

**Sewage sludge acidogenic fermentation for organic resource
recovery towards carbon neutrality: an experimental survey
testing the headspace influence**

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Abstract

Volatile fatty acids (VFAs) produced by acidogenic digestion of sewage sludge are very interesting bio-products which can contribute to carbon neutrality of wastewater treatment plants. Studies on the production of VFAs from sewage sludge from fermenters with membrane are limited. In view of above, VFAs from a fermenter pilot plant equipped with a membrane bioreactor and fed with real sewage sludge has been monitored. The effect of headspace volume (HdV) on VFA production was studied for the first time to elucidate the optimal operation conditions. Specifically, three fermenter HdV values (namely, 20, 40 and 60% of the total volume) have been investigated. Results revealed that the HdV of 20% ensured the highest sCOD production (900 mgCOD/L) and VFA/COD ratio (45.4%). High value of HdV (namely, 40 and 60%) strongly decreased the acidogenic fermentation performance in terms of VFA production.

Keywords: Anaerobic digestion; Headspace effects, Resource recovery from wastewater; Sewage sludge; Volatile fatty acids.

1. Introduction

Large amount of sewage sludge is every year produced in Europe by urban wastewater treatment plants (WWTPs) (12 million tons) (Bianchini et al., 2016; Nieto et al., 2021). Due to the grow of global population and the requirements of increasing wastewater treatment worldwide, the amount of sewage sludge is expected to increase in the future (Kubonova et al., 2021). Sewage sludge is usually stabilized by using anaerobic digestion (AD) before its

final disposal (usually in the solid waste landfill) or land application. AD represents a multi-step process (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) which allows to transform organic compounds into carbon dioxide (CO₂) and methane (CH₄) by means of the growth of anaerobic microorganisms (Garrido et al., 2013). Therefore, during AD energy and added-value bio-products (such as volatile fatty acids – VFAs) can be produced according to the concept of biorefinery (Yang et al., 2015). In view of increasing the opportunity of recovering as much as possible bio-products from AD, the multi-step processes can be interrupted to the acidogenesis (Nabaterega et al., 2021). Indeed, the acidogenic fermentation allows the production of high value chemicals such as VFAs that can be used as renewable carbon source (Liu et al., 2020). For example, VFA-rich streams can be used as feeding for polyhydroxyalkanoates (PHA) production and consequently bioplastic (Moretto et al., 2020). Several operation conditions, initial sewage sludge features and pre-treatments have been investigated in literature (Atasoy et al., 2018; Reyhanitash et al., 2019; Aghapour Aktij et al., 2020; Presti et al., 2021). Previous studies demonstrated that VFAs depend by several factors: hydraulic retention time (HRT), temperature, pH, nutrients availability, headspace pressure (HP) and plant schemes. Among these factors few studies focused on the mitigation of HP on VFAs production using environmental sustainable solutions. Indeed, previous studies employ hydrogen (H₂) and carbon dioxide (CO₂) flushed in the headspace (Koch et al., 2015). Recently, Li et al. (2019) studying batch test experiments using vacuum pump for HP control, found that VFA production increases under low HP (around 0.02 MPa). Such results were related to the capability (under low HP values) of the protein compounds to be degraded under acetogenesis fermentation, thus favouring the short-chain VFA production (Li et al., 2019). Despite the beneficial effects played by H₂ and CO₂ in attenuating the HP effects for

producing VFAs, those gases are strongly in contrast with the concept of environmental sustainability (Yan et al., 2017). Very recently, García-Depraect et al. (2022) in view of producing VFA from bioplastic fermentation have applied helium gas flushing to guarantee anaerobic conditions within the fermenter. However, the fermenter volume adopted by García-Depraect et al. (2022) has a bench scale (1 L working volume). Therefore, the cost sustainability of adopting helium gas flushing has been neglected García-Depraect et al. (2022) despite it is quite expensive (on average 10 € m⁻³) (Kornbluth, 2018). Moreover, especially CO₂ is among the most important greenhouse gas emissions (IPCC, 2022). Therefore, strategies for improving acidogenesis fermentation without using chemicals, gases or compounds that may compromise the environmental or economic sustainability of the process are required. Despite the effort performed in literature, as far as authors are aware, there is a lack of studies regarding VFA production from real sewage sludge from fermenters in operation without flushing gases (i.e., CO₂, H₂) for mitigating the HP effects. Thus, to gain insights about best operation conditions, here the role of the headspace volume percentage on the short-chain VFA production has been investigated by using a fermenter pilot plant equipped with an ultrafiltration membrane for solid/liquid separation. The novelty of the study is the improvement of the acidogenesis fermentation performance avoiding the adoption of external chemicals to control HP that can have negative environmental impacts.

2. Materials and methods

2.1. Pilot plant description

A fermentation pilot plant was built at the Water Resource Recovery Laboratory at Palermo University (Mannina et al., 2021) (Figure 1). The pilot plant was composed of a fermenter,

an ultra-filtration unit and a permeate tank. The fermenter was a covered Continuous Stirred Tank Reactor (CSTR) ($V= 225$ L) equipped with a mechanical stirrer and liquid sampling ports. The fermenter was also equipped of gas sampling ports installed in the cover. Two probe ports were installed inside the reactor. An ultra-filtration unit ($V= 43$ L) with an hollow fibre membrane (porosity = $0.03\ \mu\text{m}$, surface area: $1.4\ \text{m}^2$) was connected to the fermenter. Once the fermentation experiment ended, the fermented sludge was filtered by the membrane with a net initial flowrate of $13.2\ \text{L/h}$ (9 min filtration at flow rate of $18\ \text{L/h}$, 1 min backwashing at flow rate of $30\ \text{L/h}$). During membrane filtration, the off-gas produced inside the ultrafiltration tank was continuously recirculated inside the membrane by using a Gilian GilAir Plus (Recom Industriale) set at gas flow rate of $5\ \text{LPM}$. The gas recirculation allowed the reduction of membrane fouling by increasing the scouring action on the membrane surface.

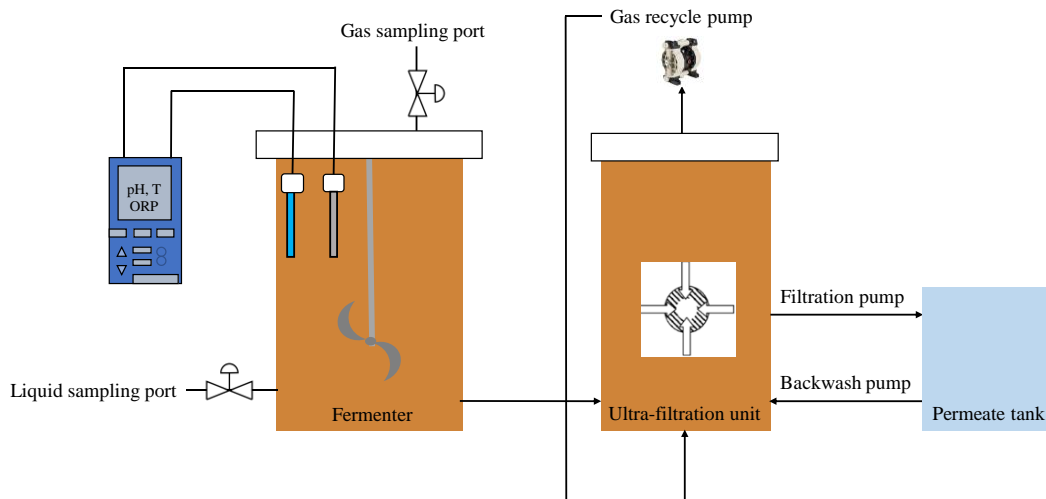


Figure 1. Fermentation pilot plant layout

2.2. Analytical methods

Chemical oxygen demand (COD), total and volatile suspended solids (TSS, VSS), ammonium (NH_4^+ -N) and phosphate (PO_4^{3-} -P) concentrations were measured three times per week by using the standard methods (APHA, 2012). VFA measurements were performed by treating filtered (0.45 μm) fermented sludge samples with dimethyl carbonate (DMC-OEI) according to Ghidotti et al (2019). Treated samples were analysed with an Agilent Technologies 7820A gas chromatograph (GC) equipped with a flame ionization detector (FID) and a DB FFAA column (30 m x 0.25 mm x 0.25 μm). Formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, hexanoic and n-heptanoic acids were analysed according to the protocol proposed by Montiel-Jarillo et al. (2021). Finally, VFA concentrations were converted into COD by using the conversion factors as proposed by Yuan et al. (2011). Carbon dioxide (CO_2) concentration have been measured in the headspace gas by using the GC equipped with an Electron Capture Detector (ECD).

During the membrane operation, the transmembrane pressure (TMP) was continuously monitored by using a vacuum gauge indicating TMP both during filtration and backwashing. Furthermore, during membrane operation the net extracted permeate volume was measured in view of calculating the total membrane resistance (R_T) as the ratio between the average TMP and the average permeate flux, this latter multiplied to the permeate viscosity (Judd and Judd, 2008). The COD solubilization was estimated according to Equation 1 (Mohammad Mirsoleimani Azizi et al., 2021):

$$\text{COD solubilization} = \frac{sCOD_t - sCOD_0}{TCOD_0} \quad (1)$$

where (t) and (0) refer to generic and initial time, respectively.

2.3. Experimental campaign

The pilot plant fermenter was fed with real sewage sludge produced from a biological pilot plant for carbon and nitrogen removal installed at the Water Resource Recovery at Palermo University Campus (Mannina et al., 2021). In Table 1 the main features of the sewage sludge are reported. Three different fermenter pilot plant experiments (T1, T2 and T3) were carried out. The headspace volume percentage (with respect to the total volume) for T1, T2 and T3 was respectively equal to 60, 40 and 20%.

Table 1. Sewage sludge features.

Parameters	T1	T2	T3
pH	7.21	7.15	6.6
Total Suspended solids, TSS (g/L)	4.5	4.8	4.27
Volatile Suspended Solids, VSS (g/L)	4.33	3.91	3.64
Total Chemical Oxygen Demands, TCOD (mg/L)	7340	4801	6201
Soluble Chemical Oxygen Demands, sCOD (mg/L)	99	37	103
Ammonium, NH ₄ ⁺ -N (mg/L)	6.5	28.20	19.81
Phosphate, PO ₄ ³⁻ -P (mg/L)	6.1	5.21	5.20
EPS proteins (mg/g VSS)	136.9	96.1	15.9
EPS carbohydrates (mg/g VSS)	16.9	9.8	4.7

The VSS concentration for each fermenter pilot plant experiments was around 4.3 gVSS L⁻¹ (more precisely the VSS concentration was equal to 4.34, 4.80 and 4.27 gVSS L⁻¹ for T1, T2 and T3 respectively).

The duration of each acidogenic fermentation experiment was 15 days. pH, temperature and Oxidation Reduction Potential (ORP) have been continuously monitored inside the fermenter by using a WiFi - Multi 3630 IDS “WTW” (Xylem brand) and related probes.

3. Results and Discussion

3.1. sCOD, nutrients and SMP concentrations

Figure 2a and 2b reports sCOD concentration trend and COD solubilization. The highest sCOD concentration was recorded for T3 revealed during day 8 (900 mg COD/L and 12.85% of the initial total COD in term of solubilisation). T2 and T1 reached the COD peak both on day 11 (615 and 146 mg COD/L, respectively). T1 maintained the highest pH value during the test while the lowest value was recorded for T3. This latter result shows the different hydrolysis rate and its relative sCOD production: different headspace volume influenced CO₂ solubility in the supernatant thus rapidly decreasing pH in T3 (Figure 2e) (Nabaterega et al., 2021). Indeed, in T3 acidic pH was rapidly reached (2nd-3rd day) thus enhancing the hydrolysis step and reaching sCOD production peak 3 days before T1 and T2 (Ma et al., 2016). However, COD solubilisation was low in all the experiments (Fig. 2b) which could be correlated to the low EPS concentration. T3, which had the lowest EPS concentration of all the experiments, showed the highest COD solubilisation (13%), while T2 and T1 reached 10 and 1% of COD solubilisation, respectively. These results confirm that in T3 the

hydrolysis step was enhanced compared to the other experiments (T1 and T2). Indeed, during test T3 the highest values of sCOD concentration and COD solubilisation were achieved despite the low EPS fraction availability.

Figure 2 also shows the NH_4^+ (c) and PO_4^{3-} (d) concentrations trend during the experiments.

T3 revealed the highest NH_4^+ concentration (70.2 mg NH_4^+ /L).

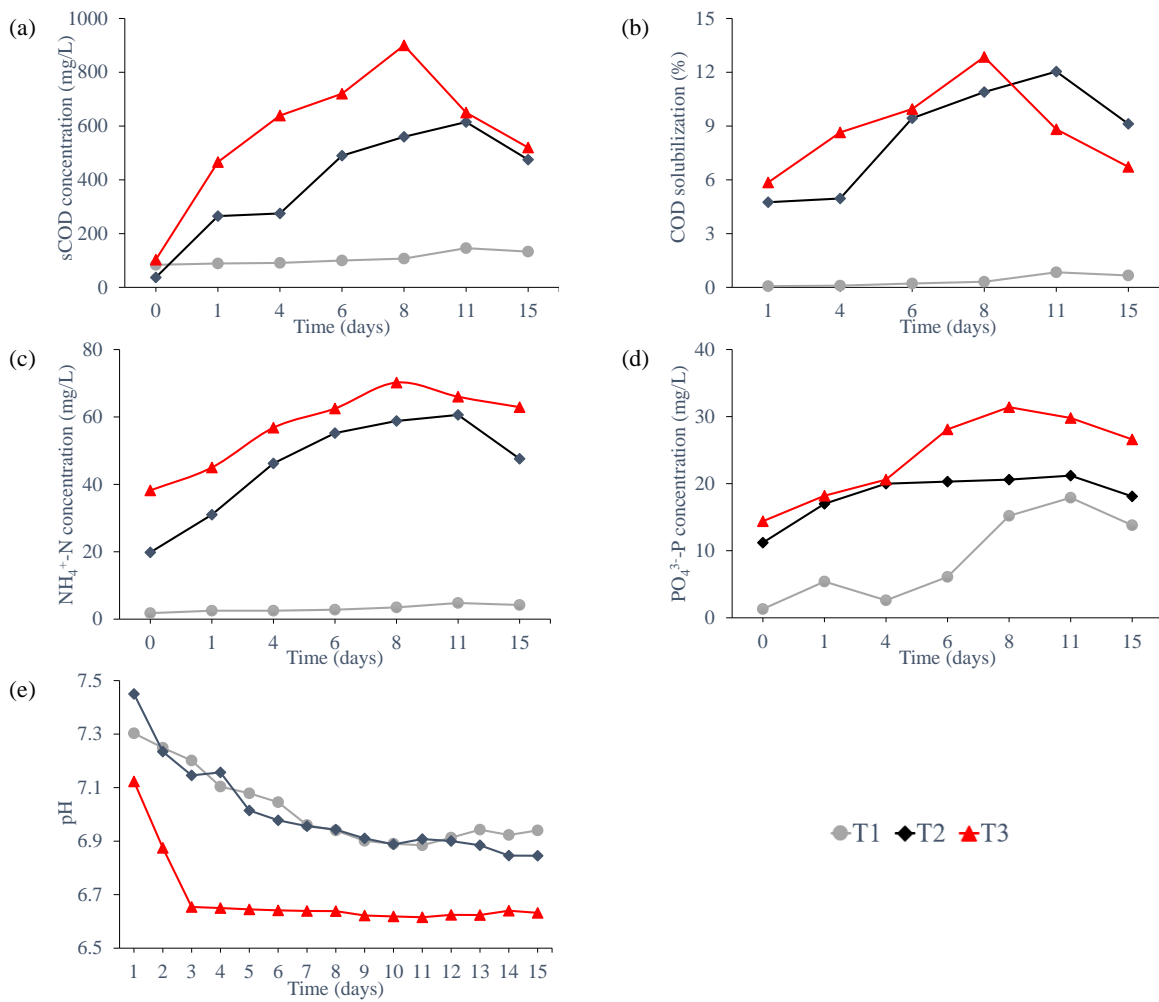


Figure 2. sCOD (a), COD solubilization (b), ammonium (a) and phosphate (b) concentration trends during the experiments.

During T2 showed similar NH_4^+ concentration to T3 was achieved (60.6 mg NH_4^+ /L). Conversely, during T1 very low NH_4^+ concentration was obtained (4.8 mg NH_4^+ /L). These results suggest according to Feng et al. (2019) an effective protein hydrolysis during T2 and T3. In terms of phosphate release, T3 revealed the highest concentration (31.4 mg/L).

In order to corroborate the previous statement related with the protein hydrolysis, Figure 3 shows how soluble proteins and carbohydrates concentration varied from the start to the end of the fermentation experiments. T3 reached the highest soluble proteins and carbohydrates concentrations at the end of the test (46.1 and 15.6 mg/g VSS), followed by T2 (30.8 and 12.1 mg/g VSS) and T1 (22.5 and 7.2 mg/g VSS).

sCOD, nutrients and SMP concentrations during the experiments highlight the effect of the HdV in the acidogenic fermentation process. Low HdV shifted CO_2 gas-liquid equilibrium towards its solubilisation in the supernatant liquid thus leading to carbonic acid formation, whose subsequent ionization produces hydrogen ions (H^+) (Li et al., 2029). Similar results were found by other authors investigating the VFA production from organic waste (Iglesias-Iglesias et al., 2019; Lü et al., 2021). Free H^+ ions were generated mostly in the first days of the experiment since acidic conditions were rapidly reached in T3 (Figure 2e), thus enhancing the organic matter disruption.

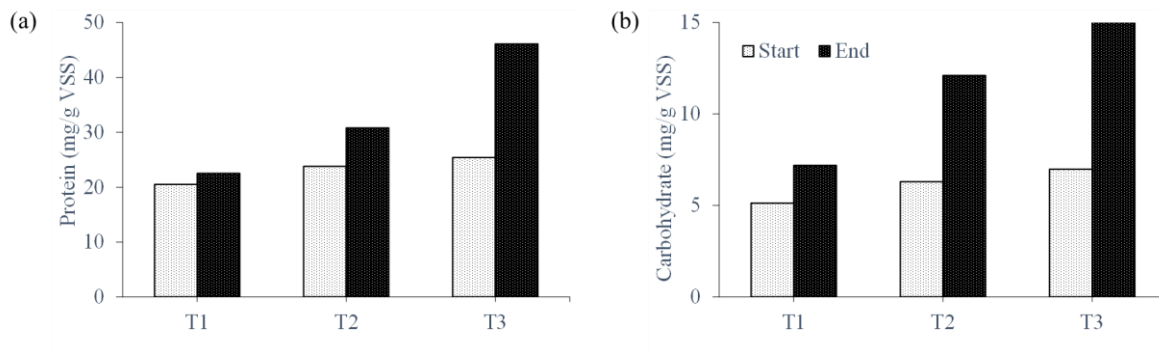


Figure 3. Protein (a) and carbohydrate (b) concentration at the start and end of T1-T3.

3.2. Effect of the headspace volume on the acidogenic fermentation

VFAs concentrations during the experiment are reported in Figure 4a. By analysing Figure 4a one can observe that the trend was similar as sCOD production. T3 reached the highest VFA concentration (409 mg COD/L) and VFA/sCOD (45.4 %) on day 8. A slight decrease from T3 was registered for T2 (198 mg COD/L and 31.2 %) on day 11, as expected since higher pH in T2 compared to T3 may have worsened the organic matter disruption. T1 reached the VFAs concentration peak at the 11st day (30 mg COD/L). Finally, none of the experiments reached at least 30% of VSS reduction or a VFA yield higher than 200 mg COD/g VSS (Fig. 4b).

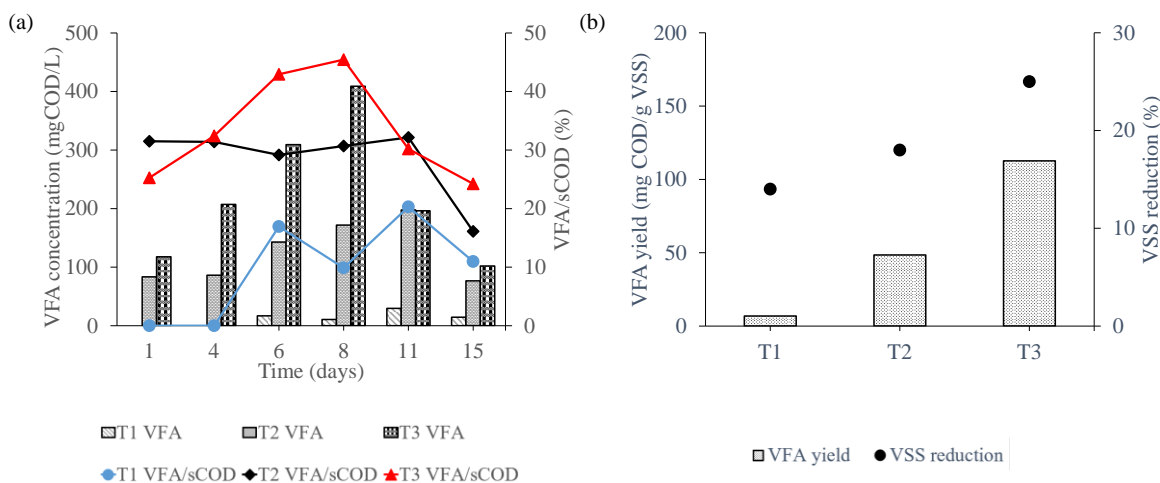


Figure 4. VFA concentrations, VFA /sCOD ratio (a) and VFA yields and VSS reduction (b) for T1-T3

Figure 5 a-c shows VFA composition during the experiments. The predominant species of VFA was acetic acid both in T2 (average 96.6 %) and T3 (92.43%).

Butyric and iso- butyric acid accounted for 2.5 and 0.9 % for T2 respectively, while lower amounts were measured in T3 (0.8 and 3.3% respectively). Propionic acid was the second most abundant acid in T3 (3.5%) while it was the predominant acid in T1 (33.8%). Compared to other experiments, T1 showed lower amounts of acetic acid (33.72%) and was also the only experiment where iso valeric acid was detected (32.52%).

As already discussed for COD, headspace volume clearly affected not only the fermentation process but also its rate. T3 reached the highest VFA concentration faster than T1 and T2, thus assuming that lower pH value enhanced acidogenesis as well (Li et al., 2017). Also, T1 trend shows a probable methanogenic activity that hindered the VFA production, especially on day 8. Methane measurements were not performed to confirm these results, but CO₂ concentration started increasing after day 6 (5.67, 10.21, 12.29, 15.64 and 26.82% for days 4, 6, 8, 11 and 15 respectively). Also, propionic acid was the only VFA produced in the first

days of experiment (Fig, 6a). These results suggest, according to previous literature, that hydrogenogenic and acetate methanogenic archaea activity could be possible (Lyberatos and Skiadas, 1999). Propionic acid was consumed rapidly to produce acetate and hydrogen. Indeed, during day 8 acetate was detected and no propionic acid was measured. After day 11, methanogenic activity led to acetate consumption and CO₂ production.

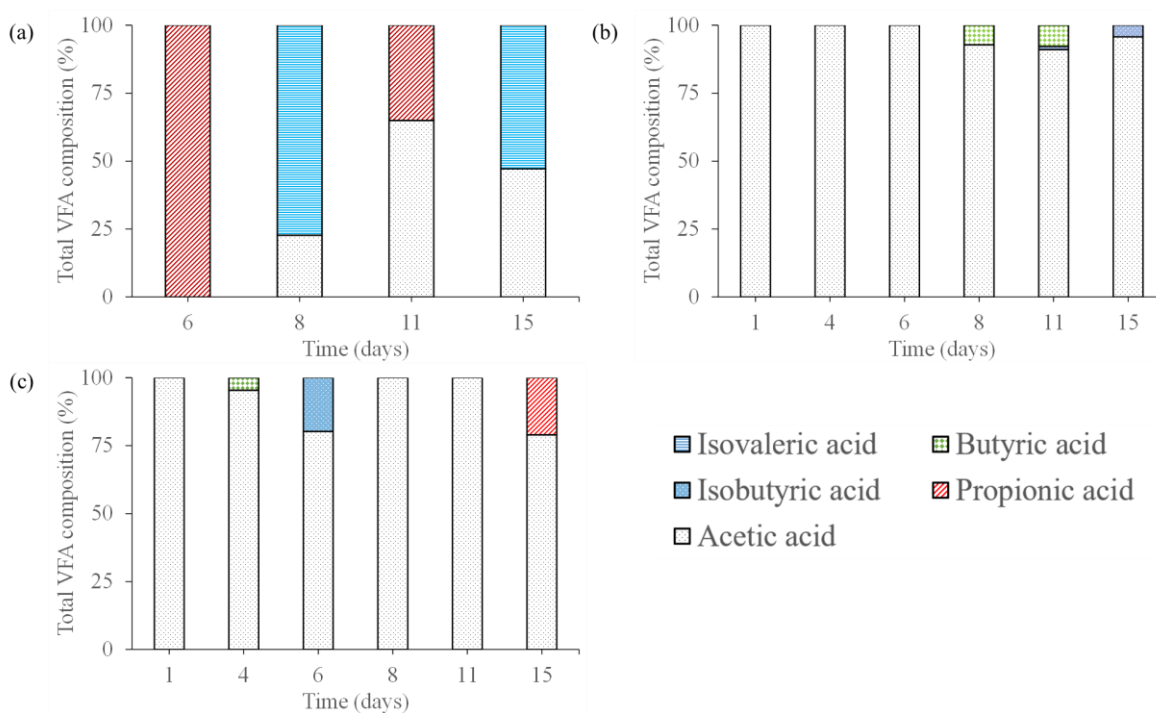


Figure 5. VFA composition during the experimentation period for T1 (a), T2 (b) and T3 (c).

Considering the effect of different carbon sources (VFAs) in the mixed microbial community (MMC) PHA production process (Tu et al., 2020), T3 showed the best composition compared to other experiments but, despite the encouraging results obtained, mere sewage sludge fermentation without pre-treatment or other substances addition isn't enough to convert all the organic matter into VFA and more research is needed to optimize the pilot scale process.

3.3 Membrane fouling

Table 3 summarizes the results in terms of average initial and final parameters related to the membrane filtration and fouling. By analysing data of Table 3 one can observe that the membrane fouling on average increases with the decrease of the headspace. Indeed, as reported in Table 3 the average final TMP value during filtration increased from 0.8 bar (for T1), till to 0.95 bar (for T3) with a value of 0.95 bar for T2. Consequently, the average final net permeate flow rate decreased from 12 L h⁻¹ (for T1) to 4.3 L h⁻¹ (for T3). These data are mainly due to the increase, with the reduction of the headspace, of the filtration duration time, without any membrane cleaning excepting for the backwashing cleaning (every 9 min filtration). Moreover, with the decrease of the headspace volume the amount of the total soluble (both protein and carbohydrate) concentration increased (Figure 3a-b) likely due to the sludge deflocculation effect during the acidogenic fermentation (Li et al., 2019a).

Table 3. Initial and final parameters related with membrane filtration and fouling

Parameters	Unit	T1	T2	T3
Average initial net permeate flow rate	L h ⁻¹	13.2	13.2	13.2
Average final net permeate flow rate	L h ⁻¹	12	5.6	4.3
Average initial TMP	bar	0.11	0.11	0.11
Average final TMP	bar	0.8	0.95	0.98
Filtration duration time	day	1	2.5	4

Indeed, the highest TMP value and the lowest average final flow rate were achieved for experiment T3 (0.98 bar and 4.27 L h⁻¹, respectively) during which the highest soluble SMP was obtained.

Figure 6 shows the results obtained in terms of initial and final R_T for each fermentation experiment.

Consistently with the results discussed above, there was an increase of R_T with the decrease of the headspace volume. Indeed, R_T value was equal to 35.9 10¹² m⁻¹, 88 10¹² m⁻¹ and 96 10¹² m⁻¹ T1, T2 and T3, respectively.

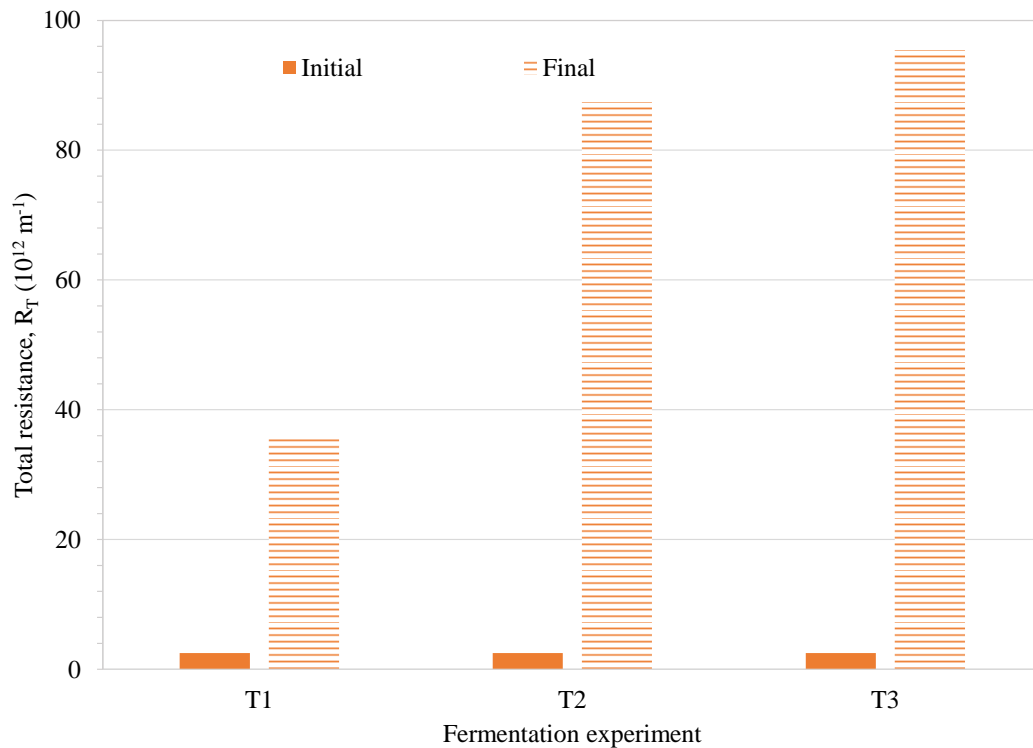


Figure 6. Initial and final total resistance R_T obtained for each fermentation experiment

This result could have important economic implications in terms of operational costs, because decreasing the headspace volume the availability of fermentation volume increases (resulting in a highest sludge volume that can be subjected to formation). However, the filtration time and the membrane fouling increase with the headspace volume decreasing thus influencing the electric power requirement and the potential membrane life duration. This issue is out of the scope of this manuscript and will be tested using different membrane types in the future.

4. Conclusions

Sewage sludge was tested in fermentation experiments investigating the influence of headspace volume and therefore pH with the aim of enhancing VFA production improving the process environmental sustainability. Results showed that low HdV enhanced organic matter disruption by an increase in sCOD production (from 146 to 615 and 900 mgCOD/L for 60, 40 and 20% HdV, respectively) and acidogenesis (112 mg VFA/g VSS). Also, high HdV (60%) inhibited VFAs production which led to CO₂ production. Future research will focus on maximizing the COD and VFA production of this sludge, investigating inexpensive and environmentally friendly pre-treatment.

Acknowledgments

This work was funded by the project “Achieving wider uptake of water-smart solutions—WIDER UPTAKE” (grant agreement number: 869283) financed by the European Union’s Horizon 2020 Research and Innovation Programme, in which the last author of this paper, Giorgio Mannina, is the principal investigator for the University of Palermo. The Unipa project website can be found at: <https://wideruptake.unipa.it/>.

5. References

1. Aghapour Aktij, S.; Zirehpour, A.; Mollahosseini, A.; Taherzadeh, M.J.; Tiraferri, A.; Rahimpour, A. Feasibility of membrane processes for the recovery and purification of bio-based volatile fatty acids: A comprehensive review. *J. Ind. Eng. Chem.* 2020, 81, 24–40.
2. Atasoy, M.; Owusu-Agyeman, I.; Plaza, E.; Cetecioglu, Z. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresour. Technol.* 2018, 268, 773–786.
3. APHA/AWWA/WEF, 2012. Standard Methods for the Examination of Water and Wastewater. Standard Methods 541.
4. Bianchini, A.; Bonfiglioli, L.; Pellegrini, M.; Saccani, C., 2016. Sewage sludge management in Europe: A critical analysis of data quality. *Int. J. Environ. Waste Manag.* 18, 226–238.
5. Feng, L.Y., Wang, H., Chen, Y.G., Wang, Q., 2009. Effect of solids retention time and temperature on waste activated sludge hydrolysis and short-chain fatty acids accumulation under alkaline conditions in continuous-flow reactors. *Bioresour. Technol.* 100 (1), 44-49
6. García-Depraect, O; Lebrero, R., Rodriguez-Vega, S., Aragao Borner, R., Borner, T., Munoz, R., 2022. Production of volatile fatty acids (VFAs) from five commercial bioplastics via acidogenic fermentation. *Bioresource Technology* 360, 127655
7. Garrido, J.M.; M. Fdz-Polanco, F. Fdz-Polanco, 2013. Working with energy and mass balances: a conceptual framework to understand the limits of municipal wastewater treatment, *Water Sci. Technol.* 67 2294–2301
8. Ghidotti, M., Fabbri, D., Torri, D., Piccinini, S., 2018. Determination of volatile fatty acids in digestate by solvent extraction with dimethyl carbonate and gas chromatography-mass spectrometry. *Analytica Chimica Acta* 1034, 92-101.
9. Iglesias-Iglesias, R.; Campanaro, S.; Treu, L.; Kennes, C.; Veiga, M.C. Valorization of sewage sludge for volatile fatty acids production and role of microbiome on acidogenic fermentation. *Bioresour. Technol.* 2019, 291, 121817.
10. IPCC, 2022: Climate Change 2022: Impacts, Adaptation, and Vulnerability. Contribution of Working Group II to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [H.-O. Pörtner, D.C. Roberts, M. Tignor, E.S. Poloczanska, K. Mintenbeck, A. Alegría, M. Craig, S. Langsdorf, S. Löschke, V. Möller, A. Okem, B. Rama (eds.)]. Cambridge University Press. In Press.
11. Judd, S. C. Judd, *The MBR Book*, Second Edition, Butterworth-Heinemann, Oxford, 2011.

12. Koch, K., Fernández, Y.B., Drewes, J.E., 2015. Influence of headspace flushing on methane production in Biochemical Methane Potential (BMP) tests. *Bioresour. Technol.* 186, 173–178.
13. Kornbluth, Phil (31 August 2018). "BLM reaps surprising windfall from FY 2019 Crude Helium Auction". *gasworld*.
14. Kubonova, L.; Janakova, I.; Malikova, P.; Drabinova, S.; Dej, M.; Smelik, R.; Skalny, P.; Heviankova, S. Evaluation of Waste Blends with Sewage Sludge as a Potential Material Input for Pyrolysis. *Appl. Sci.* 2021, 11, 1610. <https://doi.org/10.3390/app11041610>
15. Li, L., Wang, Y., Li, Y., 2019. Effects of substrate concentration, hydraulic retention time and headspace pressure on acid production of protein by anaerobic fermentation. *Bioresource Technology* 283, 106–111.
16. Li, X., Peng, Y., Li, B., Wu, C., Zhang, L., 2017. Effects of alkali types on waste activated sludge (WAS) fermentation and microbial communities, *Chemosphere* 186 864–872, <https://doi.org/10.1016/j.chemosphere.2017.08.017>.
17. Li, L., Chen, C., Chen, Y., Liu, H., Liu, R., Yang, D., Dong, B., Dai, X., 2021. Secondary acidogenic fermentation of waste activated sludge via voltage supplementation: Insights from sludge structure and enzymes activity. *Sci Total Environ* 25, 797:149161. doi: 10.1016/j.scitotenv.2021.149161.
18. Liu, Hongbo, Li, Y., Fu, B., Guo, H., Zhang, J., and Liu, He, 2020, Recovery of volatile fatty acids from sewage sludge through anaerobic fermentation, in *Current Developments in Biotechnology and Bioengineering*, Elsevier, 151–175.
19. Lü, F.; Wang, Z.; Zhang, H.; Shao, L.; He, P. Anaerobic digestion of organic waste: Recovery of value-added and inhibitory compounds from liquid fraction of digestate. *Bioresour. Technol.* 2021, 333, 125196.
20. Lyberatos, G., I.G. Skiadas, Modelling of anaerobic digestion—a review, *Global Nest: Int J*, 1 (1999), pp. 63-76.
21. Ma, H., Chen, X., Liu, H.e., Liu, H., Fu, B.o., 2016. Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: Alkaline or neutral pH? *Waste Manage.* 48, 397–403.
22. Mannina G., Alduina R., Badalucco L., Barbara L., Capri F.C., Cosenza A., Di Trapani D., Gallo G., Laudicina V.A., Muscarella S.M., Presti D., 2021. Water resource recovery facilities (Wrrfs): The case study of palermo university (Italy). *Water*, 13 (23),3413 <https://doi.org/10.3390/w13233413>.
23. Mohammad Mirsoleimani Azizi, S., Dastyar, W., Meshref, M., Maal-Bared, R., Ranjan Dhar, B., 2021. Low-temperature thermal hydrolysis for anaerobic digestion facility in

- wastewater treatment plant with primary sludge fermentation. *Chem. Eng. J.* 426 (March) <https://doi.org/10.1016/j.cej.2021.130485>.
24. Montiel-Jarillo, G., Gea, T., Artola, A., Fuentes, J., Carrera, J., and Suarez-Ojeda, M.E., 2021, Towards PHA Production from Wastes: The Bioconversion Potential of Different Activated Sludge and Food Industry Wastes into VFAs Through Acidogenic Fermentation: Waste and Biomass Valorization, no. 08130
 25. Moretto, G., Russo, I., Bolzonella, D., Pavan, P., Majone, M., and Valentino, F., 2020, An urban biorefinery for food waste and biological sludge conversion into polyhydroxyalkanoates and biogas: *Water Research*, v. 170, p. 115371.
 26. Nabaterega, R., V. Kumar, S. Khoei, C. Eskicioglu, A review on two-stage anaerobic digestion options for optimizing municipal wastewater sludge treatment process, *J. Environ. Chem. Eng.* 9 (2021), <https://doi.org/10.1016/j.jece.2021.105502>.
 27. Presti, D., Cosenza, A., Capri, F.C., Gallo, G., Alduina, R., Mannina, G., 2021. Influence of volatile solids and pH for the production of volatile fatty acids: batch fermentation tests using sewage sludge. *Bioresour. Technol.* 342, 125853.
 28. Reyhanitash, E.; Fufachev, E.; van Munster, K.D.; van Beek, M.B.M.; Sprakel, L.M.J.; Edelij, C.N.; Weckhuysen, B.M.; Kersten, S.R.A.; Bruijninx, P.C.A.; Schuur, B. Recovery and conversion of acetic acid from a phosphonium phosphinate ionic liquid to enable valorization of fermented wastewater. *Green Chem.* 2019, 21, 2023–2034.
 29. Tu, W., Zou, Y., Wu, M., Wang, H., 2020. Reducing the effect of non-volatile fatty acids (non-VFAs) on polyhydroxyalkanoates (PHA) production from fermented thermally hydrolyzed sludge. *Int. J. Biol. Macromol.* 155, 1317–1324.
 30. Yan, B.H., Selvam, A., Wong, J.W.C., 2017. Influence of acidogenic headspace pressure on methane production under schematic of diversion of acidogenic off-gas to methanogenic reactor. *Bioresour. Technol.* 245, 1000–1007
 31. Yang, X., Choi, H.S., Park, C., Kim, S.W., 2015. Current states and prospects of organic waste utilization for biorefineries. *Renew. Sustain. Energy Rev.* 49, 335–349.
 32. Yuan, Q., Sparling, R., Oleszkiewicz, J.A., 2011. VFA generation from waste activated sludge: Effect of temperature and mixing. *Chemosphere* 82 (4), 603–607.