Supplementary Materials

Table S1: All media components of Beefy-9, their concentrations, yearly demand, and projected costs.

Components	Concentration (mg/L)	Yearly Industry Demand (kg)	Cost per mass ($/g)	Cost per volume ($/L media)	Cost Contribution ($/kg meat)
Amino Acids					
Glycine	18.75	19,257	0.84	0.02	0.16
L-Alanine	4.45	4,570	1.90	0.01	0.09
L-Arginine hydrochloride	147.50	151,488	0.26	0.04	0.40
L-Asparagine-H2O	7.50	7702	1.42	0.01	0.11
L-Aspartic acid	6.65	6,829	1.51	0.01	0.10
L-Cysteine hydrochloride-H2O	17.56	18,034	0.88	0.02	0.16
L-Cysteine 2HCl	31.29	32,136	0.63	0.02	0.20
L-Glutamic acid	7.35	7,548	1.43	0.01	0.11
L-Glutamine	365.00	374,871	0.16	0.06	0.60
L-Histidine hydrochloride-H2O	31.48	32,331	0.63	0.02	0.20
L-Isoleucine	54.47	55,943	0.46	0.03	0.26
L-Leucine	59.05	60,646	0.44	0.03	0.27
L-Lysine hydrochloride	91.25	93,717	0.35	0.03	0.32
L-Methionine	17.24	17,706	0.89	0.02	0.16
L-Phenylalanine	35.48	36,439	0.59	0.02	0.21
L-Proline	17.25	17,716	0.89	0.02	0.16
L-Serine	26.25	26,959	0.70	0.02	0.19
L-Threonine	53.45	54,895	0.47	0.03	0.26
L-Trypthophan	9.02	9,263	1.28	0.01	0.12
L-Tyrosine	55.79	57,298	0.46	0.03	0.26
L-Valine	52.85	54,279	0.47	0.02	0.26
Vitamins					
Ascorbic acid 2-phosphate	200.00	205,408	0.0020	0.0004	0.0041
Biotin	0.00	3.59	0.0100	0.0000	0.0000
Choline chloride	8.98	9,222	0.0001	0.0000	0.0000
D-Calcium pantothenate	2.24	2,300	0.0000	0.0000	0.0000
Folic Acid	2.65	2,721	0.0380	0.0001	0.0010
Niacinamide	2.02	2,074	0.0010	0.0000	0.0000
Pyridoxine hydrochloride	2.01	2,067	0.0150	0.0000	0.0003
Riboflavin	0.22	224	0.0050	0.0000	0.0000
Thiamine hydrochloride	2.17	2,228	0.0010	0.0000	0.0000
Vitamin B12	0.68	698	0.0200	0.0000	0.0001

i-Inositol	12.60	12,940	0.0050	0.0001	0.0006
Inorganic Salts					
Sodium selenite	0.02	20.54	0.0180	0.0000	0.0000
Calcium chloride	116.60	119,753	0.0001	0.0000	0.0001
Cupric sulfate	0.00	1.34	0.0300	0.0000	0.0000
Ferric nitrate	0.05	51.35	0.0100	0.0000	0.0000
Ferric sulfate	0.42	428	0.0002	0.0000	0.0000
Magnesium chloride	28.64	29,414	0.0001	0.0000	0.0000
Magnesium sulfate	48.84	50,160	0.0001	0.0000	0.0001
Potassium chloride	311.80	320,232	0.0003	0.0001	0.0010
Sodium bicarbonate	2,438.00	2,503,932	0.0002	0.0005	0.0050
Sodium chloride	69,95.50	7,184,685	0.0001	0.0003	0.0036
Sodium Phosphate monobasic	62.50	64,190	0.0005	0.0000	0.0003
Zinc sulfate	0.43	443	0.0005	0.0000	0.0000
Carbohydrates					
D-glucose (Dextrose)	3,151.00	3,236,215	0.0440	0.1386	1.4239
Sodium pyruvate	55.00	56,487	0.0010	0.0001	0.0006
Lipids					
Linoleic acid	0.04	43.14	0.0100	0.0000	0.0000
Lipoic acid	0.11	107	0.0600	0.0000	0.0001
Growth Factors/Hormones/Proteins					
Insulin (human, recombinant)	20.00	20,540	5.9	0.1177	1.2091
Transferrin (human, recombinant)	20.00	20,540	5.9	0.1177	1.2091
FGF2	0.01	5.14	7,433.8	0.0372	0.3817
TGF-β3	0.00	0.10	215,785.3	0.0216	0.2216
rAlbumin	800.00	821,635	0.2	0.1966	2.0190
NRG1	0.00	0.10	215,785.3	0.0216	0.2216
Other					
Phenol red	8.10	8,319	0.001	0.0000	0.0001
Putrescine 2HCl	0.08	83.19	2.780	0.0002	0.0023
Thymidine	0.37	374	0.030	0.0000	0.0001
Hypoxanthine	2.39	2,454	0.030	0.0001	0.0007

Fermentation Operation Assumptions

To meet the required OUR, a power input of 500 W/m^3 was chosen for all STR reactors, and this value is also on the upper limit for typical mammalian cell culture (Nienow, 2015). Similarly, a sparging of 0.01 vvm was chosen for all STR reactors to target the maximum value for typical mammalian cell culture (Nienow, 2015). For each reactor, calculations were performed to calculate the following: reactor dimensions, impeller number (n) and diameter (D_i), power number (N_p), gassed and ungassed power (P_g and P_o), aeration number (N_a), and agitation rate (N). From these data, important scale-up parameters were calculated, namely a mixing parameter ($N*D_i^3/V$) and a shear parameter ($N*D_i$). Since the power input per volume was set constant across all sized STRs, the mixing and shear parameters must change. At the 100 L scale, the mixing parameter is 0.33 and the shear parameter is 1.03. Scaling up to 170,000 L WV, the mixing parameter decreases by a factor of ~0.25 to 0.08, and the shear parameter increases by a factor of ~2 to 2.15. For the 260,000 L ALR, a power input of 50 W/m^3 and a superficial gas velocity of 3 cm/s (0.1 vvm) was chosen in alignment with the CFD work (Li et al., 2020).

Oxygen Transfer Rate and Oxygen Uptake Rate

$$\frac{dC_L}{dt} = OTR - OUR \qquad \textbf{Equation S1}$$

Equation S1 shows that the change in oxygen concentration in the liquid is defined by the difference between the oxygen transfer rate (OTR) and the oxygen uptake rate (OUR). In order to meet the oxygen demands of the cells, it is necessary for OTR to be greater than or equal to OUR.

$$OUR = \frac{\mu X}{Y_{X/O}} = \frac{biomass\ growth\ rate * max\ biomass\ concentration}{yield\ of\ biomass/oxygen}$$

Equation S2

For OUR calculations, the specific growth rate, μ, is 0.0292 hr^{-1} as used in the simulation. The maximum biomass concentration at the end of fermentation, X, is 30 g FW/L. Finally, the yield of biomass on oxygen, $Y_{X/O}$, is 2.65. This results in an OUR of ~10.3 mmol/L/hr.

$$OTR = k_l a(C^* - C_L) = mass\ transfer\ coeff. * (max\ O_2\ conc. - setpoint\ O_2\ conc.) \qquad \textbf{Equation S3}$$

$$k_L a = A\left(P_g/V\right)^{\alpha} (v_s)^{\beta} \qquad \textbf{Equation S4}$$

Equation S4 shows the general form of a k_La correlation with a dependence on gassed power, P_g/V, and the fluid velocity. For OTR calculations for the STR reactors, the mass transfer coefficient was calculated from a correlation by Van't Reit, 1979, which assumes a non-coalescing aqueous system and uses 0.002 for A, 0.7 for α, and 0.2 for β (Van't Riet, 1979). For a 100 L WV STR, the k_La was calculated to be about 94 hr^{-1}. Using equation S3, C*, the maximum possible O_2 concentration, is assumed to be about 6.7 mg/L (Anon, 2013; Anon, 2022). The dissolved oxygen setpoint, C_L, was set at 35% or 2.5 mg/L. OTR for this small scale of 100 L WV was thus calculated to be ~12.8 mmol/L/hr, meeting the OUR of ~10.3 mmol/L/hr. Scaling up to 200,000 L STR, the OTR was calculated to be ~21.0 mmol/L/hr.

For the 260,000 L ALR case, a correlation specifically for sparge type column is used with a A of 0.32, a β of 0.7, and an α of zero or rather the OTR has no dependence on the power input (Heinen and Van't Riet, 1982). This resulted in a OTR of ~13.5 mmol/L/hr, which comfortably meets the 10.3 mmo/L/hr OUR.

High Temperature Short Time Sterilization Design
Example for 42K L scenario:
1 contamination per 50 years
Total medium: 330,967 L/batch, Set sterilization time: 30 hours
6,150 batches per 50 years, Probability of contamination: 1/6,150 = 1.626×10^{-4}
Assume initially 10^6 spores/mL medium
$n_{v0}/n_v = (10^6$ spores/mL x 10^3 mL/L x 330,967 L/batch) / $1.626 \times 10^{-4} = 2.04 \times 10^{18}$
$\ln(n_{v0}/n_v) = 42$
Flow rate $F = (D^2/4) \times u$ = 330,967 L / (30 hr x 60 min/hr) = 184 L/min
Set pipe diameter D = 3 in = 7.62 cm
Linear flow rate u = 67 cm/s
Reynolds number $Re = (D \times u \times \rho) / \mu$ = (7.62 cm x 67 cm/s x 1 g/cm^3) / 0.01 g/cms = 51,200 so turbulent flow regime
Peclet number (convention over diffusion) for turbulence, $Pe_z = 3.33(L/D)$
Damkohler number (reaction rate over convection), $Da = kL/u$
$n_v(L)/n_v = \exp(-Da + Da^2/Pe_z)$
$k^2 \times L / u^2 - (3.33k \times L) / (D \times u) + (\ln(n_{v0}/n_v) \times 3.33) / D = 0$
B. stearothermophilus spores: k = 136 min^{-1} = 2.27 s^{-1} @ T = 140 C
Length of pipe L = 13.5 m

Table S2. Clean-in-place procedures used in the process simulation models. LPM, liter per minute per meter circumference; RO, reverse osmosis; WFI, water-for-injection.

Step	Material	Flow Rate	Time (minutes)	Temperature (°C)
Pre-rinse	Potable water	15 L/min-m	15	25
Alkaline wash	NaOH (0.5M)	15 L/m^2 filter	30	25
Post-rinse	USP water	15 L/m^2 filter	15	25
Acid rinse	Citric acid (5% w/w)	15 L/m^2 filter	30	25
Water rinse	USP water	15 L/m^2 filter	15	25

Table S3. Sizes and prices of each unit, and overall seed train design for each scenario

Scenario 1: 42,000 L Production Reactor						
Unit Description	Volume (L) or throughput (L/hr)	Unit Name	Number of Parallel Equipment	Stagger Factor	Single Equipment Purchase Cost (PC)	Total PC of all Equipment in Step
media tank	22,266,600	V0	1	1	2,219,000	2,219,000
HTST sterilizer	186 L/min	ST0	1	1	500,000	500,000
seed tanks	124	R1	1	1	28,000	28,000
	628	R2	1	1	73,000	73,000
	3,188	R3	1	1	193,000	193,000
	16,190	R4	1	1	511,000	511,000
	16,435	R5	5	1	516,000	2,580,000

production reactor	39,546	R6	10	6	902,000	54,120,000
centrifuge	82,967	C1	1	1	989,000	989,000
Scenario 2: 210,000 L Production Reactor						
Unit Description	Volume (L) or throughput (L/hr)	Unit Name	Number	Stagger factor	Single Equipment Purchase Cost (PC)	Total PC of all Equipment in Step
media tank	1,869,754	V0	1	1	5,854,000	5,854,000
HTST sterilizer	29,580	ST0	1	1	677,000	677,000
seed train	123	R1	1	1	27,000	27,000
	624	R2	1	1	72,000	72,000
	3,164	R3	1	1	192,000	192,000
	16,068	R4	1	1	509,000	509,000
	40,777	R5	2	1	809,000	1,618,000
	82,830	R6	5	1	1,361,000	6,805,000
production reactor	210,268	R7	10	5	2,380,000	119,000,000
centrifuge	418,007	C1	1	1	4,248,000	4,248,000
Scenario 3: 260,000 L Production Reactor						
Unit Description	Volume (L) or throughput (L/hr)	Unit Name	Number	Stagger factor	Single Equipment Purchase Cost (PC)	Total PC of all Equipment in Step
media tank	2,318,343	V0	1	1	6,661,000	6,661,000
HTST sterilizer	610	ST0	1	1	723,000	723,000
seed tanks	153	R1	1	1	31,000	31,000
	773	R2	1	1	82,000	82,000
	3,923	R3	1	1	218,000	218,000
	19,923	R4	1	1	579,000	579,000
	50,560	R5	2	1	1,012,000	2,024,000
	85,585	R6	6	1	1,388,000	8,328,000
production reactor	260,715	R7	10	5	312,000	15,600,000
centrifuge	518,294	C1	1	1	5,223,000	5,223,000

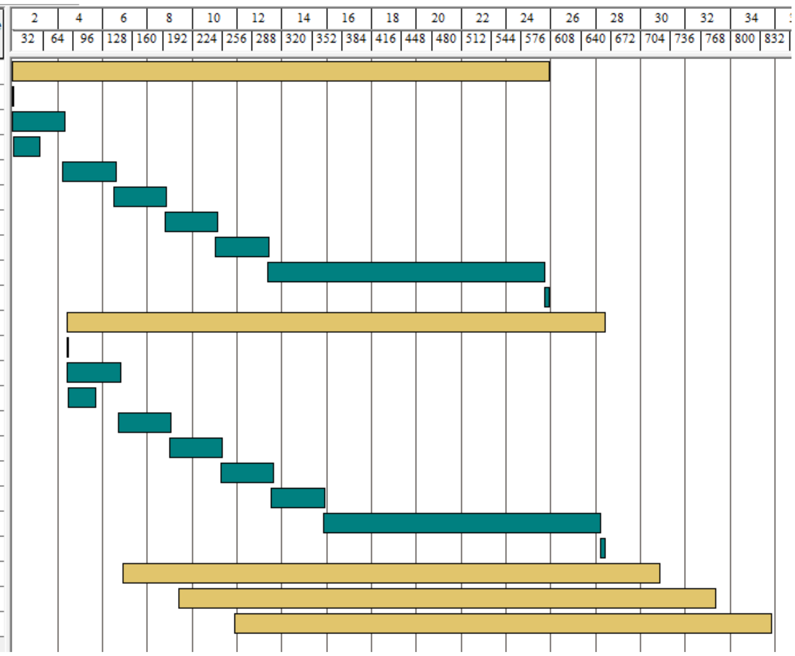
Table S4. Process simulation operating expenditures (OPEX) and capital expenditures (CAPEX) assumptions. Parameters. UF/DF, ultrafiltration/diafiltration.

Expense Type	Parameter	Value
OPEX	Labor Cost	Basis: total labor cost (TLC) = basic labor rate x (1 + benefits(0.4) + supervision(0.2) + supplies(0.1) + administration(0.6))
	Labor Types	Upstream Operator • Basic rate = \$20/hour; TLC = \$46/hour • Time utilization = 60%

		Downstream Operator • Basic rate = \$25/hour; TLC = \$57.5/hour • Time utilization = 60%
	Laboratory / Quality Assurance / Quality Control	Basis: % total labor cost (15%)
	Utility	Electricity: \$0.1/kW-h Steam: \$12.00/MT Chilled water: \$0.40/MT Biowaste disposal: \$0.02/kg
	Water	Potable water: \$0.055/L USP purified water: \$0.0004/L
	Facility-Dependent Costs	Basis: maintenance, depreciation, insurance, local taxes, factory expense
	Maintenance Cost	Basis: % equipment purchase cost (section dependent)
CAPEX	Unlisted Equipment	Basis 20% of the total equipment costs are devoted to overlooked equipment and accessories (e.g. integrity testing equipment) that are not explicitly included in the model
	Direct Fixed Capital (DFC)	Basis: 1.2 x listed equipment purchase cost (20% for unlisted equipment) + direct cost factors* (piping, instrumentation, insulation, electrical facilities, buildings, yard improvement, auxiliary facilities) + indirect cost factors* (engineering, construction) + other cost factors* (contractor's fee, contingency) *Note: see model for associated calculations
	Working Capital (WC)	Basis: 30 days raw materials, labor, utilities, waste treatment
	Startup Costs	Basis: % DFC (section dependent) Neglected: upfront research and development, upfront royalties, land purchase cost

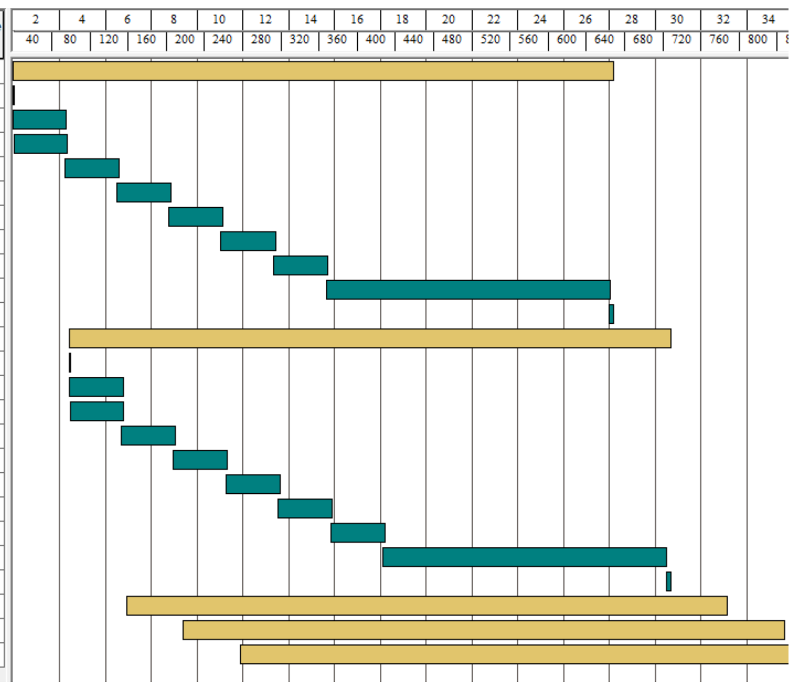
Figure S1. Equipment Gantt Charts for each scenario of A) 42,000 L, B) 210,000 L, and C) 260,000 L production reactors. Five batches are shown for each, with the first and second batch expanded to show the duration of each equipment. Part D) shows the Gantt Chart for the 260,000 L without staggered reactors, which demonstrated the beneficial effect staggering has on scheduling. In the no staggering case, the next batch cannot start until the production step ends.

A)



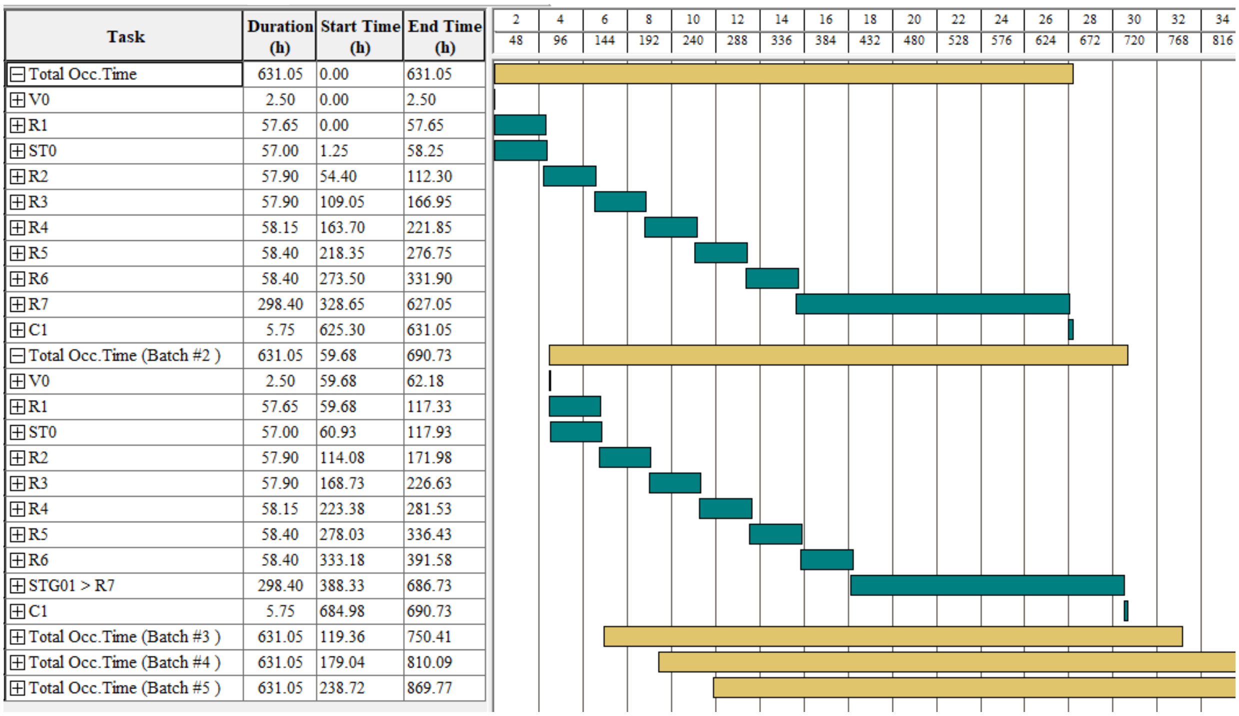
Task	Duration (h)	Start Time (h)	End Time (h)
⊟ Total Occ.Time	576.15	0.00	576.15
⊞ V0	2.50	0.00	2.50
⊞ R1	57.65	0.00	57.65
⊞ ST0	30.00	1.25	31.25
⊞ R2	57.90	54.40	112.30
⊞ R3	57.90	109.05	166.95
⊞ R4	58.15	163.70	221.85
⊞ R5	58.40	218.35	276.75
⊞ R6	298.65	273.50	572.15
⊞ C1	5.75	570.40	576.15
⊟ Total Occ.Time (Batch #2)	576.15	59.73	635.88
⊞ V0	2.50	59.73	62.23
⊞ R1	57.65	59.73	117.38
⊞ ST0	30.00	60.98	90.98
⊞ R2	57.90	114.13	172.03
⊞ R3	57.90	168.78	226.68
⊞ R4	58.15	223.43	281.58
⊞ R5	58.40	278.08	336.48
⊞ STG01 > R6	298.65	333.23	631.88
⊞ C1	5.75	630.13	635.88
⊞ Total Occ.Time (Batch #3)	576.15	119.46	695.61
⊞ Total Occ.Time (Batch #4)	576.15	179.19	755.34
⊞ Total Occ.Time (Batch #5)	576.15	238.92	815.07

B)



Task	Duration (h)	Start Time (h)	End Time (h)
⊟ Total Occ.Time	631.05	0.00	631.05
⊞ V0	2.50	0.00	2.50
⊞ R1	57.65	0.00	57.65
⊞ ST0	57.00	1.25	58.25
⊞ R2	57.90	54.40	112.30
⊞ R3	57.90	109.05	166.95
⊞ R4	58.15	163.70	221.85
⊞ R5	58.40	218.35	276.75
⊞ R6	57.90	273.50	331.40
⊞ R7	298.40	328.65	627.05
⊞ C1	5.75	625.30	631.05
⊟ Total Occ.Time (Batch #2)	631.05	59.68	690.73
⊞ V0	2.50	59.68	62.18
⊞ R1	57.65	59.68	117.33
⊞ ST0	57.00	60.93	117.93
⊞ R2	57.90	114.08	171.98
⊞ R3	57.90	168.73	226.63
⊞ R4	58.15	223.38	281.53
⊞ R5	58.40	278.03	336.43
⊞ R6	57.90	333.18	391.08
⊞ STG01 > R7	298.40	388.33	686.73
⊞ C1	5.75	684.98	690.73
⊞ Total Occ.Time (Batch #3)	631.05	119.36	750.41
⊞ Total Occ.Time (Batch #4)	631.05	179.04	810.09
⊞ Total Occ.Time (Batch #5)	631.05	238.72	869.77

C)



D)

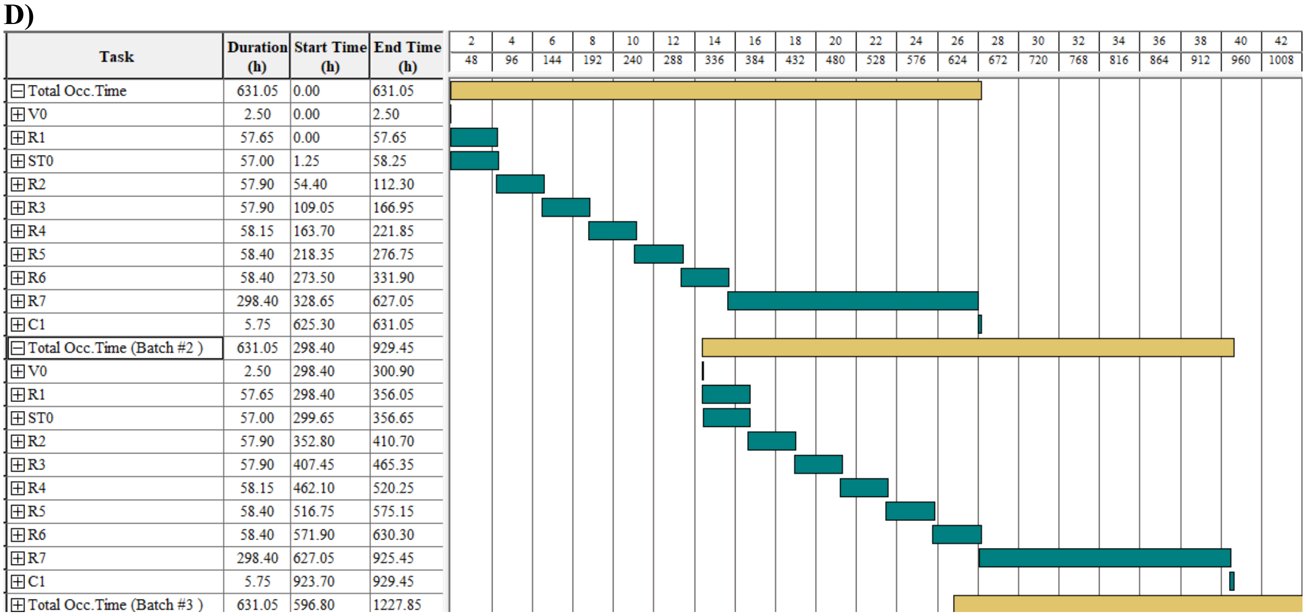


Table S5. Comparison of different staggering modes for the main production reactor in each of the three scenarios.

	Scenario 1: 42,000 L Production Reactor					
Number of staggered sets	1	2	3	4	5	6
Cycle Time (days)	12.4	6.2	4.2	3.1	2.5	2.4
# Batches	25	50	74	99	123	126

COGS ($/kg)	40.6	34.0	32.1	31.0	30.4	33.2
	Scenario 2: 210,000 L Production Reactor					
Number of staggered sets	1	2	3	4	5	6
Cycle Time (days)	12.4	6.2	4.1	3.1	2.5	2.4
# Batches	25	49	74	98	123	125
COGS ($/kg)	26.6	23.1	21.8	21.2	20.8	22.3
	Scenario 3: 260,000 L Production Reactor					
Number of staggered sets	1	2	3	4	5	6
Cycle Time (days)	12.4	6.2	4.1	3.1	2.5	2.4
# Batches	25	49	74	98	123	125
COGS ($/kg)	18.6	15.2	14.0	13.4	13.0	13.1

Supplementary References

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