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## BACKGROUND & AIM

Volatile fatty acids (VFA) are key intermediates in the conversion of organic waste into valuable chemicals and biofuels. High ammonia concentrations may inhibit VFA-degrading microorganisms and photofermentation processes. Zeolite addition can enhance fermentation by improving microbial activity and reducing ammonia accumulation.

### AIM OF THIS STUDY

To optimize VFA production and minimize ammonia accumulation during acidogenic fermentation by evaluating the combined effects of organic loading (OL) and zeolite addition under pH-uncontrolled conditions.

## SUBSTRATES

Mixture of organic waste representative of a crew of astronauts (MELISSA project), in a TS ratio of 4.5 : 1 : 6 : 6 : 6 (WAS : TP : L : RB : W).



Table 1. Physicochemical characterization of the substrates.

Substrate	TS (gTS/kg)	TVS (gTVS/kg)	COD (gCOD/kgTS)
Red beet (RB)	98.0 ± 0.2	89.2 ± 0.4	1130 ± 70
Lettuce (L)	77.5 ± 0.3	68.1 ± 0.1	902 ± 40
Straw (W)	89.7 ± 3	85.5 ± 3	1090 ± 50
Toilet paper (TP)	95.3 ± 3	94.6 ± 1	1059 ± 40
WAS	48.1 ± 0.1	35.3 ± 0.1	1046 ± 63

## ZEOLITE



- Ammonium removal and pH buffering
- Microorganisms immobilization
- Higher VFA yield and stability

## WHY VFAs?

- Valuable platform chemicals for biotechnological processes
- Key step for sustainable waste biorefineries
- Enables coupling with AD and hydrogen (photo)fermentation
- Essential for bioregenerative life-support systems in space missions

## EXPERIMENTAL SETUP

- Anomonic anaerobic batch system (Nuair®; Anaerobic Technology, UK)
- 1 L reactors (0.4 L operative volume)
- Mesophilic temperature: 37.0 ± 0.1 °C
- Duration: 14 days
- Tests performed in triplicate
- Periodical monitoring of VFA and NH<sub>4</sub><sup>+</sup>-N

### EXPERIMENTAL DESIGN

#### EXP 1 – Effect of OL (Control – no zeolite)

OL = 20, 25, 30, 40, 50 gTVS/L

#### EXP 2 – Effect of zeolite dose (OL fixed)

OL = 40 gTVS/L, Zeolite = 0, 5, 10, 15, 20 g/L

#### EXP 3 – Effect of OL (Zeolite fixed)

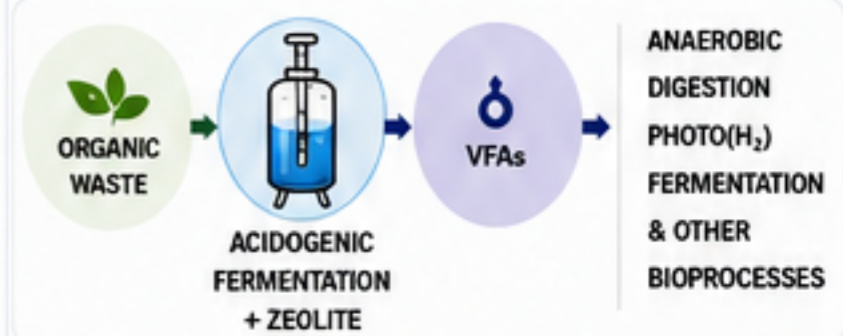
Zeolite = 15 g/L; OL = 20, 25, 30, 40, 50 gTVS/L

### ANALYTICAL METHODS

- COD, sCOD, TS, TVS, NH<sub>4</sub><sup>+</sup>-N (Standard Methods)
- VFA analysis by HPLC (Agilent 1100 Series, DAD)
- Samples centrifuged (9000 rpm, 10 min) and filtered (0.2 µm) before analysis

## KEY FINDINGS

- VFA production increased with OL, with the highest yields at OL = 40 gTVS/L (EXP 1).
- Zeolite addition enhanced VFA concentration and yield in a dose-dependent manner, peaking at 15–20 g/L (EXP 2).
- Fixing zeolite at 15 g/L, the highest VFA concentration was achieved at OL = 50 gTVS/L (EXP 3).
- Zeolite reduced ammonia yields by ~6% at OL 40 and 50.
- Optimal conditions: OL = 30–40 gTVS/L with 15 g/L zeolite → highest yield (~0.8 gCOD/gVS) and lower ammonia production.



## RESULTS

### EXP 1 – Effect of Organic Loading (No Zeolite)

VFA concentration (gCOD/L)

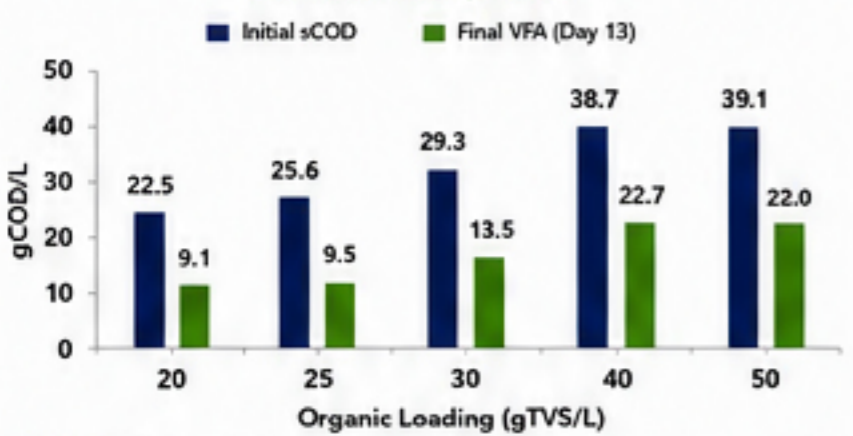


Table 2. Changes in Ammonia and VFA (EXP 1)

OL (gTVS/L)	Ammonia content (mg NH <sub>4</sub> <sup>+</sup> -N/L)	VFA content (gCOD/L)	Ammonia yield (mg NH <sub>4</sub> <sup>+</sup> /gTVS)	VFA yield (gCOD/gTVS)
20	138.5	9.1	6.34	0.45
25	183.5	9.5	7.34	0.38
30	256.2	13.5	8.54	0.45
40	290.2	22.7	7.25	0.57
50	339.4	22.0	6.79	0.44

### EXP 2 – Effect of Zeolite Dose (OL = 40 gTVS/L)

VFA concentration (gCOD/L)

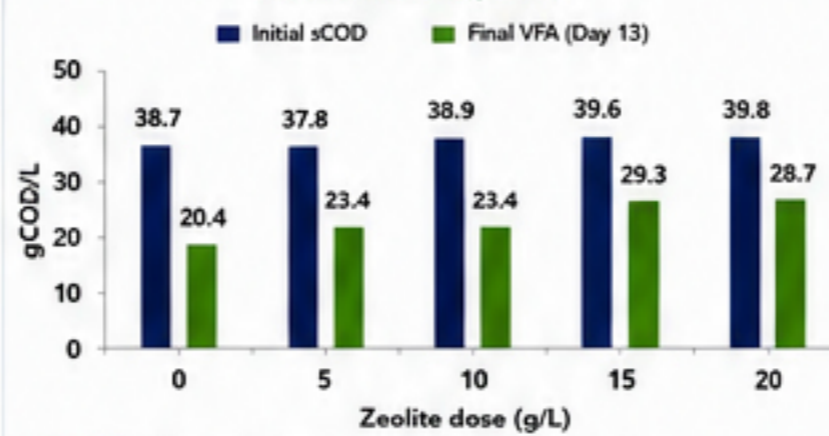


Table 3. Changes in Ammonia and VFA (EXP 2)

Zeolite (g/L)	Ammonia content (mg NH <sub>4</sub> <sup>+</sup> -N/L)	VFA content (gCOD/L)	Ammonia yield (mg NH <sub>4</sub> <sup>+</sup> /gTVS)	VFA yield (gCOD/gTVS)
0 (Z=0)	402.7	20.4	10.1	0.51
5 (Z=5)	342.2	23.4	8.6	0.59
10 (Z=10)	381.2	23.4	9.5	0.59
15 (Z=15)	298.8	29.3	7.4	0.73
20 (Z=20)	283.6	28.7	7.1	0.72

### EXP 3 – Effect of Organic Loading (Zeolite = 15 g/L)

VFA concentration (gCOD/L)

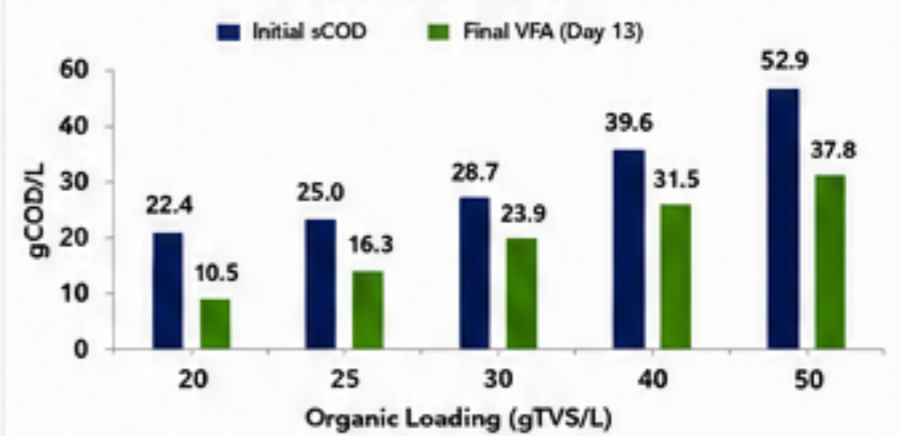


Table 5. Changes in Ammonia and VFA (EXP 3)

OL (gTVS/L)	Ammonia content (mg NH <sub>4</sub> <sup>+</sup> -N/L)	VFA content (gCOD/L)	Ammonia yield (mg NH <sub>4</sub> <sup>+</sup> /gTVS)	VFA yield (gCOD/gTVS)
20	90	10.5	4.5	0.53
25	161	16.3	6.4	0.65
30	250	23.9	8.3	0.80
40	273	31.5	6.8	0.79
50	319	37.8	6.4	0.76

## YIELD & FERMENTATION RATE COMPARISON

Experiment	OL (gTVS/L)	Zeolite (g/L)	VFA (gCODVFA/gTVS)	Fermentation rate (gCOD/VFA/L-d)
EXP 1	20	0	0.45	0.95
	25	0	0.38	1.15
	30	0	0.45	1.25
	40	0	0.57	2.20
	50	0	0.44	2.21
EXP 2	40	0	0.51	2.04
	40	5	0.59	2.35
	40	10	0.59	2.24
	40	15	0.73	2.93
	40	20	0.72	2.87
EXP 3	20	15	0.53	1.05
	25	15	0.65	1.63
	30	15	0.80	2.39
	40	15	0.79	3.15
	50	15	0.76	3.78

## CONCLUSION

- Zeolite addition significantly enhanced VFA production and yield in a dose-dependent manner, with optimal performance at 15–20 g/L.
- The highest VFA concentration was achieved at OL = 50 gTVS/L with 15 g/L zeolite (37.8 gCOD/L), while the highest yield (~0.8 gCOD/gTVS) was obtained at OL 30–40 gTVS/L with 15 g/L zeolite.
- Zeolite slightly reduced ammonia yields (~6%) but did not significantly remove TAN.
- Optimal condition for balancing high yield, high VFA concentration, and lower ammonia production: **OL = 30–40 gTVS/L with 15 g zeolite/L.**
- These results support the integration of acidogenic fermentation with downstream processes such as photofermentation for hydrogen production in sustainable biorefineries and space missions.

## ACKNOWLEDGEMENTS

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