N-CAPROIC ACID PRODUCTION FROM FOOD WASTE VIA TWO-STAGE ANAEROBIC FERMENTATION

Samuel G. Arhin[1]*, Alessandra Cesaro[1], Francesco Di Capua[2] & Giovanni Esposito[1]

[1] *Department of Civil, Architecture and Environmental Engineering, University of Naples Federico II, Via Claudio 21, 80125, Naples, Italy,*
[2] *School of Engineering, University of Basilicata, via dell'Ateneo Lucano 10, 85100, Potenza, Italy.*
** Corresponding author's email address: emailadress@emailadress.com*

Highlights

- Enhanced lactic acid production was attained in the first-stage bioreactor, reaching 43.5 g/L
- High *n*-caproic acid titer (14.7 g/L) and productivity (4.9 g/L.d) were attained in stage-two
- Lactic acid exhibited a more pronounced stimulatory effect on *n*-caproic acid than ethanol.

Introduction

The global rise in population and swift socioeconomic shifts have led to a significant surge in food waste generation (Arhin et al., 2023a, 2025). This situation presents both a challenge and an opportunity to develop environmental systems that can efficiently and effectively utilize the resource potential of food waste within a circular economy framework. Anaerobic fermentation with mixed microbial culture has attracted significant attention for food waste valorization due to its comparatively lower capital and operational costs compared to pure culture fermentation.

Lactic acid is one potential high-value product from the acidogenic fermentation (AF) of food waste by anaerobic microbiomes that has numerous industrial applications, including the food and beverage industries, pharmaceuticals, and in the synthesis of biodegradable plastics. The utility of lactic acid depends greatly on its nature and selectivity, which is influenced by the metabolic pathway assumed by the fermenting microbiome. Typically, the homo-lactic fermentation route is desirable during lactic acid fermentation because this pathway produces only lactic acid, precluding antagonistic pathways that may lead to the synthesis of volatile fatty acids (VFAs) or ethanol. Yet, the wide and complex spectrum of anaerobic microbiomes makes it challenging to steer fermentation reactions towards homo-lactic synthesis.

Recently, the utilization of food waste-derived lactic acid as an electron donor in chain elongation (CE) reactions to generate medium-chain fatty acids (MCFAs) such as *n*-caproic acid is increasingly gaining attention as an economically viable alternative route (Contreras-Dávila et al., 2020). *n*-Caproic acid is a platform chemical with numerous industrial applications, including aviation fuels, plasticizers, agrochemicals, fragrances, etc., (Arhin et al., 2023b). Its high hydrophobicity resulting from its higher carbon-to-oxygen ratio grants it better separability than lactic acid. In the lactic acid-driven CE pathway, lactate dehydrogenase catalyzes lactic acid conversion into pyruvate followed by pyruvate oxidation into acetyl-coenzyme A (acetyl-CoA). The 2-carbon acetyl-CoA molecule is then added to an electron acceptor (e.g., VFAs) via the cyclic reverse β-oxidation (RBO) or fatty acids biosynthesis (FAB) pathway, elongating the carbon length of the electron acceptor with two carbons (C2) per cycle. Because by-products such as VFAs and ethanol generated alongside lactic acid via hetero-lactic fermentation are also vital intermediates in *n*-caproic acid production, hetero-lactic fermentation could be tolerated when lactic acid is to be used for *n*-caproic acid production, guaranteeing operational flexibility during lactic acid fermentation.

Although *n*-caproic acid could be generated from food waste via a single-stage CE, the two-stage approach whereby a lactic acid-rich broth is generated in the first-stage bioreactor followed by CE in the second-stage bioreactor has been demonstrated to outperform single-stage setups as the operational conditions for the two main functional groups (i.e., acidogenic bacteria and chain elongators) could be optimized separately (Duber et al., 2025). Nonetheless, limited information exists on the systematic optimization of two-stage CE systems, especially operational parameters for enhancing lactic acid yield via the hetero-lactic pathway for subsequent *n*-caproic acid synthesis. A key approach to control and influence the metabolic pathway during AF and CE is by

manipulating environmental conditions such as temperature, pH, hydraulic retention time (HRT), and organic loading rate (OLR). Therefore, this study aimed to maximize lactic acid production from mixed food waste for subsequent utilization for *n*-caproic acid production by exploring the influence of various environmental conditions, including initial pH adjustment, OLR, and HRT on lactic acid and n-caproic acid production.

Material and Methods

Food waste from a food retail market in Naples (Campania, Italy) was used as a substrate in this study. It was comprised of fruits and vegetables waste (79 %), stale bread (6 %), pasta, rice, cereal, and flour residues (5%), meat residues (8 %), and cheese and diary residues (2 %) as described in previous studies (Arhin et al., 2023a, 2025). The food waste was homogenized into a uniform slurry upon receipt with a kitchen food processor and stored at – 20 ◦C. The total solid (TS) and volatile solid (VS) content of the food waste were 20.2 ± 0.1% and 19.2 ± 0.1%, respectively.

The inoculum used in the AF and CE bioreactors originated from the anaerobic digester of Mercato San Severino municipal wastewater treatment plant (MSSMWTP, Campania, Italy). The characteristics of the mesophilic digestate used as inoculum, including TS and VS were 10.0 ± 0.0% and 2.2 ± 0.0%.

The experimental setup consisted of a two-stage anaerobic fermentation system operated in semi-continuous and continuous mode. Specifically, the first-stage bioreactor used for AF was a 5 L glass jacket continuously stirred tank reactor (CSTR) with an effective volume of 2 or 3 L, depending on the operating condition (**Table 1**). In the start-up phase (period A0), the food waste was feed directly into the AF bioreactor without pH control at an HRT of 10 d, OLR of 16.4 g COD/L.d, and a mesophilic temperature of 35 °C. Afterward, the influent pH was increased to 9.0 in period AI while maintaining the other parameters. Lastly, in period AII, the HRT was reduced from 10 to 5 d and the working volume was increased to 3 L. The AF bioreactor was operated in the semi-continuous mode with intermittent sampling and feeding every 3.5 d. Thus, depending on the HRT, a specific amount of the fermentation broth was discharged every 3.5 d, and an equal amount of fresh food waste was added to the bioreactor.

Table 1. Operating conditions of the acidogenic fermentation bioreactor.

Period	Influent pH	HRT (d)	OLR (g COD/L.d)	Volume (L)	Temperature (°C)
A0 (startup)	5.5 (original pH of food waste)	10.0	16.4	2.0	35
AI	9.0	10.0	16.4	2.0	35
AII	9.0	5.0	32.7	3.0	35

The discharged lactic acid-rich effluent from the AF bioreactor was used as the influent for the second-stage (CE) bioreactor after centrifugation (5000 rpm for 10 min) and microfiltration (1.5 µm) to reduce the solids content. The CE bioreactor was also a 3 L glass jacket CSTR bioreactor operated in continuous mode with an effective volume of 1L. The CE bioreactor begun operating after stable lactic acid production was attained in the AF bioreactors (i.e. period I). The operating conditions of the CE bioreactor are also shown in **Table 2**. As portrayed in **Table 2**, the influent pH in period I of the CE bioreactor (BI) was 5.5 and that of period II (BII) was 5.0 while all the other parameters, including HRT and temperature were the same.

Table 2. Operating conditions of the acidogenic fermentation bioreactor.

Period	Influent pH	HRT (d)	OLR (g COD/L.d)	Volume (L)	Temperature (°C)
BI	5.5	3.0	20.0	1.0	35
BII	5.0	3.0	20.0	1.0	35

The biogas produced in the AF and CE bioreactors were collected in 10 L gas bags connected to the bioreactors and the gas volume was determined by the water displacement method. The gas volume was normalized at standard temperature and pressure (Sun et al., 2021; Yang et al., 2022).The gas composition was also analyzed with a QCA 2.0 gas analyzer (Hiden Analytical, UK). Organic acids and alcohols were analyzed by a high-

performance liquid chromatography (Nexera Series, Shimadzu, Japan) equipped with a photo-diode array detector (SPD-M40), a refractive index detector (RID-20A), and a Rezex ROA-organic acid H+ (8 %) column (Phenomenex, USA). The mobile phase was 1 mM H2SO4 solution at a flow rate of 0.8 mL/min. The oven temperature was maintained at 50 ◦C. Samples for carboxylic acid and alcohols analysis were centrifuged (10,000 rpm for 10 min) and microfiltered 0.22 µm (MCE syringe filter, Labfil, China) before analysis. The TS and VS content of the substrate and inoculum were also analyzed according to standard methods (Arhin et al., 2023a).

Results and Discussion

Figure 1 shows the performance of the AF biorector in terms of the pH, carboxylic acids and ethanol production. As shown in **Figure 1a**, in the start-up stage (period A0), the pH decreased continously from 6.50 to 3.85 by day 10, stabilizing thereafter around pH 4. Even though the influent pH was increased to 9.0 in periods AI and AII, the pH remained stable around pH 4 with no notewothy variation from period A0. Considering that the oprational pH was not controlled, a possible explaination for this occurance is that after each feeding cycle, fermentation contnued until the systems pH approached the pKA of the predominnant carboxylic acid. Afterwards acidification stalled to prevent the accumulation of undissociated acids that can be toxic to the microorganisms (Candry et al., 2020).

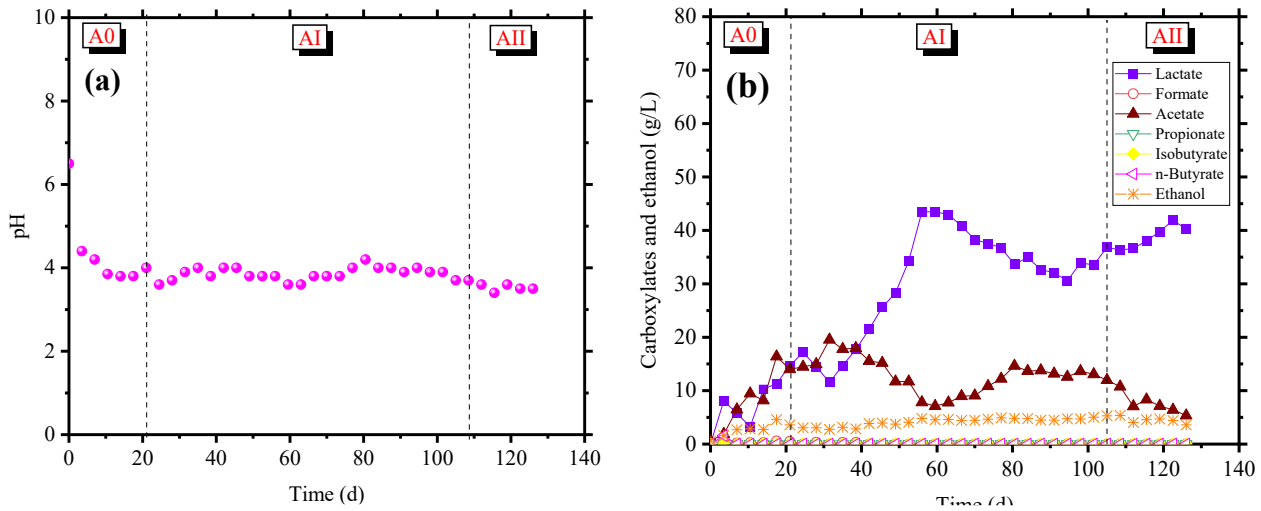

Figure 1. (a) pH profile and (b) carboxylic acids and ethanol production in the acidogenic fermentation bioreactor for generating electron donors and acceptors from food waste for subsequent utilization as reactors for n-caproic acid production.

As depicted in **Figure 1b**, lactic acid was the main metabolic product in the AF bioreactor. As a weak acid, lactic acid has a pKA value of 3.86, which could explain why the opeartional pH stabilized around pH 4.0. In period A0, the lactic acid and acetic acid concentrations were quite similar, reaching 14.6 and 14.0 g/L, respectively. When trhe influent pH was increased to 9.0 in period AI, an increase in lactic acid concentration was observed, reaching 43.5 g/L (i.e., 4.4 g/L.d) on day 56. Meanwhile, acetic acid concentration decreased to 7.8 g/L on day 56. After day 63, a potential oxidation of lactic acid into acetic acid occured, resulting in maginal drops in the concentration of lactic acid to 30.6 g/L, while acetic acid increased to 12.6 g/L on day 95.By reducing the HRT from 10 d to 5 d in period AII, the lactic acid concentration gradually retored to its peak, reaching 41.9 g/L (i.e., 8.4 g/L.d) around day 123. In contrast, acetic acid decreased again in period AII to 5.3 g/L on day 126. Overall, the results of the AF bioreactor depict that the pH and HRT are crucial process levers for regulationg the formation of lactic acid. The highest productivity of lactic acid (i.e., 8.4 g/L.d) was observed in period AII by increasing the initial pH to 9.0 and decresing the HRT to 5 d. The lactid acid productivity at an HRT of 5 d was twice that observed at HRT of 10 d, which is consistent with past studies suggesting that shorter HRT and higher OLR are more beneficial for lactic

acid accumulation (Tang et al., 2016). The results also conforms to past studies suggesting that the initial pH is crucial in anaerobic processes (Zhao et al., 2021).

It is worth noting that besides lactic acid and acetic acid, ethanol – a well known electron donor for *n*-caproic acid production (L. Wu et al., 2023; Q. Wu et al., 2018) – was slso detected in an appreciable concentration in the AF biorector. Ethanol can be formed via various metaboic pathways during fermentation, including pyruvate decarboxylation into acetaldehyde followed by acetaldehyde reduction using nicotinamide adenine dinucleotide (NADH) or flavin adenine dinucleotide ($FADH_2$) as electron donors. Alternatively, ethnol could also be formed via acetic acid conversion into acetyl-CoA followed by acetyl-CoA reduction into acetaldehyde. As depicted in **Figure 1b**, no notable vcariations in the ethanol concentration was observed in the different periods of fermentation, with concentration around 5 g/L detected in periods A0, AI, and AII. Decrease in the acetic acid concentration during peak lactic acid production in periods AI and AII had no apparent effects on ethanol production, suggesting that ethanol was likely formed via pyruvate decarboxylation rather than acetic acid reduction, considering that the food waste had high carbohydrates content for pyruvate synthesis.

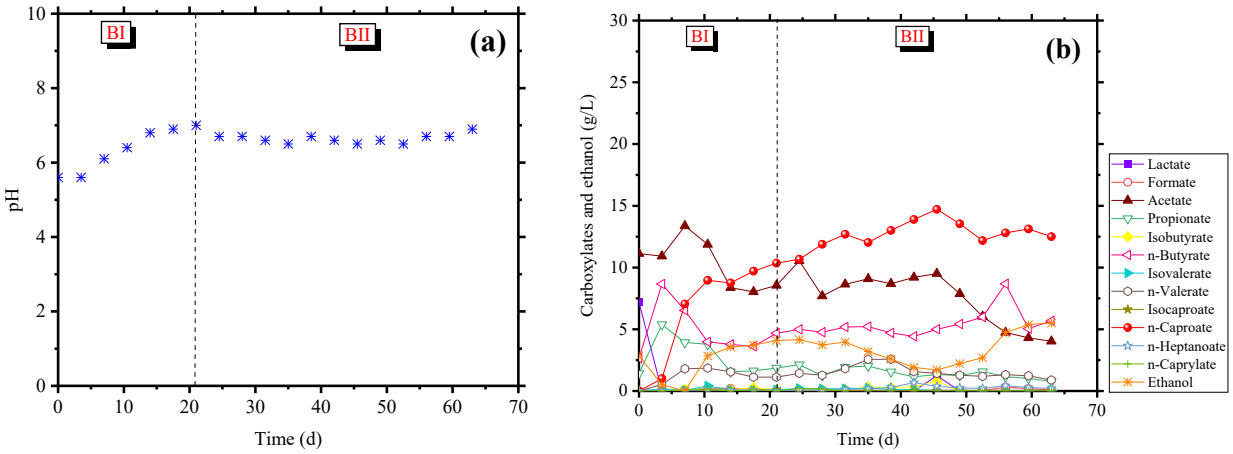

Figure 2. (a) pH profile and (b) carboxylic acids and ethanol production in the chain elongation bioreactor for generating *n*-caproic acid for the lactic acid-rich effluent from food waste acidogenic fermentation.

Figure 2 also shows the pH, carboxylic acids, and ethanol production in the CE biorector. As displayed in **Figure 2a,** the pH in the CE biorector increased gradually to 7.0 on day 21 and remained quite stable thereafter, even though the influent pH in period BI was 5.5 and that of BII was 5.0. Previous studies demonstrated that that lactic acid-driven CE is a net proton consuming process (Arhin et al., 2025). This could explain the net increase in the systems pH to around neutrality during fermentation.

As depicted in **Figure 2b**, the supplied lactic acid was completely consumed in the CE bioreactor. On the contrary, ethanol consumption, that could cause a net decrease in pH due to protons release, was supressed. Ethanol accumulation in period BI begun on day 10, reaching 4 g/L. Coisdering that the AF effluent contained about 5 g/L of ethanol, this results suggest that only about 1 g/L of the supplied ethanol was consumed in the CE phase. It should be mention that ethanol consumption improved after day 32, with only 1.7 g/L detected in the effluent of the CE bioreactor on day 46. However, ethanol begun to accumulate again, increasing to 5.5 g/L on day 63.

Regarding *n*-caproic acid production, it was observed that *n*-caproic acid concentration increased to a peak value of 14.7 g/L (4.9 g/L.d) on day 46. Overall, sustained generation of n-caproic acid above the solubility threshold of 10.8 g/L was observed after day 28. These results highlight that the microbiome used in this study could efficiently convert lactic acid to *n*-caproic acid. Besides *n*-caproic acid, acetic and butyric acid were the two notable co-products detected in the CE phase. Meanwhile odd-chain carboxylic acids, including propionic acid

and valeric acid and brached-chain caboxylic acids such as isobutyric acid and isovaleric acid acid made up a negligble proportion of the product spectrum with their concencention not exceeding 2 g/L throughout the CE phase.

Conclusion

In this study, a two-stage anaerobic fermentation process was used to convert food waste into high-value industrial biochemicals, including *n*-caproic acid. In the first-stage bioreactor, manipulating operating parameters such as influent pH and HRT impacted lactic acid generation. The highest lactic acid concentration of 43.5 g/L (4.4 g/L.d) was obtained by increasing the influent pH to 9.0 at an HRT of 10 d. Reducing the HRT from 10 d to 5 d doubled lactic acid productivity, reaching 8.4 g/L.d. Besides lactic acid, ethanol and acetic acid – an electron acceptor for the CE process - were the other key metabolites formed in the AF bioreactor.

In the second-stage bioreactor, lactic acid was efficiently converted into *n*-caproic acid via CE with limited carbon losses to the formation of odd-chain and branch-chain carboxylic acids. The concentration of *n*-caproic acid exceeded its solubility threshold during steady-state operations, attaining a peak value of 14.7 g/L (4.9 g/L.d). Lactic acid consumption maintained the operating pH around pH 7, circumventing the need for additional alkalinity supply for pH control. The pH self-regulating behavior of the CE system to near neutral pH also suppressed the accumulation of undissociated *n*-caproic acid and its potential toxicity to the microbiome.

Overall, the results are useful for the thrash-to-cash concept, providing useful insights on high-value *n*-caproic acid biosynthesis from food waste streams.

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References

Arhin, S. G., Cesaro, A., Capua, F. Di, & Esposito, G. (2025). Pivotal role of lactate-to-ethanol ratio in medium-chain fatty acids production without operational pH adjustment. *Chemical Engineering Journal*, *516*, 163675. https://doi.org/10.1016/j.cej.2025.163675

Arhin, S. G., Cesaro, A., Di Capua, F., & Esposito, G. (2023a). Acidogenic fermentation of food waste to generate electron acceptors and donors towards medium-chain carboxylic acids production. *Journal of Environmental Management*, *348*, 119379. https://doi.org/10.1016/j.jenvman.2023.119379

Arhin, S. G., Cesaro, A., Di Capua, F., & Esposito, G. (2023b). Recent progress and challenges in biotechnological valorization of lignocellulosic materials: Towards sustainable biofuels and platform chemicals synthesis. In *Science of the Total Environment* (Vol. 857). Elsevier B.V. https://doi.org/10.1016/j.scitotenv.2022.159333

Candry, P., Radić, L., Favere, J., Carvajal-Arroyo, J. M., Rabaey, K., & Ganigué, R. (2020). Mildly acidic pH selects for chain elongation to caproic acid over alternative pathways during lactic acid fermentation. *Water Research*, *186*. https://doi.org/10.1016/j.watres.2020.116396

Contreras-Dávila, C. A., Carrión, V. J., Vonk, V. R., Buisman, C. N. J., & Strik, D. P. B. T. B. (2020). Consecutive lactate formation and chain elongation to reduce exogenous chemicals input in repeated-batch food waste fermentation. *Water Research*, *169*. https://doi.org/10.1016/j.watres.2019.115215

Duber, A., Zagrodnik, R., Gutowska, N., Brodowski, F., Dąbrowski, T., Dąbrowski, S., Łężyk, M., & Oleskowicz-Popiel, P. (2025). Single- vs. two-stage fermentation of an organic fraction of municipal solid waste for an enhanced medium chain carboxylic acids production – The impact of different pH and temperature. *Bioresource Technology*, *415*. https://doi.org/10.1016/j.biortech.2024.131697

Sun, H., Yang, Z., Shi, G., Arhin, S. G., Papadakis, V. G., Goula, M. A., Zhou, L., Zhang, Y., Liu, G., & Wang, W. (2021). Methane production from acetate, formate and H2/CO2 under high ammonia level: Modified ADM1 simulation and microbial characterization. *Science of the Total Environment*, *783*. https://doi.org/10.1016/j.scitotenv.2021.147581

Tang, J., Wang, X., Hu, Y., Zhang, Y., & Li, Y. (2016). Lactic acid fermentation from food waste with indigenous microbiota: Effects of pH, temperature and high OLR. *Waste Management*, *52*, 278–285. https://doi.org/10.1016/j.wasman.2016.03.034

Wu, L., Wei, W., Chen, Z., Chen, X., & Ni, B. J. (2023). Long-chain alcohol production in open culture anaerobic fermentation. In *Chemical Engineering Journal* (Vol. 452). Elsevier B.V. https://doi.org/10.1016/j.cej.2022.139225

Wu, Q., Guo, W., Bao, X., Meng, X., Yin, R., Du, J., Zheng, H., Feng, X., Luo, H., & Ren, N. (2018). Upgrading liquor-making wastewater into medium chain fatty acid: Insights into co-electron donors, key microflora, and energy harvest. *Water Research*, *145*, 650–659. https://doi.org/10.1016/j.watres.2018.08.046

Yang, Z., Sun, H., Kurbonova, M., Zhou, L., Arhin, S. G., Papadakis, V. G., Goula, M. A., Liu, G., Zhang, Y., & Wang, W. (2022). Simultaneous supplementation of magnetite and polyurethane foam carrier can reach a Pareto-optimal point to alleviate ammonia inhibition during anaerobic digestion. *Renewable Energy*, *189*, 104–116. https://doi.org/10.1016/j.renene.2022.02.092

Zhao, Q., Arhin, S. G., Yang, Z., Liu, H., Li, Z., Anwar, N., Papadakis, V. G., Liu, G., & Wang, W. (2021). pH regulation of the first phase could enhance the energy recovery from two-phase anaerobic digestion of food waste. *Water Environment Research*, *93*(8), 1370–1380. https://doi.org/10.1002/wer.1527