Electronic Supplementary Information

Liquid-liquid extraction for in situ carboxylic acid recovery via continuous membrane-based emulsion separations

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Supporting Data

Table S1. Surface hydrophilicity of the pristine, emulsion filtered, and fouled membranes.

Membrane	Sessile drop (SD) water contact angle[a] (°)	Free energy of hydration (ΔG_{iw})[b] (mJ/m^2)
Hydrophobic PTFE membrane	125.1±6.5	-30.8
Hydrophilic PTFE membrane	59.2±2.5	-109.1
Hydrophobic PTFE membrane after emulsion filtration[c]	74.0±0.7	-92.1
Hydrophobic PTFE membrane after organic fouling[d]	59.8±3.2	-108.4
Hydrophilic PTFE membrane after organic fouling[d]	42.2±3.1	-125.6

[a]The reported SD water contact angle was the average measurements of at least five locations of each membrane sample, with ten continuous measurements for each location.

[b]The surface free energy of hydration (ΔG_{iw}) was then calculated from the Young-Dupré equation, $\Delta G_{iw} = -\gamma_w (1+\cos\theta_w)$, where γ_w is the liquid water surface tension and θ_w is the water contact angle. It is noted that surfaces are considered more hydrophilic when ΔG_{iw} has greater negative values.

[c]The hydrophobic PTFE membrane was filtered with an emulsion prepared by mixing 300 mL 10 g/L butyric acid aqueous solution with 150 mL organic phase. The membrane filtration was carried out at feed flow rate of 140 mL/min for 1 hr and membrane phase breakthrough was observed.

[d]Membrane was filtered with an emulsion prepared by mixing 300 mL *C. tyrobutyricum* fermentation broth (pre-filtered) with 150 mL organic phase. The membrane filtration was carried out at feed flow rate of 30 mL/min for 1 hr.

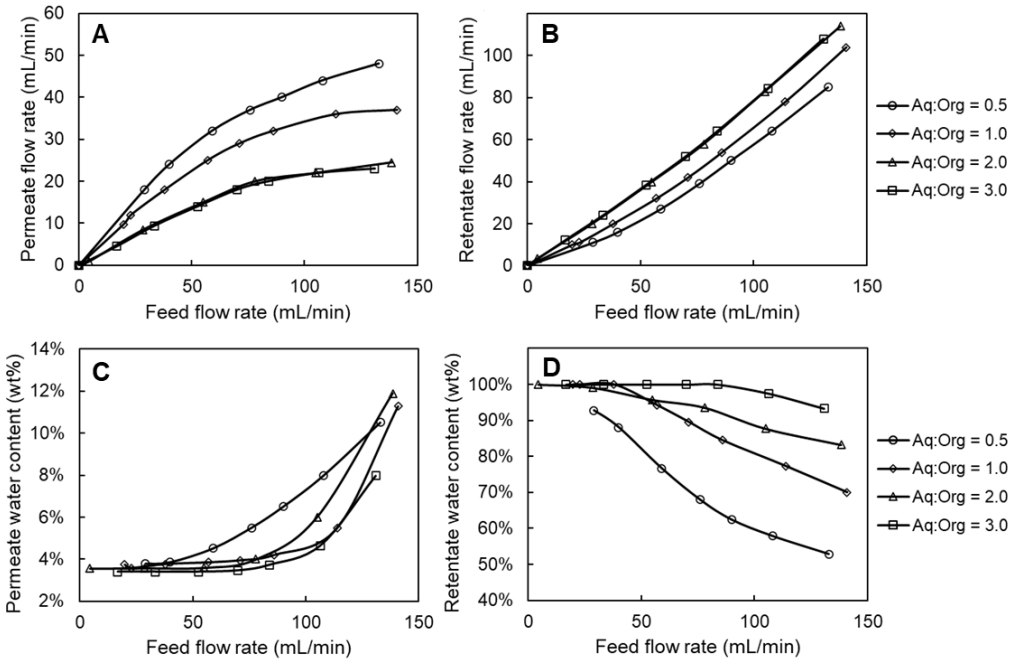


Figure S1. MBES phase separation efficiency (*PS*) of the hydrophobic PTFE membrane calculated by (**A**) permeate and (**B**) retentate flow rates, and (**C**) permeate and (**D**) retentate water content as affected by the aqueous:organic phase volume ratio and feed flow rate. (Note: The emulsified mock broth was prepared by mixing 10 g/L model butyric acid solution as the aqueous phase and 70 vol% Cyanex 923 and 30 vol% mineral oil as the organic phase at different phase volume ratio with a stir rate of 650 rpm for 3 min.)

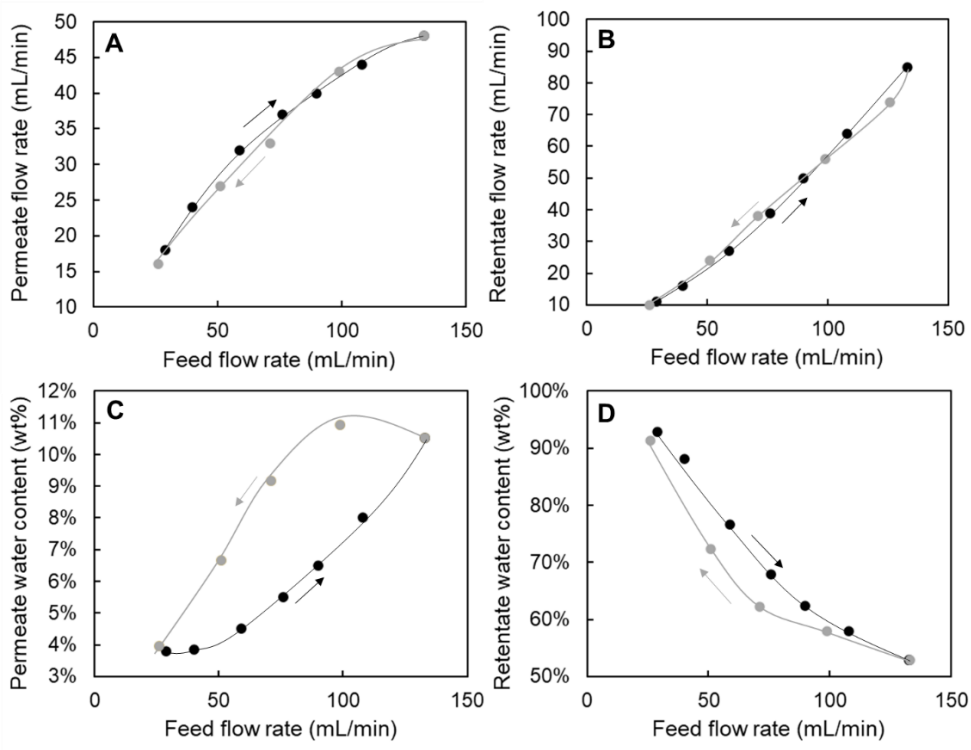


Figure S2. Hydrophobic PTFE membrane (**A**) permeate and (**B**) retentate flow rates and (**C**) permeate and (**D**) retentate water content affected by the feed flow rate and their reversibility. (Note: the emulsified mock broth was prepared by mixing 100 mL 10 g/L butyric acid aqueous solution with 200 mL organic phase.)

Table S2. MBES phase separation efficiency[a] at an aqueous:organic phase volume ratio $\emptyset = 0.5$ as indicated by the flow rates and KF water contents in the feed, permeate, and retentate streams.[b]

Volumetric flow rate (mL/min)			Water content (%)		
Feed	Permeate	Retentate	Feed	Permeate	Retentate
0	0	0	37.55%		
29	18	11	37.55%	3.78%	92.80%
40	24	16	37.55%	3.85%	88.09%
59	32	27	37.55%	4.51%	76.70%
76	37	39	37.55%	5.50%	67.95%
90	40	50	37.55%	6.50%	62.39%
108	44	64	37.55%	8.00%	57.86%
133	48	85	37.55%	10.51%	52.81%
126	52	74	37.55%	14.27%	53.90%
99	43	56	37.55%	10.93%	57.99%
71	33	38	37.55%	9.18%	62.18%
51	27	24	37.55%	6.66%	72.30%
26	16	10	37.55%	3.97%	91.27%
Thermodynamic Limit					
Emulsion water content		*37.55%*	*Organic phase water content*		*3.80%*

[a] Phase separation efficiency is defined as $PS = 1 - C_{w,p}/C_{w,f}$, where $C_{w,p}$ and $C_{w,f}$ are the water content (wt%) of the permeate and feed solutions, respectively.

[b] Hydrophobic PTFE membrane OB-2000-S200F was used for the phase separation efficiency test.

Table S3. MBES phase separation efficiency at phase volume ratio $\emptyset = 1$ as indicated by the flow rates and KF water contents in the feed, permeate, and retentate streams.

Volumetric flow rate (mL/min)			Water content (%)		
Feed	Permeate	Retentate	Feed	Permeate	Retentate
0	0	0	54.60%		
19.8	9.8	10	54.60%	3.74%	100.00%
23	12	11	54.60%	3.59%	100.00%
38	18	20	54.60%	3.77%	100.00%
57	25	32	54.60%	3.85%	94.24%
71	29	42	54.60%	3.95%	89.56%
86	32	54	54.60%	4.20%	84.46%
114	36	78	54.60%	5.49%	77.26%
141	37	104	54.60%	11.29%	70.00%
176	38	138	54.60%	14.75%	65.57%
Thermodynamic Limit					
Emulsion water content		*54.60%*	*Organic phase water content*		*3.75%*

Footnote same as **Table S2**.

Table S4. MBES phase separation efficiency at phase volume ratio $\emptyset = 2$ as indicated by the flow rates and KF water contents in the feed, permeate, and retentate streams.

Volumetric flow rate (mL/min)			Water content (%)		
Feed	Permeate	Retentate	Feed	Permeate	Retentate
0	0	0	70.63%		
4.5	0.9	3.6	70.63%	3.56%	100.00%
28.5	8.5	20	70.63%	3.58%	99.13%
55	15	40	70.63%	3.60%	95.77%
78	20	58	70.63%	4.00%	93.61%
105	22	83	70.63%	6.00%	87.76%
138.5	24.5	114	70.63%	11.89%	83.26%
175	27	148	70.63%	17.74%	80.28%
Thermodynamic Limit					
Emulsion water content		*70.63%*	*Organic phase water content*		*3.60%*

Footnote same as **Table S2**.

Table S5. MBES phase separation efficiency at phase volume ratio $\emptyset = 3$ as indicated by the flow rates and KF water contents in the feed, permeate, and retentate streams.

Volumetric flow rate (mL/min)			Water content (%)		
Feed	Permeate	Retentate	Feed	Permeate	Retentate
0	0	0	78.30%		
16.7	4.5	12.2	78.30%	3.40%	100.00%
33.4	9.4	24	78.30%	3.40%	100.00%
52.5	14	38.5	78.30%	3.40%	100.00%
70	18	52	78.30%	3.46%	100.00%
84	20	64	78.30%	3.73%	100.00%
106.5	22	84.5	78.30%	4.65%	97.47%
131	23	108	78.30%	7.98%	93.27%
168	24	144	78.30%	15.02%	88.84%
Thermodynamic Limit					
Emulsion water content		*78.30%*	*Organic phase water content*		*3.42%*

Footnote same as **Table S2**.

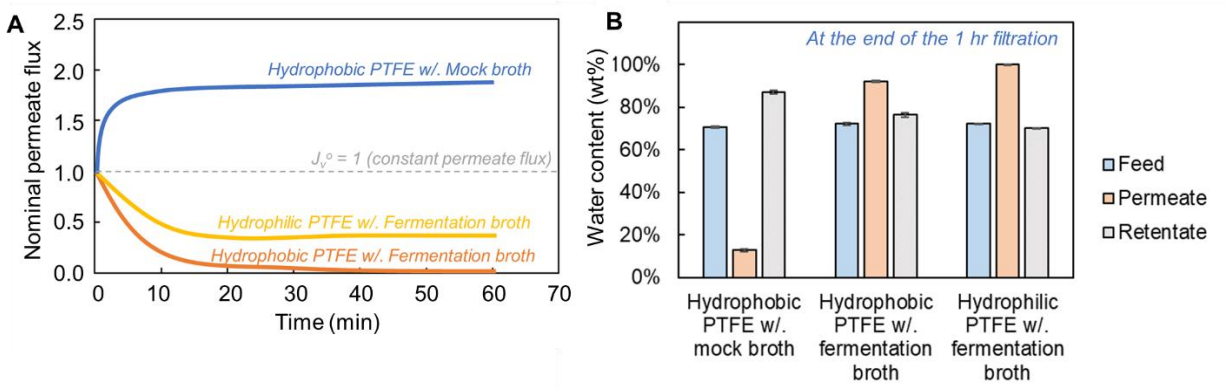


Figure S3. Wetting of both hydrophobic and hydrophilic PTFE membranes during the 1 hr filtration of both emulsified mock broth and fermentation broth as indicated by (**A**) the normalized permeate flux profile and (**B**) the final water content in the permeate and retentate streams.

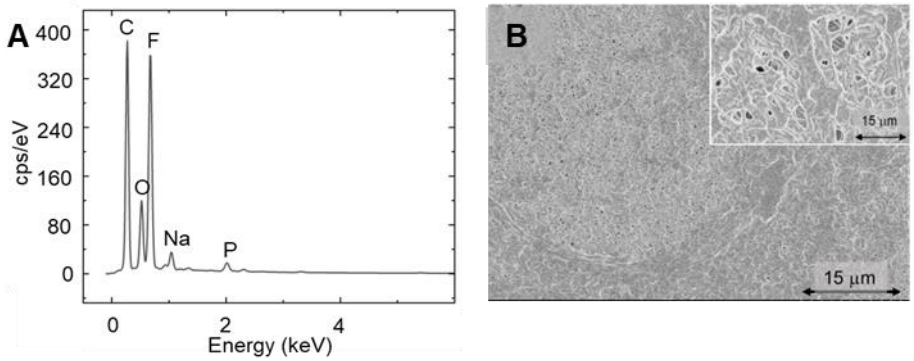


Figure S4. (**A**) EDS and (**B**) SEM results of the 1 hr emulsified fermentation broth filtered hydrophilic PTFE membrane surfaces.

Table S6. EDS results of the pristine and fouled hydrophilic membrane surfaces.

Membrane	F	C	O	C/F	O/F
Pristine hydrophilic PTFE membrane	49.3%	35.3%	9.8%	0.72	0.20
Hydrophilic PTFE membrane filtered with emulsion B[a]	38.7%	38.3%	15.3%	0.99	0.40

[a]Emulsion B is the emulsified fermentation broth.

Table S7. MBES fouling propensity as indicated by the flux decline over 1 hr filtration of different feed emulsion solutions.[a]

Aqueous	Time (min)	Volumetric flow rate (mL/min)			Normalized Flux	Water content (wt%)		
		Feed	Permeate	Retentate		Feed	Permeate	Retentate
Membrane: OB-2000-S200F								
Mock Broth	0	33.1	3.5	29.6	1.0			
Mock Broth	5	33.1	6.3	26.8	1.8			
Mock Broth	10	31.8	6.2	25.6	1.8			
Mock Broth	15	32.0	6.2	25.8	1.8			
Mock Broth	20	31.7	6.5	25.2	1.9			
Mock Broth	30	31.6	6.0	25.6	1.7			
Mock Broth	35	32.4	7.2	25.2	2.1			
Mock Broth	40	32.2	7.0	25.2	2.0			
Mock Broth	60	30.5	6.5	24.0	1.9	70.6%	3.6%	99.1%
Membrane: OB-2000-S200F								
Fermentation Broth	0	28.0	9.0	19.0	1.0			
Fermentation Broth	5	29.0	4.0	25.0	0.4			
Fermentation Broth	10	27.0	2.0	25.0	0.2			
Fermentation Broth	15	26.6	1.1	25.5	0.1			
Fermentation Broth	20	25.8	0.8	25.0	0.1			
Fermentation Broth	30	25.1	0.6	24.5	0.1			
Fermentation Broth	40	26.5	0.5	26.0	0.1			
Fermentation Broth	60	26.4	0.4	26.0	0.0	72.1%	92.0%	76.4%
Membrane: IL-2000-S200F								
Fermentation Broth	0	27.4	0.4	27.0	1.0			
Fermentation Broth	10	26.2	0.2	26.0	0.5			
Fermentation Broth	20	25.2	0.2	25.0	0.4			
Fermentation Broth	30	25.7	0.2	25.5	0.4			
Fermentation Broth	40	25.2	0.2	25.0	0.4			
Fermentation Broth	50	24.7	0.2	24.5	0.4			
Fermentation Broth	60	24.2	0.2	24.0	0.4	72.1%	100.0%	70.0%

[a]Hydrophobic PTFE membrane OB-2000-S200F and hydrophilic PTFE membrane IL-2000-S200F were used for the fouling tests. For all three tests, the feed emulsion solutions were prepared using a mixture of 70vol% Cyanex 923 + 30vol% Mineral Oil as the organic solvent.

Table S8. Fermentation broth pre-filtration and backwash for MBES fouling mitigation.

Pre-filtration	Time (min)	Permeate flow rate (mL/min)	Nomalized flux	Permeate water content (wt%)
0.2 μm MF	0	8.00	1.00	
	5	4.50	0.56	
	10	3.00	0.38	
	20	1.30	0.16	
	30	0.70	0.09	
	40	0.60	0.08	
	50	0.45	0.06	
	60	0.30	0.04	0.92
Membrane backwash cleaning efficiency			*68.60%*	*0.69*
0.1 μm MF	0	8.00	1.00	
	5	6.30	0.79	
	10	4.80	0.60	
	20	3.20	0.40	
	30	2.00	0.25	
	40	1.60	0.20	
	50	1.40	0.18	
	60	1.30	0.16	0.81
Membrane backwash cleaning efficiency			*92.16%*	*0.26*
10 kDa	0	8.00	1.00	
	5	7.70	0.96	
	10	7.50	0.94	
	20	7.30	0.91	
	30	7.10	0.89	
	40	6.90	0.86	
	50	6.80	0.85	
	60	6.70	0.84	0.55
Membrane backwash cleaning efficiency			*99.64%*	*0.09*
1 kDa	0	8.00	1.00	
	5	7.90	0.99	
	10	7.70	0.96	
	20	7.60	0.95	
	30	7.50	0.94	
	40	7.40	0.93	
	50	7.30	0.91	
	60	7.30	0.91	0.29
Membrane backwash cleaning efficiency			*100%*	*0.04*

Model prediction for counter-current multi-stage MBES system

The counter-current multi-stage MBES system (**Fig. S5**) can be used to improve the overall extraction efficiency with enhanced butyric acid concentration in the final organic phase without the expense of high solvent consumption. The extraction efficiency (*EE*) is a function of partition coefficient (K_D) and aqueous:organic phase volume ratio (∅). The equations of butyric acid extraction efficiency can be derived for different number of MBES stages.

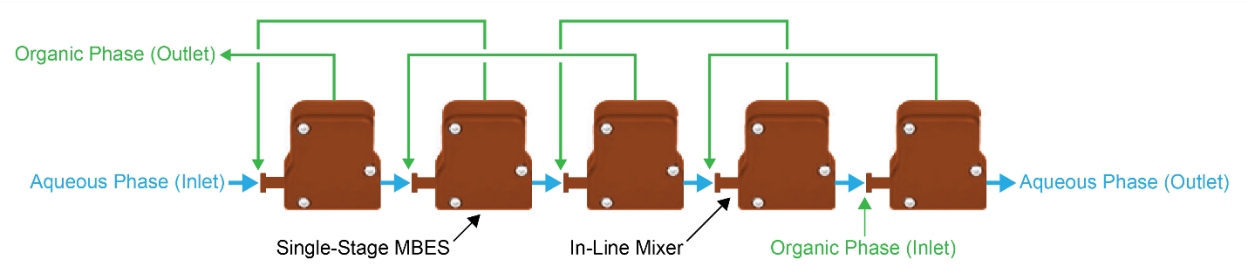


Figure S5. System configuration of a counter-current multi-stage MBES system.

$$\text{For 1 stage: } EE = 1 - \frac{\emptyset}{K_D+\emptyset} \tag{S1}$$

$$\text{For 2 stages: } EE = 1 - \frac{\emptyset^2}{(\emptyset+K_D)^2-K_D\cdot\emptyset} \tag{S2}$$

$$\text{For 3 stages: } EE = 1 - \frac{\emptyset^3}{(\emptyset+K_D)^3-2K_D\cdot\emptyset\cdot(\emptyset+K_D)} \tag{S3}$$

$$\text{For 4 stages: } EE = 1 - \frac{\emptyset^4}{(\emptyset+K_D)^4-3K_D\cdot\emptyset\cdot(\emptyset+K_D)^2+{K_D}^2\cdot\emptyset^2} \tag{S4}$$

$$\text{For 5 stages: } EE = 1 - \frac{\emptyset^5}{(\emptyset+K_D)^5-4K_D\cdot\emptyset\cdot(\emptyset+K_D)^3+3{K_D}^2\cdot\emptyset^2\cdot(\emptyset+K_D)} \tag{S5}$$

where K_D is butyric acid partition coefficient (defined as $C_{org,out}/C_{aq,out}$, where $C_{org,out}$ and $C_{aq,out}$ are the butyric acid concentrations in the organic and aqueous phase outlet streams, respectively), and ∅ is the aqueous/organic phase volume ratio (defined as Q_{aq}/Q_{org}, where Q_{aq} and Q_{org} ate the volumetric flow rates of the aqueous and organic phases, respectively, assuming volume change throughout the process due to LLE is negligible). For the organic solvent used in the present study, 70 vol% Cyanex 923 and 30 vol% mineral oil, the measured butyric acid partition coefficient is 1 at aqueous solution pH of 5. The above derived equations of butyric acid extraction efficiency can be plotted as a function of the number of membrane stages and the phase volume ratio (**Fig. S6**). For example, the butyric acid extraction efficiency ($\emptyset = 1$) can be increased from 50% to 83% by using a five-stage counter-current MBES system instead of a single-stage one.

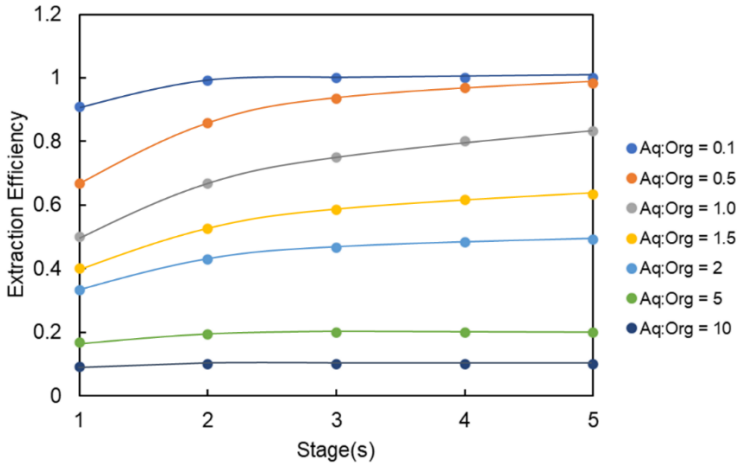


Figure S6. MBES butyric acid extraction efficiency as a function of the number of membrane stages and the phase volume ratio.

Table S9. MBES butyric acid extraction efficiency as a function of the number of membrane stages and the phase volume ratio.

	Aq:Org Phase Volume Ratio						
Stage	0.1	0.5	1	1.5	2	5	10
1	90.91%	66.67%	50.00%	40.00%	33.33%	16.67%	9.09%
2	99.10%	85.71%	66.67%	52.63%	42.86%	19.35%	9.91%
3	99.91%	93.33%	75.00%	58.46%	46.67%	19.87%	9.99%
4	99.99%	96.77%	80.00%	61.61%	48.39%	19.97%	10.00%
5	100.00%	98.41%	83.33%	63.46%	49.21%	19.99%	10.00%

TEA and LCA

Table S10. CAPEX breakdown for the overall downstream ISPR process integrated with MBES and membrane contactor for the step of liquid-liquid extraction[a].

CAPEX	MBES		Membrane contactor	
	Installed Cost	$/year over 30 years	Installed Cost	$/year over 30 years
Solvent initial Charge	$14,711,000	$490,369	$14,711,081	$490,369
Initial Membranes	$886,055	$29,535	$38,764,917	$1,292,164
Polishing Filter	$865,457	$28,849	$865,457	$28,849
Heat Exchangers	$2,437,926	$81,264	$2,437,926	$81,264
Flash Tank	$1,311,412	$43,714	$1,311,412	$43,714
Distillation Column 1	$3,637,056	$121,235	$3,637,056	$121,235
Distillation Column 2	$1,117,717	$37,257	$1,117,717	$37,257
ISPR Feed Pump	$735,810	$24,527	$735,810	$24,527
Total	**$25,702,515**	**$856,751**	**$63,581,377**	**$2,119,380**

[a]The TEA analysis (and reported costs) is in 2016 dollars and based on 2000 metric ton per day feedstock processing scale. The cell retention cost is included but with high uncertainty.

Table S11. Raw material costs and sources.

Material	Unit Cost ($/unit)	Source
Ammonium hydroxide, kg	0.42	Davis et al. (2018)[1]
Sulfuric acid, 93 wt%, kg	0.09	Basic chemical, Omaha via HGI, 2018 design report
Cyanex-923, kg	20.0	Salvachua et al. (2021)[2]
Mineral oil, kg	0.68	Salvachua et al. (2021)[2]

Table S12. OPEX breakdown for the overall downstream ISPR process integrated with MBES and membrane contactor for the step of liquid-liquid extraction.

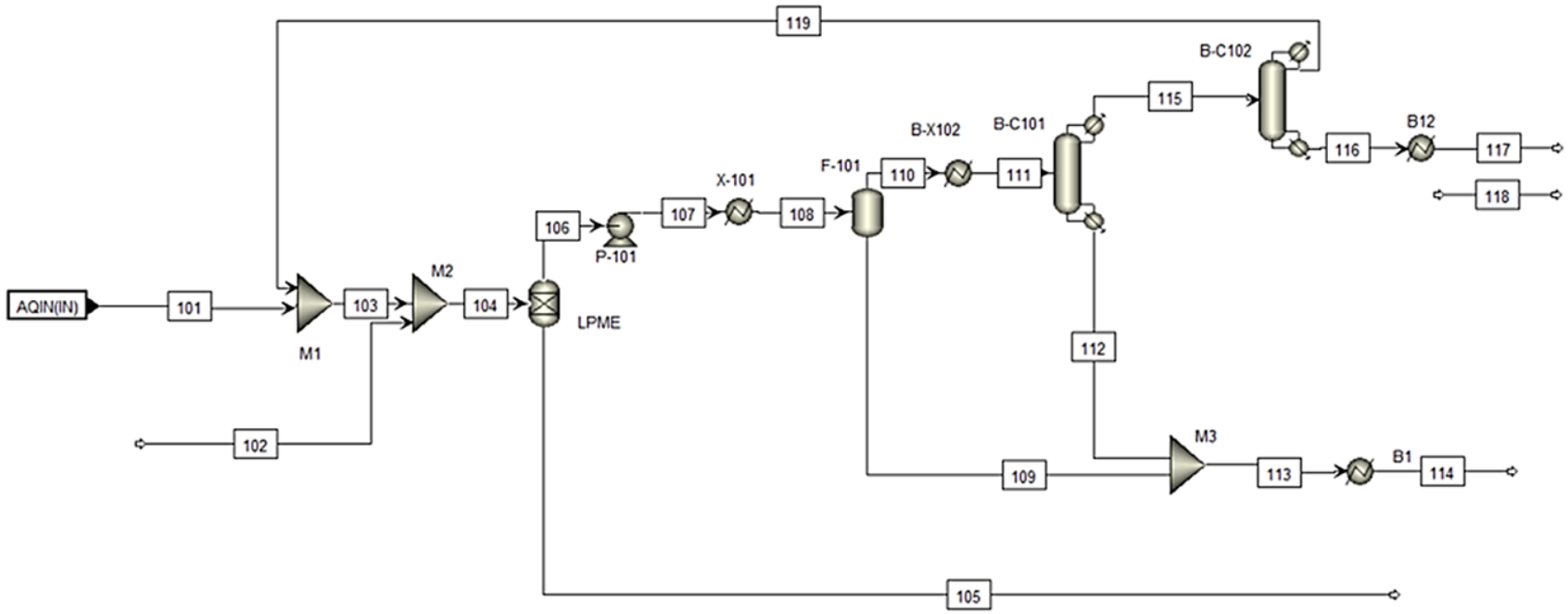
OPEX ($/year)	MBES	Membrane contactor
Membrane Replacements (after 1st year)	$886,055	$38,764,917
Cyanex-923 - Makeup	$8,597,888	
Mineral Oil - Makeup	$2,884,225	
Polishing Filter Cleaning Chemicals	$352,422	
Ammonia	$2,251,507	
Sulfuric acid, 93%	$662,872	
Electricity	$2,113,734	
Cooling	$1,273,404	
Heating	$16,488,755	
Total	**$35,510,862**	**$73,389,724**

Table S13. Parameters for liquid-liquid extraction using MBES and membrane contactor.

LLE parameter	MBES	Membrane contactor
Membrane material	Polytetrafluoroethylene	Polypropylene
Membrane type	Hydrophobic flat sheet	Hydrophobic hollow-fiber
Operating temperature	Ambient	Ambient
Butyric acid flux (g/hr/m^2)	1400	8.9

Table S14. Distillation conditions for process model simulation.

Column No.	1	2
Number of stages	6	6
Reflux ratio	1.2	0.51
Boilup ratio	2.17	1.2
Reboiler temperature (°C)	213.8	82.3
Condenser temperature (°C)	68.5	46.2



Stream Name	Units	6-0	ORGIN	ORGREC	AQ-HED	B2	B4	B5	B7	B21	B8	S11	SOLVLOSS
Stream Description		Ferm Broth In	Total Solvent to Separation	Solvent Recycle	Aqueous Waste	Flash Inlet	Flash Bottoms	Distillation #1 Inlet	Distillation #1 Bottoms	Distillation #2 Inlet	Water Distillate Recycle	Product Stream	Solvent Loss
Temperature	C	37	50	37	41	225	215	141	222	60	44	62	82
Pressure	atm	1.0	1.0	0.1	1.0	0.2	0.1	0.2	0.1	0.1	1.0	0.1	1.1
Mass Vapor Fraction		0.000	0.000	0.000	0.000	0.330	0.000	0.191	0.000	1.000	0.000	0.000	0.000
Mass Liquid Fraction		0.998	1.000	1.000	0.997	0.670	1.000	0.809	1.000	0.000	1.000	1.000	1.000
Mass Solid Fraction		0.002	0.000	0.000	0.003	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Mass Density	gm/cc	0.9816	0.7814	0.7894	0.9645	0.0012	0.6769	0.0007	0.6538	0.0001	0.9736	0.9116	0.7612
Average MW		18.28	309.91	308.02	18.05	163.88	330.69	98.11	277.73	22.93	18.18	58.11	309.91
Volume Flow	l/min	24,724	22,092	21,910	23,763	15,347,386	15,688	12,143,169	10,211	15,699,814	945	435	12
Mass Flows	kg/hr	1,456,084	1,035,700	1,037,689	1,375,109	1,116,675	637,173	479,502	400,516	78,986	55,196	23,790	548
Mass Fractions													
H2O		0.980	0.000	0.000	0.995	0.052	0.000	0.120	0.000	0.731	0.988	0.133	0.000
C17		0.000	0.300	0.299	0.000	0.278	0.138	0.464	0.556	0.000	0.000	0.000	0.300
BUTYRIC		0.015	0.000	0.000	0.000	0.019	0.000	0.044	0.000	0.269	0.012	0.867	0.000
Cyanex-923		0.000	0.700	0.699	0.000	0.649	0.861	0.368	0.440	0.000	0.000	0.000	0.700
Other		0.005	0.000	0.002	0.004	0.001	0.000	0.003	0.004	0.000	0.000	0.000	0.000
Total		1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000

Figure S7. ASPEN PLUS simulation of ISPR process diagram (*top*) and stream table (*bottom*).

Table S15. Membrane area needed for pre-filtration, MBES, and membrane contactor.

Unit operation	Needed membrane area (m^2)
Pre-filtration	350,000
MBES	14,910
Membrane contactor	2,609,252

Table S16. Life cycle inventory for carbon intensity and other environmental impact calculations.

		MBES		Membrane contactor
Product				
Butyric acid (kg/hr)		20,626		
Resource Consumption				
Electricity consumption	kWh/hr	14.44		
	g CO_2e/kg butyric acid	0.30		
Membrane consumption	m^2/yr	14,910 (PTFE)	79,285 (cellulose)	2,609,252
	kg/yr	805	4,281	140,900
	g CO_2e/kg butyric acid	1.7	0.6	21.4
Solvent makeup	kg/hr	548		
	kg CO_2e/kg butyric acid	0.20		
Environmental Impact				
Carbon Intensity (g CO_2e/kg butyric acid)		202		221

Table S17. Other LCA environmental impact calculations conducted with TRACI 2.1 US 2008 method.

Environmental Impact (per kg butyric acid)	MBES	Membrane contactor	% Improvement
Global Warming Potential (kg CO_2e)	2.02E+02	2.24E+02	8.78%
Ozone Depletion (kg CFC-11eq)	1.20E-07	1.30E-02	100.00%
Smog (kg O_3 eq)	1.18E-02	1.30E-02	8.83%
Acidification (kg SO_2 eq)	1.40E-03	1.54E-03	9.14%
Eutrophication (kg N eq)	1.45E-04	3.21E-04	54.72%
Carcinogenics (CTUh)	7.51E-09	1.17E-08	35.83%
Non Carcinogenics (CTUe)	3.33E-08	7.00E-08	52.45%
Respiratory Effects (kg OM2.5 eq)	1.53E-04	1.84E-04	17.27%
Ecotoxicity (CTUe)	3.26E-01	1.16E+00	71.86%
Fossil Fuel Depletion (MJ)	2.84E-01	3.08E-01	7.75%

Table S18. Major TEA and LCA assumptions.

No.	TEA assumptions
1	Process is simulated based on the Nth-Plant scale of processing 2000 metric ton feedstock per day
2	Process butyric acid production rate is 21,082 kg/hr
3	Continuous membrane-based solvent extraction with Aq:Org phase volume ratio of 1:1
4	When reach steady state, *C. tyrobutyricum* fermentation broth contains butyric acid titer of 15 g/L
5	When reach steady state, *C. tyrobutyricum* butyric acid productivity stabilizes at 0.3 g/L/hr
6	Membrane contactor has lifetime of 1 year during continuous membrane-based solvent extraction
7	MBES membrane has lifetime of 3 months during continuous membrane-based solvent extraction
8	Pre-filter (1 kDa UF membrane) has lifetime of 4 months during fermentation broth clarification
9	Plant lifetime is 30 years, all the major equipment has lifetime same to plant lifetime
10	Plant on-stream time factor is 0.9
11	Depreciation and income taxes were not considered
No.	LCA assumptions
1	Life cycle impact assessment method is TRACI 2.1 (v. 1.04)
2	Functional unit is 1 kg of butyric acid produced
3	Underlying process and background data for raw material inputs utilize various data sources, including Ecoinvent database, DATASMART Lice Cycle Inventory Package, the US LCI processes (USLCI), and GREET2021

Experimental

1 Materials

Butyric acid (>99%, Sigma Aldrich, Inc.) was dissolved in de-ionized water to prepare the mock broth solution. *C. tyrobutyricum* fermentation broth fed with mock deacetylation and mechanical refining (DMR) sugar[2] was used as the feed aqueous phase and the butyric acid source for downstream LLE. A mixture of Cyanex 923 (Solvay) and mineral oil (light, Sigma Aldrich, Inc.) was used as the organic phase to extract butyric acid. Solution pH was adjusted using 0.1 N HCl (ACS reagent, Fisher Scientific) and 0.1 N NaOH (50% w/w, Fisher Scientific) solutions. Liquinox (Alconox Inc.) and ethanol (KOPTEC 190 proof pure, Decon Labs, Inc.) solutions were used to clean the membrane emulsion separator system (SEP-200, Zaiput Flow Technologies) after each filtration run.

Hydrophobic and hydrophilic polytetrafluoroethylene (PTFE) membranes (OB-2000-S200F and IL-2000-S200F, Zaiput Flow Technologies) with nominal pore size of 1 μm were tested for emulsion phase separation. An Al_2O_3 ceramic membrane disc (132392815, Andritz Separation GmbH) with a pore size of 0.2 μm was used to filter out cells, cell debris, and other solid fractions of the fermentation broth. Nylon (pore size of 0.2 μm; GNWP04700, Millipore Sigma) and polycarbonate (pore size of 0.1 μm; VCTP04700, Millipore Sigma) microfiltration membranes, and ultracel regenerated cellulose ultrafiltration membrane disks with molecular weight cutoff of 10 kDa and 1 kDa (PLGC04310 and PLAC04310, Millipore Sigma) were used for fermentation broth potential foulant removal.

2 Quantitative analysis

2.1 High-performance liquid chromatography (HPLC)

The butyric acid concentration in the aqueous phase was measured before and after LLE using HPLC (Agilent1100 series) equipped with a refractive index detector. Prior to injection, samples were filtered through a 0.2 μm syringe filter (Mdi Membrane Technology Inc.). An Aminex HPX-87H (300×7.8 mm) organic acid column was used with a

mobile phase consisting of 0.01 N sulfuric acid (Ricca Chemical Company) with a flow rate of 0.6 mL/min at 65°C. For quantification of butyric acid, retention times and spectral profiles were compared with the pure standards' calibration curve. All samples were run in triplicate.

2.2 Karl Fisher (KF)

Solution water content (weight percentage) was measured using automated Karl Fisher (KF) titration (Metrohm 870KF Titrino plus, Metrohm). Before any sample analysis, the system was calibrated using a 0.1% water standard (HYDRANAL – Water Standard 10.0, Honeywell) in triplicate. KF Ipol was selected as the KF measurement method. The KF titration cell was filled with methanol (HPLC grade, Fisher Scientific) until electrodes were fully immersed. At least 30 µL retentate and 100 µL permeate samples were weighed using an analytical balance before adding to the titration cell. The sample weight was entered into the instrument before sample titration was started using CombiTitrant 5 (Merck) for KF moisture determination. Sample KF water content was reported as the average of at least three measurements.

2.3 Zetasizer

Droplet size distribution in the feed emulsion was measured at 25 °C using Zetasizer (Nano ZS, Malvern Panalytical) by dynamic light scattering (DLS) technique. Particle diffusion due to Brownian motion was measured and converted to size and size distribution using the Stoke-Einstein relationship. Each liquid sample (~1 mL) was added to a disposable cuvette (DTS0012, SARSTEDT), sealed by the lid and pushed into the cell holder. After 2 min thermal equilibration, 7-20 measurements of solute particle size distribution were taken consecutively to calculate the average solute particle size depending on the solution stability. Each measurement takes ~3 min.

2.4 Protein gel electrophoresis

Gel electrophoresis was used for the separation and analysis of proteins based on their molecular weight. For proteolysis, a 22.5 µL fermentation broth sample was mixed with 7.5 µL lithium dodecyl sulfate (LDS) tris(2-carboxyethyl)phosphine (TCEP) buffer (1x), and the mixed solution was heated to 100°C for 10 min. The boiled sample was cooled to room temperature and 25 µL were loaded onto a Bis-Tris protein gel (Bolt 4-12% Bis-Tris Plus, 1 mm × 10 well, Thermo Fisher Scientific) together with 10 µL protein standard (SeeBlue™ Plus2 Pre-stained Protein Standard, Thermo Fisher Scientific, Waltham, MA) for reference. Protein gel electrophoresis was performed using a gel system (XCell SureLock electrophoresis cell, Life Technologies) in 3-(N-morpholino)propanesulfonic acid (MOPS) sodium dodecyl sulfate (SDS) running buffer (20×, Life Technologies) diluted to 1× with de-ionized water. A voltage of 200 V was supplied to the electrophoresis cell by a power supply (EC 300 XL, Thermo Fisher Scientific) for ~32 min until the dye front was near the bottom of the gel. After the electrophoresis was complete, the power supply was turned off and the electrical leads were disconnected. Gel can be peeled off the plate by popping open the gel cassettes, cutting off the sides, and floating into water. The Pierce™ Midi Gel Power Staining Kit and the Pierce™ Power Stainer (Thermo Fisher Scientific) were then used for rapid dye staining of proteins in the peeled off gel (~7 min) and the removal of unbound stain from the gel matrix. The stained protein gel was ready to be imaged.

3 Membrane surface characterization

3.1 Surface contact angle

Surface wettability of the pristine and fouled PTFE membranes was evaluated by sessile drop (SD) water contact angle measurements using an automated drop shape analyzer (DSA20; KRÜSS GmbH). The membranes were kept in a vacuum oven (37 °C) for at least 24 hr prior to water contact angle measurement. An automation program was made to take 10 continuous SD water contact angle measurements every 2s following placement of 15 µL D.I. water drops onto sample surfaces. The previous step was repeated for at least five locations of each membrane sample, and their average was reported as the sample surface water contact angle. The surface free energy of hydration (ΔG_{iw}) was then calculated from the Young-Dupré equation, $\Delta G_{iw} = -\gamma_w(1 + cos\theta_w)$, where γ_w is the liquid water surface tension (72.1 mN/m at 20°C) and θ_w is the water contact angle. It is noted that surfaces are considered hydrophilic and hydrophobic when ΔG_{iw} has ≤ -113 and > -113 mJ/m^2, respectively.[3]

3.2 Surface topography and elemental analysis

The dry surface topography of both the top and bottom sides of the membrane was characterized via scanning electron microscope (SEM, S-4800, Hitachi, Ltd.) for both pristine and fouled membranes. Prior to imaging, membrane samples were dried in a vacuum oven at 37°C for at least 24 hr. The dried samples were sputter-coated (Q150T Plus Turbomolecular pumped coater, Quorum Technologies) to form a 5 nm Chromium film. SEM scanning was carried out with an accelerating voltage of 2.0 kV.

A quantitative analysis of the elements present in the membrane surface layer was carried out with an EDS equipped with the SEM instrument (S-4800, Hitachi, Ltd.). The EDS elementary analysis was conducted immediately after SEM images were taken using an Oxford's Ultim Max 100 mm^2 large area silicon drift detector.

4 Performance evaluation

4.1 Phase separation

Membrane performance tests were carried out using the MBES system (**Fig. S8**, SEP-200, Zaiput Flow Technologies) that can accommodate two flat-sheet PTFE coupon membranes with membrane area of 30 cm^2 each. A 600 mL feed reservoir was sitting on a magnetic stirrer (PC-410D, Corning Inc.) to allow vigorous mixing of two phases for emulsification. The feed emulsion solution was then pumped into the MBES system using a magnetic drive gear pump (GA-T23-DEMSE, MICROPUMP). The pump speed and direction can be adjusted using a control box (1300003, Burt Process Equipment). Both permeate and retentate streams were continuously recirculated to the feed reservoir in a total recycle mode.

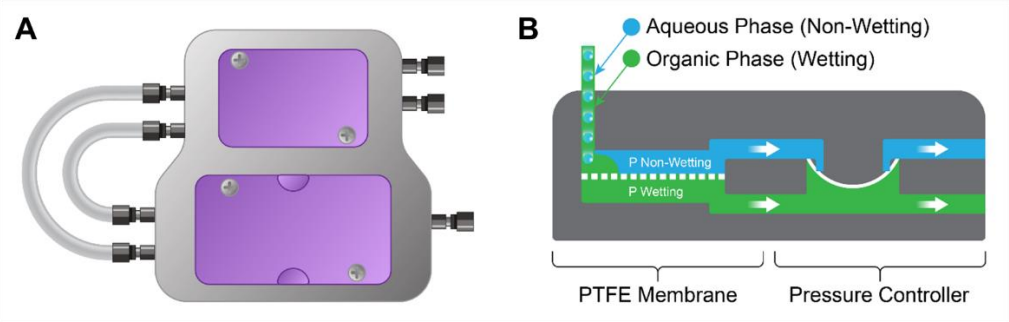


Figure S8. (**A**) The picture of the MBES system, and (**B**) the mechanism for membrane-based emulsion separation (adapted with permission from Zaiput Technology).

The phase separation efficiency of MBES system was tested using the emulsified mock broth as the feed with a wide range of feed flow rates (0-180 mL/min). The emulsified mock broth was prepared by mixing mock 10 g/L butyric acid solution with organic phase (70 vol% Cyanex 923 and 30 vol% mineral oil) at a range of phase ratio (∅) from 0.5 to 3 at a stir rate of 650 rpm for 3 min. Both permeate and retentate samples were collected for each operating condition after 20 min stabilization. The water content of the permeate and retentate samples were then measured using Karl Fisher (**Section 2.2**). The phase separation efficiency (*PS*) is calculated by: $PS = 1 - C_{w,p}/C_{w,f}$, where $C_{w,p}$ and $C_{w,f}$ are the water content of the permeate and feed solutions, respectively. Due to a small water solubility in the organic phase, complete phase separation is defined with the achieved *PS* as high as the thermodynamic limit determined with the batch LLE. The reversibility of the membrane phase breakthrough was assessed by reducing the feed flow rate, collecting and measuring the water content of the permeate samples after 20 min stabilization.

The thermodynamic limit for the phase separation efficiency was determined via batch or overlay LLE until the equilibrium was reached. Specifically, the equilibrium was achieved the mixer – settler with long contact times of the phases involved. Aqueous and organic phases with the targeted phase volume ratio were added to a temperature-controlled shaking cylinder and then manually shake the two phases to accelerate the mass transfer. Equilibrium was established by allowing the mixture to settle and phase separate for over 24 hr.

The demulsification rate of the MBES was calculated using its maximum feed flow rate with complete phase separation (for phase volume ratio of 1) and then compared to the natural emulsion separation. The emulsified

fermentation broth was prepared by mixing 200 mL pre-filtered *C. tyrobutyricum* fermentation broth with 200 mL organic phase (70 vol% Cyanex 923 and 30 vol% mineral oil) with a stir rate of 650 rpm for 3 min. The pictures of the emulsion were taken over a time period of 25 hr with measured emulsion layer water content.

Membrane solvent permeability was evaluated in a 50 mL dead-end stirred UF cell (Amicon 8050, Millipore Corporation, Burlington, MA) following a previously described protocol. The membrane (OB-2000-S200F, Zaiput Flow Technologies, Waltham, MA) was cut into 44.5 mm diameter circular coupons (with active area of 13.4 cm^2). The stirred membrane cell was fed organic phase solution from an 800 mL nitrogen pressurized feed tank. Prior to determining membrane permeability, membrane coupons were compacted with the organic solvent at 3.5 bar (~50 psi) and ~20°C for 3 h until the permeate flux stabilized. Water flux ($J_v = Q_p/A$, in which Q_p and A are the permeate flow rate and membrane area, respectively) was determined over an applied pressure range of 0-3.5 bar (0-50 psi). Water permeability (L_p) was determined from the slope of a linear plot of water flux versus transmembrane pressure (i.e., $L_p = J_v/\Delta P$, where ΔP is the applied transmembrane pressure).

4.2 Butyric acid extraction efficiency

The butyric acid extraction efficiency of MBES system was tested for both mock broth solution and *C. tyrobutyricum* fermentation broth using 70 vol% Cyanex 923 and 30 vol% mineral oil as the organic phase with phase volume ratio of 1. The pH of the mock broth solution and *C. tyrobutyricum* fermentation broth were adjusted to 5 using 0.1 N HCl and 0.1 N NaOH solutions. The feed emulsion solution was prepared by mixing both aqueous and organic phases and fed into the MBES at the maximum feed flow rate with complete phase separation. The retentate sample was collected after 20 min stabilization. The butyric acid concentrations in both original feed solution and MBES retentate were measured using HPLC (**Section 2.1**). The extraction efficiency (*EE*) can be calculated by: $EE = \frac{C_{BA,O,f}}{C_{BA,A,o} \times \emptyset} = \frac{C_{BA,A,o} - C_{BA,A,f}}{C_{BA,A,o}}$, where $C_{BA,A,o}$ is the initial butyric acid concentration in the feed aqueous phase, $C_{BA,O,f}$ is the final butyric acid concentration in the permeate organic phase, $C_{BA,A,f}$ is the final butyric acid concentration in the retentate aqueous phase, and $\emptyset$ is the aqueous:organic phase volume ratio. The MBES butyric acid extraction efficiency was then compared with the thermodynamic limit which was determined through batch LLE.

4.3 Membrane fouling and cleaning

Prior to the fouling tests, the *C. tyrobutyricum* fermentation broth was pre-filtered with an Al_2O_3 ceramic membrane disk with pore size of 0.2 μm and active membrane area of 0.034 m^2 using a dynamic crossflow filter (DCF) system (152/S-P10, Andritz) to filter out cells, cell debris, and other solid fractions of the fermentation broth. Fermentation broth protein removal was carried out with MF membranes with pore size of 0.2 μm and 0.1 μm, and UF membranes with molecular weight cutoff of 10 kDa and 1 kDa using a 50 mL dead-end stirred UF cell (Amicon 8050, Millipore Corporation) at 50 psi.

Fouling tests with both hydrophobic and hydrophilic PTFE membranes were conducted with the emulsified fermentation broth over a period of 1 hr. The emulsified fermentation broth was prepared by mixing 300 mL pre-filtered *C. tyrobutyricum* fermentation broth with 150 mL organic phase at a stir rate of 650 rpm for 3 min. Membrane fouling propensity was assessed following a previously described protocol. Briefly, permeate flux decline, quantified as $FD = 1 - J_{v,t}/J_{v,o}$, where $J_{v,t}$ and $J_{v,o}$ designate the permeate fluxes at time t and 0, respectively, was followed over the filtration period.

The clean membrane permeability coefficient, L_p, was determined using the organic solvent (**Section S2.4.2**) prior to each fouling test, and the corresponding intrinsic membrane hydraulic resistance, R_m, was determined from the relation $L_p = 1/\mu R_m$, where μ is the organic solvent viscosity (20°C in the current study). At the end of each fouling test, a determination was made of the membrane overall hydraulic resistance (R_T) being the sum of the intrinsic membrane resistance (R_m) and fouling resistance ($R_{fouling}$). It is noted the fouling resistance ($R_{fouling}$) is the sum of reversible and irreversible fouling resistances: $R_{fouling} = R_{reversible} + R_{irreversible}$.

Membrane backwash with the permeate organic phase was conducted by reversing the pump direction at ~20 mL/min for 2 min was carried out at the end of each fouling test to evaluate membrane cleaning efficacy. The resistance of the backwashed membrane was then again determined with organic solvent, thereby allowing quantification of the

combined intrinsic membrane and irreversible fouling resistances expressed as $R'_T = R_m + R_{irrev}$. Subsequently, R_{rev} and R_{irrev} were determined given the calculated values of R_T and R'_T.

5 Techno-economic analysis (TEA) and life cycle assessment (LCA)

TEA was used to determine butyric acid production cost. Aspen Plus process models that incorporated experimental data were developed to solve mass and energy balances for each unit operation. The material and energy flows from the process models allow for the estimation of the associated capital and operating costs. The equipment costs were estimated based on the scale of butyric acid production rate of 21.7 t/hr.

Greenhouse gas (GHG) emissions are represented in grams of carbon dioxide equivalent (CO_2e) using a 100-year GHG emission factor. Fossil energy demand (FED) is determined based on the method published by Ecoinvent version 3.3 and expanded by PRé Consultants for raw materials available in the SimaPro 9.5 software. The input inventory that captures the impacts of input raw materials and energy provides the necessary information required to perform the LCA modeling to quantify greenhouse gas (GHG) emissions and fossil energy consumption. We used the DATASMART Life Cycle Inventory Package, which is a dataset representative of the North American region provided containing expanded modified Ecoinvent processes to be reflective of U.S. conditions and the US LCI processes (USLCI) to account for embodied emissions and energy flows. The GHG and FED basis values for electricity are applied consistently with the values utilized in GREET 2021. The factors are used to convert the life cycle inventory to the partial life cycle GHG emissions and FED which are expressed in CO_2e and megajoule (MJ) per kg butyric acid, respectively. Other impact categories were also quantified and assessed using TRACI 2.1 (v.104).

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