Influence of Organic Loading and Zeolite Addition on Organic Waste Fermentative VFA Production

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Volatile fatty acids (VFA) are key intermediates in various biotechnological processes, with high economic value and broad application ranges (Kleerebezem et al., 2015). Maximizing the fermentative conversion of organic waste into VFAs may play an important role in the development of sustainable waste management strategies, as it opens opportunities for coupling with several biotechnology processes, such as two-phase anaerobic digestion and photofermentative hydrogen production (Asunis et al., 2022).

VFA production yields are affected by several factors, including substrate composition and biodegradability, microbial community, operational conditions, the presence of inhibitory compounds, and the supplementation of nutrients or additives. Ammonium release, for instance, is one of the main inhibitory compounds of the process, as it can, on the one hand, inhibit microbial activity and decrease VFA yield (Qin et al., 2025), and on the other hand, hinder the opportunity for coupling the system with other biotechnological processes (e.g., photo-fermentation, (Niño-Navarro et al., 2020)). In this regard, the application of zeolites during acidogenic fermentation has gained the interest of many authors in recent years, due to their capacity of microorganisms' immobilization, which improves substrate conversion yields, and their cation exchange properties, which enhance ammonia removal and pH buffer in the system (Paritosh et al., 2020).

In this study, the influence of zeolite addition (0 – 20 g/L) and organic loading (10 – 50 g_{TVS}/L) on VFAs production has been investigated. The fermentation test was performed using as substrates a mixture of organic waste composed of Waste Activated Sludge (WAS), Toilet Paper (TP), Lettuce (L), Red Beet (RB), and Wheat straw (W), in a TS ratio of 4.5 : 1 : 6 : 6 : 6 (WAS : TP : L : RB : W). The substrates used in this study and their respective ratio were selected as a possible scenario of waste generated by a crew of astronauts (MELiSSA project, contract No. 19297/05/NL/SFe), to investigate the possible application of fermentative VFA production as sustainable waste management strategy on long-term space missions, in agreement with the framework within this study is conducted. The substrates were purchased at a grocery shop, then individually blended using a kitchen blender until complete homogenization. The WAS was collected from the static gravimetric thickener of a local municipal wastewater treatment plant (WWTP, VERITAS spa, Fusina, Italy). No inoculum was used, relying on the microorganisms naturally present in the treated substrates. Details on the composition and physicochemical characteristics of the substrates are provided in Table 1.

Table 1. Physicochemical characterization of the substrates used during the test.

	TS (g_{TS}/kg)	TVS (g_{TVS}/kg)	COD (g_{COD}/kg)
LW	77.5 ± 0.3	89.2 ± 0.4	902 ± 40
RB	98.0 ± 0.2	68.1 ± 0.1	1130 ± 70
W	897 ± 3	855 ± 3	1090 ± 50
TP	953 ± 3	946 ± 0.2	1059 ± 40
WAS	48.1 ± 0.1	35.3 ± 0.1	1046 ± 63

The operative conditions tested during the experiment are reported in Table 2.

Table 2. Operative conditions investigated in this study.

	OL (g_{TVS}/L)	zeolite ($g_{zeolite}$/L)
Test 1	20, 25, 30, 40, 50	-
Test 2	40	0, 5, 10, 15, 20

Test 3	20, 25, 30, 40, 50	15

The fermentation tests have been carried out in triplicate using an automatic anaerobic batch system (Nautilus, Anaero Technology, Cambridge, UK) at mesophilic temperature (37.0 ± 0.1 °C) on 1 L reactors (0.4 L of operative volume). Each test was run for 14 days, periodically monitoring VFA production throughout the experiment.

The highest VFA concentration was achieved by combining the higher OL of 50 g_{TVS}/L and the dosage of 15 $g_{zeolite}$/L, reaching a concentration of 36 ± 5 $g_{VFA(COD)}$/L. The highest conversion yield was obtained dosing the same amount of zeolite at OL 30 g_{TVS}/L, with a yield of 0.89 ± 0.08 $g_{VFA(COD)}/g_{TVS}$, while the highest COD/NH_4^+ ratio, an important parameter to consider when aiming at coupling the fermentative step with subsequent biological processes, was obtained at OL of 40 g_{TVS}/L dosing 20 $g_{zeolite}$/L. Under this latter condition, a concentration of 29 ± 4 $g_{VFA(COD)}$/L and a conversion yield of 0.74 ± 0.10 $g_{VFA(COD)}/g_{TVS}$ were obtained. Overall, the dosage of zeolite was observed to be a successful solution for increasing the COD/NH_4^+ ratio of the produced fermentative effluent, facilitating the integration of fermentative waste valorization with various downstream biotechnological processes, in which high ammonium concentration is often a limiting factor.

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