FEMS Microbes, 2026, **7**, xtag035
https://doi.org/10.1093/femsmc/xtag035
Published: 18 June 2026
Research article – Microbes and Environment

Acetate to caproate: metagenomic insights into functional shifts in a methane-arrested anaerobic bioreactor

Laura G. Schaerer [1,*], Ryan S. Anderson [1], Joshua Chan [2], Susan K. De Long [1]

[1]Department of Civil and Environmental Engineering, Colorado State University, Fort Collins, CO 80523, United States
[2]School of Biomedical and Chemical Engineering, Colorado State University, Fort Collins, CO 80523, United States
*Corresponding author. Colorado State University, Department of Civil and Environmental Engineering, 700 Meridian Ave, Fort Collins, CO 80523, USA. E-mail: laura.schaerer@colostate.edu
Editor: Jeremy Burton

Abstract

Methane-arrested anaerobic digestion (AAD) is a waste management strategy that produces carboxylic acid precursors to industrial products (fuels, bio-based polymers, and pharmaceuticals) from organic wastes. A major challenge preventing application of AAD is highly variable product profiles resulting from an inability to control the microbial communities underlying waste decomposition and product biosynthesis. Over time, lactic acid bacteria (LAB) often dominate AAD bioreactors and overproduce shorter chain acids causing acidosis. Here an AAD bioreactor where caproic acid production increased from an average of 3.9 g/l to an average of 12.3 g/l when the feedstock was switched from manure and paperboard to food waste. Time series shotgun metagenomics is used to investigate how microbial dynamics drive performance shifts. The dominant LAB shifted from *Lactobacillus amylovorus* spp. to *Lactiplantibacillus pentosus* spp. following the feedstock switch, corresponding with increased diversity and relative abundance (26.2%) of *Caproicibacter* spp. (putative chain elongator). Additionally, *L. amylovorus* MAGs encoded biosynthesis genes to produce the bacteriocin helveticin often produced by LAB to target closely related species. *Lactiplantibacillus pentosus* MAG.84 encodes bacteriocin-degrading enzymes and helveticin resistance genes, suggesting putitive mechanisms for bacteriocin resistance. These results suggest that bacteriocins may be an underappreciated mechanism for shaping microbial community dynamics in AAD.

Keywords methane-arrested anaerobic digestion, chain elongation, metagenomics, bacteriocin, lactic acid bacteria, caproic acid, circular economy

Introduction

Nearly one third of food produced in the United States is wasted, and nearly a quarter of the organic matter in landfills is wasted food (Lettuce Not Waste: New EPA Research Highlights Food Waste Contributions to Climate Change | US EPA, n.d., Quantifying Methane Emissions from Landfilled Food Waste | US EPA, n.d.). In 2023, it was estimated that the waste sector is responsible for ∼18% of methane emissions globally (Maasakkers et al. 2022). When organic matter decomposes under low-oxygen conditions, methane (a potent greenhouse gas) is produced (Lettuce Not Waste: New EPA Research Highlights Food Waste Contributions to Climate Change | US EPA, n.d., Quantifying Methane Emissions from Landfilled Food Waste | US EPA, n.d.). Looking forward, technologies are needed to incorporate carbon from organic waste streams (food, manure, agricultural crop residues, and yard trimmings) into value-added products to promote a circular economy instead of contributing to climate change (Wiatrowski et al. 2024). Anaerobic digestion (AD) has been used for centuries to produce energy from waste in the form of biogas (Abbasi et al. 2012). Methane-arrested anaerobic digestion (AAD) is an emerging technology, which produces valuable carboxylic acids from organic waste streams.

Carboxylic acids are highly valued precursors to many products across industries; the carboxylic acid market is predicted to grow from an estimated $2 billion USD in 2025, to $3.76 billion USD by 2032 (Carboxylic Acid Market Size, Competitors & Forecast to 2032, n.d.). End products include sustainable fuels, biopolymers, cosmetics, and pharmaceuticals (Carboxylic Acid Market Size, Competitors & Forecast to 2032, n.d.). Longer chain acids are more highly valued compared to shorter chain acids (Kim et al. 2019). For example, acetic acid is valued at ∼$550 USD/ton, while caproic acid is valued at ∼$2700 USD/ton (Kim et al. 2019, Debergh and Van Dael 2022). Despite the potential for high earnings from AAD systems, widespread application of this technology is currently limited by low yields of desired high-value acids and frequent system failures (Goux et al. 2015). AAD employs a diverse community of microorganisms to convert organic waste into carboxylic acids (Abbasi et al. 2012, Wiatrowski et al. 2024).

Received: 13 February 2026. **Revised:** 28 April 2026. **Accepted:** 26 May 2026

Generally, three metabolic processes are necessary for carboxylic acid biosynthesis: hydrolysis, fermentation, and chain elongation (Giduthuri and Ahring 2023). First, hydrolysis breaks apart complex organic polymers into more digestible monomers, such as the conversion of cellulose into sugar. Fermentation is microbial metabolism using an internal organic electron acceptor instead of more energetically favorable external electron acceptors like oxygen, iron, or sulfur (Madigan and Parker 2015). Fermentation products including ethanol, acetate, lactate, butyrate, and others are used as building blocks for chain elongation (Ziels et al. 2019). The β-oxidation cycle typically catabolizes fatty acids to produce energy; however, certain microorganisms can operate the β-oxidation cycle in the reverse (anabolic) direction, elongating the hydrocarbon tail by two carbon atoms per cycle (Aboulnaga et al. 2013, Buckel and Thauer 2018, Scarborough et al. 2020). Electron bifurcation enables bacteria to couple a favorable reaction with an unfavorable one; in reverse β-oxidation (rBOX) the reduction of crotonyl-CoA (spontaneous) and the reduction of ferredoxin (non-spontaneous) are coupled. Reduced ferredoxin oxidizes the RNF complex, creating an ion motive force which powers an ATP synthase, producing ATP (Aboulnaga et al. 2013, Buckel and Thauer 2018, Scarborough et al. 2020). For AAD to work, hydrolyzers, fermenters, and chain elongators must work together at similar rates so intermediate products do not accumulate to inhibitory levels.

In AAD, the desired microbial metabolisms are enriched using selective environmental conditions; operational parameters (e.g. pH, temperature, and hydraulic retention time) are known to be strong drivers of microbial dynamics and system outputs (Jiao et al. 2017, Wu et al. 2023). Because chain elongation yields very little energy compared to other metabolisms, the growth of chain elongators is typically slow and vulnerable to competition from other more favorable metabolisms (Joshi et al. 2021). Lowering pH inhibits both methanogenesis and excessive ethanol oxidation, thereby reducing competition (Qiu et al. 2023, Quintela et al. 2024). Stable production of desirable carboxylic acids in economically viable concentrations remains challenging due to stochastic (random) microbial community assembly, underappreciated microbial dynamics driving system outcomes, and imbalance between metabolic processes (Fernandez et al. 2000, Goux et al. 2015, Wu et al. 2023, Wiatrowski et al. 2024, Liu et al. 2025).

Overproduction of small, low-value carboxylic acids (like acetic or lactic acids) is another cause of system failure; fermenters can grow more quickly than chain elongators, and too much buildup of smaller acids can become inhibitory, leading to acidification and collapse of the metabolic network, and/or process failure (Gu et al. 2018). Lactic acid bacteria (LAB) are fermenters that are used in silage (Contreras-Govea et al. 2013) and food preservation (Marco et al. 2017, Einson et al. 2018). LAB often dominate AAD bioreactors as they tend to have high growth rates (Rezvani et al. 2017), flexible metabolisms (Rezvani et al. 2017), and are known producers of bacteriocins (Mokoena 2017). Bacteriocins are antimicrobial peptides which have largely underappreciated impacts on microbial community dynamics (Todorov 2009, Mokoena 2017). System takeover by *Lactobacillacae* spp. and overproduction of lactic acid is a recognized cause of system failure in anaerobic digestion of food waste (Gu et al. 2018).

This study builds on prior work by Anderson et al. (2026) , where the overall composition of volatile carboxylic fatty acids (VFAs) increased in value after the feedstock was switched from manure and paperboard to food waste. Prior to the feedstock switch, acetic acid was the dominant product; following the feedstock switch, caproic acid production dramatically increased from an average of 3.9 g/l before the switch, to an average of 12.3 g/l. This unexpected result is particularly exciting as the market value of caproic acid is nearly five times that of acetic acid (Kim et al. 2019, Debergh and Van Dael 2022). By investigating the microbial dynamics driving this change in product profile we seek to uncover basic knowledge that can be to the basis of future studies eventually leading to future AAD systems with more predictable outcomes. Here we build on the prior study by Anderson et al. (2026), using timeseries shotgun metagenomics to generate mechanistic hypotheses to explain the microbial dynamics and improved performance observed previously. The present study considers three hypotheses: (1) The microbial community will be functionally redundant before and after the feedstock change (in terms of polymer degradation and fermentation genes), (2) Micro-aeration during feeding favors aerotolerant microbes over non-aerotolerant microbes, and (3) Bacteriocin-producing and/or bacteriocin-degrading genes will be encoded by microorganisms dominant in the final (caproate-producing) microbial community. While this present case study is limited in its ability to definitively answer these hypotheses, we hope that the ideas findings presented here will be a starting point for future mechanistic work in AAD.

Materials and methods

Experiment design and sample collection

A fed-batch bioreactor was maintained at pH 5.0 $\pm$ 0.2 by automated additions of acid (5 N HCl) or base (5 N NaOH) for 126 days at 35°C. Deviations from the set pH occurred due to technical malfunctions during the experiment; samples selected for this present study were chosen from a time period with no large pH fluctuations. A previous study by Anderson et al. (2026) investigated the effect of feedstock change on production of volatile products (acetic acid, lactic acid, formic acid, propanoic acid, butyric acid, valeric acid, caproic acid, heptanoic acid, isovaleric acid, and ethanol). A bioreactor from the Anderson et al. study is investigated in depth here with metagenomic sequencing. Bioreactor operation details are described in depth in Anderson et al., but briefly the bioreactor was inoculated with cattle rumen fluid and fed homogenized grass (Table S3) every 14 days until day 56, when the feedstock was switched to a slurry of cattle manure and paperboard (National Overview: Facts and Figures on Materials, Wastes and Recycling | US EPA, n.d.) (Table S4); on day 70 the feedstock was switched to leachate food waste (Table S5). Our feeding protocol consisted of allowing solids in each bioreactor to settle for 2 hours prior to drawing off 200 ml of liquid and replacing with 200 ml of fresh feedstock. Triplicate samples were collected and immediately centrifuged (10 000 $\times$ g for 10 min), decanted, and frozen separately at -80°C. The pellet was used for DNA extraction, and the supernatant was used for high performance liquid chromatography (HPLC) to quantify select metabolites. Samples from days 45, 51, 57, 70, 77, and 81 were chosen for metagenomic sequencing based on the product profile measured by the HPLC. We acknowledge that this study has intrinsic limitations since it is based on six single-replicate timepoints from a single bioreactor. Despite these

limitations, we believe that this study will bring attention to underappreciated yet important dynamics in engineered microbial systems.

Volatile fatty acid quantification

Liquid chromatography was used to quantify production of volatile fatty acids including: acetic acid, lactic acid, formic acid, propanoic acid, butyric acid, valeric acid, caproic acid, heptanoic acid, isovaleric acid, and ethanol. Briefly, in-house standards were made from stock solutions to create standard curves for each acid ($R^2 > 0.995$). Samples and standards were run on a high-performance liquid chromatograph Agilent 1200 series with an Aminex HPX-87H column (Analysis of sugars, small organic acids, and alcohols by HPLC-RID V.2, n.d.). Data quality control was performed in Excel, and analysis was performed in R (Team 2013). To determine whether there was a statistically significant difference between the amount of each product produced before the feedstock change versus after the feedstock change, we performed a Kruskal–Wallis test with a Dunn post-hoc test, using the Benjamini–Hochberg method to adjust p-values for multiple comparisons (Kruskal and Wallis 1952, Dunn 1964, Benjamini and Hochberg 1995). All statistics were performed in R using the dunn.test and FSA (Ogle et al. 2020) packages.

Metagenomic sequencing and analysis

To obtain sufficient DNA for metagenomic sequencing, pellets were extracted manually with a Zymo Quick-DNA Fecal/Soil extraction kit (Irvine, CA). Samples were sent to Novogene Corporation, Inc. (Sacramento, CA) for metagenomic sequencing. Raw sequencing files were assessed for quality using fastqc (Babraham Bioinformatics—FastQC A Quality Control tool for High Throughput Sequence Data, n.d.) and quality filtered using sickle (GitHub—najoshi/sickle: Windowed Adaptive Trimming for fastq files using quality, n.d.). Raw data has been submitted to the SRA under PRJNA1423891. Then forward and reverse files were interleaved using BBMap (Bushnell 2014), and then were assembled using MEGAHIT (Li et al. 2015). Assembled reads were indexed and mapped using Bowtie2 (Langdon 2015) and SAMtools (Danecek et al. 2021) before binning was performed with MetaBat2 (Kang et al. 2019). Bins were dereplicated at 99% identity using Galah (Parks et al. 2020a, 2020b). Quality was assessed with CheckM2 (Parks et al. 2015, GitHub—chklovski/CheckM2: Assessing the quality of metagenome-derived genome bins using machine learning, n.d.) using the following criteria to define quality thresholds: high (completeness >90%, contamination < 5%), medium (completeness ≥ 50%, contamination < 5%), low (completeness < 50%, contamination < 10%), and contaminated (contamination ≥ 10%). Taxonomy was assigned using GTDB-tk (database release 220) (Chaumeil et al. 2019). Genes were annotated with Prokka (Seemann 2014) and metaErg (Dong and Strous 2019). Relative abundances were estimated using CoverM (Aroney et al. 2025). All raw results were further analyzed with custom pipelines in R (Team 2013) and tidyverse (Wickham et al. 2019), available on github at https://github.com/lgschaer/Feedstock-Switch. All data figures were generated in R using ggplot2 (Wickham et al. 2019), or created/edited in BioRender (Understanding the importance of citing BioRender—BioRender Help, Schaerer 2026).

Results

During this experiment, the overall concentration of carboxylic acids and ethanol more than doubled (average total production of 17.26 g/l before the switch compared to an average total production of 43.40 g/l after the switch) (Table S1). The overall product profile improved considerably following the feedstock switch as well with marked increases in the more desirable acids including valeric (23.7% increase), caproic (31.7% increase), and heptanoic acids (7% increase) (Fig. 1A). From day 45 to day 70, acetic acid was the primary product produced by the microbial community (average 6.8 g/l); then around day 74 lactic acid production dramatically increased to 26.7 g/l on day 73, followed by increased production of valeric (average 8.2 g/l), caproic (average 12.27 g/l), and heptanoic acids (average 1.28 g/l) until day 100 (Table S1). A Kruskal–Wallis test determined that the shifts in production were statistically significant (chi-squared = 93.7, degrees of freedom = 17, and *P*-value = 1.3e-12). However, a Dunn post-hoc test with Benjamini–Hochberg correction for multiple comparisons revealed that only the production of ethanol was statistically different before and after the feedstock switch (Table S2). Although the statistic was not significant, there was a visible increase in the production of valeric, caproic, and heptanoic acids. Because the production of longer acids did not increase until three sampling points after the feedstock switch, we expect that this trend could be biologically meaningful, despite the lack of statistical significance.

To survey the bacterial and archaeal taxa driving the changes in productivity, metagenome assembled genomes (MAGs) were obtained from the six metagenomic samples. The final set of 112 dereplicated and quality filtered (high and medium quality) MAGs represent 34 taxonomic families (Fig. S1). When mapped to the final set of dereplicated MAGs, the quality trimmed and filtered sequencing reads represent >90% of the relative abundance per sample.

The overall change in MAG abundances during this experiment highlights a shift from predominantly Gram-negative to predominantly Gram-positive taxa. Additionally, we observed a decrease in the number of dominant genera present after manure was fed and recovery of diversity after the food waste was fed; there were a total of 16 unique genera (> 5% relative abundance) in the grass-fed bioreactor, dropping to 9 unique genera (> 5% relative abundance) in the manure-fed bioreactor which increased to 17 unique genera (> 5% relative abundance) in the food waste-fed bioreactor (Fig. 1B, Table S7). On days 45, 51, and 57, the microbial community was dominated by *Prevotella* (> 50% relative abundance). On day 70, following the feedstock change, the relative abundance of *Lactobacillus amylovorus* spp. increased to ∼40% relative abundance. Then by days 77 and 81, *L. amylovorus* had decreased substantially, and *Lactiplantibacillus pentosus* had increased in abundance. Prior to the feedstock switch, *L. pentosus* was not detected. Interestingly, *Caproicibacter* MAG.64 was present at ∼15% relative abundance on day 45; however, it decreased in abundance and eventually disappeared from the dominant microbial community on day 70; *Caproicibacter* was not part of the dominant community again until day 81 when *Caproicibacter* MAG.93 increased to ∼20% relative abundance. Likewise, the dominant product shifted to lactate when the community became dominated by members of the *Lactobacillus* and later the *Lactiplantibacillus*, both belonging to the family *Lactobacillaceae* (Fig. 1).

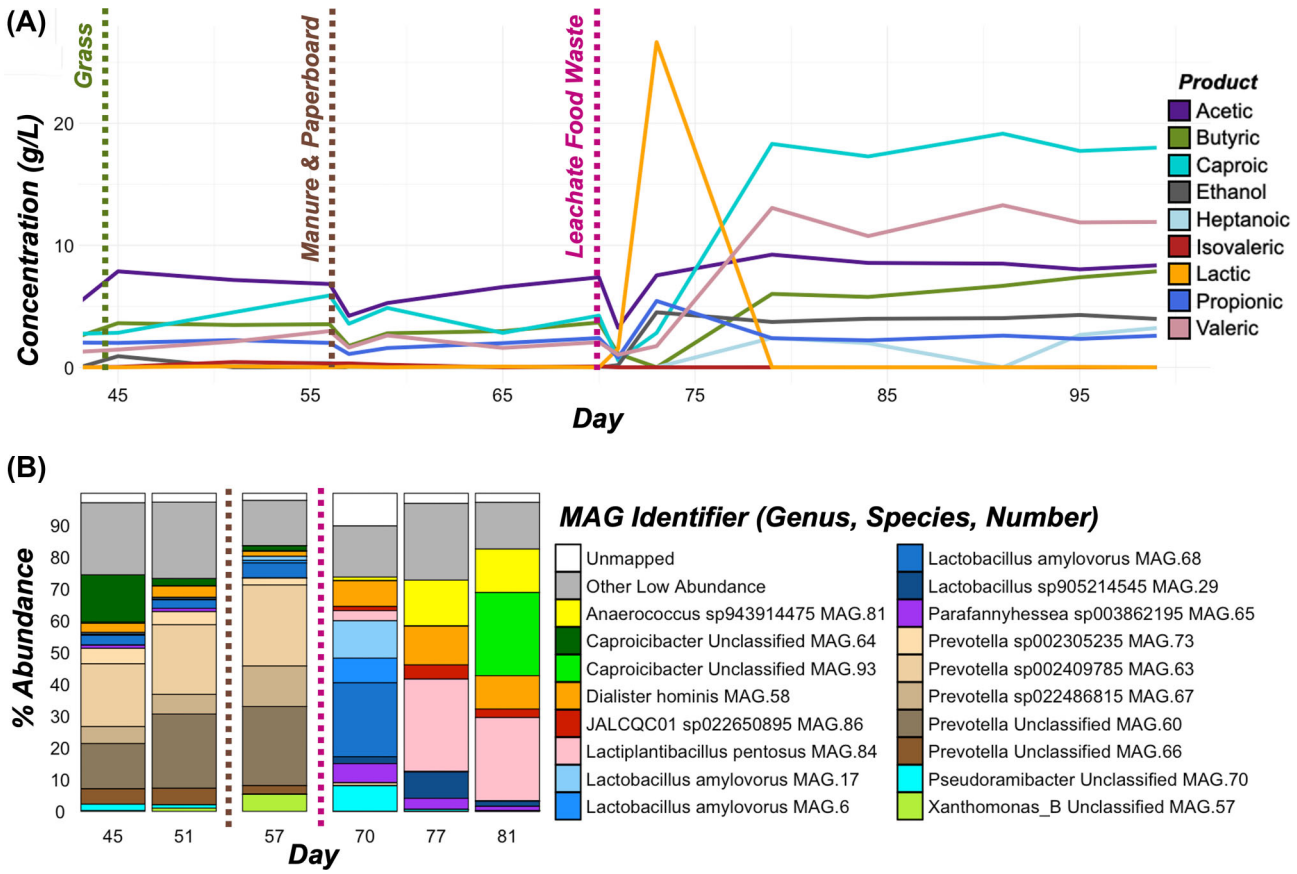

Figure 1 (A) Production of volatile carboxylic acids and ethanol produced between days 45 and 100 of the experiment. Vertical lines indicate when bioreactor was fed with grass (green), manure and paperboard (brown), and leachate food waste (pink). (B) Relative abundance of metagenome assembled genomes (MAGs) for six time points.

To investigate the hypothesis that microorganisms added with the food waste alleviated functional limitations within the microbial community, we counted functional genes for natural polymer biodegradation (cellulose, chito-oligosaccharides, fructans, galacturonan, hemicellulose, peptidoglycan, and polysaccharides) and for production of certain fermentation products (acetate, butyrate, ethanol, formate, lactate, propionate, and succinate) in the MAGs for both the dominant microbial community (present at >3% relative abundance) and all MAGs both before and after the feedstock switch (Fig. 2, Table S6). Chain elongation products (heptanoate, caproate, valerate, and sometimes butyrate) were not included here since the products are all generated from the same four core enzymes of the beta oxidation cycle. The goal of this analysis was to compare the functional potential of the organisms that are dominant in the starting microbial community to the microorganisms that are dominant after the feedstock switch; RNA analyses were outside the scope of this project, so our analysis focused on shotgun metagenomic DNA sequencing analyses and HPLC quantification of products.

We found that the whole microbial community had very little variance in functional potential before and after the feedstock switch; however, the dominant microbial community showed remarkable shifts in functional potential before and after the feedstock switch. The pre-switch dominant community encoded higher numbers of cellulose and hemicellulose degrading genes relative to the post-switch dominant community which encoded higher numbers of chito-oligosaccharide degrading genes. Likewise, higher numbers of acetate-producing genes were encoded by the pre-switch community relative to the post-switch community, which encoded more genes for ethanol, formate, and lactate production (Fig. 2).

To test the hypothesis that microaeration (small amounts of oxygen added with the feedstock) impacted the microbial community composition, we searched each MAG for its ability to encode genes involved in grow and energy production under aerobic and anaerobic conditions. We looked for genes used to sense oxygen and switch metabolism between aerobic and anaerobic (facultative) (Fig. 3, O_2 Req., Fig. S2). We found that all MAGs had some genomic evidence of being able to perform anaerobic fermentation. Some known obligate anaerobic taxa were identified as having some aerobic metabolism genes, due to encoding partial TCA cycles, which is not unusual for anaerobes (Zhou et al. 2009). Additionally, to test our hypothesis that highly abundant microorganisms directly following the feedstock switch are auxotrophic for certain nutrients (alleviated by the addition of nutrient-rich food waste, allowing for rapid growth), we estimated pathway completeness for amino acid biosynthesis. We found that the *L. amylovorus* MAGs and *L. pentosus* MAG.84 lack pathways for biosynthesis of several amino acids each (Fig. 3, Fig. S4).

Finally, we hypothesized that bacteriocin production, particularly by *Lactobacillaceae* spp. would shape microbial community dynamics. Results show that many MAGs encode genes for both

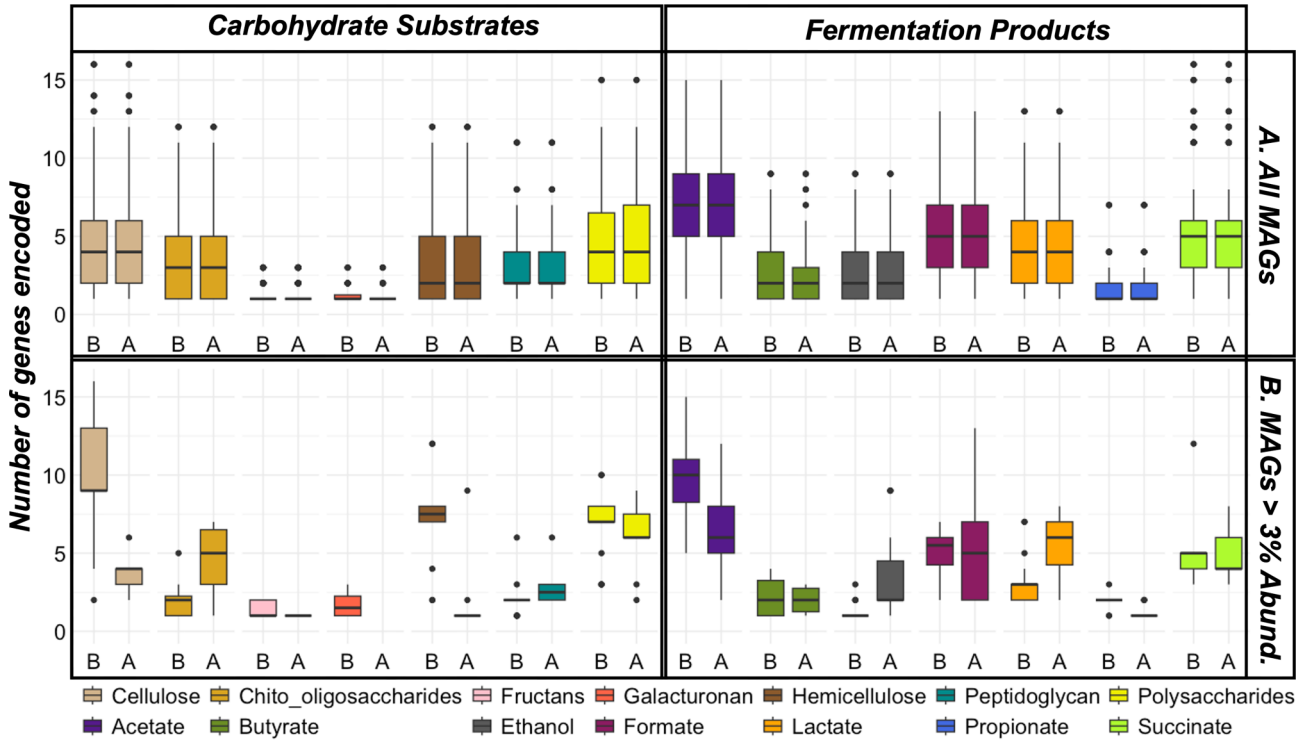

Figure 2 Summary of functional genes before and after the feedstock switch from manure and paperboard to food waste (denoted on the *x*-axis with "B" for before and "A" for after). Genes are shown for carbohydrate biodegradation (cellulose, chito-oligosaccharides, fructans, galacturonan, hemicellulose, peptidoglycan, and polysaccharides) and production of fermentation products (acetate, butyrate, ethanol, formate, lactate, propionate, and succinate) encoded by (All MAGs) All MAGs (n = 112 before the switch, and n = 109 after the switch) and (Dominant MAGs) versus MAGs present at greater than 3% relative abundance (n = 9 before the switch, and n = 11 after the switch).

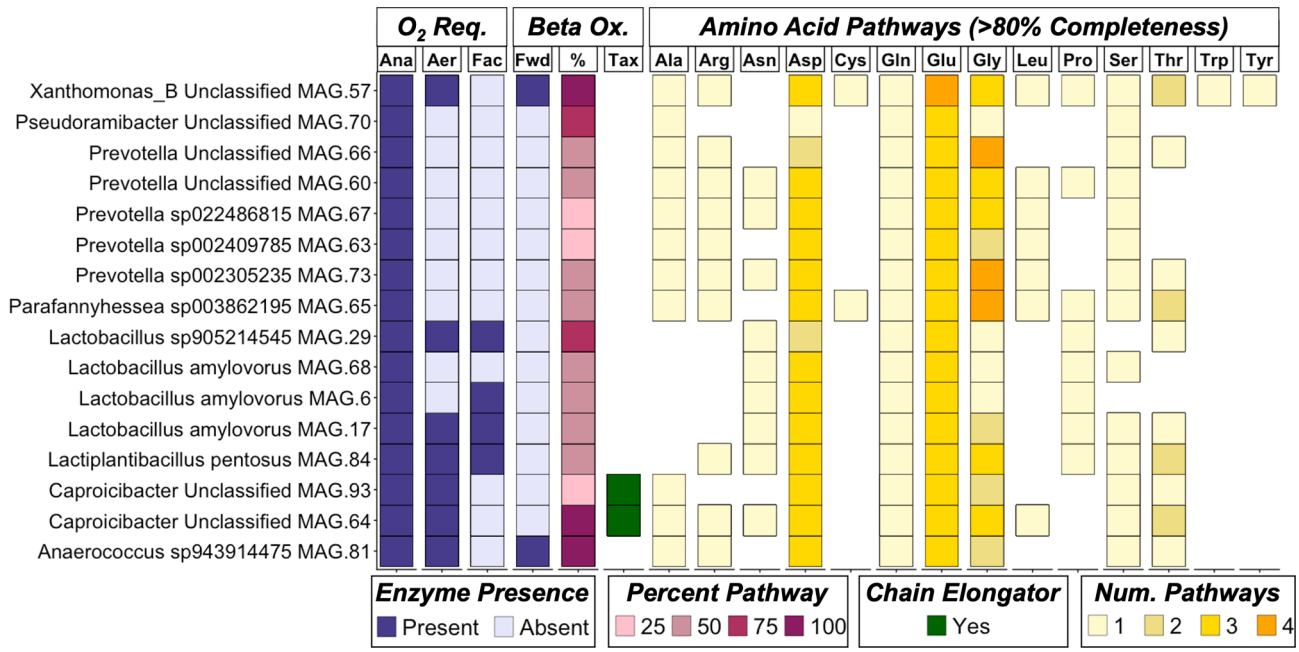

Figure 3 Oxygen requirements, beta oxidation capabilities, and amino acid synthesis potential of MAGs present at >3% relative abundance. Oxygen Requirements indicates whether >1 enzyme is present for anaerobic respiration genes (Ana), aerobic respiration genes (Aer), or facultative genes (Fac). Beta oxidation indicates MAGs encoding initiation enzymes typically associated with fatty acid catabolism (Fwd), % pathway completeness (%), and whether taxa are from known chain elongating genera (Tax). Amino Acid Pathways indicates MAGs encoding genes for amino acid biosynthesis. Color saturation indicates the number of different pathways encoded by each MAG. Only pathways >80% completeness are shown.

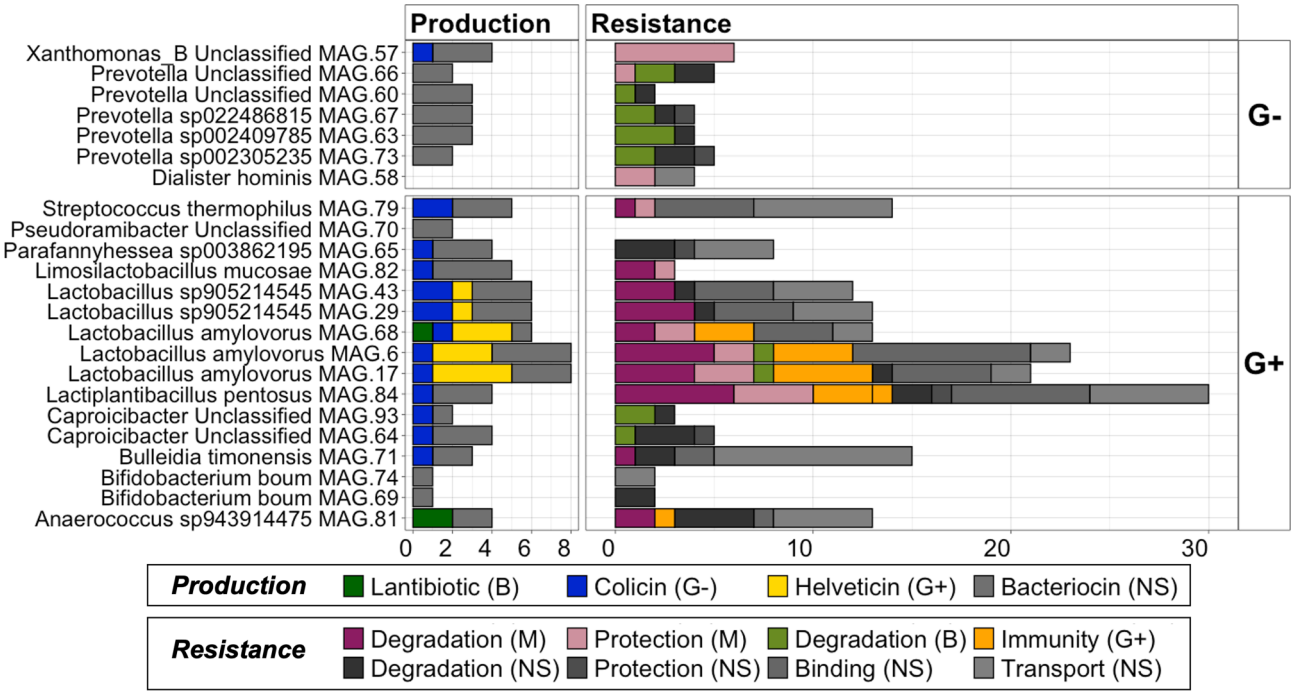

Figure 4 Bacteriocin production and resistance genes. *X*-axis is the number of genes encoded by each MAG in each category. MAGs are divided in to Gram-negative (G-, top panel) and Gram-positive (G+, bottom panel).

bacteriocin production and resistance (Fig. 4). The *Lactobacillus* MAGs encode more bacteriocin genes than other genera of microorganisms (average seven genes encoded per MAG, Table S8), and *Lactiplantibacillus* MAG.84 encodes more bacteriocin degrading genes than any other genus (30 genes, Table S8). A Wilcoxon rank sum test was run to determine whether there was a statistically significant difference between the number of putative bacteriocin production and bacteriocin degrading genes encoded by MAGs abundant before the feedstock switch and after the feedstock switch. The test showed no statistical significance for both bacteriocin production (P-value = 0.1399) and bacteriocin resistance genes (P-value = 0.1097); however, the lack of significance could be due to small sample sizes (Before: n = 9, After: n = 8). Future validation studies with additional replication would be a valuable addition to the scientific community.

Discussion

Results presented in this manuscript are six single-replicate time-series samples from a single bioreactor, thus there are inherent limitations in the conclusions we are able to infer from these data. In transparency, this study was performed nearly a year after the original experiment, thus, it is a hypothesis-generating case study of an interesting phenomenon that was observed. Because of the post-hoc nature of this study, we were unable to collect measurements of activity (RNA, direct measurements of bacteriocins, etc.), or measure other parameters such as oxygen or nutrient concentrations. Despite notable limitations, these data show temporal association between microbial abundances, system outputs, and changes in gene content. This work serves as a starting point for future investigations to improve AAD systems and the circular economy.

VFA production from organic waste relies on microorganisms capable of reversing the beta oxidation cycle to produce the desired medium chain carboxylic acids (Xu et al. 2020, Palomo-Briones et al. 2022). Many of these taxa are from the family *Clostridiales* and include genera such as the *Caproicibacter* and *Caproiciproducens* which are both known caproic acid (caproate) producers (Flaiz et al. 2020, Palomo-Briones et al. 2022). The *Caproicibacter* are Gram-positive strict anaerobes, which are known chain elongators that produce caproic acid (Flaiz et al. 2020). *Caproicibacter* MAG.64 has the complete beta oxidation pathway needed for chain elongation, thus based on taxonomy and the presence of beta oxidation genes, we have identified this MAG as a putative chain elongator. We also identified *Caproicibacter* MAG.93 as a putative chain elongator based on taxonomy, despite it lacking the beta oxidation pathway (Fig. 3, Beta Ox., Fig. S3). We acknowledge that this is tentative without additional work; future studies could interrogate whether these organisms are true chain elongators. While additional MAGs, *Xanthamonas* MAG.57 and *Anaerococcus* MAG.81 encode the beta oxidation pathway, the degrative thiolase that is also encoded by these MAGs has been associated with the catabolic (forward) direction of beta-oxidation (Bhaskar et al. 2020, Xu et al. 2020); thus, based on their genomes and previous literature, these organisms are putative fatty acid degraders (Flaiz et al. 2020). These speculations could be confirmed by future mechanistic studies.

The genera *Prevotella*, *Lactiplantibacillus*, *and Lactobacillus* are the dominant putative fermenters observed during the course of this experiment (Fig. 1). *Prevotella* are Gram-negative obligate anaerobes and putative producers of metabolic end products such as acetate, formate, and succinate (Takahashi and Yamada 2000a, 2000b), matching our observation that acetic acid (acetate) was the dominant product while the community was predominantly *Prevotella* (Fig. 1). Additionally, more of the dominant MAGs

pre-switch encode genes to produce acetate compared to after the switch when *Lactobacillaceae spp.* become more abundant (Figs. 1 and 2).

The *Lactobacillaceae* are Gram-positive facultative anaerobes, known to frequently dominate microbial communities due to their robust metabolism (high growth rates, aerotolerant traits, etc.), and ability to produce warfare molecules targeting competing taxa (Todorov 2009, Contreras-Govea et al. 2013, Mokoena 2017). Thus, there are several potential explanations for the increased abundance of *Lactobacillaceae* following the addition of food waste and contributing to the increased abundance of lactate producing genes (Fig. 2).

First, we found that the *L. amylovorus* MAGs and *L. pentosus* MAG.84 lack pathways for biosynthesis of several amino acids, although more complete genomes or pure culture work are needed to tease out whether these pathways are truly absent from the genome, or simply absent from the MAG (Fig. 3, Amino Acid Pathways, Fig. S4). Other LAB including *Lactobacillus helveticus* and *L. amylovorus* are known to be auxotrophic for multiple amino acids (Christiansen et al. 2008, Singh et al. 2018); thus, we speculate that the addition of nutrient-rich food waste could have alleviated nutrient limitations allowing these LAB to thrive. This would need further confirmation from replicated mechanistic studies.

Second, *Lactobacillaceae* MAGs from this study encoded genes to sense oxygen in their environment and switch metabolism accordingly. This is aligned with previous literature (Suissa et al. 2022, Walter and O'Toole 2023) where *Lactobacillaceae* spp. were identified as facultative anaerobes. The strict anaerobe *Anaerococcus* (Morand et al. 2021) slowly increased in relative abundance over time after the feeding and suspected micro-aeration event on day 70 (Figs. 1 and 3, Table S6). Thus, we observed that with the addition of food waste, competitive dynamics could have shifted to favor aerotolerant and facultative microorganisms, although, this is speculative without replication and direct oxygen measurements.

Thirdly, we investigated whether putative bacteriocin producing genes could have shaped dynamics within this bioreactor, as in previous studies (Riley and Gordon 1999, Todorov 2009, Mokoena 2017, Karkaria et al. 2020, Arbulu and Kjos 2024). We found that *Lactobacillus* MAGs encoded more bacteriocin producing genes than other genera of microorganisms (average seven genes encoded per MAG, Table S8), and *Lactiplantibacillus* MAG.84 encodes more bacteriocin degrading genes than any other genus (30 genes, Table S8) (Fig. 4). It is plausible that *Lactiplantibacillus* offers a benefit to other community members by degrading bacteriocins, potentially enabling the re-establishment of putative chain elongator *Caproicibacter* MAG.93, although this is speculative without further investigation.

Conclusions

We found that switching the feedstock from manure and paperboard to food waste resulted in a fundamentally reshaped the microbial community within an AAD fed-batch bioreactor. Shifts in the microbial community coincided with positive changes in bioreactor performance, although future studies will be required to determine if the change in performance was causal or simply an interesting correlation. At the beginning of the experiment, *Prevotella* spp. dominated the community but were briefly replaced by *L. amylovorus* following the switch to food waste. Possible microaeration occurred with the feeding of the food waste, which may have selected against the growth of less aerotolerant anaerobes. *Lactiplantibacillus pentosus* then increased in abundance, correlating with increased microbial diversity, including the re-establishment of *Caproicibacter* spp. (putative chain elongator). Re-establishment of *Caproicibacter* spp. coincided with 23.7%, 31.7%, and 7% higher production of valeric, caproic, and heptanoic acids, respectively (Table S1), although without support from further mechanistic studies it is possible that these trends are simply correlative. While community functional potential remained broadly conserved, dominance patterns and metabolic outputs changed markedly, highlighting the importance of community structure rather than gene inventory alone. Genomic evidence suggests that bacteriocin production and resistance could be a key underappreciated mechanism mediating these shifts, although future work measuring expression of key bacteriocin-related genes and direct measurements of bacteriocins are needed to explore these potential effects. Our results, while preliminary, suggest that *L. pentosus* could potentially mitigate the effects of antimicrobial peptides through bacteriocin degradation or resistance, offering exciting potential to drive microbial community composition and bioreactor outcomes through the addition of microorganisms with strategic metabolic abilities, although this idea warrants future testing to determine if it is plausible. This concept is in line with other recent studies which have suggested that keystone functional guilds maintain structure and function in microbial communities (Cottee-Jones and Whittaker 2012, Banerjee et al. 2018, Carlström et al. 2019, Liu et al. 2025).

Acknowledgements

The authors thank Amanda Schmidt for contributions to sample preparation and HPLC analysis.

Author contributions

Laura G. Schaerer (Conceptualization, Bioinformatics analysis, Formal analysis, Investigation, Data curation, Visualization, Writing—original draft), Ryan S. Anderson (Conceptualization, Methodology, Investigation, Data curation, Writing—review & editing), Joshua Chan (Conceptualization, Supervision, Funding acquisition, Project administration, Writing—review & editing), and Susan K. De Long: Conceptualization, Supervision, Funding acquisition, Project administration, Writing—review & editing)

Supplementary material

Supplementary material is available at *FEMSMC Journal* online.

Conflicts of interest

The authors declare they have no conflicts of interest.

Funding

This work was supported by a United States Advanced Research Projects Agency Energy (ARPRA-E) grant (21/CJ000/07/06) and by the National Science Foundation (2426904).

References

Abbasi T, Tauseef SM, Abbasi SA. A brief history of anaerobic digestion and "Biogas. " In: Abbasi T, Tauseef SM, Abbasi SA , *Biogas Energy*. New York, NY: Springer New York, 2012;11–23. https://doi.org/10.1007/978-1-4614-1040-9_2.

Aboulnaga EH, Pinkenburg O, Schiffels J *et al.* Effect of an oxygen-tolerant bifurcating butyryl coenzyme a sehydrogenase/electron-transferring flavoprotein complex from clostridium difficile on butyrate production in *Escherichia coli*. *J Bacteriol* 2013;**195**:3704–13. https://doi.org/10.1128/JB.00321-13.

Anderson, R. Sharvelle S, De Long SK *et al.* Design, Preparation and characterization of nationally representative synthetic food waste for reproducible waste valorization research. *Methods Protoc* 2026;**9**:93. https://doi.org/10.3390/mps9030093.

Arbulu S, Kjos M. Revisiting the multifaceted roles of bacteriocins: the multifaceted roles of bacteriocins. *Microb Ecol* 2024;**87**:41. https://doi.org/10.1007/s00248-024-02357-4.

Aroney STN, Newell RJP, Nissen JN *et al.* CoverM: read alignment statistics for metagenomics. *Bioinformatics* 2025;**41**:btaf147. https://doi.org/10.1093/bioinformatics/btaf147.

Banerjee S, Schlaeppi K, Van Der Heijden MGA. Keystone taxa as drivers of microbiome structure and functioning. *Nat Rev Micro* 2018;**16**:567–76. https://doi.org/10.1038/s41579-018-0024-1.

Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J R Stat Soc Ser B Stat Methodol* 1995;**57**:289–300. https://doi.org/10.1111/j.2517-6161.1995.tb02031.x.

Bhaskar S, Steer DL, Anand R *et al.* Structural basis for differentiation between two classes of thiolase: degradative vs biosynthetic thiolase. *J Struct Biol X* 2020;**4**:100018. https://doi.org/10.1016/j.yjsbx.2019.100018.

Buckel W, Thauer RK. Flavin-based electron bifurcation, ferredoxin, flavodoxin, and anaerobic respiration with protons (Ech) or NAD+ (Rnf) as electron acceptors: a historical review. *Front Microbiol* 2018;**9**:401. https://doi.org/10.3389/fmicb.2018.00401.

Bushnell B. BBMap: A Fast, Accurate, Splice-Aware Aligner. Lawrence Berkeley National Laboratory. LBNL Report #: LBNL-7065E. 2014. Retrieved from https://escholarship.org/uc/item/1h3515gn.

Carlström CI, Field CM, Bortfeld-Miller M *et al.* Synthetic microbiota reveal priority effects and keystone strains in the Arabidopsis phyllosphere. *Nat Ecol Evol* 2019;**3**:1445–54. https://doi.org/10.1038/s41559-019-0994-z.

Chaumeil PA, Mussig AJ, Hugenholtz P *et al.* GTDB-Tk: a toolkit to classify genomes with the genome taxonomy database. *Bioinformatics* 2019;**36**:1925–7. https://doi.org/10.1093/bioinformatics/btz848.

Christiansen JK, Hughes JE, Welker DL *et al.* Phenotypic and genotypic analysis of amino acid auxotrophy in *Lactobacillus helveticus* CNRZ 32. *Appl Environ Microb* 2008;**74**:416–23. https://doi.org/10.1128/AEM.01174-07.

Contreras-Govea FE, Muck RE, Broderick GA *et al.* Lactobacillus plantarum effects on silage fermentation and in vitro microbial yield. *Anim Feed Sci Technol* 2013;**179**:61–68. https://doi.org/10.1016/j.anifeedsci.2012.11.008.

Cottee-Jones HEW, Whittaker RJ. perspective: The keystone species concept: a critical appraisal. *Front Biogeogr* 2012;**4**. http://dx.doi.org/10.21425/F5FBG12533. Retrieved from https://escholarship.org/uc/item/15d2b65t.

Danecek P, Bonfield JK, Liddle J *et al.* Twelve years of SAMtools and BCFtools. *GigaScience* 2021;**10**:giab008. https://doi.org/10.1093/gigascience/giab008.

Debergh P, Van Dael M. Production of caproic acid from acetate and ethanol through microbial chain elongation: a techno-economic assessment. *Bioresour Technol Rep* 2022;**18**:101055. https://doi.org/10.1016/j.biteb.2022.101055.

Dong X, Strous M. An integrated pipeline for annotation and visualization of metagenomic contigs. *Front Genet* 2019;**10**:999. https://doi.org/10.3389/fgene.2019.00999.

Dunn OJ. Multiple comparisons using rank sums. *Technometrics* 1964;**6**:241–52. https://doi.org/10.1080/00401706.1964.10490181.

Einson JE, Rani A, You X *et al.* A vegetable fermentation facility hosts distinct microbiomes reflecting the production environment. *Appl Environ Microb* 2018;**84**. https://doi.org/10.1128/AEM.01680-18.

Fernandez AS, Hashsham SA, Dollhopf SL *et al.* Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. *Appl Environ Microb* 2000;**66**:4058–67. https://doi.org/10.1128/AEM.66.9.4058-4067.2000.

Flaiz M, Baur T, Brahner S *et al.* Caproicibacter fermentans gen. nov., sp. nov., a new caproate-producing bacterium and emended description of the genus caproiciproducens. *Int J Syst Evol Microbiol* 2020;**70**:4269–79. https://doi.org/10.1099/ijsem.0.004283.

Giduthuri AT, Ahring BK. Current status and prospects of valorizing organic waste via arrested anaerobic digestion: production and separation of volatile fatty acids. *Fermentation* 2023;**9**:13. https://doi.org/10.3390/fermentation9010013.

Goux X, Calusinska M, Lemaigre S *et al.* Microbial community dynamics in replicate anaerobic digesters exposed sequentially to increasing organic loading rate, acidosis, and process recovery. *Biotechnol Biofuels* 2015;**8**:122. https://doi.org/10.1186/s13068-015-0309-9.

Gu XY, Liu JZ, Wong JWC. Control of lactic acid production during hydrolysis and acidogenesis of food waste. *Bioresour Technol* 2018;**247**:711–5. https://doi.org/10.1016/j.biortech.2017.09.166.

Jiao PX, Liu FZ, Beauchemin KA *et al.* Impact of strain and dose of lactic acid bacteria on in vitro ruminal fermentation with varying media pH levels and feed substrates. *Anim Feed Sci Technol* 2017;**224**:1–13. https://doi.org/10.1016/j.anifeedsci.2016.11.005.

Joshi S, Robles A, Aguiar S *et al.* The occurrence and ecology of microbial chain elongation of carboxylates in soils. *ISME J* 2021;**15**:1907–18. https://doi.org/10.1038/s41396-021-00893-2.

Kang DD, Li F, Kirton E *et al.* MetaBAT 2: an adaptive binning algorithm for robust and efficient genome reconstruction from metagenome assemblies. *PeerJ* 2019;**7**:e7359. https://doi.org/10.7717/peerj.7359.

Karkaria BD, Fedorec AJ, Barnes CP. Automated design of synthetic microbial communities. *Zenodo* 2020. https://doi.org/10.5281/ZENODO.4286040.

Kim H, Jeon BS, Sang BI. An efficient new process for the selective production of odd-chain carboxylic acids by simple carbon elongation using megasphaera hexanoica. *Sci Rep* 2019;**9**:11999. https://doi.org/10.1038/s41598-019-48591-6.

Kruskal WH, Wallis WA. Use of Ranks in One-Criterion Variance Analysis. *J Am Statist Assoc* 1952;**47**:583–621. https://doi.org/10.1080/01621459.1952.10483441.

Langdon WB. Performance of genetic programming optimised Bowtie2 on genome comparison and analytic testing (GCAT) benchmarks. *BioData Mining* 2015;**8**:1–1. https://doi.org/10.1186/s13040-014-0034-0.

Li D, Liu CM, Luo R *et al.* MEGAHIT: an ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. *Bioinforma Oxf Engl (England)* 2015;**31**:1674–6.

Liu X, Wang M, Liu B *et al.* Keystone taxa mediate the trade-off between microbial community stability and performance in activated sludges. *Nat Water* 2025;**3**:723–33. https://doi.org/10.1038/s44221-025-00451-6.

Maasakkers JD, Varon DJ, Elfarsdóttir A *et al.* Using satellites to uncover large methane emissions from landfills. *Sci Adv* 2022;**8**:eabn9683. https://doi.org/10.1126/sciadv.abn9683.

Madigan MT, Parker J. Churchman K . *Brock Biology of Microorganisms* 14th edn, San Francisco, CA: Pearson Education, Inc., 2015.

Marco ML, Heeney D, Binda S *et al.* Health benefits of fermented foods: microbiota and beyond. *Curr Opin Biotechnol* 2017;**44**:94–102. https://doi.org/10.1016/j.copbio.2016.11.010.

Mokoena MP. Lactic acid bacteria and their bacteriocins: classification, biosynthesis and applications against uropathogens: a mini-review. *Molecules* 2017;**22**:1255. https://doi.org/10.3390/molecules22081255.

Morand A, Tall ML, Kuete Yimagou E *et al.* Anaerococcus urinimassiliensis sp. nov., a new bacterium isolated from human urine. *Sci Rep* 2021;**11**:2684. https://doi.org/10.1038/s41598-021-82420-z.

Ogle DH, Wheeler P, Dinno A. FSA: fisheries stock analysis. 2020. https://github.com/fishR-Core-Team/FSA *(17 June 2026, date last accessed)*.

Palomo-Briones R, Xu J, Spirito CM *et al.* Near-neutral pH increased n-caprylate production in a microbiome with product inhibition of methanogenesis. *Chem Eng J* 2022;**446**:137170. https://doi.org/10.1016/j.cej.2022.137170.

Parks DH, Chuvochina M, Chaumeil PA *et al.* A complete domain-to-species taxonomy for Bacteria and Archaea. *Nat Biotechnol* 2020;**38**:1079–86. https://doi.org/10.1038/s41587-020-0501-8.

Parks DH, Chuvochina M, Chaumeil PA *et al.* Author correction: a complete domain-to-species taxonomy for bacteria and archaea. *Nat Biotechnol* 2020;**38**:1098–. https://doi.org/10.1038/s41587-020-0539-7.

Parks DH, Imelfort M, Skennerton CT *et al.* CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res* 2015;**25**:1043–55. https://doi.org/10.1101/gr.186072.114.

Qiu S, Zhang X, Xia W *et al.* Effect of extreme pH conditions on methanogenesis: methanogen metabolism and community structure. *Sci Total Environ* 2023;**877**:162702. https://doi.org/10.1016/j.scitotenv.2023.162702.

Quintela C, Peshkepia E, Grimalt-Alemany A *et al.* Excessive ethanol oxidation versus efficient chain elongation processes. *Waste Biomass Valor* 2024;**15**:2545–58. https://doi.org/10.1007/s12649-023-02323-0.

Rezvani F, Ardestani F, Najafpour G. Growth kinetic models of five species of Lactobacilli and lactose consumption in batch submerged culture. *Braz J Microbiol* 2017;**48**:251–8. https://doi.org/10.1016/j.bjm.2016.12.007.

Riley MA, Gordon DM. The ecological role of bacteriocins in bacterial competition. *Trends Microbiol* 1999;**7**:129–33. https://doi.org/10.1016/S0966-842X(99)01459-6.

Scarborough MJ, Myers KS, Donohue TJ *et al.* Medium-chain fatty acid synthesis by " *Candidatus* Weimeria bifida" gen. nov., sp. nov., and " *Candidatus* Pseudoramibacter fermentans" sp. nov. *Appl Environ Microb* 2020;**86**:e02242–19. https://doi.org/10.1128/AEM.02242-19.

Schaerer L. *Created in BioRender*. 2026. https://BioRender.com/5bhqkug.

Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinforma Oxf Engl (England)* 2014;**30**:2068–9.

Singh P, Chung HJ, Lee IA *et al.* Elucidation of the anti-hyperammonemic mechanism of *Lactobacillus amylovorus* JBD401 by comparative genomic analysis. *BMC Genomics [Electronic Resource]* 2018;**19**:292. https://doi.org/10.1186/s12864-018-4672-3.

Suissa R, Oved R, Maan H *et al.* Context-dependent differences in the functional responses of *Lactobacillaceae* strains to fermentable sugars. *Front Microbiol* 2022;**13**:949932. https://doi.org/10.3389/fmicb.2022.949932.

Takahashi N, Yamada T. Pathways for amino acid metabolism by *Prevotella* intermedia and *Prevotella* nigrescens. *Oral Microbiol Immunol* 2000a;**15**:96–102. https://doi.org/10.1034/j.1399-302x.2000.150205.x.

Takahashi N, Yamada T. Glucose metabolism by *Prevotella* intermedia and *Prevotella* nigrescens. *Oral Microbiol Immunol* 2000b;**15**:188–95. https://doi.org/10.1034/j.1399-302x.2000.150307.x.

Team RC. *R: A Language and Environment for Statistical Computing*. 2013.https://www.R-project.org/ (17 June 2026, date last accessed).

Todorov SD. Bacteriocins from Lactobacillus plantarum production, genetic organization and mode of action: produção, organização genética e modo de ação. *Braz J Microbiol* 2009;**40**:209–21. https://doi.org/10.1590/S1517-83822009000200001.

Walter J, O'Toole PW. Microbe Profile: the *Lactobacillaceae*. *Microbiology* 2023;**169**:1–5. https://doi.org/10.1099/mic.0.001414.

Wiatrowski MR, Miller JH, Bhatt A *et al.* Economic and sustainability prospects for wet waste valorization: the case for sustainable aviation fuel from arrested anaerobic digestion. *Renewable Energy* 2024;**232**:121063. https://doi.org/10.1016/j.renene.2024.121063.

Wickham H, Averick M, Bryan J *et al.* Welcome to the Tidyverse. *JOSS* 2019;**4**:1686. https://doi.org/10.21105/joss.01686.

Wu H, Scheve T, Dalke R *et al.* Scaling up carboxylic acid production from cheese whey and brewery wastewater via methane-arrested anaerobic digestion. *Chem Eng J* 2023;**459**:140080. https://doi.org/10.1016/j.cej.2022.140080.

Xu Y, Zhu Y, Li X *et al.* Dynamic balancing of intestinal short-chain fatty acids: the crucial role of bacterial metabolism. *Trends Food Sci Technol* 2020;**100**:118–30. https://doi.org/10.1016/j.tifs.2020.02.026.

Zhou Y, Pijuan M, Zeng RJ *et al.* Involvement of the TCA cycle in the anaerobic metabolism of polyphosphate accumulating organisms (PAOs). *Water Res* 2009;**43**:1330–40. https://doi.org/10.1016/j.watres.2008.12.008.

Ziels RM, Nobu MK, Sousa DZ. Elucidating syntrophic butyrate-degrading populations in anaerobic digesters using stable-isotope-informed genome-resolved metagenomics. *mSystems* 2019;**4**:e00159–19. https://doi.org/10.1128/mSystems.00159-19.

Received: 13 February 2026. **Revised:** 28 April 2026. **Accepted:** 26 May 2026