Impact of organic loading rate and microbial migration on acidogenic co-fermentation of waste activated sludge and food waste

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Introduction

Mixed culture fermentation is an emerging biotechnology to convert organic waste into readily assimilable organic compounds such as volatile fatty acids (VFA), lactic acid and alcohols. In wastewater treatment plants, co-fermentation of waste activated sludge (WAS) with food waste (FW) provides an opportunity to boost the yield of VFA and alcohols (XOH) (Perez-Esteban et al., 2022). It has been demonstrated that the organic loading rate (OLR) is one of the operating parameters, along with pH and temperature, with the largest influence on the microbial consortium (Xin et al., 2018) and consequently on the fermentation yield and product profile (Rincón et al., 2008).

The objective of this research is to assess the resilience in the fermentation process in response to OLR disturbance. Furthermore, the impact of microbial migration on fermentation will be evaluated by feeding different WAS collection batches.

Materials and method

WAS (WAS_A and WAS_B) was collected from the same municipal WWTP's secondary clarifier, but at different collection periods. Synthetic FW was prepared to mimic the composition of household biowaste as reported by Vidal-Antich et al. (2021).

Two 4-L jacketed fermenters equipped with mechanical stirrers at 35 °C were used. They were manually fed daily with a 70:30 WAS:FW mixture (%VS basis). pH was not controlled. Two long-term disturbances were applied to fermenters, one increasing the OLR by increasing the amount of FW in the feedstock and the other changing the WAS, the main substrate collected from different time periods but the same WWTP. Control fermenter (C) operated at 11 gVS/(L·d) (HRT 3.3 days) with two WAS (A and B) (C_11A and C_11B, respectively). The tested fermenter (T) had four stages (T_11A, T_18A, T_18B, and T_11B) with varying OLR (11 and 18gVS/(L·d)) and WAS (A and B) during its operation. Changes in WAS or OLR were made after stability was achieved for over 3 consecutive HRTs. All analytical procedures were performed following the Standard Methods (APHA, 2017). DNA extraction was performed following the MiDAS field guide and the FastDNA® spin kit for soil. The V3-V4 region of 16S rRNA was sequenced by Novogene. Taxonomy was assigned to each amplicon sequencing variant (ASV) using MiDAS 5.1 database. Microbiology analyses were carried out with R studio v. 4.2.2.

Results and discussion

The OLR disturbance did not lead to a deterioration of the process. For example, fermentation yield before the OLR increase (stage T_11A) was 277 ± 15 mgCOD/gVS, and after the disturbance, it reverted to the original OLR, in stage T_11B, obtaining 234 ± 7 mgCOD/gVS. However, increasing the OLR caused a different fermentation product profile (Figure 1). The fermentation product profile was dominated by acetic, butyric and propionic acid at 11 gVS/(L·d), in line with other studies (Vidal-Antich et al., 2021), showing the reproducibility of the anaerobic co-fermentation process. At 18 gVS/(L·d), the fermentation product profile was dominated by acetic acid, ethanol and caproic acid. The shift in product profile may be the result of two potential mechanisms: the conversion of butyric acid into acetic acid and ethanol, and/or an increased generation of NADH (reducing agents) due to the applied OLR, which changed the fermentation metabolic fluxes to produce more reduced compounds such as ethanol (Vemuri et al., 2007).

After the OLR disturbance, the process was reversed, returning to the initial VFA profile. As shown Figure 2A, after the OLR disturbance of the process, stage T_11B started to revert towards the previous conditions (T_11A) regardless of the variation in the WAS. This fact demonstrates the resilience of the fermentation process. In the C fermenter, it was observed that after changing the WAS from WAS_A to WAS_B (same WWTP but different period) a depletion on acetic acid concentration was observed, but this consumption was not detected in fermenter

T. Therefore, the fermentation process has been shown to be resilient and it is possible that this acetic acid consumption was mitigated by increasing the OLR in fermenter T.

Immigrating species from the WAS had a noticeable impact on the microbial community structure. The WAS change generated a larger shift in the microbiological structure than the OLR disturbance, as seen by the clusters' proximity in the PCA plot (Figure 2B). Thus, in terms of microbiological structure, the WAS disturbance has a larger impact than the OLR disturbance. This demonstrates that microbes immigrating from WAS had their impact on the fermenter's microbial community, while more functional research is required to identify their role in the fermentation process. However, the most significant impact on microbial immigration is the entrance of undesirable species in the fermenters. A clear example would be acid-consuming microorganisms. Species midas_s_9557, (genus *Methanosarcina*) is a mixotrophic archaea capable of acetate consumption and resistant to high VFA concentrations (De Vrieze et al., 2012). This microorganism grew in particularly during stage C_11B concomitant to the acetic acid consumption. Species midas_s_9557, was also present in very low abundance (< 0.05%) at stages T_18B and T_11B, but it did not proliferate further to observe an acetic acid net consumption.

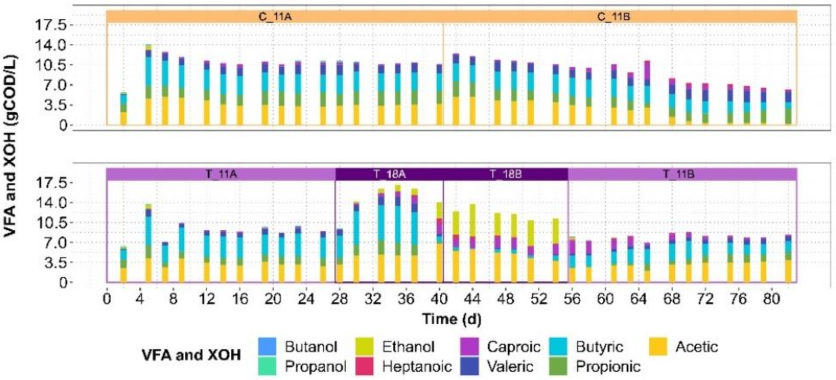

Figure 1. VFA and XOH product profile evolution for each fermenter and stage.

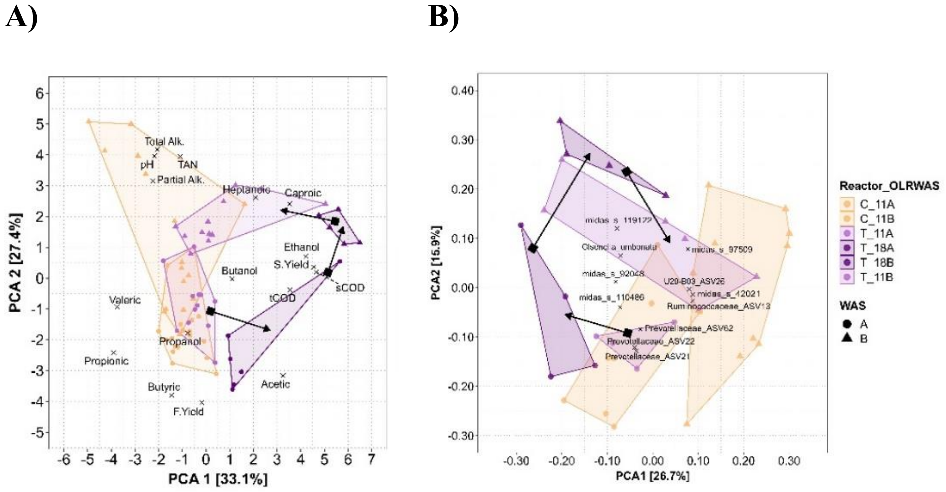

Figure 2. A) PCA of process variables and B) PCA of growing microorganisms for each fermenter and stage. Orange colour represents the control fermenter, purple represents the test fermenter where light purple is the operation at 11gVS/(L·d) and dark purple 18gVS/(L·d). The marker shape represents the two WAS batches and arrows indicate the movement of the clusters upon the operational change.

Conclusions

An increase in OLR affected the fermentation product profile in WAS and FW co-fermentation while maintaining the fermentation yield. Following the OLR perturbation, the process recovered to its original performance, illustrating the fermentation process robustness. The WAS disturbance had a greater impact on the microbial structure than OLR disturbance. Methanogenic archaea imported from the WAS thrived in the control fermenter, resulting in a net acetic acid consumption. However, the OLR disturbance mitigated their development.

References

APHA. (2017). Standard methods for the examination of water and wastewater / American Public Health Association, American Water Works Association, Water Environment Federation (22nd edition). American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF), Washington (D.C.), U.S.

De Vrieze, J., Hennebel, T., Boon, N., & Verstraete, W. (2012). Methanosarcina: The rediscovered methanogen for heavy duty biomethanation. Bioresource Technology, 112, 1–9. https://doi.org/10.1016/j.biortech.2012.02.079

Perez-Esteban, N., Vinardell, S., Vidal-Antich, C., Peña-Picola, S., Chimenos, J. M., Peces, M., Dosta, J., & Astals, S. (2022). Potential of anaerobic co-fermentation in wastewater treatments plants: A review. Science of The Total Environment, 813, 152498. https://doi.org/10.1016/j.scitotenv.2021.152498

Rincón, B., Sánchez, E., Raposo, F., Borja, R., Travieso, L., Martín, M. A., & Martín, A. (2008). Effect of the organic loading rate on the performance of anaerobic acidogenic fermentation of two-phase olive mill solid residue. Waste Management, 28(5), 870–877. https://doi.org/10.1016/j.wasman.2007.02.030

Vemuri, G. N., Eiteman, M. A., McEwen, J. E., Olsson, L., & Nielsen, J. (2007). Increasing NADH oxidation reduces overflow metabolism in Saccharomyces cerevisiae. Proceedings of the National Academy of Sciences of the United States of America, 104(7), 2402–2407. https://doi.org/10.1073/pnas.0607469104

Vidal-Antich, C., Perez-Esteban, N., Astals, S., Peces, M., Mata-Alvarez, J., & Dosta, J. (2021). Assessing the potential of waste activated sludge and food waste co-fermentation for carboxylic acids production. Science of The Total Environment, 757, 143763. https://doi.org/10.1016/j.scitotenv.2020.143763

Xin, X., He, J., & Qiu, W. (2018). Volatile fatty acid augmentation and microbial community responses in anaerobic co-fermentation process of waste-activated sludge mixed with corn stalk and livestock manure. Environmental Science and Pollution Research, 25(5), 4846–4857. https://doi.org/10.1007/s11356-017-0834-0

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