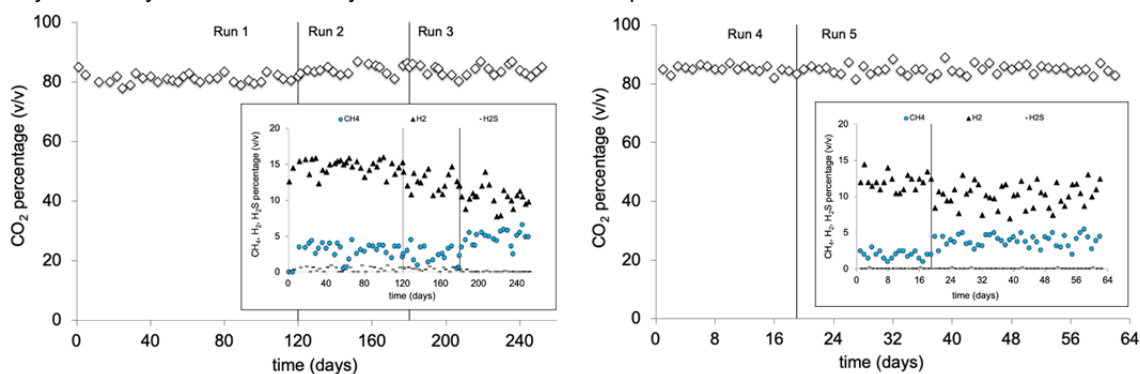

Figure 1: Composition of the gas mixture in the acidogenic CSTR fermenter for the five runs

The different gas phase composition and the LFL (%) calculated for each fermentation trial are reported in Table 2. Run 1 can be considered the starting point, at which the LFL was the lowest and equal to 22.5%. Run 2 was characterized by a decrease of the FWs. This led to an increase of LFL (a growing amount of flammable mixture is required for the ignition) to 26.3%, because flammable gases (mainly H_2 and CH_4) concentrations decreased and CO_2 concentration increased. Switching to mesophilic T (Run 3-4-5), H_2S concentration was extremely low or absent (0.04-0.06 %), with one order of magnitude lower than levels achieved under thermophilic conditions. This evidence contributed to the increase of the safety related to the fermenter. Run 3 was extremely similar to Run 2 for LFL value (27%) due to opposite effects: the decrease of H_2 (from 12.6 to 10.4%) and H_2S (from 0.5 to 0.06%), and the increase of CH_4 (from 2.4 to 4.8%) concentrations. Run 4 and 5 were characterized by the maximum concentration of CO_2 (roughly 85%), even though H_2 and CH_4 concentrations were different. In Run 4, higher H_2 concentration was observed (12.1%), whereas Run 5 exhibited higher CH4 level (3.9%). In practice, both gases had opposite effects and caused an exiguous increase of LFL from Run 4 (28.7%) to Run 5 (29.1%). As a general consideration, the large

concentration of CO_2 (always greater than 80% v/v) contributed to have particularly high LFL, in accordance with other literature data where the presence of 25% CO_2 and the CH_4/H_2 ratio of 10/1 mixture did not cause explosions (Ma et al., 2010). In addition, variation of fermenter operating parameters caused a continuous LFL growth, which corresponded to a safer process operation. Run 1 was the most hazardous scenario, being characterized by the lowest LFL (highest H_2 and lowest CO_2 concentrations). Run 5, which corresponded to optimized acidification condition, was the less hazardous scenario (highest LFL), which was generated by an extremely high CO_2 and the lowest H_2 concentration. Therefore, it is important to highlight that the optimization of fermenter operating conditions has improved both fermentation performance (in terms of VFA production) and safety level. The T decrease had also positive outcomes in terms of fermentative process safety, because it caused a LFL increase. The variation or increase of LFL from one Run to another was extremely correlated to the change of T and gas distribution. The highest LFL increase (almost 17%) was detected from Run 1 to Run 2 (H_2 and CH_4 concentrations decreased and CO_2 concentration increased), whereas the other variations did not exceed 6.3%. This was due to the following phenomena: low variation of CO_2 concentration (it did not exceed 1.2%) and balanced opposite trend of H_2 and CH_4 concentration.

Table 2: Gas phase composition in the acidogenic fermenter and related LFL

Run	CO_2 (%)	CH_4 (%)	H_2 (%)	H_2S (%)	LFL (%)
1	81.1 ± 0.9	2.9 ± 0.6	14.7 ± 0.4	0.50 ± 0.02	22.5
2	84.3 ± 0.9	2.4 ± 0.4	12.6 ± 0.5	0.40 ± 0.01	26.3
3	84.0 ± 0.7	4.8 ± 0.5	10.4 ± 0.4	0.06 ± 0.01	27.0
4	85.0 ± 0.8	2.0 ± 0.3	12.1 ± 0.5	0.04 ± 0.01	28.7
5	84.9 ± 0.6	3.9 ± 0.4	10.2 ± 0.8	0.04 ± 0.01	29.1

3.3 Safety aspects of the acidogenic fermentation reactor

Though the formation of potentially explosive atmospheres is extremely improbable in the fermentation reactor, some technical recommendations have to be adopted to ensure an adequate safety level: monitoring O_2, H_2, CH_4 and H_2S concentrations in gas phase in order to avoid the flammability field of gaseous mixture; avoid air and/or ignition sources in the fermenter. Furthermore, in order to ensure a continuous O_2 and flammable substances monitoring, the duplication (or triplication) of sensors and control apparatus is recommended. Gases detectors need to have specific qualifications (explosion-proof and intrinsically safe) and to be characterized by high sensitivity, long term stability and fast response. A flammable gas detector can be used to trigger alarms if a specified concentration of the flammable mixture is exceeded. If a specified mixture concentration or set point is exceeded, the detection system must trigger an alarm. The alarm should not stop or reset unless a deliberate action is taken. The alarm should be both audible and visible. The detector needs to be set to a low level in order to ensure the health and safety of the workers, but also at high enough level to prevent false alarms. Hence, it is recommended to set two alarm levels: low-level alarm and high-level alarm. The low-level alarm can act as a warning of a potential problem requiring investigation and it should be set as low as practicable, preferably no higher than 10-15% of gaseous mixture LFL. The high-level alarm can trigger an emergency response such as evacuation or shutdown and it should be no more than 50% LFL. An increase of pressure (P) inside the fermenter, due to an excessive heat exchange or failure of valve and T-control system, can also lead to explosive risks. The pilot CSTR utilized in this work was equipped with a hydraulic seal for preventing dangerous overpressures. In case of a full-scale plant design, it is recommended to install safety valves (SV; in duplicate) against overpressures since it allows to decrease the P inside the fermenter in a shorter time compared to the hydraulic seal. SV operation pressure must be lower than fermenter design P in order to preserve its structural resistance. Because of the formation of a flammable gaseous mixture in the fermenter, it is recommended that the SV vent is conveyed to a blowdown tank, in which nitrogen has to be injected to avoid the possible creation of explosive atmospheres. In order to avoid the possible explosive mixtures ignition, all devices and meters (T, P and level transmitters, etc.) have to be certified for use in potentially explosive atmospheres, according to Directive 2014/34/EU.

4. Conclusions

The VFA production from FWs-SS as renewable feedstock of urban origin, has been extensively demonstrated at pilot scale under different process conditions. FWs content below 40% v/v in the mixture ensured more stability in the acidification process performed without pH control whereas higher FWs content led to higher but not constant VFA production with a serious problem in terms of process management and control. The feedstock thermal pre-treatment associated to the mesophilic fermentation was the best compromise for a stable and sufficiently high VFA production from the solubilized organic matter. From the

safety point of view, although the explosive risk was extremely low due to the anaerobic medium, oxygen cannot be completely absent, and its concentration needs measured rigorously throughout the whole process. The LFL can be considered as a suitable tool for assessing the explosive risk of gas and gaseous mixture inside the fermenter and the lowest LFL corresponds to extremely flammable compounds. The high CO_2 (inert gas) concentration measured in every run (it always exceeds 80%) is extremely favourable in order to improve the safety level of acidogenic fermentation (inert gases cause an increase of gaseous mixtures LFL). The flammable gases produced during the fermentation of organic waste and wild sludge are essentially CH_4, H_2S and H_2. Since H_2S is produced in a very low percentage, the process hazard mainly depends on the percentage of CH_4 and H_2 in the mixture and on the presence of potential ignition sources. In an open or closed environment, the set-up of alarm thresholds and the gas detectors presence are essential to ensure the workers safety and minimize damages.

Acknowledgments

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