

Supplementary material

Table S1. Annual operating hours for each equipment for Scenario I, II and III

Operation	Processing time per batch ^b			Number of batches per year ^d (1 year = 7884 operation hours)			Annual operating hours ^e		
	I	II	III	I	II	III	I	II	III
Shake flask	16	32	16	149.2	130.1	157.2	2387.28	4176.95	2522.88
Seed fermentation ^a	19.5	38	19				2909.50	4960.13	2995.92
First-stage fermentation ^a	21	40.6	20.3				3133.31	5299.51	3200.90
Second-stage fermentation ^a	32.61	60.4 ^c	30.2				4865.58	7884.00	4761.94
Production-stage fermentation ^a	52.84 ^c	50	50 ^c				7884.00	6526.49	7884.00
Centrifugation	12	12	12				1790.46	1566.36	1892.16
Filtration	12	12	12				1790.46	1566.36	1892.16
Solvent extraction	12	12	12				1790.46	1566.36	1892.16
Distillation	48	48	48				7161.85	6265.43	7568.64
Freeze-drying	12	12	12				1790.46	1566.36	1892.16

^a The sterilization was performed inside fermenter for all fermentation processes. Therefore, the sterilization processing time has been included in the calculation of the processing time of each fermentation step in SuperPro Designer 8.5[®]

^b The value shown here is the processing time per batch of equal rhamnolipid production quantity for the bottleneck process in each scenario. The values were calculated from SuperPro Designer 8.5®

^c The bottleneck operation i.e., the one with the longest operating hours per batch for each Scenario is indicated.

^d The number of batches per year was calculated by dividing 7884 operation hours by the bottleneck processing time. For example, batches per year in Scenario I = $7884 / 52.84 = 149.2$ batches per year.

^e The annual operating hours was calculated by multiplying the processing time per batch using each equipment by the number of batcher per year.

<i>Total equipment cost (TEC)</i>					\$9,311,529.24	\$9,174,280.19	\$9,613,890.88
Direct cost (DC)					\$39,386,005.99	\$38,716,335.59	\$40,379,110.37
Indirect cost (IC)					\$7,089,481.08	\$6,968,940.41	\$7,268,239.87
Total direct and indirect cost (TDIC)					\$46,475,487.07	\$45,685,275.99	\$47,647,350.24
Contractor's fee					\$2,323,774.35	\$2,284,263.80	\$2,382,367.51
Contingency					\$4,647,548.71	\$4,568,527.60	\$4,764,735.02
Fixed Capital Investment (FCI)					\$53,446,810.12	\$52,538,067.39	\$54,794,452.78
Working capital					\$9,431,790.02	\$9,271,423.66	\$9,669,609.31
<i>Total Capital Investment (TCI)</i>					\$62,878,600.15	\$61,809,491.05	\$64,464,062.09

Table S3. Estimation of utility usage in each operation

Operation	Annual feedstock capacity (kg/year)			Electricity (kWh)			Steam (MT)			Cooling water (m ³)		
	I	II	III	I	II	III	I	II	III	I	II	III
Shake flask	3133	2741	3311	1323.35	1221.33	1367.95	0.00	0.00	0.00	0.00	0.00	0.00
Seed fermentation	31333	27411	33113	4653.52	4181.40	4863.80	0.51	0.46	0.54	0.00	0.00	0.00
First-stage fermentation	313331	274113	331128	5687.32	5248.84	5879.00	8.76	8.08	9.05	0.00	0.00	0.00
Second-stage fermentation	3133308	2741126	3311280	27721.64	24909.19	28974.31	42.70	38.37	44.63	0.00	0.00	0.00
Production-stage fermentation	31333081	27411260	33112800	49799.55	44747.23	52049.87	392.10	352.32	409.82	0.00	0.00	0.00
Centrifugation	31333081	27411260	33112800	274217.84	253076.17	283459.74	0.00	0.00	0.00	0.00	0.00	0.00
Filtration	31333081	27411260	33112800	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Solvent extraction	52031714	45519139	54987116	41565.25	38360.65	42966.12	0.00	0.00	0.00	0.00	0.00	0.00
Distillation	20698633	18107879	21874316	0.00	0.00	0.00	34419.33	31765.66	35579.36	2208935.11	2038630.42	2283382.36
Freeze-drying	313331	274113	331128	5589816.12	4890176.27	5907323.51	0.00	0.00	0.00	0.00	0.00	0.00
Sterilization	34814186	30456651	36791632	3512751.42	3073076.13	3712275.69	0.00	0.00	0.00	0.00	0.00	0.00
Total				9507536.01	8334997.20	10039159.99	34863.40	32164.89	36043.39	2208935.11	2038630.42	2283382.36

MT: metric tons

Table S4. Estimation of labor cost in each operation

Operation	Annual operating hours ^b			Operators/unit	Quantity in each scenario (unit)			Salary/hour/operator	Labor Cost (US\$) in each scenario ^c		
	I	II	III		All scenarios	I	II		III	All scenarios	I
Shake flask	2387.28	4176.95	2522.88	1	1	1	2	\$17.68	\$42,206.92	\$73,848.13	\$89,208.54
Seed fermentation	2909.50	4960.13	2995.92	1	1	1	2		\$51,439.68	\$87,694.65	\$105,935.14
First-stage fermentation	3133.31	5299.51	3200.90	1	1	1	2		\$55,396.58	\$93,694.81	\$113,183.33
Second-stage fermentation	4865.58	7884.00	4761.94	1	1	1	2		\$86,022.97	\$139,388.34	\$168,381.11
Production-stage fermentation	7884.00	6526.49	7884.00	1	1	2	2		\$139,388.34	\$230,775.39	\$278,776.67
Centrifugation	1790.46	1566.36	1892.16	0.5	1	1	1		\$15,827.59	\$13,846.52	\$16,726.60
Filtration	1790.46	1566.36	1892.16	0.25	1	1	1		\$7,913.80	\$6,923.26	\$8,363.30
Solvent extraction	1790.46	1566.36	1892.16	0.5	1	1	1		\$15,827.59	\$13,846.52	\$16,726.60
Distillation	7161.85	6265.43	7568.64	1	1	1	1		\$31,655.19	\$27,693.05	\$33,453.20
Freeze-drying	1790.46	1566.36	1892.16	0.5	1	1	1		\$63,310.37	\$55,386.09	\$66,906.40

^a The sterilization was performed inside fermenter for all fermentation processes. Therefore, the sterilization processing time has been included in the calculation of the processing time of each fermentation step in SuperPro Designer 8.5®. This means that the labor cost of the sterilization process is also already included in the fermentation step.

^b Annual operating hours were taken from data in Table S1.

^c Labor cost (\$) = Annual operating (hours) * (operators/unit) * quantity of equipment (unit) * operator salary (\$/hour/operator).

Table S5. Summary of operating hours, temperature, utilities cost, and labor cost in each operation

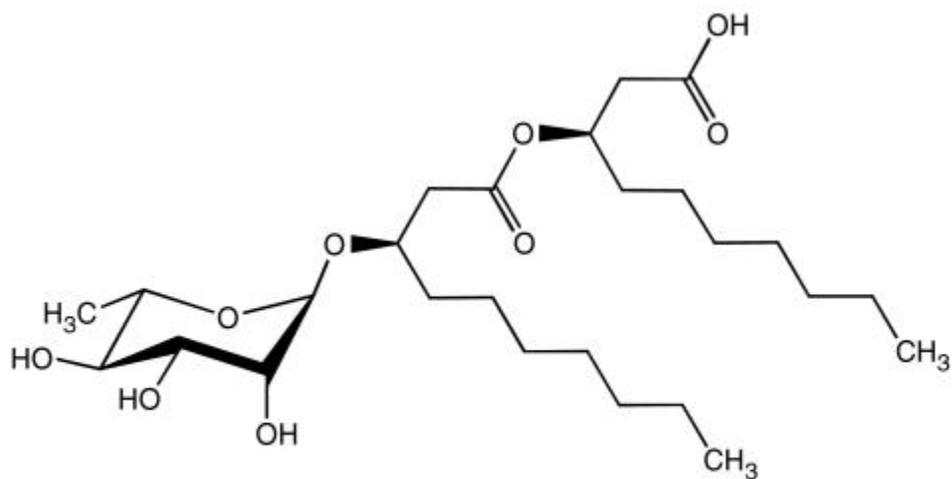
Operation	Annual operating hours ^a			Temperature (°C)			Utilities cost ^b (US\$)			Labor Cost (US\$) in each scenario		
	I	II	III	I	II	III	I	II	III	I	II	III
Shake flask	2387.28	4176.95	2522.88	55	55	55	\$161.45	\$149.00	\$166.89	\$42,206.92	\$73,848.13	\$89,208.54
Seed fermentation	2909.50	4960.13	2995.92	55	55	55	\$573.90	\$515.68	\$599.84	\$51,439.68	\$87,694.65	\$105,935.14
First-stage fermentation	3133.31	5299.51	3200.90	55	55	55	\$798.97	\$737.37	\$825.90	\$55,396.58	\$93,694.81	\$113,183.33
Second-stage fermentation	4865.58	7884.00	4761.94	55	55	55	\$3,894.40	\$3,499.30	\$4,070.38	\$86,022.97	\$139,388.34	\$168,381.11
Production-stage fermentation	7884.00	6526.49	7884.00	55	55	55	\$10,780.73	\$9,686.99	\$11,267.88	\$139,388.34	\$230,775.39	\$278,776.67
Centrifugation	1790.46	1566.36	1892.16	25	25	25	\$33,454.58	\$30,875.29	\$34,582.09	\$15,827.59	\$13,846.52	\$16,726.60
Filtration	1790.46	1566.36	1892.16	25	25	25	\$0.00	\$0.00	\$0.00	\$7,913.80	\$6,923.26	\$8,363.30
Solvent extraction	1790.46	1566.36	1892.16	25	25	25	\$5,070.96	\$4,680.00	\$5,241.87	\$15,827.59	\$13,846.52	\$16,726.60
Distillation	7161.85	6265.43	7568.64	70	70	70	\$479,300.00	\$442,346.88	\$495,453.74	\$31,655.19	\$27,693.05	\$33,453.20
Freeze-drying	1790.46	1566.36	1892.16	-65	-65	-65	\$681,957.57	\$596,601.51	\$720,693.47	\$63,310.37	\$55,386.09	\$66,906.40

Sterilization				121	121	121	\$428,555.67	\$374,915.29	\$452,897.63			
Total costs							\$1,644,548.23	\$1,464,007.31	\$1,725,799.68	\$508,989.02	\$743,096.77	\$897,660.89

^a Annual operating hours were taken from data in Table S1.

^b Utilities cost were calculated by adding the electricity, steam, and cooling water costs based on the utility usage in Table S3 and its unit prices specified in Table 1.

(A)



(B)

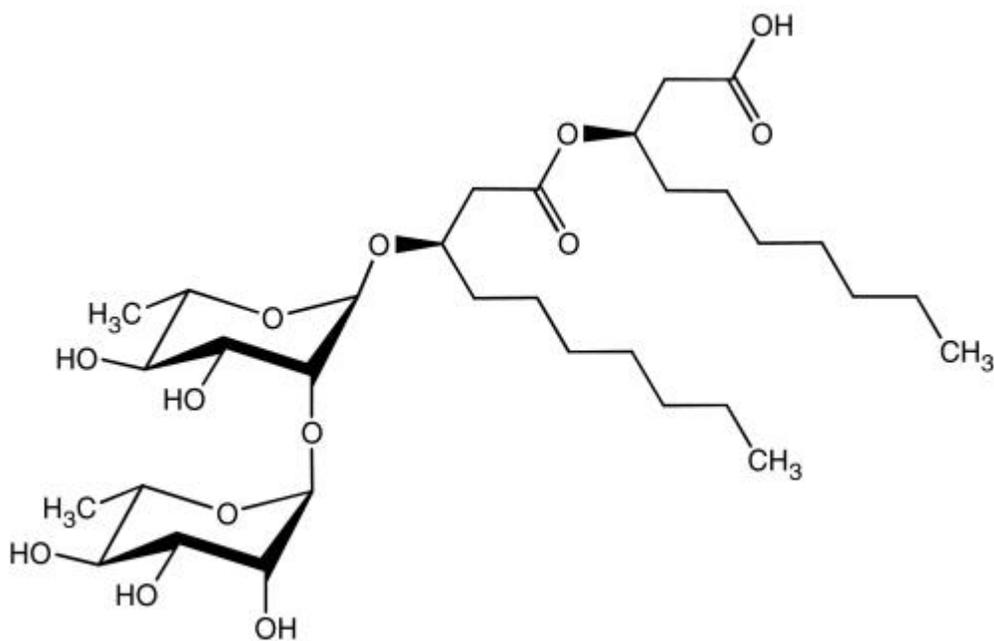


Figure S1. Examples of rhamnolipids structures. (A) Mono-rhamnolipids and (B) Di-rhamnolipids. Reproduced from Abdel-Mawgoud, *et al.* [9]. Open Access under Creative Commons Attribution License.