

# The Other Lignocellulosic Leftover: Conversion Residue Valorization with a Microbial Community

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<https://www.glbrc.org/research/highlights/microbial-community-members-play-distinct-roles-biosynthesis>

## Project Goals:

The carboxylate platform has emerged as a promising technology to produce carboxylic acids from complex organic wastes (1). Limitations persist in the ability to direct production from short-chain (C1-C5) products to medium-chain (C6-C12) products, which have higher value, are more energy dense, and are easier to recover. I will discuss implementing the carboxylate platform on a waste stream from lignocellulosic biorefining to recover additional carbon and energy from biorefinery "leftovers." We evaluated process stability and economics, and performed metagenomic, metatranscriptomic, and thermodynamic analyses of the microbial community to reconstruct metabolic networks. Lastly, we propose strategies to further improve our understanding of industrial microbiome systems and increase production of valuable products from conversion residue.

## Abstract:

Starting with an inoculum from a wastewater treatment digester, we enriched a community of microorganisms that produced a variety of volatile fatty acid end products, including medium chain fatty acids (MCFAs) (Figure 1, adapted from (2)). In total, the microbial community converted 18% of the reducing equivalents (measured as chemical oxygen demand, COD) to the MCFAs hexanoic and octanoic acid. Based on this data, we performed a techno-economic analysis of valorizing conversion residue and determined that the minimum ethanol selling price could be reduced by 18% in a biorefinery producing MCFAs as a coproduct to ethanol.

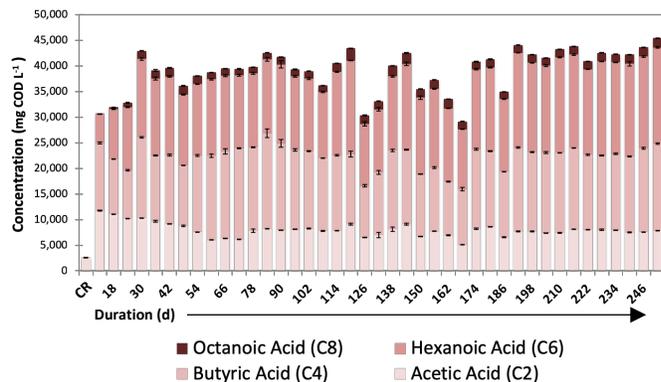


Figure 1. Production of MCFAs from organic material in conversion residue (CR). The microbial community converted xylose and other carbohydrates in conversion residue to acetic acid, butyric acid, hexanoic acid, and octanoic acid.

Amplicon sequencing of the 16s rRNA gene revealed a stable community consisting of members related to five key genera making up > 95% of the sequenced reads: *Lactobacillus*, *Olsenella*, *Atopobium*, *Roseburia*, and *Pseudoramibacter*. To elucidate the roles of these community members, we performed metagenomic and metatranscriptomic analyses. This resulted in the recovery of 10 metagenome-assembled genomes: five related to *Lactobacillus* (LAC1-5), three related to the *Coriobacteriaceae* (COR1-3), a member of the *Eubacteriaceae* (EUB1), and a member of the *Lachnospiraceae* (LCO1). Based on highly abundant transcripts, we reconstructed the active metabolic networks in each of these populations. We then performed thermodynamic analyses to refine the predicted roles of each population. In total, we predict the flow of substrates in conversion residue to MCFAs as shown in Figure 2 (adapted from (3)). Strategies to increase production of MCFAs include constructing a synthetic community to direct more carbon to lactate (rather than acetate) and utilizing hydrogen gas as a supplemental electron donor to further elongate short-chain products.

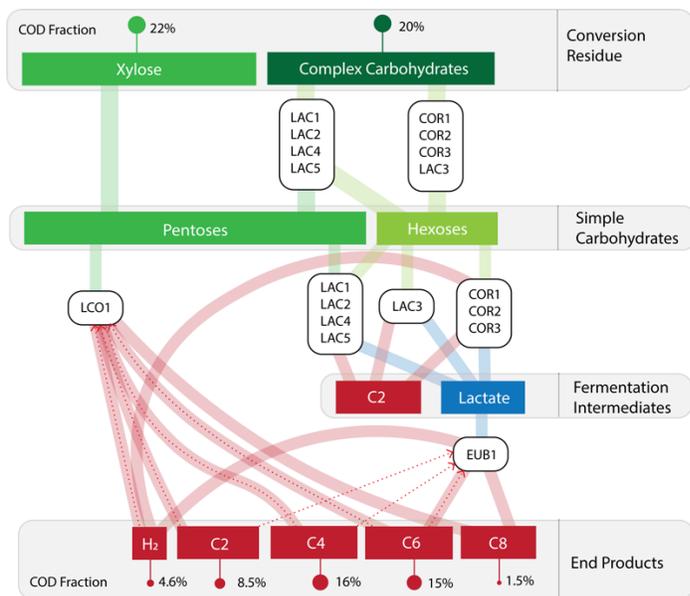


Figure 2. Based on thermodynamic and transcriptomic analyses we proposed functions of individual MAGs within the community. In total, the microbiome converts carbohydrates remaining in conversion residue to a variety of monocarboxylic acid products, including MCFAs. Lactate is expected to be a key intermediate. Future work will develop strategies to drive production of hexanoate and octanoate over acetate and butyrate.

## References

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2. Scarborough MJ, *et al.* (2018) Increasing the economic value of lignocellulosic stillage through medium-chain fatty acid production. *Biotechnology for Biofuels* 11(1):200.
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