Supplementary Information

Accumulation of high-value bioproducts *in planta* can improve the economics of advanced biofuels

Minliang Yang[a,b], Nawa Raj Baral[a,b], Blake A. Simmons[b,c], Jenny C. Mortimer[d,e], Patrick M. Shih[d,e,f*], Corinne D. Scown[a,b,g*]

[a] Life-cycle, Economics, and Agronomy Division, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
[b] Biological Systems and Engineering Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
[c] Deconstruction Division, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
[d] Environmental Genomics & Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
[e] Feedstocks Division, Joint BioEnergy Institute, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
[f] Department of Plant Biology, University of California, Davis, California 95616, United States
[g] Energy Analysis and Environmental Impacts Division, Lawrence Berkeley National Laboratory, Berkeley, California 94720, United States
*Co-corresponding authors, E-mail: pmshih@ucdavis.edu; cdscown@lbl.gov.

www.pnas.org/cgi/doi/10.1073/pnas.2000053117

S1. Value-added bioproducts extraction process.

In this study, five value-added bioproducts (limonene, artemisinin, PHB, latex, and cannabidiol) have been selected to explore their potential impact on biorefinery economics.

The average market price of limonene used in this study is $4.28/kg (1) in 2015; this price has been highly volatile because it is co-produced from an agricultural product (citrus), ranging from as low as $0.40/kg (2) about a decade ago to $11.0/kg (2) in 2011. Several methods have been reported to extract limonene, such as steam explosion (3) and dilute acid process (4, 5). However, these methods are performed under harsh conditions, which are energy-intensive (6). These extraction methods are also undesirable because they may prematurely depolymerize the biomass feedstock, making bioproduct separation and purification more challenging (7, 8). Wikandari et al. employed hexane to extract limonene under mild conditions from citrus waste, which reduced energy inputs and increased product yield relative to harsher techniques (6). Negro et al. further confirmed that using hexane as the solvent in limonene extraction from orange peel resulted in higher limonene recovery (9). Therefore, we adopted hexane as the extraction method in limonene extraction in this study. However, hexane is toxic to microorganisms (concentration of 13 g/L was used in toxicity test (6)), which may inhibit ethanol production (6, 10). Thus, hexane residue should be evaporated as much as possible prior to downstream microbial conversion of biomass-derived sugars or lignin intermediates. Wikandari et al. found that hexane evaporation at 50°C for 10 minutes could reduce hexane concentration below toxicity concentration (6). Briefly, limonene is extracted from biomass sorghum by commercial hexane at 20°C for 30 minutes; the mass ratio of biomass sorghum and commercial hexane is 1: 2 on dry basis (6). After extraction, the mixture of commercial hexane and limonene is evaporated to remove hexane, which is then condensed and recycled (6). The purity of limonene obtained through extraction is assumed to be 98% as the purity of commercial limonene is about 90 - 98% (11).

The market price of artemisinin increased dramatically to $1,100/kg (12) in 2004 - 2005 due to unstable supply of *A.annua*; with appropriate mechanisms to reduce the price fluctuation, such as a system of guaranteed demand, the market price of artemisinin is estimated sustainably to be $250/kg - $300/kg (12) although a lower market price of $170/kg in 2007 was reported (13). Using hexane to extract artemisinin from *A.annua* is widely used in main *A.annua* growing and processing countries including China and Vietnam (14). Even though using hexane as the extraction solvent has advantages like low capital investment and simplicity technician, the extraction efficiency of artemisinin in large scale processing is fairly low (62 - 70%) (14). Future research is needed in developing more favorable extraction method, which should be more environmentally friendly and higher artemisinin recovery rate (14). Industrial artemisinin purification involves filtration through silica filter or activated carbon filter to remove wax and other impurities and crystallization purified artemisinin (15). In this study, artemisinin is extracted from biomass sorghum by commercial hexane at 40°C for 8 hours; the mass ratio of biomass sorghum and commercial hexane is 1: 4 on dry basis (14). After extraction, the mixture of commercial hexane and artemisinin is evaporated to remove hexane, which is then condensed and recycled (6). Artemisinin is further filtered through gel filtration in order to remove impurities (15). After filtration, artemisinin is condensed for crystallization (15). The purity of artemisinin obtained through extraction is assumed to be 99% (15).

In this study, the market price of polypropylene (PP) was used as the price of PHB because eventually PHB produced in a biorefinery will compete with conventional plastics. In 2017, PP was sold in the range of \$1.7–\$3.8/kg in the U.S. with an average price of \$3.5/kg (16). Currently, most PHBs are extracted by halogenated organic solvents like chloroform (17). However, chloroform is hazardous to water resources and likely to be carcinogenic to humans (18). Thus, a non-halogenated organic solvent suitable for industrial application should be developed. Even though the optimization of the process conditions of PHB extraction using non-toxic solvents on industrial scale is still needed, using butyl acetate, a non-halogenated solvent, in bench scale exhibited high PHB recovery rate and purity (17). In this study, we employed this method as it has the potential to be used in the future industry plant. Similar to hexane, lab results indicated that when butyl acetate concentrations > 4 g/L are toxic to *E.coli* and should be maintained below 2.5 g/L (19). Therefore, butyl acetate should be removed as much as possible after extraction in order to have minimum impact on downstream fermentation process. Briefly, PHB is extracted from biomass sorghum by butyl acetate at 103°C for 30 minutes; the ratio of biomass sorghum and butyl acetate is 1g (dry basis): 100mL (17). After extraction, the mixture of butyl acetate and PHB is centrifuged and distilled to remove butyl acetate, which is then condensed and recycled. PHB is further filtered through membrane filtration and acetone is added to precipitate PHB; the precipitated PHB is washed with water and stored in tanks (17). The purity of PHB obtained through extraction is assumed to be 99% (17).

The market price of natural latex from 2010 to 2018 varies between \$1.57/kg - \$4.82/kg with an average price of \$2.57/kg (20). It is not practical to collect latex in industry by tapping as in natural rubber tree; therefore, a flow method that parallels commercial extraction process is developed since latex can be extracted by flowing latex in extraction medium (21, 22). In this study, the flow method using aqueous extraction solvent is also chosen in latex extraction process. Briefly, latex is extracted from biomass sorghum by ice cold extraction buffer (0.1%

Na_2SO_3, 0.2% NH_3 and 0.1% casein) at room temperature for 20 minutes; the ratio of biomass sorghum and extraction buffer is 1g (dry basis): 2mL (21). After extraction, the mixture of extraction buffer and latex is centrifuged for five times in order to collect latex as much as possible (21). The purity of latex obtained through extraction is assumed to be 99% (21).

The market price of cannabidiol (CBD) is obtained from hemp-derived CBD oil reported in 2018 (23). Industry cannabidiol extraction technologies include thermal extraction, alcohol extraction and supercritical carbon dioxide extraction (24). However, thermal process like pyrolysis(25) and supercritical carbon dioxide process (26) have been used in biomass pretreatment. In this study, methanol is chosen as the major extraction solvent because methanol achieved higher cannabidiol recovery compared to ethanol (27). Briefly, cannabidiol is extracted from biomass sorghum by a mixture of methanol and hexane (9:1) at room temperature for 30 minutes (28, 29); the ratio of biomass sorghum and extraction solvent is 15mg (dry basis): 1mL (28). After extraction, the mixture of extraction solvent and cannabidiol is centrifuged to collect cannabidiol; the extraction solvent is further distilled to separate methanol and hexane. The purity of cannabidiol obtained through extraction is assumed to be 99% (30).

Table S1. Current market price ($/kg) and probability distribution of selected bioproducts.

Compound	Market price ($/kg)				Source
	Average	Minimum	Maximum	Distribution	
Artemisinin	250	170	1,100	Triangular	(12, 13)
Cannabidiol	90	60	200	Triangular	(23)
Limonene	4.28	0.40	11.00	Triangular	(1, 2)
PHB	3.48	1.70	3.80	Triangular	(16)
Latex	2.59	1.57	4.82	Triangular	(20)

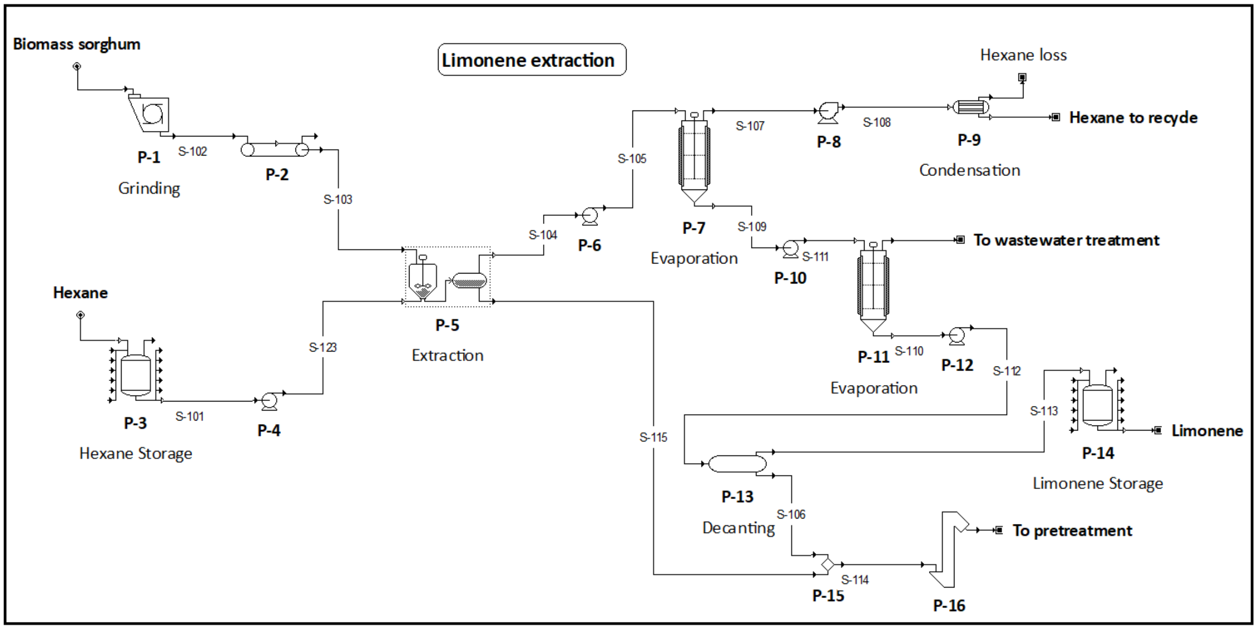


Figure S1. An overview of limonene extraction process.

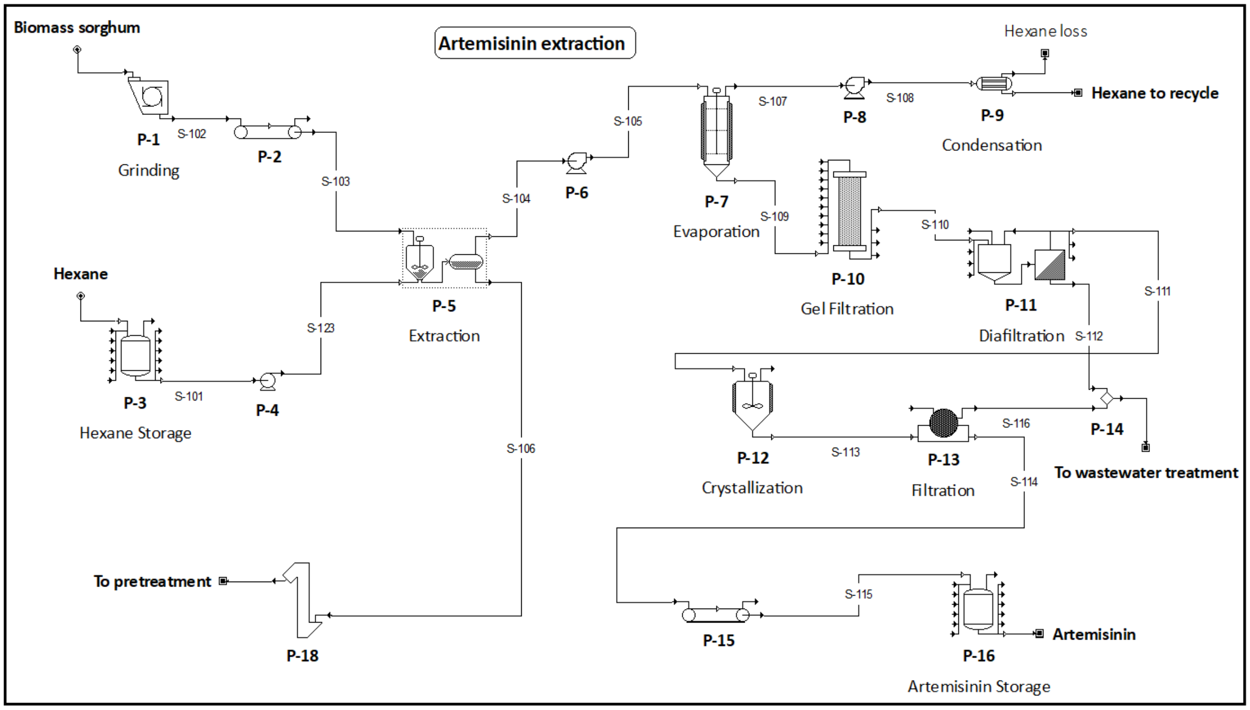


Figure S2. An overview of artemisinin extraction process.

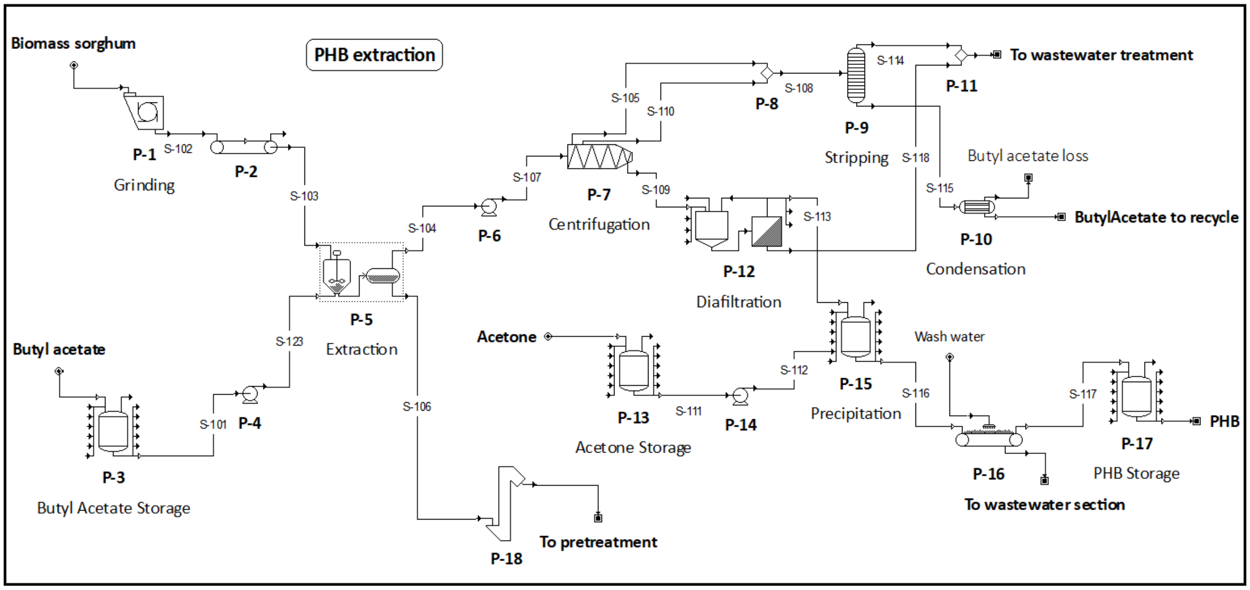


Figure S3. An overview of PHB extraction process.

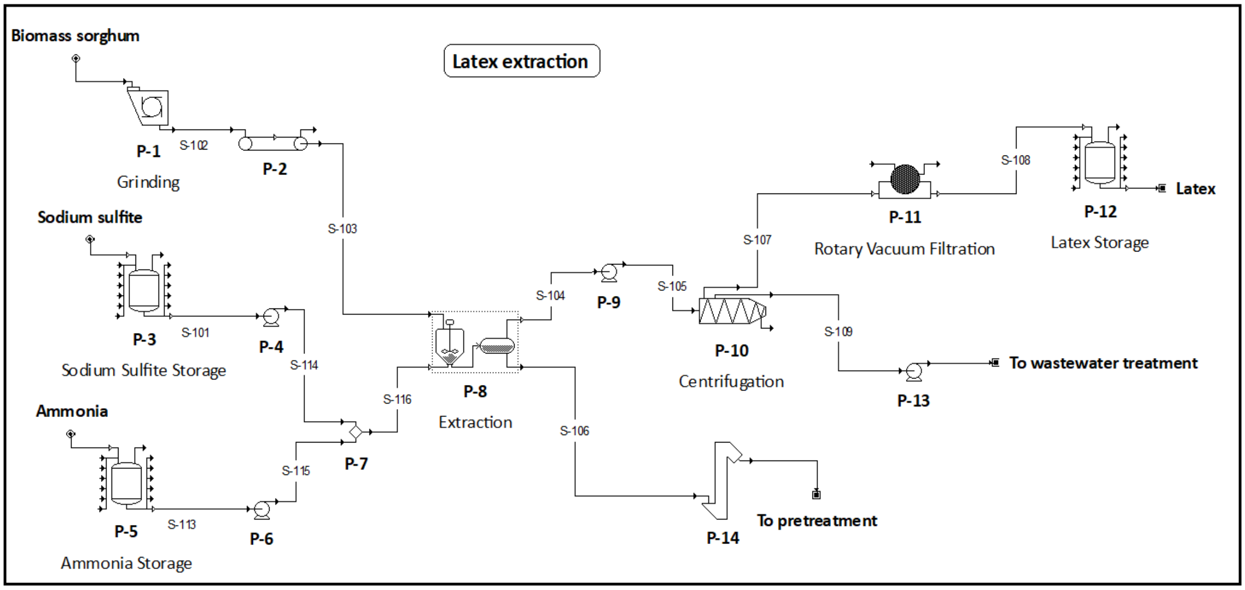


Figure S4. An overview of latex extraction process.

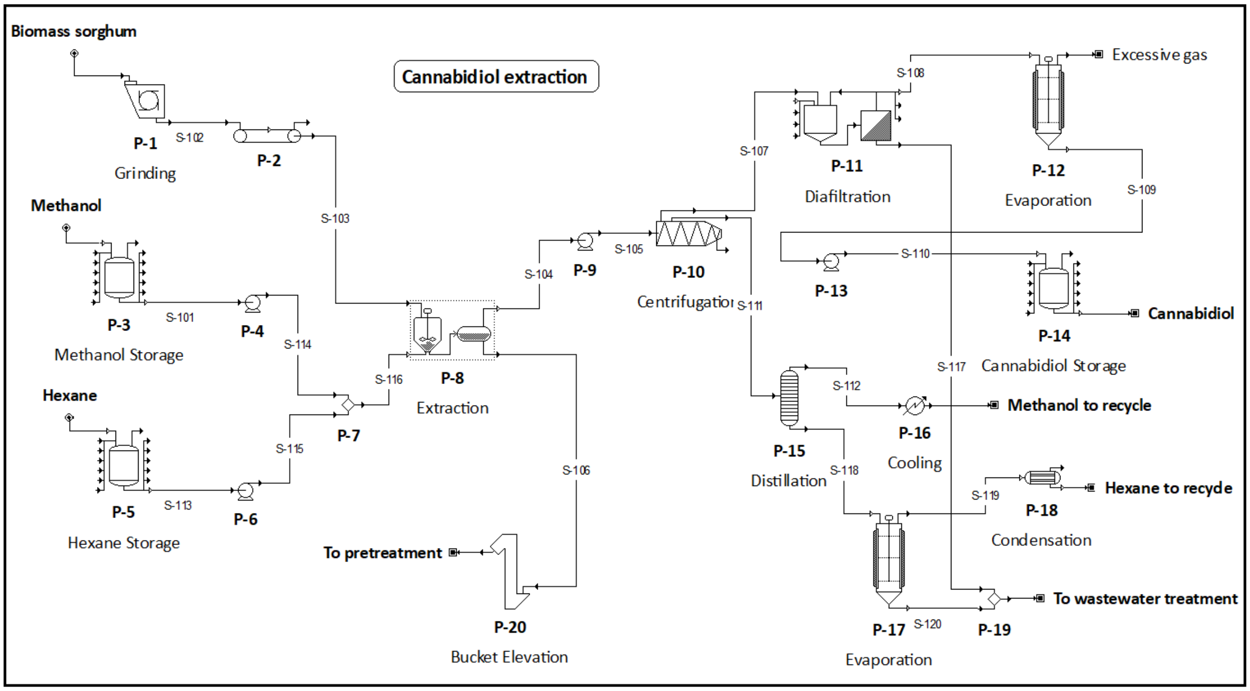


Figure S5. An overview of cannabidiol extraction process.

S2. Bioethanol production using the integrated one-pot high gravity ionic liquid pretreatment process.

In this study, biomass sorghum is chosen as the representative of bioenergy crops since it is a promising near-term feedstock for biofuel production due to its high yield (up to 24.8 metric ton per hectare) (31) and drought tolerance characteristics (32). Among all pretreatment methods, we selected ionic liquid (IL) pretreatment method in this study because it is proven to be effective for biomass pretreatment (33) and most ILs are green solvents due to low flammability and low toxicity (34). Additionally, using [Ch][Lys] has the potential to reduce greenhouse gas emissions by around 45% per megajoule of fuel (35). Potential challenges in reducing minimum ethanol selling price associated with IL pretreatment process include high IL purchasing price and IL recycling rate (36).

Table S2. Input parameters used for the techno-economic modeling and sensitivity analyses.

Parameter	Unit	Average	Minimum	Maximum
Biomass sorghum price (37)	$/dry metric ton	95.0	76.5	114.7
Structural composition of biomass sorghum (38)				
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		
Value-added compound extraction process				
Limonene				
Extraction time (6)	min	10	10	30
Extraction temperature (6)	°C	20	20	40
Solvent price (39)	$/kg	0.4	0.2	0.5

Parameter	**Unit**	**Average**	**Minimum**	**Maximum**
Solvent to biomass ratio (6)	g/g (dry basis)	2	1	4
Limonene loss during extraction	%	5	1	10
Biomass sorghum loss during extraction	%	1	1	10
Artemisinin				
Extraction time (14)	h	8	8	10
Extraction temperature (14)	°C	40	30	40
Solvent price (39)	$/kg	0.4	0.2	0.5
Solvent to biomass ratio (14)	g/g (dry basis)	4	2	5
Artemisinin loss during extraction	%	5	1	10
Biomass sorghum loss during extraction	%	1	1	10
PHB				
Extraction time (17)	min	15	30	60
Extraction temperature (17)	°C	103	50	103
Solvent price (39)	$/kg	1.08	0.80	1.20
Solvent to biomass ratio (17)	mL/g (dry basis)	100	50	100
PHB loss during extraction	%	5	1	10
Biomass sorghum loss during extraction	%	1	1	10
Latex				
Extraction time (21)	min	30	15	45

Parameter	**Unit**	**Average**	**Minimum**	**Maximum**
Extraction temperature (21)	°C	20	20	30
Solvent price (39, 40)	$/kg	1.15	0.86	1.44
Solvent to biomass ratio (21)	mL/g (dry basis)	2	1	4
Latex loss during extraction	%	5	1	10
Biomass sorghum loss during extraction	%	1	1	10
Cannabidiol				
Extraction time (28)	min	30	15	45
Extraction temperature (28)	°C	20	20	30
Solvent price (39, 40)	$/kg	0.64	0.36	0.80
Solvent to biomass ratio (28)	L/g (dry basis)	0.07	0.01	0.15
Cannabidiol loss during extraction	%	5	1	10
Biomass sorghum loss during extraction	%	1	1	10
IL pretreatment process (33, 41)				
Solid loading rate	wt.%	30		
IL to biomass ratio	kg/kg (dry basis)	0.29		
IL cost	$/kg	2.00	1.43	5.00
Pretreatment time	h	3.00	1.00	5.00
Enzymatic hydrolysis and fermentation process (33, 41, 42)				
Enzyme loading rate	mg/g glucan	20.0		
Enzyme price	$/kg	4.29		
Glucan to glucose	wt.%	90.0	80.0	95.0

Parameter	**Unit**	**Average**	**Minimum**	**Maximum**
Xylan to xylose	wt.%	90.0	80.0	95.0
Hydrolysis time	h	72.0		
Corn steep liquor price	\$/kg	0.06		
Diammonium phosphate price	\$/kg	0.97		
Glucose conversion	wt.%	95	85	95
Xylose conversion	wt.%	85	75	85
Fermentation residence time	h	36	24	48
Product recovery, wastewater treatment, and lignin utilization process				
IL recovery (33)	wt.%	99.0	99.9	90.0
Natural gas price (43)	\$/m^3	0.16		
Electricity price (44)	\$/kWh	0.057		
Cooling water (39)	\$/ton	0.05		
Chilled water (39)	\$/ton	0.40		
Economic evaluation (42)				
Daily feedstock processed	dry metric ton/day	2,000		
Feedstock moisture content	wt.%	20.0		
Annual operating time	h	8,410		
Total capital investment	\$		-25%	+25%
Interest rate	%	10	5	15
Plant life	y	30		

Table S3. Installation multiplier for various equipment employed in developing technoeconomic models in *SuperPro Designer* (42).

Equipment	**Multiplier**
Aerobic digester	1.7
Anaerobic digester	1.7
Boiler	1.8
Bucket elevator	1.7
Centrifuge	1.7
Clarifier	1.7
Compressor	1.6
Condenser	1.7
Conveyor	1.7
Crystallizer	1.7
Distillation column, stainless steel	2.4
Extractor	1.7
Evaporator	1.7
Fermenter	1.7
Filters	1.7
Grinder	1.7
Heat exchanger, stainless steel	2.2
Pretreatment reactor	1.5
Pumps, stainless steel	2.3
Tanks, stainless steel	1.5
Tanks, storage, stainless steel	1.8
Turbogenerator	1.8

Table S4. Assumptions in quantifying total capital investment (TCI) (in 2018 million dollars) of biorefineries with co-production with various value-added bioproducts.

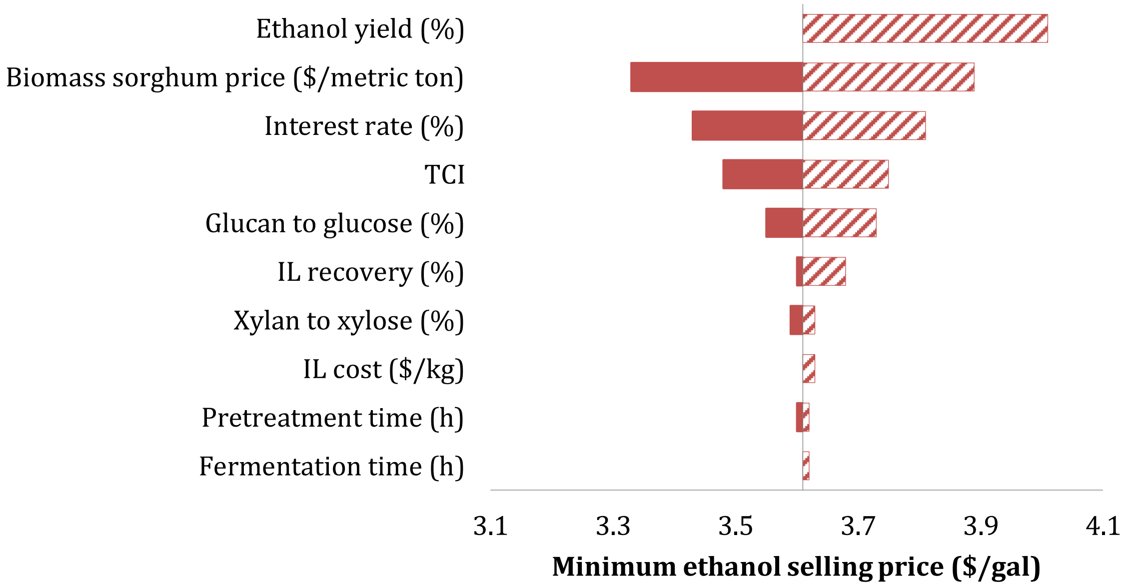
Parameters	Assumptions	Sources
1. Fixed capital investment (FCI)	TDC + TIC	
1) Total direct cost (TDC)	PC+W+S+P	
Installed equipment cost (PC)	Equipment purchase cost × Installation multiplier	(39) Table S3
Warehouse cost (W)	0.04 × IPC (excl. feedstock handling)	(39)
Site development cost (S)	0.09 × IPC (excl. feedstock handling)	(39)
Additional piping cost (P)	0.045 × IPC (excl. feedstock handling)	(39)
2) Total indirect cost (TIC)		
Prorateable expenses	0.10 × TDC	(39)
Field expenses	0.10 × TDC	(39)
Home office & construction fee	0.20 × TDC	(39)
Project contingency	0.10 × TDC	(39)
Other costs	0.10 × TDC	(39)
2. Land cost	$1,800,000	(39)
3. Working capital	0.05 × FCI	(39)
4. Start-up and validation cost	0.05 × FCI	(39)

Table S5. Total capital investment and annual operating cost (in 2018 million dollars) of biorefineries with co-production with various value-added bioproducts. In the base case scenario, the non-engineered biomass sorghum feedstock is utilized and no value-added bioproduct is produced.

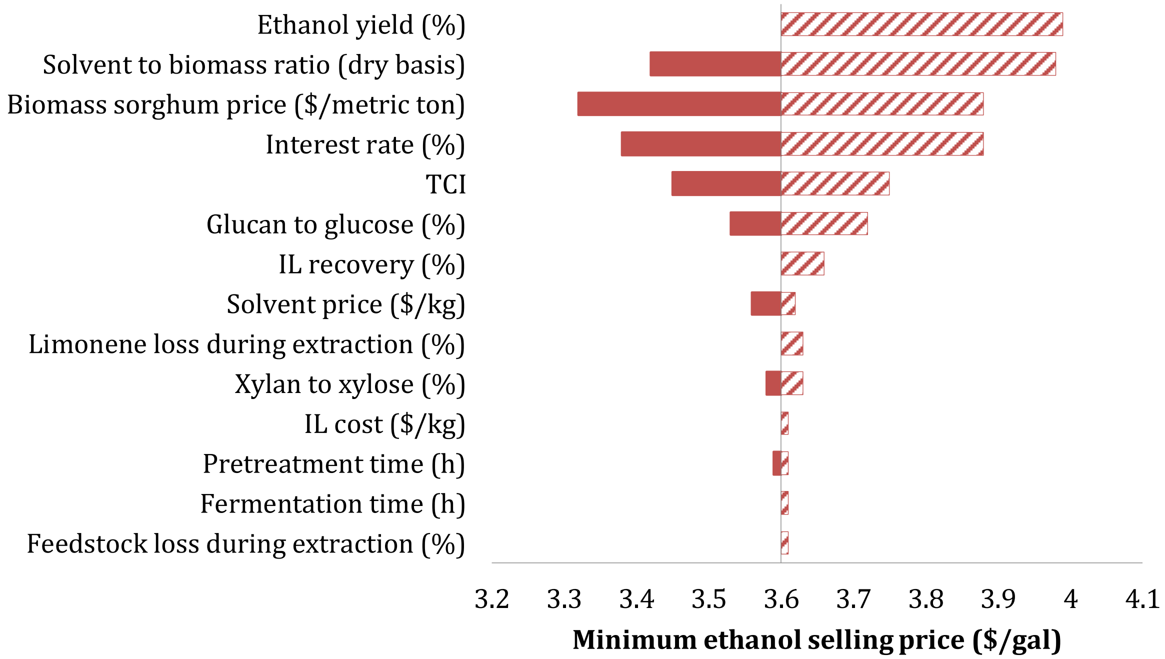
	Base case	Bioproduct				
		Limonene	Artemisinin	PHB	Latex	Cannabidiol
Total capital investment						
Feedstock handling	11	11	11	11	11	11
Extraction	0	16	27	15	19	12
Pretreatment	18	18	18	17	18	17
Fermentation	26	26	27	27	27	27
Recovery	28	28	28	26	28	28
Wastewater treatment	61	73	73	73	105	73
Utility	82	90	97	89	83	84
Annual operating cost						
Feedstock cost	84	84	84	84	84	84
Process materials cost	40	53	64	80	44	55
Utilities cost	62	83	99	83	70	70
Facility related cost	6	6	7	6	6	6

S3. Sensitivity analyses of biorefineries with co-production with value-added bioproducts on minimum ethanol selling price (MESP).

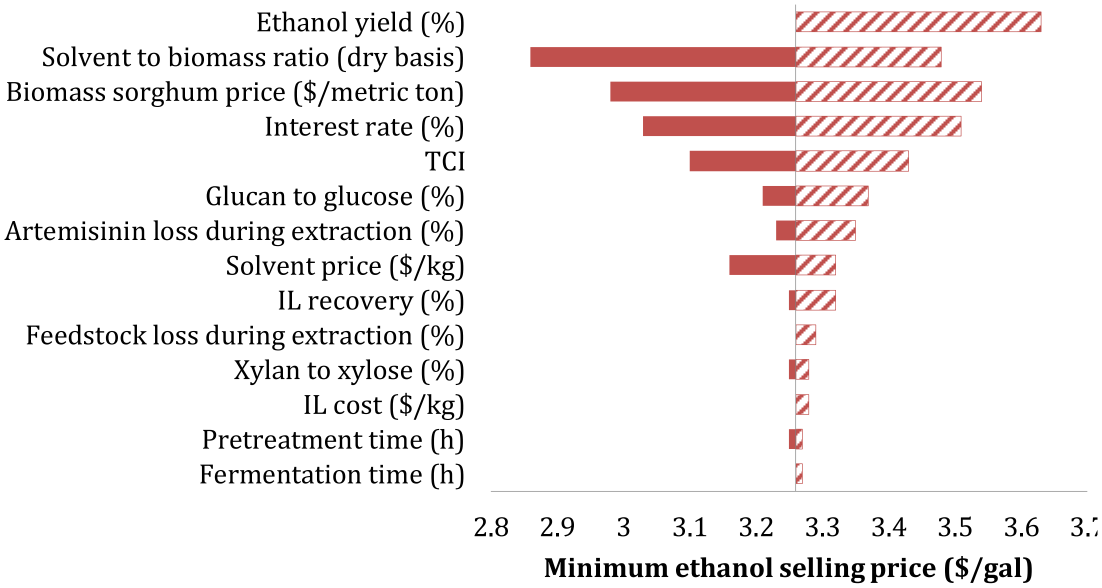
a. Base case scenario



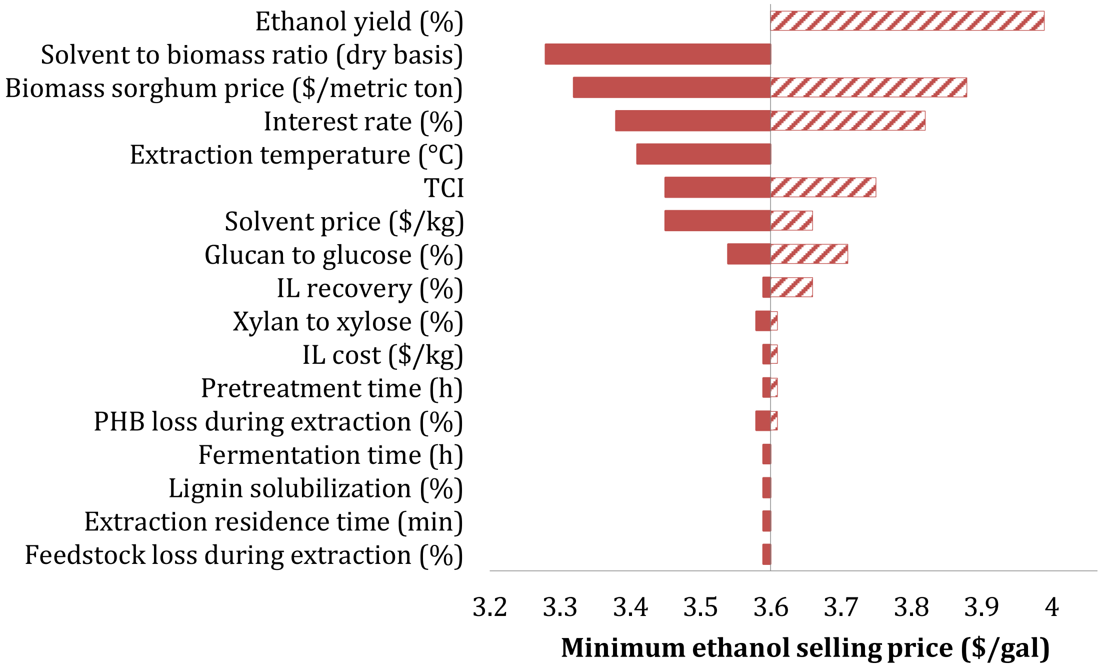
b. Biorefinery with co-production of limonene



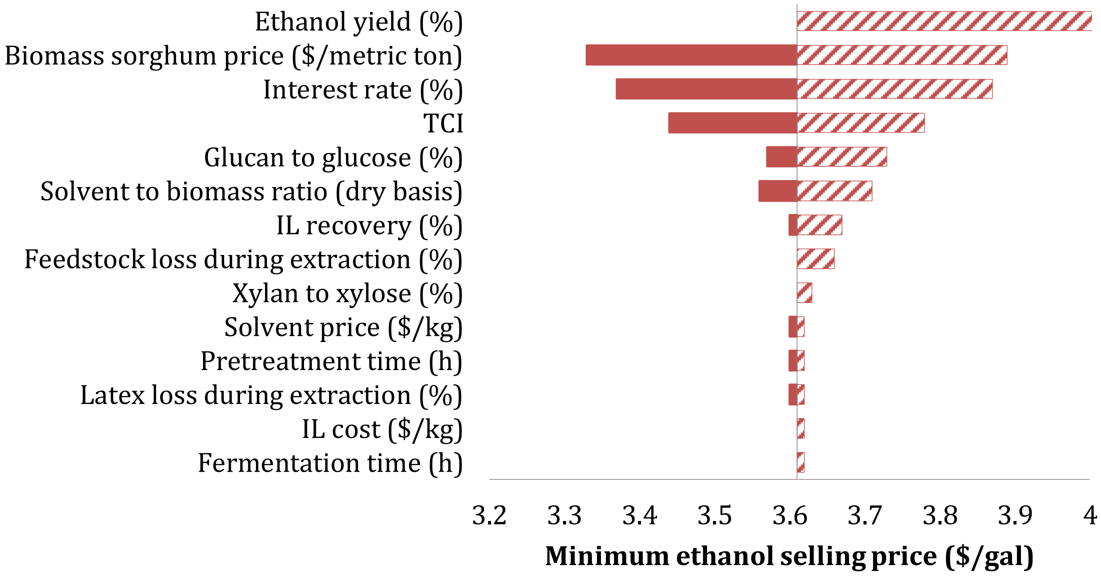
c. Biorefinery with co-production of artemisinin



d. Biorefinery with co-production of PHB



e. Biorefinery with co-production of latex



f. Biorefinery with co-production of cannabidiol (CBD)

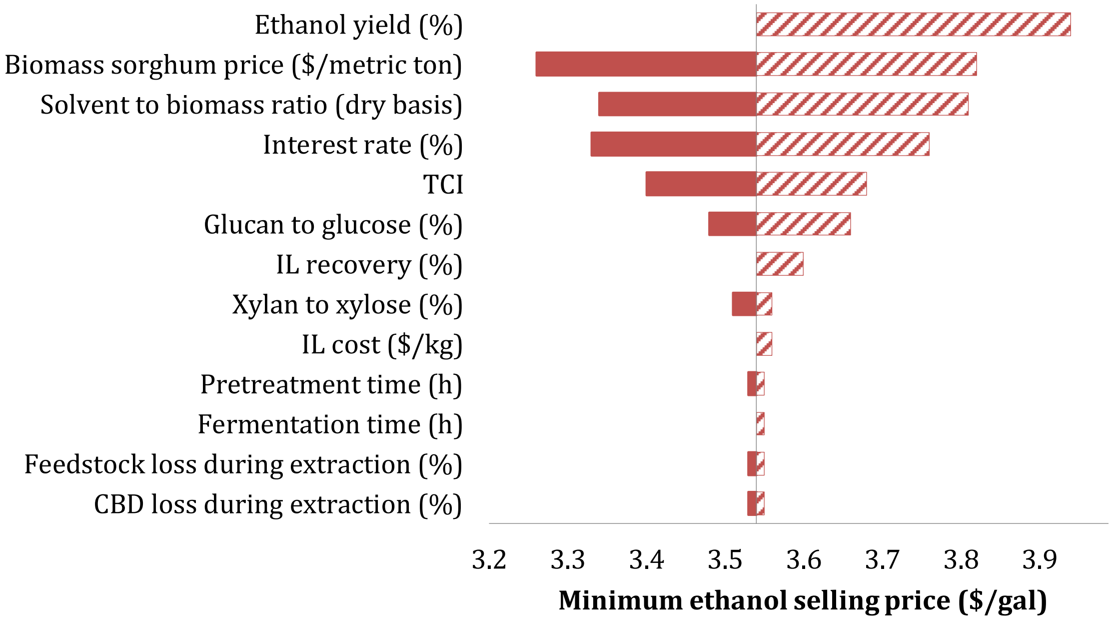


Figure S6. Sensitivity analyses of biorefineries with co-production of various bioproducts. The minimum ethanol selling price is calculated based on 90% extraction efficiency. In base case scenario, no compound is extracted from biomass sorghum as a coproduct. TCI refers to total capital investment. IL refers to ionic liquid; in this study, [Ch][Lys] is used in IL pretreatment process.

S4. Prediction of minimum selling price of any bioproduct of interest with different *in planta* accumulation level in bioenergy crops for reaching MESP parity ($3.61/gal) and MESP target ($2.50/gal).

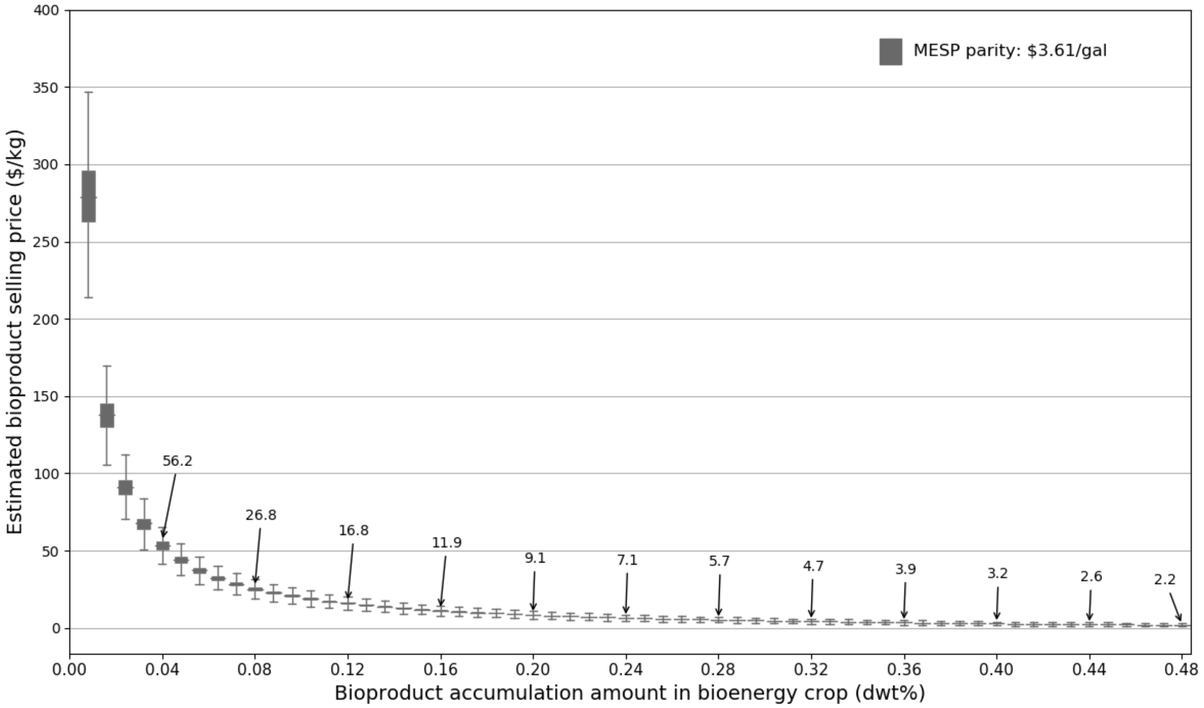


Figure S7. Selling price of any bioproduct of interest with different *in planta* accumulation amount in the minimum ethanol selling price (MESP) parity of $3.61/gal.

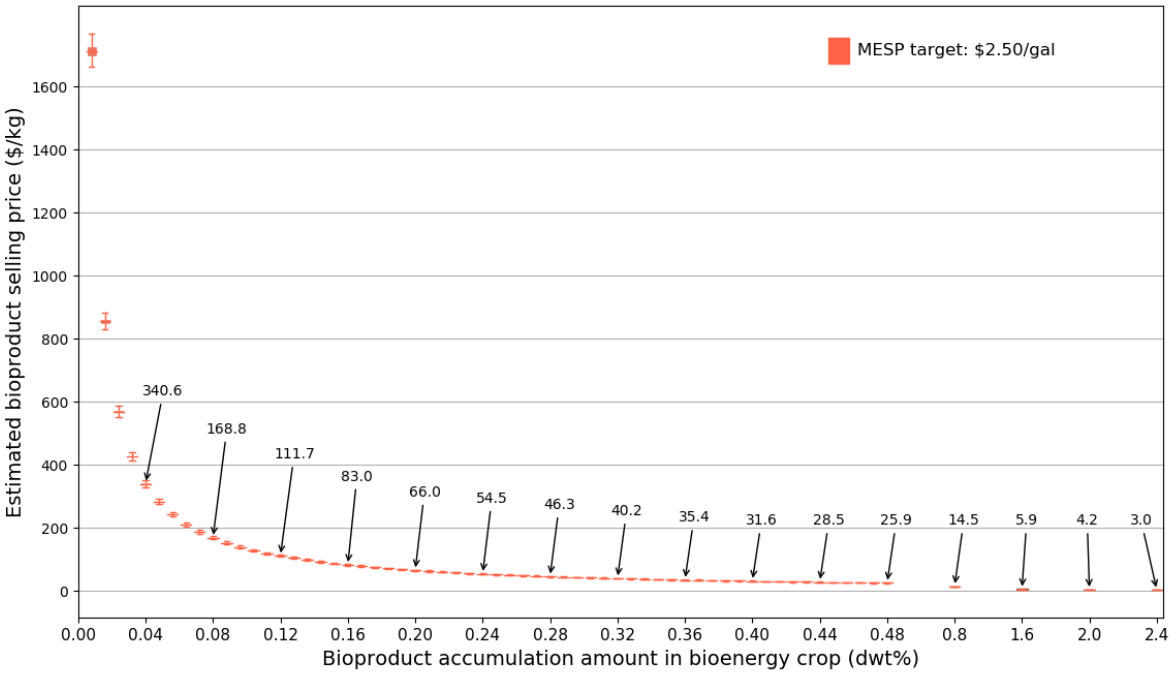


Figure S8. Selling price of any bioproduct of interest with different *in planta* accumulation amount in the targeted minimum ethanol selling price (MESP) of $2.50/gal.

S5. Example of *in planta* accumulation of an extremely high-value bioproduct.

Vinblastine, a chemotherapy medication first approved by the U.S. Food and Drug Administration in 1961, is included in this study because of its extremely high market price ($2 million/kg) (45), which is due to its low mass fraction in its natural source, *C. roseus*, and costly process required to separate vinblastine from the large number of alkaloids present in *C.roseus* (46). Semi-synthetic and microbial routes have been demonstrated, and may ultimately reduce production costs, but isolation from *C. roseus* remains the conventional production route (47).

In this study, vinblastine is assumed to be produced *in planta* in a hypothetical engineered plant without any other compositional changes, so this idealized case is not subject to many of the challenges of extraction from *C.roseus*. Traditional vinblastine extraction methods include hot water extraction, supercritical carbon dioxide extraction, and solvent extraction using methanol or dichloromethane (48). Solvent extraction method is chosen for this study because of the potential for hot water (49) and supercritical carbon dioxide (26) to prematurely depolymerize the biomass (both are biomass pretreatment methods). Dichloromethane is chosen as the extraction solvent because of its high efficiency and the fact that it is widely used in the pharmaceutical industry (48, 50). The extraction process of vinblastine from biomass sorghum is shown in Fig. S9. Briefly, vinblastine is extracted from biomass sorghum by dichloromethane at 39°C for 16 hours; the ratio of biomass sorghum and dichloromethane is 10mg (dry basis): 7mL (48). Three stages of extraction cycle are used due to its higher extraction efficiency (51). After extraction, dichloromethane is distilled from the mixture and recycled. Vinblastine is further filtered through gel filtration in order to remove other impurities, as employed in artemisinin purification. After filtration, vinblastine is crystallized, filtered and stored in tanks (52). The purity of vinblastine obtained through extraction is assumed to be 99% (53) .

Similar to biorefinery with co-production of artemisinin, vinblastine requires higher capital investment in the recovery process as well (~$26 million $). Previously reported production costs of vinblastine from *C. roseus* are high due to its low mass fraction in *C. roseus* and costly separation process required to isolate vinblastine from other alkaloids present in the plant (46). In this study, we assume the biomass, sorghum, can be engineered to accumulate only vinblastine, which reduces its separation cost. However, this simplifying assumption may not hold true in practice, in which case vinblastine recovery costs may be significantly higher than estimated in our results. Because of its extremely high market value ($2 million/kg), a small fraction of vinblastine accumulation *in planta* (< 0.01 dwt%) is sufficient to achieve the targeted ethanol selling price of $2.50/gal.

Vinblastine production, similar to cannabidiol, has not been reported as a product of any engineered plants. *C. roseus* is the major natural source of vinblastine. The leaves of *C. roseus* contain about 0.0011 dwt% vinblastine; with somatic embryo-generated plants, vinblastine yield in *C. roseus* leaves could increase to 0.0013 dwt% (54). Even if these low accumulation rates could be achieved in a bioenergy crop, particularly in the absence of other alkaloids that complicate the separations process, vinblastine would be an economically viable co-product at cellulosic biorefineries due to its extremely high market price. As for the impact on the global market size, vinblastine is an extreme case, as a small fraction of output from a single biorefinery would meet global market demand.

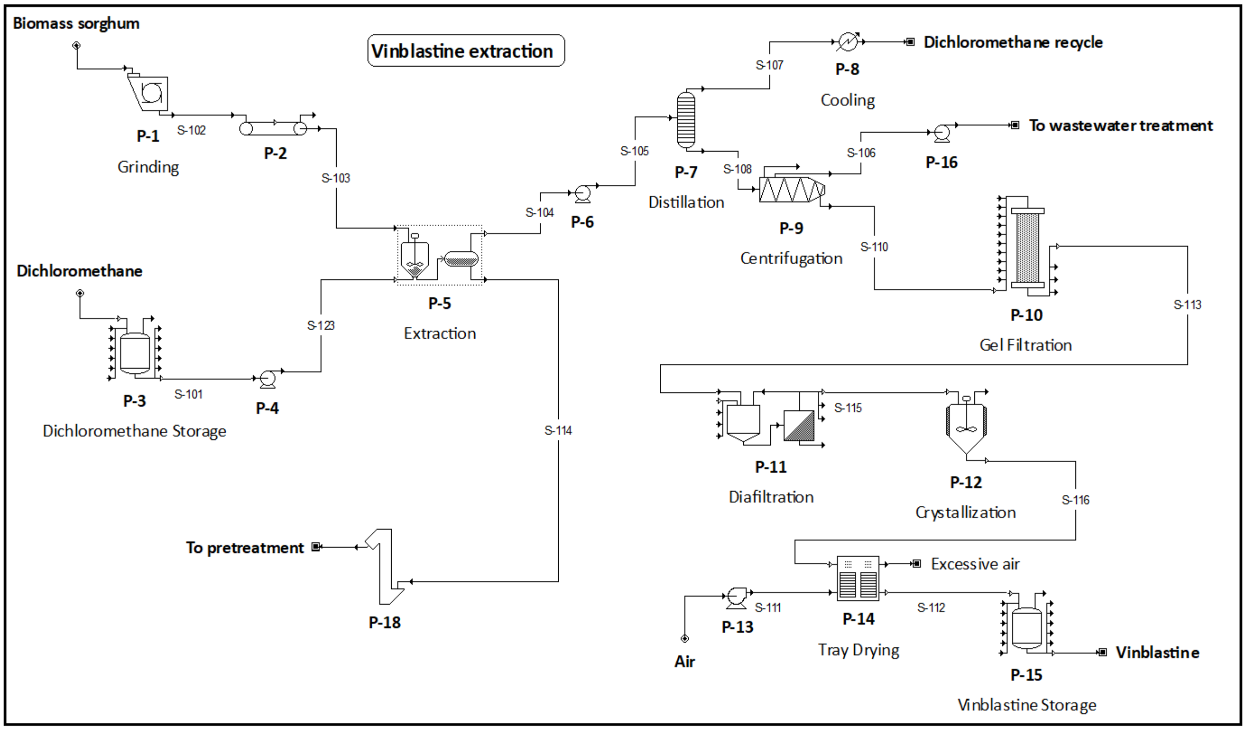


Figure S9. An overview of vinblastine extraction process.

References

1. Porter M, Haynes M. Biorenewable Insights Isoprene and Isoprenoids. Nexant Inc.; 2016 p. 151.
2. Ciriminna R, Lomeli-Rodriguez M, Demma Carà P, Lopez-Sanchez JA, Pagliaro M. Limonene: a versatile chemical of the bioeconomy. Chem Commun. 2014 Dec 18;50(97):15288–96.
3. Forgács G, Pourbafrani M, Niklasson C, Taherzadeh MJ, Hováth IS. Methane production from citrus wastes: process development and cost estimation. J Chem Technol Biotechnol. 2012 Feb;87(2):250–5.
4. Pourbafrani M, Forgács G, Horváth IS, Niklasson C, Taherzadeh MJ. Production of biofuels, limonene and pectin from citrus wastes. Bioresour Technol. 2010 Jun;101(11):4246–50.
5. Lohrasbi M, Pourbafrani M, Niklasson C, Taherzadeh MJ. Process design and economic analysis of a citrus waste biorefinery with biofuels and limonene as products. Bioresour Technol. 2010 Oct;101(19):7382–8.
6. Wikandari R, Nguyen H, Millati R, Niklasson C, Taherzadeh MJ. Improvement of biogas production from orange peel waste by leaching of limonene. Biomed Res Int. 2015 Mar 19;2015:494182.
7. Sipos B, Réczey J, Somorai Z, Kádár Z, Dienes D, Réczey K. Sweet sorghum as feedstock for ethanol production: enzymatic hydrolysis of steam-pretreated bagasse. Appl Biochem Biotechnol. 2009 May;153(1–3):151–62.
8. Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, et al. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol: Dilute-Acid Pretreatment and Enzymatic Hydrolysis of Corn Stover. Golden, CO (United States): National Renewable Energy Laboratory (NREL); 2011 Mar.
9. Negro V, Ruggeri B, Mancini G, Fino D. Recovery of D-limonene through moderate temperature extraction and pyrolytic products from orange peels. J Chem Technol Biotechnol. 2017 Jun;92(6):1186–91.
10. Aono R, Kobayashi H, Joblin KN, Horikoshi K. Effects of Organic Solvents on Growth of *Escherichia coli* K-12. Biosci Biotechnol Biochem. 1994 Jan;58(11):2009–14.
11. PubChem. D-Limonene [Internet]. [cited 2019 Aug 2]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/___-Limonene#section=Formulations-Preparations
12. Kindermans J-M, Pilloy J, Olliaro P, Gomes M. Ensuring sustained ACT production and reliable artemisinin supply. Malar J. 2007 Dec;6(1):125.
13. UNITAID. Independent final review of the assured artemisinin supply system [Internet]. 2013 [cited 2019 Dec 2]. Available from: http://www.unitaid.org/assets/End-of-project-evaluation-Assured-artemisinin-supply-system.pdf
14. Lapkin AA, Plucinski PK, Cutler M. Comparative assessment of technologies for extraction of artemisinin. J Nat Prod. 2006 Nov;69(11):1653–64.
15. Piletska EV, Karim K, Cutler M, Piletsky SA. Development of the protocol for purification of artemisinin based on combination of commercial and computationally designed adsorbents. J Sep Sci. 2013 Jan;36(2):400–6.
16. Polypropylene Production Capacity, Market and Price [Internet]. [cited 2019 Nov 24]. Available from: https://www.plasticsinsight.com/resin-intelligence/resin-prices/polypropylene/
17. Aramvash A, Gholami-Banadkuki N, Moazzeni-Zavareh F, Hajizadeh-Turchi S. An Environmentally Friendly and Efficient Method for Extraction of PHB Biopolymer with Non-Halogenated Solvents. J Microbiol Biotechnol. 2015 Nov;25(11):1936–43.

18. U.S. EPA. Chloroform [Internet]. [cited 2019 Jul 29]. Available from: https://www.epa.gov/sites/production/files/2016-09/documents/chloroform.pdf
19. Wilbanks B, Trinh CT. Comprehensive characterization of toxicity of fermentative metabolites on microbial growth. Biotechnol Biofuels. 2017 Nov 30;10:262.
20. Rubber price per pound 2019 | Statistic [Internet]. [cited 2019 Jun 10]. Available from: https://www.statista.com/statistics/727582/price-of-rubber-per-pound/
21. Buranov AU, Elmuradov BJ. Extraction and characterization of latex and natural rubber from rubber-bearing plants. J Agric Food Chem. 2010 Jan 27;58(2):734–43.
22. Cornish K, Chapman MH, Nakayama FS, Vinyard SH, Whitehand LC. Latex quantification in guayule shrub and homogenate. Ind Crops Prod. 1999 Sep;10(2):121–36.
23. Carcer GAD, Murphy S, Kagia J, Ooyen C, McCoy JJ. The CBD Report 2018 industry outlook. New Frontier Data; 2018.
24. Moss A. Cutting Edge Cannabidiol Extraction Technologies Used by Industry Leaders [Internet]. 2018 [cited 2019 Aug 5]. Available from: https://code.likeagirl.io/cutting-edge-cbd-extraction-technologies-bc2585e48763
25. Ou L, Kim H, Kelley S, Park S. Impacts of feedstock properties on the process economics of fast-pyrolysis biorefineries. Biofuels, Bioprod Bioref. 2018 May;12(3):442–52.
26. Gu T, Held MA, Faik A. Supercritical CO2 and ionic liquids for the pretreatment of lignocellulosic biomass in bioethanol production. Environ Technol. 2013 Aug;34(13–16):1735–49.
27. Chang C-W, Yen C-C, Wu M-T, Hsu M-C, Wu Y-T. Microwave-Assisted Extraction of Cannabinoids in Hemp Nut Using Response Surface Methodology: Optimization and Comparative Study. Molecules. 2017 Nov 3;22(11).
28. Ambach L, Penitschka F, Broillet A, König S, Weinmann W, Bernhard W. Simultaneous quantification of delta-9-THC, THC-acid A, CBN and CBD in seized drugs using HPLC-DAD. Forensic Sci Int. 2014 Oct;243:107–11.
29. Patel B, Wene D, Fan ZT. Qualitative and quantitative measurement of cannabinoids in cannabis using modified HPLC/DAD method. J Pharm Biomed Anal. 2017 Nov 30;146:15–23.
30. Abcam. Cannabidiol [Internet]. [cited 2019 Aug 2]. Available from: https://www.abcam.com/--cannabidiol-natural-cannabinoid-ab120448.html
31. Gill JR, Burks PS, Staggenborg SA, Odvody GN, Heiniger RW, Macoon B, et al. Yield Results and Stability Analysis from the Sorghum Regional Biomass Feedstock Trial. Bioenerg Res. 2014 Sep;7(3):1026–34.
32. Lamm FR, Stone LR, Brien DMO. Crop production and economics of corn, grain sorghum, soybean, and sunflower under irrigated and dryland. Appl Eng Agric. 2007;(23):737–46.
33. Xu F, Sun J, Konda NVSNM, Shi J, Dutta T, Scown CD, et al. 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. 2016;9(3):1042–9.
34. Zhu S, Wu Y, Chen Q, Yu Z, Wang C, Jin S, et al. Dissolution of cellulose with ionic liquids and its application: a mini-review. Green Chem. 2006;8(4):325.
35. Neupane B, Konda NVSNM, Singh S, Simmons BA, Scown CD. Life-Cycle Greenhouse Gas and Water Intensity of Cellulosic Biofuel Production Using Cholinium Lysinate Ionic Liquid Pretreatment. ACS Sustain Chem Eng. 2017 Nov 6;5(11):10176–85.
36. Klein-Marcuschamer D, Simmons BA, Blanch HW. Techno-economic analysis of a lignocellulosic ethanol biorefinery with ionic liquid pre-treatment. Biofuels, Bioprod Bioref. 2011 Sep;5(5):562–9.

37. Baral N, Scown C. Biomass sorghum logistic supply cost. 2018.
38. Dahlberg J, Wolfrum E, Bean B, Rooney WL. Compositional and agronomic evaluation of sorghum biomass as a potential feedstock for renewable fuels. J Biobased Mat Bioenergy. 2011 Dec 1;5(4):507–13.
39. Intelligen. SuperPro Designer . Intelligen; 2018.
40. ICIS. Chemical profile: Sodium sulfite [Internet]. [cited 2019 Jun 18]. Available from: https://www.icis.com/explore/resources/news/2008/03/10/9106600/chemical-profile-sodium-sulfite/
41. Baral NR, Kavvada O, Mendez-Perez D, Mukhopadhyay A, Lee TS, Simmons BA, et al. Techno-economic analysis and life-cycle greenhouse gas mitigation cost of five routes to bio-jet fuel blendstocks. Energy Environ Sci. 2019;12(3):807–24.
42. Humbird D, Davis R, Tao L, Kinchin C, Hsu D, Aden A, et al. Process Design and Economics for Biochemical Conversion of Lignocellulosic Biomass to Ethanol. National Renewable Energy Laboratory; 2011 May. Report No.: NREL/TP-5100-47764.
43. U.S. Energy Information Administration. Natural Gas Price (Dollars per Million Btu) [Internet]. [cited 2019 Jun 17]. Available from: https://www.eia.gov/dnav/ng/hist/rngwhhdM.htm
44. U.S. Energy Information Administration. Electricity Data [Internet]. [cited 2019 Jun 17]. Available from: https://www.eia.gov/electricity/data/browser/#/topic/7?agg=0
45. Alam P, Sharaf-Eldin MA. Limited production of plant derived anticancer drugs vinblastine and vincristine. Planta Med. 2016 Mar 17;82(05).
46. Heijden R, Jacobs D, Snoeijer W, Hallard D, Verpoorte R. The catharanthus alkaloids:pharmacognosy and biotechnology. CMC. 2004 Mar 1;11(5):607–28.
47. Muñoz-Rojas J. Importance of producing economic compounds to combat cancer. Microb Biotechnol. 2017 Jul;10(4):683–4.
48. Verma A, Hartonen K, Riekkola M-L. Optimisation of supercritical fluid extraction of indole alkaloids from Catharanthus roseus using experimental design methodology--comparison with other extraction techniques. Phytochem Anal. 2008 Feb;19(1):52–63.
49. Yan L, Ma R, Li L, Fu J. Hot water pretreatment of lignocellulosic biomass: an effective and environmentally friendly approach to enhance biofuel production. Chem Eng Technol. 2016 Oct;39(10):1759–70.
50. Jaber-Vazdekis N el, Gutierrez-Nicolás F, Ravelo ÁG, Zárate R. Studies on tropane alkaloid extraction by volatile organic solvents: dichloromethane vs. chloroform. Phytochem Anal. 2006 Mar;17(2):107–13.
51. Yang L, Wang H, Zu Y, Zhao C, Zhang L, Chen X, et al. Ultrasound-assisted extraction of the three terpenoid indole alkaloids vindoline, catharanthine and vinblastine from Catharanthus roseus using ionic liquid aqueous solutions. Chemical Engineering Journal. 2011 Aug;172(2–3):705–12.
52. Guanasekera SP. Process of isolating vinblastine from the plant Catharanthis roseus. Canada; 4749787, 1988.
53. Cell Signaling Technology. Vinblastine [Internet]. [cited 2019 Aug 2]. Available from: https://www.cellsignal.com/1/1/7360-chemical-modulators-vinblastine-5-mg-14255.html
54. Aslam J, Mujib A, Fatima Z, Sharma MP. Variations in vinblastine production at different stages of somatic embryogenesis, embryo, and field-grown plantlets of Catharanthus roseus L. (G) Don, as revealed by HPLC. In Vitro CellDevBiol-Plant. 2010 Aug;46(4):348–53.