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FEEDSTOCK-CONVERSION INTERFACE CONSORTIUM

Techno-Economic Case Study: Fermentation Cost Impacts from Selected Critical Material Attributes

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Disclaimer

About the Feedstock-Conversion Interface Consortium

The Feedstock-Conversion Interface Consortium (FCIC) develops first-principles-based knowledge and tools to understand, quantify, and mitigate the effects of feedstock and process variability across the bioenergy value chain, from the field and forest through downstream conversion. The FCIC is a collaborative and coordinated effort involving researchers in many different disciplines. It is led by the U.S. Department of Energy's Bioenergy Technologies Office (BETO) and includes researchers from nine national laboratories: Argonne National Laboratory, Idaho National Laboratory, Lawrence Berkeley National Laboratory, Los Alamos National Laboratory, National Energy Technology Laboratory, National Renewable Energy Laboratory, Oak Ridge National Laboratory, Pacific Northwest National Laboratory, and Sandia National Laboratories.

Research within the FCIC focuses on two complementary conversion pathways: (1) the low-temperature conversion of corn stover to fuels and chemicals using deacetylation and mechanical refining, enzymatic hydrolysis, and biological upgrading of the sugar- and lignin-rich streams; and (2) the high-temperature conversion of pine residues to fuels using catalytic fast pyrolysis and hydrotreating. Each pathway covers three sequential process areas—biomass harvest and storage, preprocessing, and conversion.

The FCIC is organized into eight collaborative tasks working in each of these process areas. The Feedstock Variability task investigates biomass attribute variations that originate in the harvest and storage process area; the Preprocessing, Materials Handling, and Materials of Construction tasks investigate the effects of biomass variability in the preprocessing area; and the High-Temperature Conversion and Low-Temperature Conversion tasks investigate the effects of biomass variability in the conversion process area. Two supporting tasks (Crosscutting Analyses and Scientific Data Management) support all FCIC research.

> The Feedstock-Conversion Interface Consortium uses first-principles-based science to de-risk biorefinery scale-up and deployment by understanding and mitigating the impacts of feedstock variability on bioenergy conversion processes.
>
> **energy.gov/fcic**

Availability

This report is available electronically at no cost from http://www.osti.gov.

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List of Acronyms

CMA	critical material attribute
DMR	deacetylation and mechanical refining
FCIC	Feedstock-Conversion Interface Consortium
ISPR	*in situ* product recovery
LT	low temperature
MFSP	minimum fuel selling price
NREL	National Renewable Energy Laboratory
SOT	state of technology
TEA	techno-economic analysis

Executive Summary

This report summarizes analysis conducted to support a case study under the Feedstock-Conversion Interface Consortium (FCIC) focused on techno-economic analysis (TEA) modeling to quantify the economic implications of biomass hydrolysate substrate variability on fermentation performance and resultant biorefinery fuel yields. It is known that fermentation inhibitors or byproducts, as may either come from constituents in the biomass feedstock or imparted through biorefinery processing operations, can detrimentally impact fermentation rates and yields. However, it is often difficult to isolate fermentation impacts to individual components given the complex nature of biomass, varying simultaneously in multiple attributes from one lot to another.

For this study, we worked with FCIC researchers to obtain data on key fermentation performance metrics, namely productivity rates and conversion yields, across a number of material attribute species previously selected by the researchers as “critical” attributes that may be found in hydrolysate used as microbial carbon sources during fermentation. Critical attributes were identified as compounds that had the most dramatic effect on microbial growth (done under prior FCIC experimental work) at concentrations seen in at least a subset of analyzed hydrolysates. Namely, the evaluated components included lactate (lactic acid, a common contamination byproduct), ammonium sulfate (a common salt species formed during certain upstream processing steps), and coumarate (a key lignin constituent typically observed in deacetylation black liquor, which may be present in residual pretreated solids sent to fermentation depending on the degree of solids washing employed following deacetylation). These components were spiked into mock hydrolysate sugars to study their impacts on fermentation performance in isolation, evaluated at concentrations corresponding to 25% and 75% cell growth inhibition, in comparison to a control case serving as a baseline reference point.

The biorefinery modeled in this study reflected fermentation of hydrolysate sugars to carboxylic acid intermediates via *Clostridium tyrobutyricum*, with the acid products subsequently upgraded to hydrocarbon fuels through a series of catalysis steps (outside the scope of FCIC research). Due to resource constraints, the experiments performed here were not coupled with *in situ* product recovery (ISPR), which impedes fermentation performance but still provides insightful trends in fermentation impacts from the selected critical material attributes. For this pathway, on a relative basis compared to the control case, the lactate component was seen to substantially reduce minimum fuel selling price (MFSP) over increasing concentrations, first reducing by 17% and then further by 37% relative to the control case between the 25% and 75% cell growth inhibition levels, respectively. These results were unexpected and at odds with microtiter assays that demonstrated lactate-mediated inhibition of cell growth. In fact, these data imply that lactate is not a problematic component over the range of concentrations studied, but rather serves as an additional convertible substrate boosting fermentation product yields. Additionally, ammonium sulfate was also seen to more marginally improve MFSPs under lower concentrations, translating to an MFSP reduction of 9% relative to control for the 25% cell growth inhibition case, but then becoming more inhibitory with lower yields at the 75% inhibition level, increasing MFSP by 10% relative to the control case. Again, this suggests that at lower concentrations the ammonium may favorably improve fermentation performance in fed-batch but rapidly becomes inhibitory with increasing concentrations. The coumarate component was seen to modestly inhibit sugar fermentation yields, with MFSP increasing by 7% and 4% relative to control for the 25% and 75% cell growth inhibition concentrations, respectively.

Overall, the trends in MFSP closely followed fuel yields, in turn tied to fermentation process yields and particularly yield/selectivity to butyric acid product, with minimal economic impact from variations in fermentation productivity. This reiterates that yields strongly dictate overall biorefinery economics, varying up to 61% higher than control for the best case with lactate addition, or reducing by as much as 9% for the ammonium sulfate 75% inhibition case. The data set and modeling presented here also highlights the need for improved understanding of the differential in fermentation metrics between volumetric scales and between fermentation modes. Moving forward, further opportunities may exist for FCIC researchers to expand on this initial set of material attribute species to consider additional components also frequently present in deconstructed biomass hydrolysate that may be problematic or (as in the case of lactate) beneficial to fermentation performance.

Table of Contents

List of Figures

List of Tables

Introduction

The Feedstock-Conversion Interface Consortium (FCIC) focuses on understanding how variability in feedstock attributes impacts biorefinery performance. For low-temperature (LT) conversion via biochemical processing of corn stover, one element within this overall scope is fermentation performance dependencies on specific constituents in hydrolysate substrates that may reduce fermentation titers, rates, or yields. Some such components may be native to the biomass feedstock, while others could either be added exogenously or formed as reaction byproducts in upstream operations (e.g., pretreatment). This case study seeks to quantify impacts to biorefinery economics as measured by the minimum fuel selling price (MFSP), attributed to data collected on fermentation of hydrolysate sugars to carboxylic acids (subsequently to be upgraded through catalysis steps to hydrocarbon fuels), over varying ranges of selected species deemed as critical material attributes (CMAs). Through this approach, as metrics such as fermentation productivity rates and product yields decrease with increasing levels of these inhibitory species, such metrics can be translated to changes in MFSP to understand the degree of impact on overall biorefinery economics.

A simplified diagram of the LT conversion process is depicted in Figure 1, highlighting the key operations for which experimental data inputs were furnished for techno-economic analysis (TEA) modeling. In brief, the National Renewable Energy Laboratory's (NREL's) 2018 biochemical design report forms the general basis for the integrated biorefinery process as leveraged for the present modeling efforts (Davis et al. 2018), consisting of deacetylation and mechanical refining (DMR) pretreatment, batch enzymatic hydrolysis, hydrolysate clarification/solids removal, fermentation of clarified sugars to intermediates, and catalytic upgrading of those intermediates to final hydrocarbon fuels. This case study focuses only on data collected for the fermentation step. Additionally, the aforementioned design report included consideration for lignin valorization via deconstruction and bioconversion of lignin to value-added coproducts (adipic acid), for which experimental data were also collected in this FCIC effort to understand similar impacts through bioconversion of lignin monomers. However, to simplify this case study (and because the economics for lignin deconstruction/upgrading are presently less attractive than lignin combustion), the TEA presented here simply routes lignin and other waste biomass to the boiler for heat and power generation.

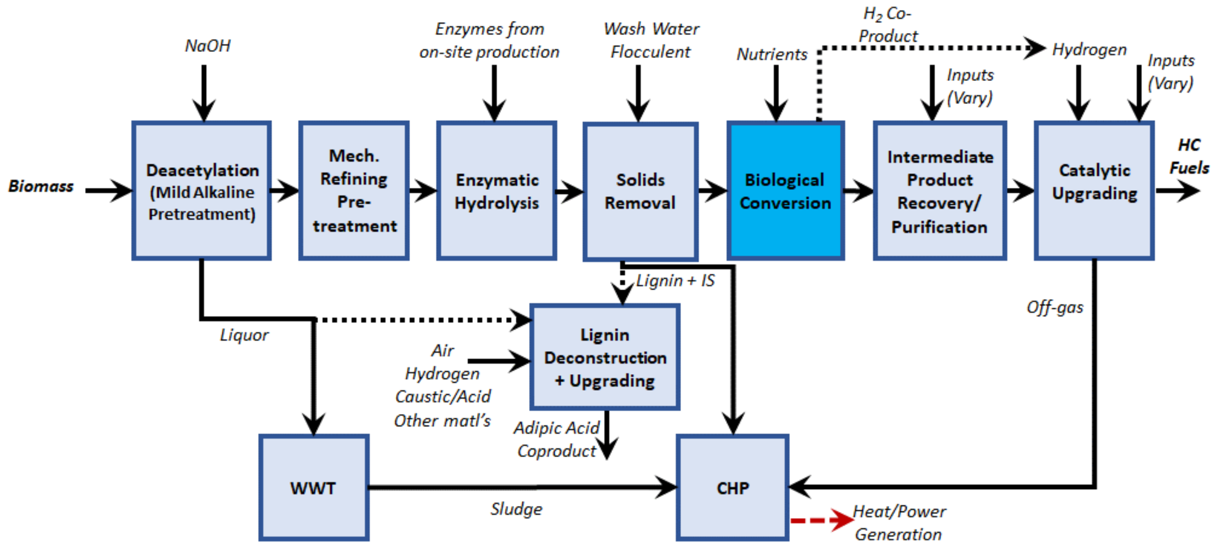

Figure 1. Block diagram schematic of framework LT conversion biorefinery process as modeled. Blue highlighted box represents the primary scope of this study. To simplify the analysis, this assessment assumes routing all lignin and residual solids/off-gases to combustion for combined heat and power (CHP) rather than upgrading of lignin and deacetylation liquor (solubilized lignin) to coproducts.

Methods

As noted above, NREL's 2018 biochemical design case was utilized as the starting framework for TEA modeling conducted here (Davis et al. 2018). However, as highlighted in Figure 2, some details of that framework were modified to reflect the operations as performed experimentally, as well as the sequence of the operations. In summary, the key modifications incorporated into the models relative to the projected design case details include:

- Deacetylation: Replaced continuous counter-current deacetylation operation (design case target approach) with standard batch deacetylation (FCIC experimental basis).
- Hydrolysis/hydrolysate clarification: FCIC model only reflects standard batch enzymatic hydrolysis (no continuous enzymatic hydrolysis employed), followed by hydrolysate solids removal (not used in either design case pathway). For the latter step, the TEA model assumes the use of a vacuum belt filter with wash water and flocculant, following parameters as utilized in NREL's 2019 state of technology (SOT) benchmarks for this operation (Davis, Bartling, and Tao 2020).
- Sugar fermentation: TEA models are based only on the acids fermentation pathway (the second design case pathway via 2,3-butanediol upgrading to fuels is not included in this study). We note that this is not a reflection of a broader preference for one pathway over another in the LT Conversion platform, but strictly a decision to focus limited resources. The TEA models maintain the use of fed-batch fermentations coupled with *in situ* product recovery (ISPR) for acid removal via pertractive membranes reflecting 2019

SOT parameters for pertraction and downstream catalytic upgrading (outside of FCIC's scope). However, as ISPR is a resource-intensive step to conduct experimentally, the fermentation data were collected using pH control without ISPR, which hinders fermentation performance.

- Lignin upgrading: To simplify this report, lignin valorization via deconstruction and upgrading to coproducts is not included here. Rather, solid residual lignin is routed to combustion for combined heat and power generation, while DMR liquor is routed to wastewater treatment.

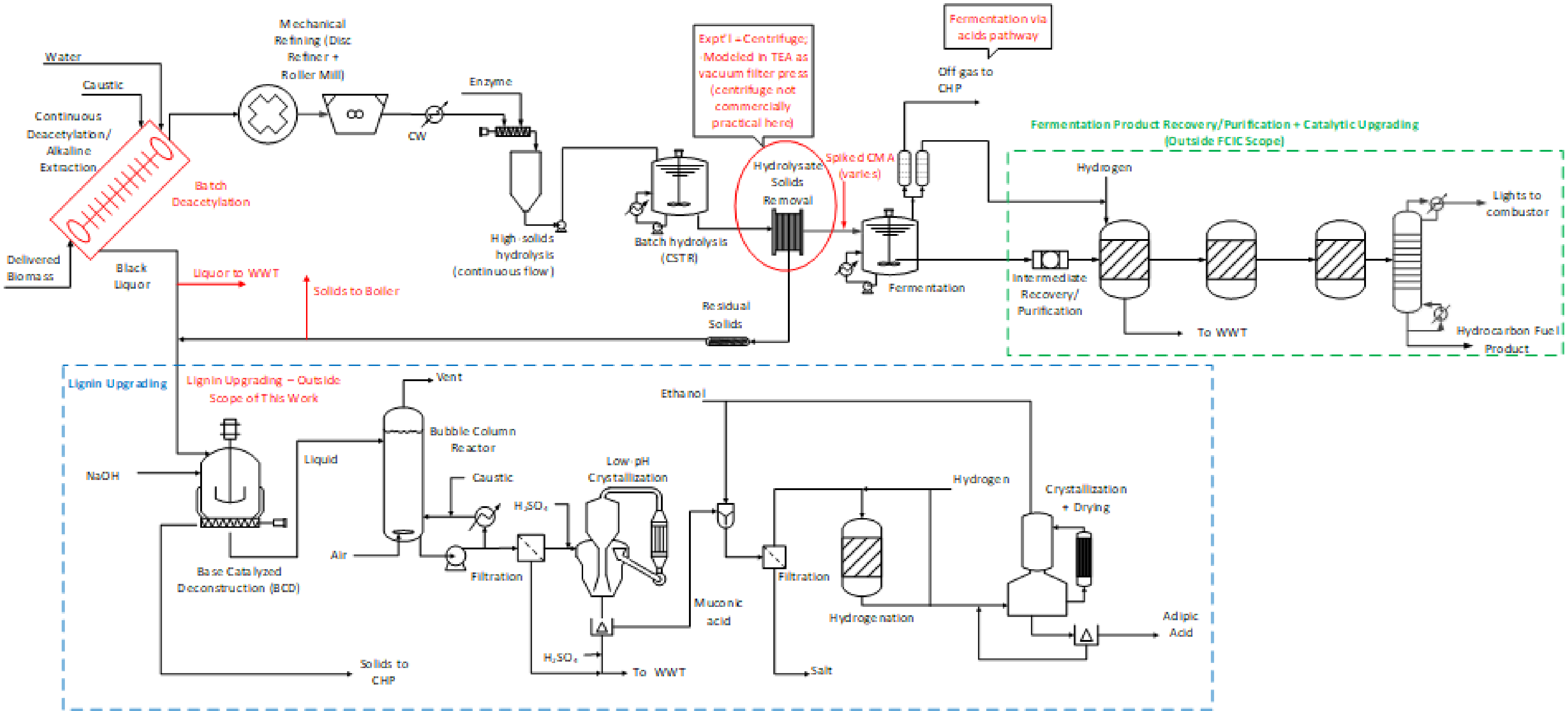

Figure 2. Process flow diagram of 2018 NREL design report framework for the LT conversion process. Modifications from design case models reflecting FCIC operational details for this case study are highlighted in red.

NREL's 2019 SOT model was used as the basis framework for this exercise (Davis, Bartling, and Tao 2020), maintaining all operating conditions and conversions consistent with that framework for unit operations outside the scope of experimental focus for this study (DMR pretreatment, enzymatic hydrolysis, hydrolysate clarification, pertractive membrane acid recovery, and acid catalytic upgrading steps to fuels)—key details are summarized in Table 1. Feedstock costs were also set consistent with 2019 SOT benchmarks furnished from Idaho National Laboratory, at $81.37/dry ton (Davis, Bartling, and Tao 2020). For the fermentation step, data were input to the models for hydrolysate sugar conversions, yields to butyric acid versus acetic acid (acetic acid is less favorable in terms of *in situ* membrane recoveries and subsequent carbon yields through catalytic upgrading), sugar diversions to biomass growth, and acid productivity rates. These data were collected in duplicate and then averaged for a control case plus three previously selected CMA species, which had been prioritized as "critical" from prior FCIC experimental work based on exhibiting highest impact to cell growth rates at concentrations that may be realistically present in biomass hydrolysates. The three selected CMA species included lactate (lactic acid is a known product indicating contamination in the system), ammonium sulfate (may be formed from acid/base reactions in pretreatment/conditioning), and coumarate (a key lignin monomer released during DMR pretreatment that could remain with hydrolysate solids if solids are not subjected to a water wash step following deacetylation).

To isolate fermentation impacts to only each CMA of interest, mock sugars were utilized in the fermentation trials at ratios matching typical levels in corn stover hydrolysate, with each CMA species spiked into the mock substrate at concentrations corresponding to 25% and 75% cell growth inhibition (EC25 and EC75, respectively), established from previous FCIC experimental work. As noted above, the experiments performed here were not coupled with ISPR, which reduces overall productivity metrics compared to the design as modeled with ISPR included (and as utilized for SOT benchmark cases). As the researchers were not equipped to perform parallel ISPR-coupled fermentations, fed-batch fermentations with pH control (excluding ISPR) were used to understand overall impacts of CMAs, but recognizing that the TEA results accordingly would not be as favorable as presented in SOT cases (Davis, Bartling, and Tao 2020).

Table 1. Key Model Parameters Outside Experimental Scope of Present Study, Fixed Constant in All Cases as Consistent with 2019 SOT (see Davis, Bartling, and Tao [2020] for additional details)

Parameter	Value
Feedstock glucan content (dry wt %)	35.1%
Feedstock xylan content (dry wt %)	19.5%
Feedstock lignin content (dry wt %)	15.8%
Feedstock ash content (dry wt %)	4.9%
Feedstock arabinan content (dry wt %)	2.4%
Deacetylation NaOH loading (g/kg dry biomass)	80
Enzymatic hydrolysis total solids loading (wt %)	20%
Hydrolysis glucan to glucose (%)	84%
Hydrolysis xylan to xylose (%)	82%
Hydrolysis enzyme loading (mg/g cellulose)	12
Sugar loss through solid/liquid separation (%)	5%
In situ acid product recovery from fermentation (wt %)	76% acetic, 98% butyric
Acid upgrading ketonization – conversion (%)	100%
Acid upgrading condensation – conversion (%)	92%
Acid upgrading hydrotreating – conversion (%)	100%

Results and Discussion

Table 2 summarizes the resulting fermentation performance quality attributes measured experimentally for the bioconversion step, following pretreatment and enzymatic hydrolysis fixed at the conditions described above. Each case in Table 2 represents an average of duplicate runs. The second fermentation run was lost for the ammonium sulfate EC75 case due to a malfunctioning bioreactor and had to be rerun at a different time using a new control case. To present this data set consistently with the others, the results were scaled by ratio between the new control versus the second control case otherwise used as the reference basis for all other secondary duplicate runs. The result was then averaged along with the first ammonium sulfate EC75 data set (completed normally) to provide the results for this CMA case as shown in the table.

Notably, relative to the control baseline, overall glucose utilization was reduced for all but one CMA case (coumarate EC25), while overall xylose utilization was improved for all cases. Resulting acid process yields (defined as grams of total acids produced per gram of total available sugars to fermentation) were more nuanced, increasing in three cases and decreasing in three others, with the remainder of consumed sugar that does not go to acids production otherwise diverted to cell growth in the models. Compared to the control case, lactate was seen to improve overall process yields, driven particularly by butyric acid yield with no appreciable production of acetic acid. This trend was reflected first moving from the control to the lactate EC25 case (minimally increasing from 0.26 to 0.27 g/g overall process yield to acids, though entirely concentrated in the more favorable butyric acid component), and further improved moving to lactate EC75 (further increasing process yields to 0.40 g/g). This indicates that lactic acid in fact has a beneficial effect on fermentation yields in this case, likely by acting as an additional convertible substrate, at least up to the point of reaching cell growth inhibition. Additionally, ammonium sulfate also showed a beneficial effect on overall acid yields, at least in lower levels, increasing overall process yield from 0.26 to 0.28 g/g (again concentrated more on preferentially boosting butyric acid yield versus acetic acid), though this trend was reversed in moving to higher concentrations at the EC75 point, reducing process yields to 0.23 g/g. Finally, coumarate generally had a minimal impact with slightly reduced overall process yields, first declining from 0.26 to 0.24 g/g in the EC25 case before slightly recovering to 0.25 g/g in the EC75 case. Trends in productivity were also variable, reducing relative to the control case particularly for the lactate cases and for the ammonium sulfate EC75 case, but with more minimal impacts observed for ammonium sulfate EC25 and both coumarate cases (although overall TEA impacts to productivity variations are largely outweighed by impacts to yields, as evidenced further below).

Table 2. Fermentation Performance Metrics Spanning the Cases Evaluated. $(NH_4)_2SO_4$ = ammonium sulfate; AA = acetic acid; BA = butyric acid.

Case	Percent Glucose Utilization	Percent Xylose Utilization	AA Metabolic Yield (g/g sugar) [a]	BA Metabolic Yield (g/g sugar) [a]	AA Process Yield (g/g sugar) [b]	BA Process Yield (g/g sugar) [b]	Total Acids Process Yield (g/g sugar) [b]	Avg. BA Productivity (g/L/h)
1 - Control	97.8	25.7	0.06	0.30	0.04	0.21	0.26	0.41
2 - Lactate EC25	89.0	40.1	0.00	0.31	0.00	0.27	0.27	0.23
3 - Lactate EC75	83.3	46.8	0.00	0.35	0.00	0.40	0.40	0.34
4 - $(NH_4)_2SO_4$ EC25	92.9	37.6	0.07	0.32	0.05	0.23	0.28	0.39
5 - $(NH_4)_2SO_4$ EC75	90.3	33.6	0.05	0.28	0.03	0.19	0.23	0.32
6 - Coumarate EC25	99.1	34.7	0.06	0.26	0.04	0.20	0.24	0.42
7 - Coumarate EC75	96.2	44.5	0.06	0.26	0.04	0.20	0.25	0.38

[a] Metabolic yield = grams produced per gram of sugar utilized.

[b] Process yield = grams produced per gram of sugar sent to fermentation. Process yield is the metric of focus in this report as the primary driver on fermentation economics (encompassing both sugar utilization and metabolic yield).

As can be seen in Figures 3 and 4, varying effects on biorefinery MFSP and fuel yield were seen for the three CMAs tested. For lactate, MFSPs were lower than the control for both 25% (EC25) and 75% (EC75) cell growth inhibition cases by 17% and 37%, respectively. While the ammonium sulfate EC25 case also reflected an MFSP slightly below the control (9% lower), the

EC75 case for this CMA was higher by 10%. Both coumarate cases also incurred MFSP penalties relative to the control, at 7% and 4% above the control, respectively, for the EC25 and EC75 cell growth inhibition cases. These trends in MFSP largely follow the overall process yields—higher process yields translate to lower MFSPs. In particular, variances in butyric acid production relative to acetic acid most strongly drive overall fuel yields and thus economics, with butyric acid representing a more favorable intermediate than acetic acid for downstream recovery and catalytic upgrading (Davis et al. 2018; Davis, Bartling, and Tao 2020). Thus, in the case of lactate, overall process yields are improved relative to the control baseline, but the savings in MFSP are particularly magnified as only butyric acid is produced in these cases; likewise, the ammonium sulfate EC25 case reflects incrementally higher butyric acid than acetic acid production relative to the control (with a correspondingly lower MFSP), though yielding less butyric acid than the lactate cases. As has been shown in prior TEA studies, productivity is a minimal cost driver for anaerobic fermentation pathways such as this (Davis et al. 2018; Davis, Bartling, and Tao 2020), with the lactate cases still achieving the lowest MFSPs in spite of the lowest productivity rates and the coumarate cases reflecting higher MFSPs in spite of comparable or better productivity rates compared to the control case.

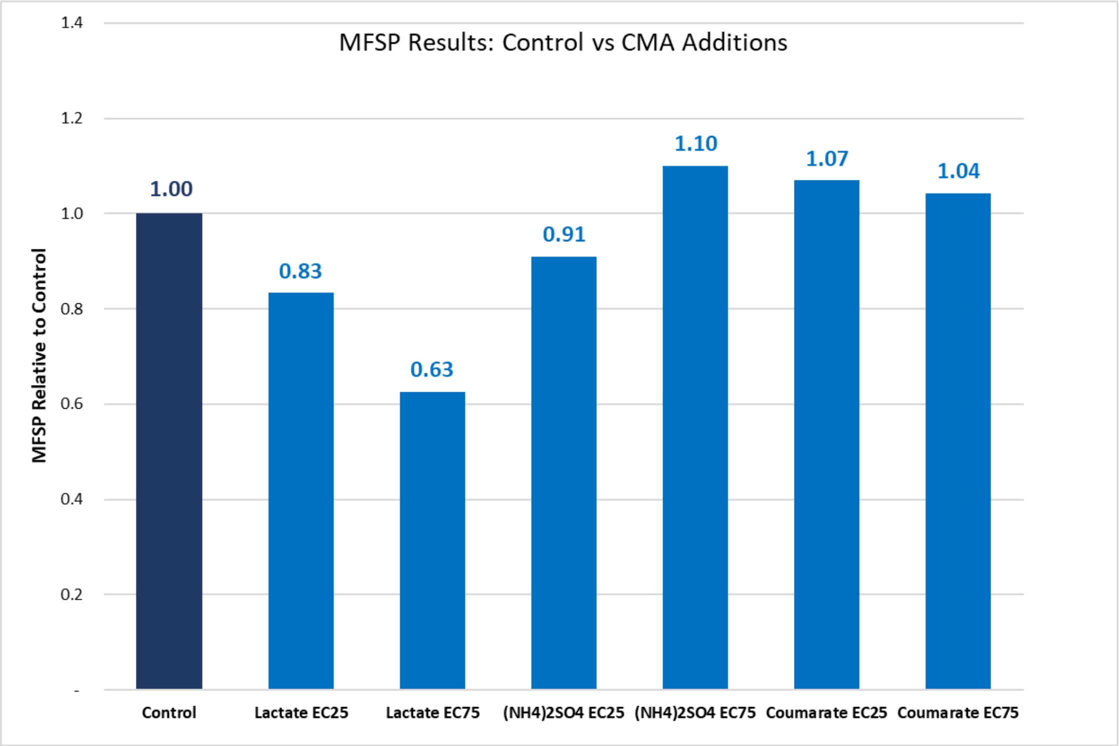

Figure 3. Relative MFSPs for control reference case in comparison to three CMAs spiked at 25% and 75% (EC25 and EC75) cell growth inhibition concentrations. (NH4)2SO4 = ammonium sulfate.

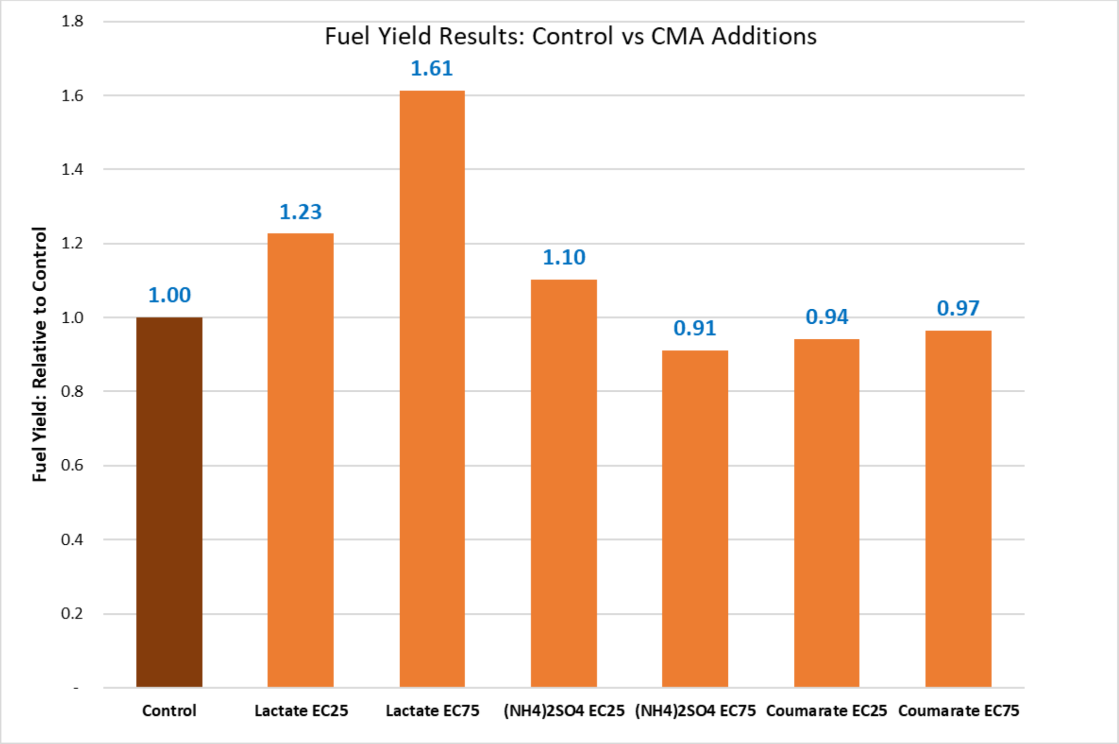

Figure 4. Relative overall fuel yields for control reference case in comparison to three CMAs spiked at 25% and 75% (EC25 and EC75) cell growth inhibition concentrations. (NH4)2SO4 = ammonium sulfate.

Conclusion and Next Steps

The work conducted in this assessment demonstrates the impact of fermentation performance due to hydrolysate media composition on overall biorefinery economics. The analysis shows that in fact, some species commonly known to be inhibitory to certain fermentation organisms may prove beneficial to a degree for the present fermentation pathway to carboxylic acids via *C. tyrobutyricum*, particularly lactate, which serves as a convertible substrate increasing yields of the more favorable butyric acid component. Notably however, in most real-world instances, lactate would only arise in significant amounts through microbial contamination and concomitant reduction of hydrolysate sugars to produce the lactate. Therefore, the process-relevant effect of lactate would need to be examined in more detail accounting for sugar loss. As an interesting side note, these data suggest that the frequent industrial contaminant, *Lactobacillus*, which produces lactic acid, may be less detrimental to *C. tyrobutyricum* than in other fermentation processes. Researchers have since seen evidence that this is indeed the case. Ammonium sulfate was also seen to improve overall yields and economics at low concentrations, but this benefit was negated at higher concentrations, while coumarate (a key lignin component typically present in deacetylation liquor) was also observed to incur detrimental impacts to fermentation yields and economics, though not dramatically so. The work also reiterated prior findings that particularly for anaerobic fermentation pathways such as this, fermentation productivity has a much smaller influence on overall biorefinery economics than yield, thus highlighting that future FCIC work on this topic should focus particularly on understanding yield dependencies on feedstock attribute variability, ideally spanning a broader set of material attribute components beyond this initial list of three species.

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