Supplementary Information (SI) for Green Chemistry.
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Supplementary Information

Bioprocess development and scale-up for *cis,cis*-muconic acid production from glucose and xylose by *Pseudomonas putida*

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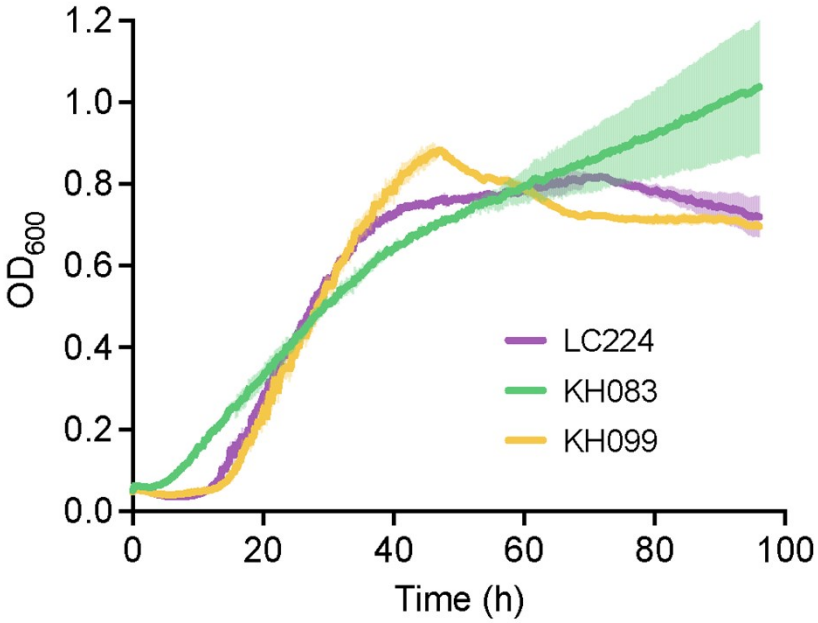
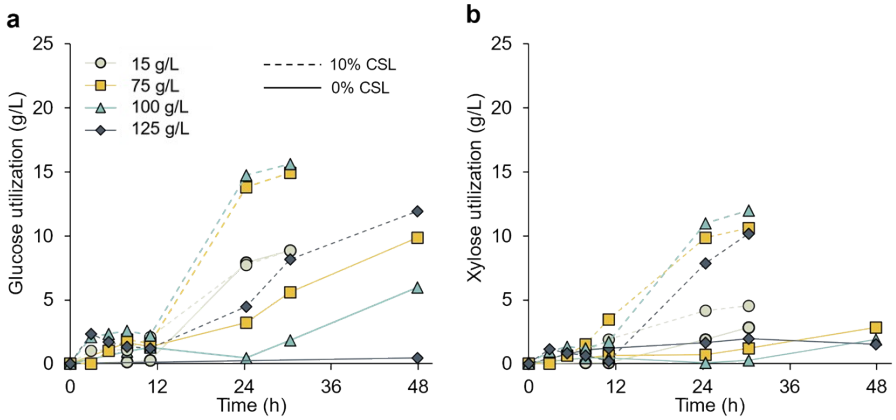
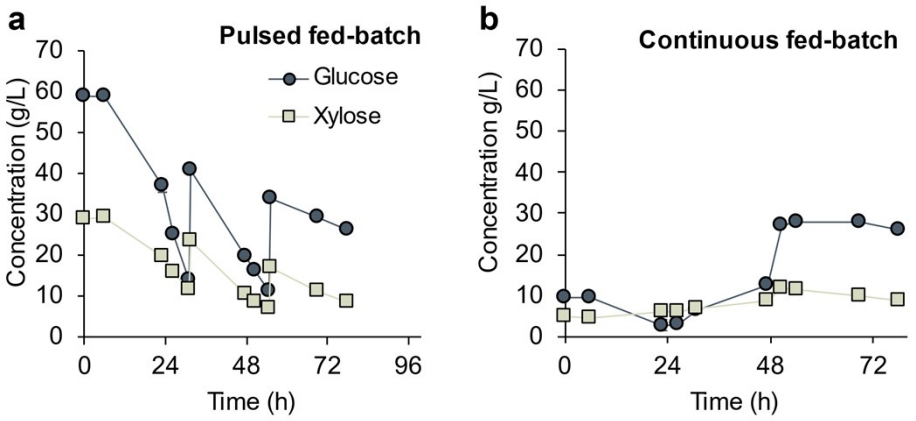
Supplementary Figures



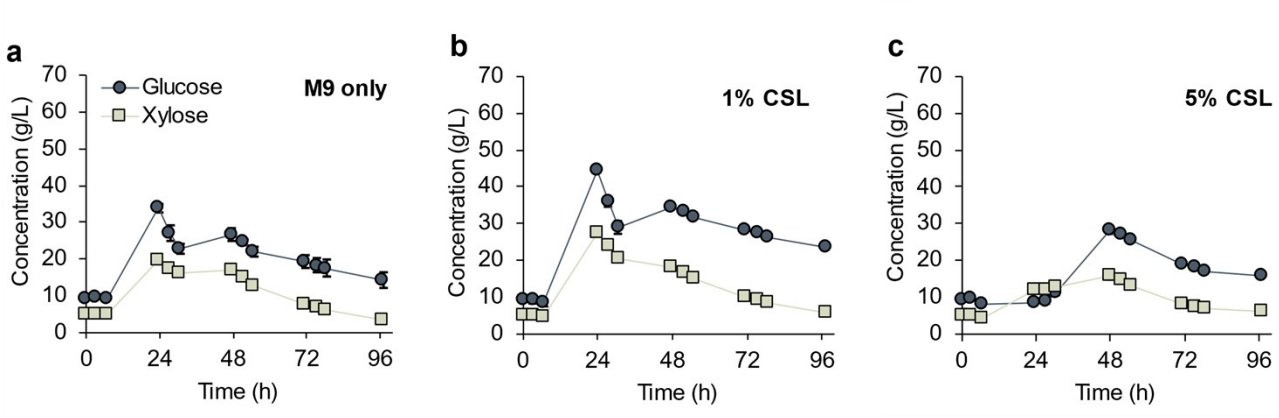
Supplementary Figure 1: Growth comparison of different *P. putida* strains. Growth curves of LC224, KH083 (LC224 Δ*gacS*), and KH099 (LC224 Δ*gntZ*) on modified M9 medium supplemented with 30 mM glucose and 15 mM xylose in a plate reader. Data represent the average of three biological replicates and error bars show the standard deviation.



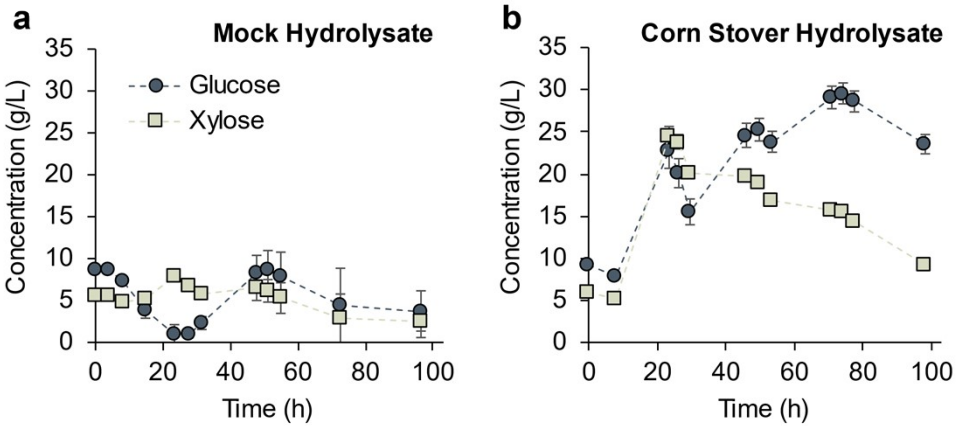
Supplementary Figure 2: Evaluation of the effect of initial sugar concentration on growth and sugar utilization by KH083 in shaken flask experiment. (a) Glucose utilization and (b) xylose utilization profiles at different starting sugar concentrations (15, 75, 100 and 125 g/L) with and without supplementation of corn steep liquor (CSL) at 10% (v/v). Shaken flask experiments were performed in duplicate, and error bars indicate the absolute error between duplicates. Sugar utilization data shown in the main manuscript corresponds to 30 h of incubation.



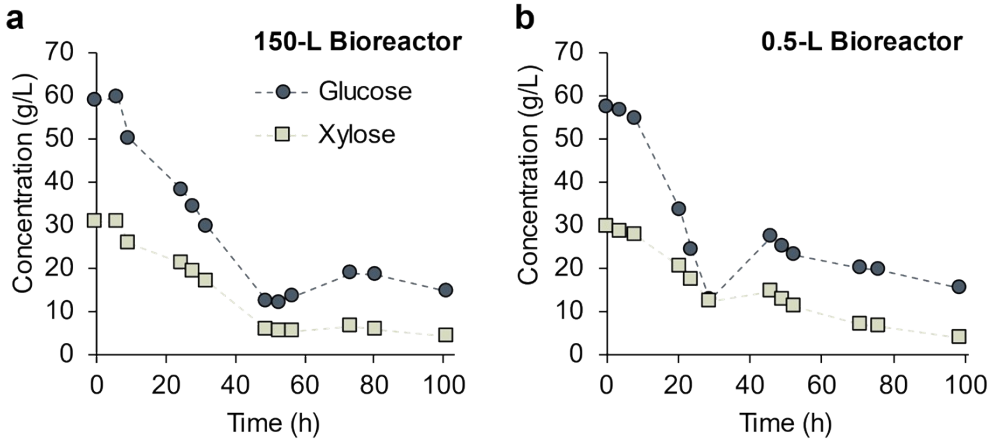
Supplementary Figure 3: Residual glucose and xylose concentration in 0.5-L bioreactors from cultivations controlled at low and high sugar concentrations. (a) Low initial sugar concentration and pulsed fed-batch and (b) low initial sugar concentration and continuous feeding in the presence of 10% (v/v) CSL. Data show the average of biological duplicates and error bars indicate the absolute error between duplicates.



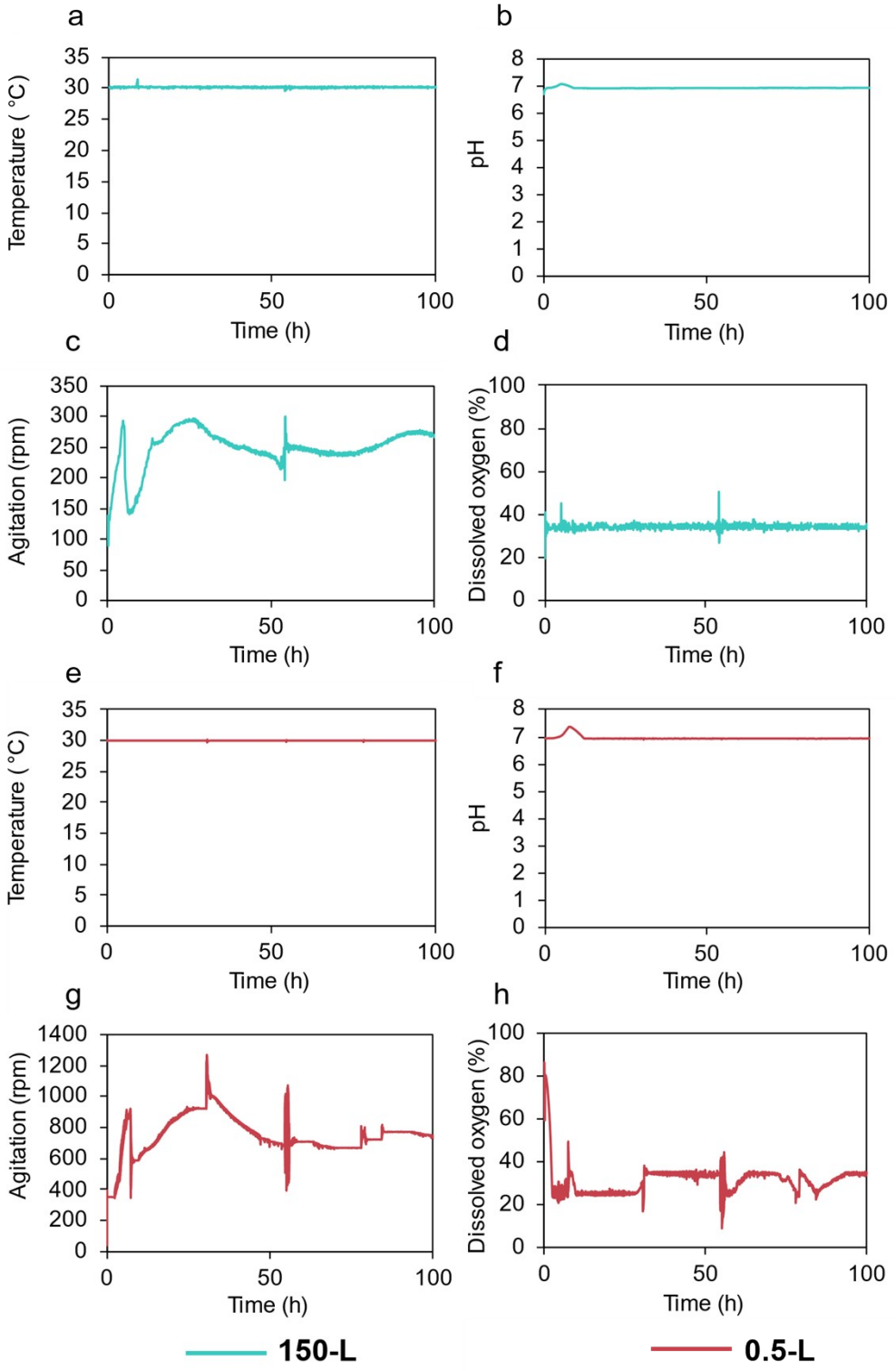
Supplementary Figure 4: **Residual glucose and xylose concentration in 0.5-L bioreactors from cultivations conducted at different concentrations of CSL.** (a) M9 only, (b) 1% CSL, and (c) 5% CSL. Data show the average of biological duplicates and error bars indicate the absolute error between duplicates.



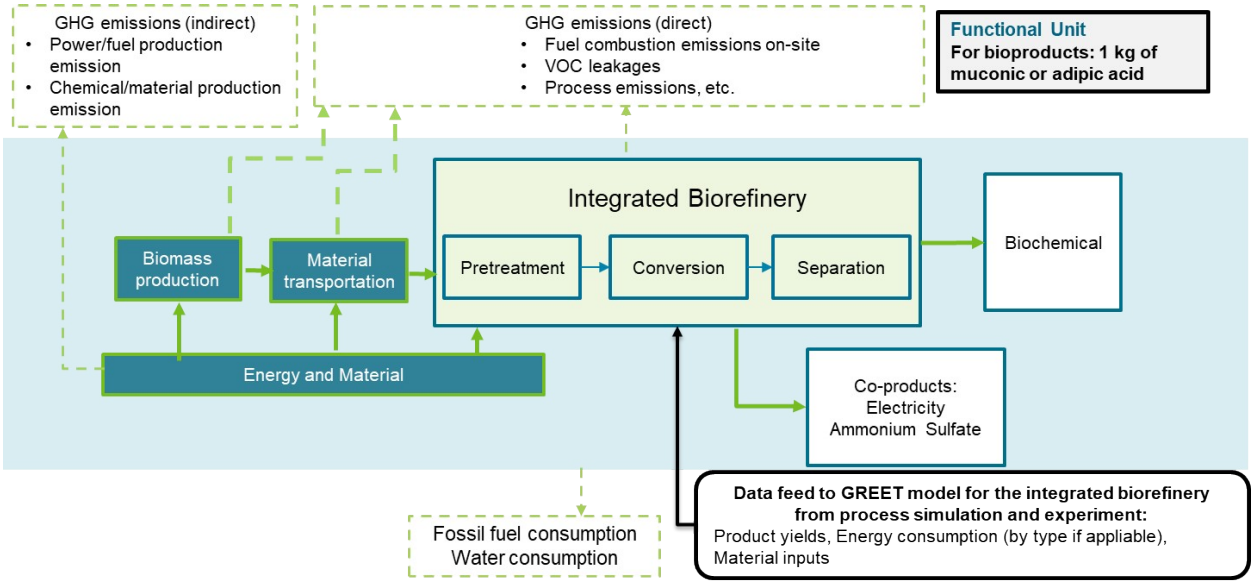
Supplementary Figure 5: Residual glucose and xylose concentration in 0.5-L bioreactors from cultivations conducted at a glucose-to-xylose ratio of 1.5:1 in mock and corn stover hydrolysates. (a) Mock hydrolysate, (b) corn stover derived sugar hydrolysate. Data show the average of biological duplicates and error bars indicate the absolute error between duplicates.



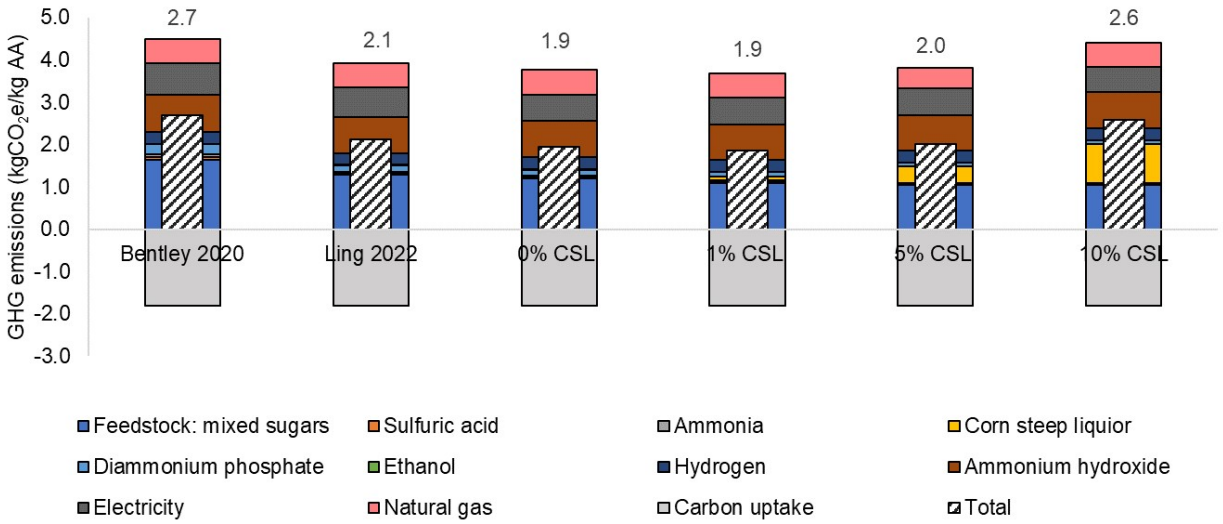
Supplementary Figure 6: Residual glucose and xylose concentration in 150-L and 0.5-L bioreactors. (a) 150-L bioreactor, (b) 0.5-L bioreactor. Data show the average of biological duplicates and error bars indicate the absolute error between duplicates for 0.5-L bioreactors. The cultivation in 150-L bioreactors was conducted in singlet.



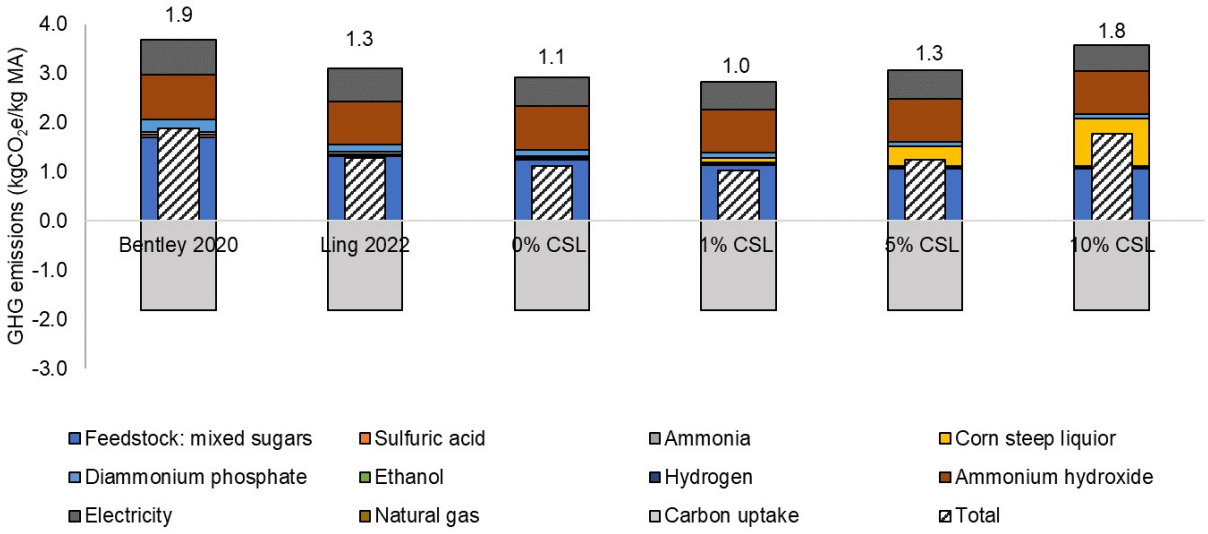
Supplementary Figure 7: Bioreactor parameters from cultivations conducted in 150-L and 0.5-L bioreactors. (a, e) Temperature, (b, f) pH, (c, g) agitation, and (d, h) DO.



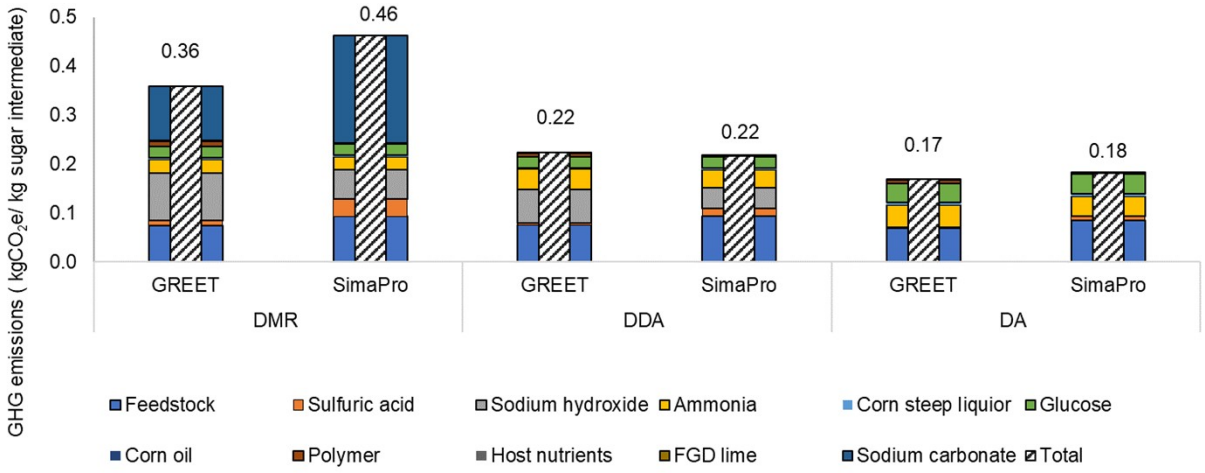
Supplementary Figure 8: Cradle-to-gate system boundary for MA and AA production. Life-cycle assessment in Figure S8 presents the system boundary for the analysis presented here. The first stage is feedstock production and logistics which includes fertilizer and agrochemical production, water and energy consumption related to farming activities, as well as storage and preprocessing of the feedstock. We leveraged the extensive background information for feedstock production and logistics that can be found in the GREET model to evaluate the emission and energy burdens of feedstock production. Then, the feedstock is transported to the conversion facility where the bioproduct (muconic acid and then adipic acid) is produced via biological conversion technologies described above. In this analysis, corn stover is the feedstock for all bio-derived pathways. The key parameters for corn stover collection, fertilizer use inputs, and transportation are from GREET (Argonne 2024).[1]



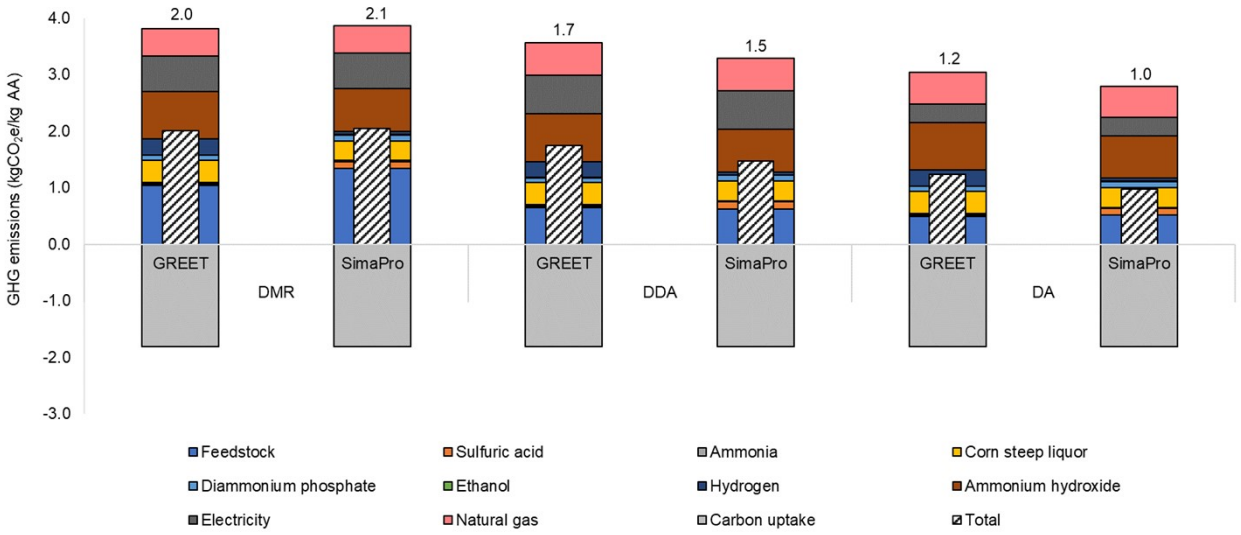
Supplementary Figure 9: GHG emission breakdown of AA. Figures S9 and S10 present the GHG emission breakdown to produce one kg AA and MA, respectively, for the six fermentation scenarios discussed in the main manuscript under baseline biorefinery model using mixed sugars from DMR pretreatment.



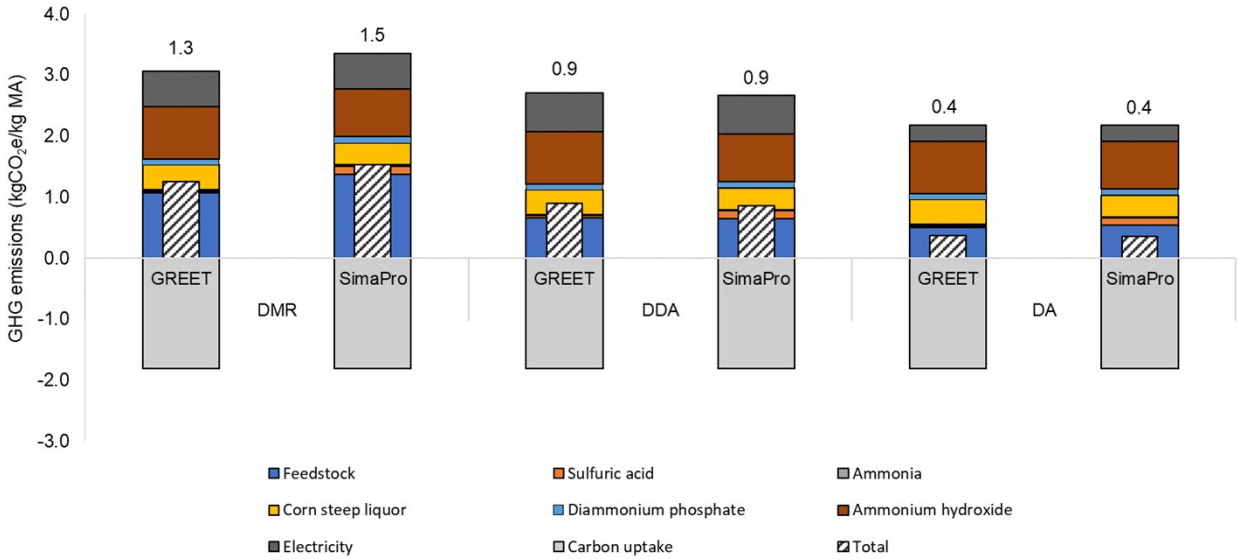
Supplementary Figure 10: GHG emission breakdown of MA.



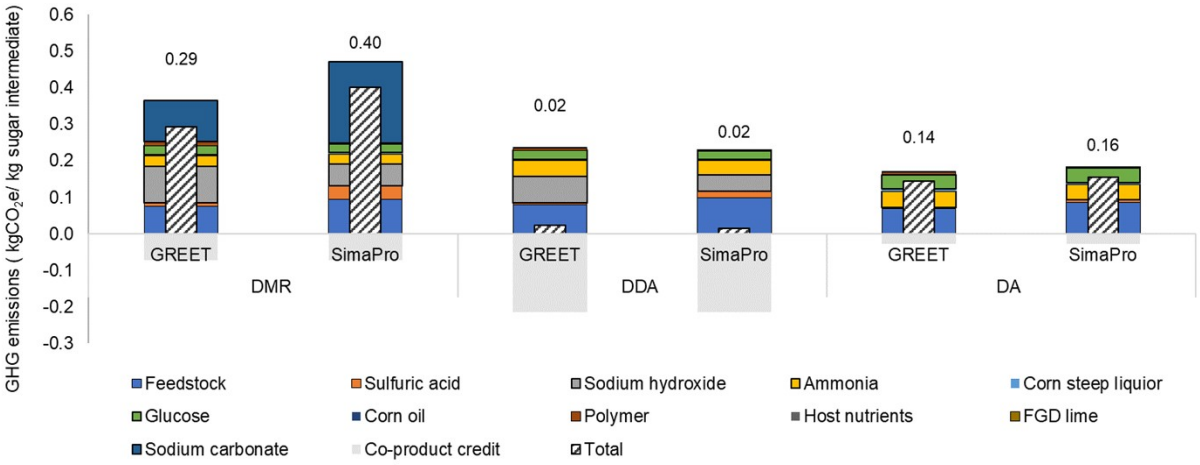
Supplementary Figure S11: Carbon intensity of mixed sugar intermediate for three pretreatment methods and LCA tools, namely, GREET and SimaPro. Co-product handling uses market-value allocation. Figure S11 shows the comparison of GREET and SimaPro GHG emission results of mixed sugar intermediate using three pretreatment methods: DMR, DDA, and DA using market allocation, which allocates the emissions and energy burdens based on the market value of mixed sugars intermediate and the co-produced electricity. For the DMR case, the amount of electricity co-produce is very low compared to the DDA (see Table S11), so the majority of the impact is attributed to the mixed sugars, while for the DDA 97% of the impact is attributed to the mixed sugar. The DDA-mixed sugar intermediate GHG emissions from the two LCA tools are comparable, with both GREET and SimaPro estimating 0.22 kg CO_{2e}/kg. A similar conclusion can be derived for DA-based mixed sugars, with both GREET and SimaPro estimating similar GHG emissions. On the other hand, there is a noticeable difference in GHG emissions between SimaPro and GREET when it comes to the DMR-mixed sugar intermediate, with SimaPro and GREET yielding 0.46 and 0.36 kg CO_{2e}/kg, respectively. The GREET-GHG emissions of mixed sugars in the DMR case are 23% lower than those of SimaPro. The major difference is attributed to the contribution of sodium carbonate, which has a different carbon intensity in each tool, and represents 31% and 47% of the total GHG emissions in the GREET and SimaPro models, respectively. Therefore, while it is evident the GHG emissions reduction from DMR to DDA and DA in both LCA tools, there is an apparent deviation between GREET and SimaPro results in the DMR case, resulting in a reduction of up to 53% and 61% using GREET and SimaPro, respectively. In both DMR and DDA pretreatment methods, however, the contributions of sodium hydroxide were higher in the GREET model compared to SimaPro, which slightly offset the contribution of the sodium carbonate, sulfuric acid, and feedstock categories in the SimaPro model. Consumption of sodium hydroxide in the DA biorefinery is zero.



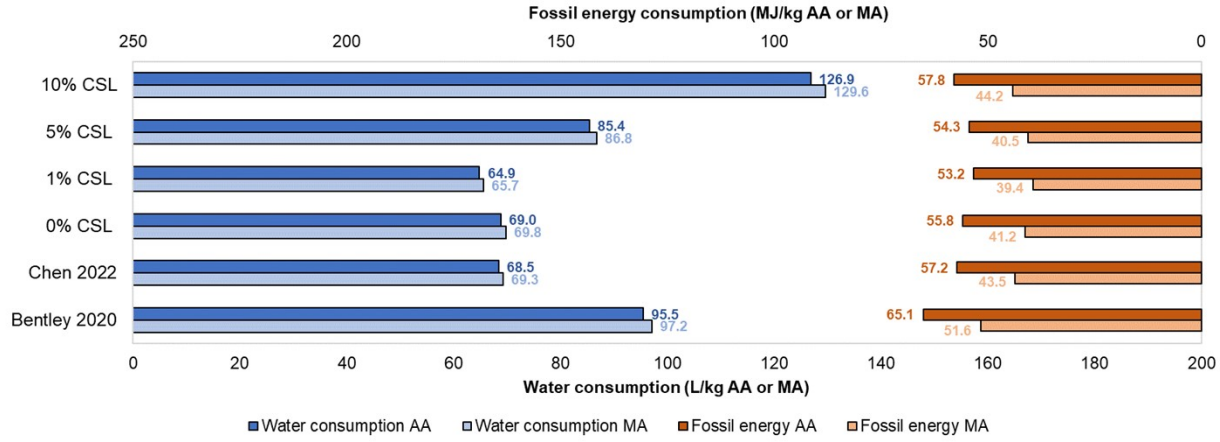
Supplementary Figure S12: AA GHG emission breakdown comparison between DMR, DDA, and DA pretreatment methods using two different LCA tools, namely, GREET and SimaPro, and co-product handling using market-value allocation. In Figures S12 and Fig S13, a comparison of GHG emissions breakdown is presented between GREET and SimaPro for AA and MA, respectively, as produced under the three assessed pretreatment methods. These figures correspond to the data presented in Table S9 and S10 for the best-case scenario (5% CSL) for both AA and MA. The GREET and SimaPro GHG emission calculations utilize identical assumptions discussed in the main manuscript. For example, the allocation method used to treat the co-product in both mixed sugar production and upgrading to AA and MA was based on the market value for the mixed sugar intermediate part. As discussed in the main manuscript, the GHG emissions resulting from DA pretreatment in comparison to DDA and DMR are progressively lower, and this trend remains consistent regardless of the LCA tool used. However, the sources of discrepancy from GREET to SimaPro in all cases are attributed to the contribution of certain chemicals including sulfuric acid, hydrogen, and ammonium hydroxide categories, which seems to be slightly higher in the GREET model compared to the SimaPro. Feedstock carbon intensity, however, is higher in SimaPro compared to the GREET (see Figure S11). This current LCA tool comparison sheds light on the aleatory uncertainty lined to the background inventory data and information used in an LCA study.



Supplementary Figure S13: MA GHG emission breakdown comparison between DMR, DDA, and DA pretreatment methods using two different LCA tools, namely, GREET and SimaPro, and co-product handling using market-value allocation. Other chemicals in this figure refer to CSL, ammonia, H_2, and ethanol.



Supplementary Figure S14: **Carbon intensity of mixed sugar intermediate for three pretreatment methods and LCA tools, namely, GREET and SimaPro, and co-product handling using displacement method.** The LCA results obtained from different tools (GREET and SimaPro) showed more variation with varying co-product handling methods. Therefore, a displacement allocation method was also evaluated to allocate the emissions and energy burdens between the products for the mixed sugars intermediate. Figure S14 compares the GHG emissions for producing mixed sugars intermediate applying the displacement method. In this case, a credit was applied for the co-produced electricity based on the avoided emissions of the U.S grid electricity. In the DMR case, the amount of co-produced electricity is very small compared to the mixed sugars than the avoided emissions do not impact the emissions, resulting in the same GHGs from the market allocation presented in Figure S11. For the DDA case, however, as more electricity was produced compared to DMR, the avoided emissions from the electricity grid lowered proportionally the total GHG emissions for both GREET and SimaPro cases, with the DA scenario achieving intermediate results due to low chemical consumption and to low electricity exports to the grid.



Supplementary Figure S15: Comparison of fossil energy use and water consumption for MA and AA production from DMR sugars using fermentation metrics from previous publications (Bentley *et al*.[2] and Ling *et al*.[3]) and those determined in this study with different CSL addition ratios and fermentation times. Fossil energy use and water consumption for petroleum-based AA are estimated in the R&D GREET model 2023 as 102.86 MJ/kg and 11.06 L/kg, respectively. **Additional LCA metrics.** The results for fossil energy use (Figure S17) are consistent with the GHG emission results for both AA and MA. Higher values of fossil energy use are expected for AA because its production involves more resources including natural gas and chemical inputs compared to MA, which production does not use natural gas, ethanol, and hydrogen. Overall, the fossil energy use of AA decreases -on average- by 44% lower than that of petroleum-based AA. On the other hand, water consumption, is significantly higher for biobased AA compared to fossil-based AA, up to eleven times higher than petroleum case for the 10% CSL case due to the high contribution of process water use during feedstock preparation and product recovery. Water consumption is slightly higher (1 to 2%) in muconic acid than AA as more process water is involved in MU production.

Supplementary Tables

Supplementary Table S1: Composition of the sugar stream derived from corn stover pretreated via DMR and enzymatically hydrolyzed. n.d.= not detected.

Component	Concentration of monomeric compounds (g/L)[1]	Concentration of monomeric sugars after hydrolysis (g/L)[2]
Sugars		
Glucose	300.4	328.6
Xylose	196.6	214.2
Arabinose	16.8	22.8
Galactose	n.d.	5.1
Cellobiose	10.77	
Glycerol	n.d.	
Ethanol	n.d.	
Furfural	n.d.	
Hydroxymethylfurfural	n.d .	
Organic acids		
Lactic acid	9.04	
Acetic acid	1.15	

[1] Concentration of monomeric sugars and organic acids in the sugar hydrolysate.
[2] Concentration of monomeric sugars in hydrolyzed (with H_2SO_4) sugar hydrolysate. Hydrolysis is conducted to identify the concentration of sugars that remain in polymeric form in the sugar hydrolysate.

Supplementary Table S2: Details for plasmids described in this study.

Plasmid	Utility	Construction details
pK18sB	Backbone for gene replacement via electroporation	Previously described in Jayakody, *et al.*[4] Genbank: MH166772.
pJH0002	Deletion of *gntZ* in strain LC224	Previously described in Bentley, *et al.*[2]
pAW052	Deletion of *gacS* in strain LC224	Two 1 kB homology regions directly flanking *PP_1650* (*gacS*) (see *pAW052_HRup* and *pAW052_HRdn* in **Supplementary Table 7**) were designed. An XbaI restriction enzyme recognition site (TCTAGA) was added between *pAW052_HRup* and *pAW052_HRdn*, and the fragment was synthesized in an pK18sB backbone by Twist Biosciences. The insert was sequence verified with oFB157, oFB191, oFB192, and oFB193 by GENEWIZ.

Supplementary Table S3: Strain construction details.

Strain	Genotype	Construction details
CJ522	*P. putida* KT2440 Δ*catRBC*::Ptac:*catA* Δ*pcaHG*::P*tac*:*aroY*:*ecdB*:*asbF* Δ*pykA*::*aroG-D146N*:*aroY*:*ecdB*:*asbF* Δ*pykF* Δ*ppc* Δ*pgi-1* Δ*pgi-2* Δ*gcd*	Previously described in Johnson, *et al.*[5]
LC224	**CJ522** *ΔhexR* Δ*ampC*::P_{xylE}:*xylE-A62V, A455V*:P_{tac}:*xylAB*:*talB*:*tktA* Δ*pgi-1*::*pgi-1* PP_1736-1737(intergenic)::Plac:*ubiC*-C22 P_{PP_2569} G→A Δ*pykF*::P_{tac}:*aroB*	Previously described in Ling, *et al.*[3]
KH083	LC224 Δ*gacS*	Deletion of *gacS* was performed using plasmid pAW052. Correct deletions were identified by colony PCR using primers oFB168 and oFB169. The PCR products were run on agarose gel, with a band of 2131 bp for the deletions, and a band of 4879 bp for the negative control.
KH099	LC224 Δ*gntZ*	Deletion of *gntZ* was performed using plasmid pJH0002. Correct deletions were identified by colony PCR using primers oKH134 and oKH135. The PCR products were run on agarose gel, with a band of 1888 bp for the deletions, and a band of 2806 bp for the negative control.

Supplementary Table S4: Oligonucleotides and synthetic DNA used in this study.

Oligo	Sequence (5'-3')
oFB157	GAAACGCCTGGTATCTTTATAGTCCTGTCGG
oFB168	GGCTCCGAGAGGAAGGCATTCTGGGTTTCCATGTG
oFB169	CCGCGTATCCTAGCATTAACCCATCACCAGGTTCTGTGGATTGC
oFB191	CTGAATGGCTTGGGCAGGTAATC
oFB192	GCATTGCAAAGGAGTTCGACCATG
oFB193	CTACCCCAACCCAGAGCTGCTG
oKH134	GTACGGAATTCTTCTGGGCCATT
oKH135	CATTACCGACGACTGGGCAA
pAW052_HRup	GGTATAACTGAGGCCGATCATCAGCAGGCAGAAGCCCACCAGCAAGATGGCCAGCTTCCAGAACAGCG AATGGCGGTCGAGCATGGTTCACTCCGCCTCGCTGGCACTGAGCACGTAACCCTTGCCCCACACGGTG CGGATCTGCCGTTCGTGATAACCGATGCCCTTGAGCTTGCGACGGATCTGGCTGACGTGCATGTCCAG GCTACGGTCATGCCGTGAATAGCCGCGCTGCAACACGTGCTGGTACAGGAATGGCTTGCTGAGGACTT CGTCGAGGTTGCGGTTGAGGATGTCCAGCAGGCGGTATTCGCTGGGGGTCAGGCCTGCCAGGCGGCTA TTCAGGCGCACGTCGCACAGCCTTTCATCAAACTGCAGCTCGCCGCAGCCGGCTTGCTCCAGCGGGCC CTGATGCCGCCGCTCCAGTGCCACGCGGCGCAGAATCGCCTCGATGCGCACCTGCAACTCGGCCATGC TGAATGGCTTGGGCAGGTAATCGTCGGCGCCGCGCTGAAAGCCGCTGATACGATCCGCCTCGGCCCCC AGCGCCGACATCATGATCACCGGTGTGGTGCTGTGCCTGCGCAACTGCGCCAAGGCGTCCAGCCCATC AAGCCCGGGCAGCAGGATGTCCATCAGCACCACATCGAAAGCCTGGCGGCCAGCCGTTTCCAGGCCCT CCAGGCCATTGCGGCACCAGGTGACCTGGCAGCCGCCACGCTGCAGCTCTTCCTGCAGATAGGCACCA AGCACCGGGTCGTCCTCGATGGCAAGGATGTTGGTGTTGTAAATAGATACAGGATTCATTGGCAACTG CCATGCATTCTCAATTGCGTGATTATTGAGCATTCGCCACCTGCCAGGCAACCGCACTGCGACCAATG CCTCGGCAACGGCGCATAACACGTACTAAAGAGATGCAATGGTGCGGCAGGCGGAGTAACTTCGCGGC TTGGCCGTTACCACTTGACGGCATCCAGGCACATCAGAGGAGGCGAGT
pAW052_HRdn	GGGGATCTGGGCCACGCTGGGCATGCCTTGACAGGCAGTGACCCACCCAAGACATGCATTGCAAAGGA GTTCGACCATGCGCGCCCTGTTGTTCAGCAGCCAGCACTACGATCAGGAAAGCTTCACCAAGGCTGCC GGCGGCACCGCCCTGGAGCTGCATTTCCAGCCCGCCCGCCTGACCCTCGACACCGCCGCCCTGGCAGA TGGCTTTGAGGTGGTCTGCGCCTTCATCAATGACGAACTCGACGCGCCGGTGCTGCAGCGCCTGGCCG CCGCTGGCACGCGGTTGATCGCCCTGCGCTCGGCCGGCTACAACCACGTCGACCTGGCCGCCGCCCAG CGCTTGGGGCTGGCCGTGGTGCGGGTGCCGGCCTACTCGCCACACGCCGTTGCCGAGCATGCCGTGGC GCTCATCCTGGCACTCAACCGGCGTCTGCATCGGGCCTACAACCGCACCCGCGAAGGTGACTTCACCC TGCACGGGCTGACCGGCTTCGACCTGCACGGGAAAACCGTCGGGGTGGTCGGTACCGGCCAGATCGGG GTCGCCTTCGCTCGCATCATGGCCGGTTTCGGCTGCCAGCTGCTGGCTTACGACCCCTACCCCAACCC AGAGCTGCTGGCCCTTGGCGCCCGCTACCTGCCCCTGCCCGAGCTATTGCGCGAAGCCCGCATCATCA GCCTGCACTGCCCACTGACCGAGCACACTCGCCACCTGATCAACGCGCAAAGCCTGGCCCAGTTGCAG CCCGGCGCCATGCTGATCAACACCGGTCGCGGTGCGTTGGTCGACACCCCGGCGCTGATCGACGCACT GAAAAGCGGCCAGCTTGGCTACCTGGGCCTGGACGTCTACGAAGAAGAAGCCCAACTGTTCTTCGAGG ACCGCTCCGACCTGCCCTTGCAAGACGATGTGCTGGCTCGGCTGCTGACCTTCCCCAACGTGATCATC ACCGCCCACCAGGCTTTCCTCACCCGCGAGGCGCTGGATGCCATTGCC

Supplementary Table S5: Main reactions for the conversion of glucose to muconic acid.

Process simulation and techno-economic assessment: Production of muconic acid through the fermentation of mixed sugars is carried out in aerobic fermenters at specific productivities, yields, and titers, as determined experimentally for the individual points in the main text of the manuscript. Reactions for biomass growth and product formation are shown in Table S5 for glucose. As both five- and six-carbon sugars are converted in the fermentation operation, the reactions for xylose and arabinose can also be derived from the equations in Table S5 by multiplying all non-sugar compounds by a factor of 5/6. In the simulations, the diversion of carbon to product formation or biomass production is modulated through the fractional conversion of reactions #1 and #2. Losses of sugars due to contamination (reaction #3) is fixed at 3% for all cases. For simplification purposes and in view of the lack of experimental data to support this claim, the funneling of carbon from corn steep liquor (CSL) either to product or *P. putida* biomass is not accounted for in the reaction set below.

Number	Reaction	Equation
#1	Glucose to product	**1** Glucose + **1.94** O_2 → **0.74** Muconic + **1.57** CO_2 + **3.78** H_2O
#2	Glucose to biomass	**1** Glucose + **0.28** NH3 + **1.77** O_2 → **4.8** *P. putida* + **1.2** CO_2 + **1.98** H_2O
#3	Glucose loss to contamination	**1** Glucose + **6** O_2 → **6** CO_2 + **6** H_2O

Supplementary Table S6: **Financial assumptions used in the TEA, based on an nth-plant design.**

Financial Assumptions	Value
Plant life	30 years
Cost year dollar	2016$
Capacity Factor	90%
Discount rate	10%
General plant depreciation	MACR
General plant recovery period	7 years
Steam plant depreciation	MACR
Steam plant recovery period	20 years
Federal tax rate	21%
Financing	40% equity
Loan terms	10-year loan at 8% APR
Construction period	3 years
First 12 months' expenditures	8%
Next 12 months' expenditures	60%
Last 12 months' expenditures	32%
Working capital	5% of fixed capital investment
Start-up time	6 months
Revenues during start-up	50%
Variable costs during start-up	75%

Supplementary Table S7: Main inputs and outputs of the baseline biorefinery design. This includes the associated purchase/selling prices (given in 2016$) for the case with the lowest minimum selling price (MSP) of adipic acid: addition of 5% (v/v) CSL to M9 medium, fermentation time of 96h, and use of DMR-based mixed sugars.

Input	Value	Price	Source
Mixed sugars [a]	41,692 kg/h	$0.489/kg	[a]
Ammonium hydroxide (NH_4OH)	10,487 kg/h	$0.27/kg	[b]
Sulfuric acid (H_2SO_4)	14,577 kg/h	$0.12/kg	[6]
Ammonia (NH_3)	118 kg/h	$0.42/kg	[7]
Diammonium phosphate (DAP)	819 kg/h	$0.36/kg	[7]
Corn steep liquor (CSL) [c]	3,536 kg/h	$68/ton	[7]
Hydrogen (H_2)	426 kg/h	$1,461/ton	[7]
Ethanol	89 kg/h	$674/ton	[7]
Process steam, 150 psig	27,189 kg/h	$13.82/1,000 kg	[6]
Cooling water	1,952,771 kg/h	$0.02/1,000 kg	[6]
Chiller water, 40° F	65 MMkcal/h	$4.71/GJ	[6]
Electricity	8,157 kW	$0.0682/kWh	[6]
Output	**Value**	**Price**	
Adipic acid	14,450 kg/h	*MSP of $2.96/kg*	-
Recoverable salt (Na_2SO_4)	18,265 kg/h	$141.27/ton	[7]

[a] Price obtained for the production of mixed sugars via a Deacetylation and Mechanical Refining (DMR) pretreatment of corn stover, further detailed in Table S11. Alternative pretreatment methods would yield mixed sugars at different prices: $0.446/kg for Deacetylation and Dilute Acid (DDA) and of $0.388/kg for Dilute Acid (DA).

[b] Estimated from the price of ammonia found in Davis et al. [7], accounting for the addition of water as a reactant to form ammonium hydroxide and a 10% charge in view of process capital costs.

[b] Both soluble and solid fractions of CSL.

Supplementary Table S8: **Breakdown of capital expenditures (CAPEX)** for the case with the lowest MSP of adipic acid: addition of 5% (v/v) CSL to M9 medium, fermentation time of 96h, and use of DMR-based mixed sugars.

	Total cost (MM$)
Total Installed Costs	**340.6**
Fermentation	*206.6*
Muconic acid recovery	*70.0*
Upgrading to adipic acid	*37.2*
Adipic acid recovery	*26.8*
Other equipment	*0.2*
Other Direct Costs	**59.6**
Total Indirect Costs	**240.2**
Fixed Capital Investment	**640.4**

Supplementary Table S9. Life-cycle inventory of the baseline biorefinery design for adipic acid production. There are two parts to the life-cycle assessment (LCA) study. The first part involves analyzing an integrated biorefinery level analysis (baseline in the main manuscript) considering the utilization of mixed sugars from a biorefinery based on the three pretreatment methods (DMR, DDA, and DA), followed by converting the sugars via fermentation and the upgrading to the final bioproduct either adipic acid (AA) or muconic acid (MA). Tables S9 and S10 present the inventory data used in this first LCA of AA and MA, respectively.

Materials and Energy Flows	**GB271 (Bentley *et al.*, 2020)**	**LC224 (Ling *et al.*, 2022)**	**0% CSL**	**1% CSL**	**5% CSL**	**10% CSL**
Products	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Acid	9,071.9	11,665.7	12,412.6	13,605.1	14,450.5	14,430.7
Resource Consumption	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Mixed sugars (glucose, xylose, arabinose)	41,692	41,692	41,692	41,692	41,692	41,692
Corn steep liquor	-	-	-	707	3,536	8,309
Ammonia	234	187	171	146	117	99
Diammonium phosphate (DAP)	1,452	1,154	1,053	917	819	815
Ammonium hydroxide	6,825	8,509	9,171	9,930	10,487	10,543
Sulfuric acid, 93%	11,102	11,102	11,102	11,102	11,102	11,102
H_2	268	344	366	401	426	426
Ethanol	57	72	75	83	89	89
Natural gas (kg/h)	1,353.4	1,740.6	1,851.8	2,029.9	1,849.7	2,153.3
Natural gas (MMbtu/hr)	71.3	91.7	97.5	106.9	97.4	113.4
Electricity import from grid	15,614	18,818	17,928	19,140	20,577	19,306
Water input	443,416	373,798	434,706	373,798	373,798	382,617
Waste Streams	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Disposal of solids	493,593	343,981	487,180	402,542	362,520	436,349

Supplementary Table S10. Life-cycle inventory for the baseline biorefinery design for muconic acid production.

Materials and Energy Flows	GB271 (Bentley *et al.*, 2020)	LC224 (Ling *et al.*, 2022)	0% CSL	1% CSL	5% CSL	10% CSL
Products	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Acid	8,797.1	11,314.1	12,038.6	13,195.1	14,015.0	13,995.4
Resource Consumption	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Mixed sugars (glucose, xylose, arabinose)	41,692	41,692	41,692	41,692	41,692	41,692
Corn steep liquor	-	-	-	707	3,536	8,309
Ammonia	234	187	171	146	117	99
Diammonium phosphate (DAP)	1,452	1,154	1,053	917	819	815
Ammonium hydroxide	6,826	8,509	9,171	9,930	10,487	10,544
Sulfuric acid, 93%	11,102	11,102	11,102	11,102	11,102	11,102
H_2	-	-	-	-	-	-
Ethanol	-	-	-	-	-	-
Natural gas (kg/h)	-	-	-	-	-	-
Natural gas (MMbtu/hr)	-	-	-	-	-	-
Electricity import from grid	14,244	17,066	16,111	17,106	18,420	17,152
Water input	443,416	373,798	434,706	373,798	373,798	382,617
Waste Streams	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**	**kg/hr**
Disposal of solids	495,502	344,423	486,962	493,593	362,520	436,984

Supplementary Table S11. Life-cycle inventory to produce mixed sugars via three pretreatment methods.
Table S11 shows the inventory for producing mixed sugar intermediate under three pretreatment methods: DMR (baseline), DDA, and DA. For this approach, we included the impacts of corn stover harvesting and processing, transportation, and deconstruction. Market allocation was used between mixed sugars and electricity produced from lignin combustion.

Pretreatment method	**DMR**	**DDA**	**DA**
Mixed sugars price ($/kg)	0.489	0.446	0.388
Electricity price ($/kWh)	0.057	0.057	0.057
Products	**Production Rate (kg/hr)**		
Sugar production rate	41,692	39,839	45,839
Co-products			
Export Electricity	6,763	19,366	2,823
Resource Consumption	**Flow Rate (kg/hr)**		
Biomass Feedstock (20% moisture)	104,167	104,167	104,167
Sulfuric Acid, 93%	9,360	4,108	2,242
Caustic (as pure)	2,000	1,417	0
Sodium carbonate (as pure)	6,667	0	0
Ammonia	523	737	871
Flocculant	324	205	228
Glucose	1,312	1,254	2,410
Corn Steep Liquor	89	85	164
Corn oil	7	7	13
Host nutrients	37	35	67
Sulfur Dioxide	9	9	16
Diammonium Phosphate	0	0	0
WWT Polymer	8	3	0
Boiler Chemicals	0	0	0
FGD Lime	109	170	262
Natural gas (93.4% eff)	0	0	0
Cooling Tower Chemicals	2	2	2
Makeup Water	214,729	209,563	199,964
Grid Electricity	0	0	0
Waste Streams	**Flow Rate (kg/hr)**		
Disposal of Ash	4,415	4,515	4,646
Air Emissions	**Flow Rate (kg/hr)**		
H2O	81,326	75,519	54,360
N2	334,137	306,654	155,509
CO2 (fossil)	0	0	0
CO2 (biogenic)	73,663	71,553	45,075
O2	46,785	38,087	11,715
NO2	59	60	34
SO2	7	10	16
CO	59	60	34
CH4	1.5	1.7	0.4

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