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Exploring Net-Zero Greenhouse Gas Emission Routes for Bio-Production of Triacetic Acid Lactone: An Evaluation through Techno-Economic Analysis and Life Cycle Assessment

Ching-Mei Wen[a], Charles Foster[b], and Marianthi Ierapetritou[a]*

[a] University of Delaware, Department of Chemical & Bio-molecular Engineering, Newark, Delaware, United States
[b] dsm-firmenich, 6480 Dobbin Rd, Columbia, Maryland, United States
* Corresponding Author: mgi@udel.edu.

ABSTRACT

Triacetic acid lactone (TAL) is a bio-privileged molecule with potential as a chemical precursor, traditionally synthesized from petroleum. Current trends are shifting towards the use of renewable biomass or CO_2-derived feedstocks to enhance sustainability. However, comprehensive studies on the techno-economic viability and carbon life cycle of such methods are limited. This study assesses TAL production from conventional glucose and a novel approach co-feeding *Yarrowia lipolytica* (*YL*) with glucose and formic acid (FA), aiming for a more cost-effective and eco-friendly process. We confront the inherent challenges in this process by exploring different technology scenarios using kinetic bioprocess modeling underpinned by techno-economic analysis (TEA) and life cycle assessment (LCA) to identify the most cost-effective and sustainable routes to TAL production. A noteworthy component of our investigation centers around the prospect of recycling and utilizing the CO_2 emitted from the YL bioreactor to eliminate greenhouse gas emissions inherent in aerobic fermentation processes. The study combines TEA and LCA to dissect the proposed TAL bio-production routes, evaluating the sustainability of the process and the implications of net-zero greenhouse gas emission manufacturing. We employed SuperPro Designer and Aspen software for process simulation and energy balance computations. The results underscore the benefits of CO_2 recycling in TAL production, with an estimated minimum selling price (MSP) slightly increasing by 6.21-7.80% compared to traditional methods, but significantly undercutting the market price of $51000/mt-TAL and achieving net-negative CO_2 emissions. This research illustrates a viable route to bio-production with net-zero emissions, providing a model for future bioprocessing and industrial practices.

Keywords: Choose an item., Choose an item., Choose an item., Choose an item., Choose an item., additional keywords separated by commas

INTRODUCTION

Energy systems across the globe are moving towards more integrated, cleaner, and sustainable processes [1]. However, attaining a carbon-free economy presents a formidable challenge, as it requires a significant reduction in emissions from hard-to-decarbonize sectors such as industrial and chemical processes. This study explores the production of triacetic acid lactone (TAL) through carbon dioxide utilization in aerobic fermentation cycles. The goal is to achieve Net-Zero emissions by identifying economically viable and environmentally friendly processes to produce chemicals. The study aims to guide the development of sustainable industrial methodologies using TAL as a case-study.

TAL is a promising molecule for sustainable chemical production, obtained through bio-production from renewable resources [2]. It has the potential to serve as a key precursor for the production of valuable chemical intermediates, as well as end products like sorbic acid [3], which have significant global demand (market size was USD 500 million in 2022, reported by Reports and Data, a market research and consulting company). Traditional synthesis methods rely on petrochemical processes that are not sustainable [4]. With the engineering *Yarrowia lipolytica* (*YL*), a yeast and model organism and naturally proficient lipid producer, to produce TAL, we can shift towards using biomass as a renewable feedstock [5-6]. This addresses the growing need for carbon-neutral production pathways. However, bioproduction involves metabolic processes that produce significant amounts of CO_2, which is typically considered waste [7]. In this study, the bioprocess emits CO_2 at concentrations greater than 99 wt.% by assuming high-purity O_2 used in providing aerobic fermentation. [8]. Rather than releasing it into the atmosphere, there is an opportunity to repurpose this emission [8]. The current trend is to convert CO_2 into value-added chemicals, which aligns with the industrial symbiosis paradigm of repurposing waste streams to reduce environmental impact [9].

One innovative approach is the electrocatalytic conversion of CO_2 into formic acid [10-11]. This process recycles carbon and transforms it into a chemical with significant utility in various industrial applications. Formic acid is an auxiliary energy source for numerous microbial species that use formate dehydrogenase enzymes (FDH) to transfers electrons to NAD+, generate NADH and release CO_2 [10-11]. This metabolic pathway is central to the co-feeding strategy used in the TAL production process with *YL* [10-11]. By co-feeding formic acid with glucose, *YL* can utilize additional reducing power for biosynthesis, contributing to the efficient production of TAL. The integration of CO_2-to-formic acid utilization in the production process has a dual benefit. It provides a supplementary energy source that enhances microbial growth and product yield and represents a strategic move toward decarbonization. The application of this technology on an industrial scale promises to reduce the carbon footprint of biochemical production and to mitigate the impact of climate change associated with industrial activities that are otherwise difficult to decarbonize.

When developing a bioprocess to supplement or replace an existing chemical product, it is crucial to conduct a techno-economic analysis. Despite the significant economic and environmental implications of developing biorefinery processes for TAL manufacture, only a few studies have examined it through techno-economic analysis (TEA) [12-13]. Based on the current retail price of $550/kg, sourced from Biosynth (bio-synth.com), and a bulk chemical price of $51,000/mt from vendors, all the scenarios are economically feasible in the proposed TAL production routes. By integrating carbon dioxide recycling into the current systems, biogenic carbon capture could be achieved, leading to a substantial reduction in greenhouse gas (GHG) emissions.

This study explores a novel route for TAL production by focusing on CO_2-to-formic acid electrocatalysis and co-feeding formic acid with glucose in the *YL* process. This approach highlights the transformative potential of recycling CO_2, which is traditionally viewed as a drawback in economics (options such as storage only add costs) [14], into a value-added input. This enhances the sustainability profile of TAL production, epitomizing the principles of a circular economy. Furthermore, the proposed model has the potential to shift toward more sustainable industrial practices, meet economic and environmental benchmarks, and contribute to the goal of achieving net-zero greenhouse gas emissions.

PROCESS OVERVIEW

The focus of the present study is to conduct a thorough comparison between two *YL* production systems, each scaled to produce 100 metric tons of TAL annually, examining their techno-economic and life cycle aspects. The first system utilizes *Yarrowia lipolytica* fed solely with glucose, while the second system involves a simultaneous feed of YL with glucose and formic acid [10-11]. This latter system utilizes electrochemical production of formic acid from carbon dioxide [15-16].

In the present study, process simulations were executed using SuperPro Designer [17], while energy balances and heat integration analyses were computed via Aspen Plus, employing the Non-Random Two-Liquid (NRTL) thermodynamic model to ensure accurate calculations of properties [18]. The scope of the investigation extends to a LCA and TEA across two distinct system configurations as exhibited in Figure 1A and 1B, further delineated into four specific scenarios. Within these scenarios, glucose serves as the primary substrate within the YL bioreactor, facilitating the biosynthesis of bio-TAL. Further explorations, as demonstrated in Figure 1B, illustrate the particular scenario in which a co-feeding strategy of formic acid and glucose is employed, showcasing an integrated method for enhancing the bio-production efficiency of TAL. It considers the sourcing of glucose and the subsequent recycling of off-gas into the three-compartment cell (TCC) reactor [15,19]. This innovative approach integrates the reactor for the electrochemical synthesis of formic acid from carbon dioxide emissions considering three carbon recycle percentage cases: 25%, 50%, and 75%.

Substrate for the TAL Biosynthesis Process

A) Production of TAL Using *Yarrowia lipolytica* with Glucose as Substrate.

The production of TAL through biotechnological routes is a promising alternative to traditional chemical synthesis. The process starts with the metabolic assimilation of glucose by *YL*, which undergoes glycolysis and enters the tricarboxylic acid (TCA) cycle and the associated biosynthetic pathways [10]. Genetic engineering has optimized YL to overproduce acetyl-CoA and malonyl-CoA, the precursors necessary for TAL formation [20]. By upregulating the genes involved in these pathways and downregulating those leading to competing pathways, *YL* can produce TAL.

(B) Cofeeding of *Yarrowia lipolytica* with glucose and formic acid.

In the pursuit of sustainable bioproduction, a significant step forward involves capturing and utilizing CO_2 emissions, which is crucial for reducing the carbon footprint of biotechnological applications. According to Noorman (2020) [10-11], an innovative approach involves the electrochemical reduction of biogenic CO_2 using renewable electricity to form valuable organic molecules that can be fed to the fermentation process. This study uses a Three Compartment Cell (TCC) reactor to electrochemically reduce CO_2, effectively converting it into formic acid with the net reaction [15,21]:

$$CO_2 + H_2O \rightarrow CHOOH + \frac{1}{2}O_2 \quad (1)$$

This method creates a closed carbon cycle, capturing emitted CO_2, reducing it to formic acid, and reintroducing it into the fermentation stage. Theoretically, this cycle could sustain cellular energy requirements (ATP) through respiration alone. Moreover, by utilizing the primary carbon source, such as glucose, predominantly for biomass and product assimilation rather than energy production, this process substantially increases biomass and product yields, thereby enhancing the overall efficiency of the primary carbon source.

The TCC reactor's innovative configuration employs a gas diffusion electrode (GDE) cathode, GDE anode, and central flow compartments [15,19]. The central compartment is flanked by a cation exchange membrane adjacent to the anode, which is coated with an IrO_2 catalyst on Toray paper [15,19], and an anion exchange membrane on the cathode side, loaded with a tin (Sn) catalyst and

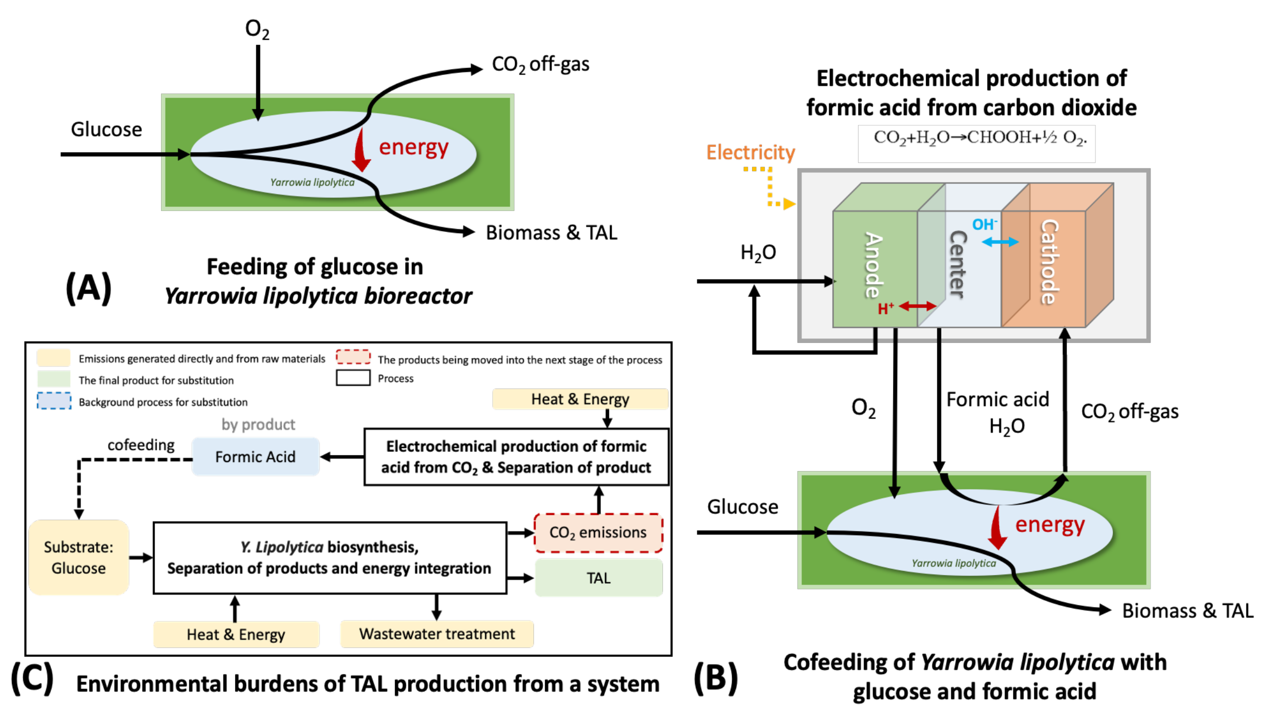

Figure 1: Comparative Models for TAL Production Scenarios: (A) TAL production via the *YL* biosynthesis process with glucose procured directly from commercial suppliers. This traditional aerobic fermentation process entails the partial oxidation of glucose to CO_2, which provides metabolic energy, while the remaining glucose serves as the carbon source for biosynthesis. (B) TAL production through the *YL* biosynthesis process, where glucose is co-fed with CO_2-derived formic acid. In this scenario, formic acid acts as an additional energy source, facilitating electron transfer, while glucose is exclusively utilized as a carbon source. The system incorporates a TCC reactor to recycle biogenic CO_2 emissions directly from the *YL* fermentation process. (C) Environmental burdens for the biosynthesis multi-routes TAL production.

polytetrafluoroethylene at 5% [15,19]. The key electrochemical reactions are delineated within their respective compartments. Notably, the TCC reactor design does not require the separation of by-products, given their minor concentrations within the effluent stream [21]. The efflux from the anode compartment predominantly comprises unconverted H_2O and the by-product O_2, where O_2 will be sent to the aerobic fermentation reactor and excess water will be recycled back to the TCC reactor [15]. The formic acid flow from the center part of the TCC is sent to a pressurized 4 bar distillation tower to remove the excess water to achieve a 70 wt. % of FA and then co-fed with glucose to the *YL* bioreactor [15].

To produce 1 kg of formic acid, the process requires an input of 0.96 kg CO_2 and 0.57 kg H_2O within the TCC reactor. The electrochemical reduction itself is powered by 4.79 kWh of electricity [15]. This co-feeding approach, where *Yarrowia lipolytica* is supplemented with both glucose and electrochemically produced formic acid, represents a shift toward more sustainable and partially decarbonized biotechnological processes.

TAL Purification Process.

Purification stands as a critical component in biorefining, with significant implications for costs. The process necessitates the removal of excess biomass, alongside organic and inorganic contaminants from the fermentation broth. Various techniques, including hexanol extraction [22], have been employed to recover TAL. However, an enhanced method utilizing charcoal adsorption has been delineated by Singh R. et al. [13], where TAL is absorbed by activated charcoal and subsequently desorbed using ethanol, which is then recovered for reuse. The selection of charcoal adsorption is predicated on its extractant reusability, providing a method that facilitates continuous operation while aligning with economic and environmental objectives, particularly leveraging the volatility of ethanol and ready availability.

Bioprocess modeling.

A bioprocess model used for this study was trained using fermentation profiles from Markham et al. [33]. A fed-batch process configuration was considered for all conditions explored. Briefly, within the system of ordinary differential equations (ODEs), the amount ATP generated from intracellular carbon catabolism at each timestep was calculated. It was then assumed that 25, 50, or 75% of the CO_2 emitted from the bioreactor could be captured and recycled as formic acid. An equal amount of formic acid was fed as was consumed by the organism (keeping the concentration in the bioreactor at zero). The molecular stoichiometry and energy generation from formic acid consumption defined by van Winden et al. [34] was used in the study. Energy generated from formic acid recycling was considered as a replacement for the same amount of energy generated via intracellular carbon catabolism, and the intracellular carbon that would have been used for catabolism to generate that energy was re-directed towards biomass and TAL in a ratio consistent with the carbon molar ratio of biomass and TAL produced under the glucose only feeding condition at each timepoint in the ODE integration.

RESULTS

TEA results.

The TEA conducted for TAL production adhered to the 2019 Chemical Engineering Equipment Cost Index and applied the discounted cash flow methodology for the determination of the Minimum Selling Price (MSP). The derived MSP was subsequently benchmarked against market prices ($50/kg) to assess economic viability. The Internal Rate of Return (IRR) served as the economic indicator for comparison among different technologies.

Capital and operational expenditures were extrapolated from existing literature and SuperPro Designer [17], with equipment costs being estimated according to size by employing a power scaling factor, of 0.6 [23]. This calculation was based on an equipment price benchmark of a 500 m³ bioreactor [35], providing a reference for cost estimations. Annual operating costs were assessed, including material, labor, utilities, and laboratory operations, along with facility expenses such as maintenance, depreciation, insurance, taxes, and overheads, with labor costs aligned with project demands. The detailed economic parameters are listed in Table 1. The TEA results, detailed in Table 2 [15,24-26], encompass capital expenditure (CapEx), operating expenditure (OpEx), the MSP of TAL, and the Internal Rate of Return (IRR) across various scenarios. Moreover, there is an additional by-product sale in the recycling scenarios examined in the TEA for TAL production. A key aspect was the conversion of all emitted CO_2 into FA. This process not only contributes to the sustainability of the operation but also adds a revenue stream, as the portion of FA not used for feeding back into the reactor is sold externally.

TEA analysis indicates that the scenario using glucose as the sole substrate for TAL production outperforms the co-feeding scenario with glucose and CO_2-derived formic acid (FA). Notably, the co-feeding approach, with a 75% carbon recycle rate, demonstrates more than double the conversion efficiency compared to the glucose-only scenario. This enhanced efficiency is attributed to the additional NADH generated by the dissimilation of formic acid, which contributes to ATP production, thus supporting growth [11]. However, the production cost for CO_2-derived FA, ranging from $0.78 to $2.63 per kg, exceeds that of glucose [21], priced at $0.33 per kg on average [27].

Table 1: Summary of economic assumptions for capital and operating cost.

TCC process		Capital investment cost		Fixed operating cost	
Cathode	Graphite block with Sn nanoparticles	General & Administrative Overheads	3% DFC [a]	Operating Labor Cost	average annual pre-taxed salary of $80,000 per employee
Anode	Titanium anode with IrO2-based catalyst coating	Contract Fee	19% DFC	Maintenance Cost	2% of installed equipment cost
Membrane	Amberlite® IR120 strong acid ion exchange resin, 620.0 – 830.0 μm and Nafion 212/115/324	General & Administrative Overheads	3% DFC	Operating Charges	25% Operating Labor Cost
Electrolyzer cost	450 $/kW	Working capital	20% annual operating cost	Operating General & Administrative Cost	20% direct production cost
Pressure, temperature	Ambient pressure, 25 C	Contingencies	37% DFC	**Time parameters**	
Residence time	4.0 min	Contract Fee	19% DFC	Project life	20 yr
Membrane lifetime	5000 hr	Other Capital Cost	71% DFC	Depreciation period	10 yr
				Annual operating time	8000 hr

In the co-feeding scenarios, the 25% carbon recycling case achieves the most favorable MSP at $30.91/kg. The lower MSP in scenarios with less formic acid co-feeding correlates with the profits from by-product sales after accounting for the amounts needed for co-feeding, the surplus FA is sold as a by-product, offsetting TAL production costs). Based on the SuperPro database, with an FA market price of $0.91 per kg, approximately 240, 448, and 648 mt/yr of FA can be sold in the 75%, 50%, and 25% carbon recycling scenarios, respectively. In these scenarios MSP is inversely correlated to TAL yield on glucose (0.30, 0.21, and 0.18 g TAL per gram of glucose for 75%, 50%, and 25% carbon recycle cases, respectively). Also worth noting, the opposite trend in TAL MSP is observed if we only produce the required amount of formic acid and release the remainder of the CO_2 to atmosphere (at $33.6, $31.74, and $31.51 per kilogram, for 25, 50, and 75% recycle scenarios, respectively).

Figure 2 illustrates the detailed cost components contributing to the MSP for each production scenario. The primary utilities impacting the process economics are identified as electricity, steam, process water, and cooling water. The energy-efficient TAL separation process, notably the adsorption and desorption steps, is highlighted for its role in reducing utility costs. However, for the electrochemical production of FA, the requirement of 4.79 kWh of electricity per kilogram of FA [15] results in a higher proportion of utility costs compared to glucose-based TAL production.

Table 2: Summary of TEA for various raw materials for a benchmark plant capacity of 100 mt TAL/y.

Stream	CapEx (MM$)	OpEx (MM$)	MSP ($/kg)[a]	IRR (%) [b]
Glucose	4.88	2.11	29.10	51.14
Glucose + Formic acid 75% carbon recycle	5.10	2.47	31.37	45.86
Glucose + Formic acid 50% carbon recycle	5.44	2.58	31.16	45.26
Glucose + Formic acid 25% carbon recycle	5.67	2.69	30.91	44.71

[a] MSP is computed when the sum of the discounted cash flow over 10 years equals zero (minimum selling price without any benefit).

[b] The internal rate of return after twenty years is based on the glucose-based MSP at a scale 0.1 kt/y, 50.00 $/kg.

The larger FA requirement to feed in the 75%, 50%, and 25% carbon recycle cases, with amounts of 95.95, 58.42, and 28.03 kg/hr, respectively causes an increase in electricity demands for the TCC reactor. However, TAL production using 75% carbon recycling of FA incurs less utility costs in the YL sections, as the cooling water demands of the YL bioreactor decrease, assuming that the reaction expends 115 kcal of heat for each mole of O_2 consumed [32]. To produce an equivalent quantity of TAL, the oxygen mole ratio required for the 75%, 50%, and 25% FA scenarios are 13.67, 15.13, and 16.59, respectively. The trade-off in utility costs between the two sections indicates that the CO_2-to-FA process is more energy-intensive. When using only glucose as a substrate in the YL bioreactor, a higher capital expenditure is required due to the lower yield and productivity of TAL (larger reactor size and more fermentation broth); they are 0.40, 0.66, 0.52, and 0.45 g-O2/kg broth/h for the glucose-based scenario and FA co-feeding with glucose with 75, 50, and 25 % carbon recycle scenarios respectively. However, when FA and glucose are co-fed, yield and productivity increase. For the 75%, 50%, and 25% carbon recycle cases, they are 0.30, 0.21, and 0.18 g TAL/g glucose, that is 0.19, 0.13, and 0.10 g-TAL/kg broth/h in productivity, respectively. In these scenarios, the increase in biomass yield not only enhances product titer but also allows the system to burn less glucose for ATP generation. This efficiency results in smaller reactor sizes, with the 75% carbon recycle case being 44% smaller than the 25% case, and less fermentation broth, further improving the overall productivity (0.19 and 0.10 g-TAL/kg broth/h respectively). Moreover, in the 25% carbon recycling scenario, capital costs for the CO_2-derived FA segment are lower due to the reduced CO_2 output from the *YL* section.

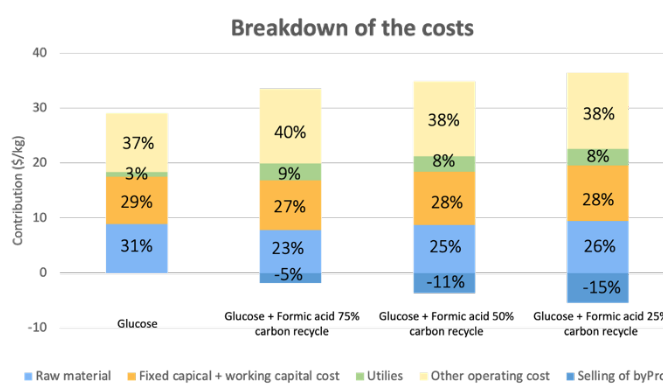

Figure 2. Cost breakdown of applying different TAL production routes.

LCA results.

The life cycle assessment (LCA) aimed to evaluate the environmental advantages of electrochemically converting CO_2 to FA and the subsequent co-feeding of glucose and FA for TAL production. The assessment, considering cradle-to-gate scope, tracked the CO_2 from its origin in the YL bioprocess through its transformation into FA and its return to the YL bioprocess in combination with glucose. The functional unit for this analysis was set at 1 kg of TAL.

Conforming to the ISO 14040 standard [28], the environmental evaluation of the CO_2-to-FA reduction was conducted using the Brightway2 framework [29] and the Ecoinvent® v3.9.1 database [30], prioritizing US data but using global data when US data was unavailable. To determine carbon footprint and LCA, emissions factors were created and used for various activities, such as grid electricity usage, measured in g CO_2/kWh consumed. These factors represent an estimate of emissions from a broad system of power generators, rather than a single point source of emissions. The ReCiPe Midpoint method (version 1.13) was employed to gauge the Global Warming Potential (GWP) of greenhouse gases over a century [31], thus ensuring a comprehensive perspective of both immediate and enduring environmental effects.

Furthermore, the LCA utilized system expansion for product allocation within multi-output processes, a method advantageous for co-products with distinct, quantifiable market values. This approach allows for the apportionment of environmental burdens proportionately to the economic value or utility of each product. In this case, the co-produced FA, derived from recycled CO_2, is considered an environmental credit, acknowledging its displacement of fossil-derived equivalents, as depicted by the blue squares in Figure 1(C). Oxygen, another by-product of electrolysis, was excluded from further utility considerations in this assessment.

The LCA results demonstrate that co-feeding *Yarrowia lipolytica* with glucose and formic acid significantly improves the greenhouse gas performance of TAL production. Notably, the scenarios with 75%, 50%, and 25% carbon recycling exhibit negative net emissions, thereby enhancing the environmental profile of the process. The 25% carbon recycling case is the most effective, with a net emission reduction to -10.25 kg CO_2-equivalents per kg of TAL (4.03 CO_2-eq per kg of TAL, if not considering the CO_2-to-FA credit), indicating an exemplary case of carbon-negative output. While if not considering the carbon credit from the by-product, FA, the 75% carbon recycling case performs the best in GWP impact. The inclusion of recycled CO_2 as a feedstock for formic acid production contributes to this improvement, showcasing a viable pathway for reducing GHG emissions in chemical manufacturing. On the other hand, the production of TAL using glucose-based methods, without recycling the CO_2 generated during fermentation, results in a higher carbon footprint. In this scenario, the carbon emissions from the fermentation process account for more than 50% of the total net emissions. This assessment solidifies the co-feeding of glucose with formic acid, particularly with high rates of carbon recycling, as a technologically and environmentally advantageous route for TAL production, aligning with global sustainability goals.

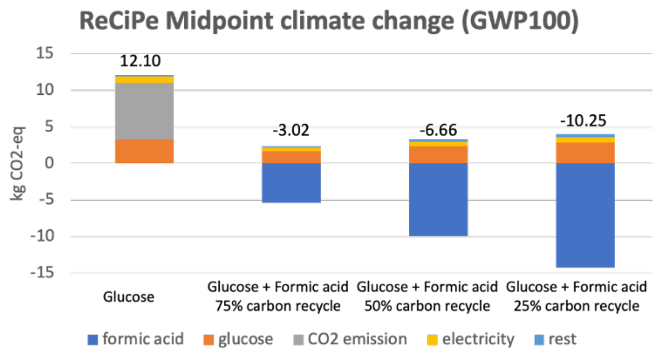

Figure 3. Lifecycle greenhouse gas emissions for glucose-based TAL and cofeeding of *Yarrowia lipolytica* with glucose and formic acid-based TAL.

DISCUSSION

The transition to sustainable energy systems is a critical endeavor in mitigating the environmental impact of hard-to-decarbonize sectors, including industrial and chemical processes. This study's LCA and TEA have shed light on a novel bioprocessing route for TAL production that integrates carbon recycling—a pivotal step towards achieving net-zero emissions.

The LCA results affirm that the co-feeding of *Yarrowia lipolytica* with glucose and formic acid significantly advances the GHG performance of TAL production. The standout 75% carbon recycling scenario not only meets but exceeds net-zero emission goals, venturing into the carbon-negative territory. This demonstrates the profound environmental benefits of incorporating CO_2-to-formic acid conversion within the TAL production cycle.

From a techno-economic perspective, the TEA findings reveal that the co-feeding approach, particularly with a high rate of carbon recycling, is economically viable. Despite the higher utility costs associated with the electrochemical generation of formic acid, this is counterbalanced by the revenue generated from the sale of excess formic acid, thereby enhancing the process's cost-competitiveness.

The capital cost analyses for the bioreactor configurations further corroborate the economic viability of the co-feeding approach. Although the initial outlay is more substantial for the glucose-only scenario due to the larger reactor requirements (4.88 MM$ CapEx of *YL* process), co-feeding with formic acid benefit the reactor sizing and utility consumption, leading to a reduction in both capital and operating expenses (3.21-4.66 MM$ CapEx of *YL* process).

Incorporating these findings, future directions will involve refining the bioprocesses and scaling the production while aligning with policy developments that support economic and environmental sustainability. This research sets a foundation for policy-backed industry practices that promote a circular economy and contribute to the global goal of a net-zero carbon future.

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