PNAS
www.pnas.org

Supplementary Information for

Comparing *in planta* accumulation with microbial routes to set targets for a cost-competitive bioeconomy

Minliang Yang, Di Liu, Nawa Raj Baral, Chien-Yuan Lin, Blake A. Simmons, John M. Gladden, Aymerick Eudes, Corinne D. Scown*

*Corresponding author: Corinne D. Scown
Email: cdscown@lbl.gov

This PDF file includes:

Table S1. Input parameters for technoeconomic modeling and uncertainty analysis in this study.

Parameter	**Unit**	**Baseline**	**Minimum**	**Maximum**
Biomass sorghum price (1, 2)	$/bone-dry tonne	95.0	60.0	120.0
Structural composition of biomass sorghum (3)				
Glucan	wt.%	36.8		
Lignin	wt.%	12.3		
Galactan	wt.%	1.0		
Sucrose	wt.%	2.0		
Xylan	wt.%	15.5		
Structural starch	wt.%	11.5		
Soluble starch	wt.%	14.8		
Ionic liquid (IL) pretreatment process (4)				
Solid loading rate	wt.%	30		
IL to biomass ratio	kg/kg	0.29		
IL cost	$/kg	2.0	1.0	5.0
IL recovery	wt.%	97	97	99
Pretreatment time	h	3	1	5
Pretreatment temperature	°C	140		
Enzymatic hydrolysis and ethanol fermentation process (4, 5)				
Enzyme loading rate	mg-protein/g-glucan	10.0		
Enzyme price	$/kg	4.29		
Glucan to glucose	wt.%	90.0	80.0	95.0
Xylan to xylose	wt.%	90.0	80.0	95.0

Hydrolysis time	h	72.0		
Corn steep liquor price	$/kg	0.10		
Diammonium phosphate price	$/kg	1.00		
Glucose utilization	wt.%	95	85	95
Xylose utilization	wt.%	85	75	85
Fermentation time	h	36	24	72
Product recovery, wastewater treatment, and utility process				
Electricity price (6)	$/kWh	0.068		
Plant system				
4-Hydroxybenzoic acid (7–9)				
Extraction time	h	6	3	12
Extraction temperature	°C	70	70	140
Extraction efficiency	%	90	70	90
Methanol market price	$/kg	0.24		
Solvent loading ratio	g/L	150	100	200
Solvent recycle rate	%	95		
4-HBA market price	$/kg	2.60		
Catechol (10, 11)				
Extraction time	h	8	4	16
Extraction temperature	°C	80	80	160
Extraction efficiency	%	90	70	90
Solvent loading ratio	g/L	60	30	120

Catechol market price	$/kg	5.00		
2-pyrone-4,6-dicarboxylic acid (7, 12–14)				
Extraction time	h	8	4	16
Extraction temperature	°C	70	70	140
Solvent loading ratio	g/L	50	25	100
Sodium chloride market price	$/kg	0.18		
Solvent recycle rate	%	95		
PDC market price (using PHB price as proxy)	$/kg	5.50		
Muconic acid (15, 16)				
Extraction time	h	6	3	12
Extraction temperature	°C	70	70	120
Solvent loading ratio	g/L	50	25	100
Solvent recycle rate	%	95		
Muconic acid market price	$/kg	1.50		
Microbial production system				
Glucose market price (17, 18)	$/kg	0.59	0.27	0.59
4-Hydroxybenzoic acid (7, 19, 20)				
Best reported titer: 36.6 g/L; yield: 0.41 mol/mol of glucose (0.31 g/g of glucose). (7, 19, 20)				
Theoretical maximum yield: 0.76 mol/mol of glucose (0.58 g/g of glucose) (20)				
Bioconversion temperature	°C	33		
Bioconversion time	h	24	24	72
Yield	g/g-glucose	0.31	0.31	0.58

NaH_2PO_4 market price	$/kg	0.20	0.10	0.40
NH_4SO_4 market price	$/kg	0.05	0.025	0.10
NH_4Cl market price	$/kg	0.07	0.035	0.14
Nitric acid market price	$/kg	0.34	0.25	0.45
$Ca(OH)_2$ market price	$/kg	0.25	0.15	0.35
Catechol (21)				
Best reported yield: 0.26 mol/mol of glucose (0.16 g/g of glucose); titer: 4.47 g/L. (21)				
Theoretical maximum yield: 0.61 mol/mol of glucose (0.38 g/g of glucose) (21)				
Bioconversion temperature	°C	32		
Bioconversion time	h	72	36	96
Yield	g/g-glucose	0.16	0.16	0.38
PDC (18)				
Best reported yield: 0.341 mol/mol of glucose (0.348 g/g of glucose); titer: 12.9 g/L. (18)				
Theoretical maximum yield: 0.83 mol/mol of glucose(0.85 g/g of glucose) [based on reaction stoichiometry]				
Bioconversion temperature	°C	30		
Bioconversion time	h	144	72	144
Yield	g/g-glucose	0.35	0.35	0.85
Muconic acid (7, 18, 22)				
Best reported yield: 0.378 mol/mol of glucose (0.298 g/g of glucose); rate: 0.1 g/L/h; titer: 12.0 g/L. (18)				
Theoretical maximum yield: 0.739 mol/mol of glucose (0.583 g/g of glucose) (18)				
Bioconversion temperature	°C	35		
Bioconversion time	h	89	72	144

Yield	g/g-glucose	0.298	0.298	0.58
Ammonia market price	$/kg	0.59		
Economic evaluation (5, 22)				
Daily biomass feedstock processed (*in planta* scenarios)	bone-dry tonne/day	2,000		
Daily glucose feedstock processed (microbial scenarios)	bone-dry tonne/day	1,000		
Biomass feedstock moisture content (*in planta* scenarios)	wt.%	20.0		
Annual operating time	h	7,920		
Total capital investment			-25%	+25%
Discount rate	%	10	5	15
Plant life	y	30		

Table S2a. Numerical results of minimum selling price (MSP: $/kg) from plant systems. S1 (base case price) refers to selling ethanol at the basecase price of $1.44/LGE as modeled in the base case biorefinery. S2 (target fuel price) refers to selling ethanol at the targeted fuel price of $0.66/LGE (23). S3 (gasoline price) refers to selling ethanol at the 1940-2020 historical average U.S. gasoline rack sales price of $0.40/LGE (24).

Plant system												
	Catechol			Muconic acid			PDC			4-HBA		
	S1	S2	S3	S1	S2	S3	S1	S2	S3	S1	S2	S3
Feedstock	183.1	137.3	121.6	131.8	101.8	90.4	2.6	2.0	1.8	2.7	2.0	1.8
IL pretreatment	60.7	45.5	40.3	45.2	34.9	27.2	0.9	0.7	0.6	0.9	0.7	0.6
Hydrolysis & fermentation	76.7	57.5	50.9	57.1	44.1	39.1	1.1	0.9	0.6	1.2	0.9	0.8
Fuel recovery	37.7	28.3	25.0	28.0	29.1	25.5	0.6	0.4	0.4	0.8	0.6	0.5
Wastewater treatment	119.7	89.7	79.5	89.3	69.0	61.2	1.8	1.4	1.2	1.8	1.4	1.2
Heat & power generation	148.5	111.3	98.6	111.6	81.8	71.7	2.2	1.3	1.2	2.4	1.8	1.2
Extraction	63.4	47.6	42.1	39.7	30.5	27.2	0.9	0.7	0.6	0.7	0.5	0.5
Electricity credit	-7.5	-7.5	-7.5	-6.1	-6.1	-6.1	0.0	0.0	0.0	-0.2	-0.2	-0.2
Bioethanol credit	-644.5	-297.7	-181.5	-478.8	-229.3	-139.8	-9.5	-4.4	-2.7	-9.8	-4.5	-2.7
MSP	36.6	212.0	269.0	18.9	155.6	200.0	0.6	3.2	4.1	0.5	3.2	4.0

Table S2b. Numerical results of minimum selling price (MSP: $/kg) from microbial routes.

Microbial system								
	Catechol		Muconic acid		PDC		4-HBA	
	Demon-strated	Theo-retical	Demon-strated	Theo-retical	Demon-strated	Theo-retical	Demon-strated	Theo-retical
Seed fermentation	1.2	0.5	0.3	0.2	0.4	0.2	0.6	0.3
Product purification	1.6	0.7	0.7	0.4	0.7	0.3	1.8	1.0
Bioconversion	2.4	1.0	1.0	0.6	1.3	0.6	1.4	0.8
Glucose	4.4	1.8	1.6	0.9	1.4	0.6	2.2	1.2
Other materials	0.8	0.3	0.2	0.1	0.7	0.3	0.5	0.3
Utilities	0.7	0.3	0.6	0.3	0.4	0.1	0.2	0.1
MSP	11.1	4.8	4.4	2.4	4.8	2.0	6.6	3.7

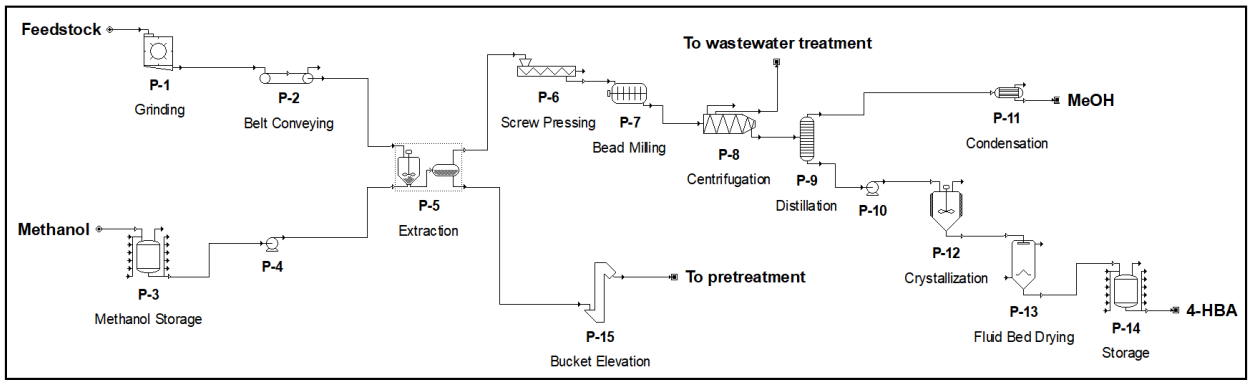

Figure S1a. Process flow diagram of 4-HBA extraction process in the plant system.

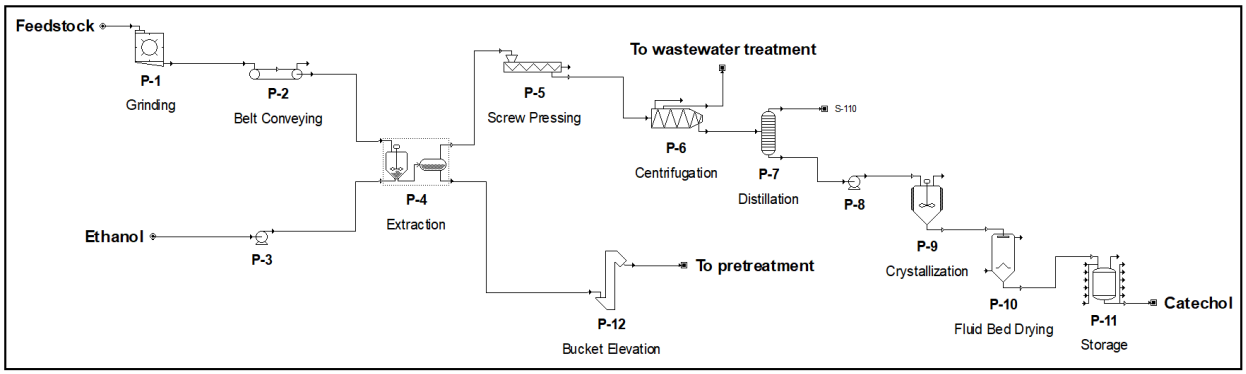

Figure S1b. Process flow diagram of catechol extraction process in the plant system.

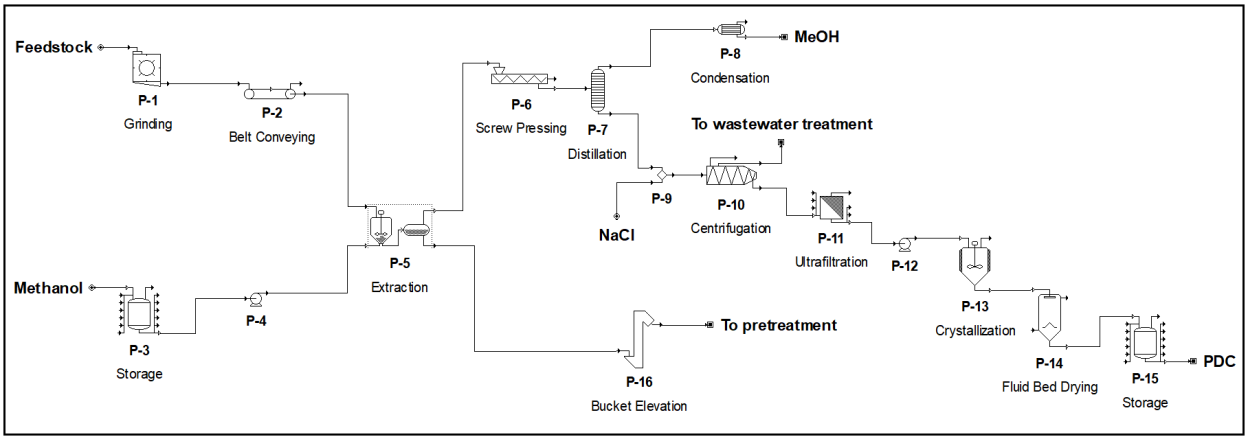

Figure S1c. Process flow diagram of PDC extraction process in the plant system.

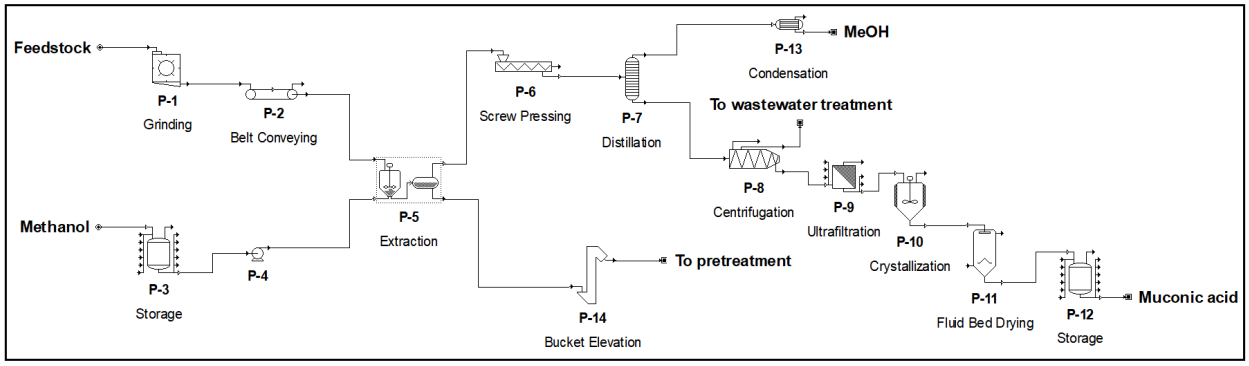

Figure S1d. Process flow diagram of muconic acid extraction process in the plant system.

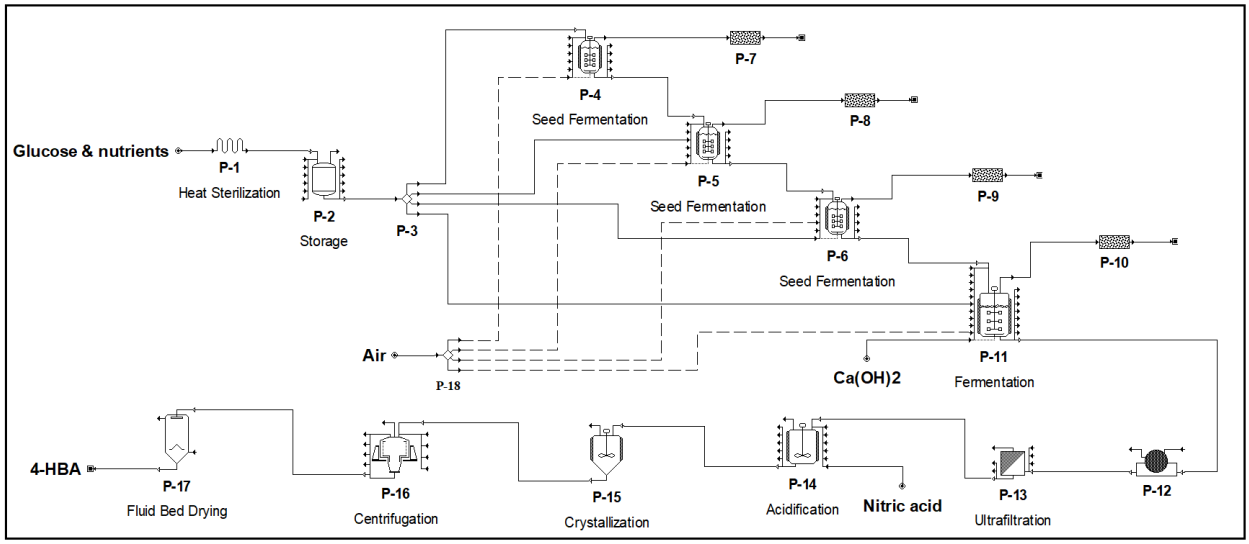

Figure S2a. Process flow diagram of 4-HBA synthesis in the microbial system.

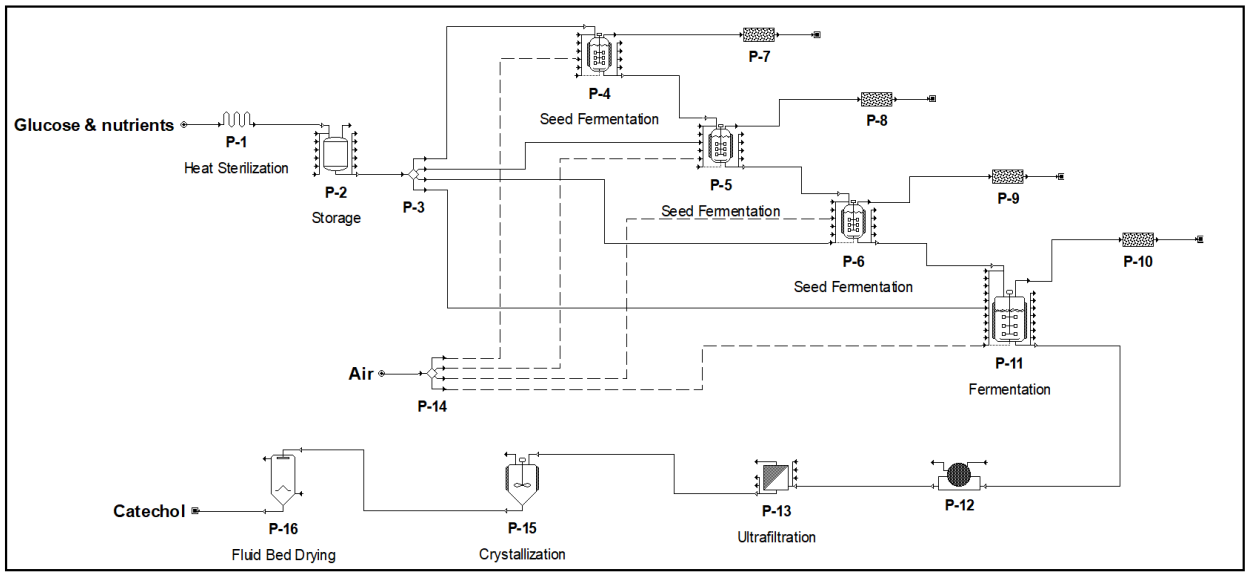

Figure S2b. Process flow diagram of catechol synthesis in the microbial system.

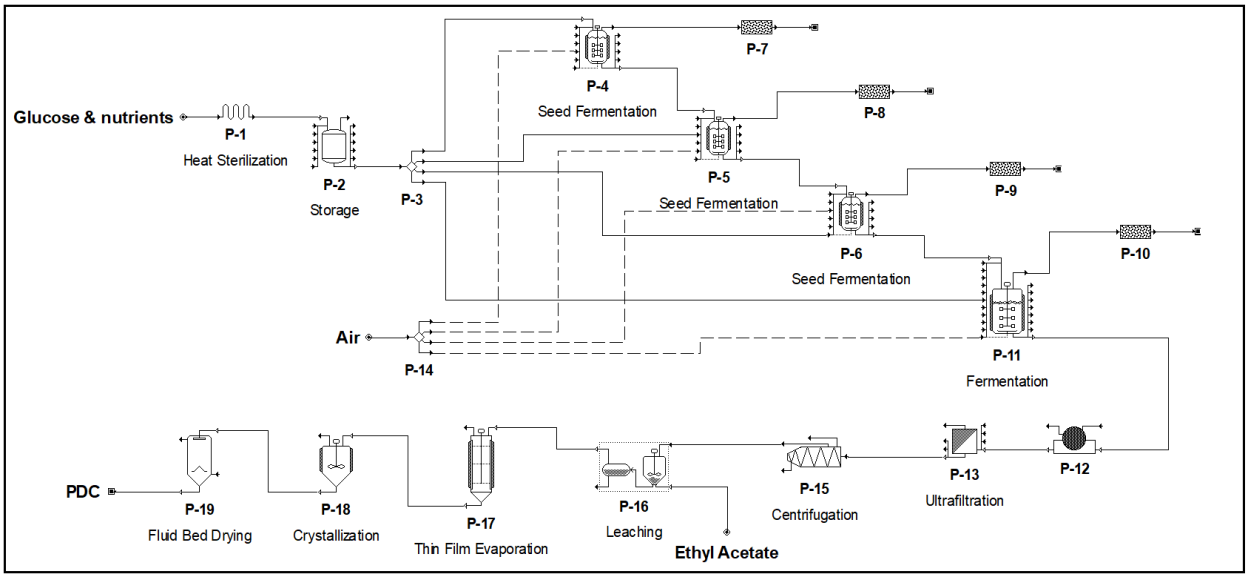

Figure S2c. Process flow diagram of PDC synthesis in the microbial system.

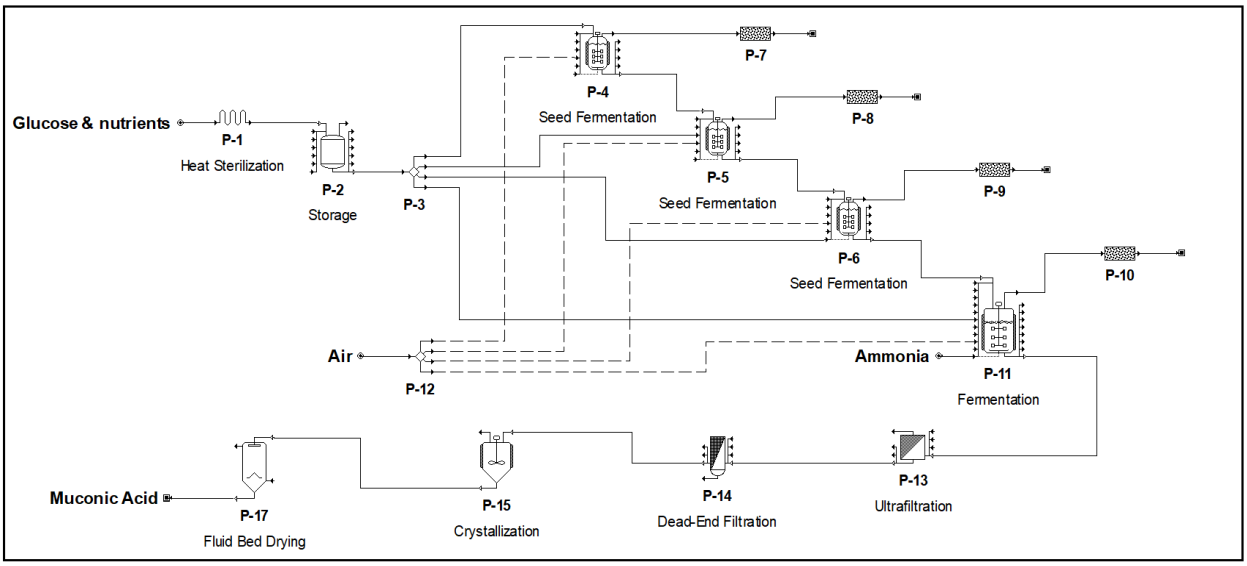

Figure S2d. Process flow diagram of muconic acid synthesis in the microbial system.

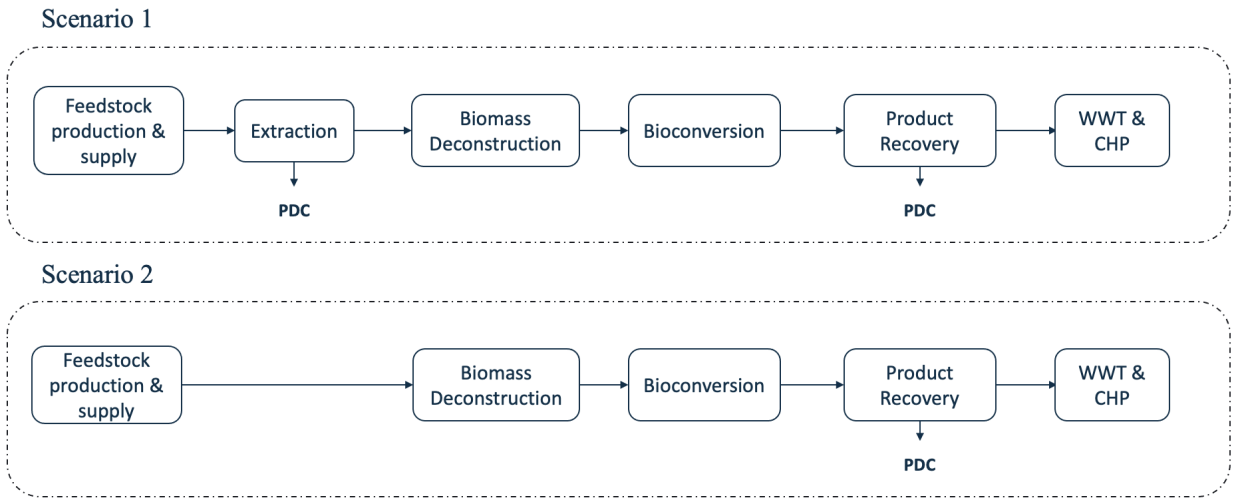

Figure S3. Two potential scenarios considered in this study for combining plant and microbial systems as a single integrated biochemical plant. This is an example considering PDC as a product. WWT: wastewater treatment. CHP: combined heat & power.

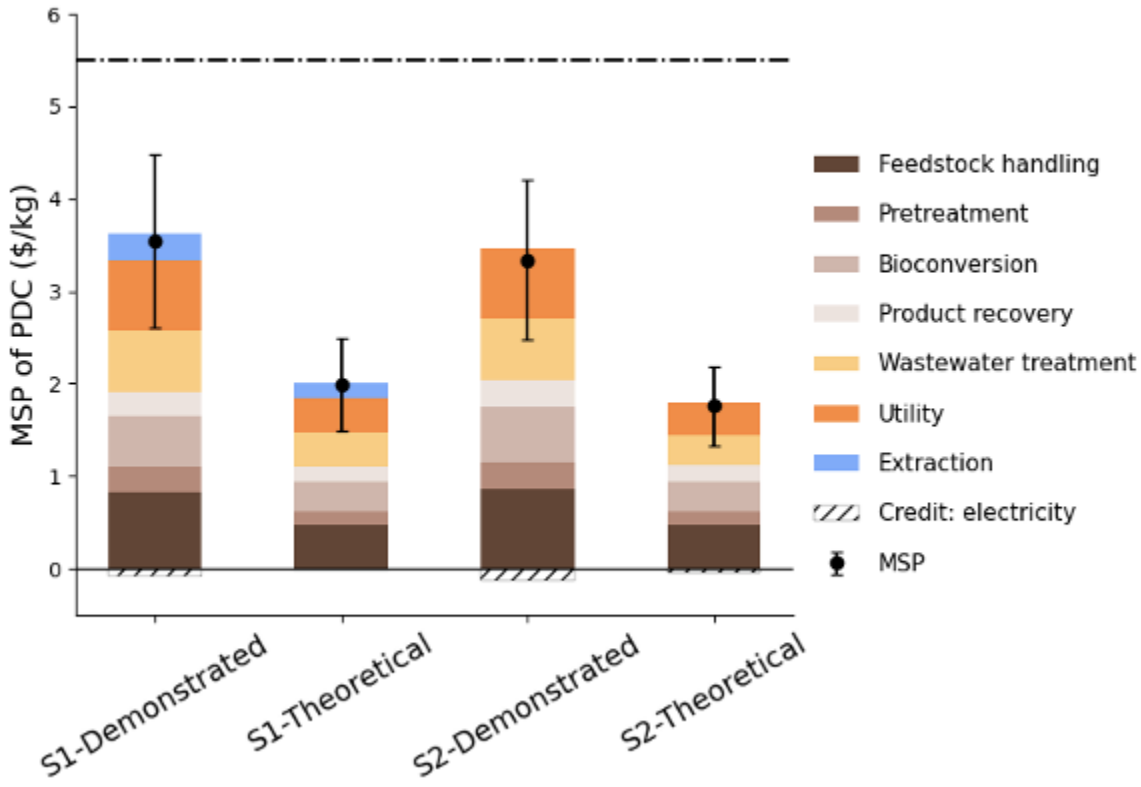

Figure S4. Technoeconomic results of combining plant and microbial systems as an integrated biochemical plant considering PDC as the product. MSP: minimum selling price.

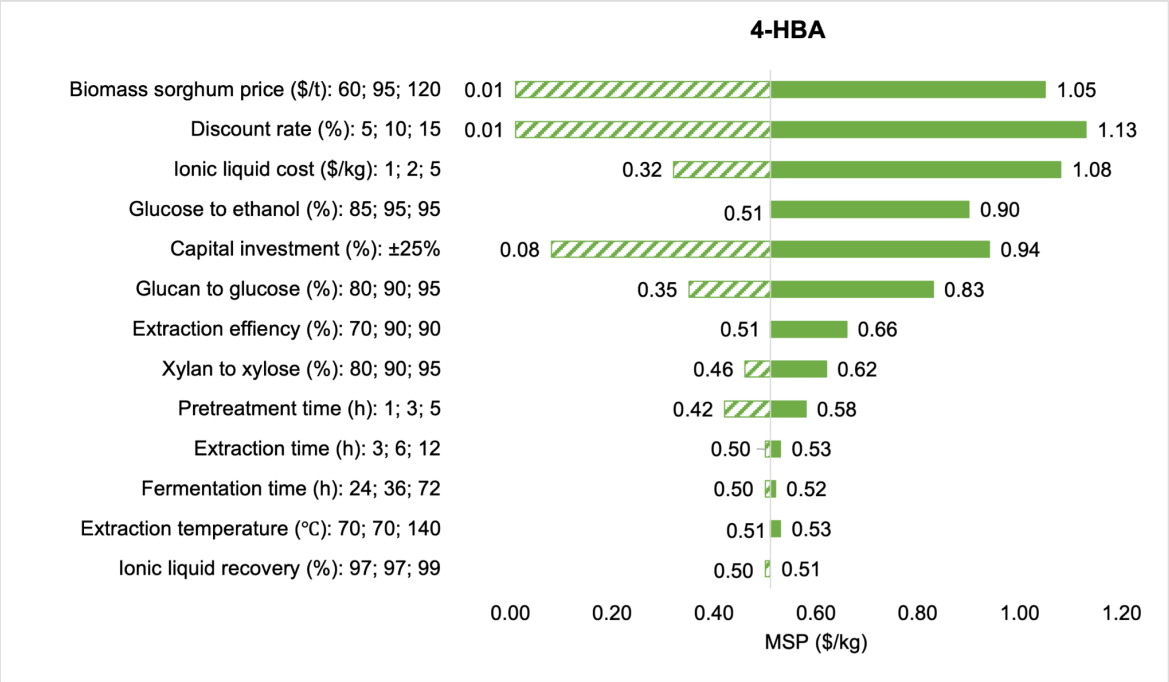

Figure S5a. Sensitivity analysis of 4-HBA production in the plant system.

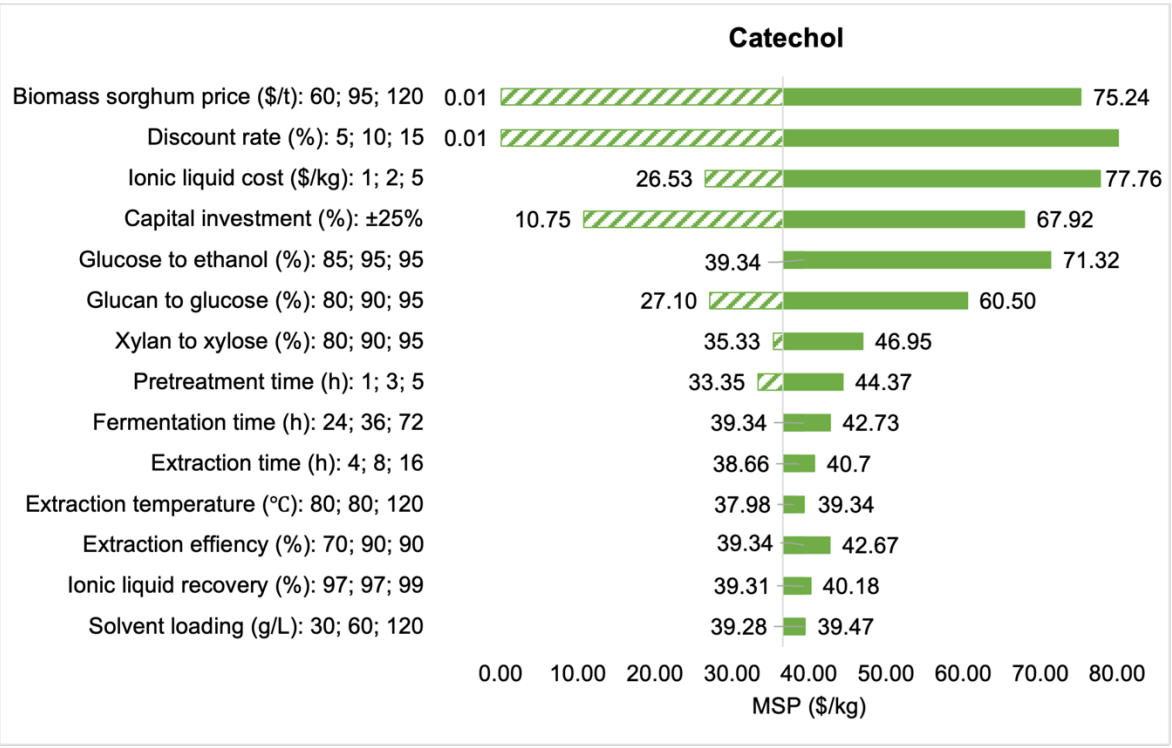

Figure S5b. Sensitivity analysis of catechol production in the plant system.

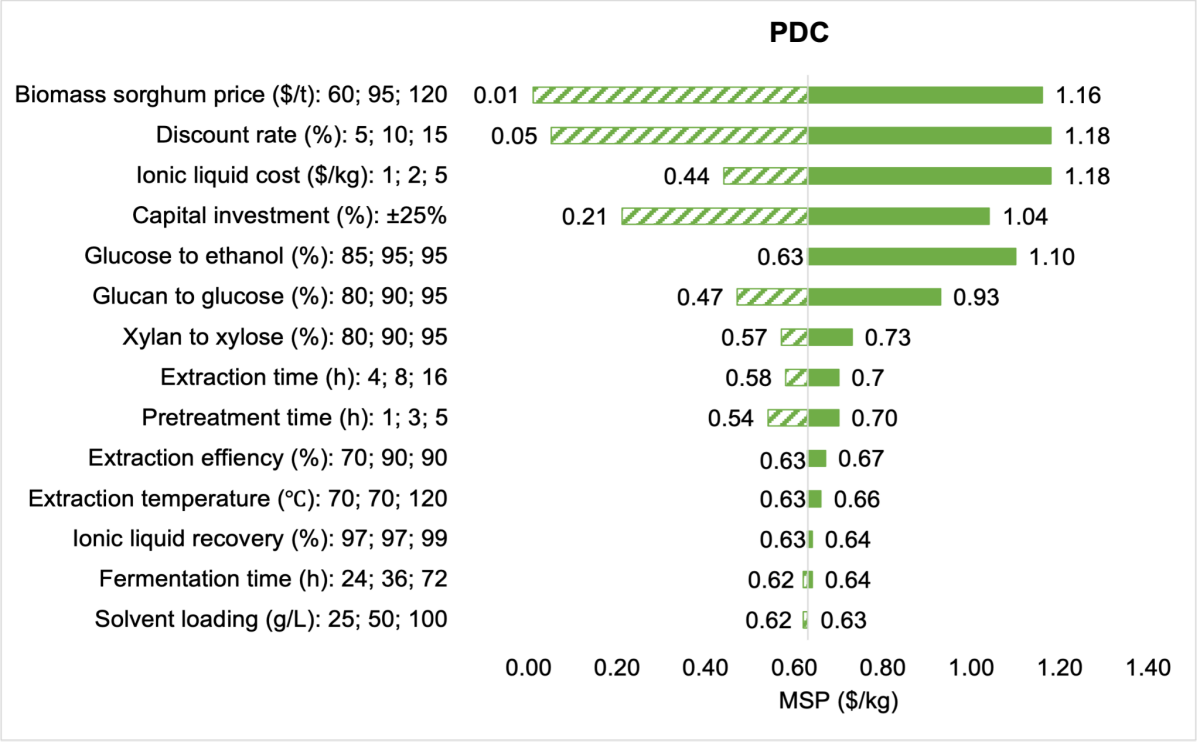

Figure S5c. Sensitivity analysis of PDC production in the plant system.

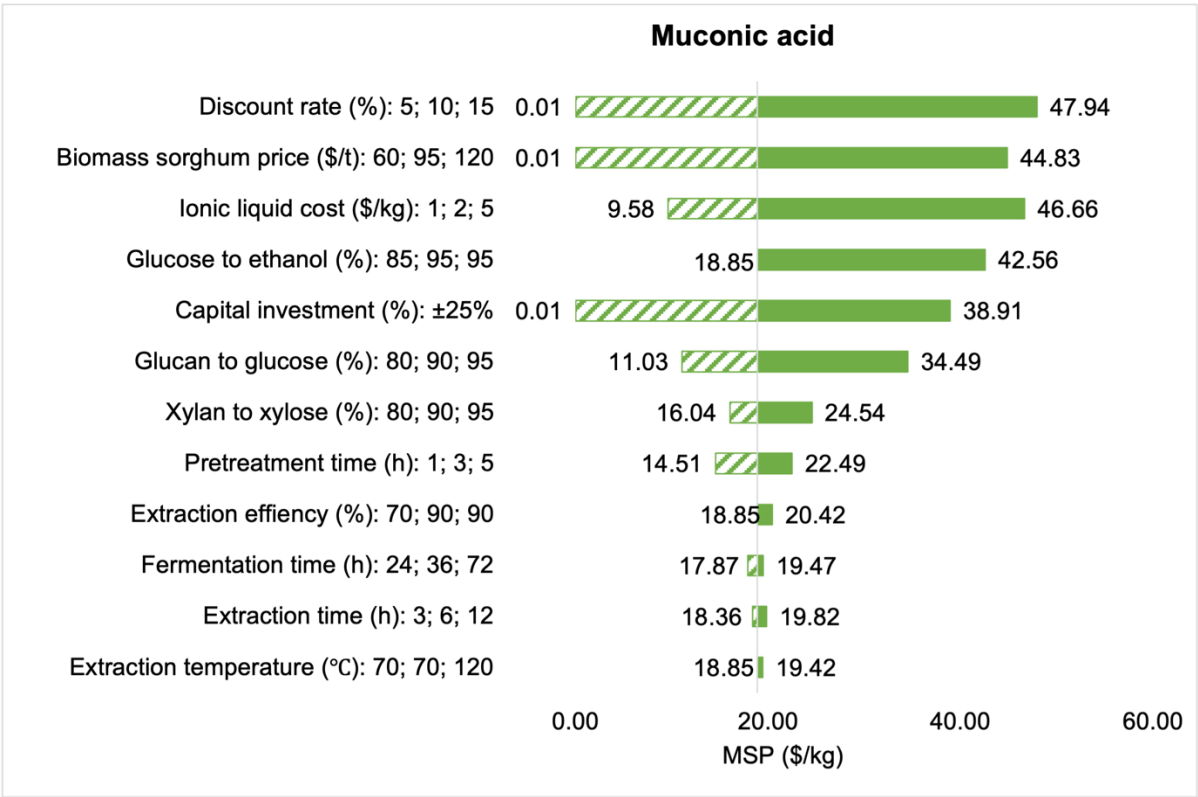

Figure S5d. Sensitivity analysis of muconic acid in the plant systems.

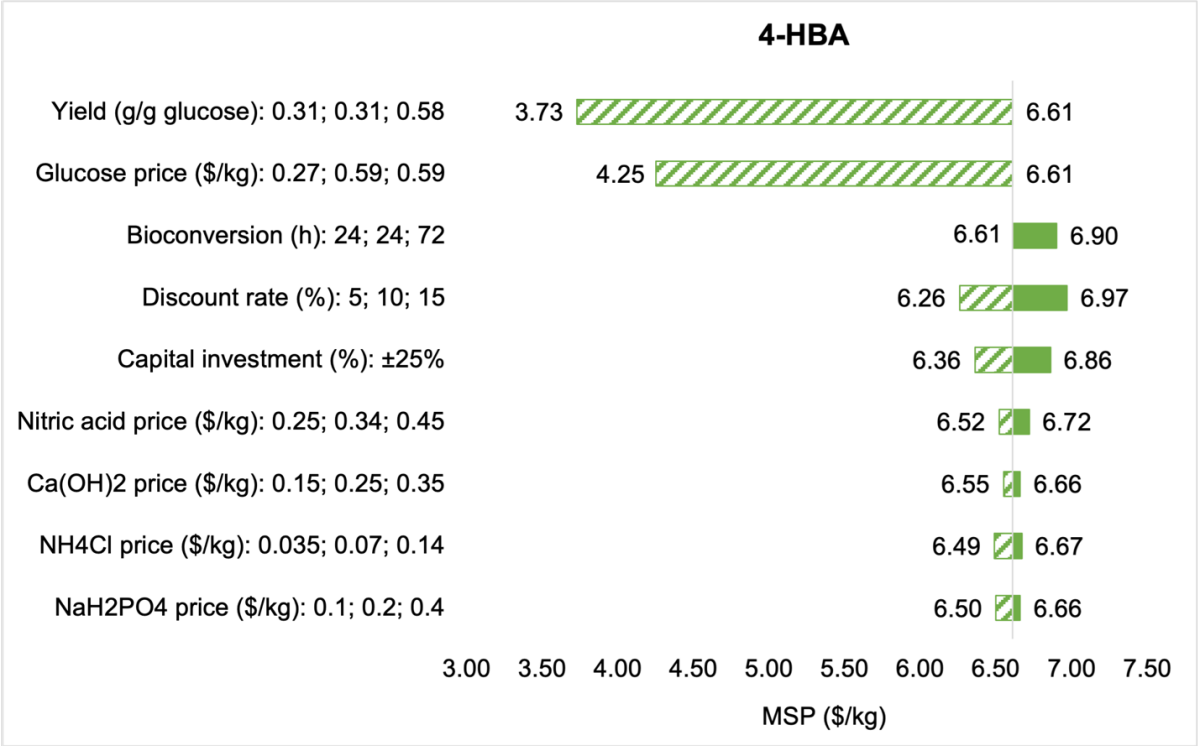

Figure S6a. Sensitivity analysis of 4-HBA production in the microbial system.

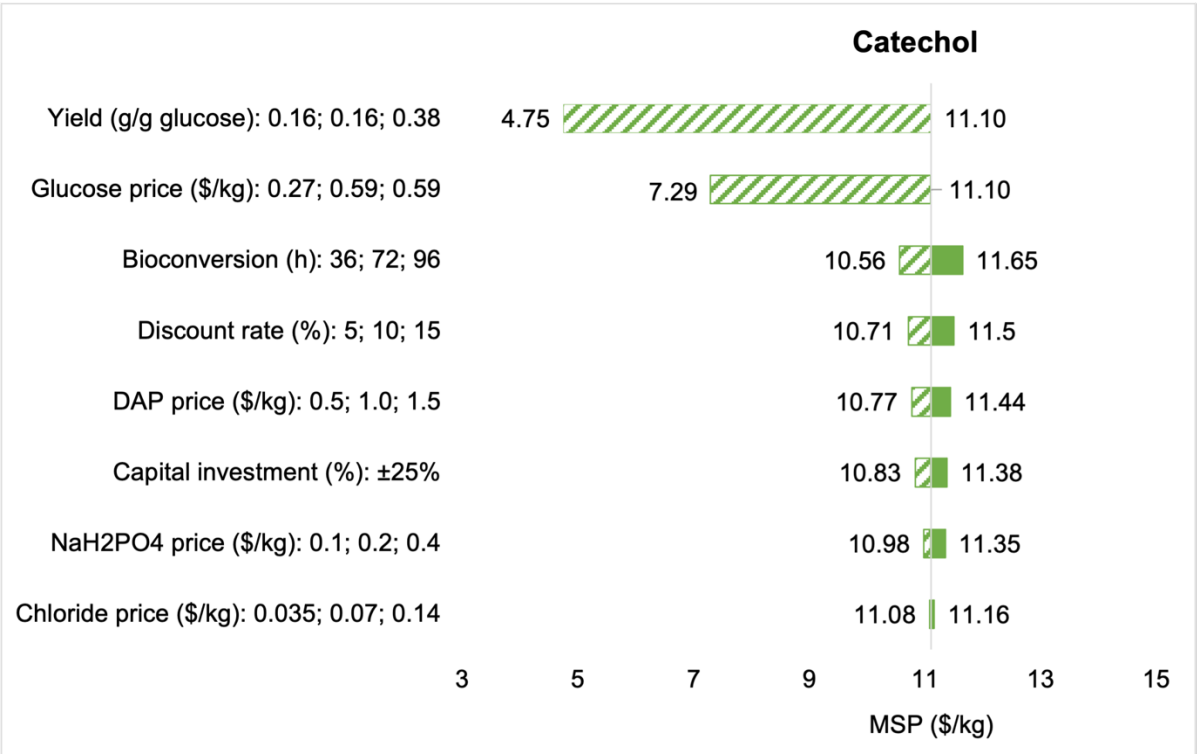

Figure S6b. Sensitivity analysis of catechol production in the microbial system.

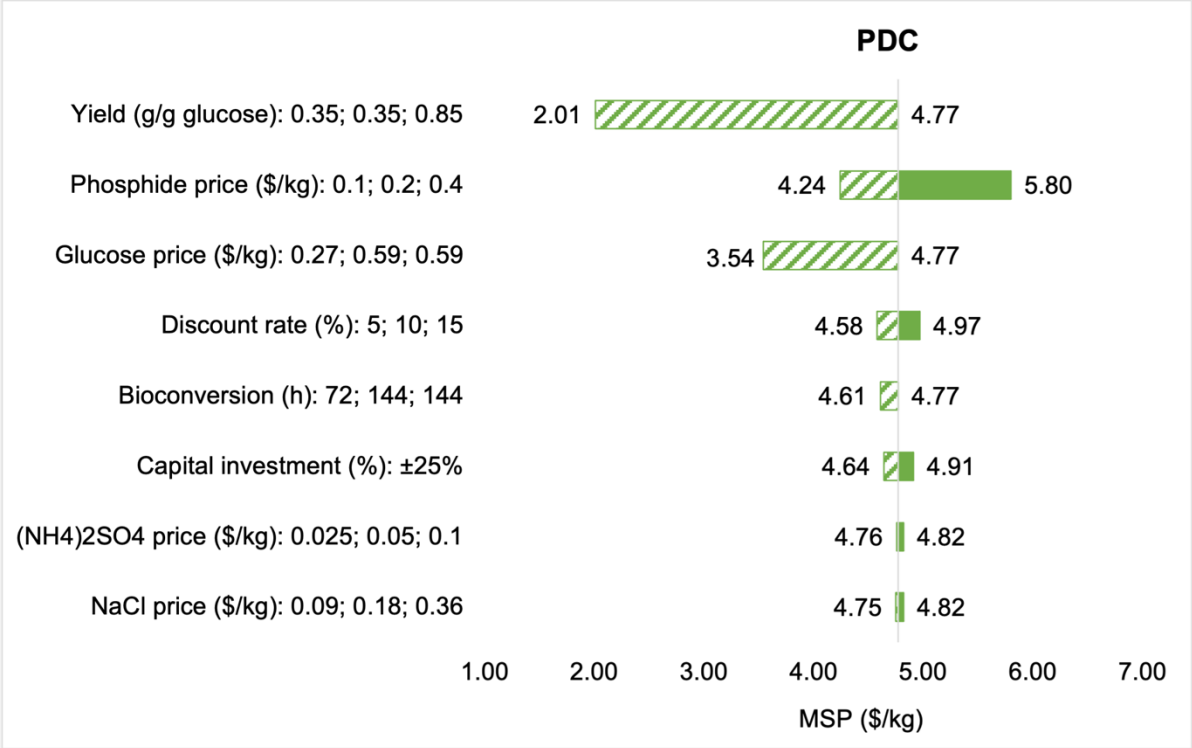

Figure S6c. Sensitivity analysis of PDC production in the microbial system.

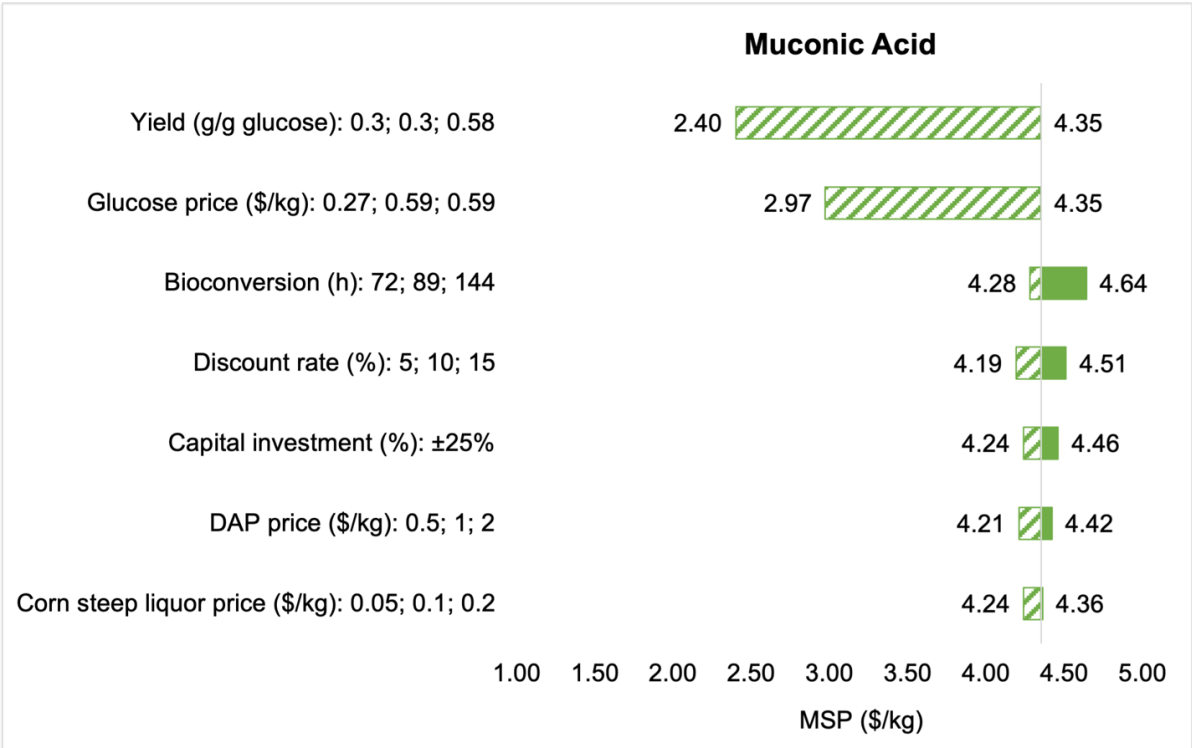

Figure S6d. Sensitivity analysis of muconic acid production in the microbial system.

SI References

1. M. Yang, N. R. Baral, A. Anastasopoulou, H. M. Breunig, C. D. Scown, Cost and Life-Cycle Greenhouse Gas Implications of Integrating Biogas Upgrading and Carbon Capture Technologies in Cellulosic Biorefineries. *Environ. Sci. Technol.* **54**, 12810–12819 (2020).

2. M. Yang, N. R. Baral, B. A. Simmons, J. C. Mortimer, P. M. Shih, C. D. Scown, Accumulation of high-value bioproducts in planta can improve the economics of advanced biofuels. *Proc Natl Acad Sci USA* **117**, 8639–8648 (2020).

3. J. Dahlberg, E. Wolfrum, B. Bean, W. L. Rooney, Compositional and agronomic evaluation of sorghum biomass as a potential feedstock for renewable fuels. *J. Biobased Mat. Bioenergy* **5**, 507–513 (2011).

4. F. Xu, J. Sun, N. V. S. N. M. Konda, J. Shi, T. Dutta, C. D. Scown, B. A. Simmons, S. Singh, Transforming biomass conversion with ionic liquids: process intensification and the development of a high-gravity, one-pot process for the production of cellulosic ethanol. *Energy Environ. Sci.* **9**, 1042–1049 (2016).

5. D. Humbird, *et al.*, “Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover” (National Renewable Energy Laboratory (NREL), 2011) https:/doi.org/10.2172/1013269.

6. U.S. EIA, “Electricity Power Annual 2019 ” (U.S. EIA, 2021).

7. Intelligen, *SuperPro Designer* (Intelligen, 2020).

8. J. O. Krömer, R. G. Ferreira, D. Petrides, N. Kohlheb, Economic Process Evaluation and Environmental Life-Cycle Assessment of Bio-Aromatics Production. *Front. Bioeng. Biotechnol.* **8**, 403 (2020).

9. R. B. McQualter, B. F. Chong, K. Meyer, D. E. Van Dyk, M. G. O’Shea, N. J. Walton, P. V. Viitanen, S. M. Brumbley, Initial evaluation of sugarcane as a production platform for p-hydroxybenzoic acid. *Plant Biotechnol. J.* **3**, 29–41 (2005).

10. A. M. Morse, T. J. Tschaplinski, C. Dervinis, P. M. Pijut, E. A. Schmelz, W. Day, J. M. Davis, Salicylate and catechol levels are maintained in nahG transgenic poplar. *Phytochemistry* **68**, 2043–2052 (2007).

11. K. Wagemann, N. Tippkötter, Eds., *Biorefineries* (Springer International Publishing, 2019).

12. D. Roland-Holst, R. Triolo, S. Heft-Neal, B. Bayrami, “Bioplastics in California: Economic Assessment of Market Conditions for PHA/PHB Bioplastics Produced from Waste Methane” (California Department of Resource Recycling and Recovery, 2013).

13. C.-Y. Lin, K. M. Vuu, B. Amer, P. M. Shih, E. E.K. Baidoo, H. V. Scheller, A. Eudes, In-planta production of the biodegradable polyester precursor 2-pyrone-4,6-dicarboxylic acid (PDC): Stacking reduced biomass recalcitrance with value-added co-product. *Metab. Eng.* **66**, 148–156 (2021).

14. M. J. Kang, H. T. Kim, M-W. Lee, K-A. Kim, T. U. Khang, H. M. Song, S. J. Park, J. C. Joo, H. G. Cha, A chemo-microbial hybrid process for the production of 2-pyrone-4,6-dicarboxylic acid as a promising bioplastic monomer from PET waste. *Green Chem.* (2020) https:/doi.org/10.1039/D0GC00007H.

15. A. Eudes, R. Berthomieu, Z. Hao, N. Zhao, V. T. Benites, E. E. K. Baidoo, D. Loqué, Production of muconic acid in plants. *Metab. Eng.* **46**, 13–19 (2018).

16. R. Kumar, *Valorization Of Lignocellulosic Biomass In A Biorefinery: From Logistics To Environmental And Performance Impact (biochemistry Research Trends)* (Nova Science Publishers, Inc., 2016).

17. USDA, USDA ERS - Sugar and Sweeteners Yearbook Tables (2021) (April 5, 2021).

18. C. W. Johnson, D. Salvachúa, N. A. Rorrer, B. A. Black, D. R. Vardon, P. C. St.John, N. S. Cleveland, G. Dominick, J. R. Elmore, N. Grundl, P. Khanna, C. R. Martinez, W. E. Michener, D. J. Peterson, K. J. Ramirez, P. Singh, T. A. VanderWall, A. N. Wilson, X. Yi, M. J. Biddy, Y. J. Bomble, A. M. Guss, G. T. Beckham, Innovative Chemicals and Materials from Bacterial Aromatic Catabolic Pathways. *Joule* (2019) https:/doi.org/10.1016/j.joule.2019.05.011.

19. Y. Kitade, R. Hashimoto, M. Suda, K. Hiraga, M. Inui, Production of 4-Hydroxybenzoic Acid by an Aerobic Growth-Arrested Bioprocess Using Metabolically Engineered Corynebacterium glutamicum. *Appl. Environ. Microbiol.* **84** (2018).

20. J. O. Krömer, D. Nunez-Bernal, N. J. H. Averresch, J. Hampe, J. Varela, C. Varela, Production of aromatics in Saccharomyces cerevisiae--a feasibility study. *J. Biotechnol.* **163**, 184–193 (2013).

21. V. E. Balderas-Hernández, L. G. Treviño-Quintanilla, G. Hernández-Chávez, A. Martinez, F. Bolívar, G. Gosset, Catechol biosynthesis from glucose in Escherichia coli anthranilate-overproducer strains by heterologous expression of anthranilate 1,2-dioxygenase from Pseudomonas aeruginosa PAO1. *Microb. Cell Fact.* **13**, 136 (2014).

22. NREL, Process Design and Economics for the Conversion of Lignocellulosic Biomass to Hydrocarbon Fuels and Coproducts:2018 Biochemical Design Case Update (2018) (July 15, 2019).

23. Bioenergy Technologies Office, “Multi-Year Program Plan” (U.S. Department of Energy, 2016).

24. U.S. EIA, U.S. Regular Gasoline Rack Sales Price by Refiners (Dollars per Gallon) (2020) (July 20, 2020).