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Techno-economic and environmental assessment of a sugarcane biorefinery: direct and indirect production pathways of biobased adipic acid

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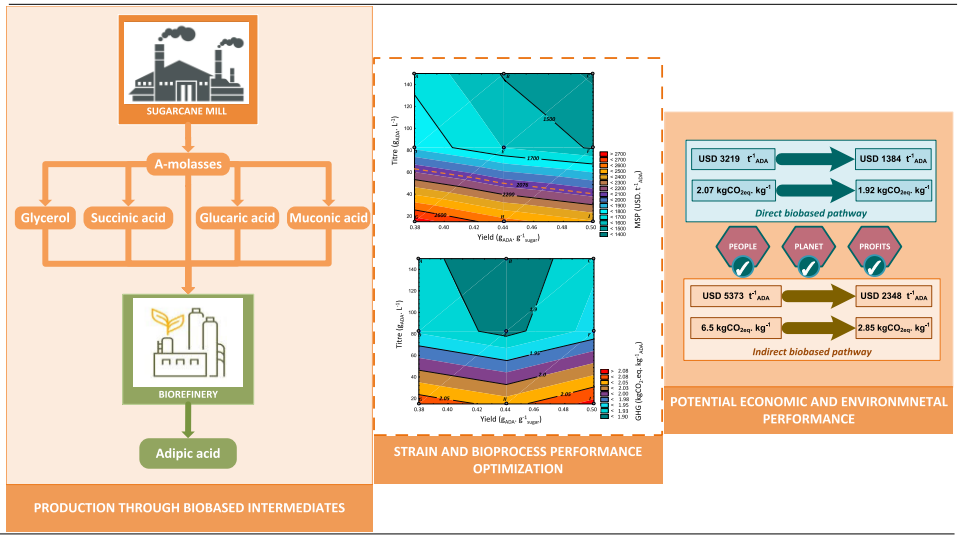
HIGHLIGHTS

- Techno-economic analysis of two promising biobased options to ADA in a biorefinery.
- Full factorial assessment of biobased ADA over a feasible fermentation space.
- Direct sugar fermentation is ultimately the best option for biobased ADA.
- Yield improvements offset the MSP of the indirect biobased route to ADA.
- Biobased ADA can significantly alleviate industrial N_2O and CO_2 emissions.

GRAPHICAL ABSTRACT



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ABSTRACT

Adipic acid (ADA) is a highly valuable industrial dicarboxylic acid used largely as a precursor of nylon 6,6 production. It is currently synthesized *via* a petrochemical process that accounts for over 80% of the global industrial N_2O emissions. Biobased ADA offers a cleaner alternative but requires technological advancements in microbe and bioprocess performance to be commercially relevant. An in-depth feasibility analysis was conducted to evaluate two biobased pathways for the production of ADA, modeled as integrated sugarcane biorefineries in Aspen Plus®. The pathways examined were: (1) direct fermentation of sugars to ADA (S1-ADA) and (2) hydrogenation of biobased cis,cis-muconic acid to ADA (S2-ccMA-ADA). The impact of improvements to key bioprocess metrics (product yield, titer, and volumetric productivity) on the minimum selling price and greenhouse gas (GHG) emissions for both pathways was also evaluated in a full-factorial study. S1-ADA demonstrated the highest feasibility potential, achieving minimum selling prices and GHG emissions that were 33.3% and 78.7% lower, respectively, than those of fossil-based production. These results were obtained under conditions of optimal strain performance and bioprocess efficiencies. However, under comparable technological advancements, the best-achievable results for S2-ccMA-ADA indicated a green premium of 13.4% alongside a 68.4% reduction in emissions compared to the fossil-based product. Consequently, the direct biobased pathway (S1-ADA) shows greater potential to compete with and eventually replace its fossil-based counterpart once optimized. This finding highlights the need to prioritize S1-ADA for further biotechnological development.

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Contents

Abbreviations

ALE	Adaptive laboratory evolution
ADA	Adipic acid
CAPEX	Capital expenditure
ccMA	Cis,cis-muconic acid
DAA	Diammonium adipate
E. coli	*Escherichia coli*
FOC	Fixed operating costs
FFD	Full factorial design
GWP	Global warming potential
GA	Glucaric acid
GHG	Greenhouse gas
HPS	High-pressure steam
LUC	Land use change
MPS	Medium-pressure steam
MSP	Minimum selling price
MAA	Monoammonium adipate
OPEX	Operating expenditure
RADP	Reverse adipate degradation pathway
RSB	Roundtable on sustainable biomaterials
TEC	Total equipment costs
TEC	Total equipment costs
VOC	Variable operating costs
WWT	Wastewater treatment

1. Introduction

Adipic acid (ADA) is ranked among the most industrially relevant dicarboxylic acids by the international energy agency (IEA) and is a primary monomer precursor of nylon 6,6 (De Jong et al., 2012). It is also used in the production of plasticizers and polyurethanes, which are central building blocks of polyvinyl chloride (Skoog et al., 2018). The worldwide annual production of fossil-based ADA was estimated at 3000 kilotonnes in 2020, representing a market share of USD 6.7 billion at a CAGR of 3–5% (Grand View Research, 2021). Commercially, it is manufactured from ketone alcohol *via* the nitric oxidation process using cyclohexane and feedstock. This process contributes to up to 80% of the annual industrial nitrous oxide (N_2O) emissions globally (Li et al., 2014). It is therefore necessary to develop cleaner ADA production routes, as N_2O has a 300 times higher global warming potential compared to CO_2 (EPA, 2017; Rios et al., 2021).

The production of ADA from renewable feedstocks such as lignocelluloses, sugars, and fatty acids has been thoroughly demonstrated. Major production pathways include direct fermentation (biological), catalytic synthesis (chemical), and hybrid routes involving the integration of biological and chemical processing methods (Skoog et al., 2018). Rennovia achieved a pilot-scale production of ADA, where glucose is oxidized to glucaric acid (GA), and GA is subsequently hydrogenated to ADA. However, this technology could not be implemented for large-scale production due to hight investment costs (Boussie et al., 2016). GA and cis,cis-muconic acid (ccMA) are the primary intermediates reported for ADA production through the hybrid process. In this approach, both intermediates are microbially synthesized from sugars and subsequently converted to ADA *via* catalytic hydrogenation (Polen et al., 2013). The direct fermentation route was attempted by Verdezyne, a US company employing a yeast capable of directly converting sugars or vegetable oils to ADA at an output of 5–15 kg per week (Picataggio and Beardslee., 2012). However, the company was liquidated after a key investor pulled out of the project in 2018.

Direct production of ADA from sugar bioconversion requires the development of an efficient microbial host to become commercially significant. While alternative synthetic pathways to ADA can be mapped out, the key enzymes essential for engineering microbes to produce ADA through potentially high-yielding metabolic pathways remain to be fully elucidated (Yu et al., 2014; Babu et al., 2015; Skoog et al., 2018). In addition, redox imbalances and cell fitness challenges arising from metabolic regulation efforts in the ADA space have not been adequately tackled (Skoog et al., 2018). However, enhanced microbial efficiency has been achieved through the deletion of genes to lactic acid and succinate formation, accompanied by genome overexpression to channel carbon flux toward ADA formation (Skoog et al., 2018). Also, the use of alternative feeding strategies, such as fed-batch cultures, has led to optimized bioprocess performances, achieving final ADA tires of up to 57.6 g. L^{-1} from glycerol (Zhou et al., 2020). Meanwhile, Zhao et al. (2018) reported the highest one-step production of ADA from glucose, achieving a concentration of 14.8 g. L^{-1} and a yield of 0.5 g. g^{-1}_{sugar} in a batch fermentation using engineered *Escherichia coli* (*E. coli*).

Similarly, biobased ccMA production by microbes such as *E. coli*, *Saccharomyces cerevisiae*, *Corynebacterium glutamicum*, and *Pseudomonas putida via* the shikimate, aromatic degradation, and phenolic pathways has been demonstrated with titers ranging between 0.01–59.2 g_{ccMA}. L^{-1} and yields of up to 0.17 g_{ccMA}. g^{-1}_{sugar}. However, the biological production of ccMA from sugars currently falls short of industrial benchmarks due to tight kinetic regulation in key metabolic pathways, which hinders productivity (Xie et al., 2014; Thompson et al., 2018; Choi

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et al., 2020). The availability of the crucial precursor phosphoenolpyruvate can lead to significant differences in the overall yield of ccMA from sugars *via* the shikimate metabolic pathway (Averesch and Krömer, 2018). Thus, scientific efforts in this field have focused on the overexpression of phosphoenolpyruvate synthase and the development of feedback-resistant enzymes rather than genome knockout techniques. Meanwhile, constraint-based metabolic modeling provides other strain reconstruction strategies that would redirect flux toward the shikimate pathway and boost overall ccMA yields (Williams et al., 2015; Kogure et al., 2016). Flux balance analyses are then applied to these alternative pathways to identify key metabolism drivers, finetune potential genome inferences, and estimate biologically theoretical limits to help form strain recombination targets (Puchałka et al., 2008; Henson and Hanly, 2014).

Cronjé et al. (2023) reviewed various biobased alternatives to bio-based ADA, including γ-valerolactone (GVL), 1,4-butanediol (1,4-BDO), GA, and 5-hydroxymethyl furfural (5-HMF) using sugarcane biomass as feedstock. The indirect biobased route (*via* ccMA) was the most economical option, with the lowest minimum selling price (MSP) of USD 2,538 t^{-1}_{ADA} and the lowest greenhouse gas (GHG) emissions compared to the direct biobased option. The direct biobased pathway to ADA is currently limited by low bioprocess performance, such as yields reaching only 50% of the theoretical maximum. However, further technological advancements could significantly reduce production costs and GHG emissions. This advantage stems from the direct pathway's reliance on fewer chemicals and its reduced number of processing steps compared to the indirect ccMA route. In any case, both pathways need improvements in bioprocess efficiency through strain engineering and process optimization, all of which demand significant investment and a prolonged development period (Nielsen and Keasling, 2016; Pothakos et al., 2018). Therefore, it is crucial to evaluate the full implications of these process developments on overall feasibility before making any investment decisions.

This study evaluated the economic and environmental performances of the direct and indirect biobased options to ADA based on current and prospective process performance. For the latter, a full factorial design (FFD) was applied to delineate the impacts of stepwise improvements in bioprocess performance parameters (in terms of yield, productivity, and titer) on the key economic (MSP) and environmental (GHG emissions) indicators of sugar-based ADA production. All scenarios were modeled in Aspen Plus ® as integrated biorefineries utilizing A-molasses from the sugarcane mill as feedstock. The main study objectives were to (i) determine the baseline technical performance required for viable commercial production of biobased ADA, (ii) determine whether strain engineering and bioprocess optimization can lead to feasible biobased ADA production, and (ii) assess whether the economic merits achievable through bioprocess development are accompanied by tangible environmental merits. Similar objectives have been achieved in previous studies involving biobased acrylic acid (Bhagwat et al., 2021) and 2,3-butanediol (Sikazwe et al., 2024) but not for biobased ADA. The overall aim of the study was to identify which biobased pathway to ADA should be prioritized for investment in microbial engineering and bioprocess development based on the overall economic and environmental benefits. **Table 1** shows a summary of key papers published on biorefinery feasibility assessments involving biobased ADA during 2020-2024.

2. Methodology

As a baseline, two process scenarios were simulated, namely, direct microbial conversion of sugars to ADA (S1-ADA) and indirect production of ADA through microbial conversion of sugars to ccMA and catalytic hydrogenation of ccMA to ADA (S2-ccMA-ADA). The baseline process models were developed using the state-of-the-art fermentation performances reported in the literature for direct conversion of sugars to ADA and muconic acid by Zhou et al. (2020) and Niu et al. (2002), respectively. Existing process models for both scenarios from Cronjé et al. (2023) were modified to include a heat transfer fluid system (hot oil) and a two-stage reactive distillation system to improve the overall energy efficiency (for S1-ADA). For S2-ccMA-ADA, the shikimate metabolic pathway to ccMA, which offers room for potential enhancements to the maximum theoretical yield, was adopted as an exemplar pathway with a view to maximize viability opportunities (Averesch and Krömer, 2018). Each coordinate fixed by the FFD in this study was simulated as a separate Aspen Plus® biorefinery, with the resulting energy and mass balance data used for economic analyses and GHG emission calculations. The statistical FFD encompassed a bioprocess space covering the full range of yield, volumetric productivity, and titer combinations achievable for the microbial conversion of sugars to ADA (S1-ADA) or ccMA (S2-ccMA-ADA), from current experimental reports to theoretical maxima. Flux balance analysis methods were used for maximum theoretical yield calculations, whereas upper limits for remaining bioprocess parameters were estimated based on values reported for commercial installations involving similar bioprocesses (Shastri and Morgan, 2004; Dellomonaco et al., 2011).

In all simulations, the sucrose in the A-molasses feedstock was inverted into glucose and fructose, with the assumption that these sugars would be consumed by the microbes to the same extent in both scenarios (Cronjé et al., 2023; Brobbey et al., 2024; Gouws et al., 2024a). The ADA biorefineries were simulated as facilities integrated into a typical sugarcane mill processing 300 t. h^{-1} of cane and yielding 27.7 t. h^{-1} crystalline sugar. All available A-molasses, comprising 54.4 wt% sucrose, 23.5 wt% glucose and fructose, and 22.1 wt% water, were redirected from the sugarcane mill to the annexed biorefinery for ADA production (Dogbe et al., 2020). The

Table 1.
Comparison of relevant feasibility studies on biobased adipic acid production published between 2020-2024.

Feedstock	Feedstock Category	Final Product	Nature of Study	Methods Used	Main Features	Reference
Sugarcane	1G and 1G2G	ADA	TEA Life cycle-GHG	DCFA RSB	Comparison of multi-biobased routes to ADA (glycerol, ccMA, GVL, 1,4-BDO, 5-HMF, and GA)	Cronjé et al. (2023)
Glucose and lignin	1G and 2G	ADA	TEA	DCFA and MCDM	Comparison of two biobased routed to ADA (glucose and lignin)	Unlu and Niu (2020)
Corn stover	2G	ADA and 1,4-BDO	TEA LCA	DCFA SimaPro 9.1	Comparison of strategies for coproducing ADA and 1,4-BDO in different ratios	Oh et al. (2022)
Lignocelluloses	2G	BO* and ADA	TEA LCA	DCFA SimaPro 9.1	Comparison of strategies for coproducing ADA and BO in different ratios	Choe et al. (2021)
Multiple (organic and inorganic)	NA	ADA	Economic	-	Comparison of multiple chemical and biobased pathways to ADA	Rios et al. (2021)
Sugarcane	1G	ADA	TEA Life cycle-GHG	DCFA and FFD RSB and FFD	Comparison of two biobased routed to ADA (glucose and ccMA)	**Current Study**

* Butane oligomers.

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diversion of A-molasses (25.4 t. h^{-1}) to the biorefinery necessitated a reconfiguration of the sugar mill from a three-step to a single-step crystallization process. This change ensured the maximum availability of sucrose-rich A-molasses but led to a 27.9% reduction in raw sugar output (Dogbe et al., 2020). Fewer sugar crystallization steps also resulted in energy savings for the sugar mill, enabling a surplus of 1.86 MW of electricity, 15.5 t. h^{-1} of high-pressure steam (HPS), and 15 t. h^{-1} of medium-pressure steam (MPS) to be supplied for biorefinery use (Dogbe et al., 2019 and 2020). Also, a thermal oil unit was on standby to supply additional HPS for scenarios requiring more HPS than what the sugarcane mill could provide, as described previously (Sikazwe et al., 2024).

Version 11 of Aspen Plus ® (Aspen Technology Inc., Cambridge, MA, USA) was the process simulator of choice with the Electrolyte Non-random Two Liquid model (ELECNRTL) adopted as the base property method due to its general versatility for fluid-based interactions. Fermenters, digesters, and general reactors were all modeled as stoichiometric process units (RSTOIC), requiring ambient operating conditions and the extent of reactant conversions as minimum inputs. Distillation columns were simulated as RADFRAC units, allowing adjustments to reflux ratios, distillate-to-feed ratios, bottoms-to-feed ratios, as well as operating temperature and pressure to achieve the target product purity of 99% ADA. Additionally, compressors, valves, and pumps were modeled as standard isentropic equipment.

2.1. Process scenarios and modeling assumptions

2.1.1. S1-ADA: adipic acid production via direct sugar conversion

- Pretreatment and fermentation

Firstly, A-molasses was sterilized at 121 °C to remove any possible microbial interferences, followed by cooling and intermittent storage at a temperature of 37 °C, as shown in **Figure 1a (**Ikram-Ul et al., 2004). The A-molasses was then diluted to approximately 630 g. L^{-1} (63 Brix) by adding process water at a rate of 24.9 g. L^{-1}, prior to enzymatic hydrolysis with invertase, assuming a 98% conversion of sucrose at an enzyme dosage of 300 mg/kg sucrose at 60 °C (Białkowska, 2016; Cronjé et al., 2023). The hydrolyzed feed stream was then split between the seed train and main fermenters, with about 2 wt% of the total stream diverted to the seed train. Recombinant strains of *E. coli* were cultured at a temperature of 37 °C using tryptone (20 g. L^{-1}), magnesium chloride (2.03 g. L^{-1}), sodium chloride (0.5 g. L^{-1}), and potassium chloride (0.186) as main sources of nutrition (Zhou et al., 2020). Upon completion of inoculation, the contents of the seed train were pumped to the main fermenter to commence ADA production. The base-case process performance in the main fermenter was modeled according to actual experimental data provided by Zhou et al. (2020), namely, a fermentation time of 72 h and ADA production of 14.8 g. L^{-1} (titre) and 0.378 g_{ADA}. g^{-1}_{sugar} (yield) at a temperature of 37 °C under aerobic conditions. These results represent the highest recorded ADA production by direct conversion of sugars *via* the reverse adipate degradation pathway (RADP). Ammonium hydroxide was continuously added to the fermenter for pH control, leading to the neutralization of ADA to a mixture of monoammonium adipate (MAA) and diammonium adipate (DAA) (Fruchey et al., 2014). The study assumes complete ADA neutralization to DAA and MAA at a ratio of 9:1, respectively.

- ADA separation and purification

After fermentation, the biomass and suspended solids were separated from the broth using a combination of microfiltration and centrifugation (**Fig.1b**) to prevent fouling of downstream equipment (Fruchey et al., 2014). Both of these units were simulated as CFuge units in Aspen Plus® with a 90% recovery assumed for each equipment (Davis et al., 2018). The clarified broth was subjected to reactive distillation (at 135 °C and 3.5 bar) in the first column, where 90% of the DAA dissociated to NH_3 and MAA, as shown in **Figure 1a** (Fruchey et al., 2014). The bottom stream from the first column entered a multi-effect evaporation chamber consisting of three flash columns operating between 0.6 bar and 0.18 bar, concentrating the broth to between 40–50 wt% adipate salts (Wooley et al., 2002). This process helped to reduce the energy requirements from boiling large water-laden process streams in the units downstream. The effluent stream from the multi-evaporation unit containing about 55 wt% MAA with some DAA in water was fed to a second reactive distillation column where MAA and DAA dissociated into NH_3 and ADA at 2 bar and 190 °C as depicted in **Figure 1b** (Fruchey et al., 2014). The overhead from both columns containing NH_3 and water was condensed as a crude alkali stream and recycled back to the fermenter as a neutralizing agent. The bottom stream from the second distillation column, containing at least 50 wt% ADA, was directed to a crystallizer operating at 30°C, resulting in the production of a solid ADA stream (Fruchey et al., 2011). The ADA crystals were centrifuged and dried at 100 °C in a fluidized bed dryer to obtain a dry final product at 99 wt% purity (Silva-Moris and Rocha, 2006).

- Wastewater treatment

Effluent streams from multi-stage evaporation, fermentation, and biomass separation were all pumped to the wastewater treatment (WWT) area for water recovery and biogas generation, as shown in **Figure 1**. The first unit in the WWT setup is the anaerobic digester, where biomass entrained in the effluents was converted to NH_3 and biogas containing chiefly methane and CO_2. The biogas was trapped and channeled to the existing boiler and/or the heat transfer fluid unit to supplement bagasse for heat generation. The discharge from the anaerobic digester was transferred to the aerobic digester, where NH_3 and unconverted biomass from the previous process were aerobically decomposed into a mixture of CO_2, nitrogen, and water. Any remaining unconverted solids settled at the bottom of the aerobic digester as sludge, with water intermittently raked out from the vessel. The effluent from the aerobic digester was subsequently treated using reverse osmosis to produce purified water, which was recycled back to the biorefinery as process water. The layout and operating guidelines for the WWT section used in this study were based on Humbird et al. (2011) and were consistent across both scenarios.

2.1.2. S2-ccMA-ADA: adipic acid production via hydrogenation of microbially-produced ccMA

- Pretreatment and fermentation

The process layout for A-molasses pretreatment, hydrolysis, and subsequent fermentation for this scenario was identical to that shown in **Figure 1a**. Mutant strains of *E. coli* assumed in this study were grown at 37 °C under anaerobic conditions in a culture medium enriched with 10 g. L^{-1} tryptone, 5 g. L^{-1} yeast extract and 10 g. L^{-1} of a salt blend comprising ammonium chloride, disodium phosphate, monopotassium phosphate, and sodium hydroxide (Niu et al., 2002). After inoculation, the culture mix was fed to the main fermenter along with the sterilized A-molasses to commence sugar fermentation to ccMA. In the baseline process performance, 36.8 g. L^{-1} ccMA was produced after 48 h at an overall yield of 0.17 g_{ccMA}. g^{-1}_{sugar}, which is the best-recorded ccMA production *via* the shikimate metabolic pathway (Niu et al., 2002). pH control was maintained through the neutralization of ccMA with sodium hydroxide, forming sodium muconate. Following fermentation, the discharge was subjected to microfiltration and centrifugation to remove biomass and suspended debris. These byproducts were either sent to the WWT section or directly combusted in the boiler or hot oil system, as depicted in **Figure 1a**.

- Production, separation, and purification of ADA

Production of ADA from ccMA *via* catalytic hydrogenation requires a very pure stream of ccMA as feedstock (Davis et al., 2018). The liquid recovered from biomass filtration was initially passed through a column packed with activated carbon to remove any coloring agents and pumped to the ccMA recovery section (**Fig. 1c**). Next, the sodium muconate in the clarified liquid stream was reacidified to ccMA with sulfuric acid to achieve a system pH of 2 for maximum ccMA protonation. Pronated ccMA in acidic media is highly insoluble and nearly crystalizes to completion. Thus, the acidified liquid stream was fed to a crystallizer operating at 15 °C, yielding ccMA crystals at a 98.8% product recovery with a purity of 99.2 wt% (Davis et al., 2018). The mother liquor from the crystallizer was neutralized before being sent to the WWT section, whereas the ccMA crystals were dried in a fluidized dryer, as shown in **Figure 1c**.

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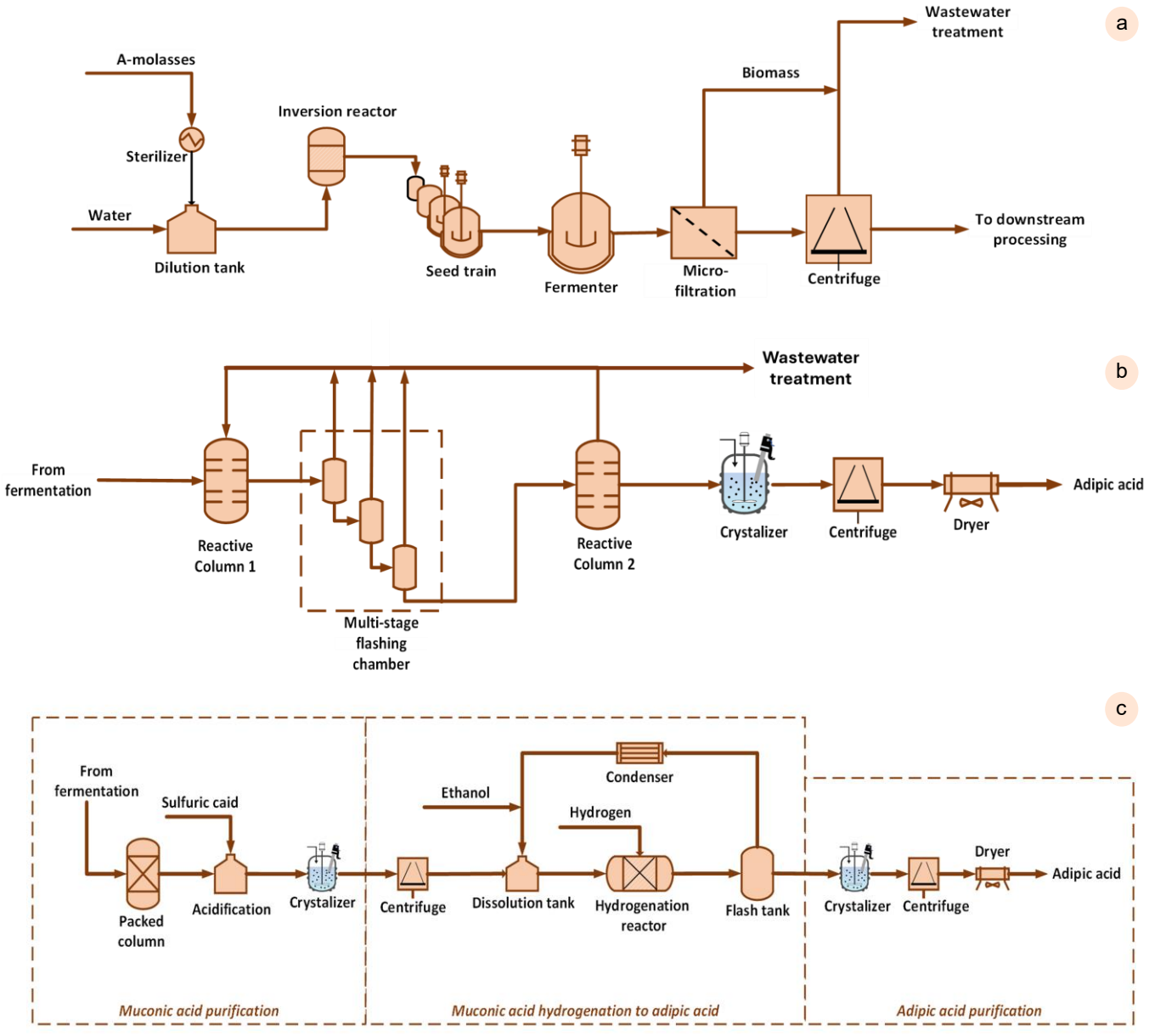

Fig. 1. Simplified process flow configuration for biobased ADA production; **(a)** pretreatment and fermentation for ADA or ccMA production, **(b)** downstream separation and purification for S1-ADA, and **(c)** downstream separation and purification for S2-ccMA-ADA.

The ccMA crystals were dissolved in pure ethanol in a mixing unit to facilitate the precipitation of entrained foreign ions, forming unwanted salts which were filtered out of the solution. An ethanol-to-ccMA mass ratio of 4:1 was used in the mixing chamber to prevent the mutual crystallization of ccMA with foreign salts (Davis et al., 2018). Lastly, the effluent from the mixing chamber was fed to a packed bed catalytic reactor where ccMA was upgraded to ADA at 40 bar and 78°C over 2% ruthenium catalyst embedded in carbon (Vardon et al., 2015 and 2016). The reactor was simulated as an RSTOIC unit in Aspen Plus ®, assuming complete conversion of ccMA by supplying excess H_2 to the ccMA feed stream at a molar ratio of 2.62:1 (Davis et al., 2018). After hydrogenation, the product stream was sent to a flash evaporator to concentrate the product to about 30 wt% ADA by expelling ethanol and water overhead. The bottom stream containing concentrated ADA was finally sent to a crystalizing unit to recover roughly 73% ADA at 15°C (Li et al., 2010). The mother liquor from this crystallizer was continuously recycled back to the flash evaporator until all the uncrystallized ADA was exhausted (Urbanus et al., 2012). The outlet from the crystallizer was centrifuged to separate the ADA crystals, which were then dried and stored as the final product with 99 wt% purity. All effluent streams from this section were directed to the WWT system, as illustrated in **Figure 1c**.

2.2. Economic assessment

The total equipment costs (TEC) were estimated using second-order capital cost correlations for biorefinery-related processes developed by Petersen et al. (2017) and based mainly on process equipment literature from Woods (2007). Energy and mass flow from each process simulation developed in Aspen Plus® provided inputs for equipment sizing and subsequent costing. Direct and indirect total costs were calculated based on

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the feedstock flow rates and heating values. These costs, along with the TEC, were used to estimate capital expenditure (CAPEX), following the methodology outlined by Davis et al. (2018). The results are presented in **Tables S1**, **S2**, and **S3** of the **Supplementary Material**. The operating expenditure (OPEX) was estimated using 2022 prices for chemicals (**Tables S4** and **S5** of the **Supplementary Material**), as well as costs for insurance, labor, and land. It comprises both fixed operating costs (FOC) and variable operating costs (VOC). A discounted cash flow analysis over a period of 25 years in real terms, under an assumed desired discount rate of 20% (real terms), was performed for each process model to determine the MSP, which is the price of ADA at which the net present value (NPV) is null. The key economic parameters used for the economic assessments across all process scenarios are summarized in **Table 2**.

Table 2.
Main economic assumptions used for CAPEX and OPEX estimations in the economic analysis.

TEA Assumptions	Value	Reference/Source
Raw materials cost (USD. t^{-1})		
A-molasses	199.6[a]	Brobbey et al. (2023)
Invertase	258833	Alibaba (2023)
Bagasse	30.0[b]	Dogbe et al. (2019)
Ammonium hydroxide	0.225	INTRATEC (2023)
Diammonium phosphate (DAP)	625	Index Mundi (2023)
Magnesium Chloride	1.79[c]	Özdemir et al. (2009)
Sodium chloride	0.045[d]	Autelitano et al. (2019)
Economic assumptions		
Cost year	2022	
Chemical Engineering Plant Index (CEPCI) for 2022	808.7	ToweringSkills (2023)
Online time (h/yr)	5000	
Plant life (yr)	25	
Construction period (yr)	2	
Discount rate (%)	20	
Income tax rate (%)	28	
Straight-line depreciation (yr)	5	
Salvage value	0	
Equity (%)	100	
% FCI spend in the second year	2	
% FCI spend in the first year	50	
% FCI spend on construction	75	
Working capital	5% of FCI	

[a] Estimated based on the lost income from reduced crystalline sugar output and the exclusion of C-molasses production (Ratshoshi et al., 2021; Brobbey et al., 2023).
[b] The bagasse price inflated to the 2022 price year using the 10-year average dollar inflation rate.
[c, d] Inflated to the 2022 value of the dollar.

2.3. Life-cycle greenhouse gas emission assessment

To assess the environmental performance of the simulated biorefinery scenarios, a cradle-to-gate life-cycle GHG emissions calculation was made from sugarcane farming to ADA dispatch using version 4.03 of the Roundtable on Sustainable Biomaterials (RSB) methodology (www.rsb.org). Numerous studies have shown that GHG emissions serve as a reliable metric for assessing the environmental performance of bioprocess scenarios (Brobbey et al., 2023; Cronjé et al., 2023; Gouws et al., 2024b). The Roundtable on Sustainable Biomaterials (RSB) methodology is internationally recognized as a robust tool for GHG calculations, particularly in processes involving biomaterials and biofuels (RSB, 2009). In this tool, a bioprocessing facility is segmented into three main processing sections, namely, feedstock cultivation, storage and transportation, and processing. The tool also accounts for emissions due to land use change (LUC) and overall water consumption for each scenario. Each process model provided energy and mass flow estimates, which are fed to the calculator for subsequent $CO_{2\text{-eq}}$ estimations using the built-in emission factors assigned to every chemical substance used in the biorefinery. The GHG bequests were finally summed up to obtain the gross carbon emission in kg $CO_{2\text{-eq}}$ per unit ADA. The sugarcane mill and biorefinery were designated processing areas 1 and 2, respectively.

The calculation of carbon footprints for bioproducts is subject to various uncertainties, primarily arising from the choice of agricultural feedstock, LUC, and the modeling methodologies employed (Brandão et al., 2022). Different crop feedstocks, for example, have distinct agronomic requirements, including nutrient demands, growth cycles, yields, and processing technologies. Each of these factors can significantly influence the total emissions associated with the product. In this study, zero emissions were attributed to LUC since the land used for producing the feedstock for ADA production has been established for at least two decades (Nieder-Heitmann et al., 2019). Previous studies have highlighted that biorefinery integrations involving LUC can potentially generate more emissions than the fossil-based products they aim to replace, primarily due to indirect LUC-related emissions (EU, 2015).

2.4. Determination of theoretical-estimates for the factorial analysis

The maximum theoretical yields of microbial ADA production from glucose or fructose *via* the RADP (S1-ADA) and ccMA from glucose *via* the shikimate pathway (S2-ccMA-ADA) were determined using a simplified metabolic flux balance methodology developed by Shastri and Morgan (2004). The method assumes all cellular metabolic conversions to be in a pseudo-steady state, implying zero net metabolite accumulation. Carbon flux is directed toward the product of interest, allowing for the possibility of zero co-product formation, provided that the minimum redox (NADH/NADPH) and energy (ATP) requirements within the cell are met (Shastri and Morgan, 2004; Töpfer et al., 2021). This assumption facilitates the linear algebraic summation of all chemical equations involved in converting the substrate to the target product, accounting for each chemical intermediate. This approach has previously been applied to 2,3-butanediol (2,3-BDO) in our earlier study and demonstrated reliability for preliminary theoretical yield estimations in the absence of genome-scale models (GEMs) (Jantama et al., 2015; Sikazwe et al., 2024).

Table 3 represents the RADP for direct microbial ADA production from glucose and fructose (Skoog et al., 2018). The theoretical maximum yield of ADA from simple sugars glucose/fructose was estimated as 0.54 $g_{ADA}.\ g^{-1}_{sugar,}$ as shown in **Eq. 12**. Further correlations were made using experimental data from Zhou et al. (2020) and Zhao et al. (2018) to account for the allocation of substrate to biomass formation, obtaining a value of 0.50 $g_{ADA}.\ g^{-1}_{sugar}$ as a realistic actual yield value to be used as an upper limit for the FFD involving S1-ADA. Likewise, **Table 4** summarizes the metabolic reactions for microbial glucose and fructose conversion to ccMA along the shikimate pathway (Xie et al., 2014). The maximum theoretical yield of ccMA from simple hexose sugars *via* the shikimate pathway was estimated as 0.68 $g_{ccMA}.\ g^{-1}{}_{sugar}$ as shown in **Equation 21**. After accounting for biomass formation using actual experimental data from Niu et al. (2002), a final value of 0.63 $g_{ccMA}.\ g^{-1}{}_{glucose}$ was calculated and used as the realistic upper limit for the FFD study for S2-ccMA-ADA.

The maximum theoretical yield of ccMA calculated above relies on a revised metabolism in which pyruvate kinase is entirely deleted, allowing all flux to be directed through the shikimate pathway. This adjustment would enable sufficient pyruvate production through the same pathway to be fed back into the TCA cycle (Averesch and Krömer, 2018). In practice, however, a portion of the substrate is expended to produce pyruvate, lowering the overall theoretical yield of ccMA. For instance, if one molecule of pyruvate is formed for each molecule of glucose introduced into the cytoplasm, the maximum theoretical yield drops to about 0.43 mol. mol^{-1} (0.34 $g_{ccMA}.\ g\text{-}1_{sugar}$), according to Niu et al. (2002). This work will demonstrate the yields at which ccMA production is commercially feasible and determine whether the Shikimate pathway is a realistic pathway worth developing in light of the above limitations to achieve the estimated maximum yield. As in our previous studies (Sikazwe et al., 2024), the theoretical upper limits for volumetric productivity and titer were assumed to be 3.0 $g_{ADA}.\ L^{-1}.h^{-1}$ and 150 $g_{ADA}.\ L^{-1,}$ respectively, which represent typical values for bioprocesses operating on a commercial scale (Dellomonaco et

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Table 3.
Stoichiometric equation showing key metabolic conversions for the formation of adipic acid from glucose *via* the reverse adipate degradation pathway.

	$[\boldsymbol{C_6H_{12}O_6} + 2NAD^+ + 2ADP + 2Pi \rightarrow 2C_3H_4O_3 + 2NADH + 2H^+ + 2ATP] \times 1.5$	Eq. 1
+	$[C_3H_4O_3 + CoASH + NAD^+ \rightarrow C_2H_3OSCoA + CO_2 + NADH + H^+] \times 3$	Eq. 2
+	$[C_2H_3OSCoA + C_2H_3OSCoA \rightarrow CoASH + C_4H_5O_2SCoA] \times 1$	Eq. 3
+	$[C_4H_5O_2SCoA + NADH + H^+ \rightarrow C_4H_7O_2SCoA + NAD^+] \times 1$	Eq. 4
+	$[C_4H_7O_2SCoA \rightarrow C_4H_5OSCoA + H_2O] \times 1$	Eq. 5
+	$[C_4H_5OSCoA + FADH_2 \rightarrow C_4H_7OSCoA + FAD] \times 1$	Eq. 6
+	$[C_4H_7OSCoA + C_2H_3OSCoA \rightarrow CoASH + C_6H_9O_2SCoA] \times 1$	Eq. 7
+	$[C_6H_9O_2SCoA + NADH + H^+ \rightarrow C_6H_{11}O_2SCoA + NAD^+] \times 1$	Eq. 8
+	$[C_6H_{11}O_2SCoA + 2H_2O \rightarrow CoASH + C_6H_{14}O_3] \times 1$	Eq. 9
+	$[C_6H_{14}O_3 + O_2 + NADPH + 2NAD^+ \rightarrow \boldsymbol{C_6H_{10}O_4} + 2NADH + H^+ + H_2O + NADP^+] \times 1$	Eq. 10
=	$1.5C_6H_{12}O_6 + 3ADP + 3Pi + 6NAD^+ + FADH_2 + NADPH \rightarrow \boldsymbol{C_6H_{10}O_4} + 3CO_2 + 6NADH + 5H^+ + 3ATP + NADP^+ + FAD$	Eq. 11
	$Yield_{max} = \frac{weight_{ADA}}{weight_{GLU}} = \frac{\left(1\times\frac{146.14g}{mol}\right)}{\left(1.5\times\frac{180.156g}{mol}\right)} = 0.54\ g_{ADA}.\ g^{-1}_{glucose}$	Eq. 12

Table 4.
Stoichiometric equation showing key metabolic conversions for the formation of ccMA from glucose *via* the shikimic metabolic pathway.

	$[1.17\boldsymbol{C_6H_{12}O_6} + 2ATP + NAD^+ \rightarrow C_3H_5O_6P + C_4H_9O_7P + 2ADP + NADH + H^+] \times 1$	Eq. 13
+	$[C_3H_5O_6P + C_4H_9O_7P \rightarrow C_7H_{13}O_{10}P + Pi] \times 1$	Eq. 14
+	$[C_7H_{13}O_{10}P \rightarrow C_7H_{10}O_6 + H_2O + Pi] \times 1$	Eq. 15
+	$[C_7H_{10}O_6 \rightarrow C_7H_8O_5 + H_2O] \times 1$	Eq. 16
+	$[C_7H_8O_5 \rightarrow C_7H_6O_4 + H_2O] \times 1$	Eq. 17
+	$[C_7H_6O_4 \rightarrow C_6H_6O_2 + CO_2] \times 1$	Eq. 18
+	$[C_6H_6O_2 + O_2 \rightarrow C_6H_6O_4] \times 1$	Eq. 19
=	$1.17C_6H_{12}O_6 + O_2 + NAD^+ \rightarrow \boldsymbol{C_6H_6O_4} + 3H_2O + CO_2 + NADH + H^+$	Eq. 20
	$\text{Yield}_{\text{max}} = \frac{\text{weight}_{muconic\ acid}}{\text{weight}_{glucose}} = \frac{\left(1\times\frac{142.11\text{g}}{\text{mol}}\right)}{\left(\mathbf{1.17}\times\frac{180.156\text{g}}{\text{mol}}\right)} = 0.68\ g_{ccMA}.\ g^{-1}_{glucose}$	Eq. 21

al., 2011). The fermentation metrics used for the FFD models developed for direct ADA production (S1-ADA) and muconic acid production (S2-ccMA-ADA) are summarized in **Table 5** and **Table 6**, respectively.

2.5. Sensitivity analysis

An initial analysis was conducted to assess the robustness of the baseline MSP to variations in key factors, including feedstock costs (A-molasses, invertase, and chemicals), CAPEX, OPEX, income tax rate, and operating hours. This analysis, performed across the baseline scenarios, aims to identify process improvement targets that could deliver the most significant economic benefits. Next, A 3^3 FFD was employed to evaluate the potential economic and environmental advantages of stepwise improvements in microbial and bioprocess performances within biologically achievable theoretical limits. This approach generated 27 unique titer-volumetric productivity-yield coordinates for both S1-ADA and S2-ccMA-ADA pathways. The baseline simulations were then updated with the new fermentation stage parameters, leaving the process configuration unchanged in both cases. Consequently, each of the 27 FFD experiments translated to a new simulation in Aspen Plus ® with unique mass and energy results, subsequently generating unique CAPEX, OPEX, MSP, and GHG emissions values. Design specifications were employed extensively in Aspen Plus ® to ensure key process units such as reactors and distillation columns achieved the same degree of reactions and product purity across all scenarios, respectively.

3. Results and Discussion

3.1. Energy and mass flow results

Table 7 summarizes the energy and mass balances for the simulated baseline scenarios, comparing them to the results reported by Cronjé et al. (2023) for similar biobased scenarios and the fossil-based pathway. S1-ADA achieved an annual ADA throughput of 38.6 kt. y^{-1}, which was 2.3 times higher than that of S2-ccMA-ADA. This finding was primarily due to the higher reported yield of ADA via the RADP, specifically 0.38 $g_{ADA}.\ g^{-1}_{glucose}$. On the other hand, the yield of ADA *via* the indirect biobased route was limited by the much lower reported yield of ccMA *via* the shikimate pathway (0.17 $g_{ccMA}.\ g^{-1}_{glucose}$), which agrees with findings from Cronjé et al. (2023). For the indirect biobased route, Cronjé et al. (2023) reported a much higher overall production rate, having assumed a higher product yield of 0.31 $g_{ccMA}.\ g^{-1}_{glucose}$, which led to an 84.9% higher ADA production rate after hydrogenation (ccMA-ADA in **Table 7**). However, the conventional fossil-based method had the highest output of ADA at 61.3 kt. y^{-1}, outperforming the biobased pathways due to a much superior ADA yield from cyclohexane (1.60 kg. kg^{-1}).

Comparing the two biobased routes, S1-ADA showed a higher overall cooling demand of 16.9 MJ. kg^{-1}_{ADA} compared to 4.0 MJ. kg^{-1}_{ADA} for S2-ccMA-ADA, which had no energy-intensive equipment. Additionally, S2-ccMA-ADA had a more concentrated process stream than S1-ADA (36.8 $g_{ccMA}.\ L^{-1}$ *vs* 14.9 $g_{ADA}.\ L^{-1}$), leading to a reduced total process water usage by 62.6%. This reduction contributed to lower heating and cooling demands

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Table 5.
Setpoints for microbial bioprocess performance metrics used in the FFD Aspen Plus® models for direct adipic acid production (S1-ADA).

Fermentation Parameters				
Scenario ID	**Yield ($g_{ADA}.g^{-1}_{glucose}$)**	**Productivity ($g_{ADA}.\ L.^{-1}h^{-1}$)**	**Final Titre ($g_{ADA}.\ L^{-1}$)**	**Fermentation Time (h)**
1	0.38	0.2	14.9	78.0
2	0.38	1.6	14.9	9.3
3	0.38	3.0	14.9	5.0
4	0.38	0.2	82.5	431.6
5	0.38	1.6	82.5	51.7
6	0.38	3.0	82.5	27.5
7	0.38	0.2	150.0	785.2
8	0.38	1.6	150.0	94.0
9	0.38	3.0	150.0	50.0
10	0.44	0.2	14.9	78.0
11	0.44	1.6	14.9	9.3
12	0.44	3.0	14.9	5.0
13	0.44	0.2	82.5	431.6
14	0.44	1.6	82.5	51.7
15	0.44	3.0	82.5	27.5
16	0.44	0.2	150.0	785.2
17	0.44	1.6	150.0	94.0
18	0.44	3.0	150.0	50.0
19	0.50	0.2	14.9	78.0
20	0.50	1.6	14.9	9.3
21	0.50	3.0	14.9	5.0
22	0.50	0.2	82.5	431.6
23	0.50	1.6	82.5	51.7
24	0.50	3.0	82.5	27.5
25	0.50	0.2	150.0	785.2
26	0.50	1.6	150.0	94.0
27	0.50	3.0	150.0	50.0

Table 6.
Setpoints for microbial bioprocess performance metrics used in the FFD Aspen Plus® models for muconic acid production (S2-ccMA-ADA).

Fermentation Parameters				
Scenario ID	**Yield ($g_{ccMA}\,g^{-1}_{glucose}$)**	**Productivity ($g_{ccMA}.\ L.^{-1}h^{-1}$)**	**Final Titre ($g_{ccMA}.\ L^{-1}$)**	**Fermentation Time (h)**
1	0.17	0.8	36.8	48.0
2	0.17	1.9	36.8	20.0
3	0.17	3.0	36.8	12.0
4	0.17	0.8	93.4	121.0
5	0.17	1.9	93.4	50.0
6	0.17	3.0	93.4	31.0
7	0.17	0.8	150.0	195.0
8	0.17	1.9	150.0	80.0
9	0.17	3.0	150.0	50.0
10	0.40	0.8	36.8	48.0
11	0.40	1.9	36.8	20.0
12	0.40	3.0	36.8	12.0
13	0.40	0.8	93.4	121.0
14	0.40	1.9	93.4	50.0
15	0.40	3.0	93.4	31.0
16	0.40	0.8	150.0	195.0
17	0.40	1.9	150.0	80.0
18	0.40	3.0	150.0	50.0
19	0.63	0.8	36.8	48.0
20	0.63	1.9	36.8	20.0
21	0.63	3.0	36.8	12.0
22	0.63	0.8	93.4	121.0
23	0.63	1.9	93.4	50.0
24	0.63	3.0	93.4	31.0
25	0.63	0.8	150.0	195.0
26	0.63	1.9	150.0	80.0
27	0.63	3.0	150.0	50.0

across all heat transfer equipment for S2-ccMA-ADA (**Table 7**). Consequently, S2-ccMA-ADA required no additional bagasse to be fed to the boiler for steam generation, whereas S1-ADA consumed 6.2 kg of bagasse and lignocelluloses per kg of ADA produced, depleting all the available bagasse. The balance was supplemented by the available lignocelluloses, making S1-ADA equally energy-sufficient in a typical sugarcane mill. The direct fermentation scenarios required two reactive distillation units for ADA separation and purification, which accounted for 81.1% of the overall heating demand (data not shown). Therefore, S1-ADA recorded an overall heating requirement 3 times that of S2-ccMA-ADA. Subsequent cooling of the overhead products in both columns also led to a higher overall cooling demand of 16.9 MJ. kg^{-1}_{ADA} for S1-ADA compared to only 4.0 MJ. kg^{-1}_{ADA} for S2-ccMA-ADA. Similarly, the fossil-based pathway demonstrated the lowest energy consumption per unit of product compared to the biobased production routes across all evaluated categories.

The use of a hot oil system in S1-ADA led to a lower overall steam demand by 28.9% compared to a comparable scenario (GLU-ADA) from Cronjé et al. (2023), as shown in **Table 7**. Furthermore, the use of a more efficient downstream processing setup in S1-ADA proposed by Fruchey et al. (2014) led to reduced overall cooling and heating demands by 46.7% and 25.8%, respectively, compared to those reported by Cronjé et al. (2023). Finally, the indirect biobased scenario from Cronjé et al. (2023), namely ccMA-ADA, reported better overall energy performance than the counterpart scenario in this study. The former assumed a more concentrated fermentation broth (nearly twice as much as S2-ccMA-ADA), which led to lower downstream energy demands, as shown in **Table 7**. Despite differences in simulation methods and results, both this study and Cronjé et al. (2023) confirm that, under current best-reported performances, the indirect biobased route to ADA is more efficient in energy and process water demands than the direct biobased method. Overall, both biobased options for ADA require significant process advancements to compete with the conventional fossil-based method in energy efficiency and ADA output.

3.2. Techno-economic assessment of baseline scenarios

The main economic results for the baseline scenarios are outlined in **Table 8**, whereas the breakdown of key processing areas for TEC and VOC is illustrated in **Figure 2**. S1-ADA recorded an MSP of USD 3219 t^{-1}_{ADA} at a green price premium of 57.8%, compared to USD 5373 t^{-1}_{ADA} for S2-ccMA-ADA at a green premium of 154%. This finding was mainly because of the lower annualized CAPEX and total OPEX for the direct biobased pathway compared to the indirect biobased pathway. The TEC (MUSD 102) for S1-ADA was significantly higher than that of S2-ccMA-ADA (MUSD 58), as shown in **Table 8**. The large overall process volumes from a low fermentation titer (14.9 $g_{ADA}.\ L^{-1}$) in the former scenario resulted in larger equipment sizes and, consequently, higher TECs for SI-ADA (**Table 8**). The WWT and fermentation areas were the most expensive sections for S1-ADA, accounting for 43.0% and 31.8% of the TEC, respectively (**Fig. 2a**). The use of a triple-effect evaporation unit significantly concentrated the fermentation broth and reduced equipment costs in the downstream separation and purification section, which accounted for only 10.3% of the TEC. For the same demand of HPS in the column reboilers, installing a hot oil system instead of a new CHP to supply HPS led to a 41.1% reduction in the TEC (data not shown), with the hot oil contributing just 10.5% to the TEC as shown in **Figure 2a**.

Conversely, the separation and purification section had the largest capital cost, contributing 67.2% to the TEC for S2-ccMA-ADA. This result is due

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Table 7.
Summary of energy and mass flow results for S1-ADA and S2-ccMA-ADA at baseline bioprocess performances compared to the best reported biobased production methods from Cronje et al. (2023), and the current fossil-based method.

Parameter	Biorefinery Scenarios				
	S1-ADA	S2-ccMA-ADA	GLU-ADA*	ccMA-ADA*	Fossil-based Method
A-molasses flow (t. h^{-1})	25.4	25.4	25.4	25.4	-
Production rate (kt. yr^{-1})	38.6	16.6	38.6	30.7	61.3
Product purity (wt%)	99.9	99.9	99.9	99.9	99.9
Overall yield (kg_{ADA}. kg^{-1}_{Feed})[a]	0.30	0.13	0.30	0.24	1.60
Total electricity demand (kWh. kg^{-1}_{ADA})	0.42	0.34	0.95	0.41	1.1E-05
Total heating (MJ. kg^{-1}_{ADA})	29.1	10.0	39.2	4.7	3.1 E-05
Total cooling (MJ. kg^{-1}_{ADA})	16.9	4.0	31.7	11.2	2.7 E-05
Overall steam usage (kg. kg^{-1}_{ADA})	13.5	4.6	19.0	2.5	-
Process water usage (kg. kg^{-1}_{ADA})	6.7	2.5	14.9	3.3	-
Bagasse and lignocelluloses usage[b](kg. kg^{-1}_{ADA})	6.2	-	5.76	-	-
Reference	**This Study**	**This Study**	Cronjé et al. (2023)	Cronjé et al. (2023)	Unlu and Niu (2020)

[a]Overall yield was based on the unit masses of A-molasses for biobased scenarios and cyclohexane for the fossil-based route.
[b]All biogas generated in each scenario was used up prior to bagasse. The sugarcane mill supplied approximately 67.4 $t \cdot h^{-1}$ of bagasse and lignocellulosic material, which could be utilized as fuel for steam generation.
* GLU-ADA and ccMA-ADA are biobased scenarios for ADA production from direct sugars conversion and hydrogenation of bio-produced ccMA respectively, based on a study by Cronje et al (2023).

Table 8.
Overall economic results for S1-ADA and S2-ccMA-ADA at baseline bioprocess performances compared to best reported biobased production methods from Cronje et al. (2023), and the current fossil-based method.

Parameters	Biorefinery Scenarios				
	S1-ADA	S2-ccMA-ADA	GLU-ADA*	ccMA-ADA*	Fossil-based method
TEC (MUSD)	102	58	64	33	20
Total Direct Cost, TDC (MUSD)	149	85	97	61	-
Total Indirect Cost, 0.45 TDC (MUSD)	67	38	43.6	27.6	-
Site development, 9% of ISBL[a] (MUSD)	5	4	5	4	-
Additional piping 4.5 % of ISBL (MUS)	3	2	2	2	-
Fixed Capital Investment (MUSD)	227	129	163	103	73
Working capital, 5% of FCI (MUSD)	11	6	8	5	3
Annualized CAPEX (USD. $t.^{-1}y^{-1}$)	235	311	169	135	59
Variable operating cost, VOC (MUSD. y^{-1})	51	47	67	45	57
OPEX (USD. t^{-1}_{ADA})	1,418	2,993	1833	1571	1726
MSP (USD. t^{-1}_{ADA})[b]	3,219	5,373	3342	2871	-
Green premium (% above fossil-based price)	57.8%	163.4%	63.8%	40.7%	-
References	**This Study**	**This Study**	Cronjé et al. (2023)	Cronjé et al. (2023)	Unlu and Niu (2020)[c]

[a] ISBL, inside the battery limits, comprises TEC from feedstock pretreatment, fermentation, and product separation & purification sections.
[b] MSPs from Cronjé et al. (2023) were updated to the 2022 dollar value.
[c] Economic parameters reported by Unlu and Niu (2020) were inflated to the base year of 2022.
* GLU-ADA and ccMA-ADA are biobased scenarios for ADA production from direct sugars conversion and hydrogenation of bio-produced ccMA respectively, based on a study by Cronje et al (2023) .

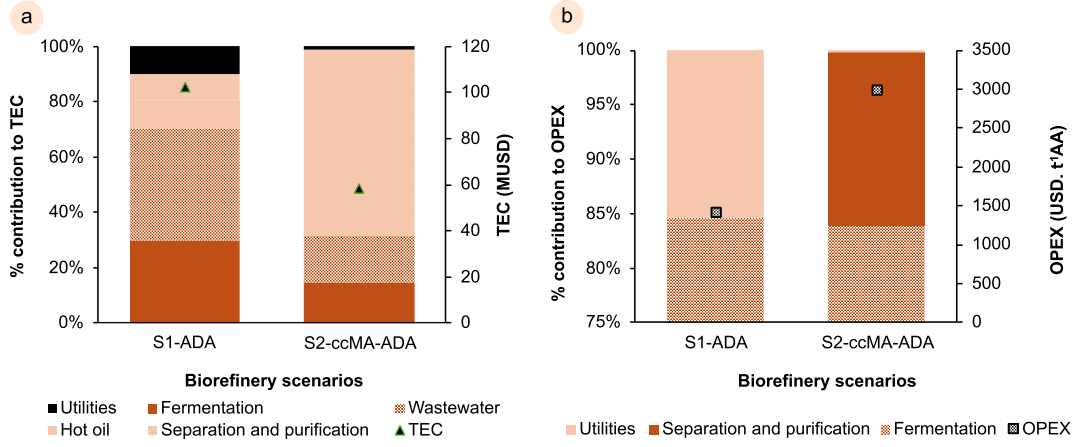

Fig. 2. Breakdown of major processing areas and their contribution to **(a)** CAPEX in terms of TEC and **(b)** OPEX in terms of VOC.

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to the increased number of processing steps, which require two crystallizers, hydrogenation and reacidification reactors, and multiple flash drums for ethanol recovery, all of which are costly units. However, the high product concentration in the fermentation broth for S2-ccMA-ADA significantly reduced the cost of the fermentation and WWT areas of the biorefinery, which accounted for only 14.7% and 17.0 % of the TEC, respectively (**Fig. 2a**). In both scenarios, raw material costs—specifically feedstock and invertase enzyme costs—were the largest contributors to the VOC, accounting for 84.7% in S1-ADA and 82.0% in S2-ccMA-ADA (**Fig. 2b**). The remaining operating costs for the former were primarily due to bagasse usage, while those for the latter were mainly attributed to sulfuric acid, ethanol, and hydrogen usage during ccMA purification and subsequent conversion to ADA (**Fig. 2b**). Similarly, raw material costs account for approximately 92.6% of the VOC for the current fossil-based process due to the excessive use of nitric acid, expensive catalysts like vanadium, and cyclohexane feedstock (**Table 8**).

In contrast, Cronjé et al. (2023) reported a lower MSP for ccMA-ADA (USD 2538 t^{-1}_{ADA}) compared to GLU-ADA (USD 2932 t^{-1}_{ADA}), as shown in **Table 8**. In the study, the indirect biobased route was designed to achieve significantly higher yields and titers of ccMA from sugars compared to the direct production method, leading to increased ADA output and reduced energy costs. Consequently, the indirect ccMA-ADA route exhibited lower capital and operating costs, resulting in a reduced MSP compared to the GLU-ADA pathway (**Tables 7** and **8**). The improved energy efficiency from the use of the hot oil and 2-stage distillation setup in the current study is reflected in a 3.8% MSP reduction in S1-ADA compared to GLU-ADA (**Table 8**). An economic assessment of multiple pathways to biobased ADA by Gunukula and Anex (2017) equally favored the direct biological pathway to the use of biobased intermediates such as GA or 6-hydroxyhexanoic acid and 1, 6-hexanediol reporting a best MSP value of USD 1360. t^{-1}_{ADA} for an ADA plant capacity of 80 kt. y^{-1} at an IRR of 10%. However, the assumptions used in this study were not based on actual experimental data.

Overall, the MSP values for both biobased fermentation pathways of sugars to ADA were significantly higher than the current fossil-based price, with green premiums of 57.8% and 163.4%, respectively (**Table 8**). The current industrial method has a much lower TEC of only MUSD 20 and a much lower OPEX (USD 1726 t^{-1}_{ADA}) due to superior economies of scale benefits compared to the biobased options (**Table 8**). Hence, biotechnological improvements, particularly those that can lead to improved product yields and titers at fermentation, may improve the economic viability of these two biobased routes. In addition, the development of microbes capable of producing ADA and ccMA directly from sucrose would eliminate the cost associated with sucrose hydrolysis and subsequently reduce the VOC by 10.9% for S1-ADA and 11.8% for S2-ccMA-ADA (data not shown). This improvement has been achieved for recombinant *E. coli* strains through both genetic manipulation and adaptive evolution of non-sucrose-utilizing strains (Sabri et al., 2013; Mohamed et al., 2019).

The robustness of the product MSP was assessed against a ±30% variation in the base value of key economic and technical parameters, as shown in **Figure 3**. In both scenarios, the MSP showed the greatest sensitivity to the total production hours, with a 42.8% reduction in MSP for a 30% increase in operating hours (6500 h). Increasing operating hours led to a higher production rate of ADA, resulting in significant economies of scale, particularly during seasons of above-average sugarcane output. Similarly, developing high-yielding microbes capable of efficiently converting sugars to ADA or ccMA would have a comparable impact, as the improved product output would enhance overall process efficiency. Additionally, a 30% reduction in feedstock costs had a more pronounced effect on reducing the MSP of S2-ccMA-ADA compared to S1-ADA (8.9% vs. 6.4%). This difference arises from the higher contribution of A-molasses costs to the variable operating costs (VOC) of ADA production in the S2-ccMA-ADA pathway.

Similarly, a 30% price reduction in total utilities (dominated by bagasse usage) reduced the MSP of S1-ADA by 1.9% compared to <0.5% for S2-ccMA-ADA due to the high bagasse consumption rate in S1-ADA (**Fig. 3**). Another key player in the process economies was the price of enzymes assumed for sucrose hydrolysis, which lowered the MSPs of S1-ADA and S2-ccMA-ADA by 1.4% and 1.9%, respectively. This could be achieved by engineering organisms capable of efficiently converting sucrose directly to ADA and ccMA and/or applying them in consortium with strains that

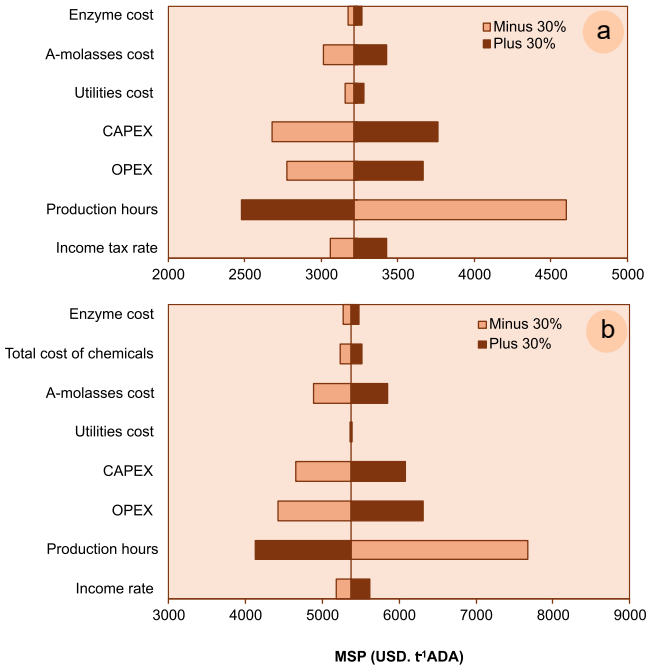

Fig. 3. Economic sensitivity analysis of the effect of a ±30% variation in key techno-economic parameters of MSP for baseline scenarios of **(a)** S1-ADA and **(b)** S2-ccMA-ADA.

naturally thrive on simple sugars (Pothakos et al., 2018). Lastly, a ±30% change in the CAPEX varied the MSP of S1-ADA by 16.8% and S2-ccMA-ADA by 13.3%, as shown in **Figure 3**. This could happen due to fluctuations in inflation rates, which have a significant bearing on the cost of key process equipment. A proactive measure would be to achieve high product titers in fermentation broths, as this is known to reduce downstream separation and purification costs (Pothakos et al., 2018; Sikazwe et al., 2024). The sensitivity analysis underscores the critical importance of improving product yield to optimize the productivity and cost-effectiveness of biobased pathways for ADA production. Achieving higher yields can be realized through advancements in microbial strain development and bioprocess efficiencies, which are pivotal for enhancing the overall performance of ADA production systems.

3.3. Environmental assessment results of baseline scenarios

Figure 4 illustrates the breakdown of key processing areas and their contributions to overall GHG emissions. S2-ccMA-ADA recorded the highest total GHG emissions of 6.50 kg_{CO2eq}. kg^{-1}_{ADA}, which is three times higher than the emissions for S1-ADA. This disparity is primarily attributed to the significant emissions associated with the extensive use of sodium hydroxide, sulfuric acid, and hydrogen in the conversion of ccMA to ADA in the S2-ccMA-ADA pathway. The main source of emissions in the production of ADA *via* S1-ADA was the use of diammonium phosphate as a nitrogen source for microbial growth, as assumed in both scenarios. Emissions associated with sugarcane cultivation contributed 58.9% and 58.7% to the total GHG emissions for S1-ADA and S2-ccMA-ADA, respectively, representing the highest contribution in both scenarios (**Fig. 4**). This was because of the usage of triazine and urea-based fertilizers, which have high CO_2 equivalents of 7.98 kg_{CO2eq}. kg^{-1} and 3.31 kg_{CO2eq}. kg^{-1} respectively. The total emissions from the sugarcane mill per unit mass of sugarcane processed were the same for both scenarios and are broken down in detail in our previous study (Sikazwe et al., 2024). Whereas the emissions for S1-ADA are comparable to those reported for the direct biobased route by Cronjé et al. (2023), the emissions for the indirect biobased scenarios differ by a 3-fold margin. This is mainly due to the higher production rate of ADA in ccMA-GLU at a yield of 0.30 g_{ccMA} $g^{-1}_{glucose}$ compared to 0.17

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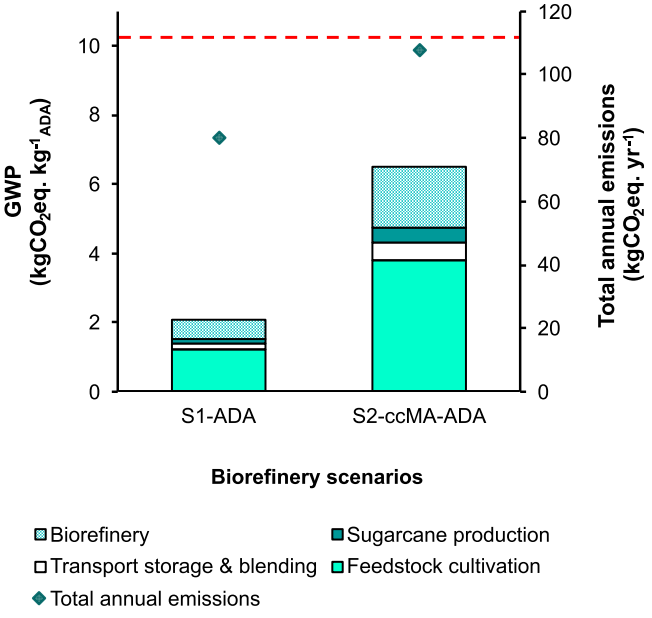

Fig. 4. GHG emission contributions associated with key processing areas of the ADA biorefineries. The red broken line represents the reported GWP for the ADA industrial fossil-based process in kg_{CO2eq}. kg^{-1} (Flederbach et al., 2019).

g_{ccMA} $g^{-1}_{glucose}$ assumed for S2-ccMA-ADA in our study, which led to reduced GHG emissions per unit mass of product.

Interestingly, the GHG emissions associated with ADA production *via* biobased methods were still significantly lower than those reported for fossil-derived ADA. Specifically, S1-ADA achieved a 77.1% reduction in emissions, while S2-ccMA-ADA recorded a 27.8% reduction. These findings highlight the potential of biobased pathways to contribute to a circular economy, making them worthwhile avenues for further development and optimization (Flederbach et al., 2019; Cronjé et al., 2023). ADA is currently manufactured using a two-stage chemical treatment of cyclohexane, emitting one mole of nitrous oxide per mole of ADA produced. Nitrous oxide abatement technologies exist and have been applied successfully in ammonia oxidation reactors in the nitric acid industry (Anderson et al., 2020). However, these technologies require drastic pressure adjustments, which reactors in the ADA process cannot tolerate. Thus, the most viable option for controlling nitrous oxide emissions in ADA production is tertiary abatement, which involves the installation of scrubbers downstream of the ketone alcohol process. However, despite many ADA industries being equipped with flue gas scrubbers, the sector still accounts for over 10 million tonnes of CO_2-equivalent emissions annually. This underscores the urgent need for more effective and comprehensive pollution control measures to mitigate the industry's environmental impact (Anderson et al., 2020; He et al., 2024). The foremost benefit of biobased ADA manufacturing would be the elimination of nitrous oxide emissions from the ADA value chain, which is equivalent to primary pollution abatement. Although the indirect biobased method involves the use of fossil-derived chemicals such as ethanol, sulfuric acid, and hydrogen, these have a much lower global warming potential (GWP) than nitrous oxide. Additionally, over 90% of the ethanol is recyclable within the process, reducing its usage and subsequent environmental impact.

3.4. Impact of strain and bioprocess optimization on energy and mass flows

The impact of improved titer and yield on the mass and energy results for both the direct and indirect ADA scenarios is shown in **Figure 5**. As with our previous studies, the volumetric productivity was excluded from the single-factor analysis due to its negligible influence on overall heating, cooling, and product output flows compared to the other two metrics (Sikazwe et al., 2024). The single-factor analysis combined effect estimates and regression coefficient results from STATISTICA to predict the impact of each fermentation parameter individually while keeping the other two constant. Results showed that the total cooling and heating demands were more sensitive to improvements in titer than yield and volumetric productivity for both scenarios. In S1-ADA, a 10% improvement in the titer of ADA reduced the cooling demand by 5.3%, which was 26 times greater than the reduction achieved by a 10% improvement in ADA yield (**Fig. 5a**). This outcome was because improving the titer resulted in reduced water-laden process streams, leading to a substantial decrease in reflux cooling duties, which constitute the largest portion of the overall cooling demand. S2-ccMA-ADA showed a similar pattern, with a 10% increase in ccMA titer reducing the cooling demand by 3.9%, compared to just 1.0% for a 10% increase in ccMA yield (**Fig. 5b**). The indirect biobased scenario similarly benefited from reduced water content in the process streams at higher ccMA titers, resulting in lower overall cooling requirements. At higher process concentrations, heat transfer equipment generally requires less energy input. As a result, a 10% improvement in product titers led to an 8.2% reduction in total heating duty for S1-ADA and a 2.3% reduction for S2-ccMA-ADA, as illustrated in **Figures 5c–d**.

S1-ADA involved two highly endothermic processes: microbial ADA production and subsequent neutralization with sodium hydroxide. The heat demand from these processes increases as ADA output rises at higher yields. This was illustrated by a region of inflection, where improving ADA yield resulted in higher overall heating demands, particularly at titers above 120 g_{ADA}. L^{-1}. Furthermore, the overall impact of titer on total heating demands diminishes significantly in the inflexion region, as shown in **Figure 5c**. As a result, in S1-ADA, a 10% improvement in ADA yield resulted in a 0.48% increase in the total heating demand within the region of inflection. (**Fig. 5c**). Finally, the nearly vertical response contours across the entire product yield range show that product throughput is more sensitive to improvements in yield than to improvements in titer (**Figs. 5e–f**). Thus, bioprocess enhancements leading to a 10% increase in yield will improve the total ADA output by 8.6% for S1-ADA and 8.9% for S2-ccMA-ADA. The slight product output advantage from improving the titer by 10% was traced from the reduced level of product entrainment in effluent streams during separation and purification, which only improved the ADA output by 0.1% in both scenarios. Finally, yield improvements result in higher ADA outputs through both biobased routes, leading to reduced overall energy demands per unit mass of ADA. Scientific advancements aimed at increasing final process titers beyond 14.9 g ADA·L^{-1} for the direct biobased route and 36.8 g ccMA·L^{-1} for the indirect biobased route should be prioritized. Achieving higher titers is critical for enhancing energy efficiency in biobased ADA production by reducing water-laden process streams and minimizing energy-intensive downstream processing. In a typical sugarcane mill, biobased ADA production *via* the two baseline scenarios would be energy self-sufficient at the stated baseline process performances.

3.5. Economic merits of strain and bioprocess optimization

In this analysis, the fossil-based price of ADA served as a benchmark for assessing the economic viability of the biobased options, represented by the orange dashed contour in **Figure 6c**. Here, estimates of statistical variance were also based on the impact of a single variable while keeping the other fermentation parameters fixed. For S1-ADA, a 10% improvement in titer reduced annualized CAPEX by 2.0% and OPEX by 0.7%. In contrast, a 10% increase in yield led to reductions of 5.9% in OPEX and 1.8% in annualized CAPEX (**Figs. 6a–b**). This was attributed to a reduction in processing equipment costs at higher titers and economies of scale at higher ADA yields, which lowered the CAPEX and OPEX, respectively. Consequently, a 10% increase in yield lowered the minimum selling price (MSP) of ADA by 13.2%, making it 27 times more effective than a 10% improvement in titer alone for S1-ADA (**Fig. 6c**).

The FFD predicted that the direct biobased method requires a baseline bioprocess performance of 80 g_{ADA}. L^{-1} (titre) and 0.38 g_{AA}. g^{-1}_{sugar} (yield) at a volumetric productivity of 1.3 g_{ADA}. $L^{-1}.h^{-1}$ to achieve an MSP equivalent to the current fossil-based price of ADA. Meanwhile, the current best-reported performance for direct sugar conversion to ADA stands at 14.9 g_{ADA}. L^{-1}, 0.38 g_{AA}. g^{-1}_{sugar} and 0.2 g_{ADA} (Zhou et al., 2020). Overall, the analysis demonstrated that improving microbe and bioprocess performance alone could reduce the MSP of the direct fermentation route to

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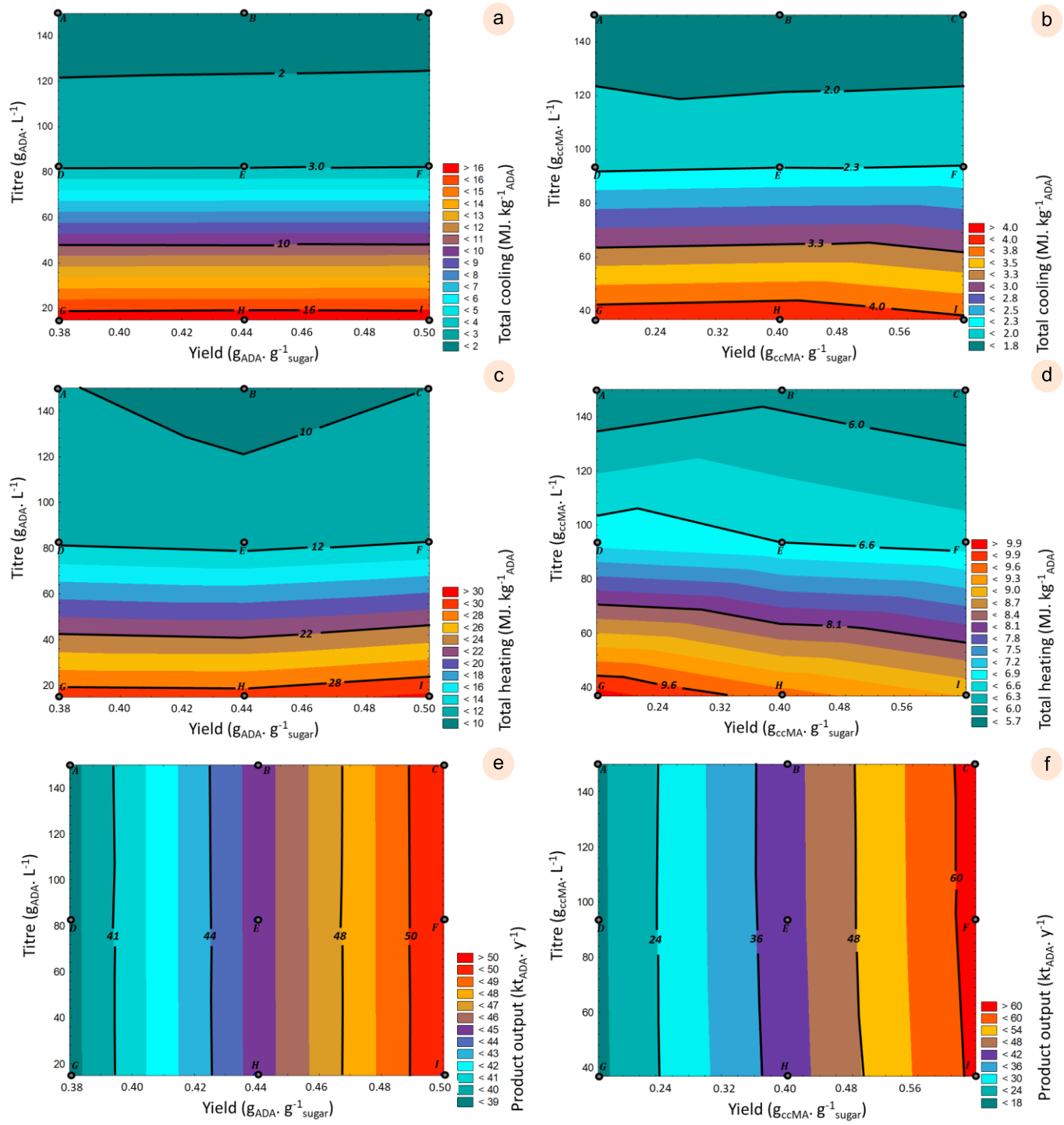

Fig. 5. Effect of bioprocess performance on cooling demand in MJ. kg^{-1}_{ADA}, heating demand in MJ. kg^{-1}_{ADA} and ADA throughput in kt. $y^{-1}$for S1-ADA: **(a, b, c)** and S2-ccMA-ADA and **(d, e, f)**). Points A-F represent the limits of the FFD study.

USD 1384 t^{-1}_{ADA}, which is 33.3% below the ADA fossil-based price (Point C in **Fig. 6c**). This justifies investment in scientific efforts aimed at engineering efficient cell factories capable of accumulating ADA at higher yields and titers.

For S2-ccMA-ADA, a 10% increase in yield reduced OPEX by 6.0%, annualized CAPEX by 3.3%, and subsequently lowered the MSP by 1.6% (**Figs. 6d–f**). Here, the benefits of economies of scale outweighed those of ccMA titer improvements, as evidenced by the much smaller reductions of 0.2% in OPEX, 0.3% in annualized CAPEX, and 0.1% in MSP for a 10% increase in ccMA titer. Conversely, the nearly vertical response contours in the direction of increasing yield indicate that improving ccMA yield has a

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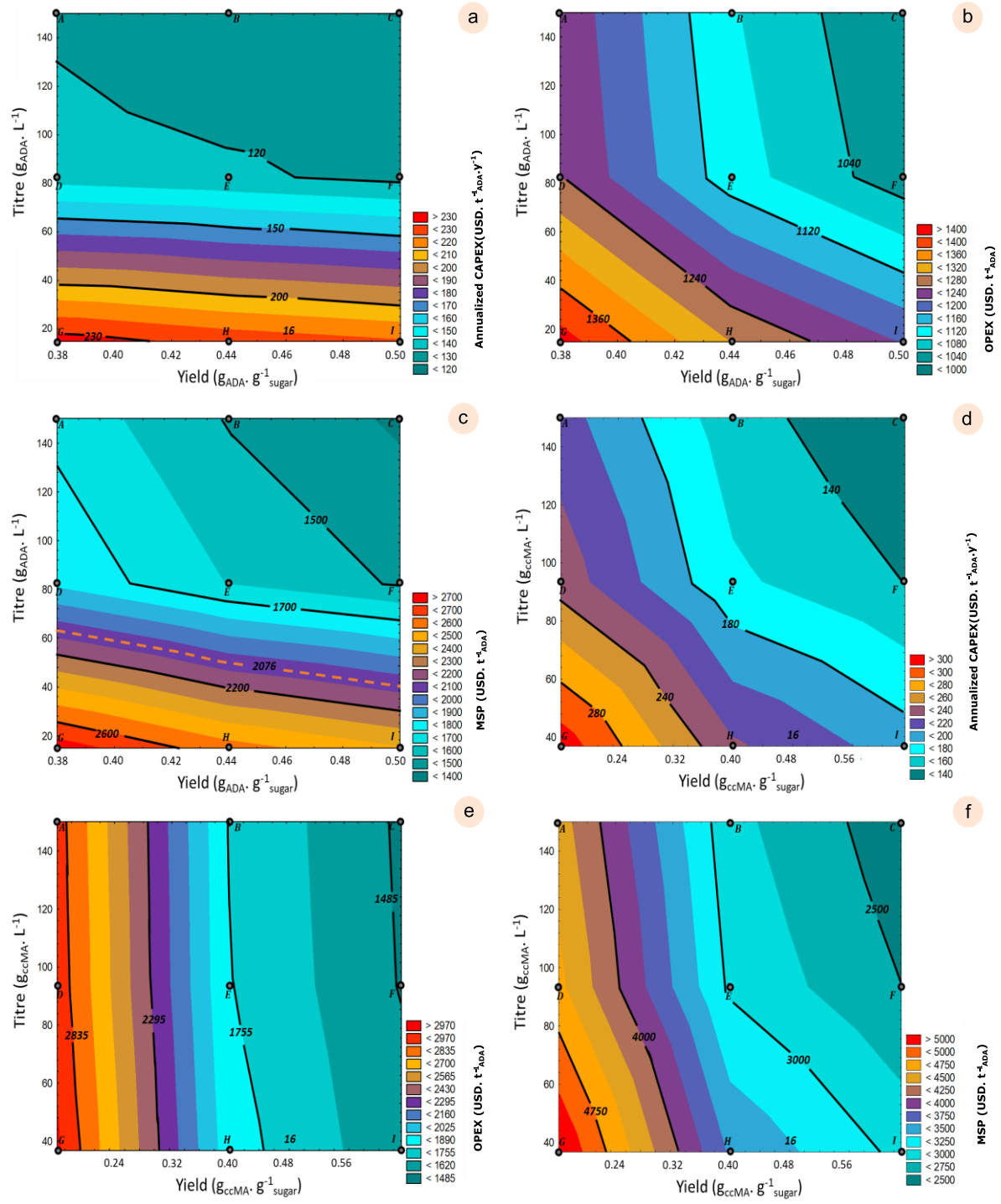

Fig. 6. Impact of bioprocess performance upgrading on economic metrics. Annualized CAPEX in USD. t^{-1}_{ADA}: (**a** and **d**), OPEX in MSP in USD. t^{-1}_{ADA}, (**b** and **e**) and MSP in USD. t^{-1}_{ADA}, and (**c** and **f**) for [1] for S1-ADA and S2-ccMA-ADArespectively. The yellow dashed contours represent the fossil-based market price of ADA, and points A-F represent the limits of the FFD study.

greater overall impact on OPEX than enhancing titer (Fig. 6e). This is because the cost of chemicals such as sulfuric acid, ethanol, and hydrogen—whose usage increases with ADA production at higher yield values—represents the largest contributor to OPEX for S2-ccMA-ADA. This offsets the economic merits obtainable from enhancing product yield for S2-ccMA-ADA. Therefore, the FFD indicated that operating the indirect biobased route at optimal bioprocess performance for ccMA production would not be enough to reduce the ADA MSP below the fossil-based price. (Fig. 6e). The lowest potential MSP S2-ccMA-ADA within the theoretical limits assumed in this study is USD 2348 t^{-1}_{ADA}, equivalent to a green price premium of 13.4% (Point C in Fig. 6f). These figures could worsen due to potential metabolic limitations in the shikimate pathway for ccMA, which could

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nearly halve the theoretical yield maximum used in this analysis (*Section 2.4*). Interestingly, if the baseline S1-ADA operated at a titer of just 48 g ADA·L^{-1}, it would achieve an economic performance comparable to the best potential scenario for S2-ccMA-ADA. This finding highlights the higher feasibility potential of S1-ADA, underscoring its promise as a more economically viable pathway with further optimization.

A similar sensitivity assessment of the effect of titer and volumetric productivity improvements on the MSP of ADA produced from unrefined corn from glucose showed that titer had a stronger influence (Gunukula and Anex, 2017). However, the observed economic advantage leveled off and became noticeably disproportionate to the individual upgrades in titer or volumetric productivity. This implies that optimizing process units along the value chain, beyond just the bioreactor, is essential for the viable production of ADA *via* the indirect biobased pathways (Gunukula and Anex, 2017). However, the previous study did not account for the effects of concurrent improvements to titer, yield, and productivity, which this study has shown to have a more significant overall economic impact when considered together, as illustrated in **Figure 6**. Thus, efforts to improve the economic prospects of the indirect biobased route should focus on reducing the number of processing stages and minimizing the use of extraction reagents during ccMA upgrading. Otherwise, any efforts to optimize the efficiency of the microbial sugars-ccMA stage will not yield tangible economic returns in the context of large-scale production. Finally, the results indicated that the bioprocess would need to be enhanced to a greater extent for S2-ccMA-ADA than for S1-ADA to achieve comparable economic viability. Therefore, research and development efforts should prioritize the direct biobased fermentation route.

3.6. Environmental merits of strain and bioprocess optimization

The environmental benefits in terms of GHG emissions reduction from improvements in microbial and bioprocess performance are shown in **Figure 7**. The single-factor analysis indicated that a 10% improvement in titer reduced GHG emissions by 12.7%, compared to just 5.2% from a 10% increase in yield alone in S1-ADA (**Fig. 7a**). Emissions from the use of chemicals for cell nutrition dominated the overall GHG emissions for the biorefinery but significantly decreased at high ADA titers, as these chemicals were dosed per unit fermenter. Similarly, the use of diammonium phosphate as a nitrogen source increased with biomass formation at higher yields, offsetting the overall environmental benefits. This effect is evident in the region of inflexion across the entire fermentation space for S1-ADA (**Fig. 7**). Nonetheless, upgrading the bioprocess would still lead to GHG emissions 14.3% below those recorded for the baseline scenario. This indicates that optimizing the economic returns for S1-ADA (**Fig. 6c**) would offset the potential best GHG emissions by about 2.1%; however, emissions would remain 78.7% lower than those of the fossil-based option (**Fig. 7b**).

For the indirect biobased route, a 10% improvement in the yield of ccMA reduced GHG emissions by 7.2%, which was 85 times greater than the reduction achieved from a 10% increase in titer alone (**Fig.7b**). Similarly, emissions from the use of ethanol, sulfuric acid, and hydrogen for downstream ccMA conversion increased with ccMA output at higher yields but not significantly enough to offset the potential environmental benefits. Hence, no region of inflexion was observed for S2-ccMA-ADA, as shown in **Figure 7b**. The results predicted a possible reduction in GHG emissions of up to 56.2% below the baseline value and 68.4% below the fossil-based numbers for S2-ccMA-ADA (Point C on **Fig. 7b**). Thus, both biobased routes to ADA demonstrated the potential for substantially lower GHG emissions compared to the fossil-based route achievable at improved microbe and bioprocess performances. However, until biobased pathways are sufficiently developed for large-scale application in an economic sense, their environmental benefits may never be harnessed. Consequently, it is crucial to develop microbes that can efficiently convert sugars into ccMA and ADA while also enhancing current bioprocesses to fully capitalize on the advantages of a circular bioeconomy, particularly for biobased ADA.

3.7. Challenges, opportunities, and future perspectives

The production of ADA in an integrated biorefinery exemplifies a circular economy model that preserves finite resources by enhancing the value of bagasse and A-molasses, two byproducts of sugarcane mills (Fazzino et al., 2023). Both the direct and indirect biobased routes showed

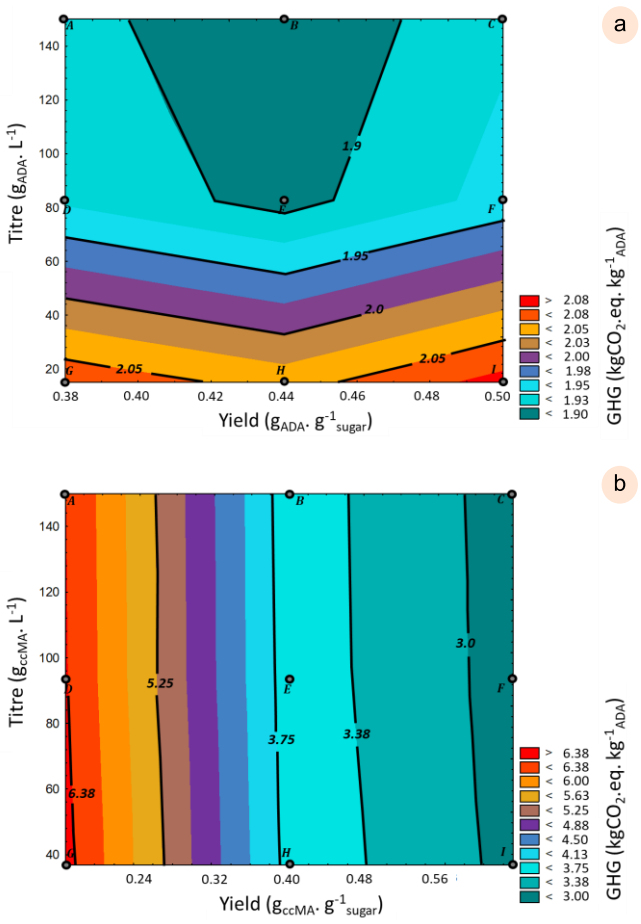

Fig. 7. Effect of bioprocess parameters upgrading on total GHG emissions in $kgCO_{2\text{-eq}}$. kg^{-1}_{AA}: **(a)** S1-ADA and **(b)** S2-ccMA-ADA. Points A-F represents the limits of the FFD study.

significantly lower GHG emissions with zero N_2O emissions compared to fossil production methods. Secondly, the integrated biorefinery assumed in this study entails the reconfiguration of a typical sugarcane mill to a single-stage crystallization process. This would improve the overall energy and exergy efficiency of the process because the crystallization stage of a sugarcane mill has a high exergetic potential (Dogbe et al., 2020). Additionally, the system's energy self-sufficiency can be improved by generating extra electricity and steam for subsequent biorefinery usage. However, retrofitting sugarcane mills with biorefineries involves substantial initial investment costs and high operating expenses, largely due to the often narrow price margin between the feedstock and the final product (Patel and Shah, 2021). Secondly, attaining energy self-sufficiency in such a facility requires transporting surplus utilities from the sugarcane mill to the biorefinery over significant distances. This would result in considerable energy losses during transport, requiring the installation of specialized equipment, which in turn adds unnecessary overhead costs.

Bio-produced ADA would also have to compete with fossil-based ADA produced using established technologies. Therefore, policymakers play a crucial role in promoting the commercial viability of biobased ADA by enforcing cleaner production regulations, subsidizing bio-based commodity chemicals, and implementing carbon pricing initiatives (Cronjé et al., 2023). Lastly, policy makers have the vital role of helping to facilitate stranger collaborations between researchers and industry on projects involving circular economies. This could be accomplished by removing organizational barriers that hinder the sharing of expertise and raising awareness of the high viability potential of bio-produced chemicals and ADA in particular (Ahmed et al., 2022). The process of scaling up a biobased ADA from laboratory to industrial scale is currently hindered by biomass availability,

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bioreactor efficiency, and downstream processing costs (Skoog et al., 2018; Cronjé et al., 2023). The competition for 1G feedstocks, such as A-molasses, with the food industry could pose challenges to commercial-scale production of biobased ADA. This necessitates exploring alternative feedstocks, such as agro-industrial waste, municipal waste, and industrial food waste (Rinke Dias de Souza et al., 2024). However, these feedstocks typically require specialized pretreatment methods, which are often energy-intensive and rely on costly catalysts, adding complexity and expense to the production process.

Enhancing bioprocess efficiency through strain engineering can significantly improve the conversion of sugars to ADA and ccMA. This would not only optimize the utilization of biomass resources but also reduce energy requirements, operating costs, and capital investments for prospective industrial-scale projects (Jarunglumlert et al., 2022). However, strain engineering is a resource-intensive and time-consuming process, which should be prioritized for scenarios with the most promising returns. Recent advancements in biotechnology have successfully scaled up the production of valuable biochemicals such as 1,3-butanediol, artemisinin, and farnesene, highlighting the potential for enhancing the commercial viability of biobased ADA. Adaptive Laboratory Evolution (ALE) has emerged as a powerful tool for developing robust industrial strains. It has been applied to expand substrate concentration ranges and improve tolerance to inhibitory process conditions in microbes like Saccharomyces cerevisiae. The successful application of ALE to ADA- and ccMA-producing strains could enable higher product titers in the final broth (Long and Antoniewicz, 2018). In addition, process modifications, such as fed-batch cultures and extractive fermentation, are known to enhance product recoveries by increasing yield and titer while minimizing substrate and product-level inhibition, respectively (Jantama et al., 2015; Badhwar et al., 2019). These approaches can further contribute to the scalability and economic feasibility of biobased ADA production.

The final yields of ADA and ccMA could be enhanced through the use of targeted genome editing technologies, which have worked notably for 2,3-BDO production in *Klebsiella oxytoca*. In this case, deletion of pyruvate kinase and redirection of flux toward shikimate production is crucial to boosting the overall yield of ccMA from sugars. Similarly, eliminating the production of acetic acid and funneling flux towards ADA could improve the yield of ADA. One way of achieving this is *via* the application of the widely emerging Clustered, regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated (Cas) systems. CRISPR-Cas systems offer a robust and highly specific toolbox for finetuning multiple metabolic fluxes, leading to efficient substrate consumption and maximal target chemical output. These advancements are currently being used to accelerate the development of high-performing cell factories for 2,3-BDO, lactic acid, itaconic acid, and 3-HP with significant success, and they could potentially be applied to ADA and ccMA in the future (Shi et al., 2022). **Table 6** shows an overall comparison between the direct and indirect biobased pathways to ADA based on the findings of this study.

4. Conclusions

A comprehensive techno-economic assessment of different biorefinery scenarios for ADA production revealed that both direct and indirect biobased pathways are currently less economically viable than the fossil-based method. However, the biobased options showed significantly lower overall GHG emissions with zero net nitrous oxide emissions compared to the petrochemical methods. The FF analysis, on the other hand, predicted that process developments leading to enhanced process titers and yields of ADA in the fermentation broth could translate to tangible economic and environmental benefits for the biobased options. The direct biobased route, for instance, showed the capacity to attain an MSP 33.3% below the fossil-based price with lesser overall GHG emissions by 78.7%. The indirect biobased route, however, could not outperform the fossil-based method from the standpoint of economic feasibility, even at optimum bioprocess efficiency. Instead, it recorded a green premium of 13.4% as the best possible MSP achievement across the entire fermentation space investigated. This was because the economic benefits of an optimized fermentation stage were offset by the operating costs from the high usage of chemicals during ccMA upgrading to ADA. The CO_2 equivalents from the usage of hydrogen, ethanol, and sulfuric acid in the indirect biobased method equally offset the environmental benefits of bioprocess optimization. However, the same pathway recorded a 68.4% potential reduction in the overall GHG emissions compared to the fossil-based option.

Future works should focus on optimizing current methods or developing novel techniques of ccMA separation from aqueous media and subsequent conversion to ADA. This would improve the chances of achieving an economically competitive indirect biobased pathway to ccMA. Policy makers and stakeholders should support biobased initiatives through increased funding towards research dealing with microbial conversion of biomass to ADA and biorefinery integration projects. Legislation and incentives (subsidies and green premiums) supporting green production must also be formulated and implemented to stimulate biobased facilities at various production scales and, subsequently, full-fledged commercial applications. Finally, policy makers should help reduce the organizational complexities than hinder meaningful collaboration between researchers and industry in the bid to achieve a circular economy.

Overall, the direct biobased route to ADA was preferred due to the lower overall energy requirements, initial investment, operating costs, and global warming potential compared to the indirect biobased route. Secondly, the same option showed the most significant upgrades in key viability indicators, from microbe and bioprocess optimization, compared to the indirect biobased option. Most importantly, process optimization within theoretical limits could upgrade the direct biobased method to match industrial scale metrics. This is critical to identifying which pathway should be prioritized for strain and bioprocess development, which are often laborious and capital-intensive. The consideration of biobased ADA production for commercial-scale applications hinges heavily on developing an efficient host capable of accumulating 80 g_{ADA}. L^{-1} (titre) and 0.38 g_{AA}. $g^{-1}{}_{sugar}$ (yield) at a volumetric productivity of 1.3 g_{ADA}. $L^{-1}.h^{-1}$ as a baseline performance *via* the RADP. The indirect biobased route, however, requires the capital and operating costs associated with ccMA upgrading to ADA to be reduced substantially to record any meaningful outcomes enhancements of bioprocess performances along the shikimate pathway for muconic acid production. Ultimately, biotechnological efforts for enhanced microbes and bioprocess efficiencies can improve the prospects of direct biobased ADA production to compete with and surpass the current fossil-based production method.

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Supplementary Material

Table S1.
Equations for estimating installation cost of major process equipment*.

$IC = purchased\ cost \times IF$	Eq. S1
$EC_{new\ cost} = (base\ cost) \times \left(\frac{New\ size}{Base\ size}\right)^{n}$	Eq. S2
$EC_{analysis\ year} = (base\ cost) \times \frac{CEPCI_{analysis\ year}}{CEPCI_{base\ year}}$	Eq. S3
$FCI = TDC + TIC$	Eq. S4
$TCI = FCI + WC$	Eq. S5
$WC = 5\% \times FCI$	Eq. S6

*Source: Davis et al. (2018); Humbird et al. (2011).
IC, installed cost; EC, equipment cost; IF, installation factor; n, scaling exponent; TDC, total direct cost; FCI, fixed capital investment; WC, working capital; TIC, total indirect cost.

Table S2.
Scaling exponents for key processing equipment*.

Equipment	Exponent (n)
Agitators	0.5
Compressors, motor-driven	0.6
Distillation columns	0.6
Heat exchangers	0.7
Inline mixers	0.5
Package quotes / skidded equipment	0.6
Pressure vessels	0.7
Pumps	0.8
Tanks, atmospheric	0.7
Solids handling equipment	0.8

*Source: Davis et al. (2018).

Table S3.
Indirect costs as a fraction of the total direct cost (TDC).

Direct costs	Description
Portable costs	10% of TDC
Field expenses	10% of TDC
Office and construction	20% of TDC
Contingency	10% of TDC
Other costs (start-up and permits)	10% of TDC

Table S4.
Cost of chemicals used in the calculation of variable operating expenditure*.

Material	Base Price	Reference
Magnesium Chloride	1.79	Özdemir et al. (2009)
Magnesium sulphate	0.210	Alibaba, (2024)
Sodium chloride	0.045	Autelitano et al. (2019)
Potassium chloride	0.475	Statista, (2023)
Ammonium hydroxide	0.225	INTRATEC (2023)
Dipotassium phosphate	1.261	ECHEMI (2023)
Disodium phosphate	0.444	ECHEMI (2024)
NaOH	0.749	Made-In-China (2024)
Ethanol	0.773	U.S. Grains Council (2024)
Sulphuric acid	0.240	Made-In-China (2024)
Hydrogen	2.0	BloombergNEF (2024)

*All costs were adjusted to the 2022 dollar value.

Table S5.
Feedstock price estimation.

	Flowrate (t. h^{-1})	Price (USD. t^{-1})	Revenue lost (USD. h^{-1})
C-molasses lost to A-molasses	11.57	145.7*	1 685.75
Sugar lost to A-molasses	10.71	303.0*	3 245.13
Total cost of diverting A-molasses	-	-	4930.88
A-molasses	25.433	193.9#	

* OECD/FAO (2019)
# A-molasses price 1 = [(11.57 ×145.70) + (10.71×303.00)]/ 25.43 = USD 193.9 t-1)
A-molasses price 2 = A-molasses price 1 × inflation factor (2019-2022).

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Table S6.
Summary of economic results for FFD scenarios for S1-ADA.

Scenario ID	TCI (USD)	FCI (USD)	TVOC (USD. h^{-1})	TPC (USD. t^{-1}_{ADA})	MSP (USD. t^{-1}_{ADA})
Case 1	226.69	215.90	50.91	1418.12	3219.47
Case 2	175.95	167.57	50.91	1391.31	2787.42
Case 3	172.33	164.12	50.91	1389.41	2756.60
Case 4	131.00	124.77	44.91	1240.60	2273.76
Case 5	75.97	72.35	44.91	1211.52	1807.78
Case 6	72.25	68.81	44.91	1209.58	1776.30
Case 7	123.31	117.44	44.49	1229.18	2201.04
Case 8	64.00	60.96	44.49	1197.83	1699.77
Case 9	60.19	57.32	44.49	1195.84	1667.94
Case 10	252.13	240.12	52.56	1278.30	3022.12
Case 11	191.69	182.56	52.56	1250.53	2574.77
Case 12	187.80	178.86	52.56	1248.76	2546.03
Case 13	146.45	139.48	45.53	1098.55	2103.84
Case 14	81.48	77.60	45.53	1068.70	1625.66
Case 15	77.49	73.80	45.53	1066.88	1596.30
Case 16	135.19	128.75	44.97	1084.00	2011.23
Case 17	68.56	65.29	44.97	1053.40	1521.15
Case 18	64.46	61.39	44.97	1051.54	1491.45
Case 19	276.65	263.48	55.28	1196.78	2898.81
Case 20	206.62	196.78	55.28	1168.18	2438.48
Case 21	202.49	192.85	55.28	1166.52	2411.39
Case 22	159.16	151.58	47.30	1015.32	1987.73
Case 23	87.17	83.02	47.30	985.95	1517.06
Case 24	82.93	78.98	47.30	984.24	1489.36
Case 25	149.79	142.66	46.55	999.59	1914.02
Case 26	72.95	69.48	46.55	968.23	1411.78
Case 27	68.60	65.33	46.55	966.47	1383.68

Table S7.
Summary of economic results for FFD scenarios for S21-ccMA-ADA.

Scenario ID	TCI (USD)	FCI (USD)	TVOC (USD. h^{-1})	TPC (USD. t^{-1}_{ADA})	MSP (USD. t^{-1}_{ADA})
Case 1	129.45	123.29	46.70	2993.22	5372.93
Case 2	124.83	118.89	46.70	2987.62	5282.11
Case 3	123.14	117.28	46.70	2985.56	5248.76
Case 4	97.44	92.80	46.75	2925.80	4690.53
Case 5	92.83	88.41	46.75	2920.27	4601.01
Case 6	91.14	86.80	46.75	2918.24	4568.14
Case 7	88.21	84.01	46.59	2900.41	4492.55
Case 8	83.60	79.62	46.59	2894.89	4403.31
Case 9	81.90	78.00	46.59	2892.87	4370.54
Case 10	218.62	208.21	66.46	1813.47	3546.78
Case 11	211.53	201.46	66.46	1809.82	3487.32
Case 12	208.93	198.98	66.46	1808.48	3465.48
Case 13	163.03	155.27	66.21	1761.59	3040.22
Case 14	155.95	148.52	66.21	1757.99	2981.92
Case 15	153.34	146.04	66.21	1756.67	2960.51
Case 16	147.69	140.65	66.20	1748.95	2905.24
Case 17	140.60	133.91	66.20	1745.36	2847.16
Case 18	138.00	131.43	66.20	1744.04	2825.84
Case 19	292.82	278.87	87.84	1521.56	3012.42
Case 20	281.40	268.00	87.84	1517.79	2951.44
Case 21	278.25	265.00	87.84	1516.75	2934.57
Case 22	217.73	207.36	87.28	1477.34	2578.04
Case 23	205.93	196.12	87.28	1473.49	2516.07
Case 24	202.66	193.01	87.28	1472.43	2498.94
Case 25	190.72	181.63	87.08	1462.92	2427.40
Case 26	178.90	170.38	87.08	1459.08	2365.58
Case 27	175.63	167.26	87.08	1458.03	2348.48

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