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Influence of temperature and hydraulic retention on the production of volatile fatty acids during anaerobic fermentation of cow manure and maize silage

Cristina Cavinato[a]*, Cinzia Da Ros[a], Paolo Pavan[a], David Bolzonella[b]

[a] University Ca' Foscari of Venice, Department of Environmental Sciences, Informatics and Statistics, via Torino 155, I-30172 Mestre, Venice, Italy. (cavinato@unive.it)

[b] University of Verona, Department of Biotechnology, Strada le Grazie 15, I-37134, Verona, Italy.

Abstract

The aim of this study was to verify the efficiency of a separate hydrolysis step by testing different working temperatures (37° to 55 °C) and hydraulic retention times (two, four and six days) and by evaluating readily biodegradable carbon production. The fermentation products included primarily acetic, propionic and butyric acids. These acids can be easily converted into biogas or can be recovered in a biorefinery approach, for example, to produce polyhydroxyalkanoates. The optimal condition was found by applying an organic loading rate of 17.9 gTVS m^{-3} with a four-day retention time at 37°C for an acidification yield of 183.2 $gCOD_{VFA}$ $kgVS_{fed}^{-1}$.

Keywords

fermentation, anaerobic digestion, maize silage, cow manure, volatile fatty acid.

* Corresponding author at: University Ca' Foscari of Venice, Department of Environmental Sciences, Informatics and Statistics, via Torino 155, I-30172 Mestre, Venice, Italy. Tel. (39) 0412348909. E-mail address: cavinato@unive.it (C. Cavinato).

1. INTRODUCTION

The objectives according to the energy policy in Europe, which strongly promotes the use of renewable sources for energy production, are to achieve a 27% rate of recovered energy from these renewable sources while simultaneously reducing by 40% the emissions of greenhouse gasses (GHG) by 2030 (European Commission, 2014). Among the solutions, anaerobic digestion (AD), which is regarded as one of the most applied and sustainable technologies, could be an optimal management answer for different types of wastes, such as agro-waste, the organic fraction of municipal solid waste and food-processing waste. Moreover, anaerobic digestion is considered a vital part of the European bio-economy as it taps into leftovers of other industries and thereby improves European resource efficiency (European Biogas Association, 2015). The production of renewable energy from agricultural feedstock has grown significantly in Europe with 11,670 anaerobic digestion plants in 2014, many of which are located in Germany and Italy. As co-digestion systems, these plants can simultaneously treat two or more substrates, an approach that balances the nutrient requirements and increases the methane production yields. As reported by Mata-Alvarez and co-authors (2014), in the rural sector, AD has been established as an important economic alternative, specifically as a renewable energy source. Hence, animal manures have become an important raw material. Manure (mainly cow manure) in the co-digestion AD process is fundamental due to its low Carbon/Nitrogen (C/N) ratio and its good buffer capacity, which can balance the C-rich substrates usually added to increase biogas production. In Germany and Italy, even if the use of energy crops is decreasing in favor of other biomasses, i.e., food processing byproducts and other organic residues, the primary C-rich feedstock remains crop silage, particularly maize silage (Linke et al. 2013), because it is easy to store and has high biogas potential. AD is a complex multi-step process that exhibits a tendency to deteriorate, especially when the composition of feedstock or the operational conditions are improperly chosen (Klimiuk et al. 2015). Maize silage is characterized by high fiber material content that consists of several components, such as cellulose, hemicellulose and lignin. During anaerobic digestion, cellulose and hemicellulose are quite easily converted into soluble compounds such as volatile fatty acids (VFA), whereas lignin limits the accessibility to hydrolytic enzymes and thus prevents degradation. An accumulation of VFA could cause an acidification of the system and a reduction in biogas yields, whereas the presence of lignin could decrease the expected yield. As biodegradation of structural carbohydrates during AD is a rate limiting process, requiring a large volume of a digester and a longer retention time results in an increase in the overall cost (construction and operational) of the system (Yu et al., 2014). Because the feedstock composition is important, i.e., the crop silage/manure volatile solids ratio, the biogas plant must be properly loaded to avoid a process breakdown. Though studies have investigated how to avoid digester overload by monitoring certain process parameters, such as propionic acid, organic loading rates, etc. (Kampmann et al., 2014, Comino et al., 2010, Linke et al., 2013), an easy way to avoid process failure and to improve the biogas yield is to pre-treat the feedstock mixture. Pre-treatment could accelerate the hydrolysis process and thereby improve the final methane conversion and hydraulic retention time reduction. In fact, in a single-stage process, the hydrolytic step can cause an imbalance in the stability of the process due to organic acid accumulation, for example, and a drop in the pH level (generally below 5 or 6). These conditions are unfavorable for methanogenic microorganisms and lead to the failure of the AD process. As the first stage reactor (pre-treatment) optimizes the (bio)chemical-physical conditions for the hydrolytic and acidogenic processes, it is possible to control the amount of readily biodegradable organic matter fed into the digester and thus avoid an uncontrolled pH drop (Ly et al., 2010). There are several ways to pre-treat lignocellulosic substrates, including chemical, mechanical and thermal technologies as well as various combinations of the three (Behera et al., 2014, Zheng et al., 2014). However, these expensive pre-treatments are applied, generally, to a sole lignocellulosic biomass that is often related with bioethanol production or bio-product recovery.

In the context of the farm scale, biological hydrolysis is a simple way to pre-treat the feedstock as it allows for the recovery of more energy from complex substrates during AD following hydrolysis, the exploitation of the manure's microorganisms, the reduction of the negative impact on the methanogenic pathway (no overload) and the enhanced creation of an anaerobic biorefinery due to the recovery of valuable products from fermentation, i.e., VFA.

The application of a two-stage process, with the production of VFA in the first phase, provides the opportunity to produce high value bio-based compounds. In general, multi-stage and temperature phased processes maximize energy recovery when slowly treating biodegradable substrates such as waste activated sludge (Bolzonella et al., 2007), straw, and other lignocellulosic substrates (Ly et al., 2010, Parawira et al. 2008). Decentralized biorefineries may further provide economic returns at the farm level, whereas the added value of products from biomass in present pathways is retained primarily by the industry, and may allow, for example, direct (re-) fertilization of the soils producing the processed biomass. The bio-economy, for the most part, depends on a reliable supply of biomass with regard to quality and price. Today's biorefineries often rely on only one specific type of biomass, e.g., wheat straw. To be more resilient to changes in the supply of raw materials (potentially due to climate change, crop seasonality and/or crop type), the development of prices and other external factors as well as biorefinery concepts with multi-input capabilities must be explored. Particularly, at the local level, this would enhance the benefits per unit of biomass and contribute to regulating the market of agricultural products while also contributing to the adaptation to climate change (Bruins and Sanders, 2012).

The aim of this study is to optimize the fermentation step as a pre-treatment of cow manure and maize silage and to improve biofuel and the production of VFA in an anaerobic biorefinery context.

2. MATERIALS AND METHODS

2.1 Substrate Characterization

The substrates used in this experiment were cow manure (CM) and maize silage (MS), which were collected from an AD plant treating livestock effluents, energy crops and other organic co-substrates (Venice, northeast Italy). Cow manure and maize silage exhibited total solids (TS) contents of 20.7% and 33.3%, respectively, with a high volatile solids (VS) fraction of 82% VS/TS for cow manure and 94% VS/TS for maize silage. These characteristics were similar to those obtained by other authors working with similar substrates (Amon et al., 2007, Sanchez et al., 2000) Cow manure could be considered an almost digested substrate, but it was mixed with wheat straw, which was added as bedding material during breeding, thus the volatile content could be partially attributed to this added substrate that is rich in lignin. The organic nitrogen content in cow manure was higher than that of maize silage, with an average of 27.8 mg g_{TS}^{-1} compared to 14.2 mg g_{TS}^{-1} for maize silage. This value influences the effective digestibility of CM such that the COD/TKN of CM was 31, a value lower than the minimum of 50 for anaerobic digestion, as suggested by Mata-Alvarez et al. (2010), whereas the value for MS COD/TKN was 70. Table 1 presents the average values obtained from the chemical-physical characterization.

Table 1. Chemical-physical characteristics of CM and MS

2.2 Fermentation experiment set-up

The acidogenic fermentation was performed in a five liter continuous stirred rank reactor (CSTR) that was maintained at a fixed temperature by an external heater. No inoculum was added because the microorganisms needed for the hydrolytic step were already present in the cow manure and sufficient to conduct the biochemical reaction. The reactor was fed in a semi-continuous manner, one time per day. The feed was a mixture of cow manure (40% on dry matter basis) and maize silage, with a total final solid content of 6 to 8%, which was maintained for each tested condition. No additional medium was added to observe the real feasibility of the proposed approach. Acid fermentation was conducted at two different working temperatures (M=37 °C and T=55 °C) and different organic loading rates (OLR) and hydraulic retention times (HRT=2, 4 and 6), as displayed schematically in Table 2.

Table 2 Operational conditions of acid fermentation

Each condition was monitored until steady state conditions were established. The feeding begins at time zero, and the steady state conditions were reached in 15 to 20 days, a sufficient time to assure acclimation to mesophilic and thermophilic temperatures without a specific inoculum (an HRT of more than five).

2.3 Analytic methods

The solid content (TS) was obtained after drying the sample at 105°C for 48 h, and the volatile solid fraction (VS) was obtained after 24 h of calcination at 550°C. The nitrogen content was analyzed using the total Kjiendhal nitrogen (TKN) method on the minced sample after drying at 105 °C for 48 h. The organic content was analyzed both in terms of particulate chemical oxygen demand (pCOD, chemical analysis on dried sample) and soluble COD (sCOD on soluble fraction). The VFA content and composition were monitored using a gas chromatograph (Carlo Erba instruments, Wigan, UK) with hydrogen as a gas carrier that was equipped with a fused silica capillary column (Supelco NUKOL™, 5 m to 0.53 mm to 0.5 mm film thickness) and a flame ionization detector (200 °C). The analyzed samples were centrifuged and filtered using a 0.45 μm membrane. The solid samples (MS and CM) were analyzed after a mild extraction, using 1 g of sample diluted in 10 g of water and mixed for 30 minutes. Other analyses were conducted, such as total phosphorus (TP) levels and stability parameters (pH, alkalinity), in accordance with the standard methods (APHA, 2012).

2.4 Data analysis

For each tested condition, the solubilization and acidification yields were evaluated, as reported by

Peces et al. (2016). The solubilization yield considers the difference between the final and initial soluble chemical oxygen demand (sCOD) in relation to the volatile solids (VS) fed into the system. The same calculation for the acidification yields considers the difference between the final and initial volatile fatty acid (VFA) production in relation to the volatile solids (VS) fed.

3. RESULTS AND DISCUSSION

The pre-fermentation step was applied to a mixture of cow manure and maize silage, at a typical ratio of 40/60%, respectively, on the basis of VS, at 35°C and 55°C while increasing the HRT and decreasing the OLR to evaluate the efficiency of the production of volatile fatty acids. The different conditions were applied for at least 10 HRTs and, in all cases, until stable steady state conditions were reached. The amount of TS and VS inside the fermentation reactor was slightly variable during the six periods tested, ranging between 65 and 87 gTS L^{-1}, due to the heterogeneity of cow manure and its wheat straw content. Considering all six periods, the average pH detected ranged between 4.6 and 5.6, an acidified pH as expected. Thus, this influenced the VFA production/composition and ammonia conversion given that because the pH was below 6, the ammonium content was not at inhibitory values but rather was between 300 and 480 mg $N\text{-}NH_4^+$ L^{-1} during the six periods. Yu and Fang (2001) observed that the ammonium content of the inlet waste must be below 5 g l^{-1} to avoid the inhibition of VFA production. Finally, low ammonia content is highly addressed, especially if the liquid fraction is used in a denitrification step.

Table 3 presents the reported average values of the chemical-physical characterization of effluents under both mesophilic and thermophilic conditions.

Table 3. Chemical-physical characterization of effluents

The fermentation step, conducted at 37° and 55°C, obviously changes the COD fractioning of the inlet substrates. Maize silage is a crop often used in agricultural biogas plants to increase biogas production. To preserve this material throughout the year, ensilage technology is applied. This technology is a natural/artificial fermentation driven by lactic acid bacteria (LAB) that converts sugars into lactic, acetic and butyric acids with a consequent decrease in pH that prohibits other degradation phenomena. The particulate COD (pCOD) content was 331.7 g kg^{-1}, while the soluble COD (sCOD) obtained after 30 min of extraction with water was 66.6 g kg^{-1}. When analyzing the liquid fraction after membrane filtration (0.45 um), a rather high amount of VFA was detected (20.04 g_{COD} kg^{-1}), and it was composed mainly of acetic acid (9.1 g_{COD} kg^{-1}) and butyric acid (6.4 g_{COD} kg^{-1}). The dissolved portion accounted for 16.7% of the total COD. Cow manure was a partially digested material mixed with wheat straw, which was used as bedding material, and it had a pCOD content of 182.6 g kg^{-1}, whereas the sCOD was 13.07 g kg^{-1} with a low fraction of VFA (0.25 g_{COD} kg^{-1}). Thus the soluble fraction was composed primarily of other soluble compounds, such as saccharides, long chain fatty acids and undetected amino acids. The dissolved portion is lower with respect to MS and accounts for 6.7% of the total COD. The percentage of $sCOD_{in}/tCOD_{in}$ of the mixture (40 to 60%) fed into the fermentation unit was 13.3%, and the $VFA_{in}/tCOD_{in}$ was 3.3%. This low ratio suggests the need to pre-treat the substrate to increase the soluble fraction that is to be readily utilized by methanogens or to be used simply to increase the conversion into VFA for PHA for the purpose of production. The main operating conditions that influence the VFA production were reviewed by Lee et al. (2014) and include pH, temperature, hydraulic retention time, solids retention time and organic loading rate. The results reported are related to different types of solid and liquid wastes, and their heterogeneity determines the possibility of providing some optimal conditions rather than a specific range of values valid for all wastes. Moreover, as there are no data about VFA production from cow manure and maize silage, the comparisons are conducted with food waste and primary or secondary sludge.

Figure 1. Solubilization and acidification yields obtained at different temperature (M=mesophilic and T=thermophilic) and applying hydraulic retention time of 2, 4 and 6 days.

Considering data reported in Fig. 1, it is possible to observe changes in the fractioning of organic matter. With respect to sCOD mass balance, ranges for both mesophilic and thermophilic temperatures allowed for the solubilization of particulate COD that accounted for approximately 13% more sCOD at 37 °C and reached approximately 17% more sCOD at 55 °C, compared with inlet sCOD. Considering the $sCOD_{out}/tCOD_{in}$ ratio, the average ratios were 26% at mesophilic temperatures and 30% at thermophilic temperatures. The solubilization yields obtained at 37 °C were 169.2 g sCOD $kgVS_{fed}^{-1}$ in the M2 period, 204.8 g sCOD $kgVS_{fed}^{-1}$ in the M4 period and 80 g sCOD $kgVS_{fed}^{-1}$ in the M6 period, whereas at 55 °C the yields were higher at 241.5 g sCOD $kgVS_{fed}^{-1}$ for T2 and 226.8 g sCOD $kgVS_{fed}^{-1}$ for T6. T4 showed the lowest solubilization yield, which is similar to the results observed in the mesophilic range (149.6 g sCOD $kgVS_{fed}^{-1}$). This value could be related to the pH. For example, when the pH is at 5.5, the solubilization yields are low, but when the pH is below 5, the solubilization yields are at their highest. With the exception of T4, it is possible to observe that increased solubilization yields are related to increased temperatures, a finding that is confirmed by past studies (Jiang et al. 2013, Peces et al. 2016).

From the perspective of VFA production, the trend was inverted. For instance, the acidification yield, as it relates to the VFA conversion, was slightly higher at mesophilic temperatures and was influenced by the HRT. The $VFA_{out}/sCOD_{out}$ at M2 and M4 indicates that approximately 60% of the sCOD was composed of VFA; however, in M6, it decreased to 42%. Moreover, at 55 °C, for T2 and T6, the percentages were 38% and 40%, respectively, but in T4, the behavior was more similar to M2 and M4, with an sCOD composition of 55% due to VFA. The maximum acidification yields obtained were 183.2 $gCOD_{VFA}$ $kgVS_{fed}^{-1}$ at mesophilic temperatures during HRT 4, whereas at thermophilic temperatures, the yields ranged from 112 to 130 $gCOD_{VFA}$ $kgVS_{fed}^{-1}$, and they exhibited greater stability. If we compare the VFA production with the 3.3% VFAin/tCODin of the mixture at 37°C, we obtain approximately 17% VFAout/tCODin in M2 and M4 and approximately 13.5% VFAout/tCODin for all 55°C periods. Jiang et al. (2013) observed the same trend with decreased acidification yields at 55°C. Peces et al. (2016) suggested that these lower acidification yields observed at 55 °C could be due to a slower adaptation to the thermophilic culture, especially considering that no inoculum was used. The yields are summarized in Table 4.

Table 4. Solubilization and acidification yields.

The yields obtained were lower when compared with those obtained from food waste fermentation. For example, Jiang et al. (2013) obtained yields of 379 and 440 $gCOD_{VFA}/kgVS_{fed}$ at 35 °C and 45 °C, respectively. However, the yields are higher than those obtained in experiments where substrates with less organic content, such as primary sludge, were used. Peces et al. (2016) obtained the maximum acidification yield of 143 $gCOD_{VFA}/kgVS_{fed}$ at 37°C and the lowest yield of 23 $gCOD_{VFA}/kgVS_{fed}$ at 55°C. Nevertheless, it must be taken into account that in this paper the fermentation was conducted in a semi-continuous way (fed once per day), whereas in most of the extant literature, the yields were obtained in batch conditions, allowing for the potential production of VFA.

3.1 VFA composition

The VFA composition is an important issue, especially if a specific volatile fatty acid must be produced. One of the previously mentioned parameters that could influence the production of VFA is the pH level that is linked to the amount of the acid compound produced during the breakdown of complex molecules and to the ammonium content of waste. The optimum pH level is strictly dependent on the type of waste used, and often it is tested under controlled conditions. Lee et al. (2014) reported that the pH level influences the composition more than it does the production, given an optimum range for acetic acid and other ranges for propionic and butyric acids. However, this is always dependent on the type of waste used. In this test, the pH level was not controlled for two reasons. First, it was important to verify the feasibility of the approach inside an agricultural biogas plant avoiding the use of chemicals, and second, the cow manure has a rather high ammonia content that improves the buffer capacity that prevents the pH from dropping below 4.5. Table 5 and Figure 2 present the VFA distribution during the six periods.

Table 5. Volatile fatty acid distribution

The pH was approximately 5.5 during the steady state of all three periods at 35 °C. Nevertheless, though the pH was constant, the composition changed with HRT, and OLR as a consequence. At HRTs of two and four days, the HAc/HBu ratios were 1.3 and 1.6, respectively, but the ratio decreased to 1.0 at an HRT of six days. This first result could suggest that increasing the HRT from two to four days allows the biomass more time to adapt and degrade the complex molecules, but that increasing the HRT to six days resulted in no VFA production. Furthermore, propionic acid reached its maximum concentration of 1,899 mgCOD l^{-1} at M2 and an HAc/HPr ratio of 2.17, whereas there was no propionic acid at M4 and 848 mgCOD l^{-1} of propionic acid at M6.

At 55 °C, the pH was lower than it was at 37°C, and it was approximately 4.7 at T2 and T6. As previously mentioned, the T4 results are similar to those in the mesophilic range with a pH of 5.4, though the VFA distribution is completely different. T2 revealed a similar concentration of M2 in terms of acetic and butyric acids with a HAc/HBu ratio of 1.5, thus confirming temperature has no influence on VFA composition at the same OLR even when the pH is lower. T4 VFA composition suggests low acetogenic activity with the accumulation of a longer VFA, such as HPe, HVal and HEpt and a low acetic acid conversion. The HAc/HBu ratio decreased to 0.4, which indicates a prevalence of butyric acid even when the HAc/HPr ratio was 0.82. During period T4, short chain VFAs greater than that of butyric acid, namely, pentanoic, caproic (and their iso-forms) and heptanoic, exhibited high concentrations compared to other experimental runs (see T2 and T6). Interestingly, the accumulation of both n- and i-valeric acids was reported in several studies, especially when treating protein-rich substrates (Bolzonella et al., 2007, Aguilar et al., 1995, Wang et al., 1999). This was ascribed to the degradation of valeric acid, a typical product of amino acid fermentation (Batstone et al., 2003). This degradation was due to environmental conditions such as pH and free ammonia concentration (Pind et al., 2003). The same mechanism applies also for n- and i-caproic acids. The T6 period exhibited the best performance in terms of butyric acid production, reaching more than 5 $gCOD_{VFA}$ l^{-1} and 2.5 $gCOD_{VFA}$ l^{-1} of acetic acid (HAc/HBu ratio of 0.5). These results confirmed that when increasing the HRT from 2d to 4d and 6d, the acetic acid production decreases in favor of butyric acid and other longer VFAs.

Figure 2. Volatile fatty acids composition during the six working periods tested.

Kim et al. (2016) reported that at pH 4.5, in general, the total VFA production is lower than it is at pH 5.5 and that the acetic acid is dominant at < pH 5 whereas butyric acid is dominant at > pH 5.5. In this experimental test, even when the pH was lower than the VFA pKa, inhibition was not observed. Thus, it is hypothesized that the difference is related to the metabolic pathways involved and to the presence of different groups of microorganism inside the initial feedstock (CM and MS).

The composition of VFA is important when the fermentation liquid is used for the production of polyhydroxyalkanoates (PHA). Thus, acetic and butyric acids are used for 3-hydroxybutyrate, whereas propionic and valeric acids are used for 3-hydroxyvaleate. Though it is essential to regulate the specific VFA production, with heterogeneous substrate such as CM and MS, it is difficult to find a specific correlation between the operational and VFA composition.

4. CONCLUSIONS

This study verified the efficiency of a separate hydrolysis step for cow manure and maize silage by testing different working temperatures (37°C to 55°C) and hydraulic retention times (two, four, six days) and evaluating the sCOD and VFA production. It was found that the solubilization yield increases when applying 55°C, but the acidification yields are higher at 37°C. The optimal condition for 3-hydroxybutyrate production was found by applying an OLR of 17.9 gTVS m^{-3} (HRT of four days at 37°C) with an acidification yield of 183.2 $gCOD_{VFA}$ $kgVS_{fed}^{-1}$ and with 44% of HAc and 29% of HBu on total VFA.

5. ACKNOWLEDGMENTS

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