INTERACTIVE CONTROL ENGINEERING
Principles and Applications

Volume 19 Control of Biological Systems and Human Performance

CHAPTER 8

Feeding an Antibody Culture Whose Appetite Outruns Its Daily Sample

Pages 202–233

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How to cite this chapter. "Feeding an Antibody Culture Whose Appetite Outruns Its Daily Sample," in *Control of Biological Systems and Human Performance*, John D. Hedengren, Ed., vol. 19 of *Interactive Control Engineering: Principles and Applications*. Advanced Process Solutions, LLC, 2026, ch. 8, pp. 202–233.

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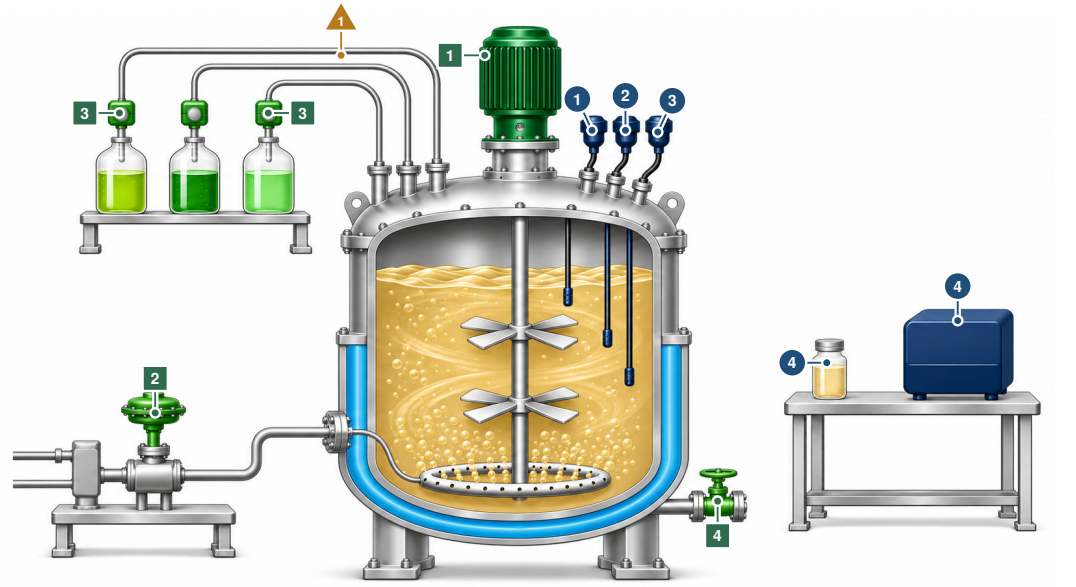
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CHAPTER 8

Feeding an Antibody Culture Whose Appetite Outruns Its Daily Sample



The controller moves 1 the agitator, which stirs the broth 2 the gas blend and its flow to the one sparger 3 three feed pumps: glucose stock, nutrient feed and base 4 the jacket valve that sets the temperature

The controller reads 1 dissolved oxygen in the broth 2 the pH of the broth 3 the temperature of the broth 4 the daily sample, drawn to the bench and read four hours later

What disturbs it 1 a weaker glucose lot hung under the same label

Notice that the three probes in the broth answer every scan, while the one sample that sees glucose, cells and product together is read four hours after it is drawn. Illustration, not to scale.

IN PLAIN TERMS Feeding Cells That Grow Hungrier Every Day

The main idea. Some medicines are grown by living cells in a tank the size of a small swimming pool. The cells are fed sugar, and they need more of it each day as they multiply.

The challenge. A sample of the broth is taken once a day, and the answer comes back hours later. By then the cells have eaten more, or much less if the broth has been cooled. A feed sized from that answer can starve them or leave sugar they turn into acid.

Why it matters. Starved cells make less medicine, and the acid has to be neutralized with something that stays in the tank and slows the cells down.

THREE WAYS TO READ THIS CHAPTER

Explore	12 min	The first page, the history box of Section 8.2, the fact box of Section 8.5, the two try-it boxes and the fact box of Section 8.6 and the key points
Study	70 min	The whole chapter with its pause points and the twelve exercises
Practice	25 min	The first page, Section 8.6, Section 8.7, Section 8.8 and the open challenge after it

The control question An antibody culture's appetite for glucose doubles with its cells, halves when the broth is cooled, and is measured directly only in a sample drawn once a day and reported four hours later. Which feed holds the glucose low but never at zero, and what does each correction of oxygen, pH and glucose leave behind in the broth?

Learning objectives

- Derive the band condition for a daily bolus and decide from measured daily uptakes whether any bolus target can hold the band.
- Explain why a feed-forward on the uptake measured between yesterday's samples overfeeds a culture that has just been cooled, and what a Raman trim or a model-based feed changes.
- Compute the base and the osmolality that lactate held at the pH setpoint costs, and the bound one sparge puts on the carbon dioxide removed.
- Analyze how the glucose target sets the lactate switch, and choose a target, a shift day and an osmolality cap from measured runs.
- Evaluate a predictive plan to the harvest against a classical feed on the same estimate.

Prerequisites Sections 1.4.3 (growth and uptake), 1.4.5 (band condition and bias update), 1.6.2 (velocity form) and 1.6.4 (scenarios and the challenge). Section 1.6.3 is needed only for the predictive controller.

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See also Volume 5, Chapter 4 for a model carried between a late analyzer and a later laboratory, and Volume 5, Chapter 10 for a soft sensor whose bias a delayed laboratory corrects. Volume 9, Chapter 4 for offset-free predictive control bought in the estimator, and Volume 9, Chapter 9 for control against a band rather than a setpoint.

8.1 Introduction

Therapeutic monoclonal antibodies are manufactured by cell culture processes [1], and the class has grown from its first commercial product in 1986 to forty-seven products approved in the United States or Europe by November 2014, with combined sales projected near $125 billion for 2020 [2]. A chemically defined fed-batch of Chinese hamster ovary (CHO) cells has reached 10 g/L of product on day 18 [3]. Such a culture is a feeding problem. Its glucose has to stay low but never reach zero against an appetite that grows with every doubling of the cells, while a sample taken once a day is the only measurement that sees the cells, the metabolites and the product together.

The culture of this chapter starts as 1300 L of medium seeded at half a million cells per milliliter. It holds 8.87 million by the temperature shift on day 5, and the glucose it consumes in one day rises from 0.53 g/L in its first day to 4.27 g/L between days 4 and 5, while the morning sample is reported four hours after it is drawn. What the culture corrects is the habit of feeding toward a comfortable target on a measurement a day old. A daily bolus to 4 g/L, sized from the morning sample, runs the glucose out on day 4.93 and leaves it outside the band of 1 to 6 g/L for 34.8 h. A continuous feed on the cells, built on the uptake measured the day before, overfeeds the culture to 6.09 g/L after the shift, because each cooled cell takes 0.0114 against the 0.0258 g per 10^9 cells per hour the feed assumes. A target of 4.5 g/L, a cautious margin above the floor, keeps the cells from ever switching from making lactate to taking it back, and the base that neutralizes the lactate drives the osmolality to 519 mOsm/kg. That culture is harvested on day 11.17 with 2.58 g/L of antibody, against 5.03 g/L on day 14 when a 2 g/L target is trimmed by an hourly Raman reading.

Feeding in proportion to an estimated demand that grows with the plant's own inventory predates the CHO platform, since a hybridoma fed-batch was fed on its on-line oxygen uptake through the stoichiometry of oxygen and glucose [4], and inferring what a culture does from what can be measured is now a field of bioprocess engineering of its own [5]. A cell therapy expanded in a bag, a yeast propagation fed on its off-gas and an aeration basin dosed to its biomass share the arrangement of a feed-forward on an estimate that drifts, a trim on a faster but indirect measurement, a correction from a slow laboratory, and a side effect of every correction that stays in the vessel. The lager fermenter of

Chapter 10 corrects its diacetyl model in the same way, by a laboratory result that arrives a day after its sample.

Feeding schemes such as glucose delivered on a rising pH [6] or on a Raman estimate [7] were proven on real cultures, whose glucose and lactate are known only when measured, so a student cannot watch a feed-forward go wrong while it happens or run one culture twice with one lever moved. This chapter finds out, on a simulated antibody culture whose every concentration is logged apart from what its probes, estimator and laboratory report, when a daily bolus, a continuous feed on an estimated consumption and a Raman-trimmed feed can hold glucose low but never at zero, and what each correction of oxygen, pH and glucose leaves behind in the broth. The kinetics, the bias update, the split range and the feeding strategies are established methods. So is the model predictive controller, which is measured as a fourth strategy against the shipped feeds and against the strongest classical feed given the same estimate. The culture has 16 states and 19 ledgers whose balances close in a growing volume, a capacitance probe that reads biovolume rather than cells and a controller that sees only its instruments, and the application offers ten cases and a challenge that isolate one decision each.

Section 8.2 traces the domain from the first hybridomas to cultures fed on their own measurements. Section 8.3 gives the model of the culture, its constants and its assumptions, and Section 8.4 derives four consequences of it: the band condition for a daily bolus, the carbon dioxide one sparge can carry, the osmolality each correction costs and the switch that sits inside the band. Section 8.5 sets out the instruments, the four loops, the feeding strategies, the estimator, the supervisor and the predictive controller of Section 8.5.6 as implemented. Section 8.6 reports the ten cases and the challenge, and Section 8.6.11 sets the predictive controller beside every shipped feed. Section 8.7 gives the real instruments, final elements and platform behind the model. Section 8.8 states the limitations and Section 8.9 concludes.

8.2 From Hybridomas to Cultures Fed on Their Own Measurements

The industry begins with a fused cell, and the first licensed antibody product followed in 1986 [1].

> ⧖ HISTORY **An Industry From One Fused Cell (1975 to 1985)**
>
> The industry begins with a fused cell. A report of 1975 described continuous cultures of fused cells that secreted antibody of a predefined specificity [8]. Within a decade such an antibody was treating patients. In a randomized multicenter trial of 123 people whose transplanted kidneys were being rejected, a murine monoclonal antibody reversed 94 % of the rejections against 75 % for high-dose steroids, and graft survival after one year was 62 % against 45 % [9]. Every dose of that antibody had to be grown. The feeding problem of this chapter is what followed the clinical result, and it has been a measurement problem from the start.

Feeding was a measurement problem from the start. In 1995 a hybridoma fed-batch closed its feed on the on-line oxygen uptake rate, with the glucose consumption estimated from the oxygen consumption by stoichiometry, and its metabolism moved from high lactate production and low oxygen consumption to the reverse while its growth phase lengthened [4]. By 2003 temperature had become a lever as well, since lowering a recombinant CHO culture from 37 to 33 °C suppressed its growth, kept its viability high for longer and raised the specific productivity of its erythropoietin about four times [10].

Titers then rose with the culture process. Rising demand drove efforts to raise titers in parallel with the construction of large bulk plants, and the higher titers cut production costs [1]. A chemically defined process reached 10 g/L on day 18 with peak densities above 20 million viable cells/mL and a peak oxygen uptake of 3.7 mmol/(L h), verified at the 100 L pilot scale in reactors configured like those of manufacturing [3]. Scale brought its own constraints. A 5000 L bioreactor had a lower oxygen transfer coefficient, a longer mixing time and a lower rate of carbon dioxide removal than vessels of 5 and 20 L, and raising its bottom sparge improved both transfers more efficiently than

Table 8.1 Signals of the Culture by Role, With Their Values at Inoculation

Symbol	Role	Start	Range
DO	CV of DIC-101	SP 40 %	SP 10–80 %
pH	CV of AIC-102	SP 7.00	SP 6.6–7.4
T	CV of TIC-103	SP 37 °C	SP 30–38 °C
$\hat{G}$	CV of FIC-201, the glucose estimate	SP 2.0 g/L	SP 0.5–8 g/L
N_a, e, Q_g	MVs, the DO sequence	73.1 rpm, 0, 15 L/min	40–140, 0–1, 15–100
y_C, F_b	MVs, the pH split range	10.6 %, 0	0–20 %, 0–2 L/h
T_{sup}	MV, jacket supply	37.5 °C	8–45 °C
F_{glc}	MV, glucose stock	0	0–40 L/h
F_{cf}	MV of the supervisor	1.63 L/h from day 3	0–8 % of V_0 a day
G_s	DV, true glucose lot	500 g/L	300–600, event 420
g	DV, capacitance gain drift	0	−5 to 10 % a day
—	DV, pH probe drift	0	±0.1 a day
—	DV, O_2 supply, Raman, chiller	full, in service, on	events
V	state, broth volume	1300 L	to 2000 L
X_v, X_d	states, cells	0.485, 0.015 $\times 10^6$/mL	—
G, L, P	states	6, 0.1, 0 g/L	—
Q, N, A	states	4, 25, 0.5 mM	—
C_O, C_C, B	states	DO 40 %, 2.27, 18 mM	—
Osm_x, T, T_j	states	208.1, 37.0 °C, 37.3 °C	—
d	state, cell diameter	14.0 µm	14.0–15.2 µm
X_{cap}	measured, capacitance	every scan	1.5 % noise
G_R, L_R	measured, Raman	hourly	0.25, 0.2 g/L noise
—	measured, daily sample	days 0, 1, …	4 h late
OUR, CER	measured, off-gas	logged only	2 % noise

raising its power input [11], while a large-scale CHO culture making an antibody-fusion protein reached 150 mmHg of carbon dioxide and 440 mOsm/kg [12].

The instruments came with a regulatory invitation. The process analytical technology initiative of the U.S. Food and Drug Administration encouraged manufacturers to apply innovative ideas to understand their processes, and in 2011 an in-line Raman probe was shown for the first time to predict glutamine, glutamate, glucose, lactate, ammonium and the viable and total cell densities in a mammalian cell culture bioreactor [13]. Radio-frequency impedance, the capacitance measurement, had already been described for the on-line measurement and control of viable cell density in manufacturing [14]. With a direct reading of the broth the feed could close on lactate itself. Raman models with prediction errors of 0.27 g/L for glucose and 0.20 g/L for lactate fed glucose only while lactate was below a setpoint of 4.0 or 2.5 g/L and glucose below 0.5 g/L, which extended the culture from 11 to 13 days and raised its harvest titer by 85 % [7]. A simpler loop read the pH, because cells take lactic acid back once glucose falls below about 1 mM and the pH rises, and glucose delivered on that rise reduced or removed the lactate accumulation at bench scale and at 2500 L and roughly doubled the final titers of eight cell lines [6].

Model-based feeding completes the sequence. A systematic development of predictive mathematical models for animal cell cultures was set out in 2010 [15], the shift of fed-batch cultures to lactate consumption became a subject of its own [16], and a nonlinear model predictive controller has controlled the glucose concentration of a fed-batch mammalian cell bioprocess [17]. One vessel in this simulator holds all of these pieces, beside a record of the true broth that no plant keeps.

8.3 The Culture as Modeled

One well-mixed broth grows from 1300 L at inoculation toward a working volume of 2000 L as the glucose stock, a complex feed and the base go in, as shown in Figure 8.1, and its signals are classified in Table 8.1. Time is in hours. Cells are in millions per milliliter (10^9 per liter), glucose, lactate and antibody in g/L, glutamine, ammonium, the amino acids, dissolved carbon dioxide and bicarbonate in mM, and per-cell rates per 10^9 cells per hour, so that a rate times the viable density is a rate per liter.

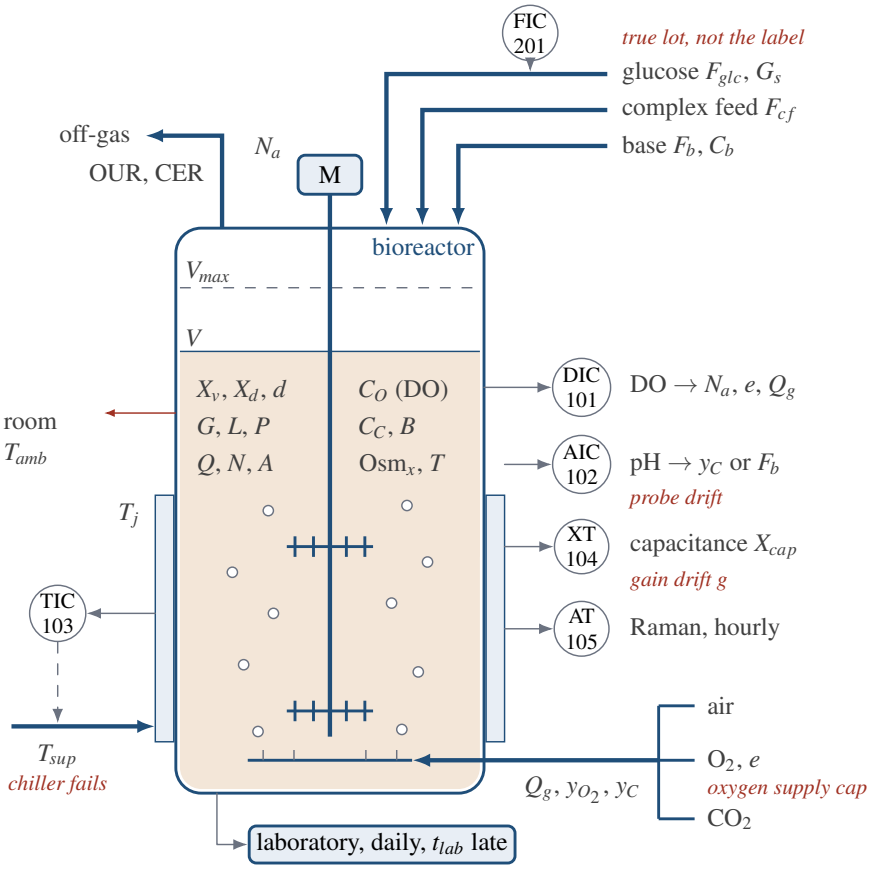


Figure 8.1 The culture, its one sparge, its feeds and what the controller reads. The broth of volume V carries viable and dead cells X_v and X_d of mean diameter d, glucose G, lactate L, antibody P, glutamine Q, amino acids N, ammonium A, dissolved oxygen C_O and carbon dioxide C_C, bicarbonate B, untracked salts Osm_x and temperature T. The agitator turns at N_a, and the sparge carries Q_g of gas whose oxygen fraction y_{O_2} follows the enrichment e and whose CO_2 fraction y_C comes from the pH loop. The feeds are the glucose stock F_{glc} at G_s, the complex feed F_{cf} and the base F_b at C_b, and the jacket holds water at T_j from a supply at T_{sup}. Red italic labels mark the disturbances. Not to scale.

8.3.1 Cells and What Slows Them

Every concentration is diluted by every addition. With the total feed $F = F_{glc} + F_{cf} + F_b$ and the dilution rate $D = F/V$, each species obeys $\mathrm{d}(VC)/\mathrm{d}t = \sum_i F_i C_{i,in} + r_C V$, which is Equation (8.1) for the volume and a concentration.

$$\frac{\mathrm{d}V}{\mathrm{d}t} = F, \quad \frac{\mathrm{d}C}{\mathrm{d}t} = \frac{1}{V}\sum_i F_i C_{i,in} + r_C - DC \tag{8.1}$$

where r_C is the net rate at which the cells make the species. The viable and dead cells follow Equation (8.2).

$$\begin{aligned} \frac{\mathrm{d}X_v}{\mathrm{d}t} &= (\mu - k_d)X_v - DX_v \\ \frac{\mathrm{d}X_d}{\mathrm{d}t} &= k_d X_v - k_{lys}X_d - DX_d \end{aligned} \tag{8.2}$$

The specific growth rate is unstructured Monod growth on glucose, glutamine and one lumped pool of amino acids, in the shape that systematic models of animal cell culture use [15], slowed by every product and condition the controller influences, as shown in Equation (8.3).

$$\begin{aligned}
\mu &= \mu_{max}\, \mathrm{e}^{\theta_\mu (T-37)}\, \frac{G}{K_G+G}\, \frac{Q}{K_Q+Q}\, \frac{N}{K_N+N} \\
&\quad \times \frac{K_L}{K_L+L}\, \frac{K_A}{K_A+A}\, f_{osm}\, f_{pH}\, f_{DO}\, f_{CO_2} \\
f_{osm} &= \frac{1}{1+\left[(\mathrm{Osm}-300)^+/K_{osm}\right]^2} \\
f_{CO_2} &= \frac{1}{1+\left[(p_{CO_2}-60)^+/K_{CO_2}\right]^2} \\
f_{pH} &= \mathrm{e}^{-[(\mathrm{pH}-7)/0.4]^2}, \qquad f_{DO} = \frac{\mathrm{DO}}{0.5+\mathrm{DO}}
\end{aligned} \tag{8.3}$$

where $(x)^+ = \max(0,x)$, T is in °C, DO in percent and $p_{CO_2} = C_C/\alpha$ in mmHg. The osmotic and carbon dioxide factors are teaching values that can be set against a case study, in which raising the osmolality from 316 to 450 mOsm/kg cut the specific growth rate by 60 % and raising p_{CO_2} from 50 to 150 mmHg at a controlled osmolality cut it by 9 % [12]. With the defaults of Table 8.2 the same changes cut it by 46 and 24 %. Death adds a base rate, starvation, ammonium, osmotic stress and a lack of oxygen, as shown in Equation (8.4).

$$\begin{aligned}
k_d &= \mathrm{e}^{\theta_d (T-37)} \Big[k_{d0} + k_{dS}\, s + k_{dA}\, \frac{a^4}{1+a^4} \\
&\quad + k_{dO}\, \frac{(\mathrm{Osm}-380)^+}{50} \Big] + 0.004\, \frac{(5-\mathrm{DO})^+}{5} \\
a &= A/K_{dA} \\
s &= \max\left(\frac{0.03}{0.03+G}, \frac{0.3}{0.3+N}, \frac{0.05}{0.05+Q} \right)
\end{aligned} \tag{8.4}$$

8.3.2 Glucose, Lactate and the Switch

Glucose comes only from the stock pump and is taken by the yield relation, growth plus a maintenance that saturates as glucose runs out, as shown in Equation (8.5).

$$\begin{aligned}
\frac{\mathrm{d}G}{\mathrm{d}t} &= \frac{F_{glc}\, G_s}{V} - q_{glc} X_v - DG \\
q_{glc} &= \frac{\mu}{Y_{xg}} + m_g\, \mathrm{e}^{\theta_m (T-37)}\, \frac{G}{0.05+G}
\end{aligned} \tag{8.5}$$

where G_s is the concentration of the stock actually on the pump, which a feed-lot disturbance can make different from the label the controller assumes. At 37 °C a cell growing at 0.022 h^{-1} takes about 0.6 ng of glucose a day and a resting one about 0.3 ng.

The cells overflow glucose to lactate while they grow fast or while glucose is plentiful, and take lactate back only when both have ended, as shown in Equation (8.6).

$$\begin{aligned}
\frac{\mathrm{d}L}{\mathrm{d}t} &= q_{lac} X_v - DL \\
q_{lac} &= Y_{lg}\, q_{glc}\, \sigma - q_{lc}\, \mathrm{e}^{\theta_m (T-37)}\, \frac{L}{K_{lc}+L}\, (1-\sigma) \\
\sigma &= 1-(1-\sigma_G)(1-\sigma_\mu) \\
\sigma_G &= \frac{1}{1+\mathrm{e}^{-(G-G_{sw})/0.25}}, \quad \sigma_\mu = \frac{1}{1+\mathrm{e}^{-(\mu-\mu_{sw})/0.003}}
\end{aligned} \tag{8.6}$$

The switch is therefore a declared condition, glucose below $G_{sw} = 2.5\,\mathrm{g/L}$ and growth below $\mu_{sw} = 0.015\,\mathrm{h^{-1}}$, standing in for a metabolic shift whose mechanism the literature treats on its own [16]. The application marks the switch at the first instant net lactate production turns into net consumption after lactate has built above 0.3 g/L, provided the glucose is at least 0.5 g/L. A culture whose glucose has run out also takes lactate back, as the only carbon left, and that flip is marked as starvation while the watch for the switch goes on.

8.3.3 Glutamine, Amino Acids, Ammonium and Antibody

The complex feed carries glutamine and the lumped amino acids, the cells take both, glutamine also decomposes in the broth, and ammonium comes from all three, as shown in Equation (8.7).

$$\begin{aligned}
\frac{\mathrm{d}Q}{\mathrm{d}t} &= \frac{F_{cf}Q_{cf}}{V} - q_{gln}X_v - k_{deg}Q - DQ \\
q_{gln} &= \frac{\mu}{Y_{xq}} + m_q\,\mathrm{e}^{\theta_m(T-37)}\frac{Q}{0.1+Q} \\
\frac{\mathrm{d}N}{\mathrm{d}t} &= \frac{F_{cf}N_{cf}}{V} - q_N X_v - DN \\
q_N &= \frac{\mu}{Y_{xN}} + m_N\,\mathrm{e}^{\theta_m(T-37)}\frac{N}{0.2+N} + 8.9\,q_P \\
\frac{\mathrm{d}A}{\mathrm{d}t} &= (Y_{aq}q_{gln} + Y_{aN}q_N)X_v + k_{deg}Q - DA
\end{aligned} \tag{8.7}$$

where $k_{deg} = k_{deg,0}\,\mathrm{e}^{0.1(T-37)}$ and the antibody consumes 8.9 mmol of amino acids per gram, about 1330 residues for a 150 kDa molecule. The antibody is made at a rate that rises as the culture is cooled, as shown in Equation (8.8).

$$\frac{\mathrm{d}P}{\mathrm{d}t} = q_P X_v - DP, \quad q_P = q_{P0}\left[1 + a_P\,(37-T)^{+}\right] f_{DO} \tag{8.8}$$

At a DO of 40 % a cell makes 26.1 pg a day at 37 °C and 46.9 pg at 33 °C, a rise of 1.8 times that is a teaching value below the fourfold rise measured for an erythropoietin line [10].

8.3.4 One Sparge: Oxygen In, Carbon Dioxide Out

Oxygen crosses from the sparge bubbles and through the surface, as shown in Equation (8.9).

$$\begin{aligned}
\frac{\mathrm{d}C_O}{\mathrm{d}t} &= k_L a\left(C^*_{air}\frac{y_{O_2}}{0.21} - C_O\right) + k_L a_s\,(C^*_{air} - C_O) \\
&\quad - q_{O_2}X_v - DC_O \\
k_L a &= k_L a_{ref}\left(\frac{N_a}{140}\right)^{\alpha_N}\left(\frac{Q_g}{100}\right)^{\beta_Q} \\
y_{O_2} &= (0.21 + 0.79\,e)(1 - y_C) \\
q_{O_2} &= q_{O_2,0}\,\mathrm{e}^{\theta_m(T-37)}\left[0.6 + 0.4\min(1, \mu/\mu_{max})\right] f_{DO}
\end{aligned} \tag{8.9}$$

where $\mathrm{DO} = 100\,C_O/C^*_{air}$, N_a is the agitator speed in rpm and Q_g the sparge flow in standard L/min. The sparge $k_L a$ is $0.33\,\mathrm{h^{-1}}$ at 40 rpm and 15 L/min, 3.10 at 140 rpm and $8.0\,\mathrm{h^{-1}}$ at 140 rpm and 100 L/min, and the enrichment e raises the driving force rather than the coefficient. The same bubbles carry carbon dioxide out. They rise through the broth and approach equilibrium with it, so the removal is the gas flow times how nearly the bubbles leave saturated, as shown in Equation (8.10).

$$\begin{aligned}
R_s &= 60\,000\,\dot{n}_g\,\varepsilon\,(y_{eq} - y_C), \quad \varepsilon = 1 - \mathrm{e}^{-\mathrm{NTU}} \\
\mathrm{NTU} &= \frac{k_{CO_2}\,k_L a\,V\,\alpha P}{60\,000\,\dot{n}_g}, \quad y_{eq} = \frac{C_C}{\alpha P}, \quad \dot{n}_g = \frac{Q_g}{22.414}
\end{aligned} \tag{8.10}$$

with R_s in mmol/h, $\dot{n}_g$ in mol/min, $\alpha = 0.0307\,\mathrm{mM/mmHg}$, $P = 760\,\mathrm{mmHg}$, and $k_{CO_2} = 0.89$ is the ratio of the carbon dioxide transfer coefficient to the oxygen one. The headspace adds $k_L a_s k_{CO_2} C_C$ per hour.

8.3.5 Bicarbonate, pH and Osmolality

Respiration makes carbon dioxide at the respiratory quotient, every lactate made spends a bicarbonate and turns it into dissolved carbon dioxide, every lactate taken back and every ammonium made returns one, and each millimole of sodium carbonate adds two bicarbonates while it consumes a carbon dioxide, as shown in Equation (8.11).

$$\begin{aligned} \frac{\mathrm{d}C_C}{\mathrm{d}t} &= \mathrm{RQ}\, q_{O_2} X_v - \frac{R_s}{V} - k_L a_s k_{CO_2} C_C \\ &\quad + r_L - r_A - r_B - D C_C \\ \frac{\mathrm{d}B}{\mathrm{d}t} &= -r_L + r_A + 2 r_B - DB, \quad \mathrm{pH} = 6.1 + \log_{10} \frac{B}{C_C} \end{aligned} \tag{8.11}$$

where $r_L = 11.1\, q_{lac} X_v$ is the lactate made in mM/h, r_A the ammonium made, the first two terms of $\mathrm{d}A/\mathrm{d}t$ in Equation (8.7), and $r_B = 1000\, F_b C_b / V$ the carbonate added. The medium's 18 mM of bicarbonate at pH 7.00 means 2.27 mM of dissolved carbon dioxide, 74 mmHg, at inoculation. Osmolality is the untracked salts plus every tracked solute, as shown in Equation (8.12).

$$\begin{aligned} \mathrm{Osm} &= \mathrm{Osm}_x + 5.55\, G + 11.1\, L + Q + A + N + B \\ \frac{\mathrm{dOsm}_x}{\mathrm{d}t} &= \frac{1}{V}\left[F_{cf}\left(\mathrm{Osm}_{cf} - Q_{cf} - N_{cf}\right) + 2000\, F_b C_b\right] \\ &\quad - D\,\mathrm{Osm}_x \end{aligned} \tag{8.12}$$

Nothing consumes Osm_x. It holds the medium's salts, the complex feed's salts and the base's sodium, and the only way it falls is dilution.

8.3.6 Heat, Jacket and Cell Size

The broth gains the cells' heat, the agitator's power and the room, and loses heat to the jacket, whose water is replaced from the supply, as shown in Equation (8.13).

$$\begin{aligned} \rho c_p V \frac{\mathrm{d}T}{\mathrm{d}t} &= 3.6\left[q_h X_v V + P_{ag}(N_a/140)^3 - UA(T - T_j)\right. \\ &\quad \left. - UA_{amb}(T - T_{amb})\right] - \rho c_p F\,(T - T_{amb}) \\ \frac{\mathrm{d}T_j}{\mathrm{d}t} &= \frac{T_{sup} - T_j}{\tau_j} + \frac{3.6\, UA\,(T - T_j)}{C_j} \end{aligned} \tag{8.13}$$

with every heat term in W, which the factor 3.6 turns into kJ/h, and with the feeds entering at room temperature. The capacitance probe responds to the volume of intact cells, and cells swell as their growth stops. The mean diameter follows a target set by the growth rate over a day, as shown in Equation (8.14).

$$\begin{aligned} \frac{\mathrm{d}d}{\mathrm{d}t} &= \frac{d^* - d}{\tau_d} \\ d^* &= d_{min} + (d_{max} - d_{min})\left[1 - \min(1, \mu/0.025)\right] \end{aligned} \tag{8.14}$$

8.3.7 Constants, Inoculation and Integration

Table 8.2 lists the constants. At inoculation the broth is the basal medium with the seed, 0.1 g/L of lactate and 0.5 mM of ammonium, its dissolved oxygen at the setpoint and its carbon dioxide at the value that puts the bicarbonate at the pH setpoint, and Osm_x is what makes the total the

Table 8.2 Constants of the Culture. Every Value Above the Last Rule Is a Slider of the Show Model Dialog, None Is Hidden, and the Last Rows Are Fixed in the Code. All Are Teaching Values

Symbol	Meaning	Value	Symbol	Meaning	Value
Vessel, medium and feeds			*Metabolism, with cells in* 10^9 *and rates per* 10^9 *cells per hour*		
V_0, V_{max}	working volume at seeding, most it holds	1300, 2000 L	Y_{xg}, m_g	cells per gram of glucose, maintenance	1.6 per g, 0.012 g
X_0, v_0	seed density (all cells), seed viability	0.5×10^6/mL, 97 %	Y_{lg}	lactate per glucose in overflow	0.45 g/g
G_0, Q_0, N_0	glucose, glutamine, amino acids	6 g/L, 4, 25 mM	q_{lc}, K_{lc}	lactate uptake after the switch, half point	0.0025 g, 0.4 g/L
B_0, Osm_0	bicarbonate, osmolality of the medium	18 mM, 290 mOsm/kg	G_{sw}, μ_{sw}	switch: glucose below, growth below	2.5 g/L, 0.015 h^{-1}
G_s	glucose stock as labeled	500 g/L	Y_{xq}, m_q	cells per mmol of glutamine, maintenance	2.5 per mmol, 0.002 mmol
Q_{cf}, N_{cf}	glutamine, amino acids in the complex feed	60, 330 mM	Y_{xN}, m_N	cells per mmol of amino acids, maintenance	1.0 per mmol, 0.0025 mmol
Osm_{cf}, C_b	complex feed osmolality, Na_2CO_3	900 mOsm/kg, 1 M	Y_{aq}, Y_{aN}	ammonium per glutamine, per amino acid	0.6, 0.04
Growth and death			$k_{deg,0}$	glutamine decomposition at 37 °C	0.003 h^{-1}
μ_{max}	growth rate at 37 °C	0.035 h^{-1}	$q_{O_2,0}$, RQ	oxygen uptake, respiratory quotient	0.25 mmol, 0.95
K_G, K_Q, K_N	half-saturations of growth	0.05 g/L, 0.1, 1.0 mM	q_{P0}	antibody per cell at 37 °C	0.0011 g
K_L, K_A	lactate, ammonium inhibition	15 g/L, 20 mM	*Gas, heat, cell size and laboratory*		
K_{osm}, K_{CO_2}	osmotic, CO_2 scales above 300, 60 mmHg	160, 160	k_La_{ref}	sparge k_La at 140 rpm, 100 L/min	8 h^{-1}
k_{d0}, k_{dS}	base death, death added by starvation	0.0006, 0.015 h^{-1}	α_N, β_Q	exponents on speed and gas flow	1.8, 0.5
k_{dA}, K_{dA}	ammonium death, its half point	0.006 h^{-1}, 10 mM	k_La_s, k_{CO_2}	headspace transfer, CO_2 ratio	0.15 h^{-1}, 0.89
k_{dO}	death per 50 mOsm/kg above 380	0.004 h^{-1}	C^*_{air}	dissolved O_2 at air saturation	0.20 mM
k_{lys}	lysis of dead cells	0.004 h^{-1}	UA, UA_{amb}, T_{amb}	jacket, room loss, room	600, 15 W/K, 22 °C
θ_μ, θ_m, θ_d	e-fold per kelvin: growth, uptakes, death	0.25, 0.09, 0.17 K^{-1}	τ_j, C_j	jacket turnover, jacket heat capacity	0.1 h, 420 kJ/K
a_P	antibody rate per kelvin below 37 °C	0.2 K^{-1}	P_{ag}, q_h	agitator power at 140 rpm, heat per 10^9 cells	250 W, 0.02 W
			d_{min}, d_{max}, τ_d	diameter growing, arrested, response	14.0, 15.2 µm, 24 h
			t_{lab}	sample drawn to reported	4 h
pK, α, P	CO_2 equilibrium, solubility, pressure	6.1, 0.0307, 760	—	switch widths, starvation mark	0.25 g/L, 0.003 h^{-1}, 0.5 g/L
ρc_p	broth heat capacity	4.0 kJ/(L K)	—	amino acids per gram of antibody	8.9 mmol

medium's 290 mOsm/kg, 208.1 mOsm/kg. The jacket water starts at the temperature that removes the vessel's net heat, and the controller starts at the DO demand that holds the setpoint against the seed's uptake, found by bisection, and at the carbon dioxide fraction that balances the starting carbon dioxide. Nineteen ledger states integrate what came in, what was made and what was used, so every balance closes in the growing volume, and in the default culture the worst of eleven balances closes to 1.4×10^{-12} relative after fourteen days.

The engine of Section 1.6.1 integrates the culture by classical Runge-Kutta steps of fourth order and a fixed 10 s, the controller executes once a minute, and the historian records every 5 min, 4032 rows for fourteen days. The speeds run from 0.1 to 48 simulated hours per second, so a whole culture takes 7 s at the top of the ladder. The default seed is 20261008, and Reset replays a culture as that section describes. Halving the step leaves the default culture's titer at 5.0334 g/L, its lactate switch on day 6.3168 and its highest osmolality at 393.231 mOsm/kg, and a step of 30 s gives the same titer and osmolality with the switch on day 6.3170. The application passes its 62 checks, among them the closure of every balance to 10^{-9} through whole cultures, the two conditions of the switch and the four-hour delay of the sample.

8.3.8 Assumptions

One well-mixed broth. A 5000 L vessel mixes more slowly than a bench one and can carry gradients of pH and dissolved oxygen [11], while this broth has none, so it cannot show a local excess of glucose at the feed point or a carbon dioxide gradient with height.

Unstructured kinetics with teaching constants. The constants of Table 8.2 are teaching values anchored in the CHO literature, not a fit to a cell line, and real lines differ several-fold in each. The real values of a process come from its own cell line, through a systematic development of predictive models of the kind set out for animal cell cultures [15], and from measured responses such as the growth and productivity of a fed-batch at scale [3], the specific productivity at a lower temperature [10], and the growth at elevated carbon dioxide and osmolality [12].

A declared switch. Equation (8.6) switches on two thresholds, a declared condition rather than a mechanism of the metabolic shift [16], so it cannot show a line that switches for another reason or one that never switches.

A shift without adaptation. The temperature factors act at once, so a culture at 33 °C grows at 37 % of its rate, dies at 51 %, takes up 70 % as much and makes 1.8 times the antibody per cell from the moment it arrives there. Real cells adapt over a day or two.

A simple buffer and an ideal gas phase. The pH ignores phosphate, the amino acids and the cells' own buffering, carbon dioxide hydrates instantly, the bubbles have no hydrostatic head, and foam, shear and antifoam are absent, which is why raising the gas flow costs nothing here that a plant would pay in foam.

A probe that reads size. The capacitance probe sees biovolume as cells of the fast-growing size, with a diameter that follows the growth rate, while real dielectric spectroscopy also depends on the membranes and the frequency. The Raman has a fixed noise and no calibration drift.

No product quality. Glycosylation, charge variants, aggregates and host-cell proteins are outside the model, although they often decide a real harvest more than the titer does.

8.4 What a Day, a Bubble and a Liter of Base Allow

Four consequences of Section 8.3, none of them an extra assumption, explain most of what the cases measure.

8.4.1 The Bolus and the Band

A bolus sized from a sample restores the glucose the sample had, not the broth's, which has fallen during the t_{lab} of the analysis. The broth therefore starts each interval below the target by what the cells took during the analysis and ends it below the target by what they took from the draw to the next report, and the difference, the consumption over the 24 h between two reports, has to fit inside the band. Once it does not, no target can hold a band $[G_{lo}, G_{hi}]$. Equation (8.15) states the condition, the band test of Equation (1.7) written for this culture's volume.

$$C_k = \frac{1}{\bar{V}} \int_{t_k}^{t_k+24} q_{glc} X_v V \, \mathrm{d}t \; > \; G_{hi} - G_{lo} = 5\,\mathrm{g/L} \tag{8.15}$$

with t_k the time of the k-th report, $\bar{V}$ the mean volume over the interval, and the dilution neglected. Fed continuously so that it eats what it wants, the default culture takes 0.53, 1.02, 1.83, 2.97 and 4.27 g/L in its first five days, 44 to 94 % more each day, and 2.92 g/L in the day after the shift, as shown in Figure 8.2. Kept at 37 °C until day 8 it takes 5.24, 5.84 and 5.93 g/L in days 5 to 8, and no daily bolus can hold the band.

🕮 **Pause.** Try Exercise 8.1 before reading on.

8.4.2 What the Gas Can Carry

Equation (8.10) bounds the carbon dioxide one sparge can remove by its flow, $R_s \le 60000\,\dot{n}_g (y_{eq} - y_C)$, whatever the agitation or the oxygen content. The bound is reached as ε approaches one, and

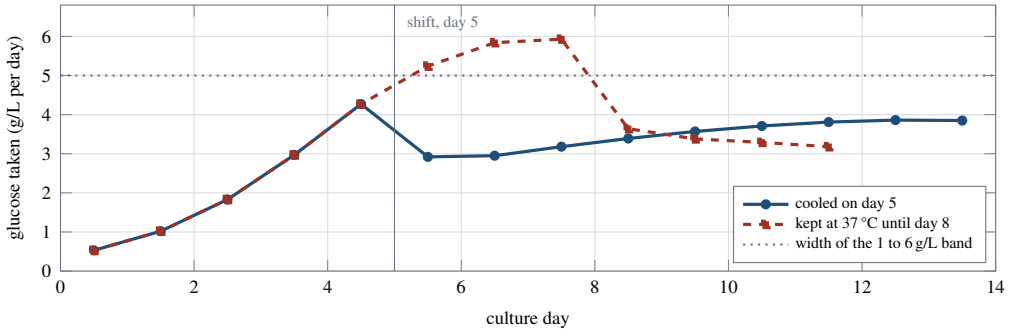


Figure 8.2 Glucose taken in each day of the culture fed continuously with the Raman trim. Cooled on day 5 (solid line, circles) the culture never takes more than 4.27 g/L in a day, and kept at 37 °C until day 8 (dashed line, squares) it takes up to 5.93 g/L, more than the band is wide (dotted line).

in the 1300 L of inoculation ε is 0.196 at 40 rpm and 15 L/min, 0.875 at 140 rpm and 15 L/min and 0.554 at 140 rpm and 100 L/min. Once the agitator is at full speed the bubbles leave the broth 87.5 % saturated, so only more gas removes more carbon dioxide, and the enrichment e, which changes neither $\dot{n}_g$ nor ε, removes none. The DO sequence therefore decides the carbon dioxide the pH loop has to live with, and through it the bicarbonate the base must supply.

🕮 **Pause.** Try Exercise 8.3 before reading on.

8.4.3 The Price of a Correction

While base is the active leg of the pH loop, holding the pH at a dissolved carbon dioxide fixed by the gas means holding the bicarbonate. Each millimole of lactate spends a bicarbonate, and half a millimole of sodium carbonate restores it while the extra carbon dioxide leaves with the gas, so a millimole per kilogram of lactate held at the setpoint costs 2 mOsm/kg, the lactate itself and one sodium, and a gram per liter costs 22 mOsm/kg. Over a whole culture the bicarbonate balance of Equation (8.11) fixes the base exactly, as half of the lactate made, less half of the ammonium made, plus half the change in the broth's bicarbonate. The default culture makes 49.3 mol of lactate net and 21.6 mol of ammonium, its bicarbonate grows from 23.4 to 50.9 mol, and it needs 27.6 mol of base, while case 7 makes 178.1 mol of lactate and 12.6 mol of ammonium, ends with 11.2 mol of bicarbonate, and needs 76.7 mol. The lactate part of the osmolality returns when the cells take lactate back, but the sodium stays, because nothing in Equation (8.12) consumes it. In the default culture Osm_x rises from 208.1 to 289.6 mOsm/kg by day 14, which is the medium's salts diluted to 143.8, the complex feed's salts at 116.3 and the sodium of the base at 29.4, and in case 7 the sodium alone is 86.8 mOsm/kg at harvest.

8.4.4 A Switch Inside the Band

After the shift the growth rate is about $0.006\,\mathrm{h}^{-1}$, far below μ_{sw}, so the glucose condition alone decides the sign of q_{lac} in Equation (8.6). The sign changes where overflow and uptake balance, and in the default culture that happens at glucose between 2.15 and 2.17 g/L, seven times from day 6.32 to day 12.52 as the trimmed feed moves the glucose across it. The useful part of the 1 to 6 g/L band is therefore 1 to about 2.15 g/L, and a target is a lever on the switch as much as on the floor.

8.5 The Controller and What It Reads

The controller executes once a minute and reads no concentration in the broth, only its instruments, its own pump commands at the stock's labeled concentration and the daily laboratory, with the settings of Table 8.3.

Table 8.3 Controller, Estimator and Supervisor Settings

Function	Setting	Default	Range
DIC-101	K_c, τ_I	0.6 % per % DO, 0.5 h	0.05–5, 0.05–5
	output 0–100 %	N_a, then e, then Q_g	or Q_g before e
AIC-102	K_c, τ_I, deadband	150 % per pH, 0.5 h, 0	5–600, 0.05–10, 0–0.2
	output ±100 %	base to 2 L/h above zero, CO_2 to 20 % below	—
	re-standardization	off	$\lambda = 1$, $\lvert b\rvert \le 0.5$
TIC-103	K_c, τ_I	6 K/K, 1.5 h	0.5–30, 0.1–10
FIC-201	strategy, target	continuous, 2.0 g/L	bolus, model-based, MPC, 0.5–8
	trim K_c, τ_I	1 L/h per g/L, 8 h	0–10, 0.5–48
MPC	κ, w_P, S, preview	3, 5, 8, on	preview off
Complex feed	rate, start	3 % of V_0 a day, day 3	0–8, 0–8
Osmolality cap	state, level	off, 390 mOsm/kg	330–460
Shift	rule, T_{low}	day 5, 33 °C at 1 K/h	or at $\hat{X}_v = 10 \times 10^6$/mL
Harvest	rule	day 14 or offline viability below 70 %	day 8–20, 40–95 %
Estimator	λ_X, Raman pull, $\hat{q}_0$	0.5 ($\lvert b\rvert \le 1.2$), 0.35, 0.02 g per 10^9 cells per hour	0–1, on or off

8.5.1 Instruments

The DO, pH and temperature probes lag the truth by 1, 0.5 and 0.5 min with 0.3 %, 0.004 and 0.02 °C of colored noise correlated over 1.2 min, and a drift disturbance walks the pH probe's offset away from the truth. The capacitance probe reads $X_{cap} = X_v (d/d_{min})^3 \, g(t) \, (1+n)$ every scan, with a gain drift g and 1.5 % of noise n correlated over 0.5 h, so it reads high as the cells swell. The Raman reports glucose and lactate every hour, 15 min after its sample, with 0.25 and 0.2 g/L of noise, close to the prediction errors of 0.27 and 0.20 g/L reported for a Raman model of a fed-batch [7]. A sample drawn at every whole day from inoculation is reported $t_{lab} = 4$ h later with the viable density, viability, glucose, lactate, ammonium, osmolality, titer, pH and p_{CO_2}, with noise of 0.04 on the logarithm of the count, 0.8 points of viability, 0.08 g/L of glucose and of lactate, 0.2 mM of ammonium, 3 mOsm/kg, 0.03 on the logarithm of the titer, 0.01 of pH and 3 mmHg. A Raman reading goes stale after three missed hours. Both the Raman and the laboratory run on the framework's shared sampled analyzer, and the off-gas uptake and evolution rates are logged with 2 % of noise and used by no loop.

> ✦ DID YOU KNOW? **A Membrane Five Nanometers Thick**
>
> What the capacitance probe senses is the thin membrane around each cell. In a study of such probes the viable cells of a culture measured 6.75 µm in radius and the dead ones 6.0 µm, around a membrane taken as 5 nm thick, about a thousandth of the cell [18].

8.5.2 Four Loops on the Shared Controller

All four loops use the framework's velocity-form law of Section 1.6.2 without derivative action and with no output bias, written for this chapter as Equation (8.16).

$$
\begin{aligned}
e_k &= \mathrm{SP}_k - \mathrm{PV}_k, \qquad P_k = K_c \, e_k \\
s_k &= s_{k-1} + (P_k - P_{k-1}), \qquad v_k = s_k + I_{k-1} \\
u_k &= \min\left(u_{max}, \max(u_{min}, v_k)\right) \\
I_k &= I_{k-1} + \left[\frac{K_c}{\tau_I} \, e_k + \frac{u_k - v_k}{\tau_I}\right] \Delta t_c
\end{aligned}
\tag{8.16}
$$

where $\Delta t_c = 1$ min. As in Equations (1.13) to (1.15), the sum s_k is never clamped, back-calculation corrects only the integral at a tracking time equal to τ_I, no rate limit applies, and a loop in MANUAL tracks the operator's output u_{op} through $I_k = u_{op} - s_k$, so the return to AUTO is bumpless. All four loops are reverse acting, with action $a = +1$ in the convention of Section 1.3: more agitation and

oxygen raise the DO, more base raises the pH, a warmer jacket supply raises the temperature and more stock raises the glucose. The application's sign checks confirm each direction. DIC-101 spreads its output over three actuators, the first third taking the agitator from 40 to 140 rpm, the second enriching the sparge from air toward pure oxygen and the last raising the gas from 15 to 100 L/min, or the gas before the oxygen in the alternative sequence, and an oxygen supply disturbance caps the enrichment. The speed such an oxygen loop needs from the agitator beneath it is the subject of Volume 3, Chapter 3. AIC-102 is a split range on an error that can be deadbanded, base above zero and carbon dioxide in the sparge below it, and TIC-103 sets the jacket supply with the supervisor's shift schedule, which ramps at 1 K/h, as its setpoint.

8.5.3 Feeding by Bolus, Rate and Platform Model

The daily bolus acts at each offline result and pumps, at the full 40 L/h, the stock that brings that sample's glucose up to the target, $(G_{SP} - G_{lab})\,V / G_s$ with G_s on the label. The continuous feed pumps the estimated consumption plus a PI trim on the glucose estimate, as shown in Equation (8.17).

$$F_{glc} = \frac{\hat{q}\,\hat{X}_v\,V}{G_s} + \mathrm{PI}\big(G_{SP} - \hat{G}\big), \qquad 0 \le F_{glc} \le 40\,\mathrm{L/h} \tag{8.17}$$

where the trim's limits move every scan so that the sum stays within the pump's range and the back-calculation of Equation (8.16) respects it. The model-based feed replaces $\hat{q}$ by the platform yield relation on the growth the corrected probe sees, $q_{model} = \hat{\mu}/Y_{xg} + m_g\,\mathrm{e}^{\theta_m (T-37)}$ with the default constants whatever the plant has been set to, so it knows a cooled culture eats less, and it scales the complex feed by $\hat{X}_v / (13 \times 10^6/\mathrm{mL})$, capped at 1.4. The fourth strategy, the predictive controller of Section 8.5.6, replaces both the feed-forward and the trim.

8.5.4 The Estimator

Each offline count corrects the capacitance probe through the framework's shared bias update, the logarithmic form of Equation (1.8), applied to the reading at the hour the sample was drawn as in Equation (8.18).

$$b \leftarrow b + \lambda_X \big[\ln X_{lab} - (\ln X_{cap}(t_s) + b)\big], \qquad \hat{X}_v = X_{cap}\,\mathrm{e}^{b} \tag{8.18}$$

The per-cell uptake $\hat{q}$ is the glucose that disappeared between the last two samples, what was there plus what the pumps delivered on the label less what is left, over the viable cell-hours their counts bracket by the trapezoid rule, averaged as $0.4\,\hat{q} + 0.6\,q$ after the first interval. The growth $\hat{\mu}$ is the log slope of $\hat{X}_v$ over the last 12 h plus a 6 h average of the dilution. The glucose estimate integrates $\mathrm{d}\hat{G}/\mathrm{d}t = F_{glc} G_s / V - \hat{q}\hat{X}_v - D\hat{G}$ with G_s on the label, moves 35 % of the way to each fresh Raman reading, and is shifted by each offline result by what it was wrong by at the hour of the draw. The milk powder dryer of Volume 5, Chapter 10 corrects the bias of its soft sensor in the same way, from a sampled laboratory result that arrives late. The osmolality estimate that feeds the cap adds what the pumps deliver, Osm_{cf} for the complex feed, $4000\,C_b$ for the base and $5.55\,G_s$ for the stock, less $5.55\,\hat{q}\hat{X}_v$ for the glucose the cells use, and is shifted by each offline osmolality in the same way. It takes Osm_{cf} and C_b at their declared 900 mOsm/kg and 1 M, and both estimates begin from the declared basal medium, 6 g/L of glucose at 290 mOsm/kg, so a medium or feed altered in the parameters is a mismatch that only the samples reveal.

8.5.5 Supervisor

The supervisor shifts the temperature on the chosen day or when $\hat{X}_v$ first reaches the chosen density, runs the complex feed, and decides the harvest on the day, at the first offline viability below the limit, or whichever comes first, with a viability rule ending the culture on day 20 at the latest. The viability rule judges a decline and acts only on a result below an earlier one, so a seed counted under the limit at inoculation is not harvested on its day-0 count, and seeds of 60 to 70 % viability fed as

in case 4 reach day 14 with 4.33 to 4.58 g/L. The optional osmolality cap scales the complex feed by $\min\{1, \max[0, (\mathrm{Osm}_{cap} - \widehat{\mathrm{Osm}})/12]\}$ and lowers the glucose target in proportion, toward 1.5 g/L at the cap. At every offline result from the second on, a bolus check multiplies the consumption measured over the last interval by the growth of $\hat{X}_v$ since, clamped to 1 to 3 and applied only while the culture is still warm, and flags that no daily bolus can hold the band when the product passes the 5 g/L of Equation (8.15).

8.5.6 A Predictive Controller Over the Rest of the Batch

The last value of the feeding strategy is a model predictive controller (MPC) [19, 20]. It is written for this culture rather than taken from the framework's option of Section 1.6.3, for the reason given below. Once an hour, at minute 15 just after the Raman reports, it decides a glucose dose u in grams per liter of broth per hour and holds it until the next decision, and the pump delivers uV/G_s with G_s on the label, as every strategy does. The DO, pH and jacket loops, the complex feed, the osmolality cap, the vessel-full stop, the shift and the harvest act as under the other strategies, the trim of Equation (8.17) tracks the applied flow, and the continuous feed stays the application's default and the starting strategy of all cases but case 10.

The controller's model is the culture's own kinetics at their declared values, written on the estimator's states rather than identified, because a fed-batch never settles at an operating point that a step test could perturb. With the state $x = [G, L, \mathrm{Osm}, X, P, V, A]$ it reads as shown in Equation (8.19).

$$\begin{aligned} \frac{\mathrm{d}X}{\mathrm{d}t} &= (\mu_m - k_d - D)X, \quad \mu_m = \mu_0\, \mathrm{e}^{\theta_\mu (T - T_m)}\, \frac{f_{osm}\, f_L\, f_A}{f^0_{osm}\, f^0_L\, f^0_A} \\ \frac{\mathrm{d}G}{\mathrm{d}t} &= u - q_m X - DG + \hat{d}_G, \quad q_m = \frac{\mu_m}{Y_{xg}} + m_g\, \mathrm{e}^{\theta_m (T - 37)} \\ \frac{\mathrm{d}L}{\mathrm{d}t} &= q_{lac} X - DL + \hat{d}_L, \qquad \frac{\mathrm{d}P}{\mathrm{d}t} = q_P X - DP \end{aligned} \tag{8.19}$$

where μ_0 is the estimator's $\hat{\mu}$ moved from the mean measured temperature of its twelve hours to the measured T_m, plus the death rate of Equation (8.4) at the estimate without its starvation term, $f_L = K_L/(K_L + L)$ and $f_A = K_A/(K_A + A)$ are the inhibition factors of Equation (8.3) with f^0 their values now, q_{lac} and q_P follow Equations (8.6) and (8.8), the osmolality follows the estimator's additions model with the base the pH loop adds for the lactate made, the ammonium follows Equation (8.7) with glutamine held at 3 mM, and every feed stops at V_{max}. The temperature and the complex feed over the horizon are the recipe's (the preview), or today's with the preview Off. Starvation, glutamine and amino-acid limits, pH, DO, carbon dioxide and cell size are left out, and the floor below stands in for starvation.

Each decision simulates Equation (8.19) over the horizon under a nominal dose $u^0 = q_m X + DG - \hat{d}_G + (G_{fl} - G)/3\,\mathrm{h}$ that holds the glucose at the plan's floor, linearizes it along that path by forward differences discretized to second order over each step, and writes every output as the nominal path plus $\Theta\delta$ in the block deviations δ from u^0. Twelve steps ahead the linear responses of glucose, osmolality and titer to a move agree with the nonlinear model's within 0.98 % and the lactate's within 8.3 %. A time-invariant model frozen at the estimate, the form the shared `LinearMPC` solves, was rejected in the build, because on day 3.5 it predicted 5.17 g/L of titer where the nonlinear model under the same plan gave 2.88 g/L, so the problem is condensed by hand for the shared dense quadratic program solver. The horizon runs to the harvest day (day 20 under a viability-only rule) in $N \le 120$ steps of $\Delta = \lceil t_{rest}/120 \rceil$ hours, 112 steps of 3 h at inoculation and 1 h over the last five days, with up to 12 graduated move blocks of $1, 1, 1, 1, 2, 2, 3, 4, 6, 9, 15$ steps and the remainder, and with less than

one hour left the last plan is held. The program is Equation (8.20).

$$\begin{aligned}\min_{\delta,s}\ & -w_P\hat{P}_N + S\Big[(u_0^0+\delta_1-u_{-1})^2 + \sum_{b=2}^{n_b}(\delta_b-\delta_{b-1})^2\Big] \\ & + \sum_i \left(\rho_i s_i^2 + \sigma_i s_i\right) \\ \text{s.t. } & 1+\kappa\sigma_G - s_{G,j} \le \hat{G}_k \le 6 + s_{G,j}, \quad \hat{G}_k \ge 0.5 - s_D \\ & \hat{L}_k \le 5 + s_L, \quad \widehat{\mathrm{Osm}}_k \le \mathrm{Osm}_{max} + s_O \\ & \hat{G}_k \le G_k^0 + 1, \quad 0 \le u_k \le u_{max}, \quad s \ge 0\end{aligned} \tag{8.20}$$

where $\hat{P}_N$ is the predicted titer at the end, u_{-1} the dose applied now, G_k^0 the nominal glucose, σ_G the standard deviation of the glucose estimate, and k runs over every $\lceil N/16 \rceil$-th step with one glucose slack $s_{G,j}$ per judged step and one slack each for depletion, lactate and osmolality over the horizon. The weights are $w_P = 5$ per g/L, $S = 8$ per $(\mathrm{g}/(\mathrm{L\,h}))^2$ and $\kappa = 3$, with $(\rho,\sigma) = (10^3, 200)$ on the band, $(10^4, 2000)$ on depletion, $(100, 20)$ on the 5 g/L lactate ceiling, a teaching value since the application has no lactate limit, and $(1, 2)$ on the 400 mOsm/kg comfort line, which becomes the cap's level when the cap is on. The pump's range, a nonzero dose after the predicted full step, and the 1 g/L trust region above the nominal path, which keeps the plan where the overflow sigmoid was linearized, are hard. With 12 moves and up to 20 slacks the program has at most 32 variables and 146 rows, and it took 5 to 96 iterations in three whole cultures. A rule fixed before the grid chose the tuning from $\kappa \in \{2,3,4\}$, $S \in \{0.5, 2, 8\}$ and $w_P \in \{5, 20, 80\}$ on case 10, keeping the settings with no hour outside the band, no osmolality above 400 and no fallback, then the highest true titer, with ties within 0.01 g/L broken by the least pump travel (18 of 27 qualified, at 5.04 to 5.06 g/L).

The estimator is offset-free in the sense of an input disturbance carried beside each state [21], the structure Volume 9, Chapter 4 gives its predictive controller for a fuel that no instrument measures. Glucose and lactate each carry a disturbance $\hat{d}$ in grams per liter per hour, propagated by Equation (8.19) every scan and corrected by steady-state Kalman gains for the hourly Raman and the daily sample. A residual e is taken against the estimate at the hour the sample was drawn, t_s, and carried to now as $\hat{G} \leftarrow \hat{G} + (\ell_G + \ell_d(t - t_s))e$ and $\hat{d}_G \leftarrow \hat{d}_G + \ell_d e$. The Raman gains $(\ell_G, \ell_d) = (0.342, 0.065)$ come from process noise of 0.03^2 and 0.02^2 per hour, which puts the glucose gain at the shipped estimator's 0.35, and the daily sample's $(0.978, 0.036)$ from a disturbance noise of 0.004^2. Titer and ammonium are reset to each offline result at its draw. Offline results arrive on the hour, inside the plan's first block, so the next decision replans on them, and a result doctored 1 g/L high moves $\hat{G}$ by 0.96 g/L on arrival against -0.16 undoctored and cuts the next dose from 0.145 to 0.014 g/(L h). The disturbances also enter the prediction, held over the horizon. The standard deviation σ_G of the applied gains' error, propagated in Joseph form, sets the floor at 1.30 to 1.40 g/L with the Raman. Without it only the daily sample corrects the estimate, σ_G grows to 0.55 g/L between results and the floor with it to 2.59 g/L, which is the fallback the case teaches.

The solver's answer is kept only when it is optimal and its doses, predicted outputs, objective and slacks are all finite, correctly sized and within the pump's range. Otherwise the continuous feed takes the pump from where the trim tracked it (a 0.0145 L/h step in the forced test), the fallback is written to the historian, and the following accepted plan returns the pump through its move penalty (0.19 L/h). No plan was rejected in any measured culture. A decision costs 0.66 ms on average in Chrome, at most 9.6 ms, or 3.2 % of a core at 48 simulated hours per second, measured beside other test runs on the same machine, so no frame guard is needed. The controller reads only its instruments, the laboratory results with their draw times, the estimator's $\hat{X}_v$, $\hat{\mu}$ and osmolality, its own commands, the recipe and the shipped limits, and the application's checks catch all 31 doctored copies of it, 25 that read something more and 6 that undo a reviewed behavior.

Table 8.4 Cases, Their Changes and Their Teaching Points

#	Change from the reference line	Teaching point
1	glucose pump in MANUAL, set to 0.20 L/h on day 3	an appetite that doubles
2	daily bolus to 4 g/L from the offline sample	one decision a day, and the variant no bolus holds
3	continuous feed without the Raman	a feed-forward the shift makes wrong
4	the reference line	feed-forward plus a direct trim
5	gas before oxygen, DO setpoint 60 % from day 2.25 to 3	one sparge, two loops
6	the reference line, shift day moved	a schedule with an interior optimum
7	glucose target 4.5 g/L	a margin above a threshold
8	pH probe drifting −0.02 per day	a drift only the laboratory sees
9	harvest only below 65 % viable	a decision on a late sample
10	feeding strategy MPC	a plan to the harvest, and its margin
C	bolus to 4.5 g/L, no Raman, no cap, probe drift from day 4, weak lot on day 6	graded challenge

8.5.7 Simulation, Estimator and Controller Models

Section 8.3 is the simulation model, which only the scorecard and the truth columns of the log can see. The controller holds three internal models, the estimator's glucose integrator with an uptake measured between samples, the platform yield relation of the model-based feed, and the additions-only osmolality model, and the bolus check holds a fourth, the consumption of the last day grown by the probe. None of them knows the cell size, the true stock or the switch, and no control model is identified from plant data. The MPC adds a fifth, Equation (8.19) at the default constants with its own Kalman estimator, which carries the declared switch of Equation (8.6) at its default thresholds but neither the cell size nor the true stock, and which is also written rather than identified.

8.6 Ten Cases and a Fourteen-Day Challenge

The cases of Table 8.4 start from one reference line, the continuous feed at 2 g/L with the Raman trim, the probe correction at 0.5, oxygen before gas, the shift on day 5, the complex feed from day 3, no osmolality cap, and the harvest on day 14 or below 70 % viable, and each changes what its row states. Every number below is a run of the engine at the default seed, stepped at the physics step of 10 s exactly as the browser steps it, with each change made at inoculation before the run and the run carried to the harvest, and Table 8.5 collects the scorecard. Every scored value is a true one, which the controller never sees. A harvest decided by a noisy daily viability can move by a day with the noise, and where it does at other seeds the text says so.

8.6.1 An Appetite That Doubles

Case 1 runs the glucose pump in MANUAL at zero and sets it on day 3 to 0.20 L/h of the 500 g/L stock. The medium's 6 g/L has carried the culture to 2.62 g/L by then, and the cells take 2.35 g/L that day and 3.62 g/L the next, while the pump supplies 1.77 g/L a day at the day-4 volume of 1357 L. The glucose is 1.37 g/L on day 4 and gone on day 4.67, with 7.63 million cells/mL and before the next sample has been drawn, and it stays near zero for 223.9 h. The cells then take lactate back as the only carbon left, which the application marks as starvation, peak at 8.24 million cells/mL on day 8.98 and begin to die. The day-13 sample reads 70.4 % viable, just above the limit, so the culture runs to day 14 and is harvested at 66.3 % viability with 3.28 g/L of antibody. At eight of ten noise seeds that sample reads below 70 % and the harvest comes on day 13.17 with 3.07 g/L. With the glucose loop returned to AUTO the same culture makes 5.03 g/L.

Table 8.5 Scorecard of Each Case and Variant, From Inoculation to Harvest, at the Default Seed. Glucose Is Scored From Day 2, and Every Value Is the True One

Case and configuration	Titer (g/L)	Harvest (day)	Outside (h)	Switch (day)	Osm (mOsm/kg)	Base (mol)
1, pump 0.20 L/h from day 3	3.28	14.00	235.6	—	407	19.6
1, glucose loop in AUTO	5.03	14.00	0	6.32	393	27.6
2, bolus to 3 g/L	4.07	14.00	104.1	5.51	399	20.0
2, bolus to 4 g/L, shipped	4.14	14.00	34.8	5.85[a]	435	39.7
2, bolus to 5 g/L	3.27	12.17	6.3	5.94[a]	490	68.1
2, bolus to 6 g/L	2.73	11.17	0.7	5.09[a]	511	75.8
2, bolus to 4, shift day 8	1.61	9.17	44.3	7.72[a]	426	42.4
2, bolus to 5, shift day 8	1.72	9.17	29.1	6.83[a]	446	52.0
2, bolus to 6, shift day 8	1.74	9.17	15.0	6.78[a]	465	62.5
3, no Raman, shipped	3.43	13.17	1.3	8.34	464	52.4
3, uncorrected probe	2.76	11.17	4.0	10.43	478	62.1
3, model-based feed	4.95	14.00	0	6.49	384	29.1
4, Raman trim, shipped	5.03	14.00	0	6.32	393	27.6
4, Raman out from day 4.5	3.12	12.17	3.8	9.29	465	55.0
4, target 1.5 g/L	5.07	14.00	0	5.10	390	26.7
4, target 2.5 g/L	3.66	13.17	0	—	467	55.9
4, target 3 g/L	2.72	11.17	0	—	501	72.3
5, gas before O_2, shipped	5.28	14.00	0	6.32	382	24.0
5, O_2 before gas	5.04	14.00	0	6.32	393	27.7
6, shift on day 3	4.33	14.00	0	5.77	359	3.1
6, shift on day 4	4.89	14.00	0	5.38	370	13.6
6, shift on day 6	4.83	14.00	0	7.26	414	38.2
6, shift on day 7	4.67	14.00	0	8.22	422	43.5
6, shift on day 8	3.57	12.17	0	7.30[a]	422	45.6
6, no shift	1.92	10.17	0	7.30	418	45.7
7, target 4.5 g/L, shipped	2.58	11.17	0	—	519	76.7
7, with the osmolality cap	4.44	14.00	0	7.31[a]	399.7	46.6
8, pH probe drift, shipped	4.50	14.00	0	6.31	405	27.2
8, re-standardized	5.02	14.00	0	6.32	394	27.7
9, harvest below 65 %	7.40	18.17	40.6	6.32	395	27.6
C, shipped	4.25	14.00	35.4	5.99[a]	430	39.5
C, suggested	5.21	14.00	0	6.30	381	27.9

[a]Lactate peaked again after the switch, once the glucose rose back above the switch's threshold, and the scorecard does not credit such a switch. Outside is the hours with the glucose outside 1 to 6 g/L after day 2.

> **TRY IT IN THE APP** **1 · Manual Feeding**
>
> Load scenario 1 and press Run. The glucose pump is set by hand to 0.20 L/h on day 3 and never moves again. Watch the glucose trend alone. It reaches zero on day 4.67 and stays near it for 223.9 h, and the culture is harvested with 3.28 g/L of antibody against 5.03 g/L with the loop in AUTO.

8.6.2 One Decision a Day

Case 2 feeds a bolus to 4 g/L at each glucose result. The day-3 sample reads 2.66 g/L and receives 3.5 L of stock, the day-4 sample 1.00 g/L and 8.2 L, and the glucose runs out on day 4.93 with 8.54 million cells/mL. Over the culture it is outside the band for 34.8 h and gone for 5.6 h, and the culture makes 4.14 g/L while its osmolality peaks at 435 mOsm/kg. A higher target trades one failure for another. At 3 g/L the cells starve for 20.1 h, while at 5 and 6 g/L the glucose stays above 2.5 g/L for 74.7 and 96.6 % of the hours after day 5, against 37.8 at 4 g/L, so the cells keep making lactate, the base drives the osmolality to 490 and 511 mOsm/kg, and the cultures are harvested on days 12.17 and 11.17 with 3.27 and 2.73 g/L. The honestly infeasible variant keeps the culture warm until day 8, where Equation (8.15) fails. At targets of 5 and 6 g/L the bolus check flags it on day 5.17, from a measured 4.07 g/L and 4.05 g/L grown by the probe to a projected 5.38 and 5.46 g/L for the next day, and targets of 4, 5 and 6 g/L leave the band for 44.3, 29.1 and 15.0 h. At 4 g/L the check never fires, because its glucose had run out and the culture ate only the 3.69 g/L it was given between the day-4

and day-5 samples. All three warm cultures are harvested on day 9.17, when the day-9 sample reads below 70 % viable, and at two to five of ten noise seeds a day later.

8.6.3 A Feed-Forward That the Shift Makes Wrong

Case 3 feeds continuously on the capacitance probe without the Raman. From day 3.5 to day 5 it holds 2.14 to 2.62 g/L. Then the culture is cooled, and the true per-cell uptake falls from 0.0224 on day 5 to 0.0114 g per 10^9 cells per hour on day 6, while the feed still uses the 0.0268 and then the 0.0258 it measured between samples before the shift. Nothing the controller reads shows the glucose rising until the day-6 result arrives on day 6.17, and by then it is at 6.09 g/L, as shown in Figure 8.3. Above the switch's threshold the cells keep making lactate, the switch waits until day 8.34, lactate reaches 7.00 g/L, the base drives the osmolality to 464 mOsm/kg, and the culture is harvested on day 13.17 at 64.8 % viability with 3.43 g/L, after a day-12 sample of 71.4 %. At the nine other seeds tried the day-12 sample reads below 70 %, and the harvest comes on day 12.17 with 3.08 to 3.13 g/L.

The probe adds an error of its own. In the default culture it reads 17, 22 and 21 % high on days 7, 10 and 14 as the cells swell, and the counts bring the corrected estimate to 7 % high on day 7 and 2 % low on day 14. In case 3 an uncorrected probe raises the glucose peak to 6.28 g/L and lowers the titer to 2.76 g/L, an ideal probe gives 5.55 and 3.53 g/L, and a probe that also drifts 4 % a day uncorrected gives 7.86 g/L, no switch at all and 2.62 g/L. The model-based feed knows that a cooled culture eats less. Without the Raman it keeps the glucose at or below 3.07 g/L after day 4, switches on day 6.49 and makes 4.95 g/L, and with the probe uncorrected 3.26 and 4.44 g/L.

> **TRY IT IN THE APP** **3 · Continuous Feed on the Capacitance Probe**
>
> Load scenario 3 and run to day 6.2. The controller's glucose estimate sits at its 2 g/L target and the last offline result reads 2.45 g/L, while the true glucose has climbed to 6.09 g/L since the culture was cooled on day 5. Nothing the controller reads shows the climb until the day-6 result arrives.

Pause. Try Exercise 8.2 before reading on.

8.6.4 A Direct Reading of the Broth

Case 4 adds the Raman, whose hourly readings pull $\hat{G}$ toward the broth. It catches the cooled culture within hours, holds the glucose between 1.74 and 2.55 g/L from day 4 to the harvest, and the switch comes on day 6.32 at a lactate peak of 4.54 g/L. The culture reaches 14.78 million cells/mL and 5.03 g/L, 9.47 kg, on day 14 at 80.9 % viability and 393 mOsm/kg. With the Raman's noise turned off, the glucose stays between 1.88 and 2.54 g/L and the pump moves 26 % less. Taken out of service on day 4.5, the Raman leaves the feed to overfeed the cooled culture as in case 3, with 6.27 g/L of glucose on day 6.17, and the culture is harvested on day 12.17 with 3.12 g/L of antibody, while taken out on day 9 it costs nothing, because by then the rate measured from the samples is right. The target is a lever on the switch of Section 8.4. At 1.5 g/L the switch comes on day 5.10 and the culture makes 5.07 g/L, while at 2.5 and 3 g/L it never comes, and the cultures are harvested on day 13.17 with 3.66 g/L and on day 11.17 with 2.72 g/L.

8.6.5 One Sparge, Two Loops

Case 5 raises the gas before the oxygen and steps the DO setpoint from 40 to 60 % on day 2.25 for eighteen hours. A step of 20 points held that long would be rare in a plant, and it serves here to isolate what one sparge does to two loops. The gas goes from 30.6 L/min to 92.6 L/min by day 2.5 and to 100 L/min by day 2.57, and the extra gas strips carbon dioxide, so the pH loop puts it back. On day 2.5 it doses 3.39 % of carbon dioxide into the larger flow, 3.13 L/min, where without the step it doses 2.74 % into 38.4 L/min, 1.05 L/min, to hold the same 31.8 mmHg and pH. The pH itself barely moves, to a peak of 7.010 and a dip to 6.972 on day 3.08, so the coupling shows in the base and the osmolality instead. With the gas first the p_{CO_2} stays between 7.7 and 13.7 mmHg from day 3 to day

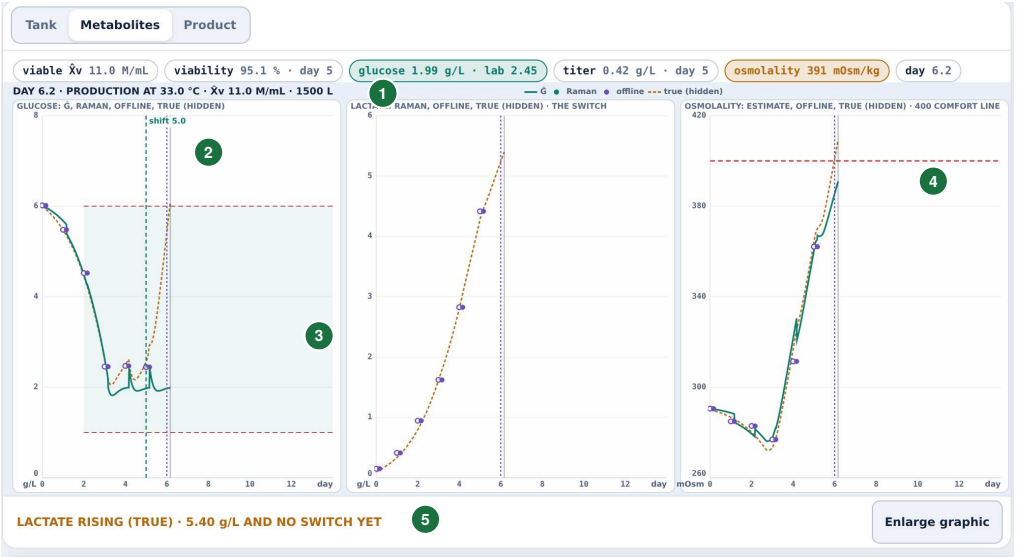


Figure 8.3 Case 3, the continuous feed on the capacitance probe without the Raman, at t = 6d 04:00 (day 6.17) in the Metabolites view, with no control changed, cropped to the plant view. Callout 1 is the badge that carries the controller's glucose estimate $\hat{G}$, 1.99 g/L against its 2.0 g/L target and the last offline result, 2.45 g/L from day 5. Callout 2 is the hidden true glucose, which has climbed to 6.09 g/L since the shift on day 5.0, and callout 3 the 1 to 6 g/L band it is about to leave, with the day-6 sample, drawn at 144 h and marked by the dashed vertical line, still at the laboratory. Callout 4 is the 400 mOsm/kg comfort line that the osmolality estimate is approaching, and callout 5 the status line, which reads lactate rising (true), 5.40 g/L and no switch yet. The badges give the corrected viable density as 11.0 million cells/mL at 1500 L, the last offline viability as 95.1 % and titer as 0.42 g/L, and the osmolality estimate as 391 mOsm/kg.

6, so 1.9 to 3.4 mM of bicarbonate holds pH 7.00 and the culture has used 23.0 mol of base by day 6. With the oxygen first the sparge never leaves 15 L/min, the p_{CO_2} runs 23.5 to 50.7 mmHg, 5.7 to 12.6 mM of bicarbonate has to be made with base, and 27.6 mol are used by day 6. Over the culture that is 382 mOsm/kg against 393 and 5.28 g/L against 5.04. With the oxygen first the same DO step moves only the enrichment, from 7 to 43 %, and the pH loop does not notice it.

8.6.6 The Shift, Early and Late

Case 6 cools the culture of case 4 on day 5, with 8.87 million cells/mL in the broth. The jacket supply falls to 28.1 °C, the broth is at 33.0 °C from day 5.18, the growth falls from 0.0223 on day 4 to 0.0059 h^{-1} on day 6 and the per-cell uptake from 0.0256 to 0.0119 g per 10^9 cells per hour. The titers for a shift on days 3, 4, 5, 6 and 7 are 4.33, 4.89, 5.03, 4.83 and 4.67 g/L, and the day-5 shift makes the most at each of ten noise seeds. Cooling early leaves too few cells, and the culture shifted on day 3 is still growing on day 14, at 15.64 million cells/mL. Cooling late leaves the warm culture making lactate, and shifted on day 7 its lactate peaks at 5.85 g/L, it needs 43.5 mol of base against 27.6, and its osmolality reaches 422 mOsm/kg, while at half of the seeds its day-13 sample already reads below 70 % and it is harvested on day 13.17 with about 4.2 g/L. Shifted on day 8 it is harvested on day 12.17 with 3.57 g/L, and never cooled on day 10.17 with 1.92 g/L. A density trigger of 8, 10 or 12 million cells/mL fires on day 4.76, 5.25 or 5.80 for 5.05, 5.01 and 4.89 g/L. It does not rescue a poor seed, because with 80 % of the seed viable the day-5 shift makes 4.77 g/L and the 10 million trigger, firing on day 5.59, makes 4.73 g/L.

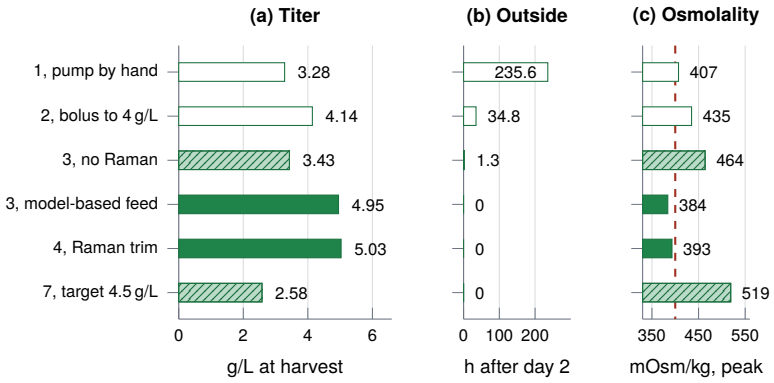


Figure 8.4 Six rows of Table 8.5 drawn as bars: the titer at harvest, the hours of true glucose outside the 1 to 6 g/L band after day 2, and the highest osmolality against the 400 mOsm/kg comfort line (dashed). White bars are the two feeds that never read the broth, hatched bars the two that read it late or not at all, and solid bars the two that read it hourly or know that a cooled culture eats less. Notice that the two tallest titers are the two lowest osmolalities, and that the target of 4.5 g/L holds the band perfectly while making the least antibody of all six.

8.6.7 A Margin Above a Threshold

Case 7 raises the target to 4.5 g/L, the setting of an operator who wants room above the floor. With the glucose at 4.27 to 5.03 g/L the cells keep overflowing it after the shift. Lactate rises from 5.57 on day 6 to 7.29 on day 8 and 8.60 g/L on day 10, ammonium from 5.00 to 7.03 mM, and the osmolality from 406 to 458 and 499 mOsm/kg, on its way to 519 mOsm/kg. Growth stops at 9.51 million cells/mL on day 6.70, the day-11 sample reports a viability below 70 %, and the culture is harvested on day 11.17 at 64.0 % with 2.58 g/L. With the osmolality cap on, the complex feed and the target come down as the estimate nears 390 mOsm/kg, the switch comes on day 7.31, the osmolality peaks at 399.7 mOsm/kg and the culture makes 4.44 g/L. Lowering the target works better, since neither 3 nor 2.5 g/L ever switches while 2 g/L switches on day 6.32. Figure 8.4 sets this case beside five others of Table 8.5.

> ✦ **DID YOU KNOW? The Comfortable Target That Costs the Most**
>
> From the simulator: a glucose target of 4.5 g/L, a cautious margin above the floor, makes 2.58 g/L of antibody, the least of any case, while a target of 2 g/L makes 5.03 g/L. The cells never stop making lactate, and the base that holds the pH drives the osmolality to 519 mOsm/kg (Table 8.5).

8.6.8 A Drift Only the Laboratory Sees

Case 8 drifts the pH probe 0.02 a day low, and the loop holds what the probe reads at 7.00. The broth goes where the probe's error takes it, 7.078 on day 4, 7.137 on day 7 while the probe reads 6.994, 7.200 on day 10 and 7.280 on day 14, and the offline results climb from 7.03 on day 2 to 7.26 on day 13 while the probe's trend stays at its setpoint. The total base hardly changes, 27.2 mol against 27.6, but the broth ends with 28.4 mM of bicarbonate against 27.1, the cells peak at 12.22 million cells/mL against 14.78, and the culture makes 4.50 g/L. Re-standardized to each offline pH through the shared bias update with a gain of one, the probe follows the laboratory, the true pH stays within 0.088 of 7.00, and the culture makes 5.02 g/L.

8.6.9 A Harvest on a Late Sample

Case 9 runs until a daily sample reports a viability below 65 %. From day 14 to day 18 the true titer is 5.03, 5.57, 6.11, 6.75 and 7.32 g/L at 80.9, 78.2, 75.6, 70.2 and 56.7 % viability. The vessel is full

Table 8.6 The Challenge With One Lever Changed at a Time

Configuration	Titer (g/L)	Outside (h)	Osm (mOsm/kg)	Viable (%)	Target
as shipped, bolus to 4.5 g/L	4.25	35.4	430	73.2	not met
bolus to 3 g/L	3.83	118.7	400	75.0	not met
bolus to 4.5 g/L with the cap	4.75	59.4	396	80.3	not met
continuous at 2 g/L, no Raman, no cap	3.41	3.6	462	65.1	not met
suggested, Raman and cap	5.21	0	381	83.3	met
suggested without the cap	5.01	0	390	81.7	met
suggested, model-based feed	5.06	0	371	84.2	met
suggested at 4.5 g/L	4.41	0	401	62.4	not met
suggested without the Raman	3.10	0	414	58.0	not met

on day 16.22 and the feeds stop, the glucose is gone on day 16.77, the day-17 sample reads 69.1 % and the day-18 sample 56.5 %, and the culture is harvested when that result arrives, on day 18.17, at 54.8 % true viability with 7.40 g/L, 14.81 kg. The limit sits at 65 % because the day-17 sample is drawn at a true 70.2 %. A 70 % limit harvests on day 17.17 at 67.7 % with 6.85 g/L, and at five of ten noise seeds a day later, while at 65 % all ten harvest on day 18.17. An 80 % limit harvests on day 15.17 at 77.8 % with 5.66 g/L, and a 75 % limit on day 17.17 at 67.7 %, more than seven points below its own limit, and at three of ten seeds each comes a day earlier.

8.6.10 The Fourteen-Day Challenge

The challenge feeds a whole culture by a daily bolus to 4.5 g/L, with no Raman and no cap, while the capacitance probe drifts 4 % a day from day 4 and a 420 g/L lot replaces the 500 g/L stock on day 6 under the same label. The target asks for at least 5.0 g/L by day 14 with the glucose between 1 and 6 g/L after day 2, the osmolality under 400 mOsm/kg, the DO above 30 %, the pH within 0.15 of 7.00 and more than 70 % viable at harvest, all scored on the truth, and a changed model parameter or disturbance voids the attempt. As shipped the glucose runs out on day 5.05 and is outside the band for 35.4 h, the osmolality peaks at 430 mOsm/kg, and the culture makes 4.25 g/L and is 73.2 % viable at harvest. Table 8.6 changes the levers one at a time. The suggested continuous feed at 2 g/L with the Raman and the cap holds 1.76 to 2.58 g/L after day 4, switches on day 6.30, keeps the pH within 0.072 and the DO above 39.2 %, and makes 5.21 g/L at 381 mOsm/kg and 83.3 %, 0.21 g/L above the target, whether the settings are revealed before the run or carried through a reset. It passes at each of ten noise seeds, with 5.16 to 5.25 g/L. The cap supplies that margin. Without it the culture passes at the default seed with 5.01 g/L at 390 mOsm/kg, only 0.01 g/L above the target, and falls short of the titer at six of the ten seeds. The Raman is necessary, since without it the feed overfeeds the cooled culture and is harvested on day 11.17 with 3.10 g/L.

8.6.11 A Plan to the Harvest, and What It Is Worth

Case 10 feeds the culture of case 4 by the MPC of Section 8.5.6. With no glucose target the plan holds the glucose between 1.09 and 2.05 g/L after day 4 on a floor of 1.30 to 1.40 g/L, where the continuous feed holds 1.74 to 2.55 g/L, so the lactate switch comes on day 5.10 at a peak of 4.18 g/L rather than on day 6.32 at 4.54 g/L. The culture makes 5.06 g/L at 81.2 % viability and 392 mOsm/kg, against 5.03 and 5.08 g/L for the continuous and model-based feeds, and the pump travels 27.8 L/h over the culture against 54.0 and 54.3 L/h. The titer the plan maximizes is its model's prediction, not a measurement. On day 7 it predicts 6.38 g/L at the harvest, a quarter above what is made, because $\hat{\mu}$ carried over the rest of the batch declines more slowly than the culture's growth, and the prediction closes to 5.38 on day 10 and 5.07 g/L on day 13. Over the first 24 h the condensed prediction of the glucose stays within 0.04 g/L of the nonlinear model under the same plan in 8 of 10 cultures (0.28 on the day-8 shift and 0.97 in case 9), while over the whole horizon the two part by up to 15.4 of glucose, 12.0 of lactate, 7.0 g/L of titer and 376.5 mOsm/kg, since the plan's deviations carry the

Table 8.7 Titer at Harvest Under Every Feeding Structure, Same Seed, Instruments and Noise. Hours of True Glucose Outside 1 to 6 g/L After Day 2 in Parentheses Where Nonzero

Culture	Bolus (shipped)	Continuous (shipped)	Model-based (shipped)	Continuous retuned[a]	Model-based on the MPC's estimate[b]	MPC	MPC, preview Off
case 10 as shipped	4.14 (34.8)	5.03	5.08	5.07	5.05	5.06	5.06
Raman out from day 4.5	4.06 (30.4)	3.12[c] (3.8)	4.95	3.95	5.06	5.01	5.01
no Raman	4.15 (35.5)	3.43[c] (1.3)	4.95	3.70[c]	5.08	4.96	4.96
shift on day 3	3.48 (0.3)	4.33	4.53	4.40	4.56	4.40	4.40
shift on day 8	1.61[c] (44.3)	3.57[c]	4.50	4.73	4.53	4.67	4.76 (1.9)
viability at harvest (%)	70.5	68.2	77.3	67.9	76.9	67.3	68.4
shift at 10×10^6 cells/mL	4.28 (52.2)	5.01	5.03	5.07	5.03	5.06	5.06
challenge, suggested levers	4.86 (55.2)	5.21	5.06	5.17	5.02	5.16	5.16
challenge, shipped levers	4.25 (35.4)	3.41[c] (3.6)	4.81	3.67[c]	4.99 (6.7)	4.88 (6.9)	4.88 (6.9)
pump travel on case 10 (L/h)	880.0	54.0	54.3	40.4	54.0	27.8	30.3

[a]The continuous feed retuned by the MPC's rule: target 1.5 g/L, gain 0.5, 8 h. The model-based feed so retuned (2 g/L, 0.5, 8 h) is within 0.01 g/L of the shipped one in every row. [b]Trimmed on the MPC's Kalman estimate, its target at the MPC's floor. [c]Harvested before day 14. In the challenge the bolus keeps 4.5 g/L and the continuous feeds 2 g/L. Every MPC run made 336 decisions, none rejected.

nonlinear model across the overflow and osmotic thresholds between the judged steps. Enforcing the trust region at every step moves no titer by more than 0.04 g/L.

Table 8.7 sets the MPC beside each shipped feed, the continuous feed retuned once by the MPC's rule (23 of 36 settings qualified) and the strongest classical feed of the MPC's signals. The MPC's clear gain is the lost Raman. Taken out on day 4.5, the Raman leaves the continuous feed to overfeed the cooled culture, which is harvested on day 12.17 with 3.12 g/L, and the retuned feed recovers only to 3.95 g/L, while the MPC keeps the glucose at 1.24 to 3.04 g/L after day 4 and makes 5.01 g/L, as shown in Figure 8.5. Its floor climbs from 1.24 toward 2.59 g/L as σ_G grows between daily results and drops back at each one. The model-based feed makes 4.95 g/L in the same culture, however, and the classical feed on the MPC's estimate makes 5.06 g/L, and with no Raman from inoculation the classical feed makes 5.08 g/L against the MPC's 4.96. The estimate, not the plan, carries the Raman-less cultures. On the day-8 shift the MPC makes 4.67 g/L but ends 67.3 % viable, under the 70 % the challenge asks, where the model-based feed makes 4.50 g/L at 77.3 %, and the retuned continuous feed makes 4.73 at 67.9 %. On the day-3 shift the model-based feeds make 4.53 and 4.56 g/L against 4.40, and at a density trigger every continuous structure lands between 5.01 and 5.07 g/L. With the challenge's suggested levers the MPC passes with 5.16 g/L, against 5.21 g/L for the suggested continuous feed. With its shipped levers, no Raman and no cap, the MPC makes 4.88 g/L with 6.9 h under 1 g/L, where the model-based feed makes 4.81 with none. The preview Off moves no titer by more than 0.09 g/L, the most on the day-8 shift, 4.76 against 4.67 g/L with 1.9 h under the band, because the hourly plan sees the shift in the measured temperature within hours. Measurably, the MPC brings a feed that survives the lost Raman where the shipped continuous feed does not (1.89 g/L more), the earliest switch of the shipped structures (day 5.10 against 5.20 to 6.32), and about half their pump travel.

8.6.12 What the Review Changed in the Plan

The MPC as first built was reviewed against every shipped feed, and three of its behaviors were changed, with the results in Table 8.8. The plan was not offset-free. Its disturbances corrected the estimate but entered neither the prediction nor the nominal dose, so under a sustained disturbance the nominal settled where $\hat{G} = G_{fl} + (3\,\mathrm{h})\hat{d}_G$. After a 300 g/L lot hung on day 6, with $\hat{d}_G$ near –0.09 g/(L h), the true glucose sat between 1.00 and 1.15 g/L from hour 178 to hour 200 under a floor of 1.24 to 1.33 g/L that the plan believed it held, and the disturbances carried in the prediction alone took that culture from 22.4 to 13.9 h under the band. The Raman's disturbance gain, 0.014 at a disturbance noise of 0.004^2, learned 63 % of the stock step in 24 h and 90 % in 42 h while the estimate ran up to 0.46 g/L above the truth, more than the margin of $3\sigma_G \approx 0.36\,\mathrm{g/L}$. The Raman

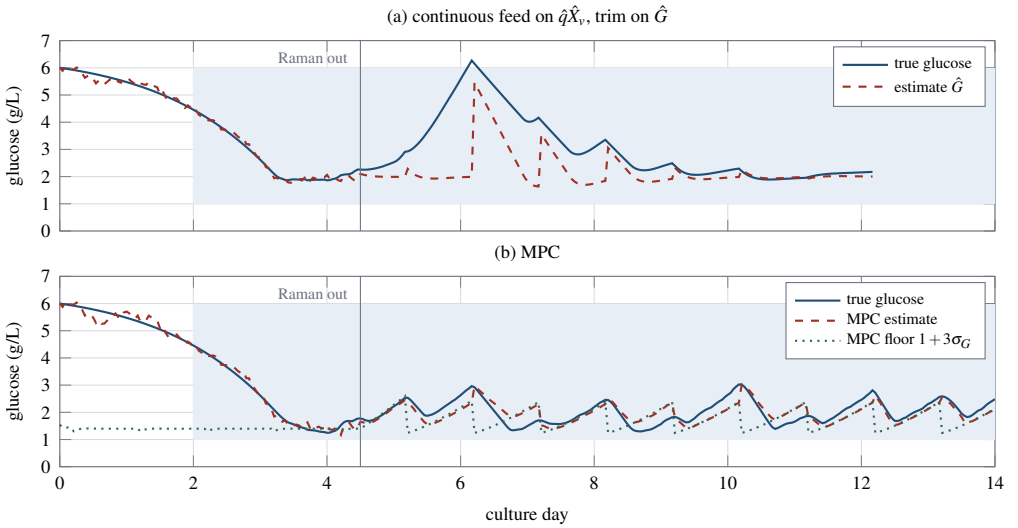


Figure 8.5 Case 10's culture with the Raman out of service from day 4.5, under the continuous feed (a) and the MPC (b), sampled hourly, with the true glucose solid, each estimate dashed and the MPC's floor dotted. The shaded box is the 1 to 6 g/L band scored after day 2. Between daily results the continuous feed's estimate sits at its 2 g/L target while the cooled culture's glucose climbs to 6.27 g/L on day 6.17, and the culture is harvested on day 12.17 with 3.12 g/L. The MPC's floor rises between daily results as its estimate ages, the true glucose stays between 1.24 and 3.04 g/L after day 4, and the culture makes 5.01 g/L.

Table 8.8 The MPC Before and After the Review: Hours of True Glucose Under 1 g/L After Day 2, Lowest True Glucose and Titer

Culture	As first built			After review		
	(h)	(g/L)	(g/L)	(h)	(g/L)	(g/L)
300 g/L lot on day 6	22.4	0.80	4.96	0	1.12	4.99
probe drift 10 % a day	2.9	0.97	5.06	0	1.05	5.10
challenge, suggested levers	4.0	0.95	5.14	0	1.14	5.16
with the drift and the lot	22.0	0.75	4.97	0	1.03	5.03
300 g/L stock, labeled so	24.9	0.83	4.97	0	1.08	5.01
challenge, shipped levers	2.3	0.98	4.87	6.9	0.94	4.88
Raman out day 2, drift, lot	17.2	0.81	4.31	30.5	0.58	4.47
no Raman, 300 g/L lot	47.1	0.50	4.92	44.9	0.49	4.93
pump travel, case 10 (L/h)			16.2			27.8

The rows below the first rule run without the Raman. No MPC row rose above 6 g/L.

gains were recomputed at 0.02^2, the shipped estimator's Raman speed, and now learn the same step 63 % in 12 h and 90 % in 13 h, with the laboratory gains and the covariance under the floor unchanged. That amendment was fixed before its single trial, with its acceptance stated in advance (no culture with the Raman leaving the band and no titer falling by more than 0.02 g/L). The pump also ran the dose on the declared 500 g/L while every shipped feed reads the label, so a 300 g/L stock correctly labeled was underdosed by 40 % and spent 24.9 h under the band. With all three changes the MPC holds the band in every culture with the Raman.

The changes have a price, reported rather than tuned away. Without the Raman the plan learns a step only from the daily sample, and a disturbance learned from the laboratory is sometimes of the wrong sign for a fresh step, which adds 4.6 and 13.3 h under the band to two of the three Raman-less

Table 8.9 Worst Cases on Case 10's Culture Under the Shipped Feeds and the MPC: Titer (g/L), Hours of Glucose Outside 1 to 6 g/L After Day 2 and Highest Osmolality (mOsm/kg), With the MPC's Lowest DO (%), Widest pH Deviation and Viability at Harvest (%)

Fault or disturbance	Continuous			Model-based			MPC					
	Titer	Out	Osm	Titer	Out	Osm	Titer	Out	Osm	DO	pH	Viable
300 g/L lot on day 6	5.01	0	382	4.94	0	360	4.99	0	378	39.2	0.081	83.6
capacitance drift 10 % a day from day 4	4.93	0	398	5.00	0	388	5.10	0	391	39.2	0.081	81.4
offline results 24 h late	4.87	0	402	5.07	0	379	5.06	0	391	39.1	0.081	81.2
seed 80 % viable	4.77	0	391	4.89	0	369	4.79	0	391	39.2	0.083	82.7
O_2 supply to 40 % on day 5	5.05	0	393	5.09	0	375	5.09	0	391	15.4	0.098	81.6
chiller failed for an hour on day 5.1	5.03	0	393	5.08	0	375	5.06	0	392	39.2	0.081	81.2
pH probe drift −0.1 a day	1.45	0	443	1.75	0	345	1.45	0	435	39.4	1.400	80.9
stock of 500 g/L labeled 300	5.03	0	394	5.04	0	377	5.07	0	393	39.2	0.083	81.2
stock of 300 g/L labeled 300	4.99	0	379	4.95	0	356	5.01	0	375	39.2	0.083	83.8
challenge, suggested levers, drift and lot	5.05	0	380	4.97	0	364	5.03	0	377	39.2	0.081	83.6
Raman out day 2, drift, 300 g/L lot	3.31	8.3	462	4.45	103.4	398	4.47	30.5	406	39.2	0.056	78.0
no Raman, 300 g/L lot	3.53	0	443	4.68	156.5	369	4.93	44.9	386	39.1	0.077	83.5
case 9, harvest below 65 % viable	7.40	40.6	395	7.68	37.2	382	7.29	36.6	395	39.2	0.123	56.8

rows of Table 8.8 and removes 2.2 h from the third. The pump travels 27.8 L/h on case 10 against 16.2, and the estimator is no longer consistent, because its σ_G of 0.08 to 0.16 g/L sits below the 0.167 g/L standard deviation of its actual error, where the first build claimed 0.115 against an actual 0.123. Raising the disturbance noise of the laboratory gains and the covariance as well, a variant tried and rejected during the build, lifts σ_G above 1 g/L between samples and sinks the Raman-less cultures, 4.98 to 2.63 g/L with the Raman out on day 4.5 and 4.95 to 2.52 with no Raman. The tuning grid was not rerun after the review.

8.6.13 Every Structure Against Every Limit

Table 8.9 runs thirteen faults and disturbances under the shipped feeds and the MPC against every limit the application checks, the 1 to 6 g/L band after day 2 and depletion, the 400 mOsm/kg comfort line, the DO at 30 and 20 %, the pH within 0.15 of 7.00, 70 % viability at harvest and the 2000 L vessel. With the Raman in service the MPC never leaves the band where the shipped feeds hold it. Its osmolality passes 400 only where the continuous feed's does too (406 against 462, 435 against 443), its lowest DO is within 0.1 points of the continuous feed's in every row (15.4 against 15.5 % when the oxygen supply falls, under the 20 % alarm for every structure that feeds continuously), and its viability is under 70 % only in case 9, which harvests on a late decline by design. Its widest pH deviation, ±0.123 at the harvest of case 9, is under the 0.15 check level. After day 16 the carbon dioxide leg of the pH loop sits at its 20 % limit under the continuous, model-based and MPC feeds alike, and the MPC's culture entered the full vessel with 0.11 g/L of lactate left to take back, against 1.89 and 1.23 g/L under the continuous and model-based feeds, which reach ±0.036 and ±0.046 then. The losses are the cultures without the Raman, 30.5 and 44.9 h under the band, against 8.3 and 0 for the continuous feed and 103.4 and 156.5 h for the model-based feed. The only depletion under the MPC is case 9's 28.9 h after the vessel fills, against 33.6 and 31.1 h. The bolus leaves the band 4.1 to 110.8 h in every row but the pH drift. No plan was rejected, and the cap, the vessel-full stop and the harvest act above the MPC unchanged.

Two older defects found during the build were fixed with no shipped or suggested run moved. A seed of 60 to 70 % viability was harvested at 4 h with no antibody on its day-0 sample, and under the decline rule it now makes 4.33 to 4.58 g/L on the continuous feed and 4.36 to 4.59 on the MPC. The glucose and osmolality estimates started at the broth's own values, so a medium set to 8 g/L or 320 mOsm/kg was known at inoculation, and both now start at the declared 6 and 290. Because 19 laboratory and Raman columns are empty until their first report, the suite's finite-column check runs under the MPC as an application test that allows them empty.

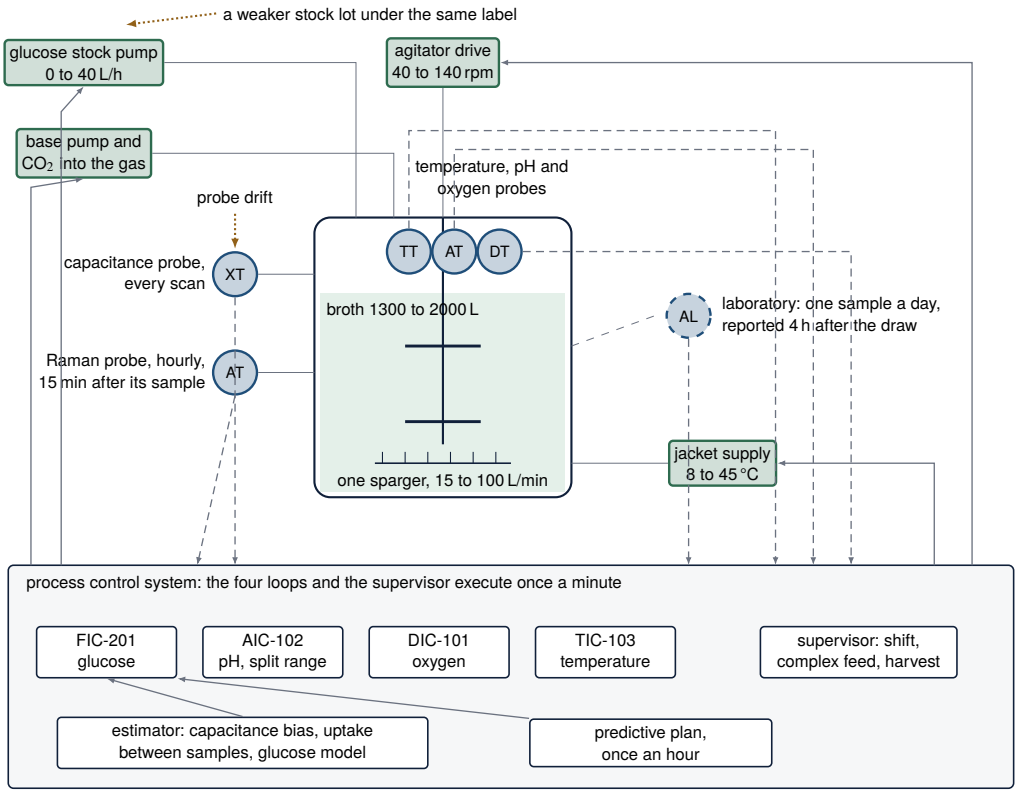


Figure 8.6 The four loops of Section 8.5 with the equipment of a culture vessel in place of the schematic's symbols. A capacitance probe (XT) and an in-line Raman probe (AT) read the broth every scan and every hour, while one sample a day goes to a laboratory (AL, dashed) and is reported four hours after the draw. The final elements are a dosing pump on the glucose stock, a base pump that shares the pH duty with carbon dioxide in the sparge gas, the agitator drive with the gas train and the jacket supply. Notice that the only instrument that sees glucose, cells, metabolites and product together is the one that answers last, and that the estimator stands between it and the feed.

8.7 Technology in Practice

The probes, the sample line and the pumps of Figure 8.1 stand for the equipment of a manufacturing culture vessel. Figure 8.6 draws the four loops of Section 8.5 with that equipment in place of the schematic's symbols.

What Measures It

Three probes stand in the broth and report every scan: a dissolved oxygen head, a glass pH electrode against a reference junction, and a temperature probe. Their lags of 1, 0.5 and 0.5 min in Section 8.5 stand for the response of a probe in a stirred broth rather than for a transmitter. Two more instruments read what the cells are doing. A capacitance probe applies a weak electric field and reports the permittivity of the broth, which follows the volume of intact cells rather than their number, while a plant otherwise learns cell physiology only from a drawn sample [18]. The in-line Raman probe reads the metabolites themselves. The chapter's hourly Raman with 0.25 g/L of noise stands for such a probe, and its four-hour laboratory for the sample that is still the reference.

TECHNOLOGY Sensor: The In-Line Raman Probe

A Raman probe stands in the broth and reads the light that molecules scatter at shifted wavelengths, so one spectrum carries glucose, lactate, cell density and product together. In a recent study a probe with a laser at 785 nm built each spectrum from 50 accumulations of 15 s, 12.5 min for one vessel, and a partial least squares model predicted glucose on a culture it had not seen with a coefficient of determination of 0.955 and a root mean square error of 0.487 g/L [22]. Such a probe fails slowly rather than suddenly. In the same cultures the spectral baseline climbed as the culture aged and stepped up at each nutrient feed, because fluorescence from the feed's aromatic amino acids masks the signal [22].

What Moves It

The glucose stock reaches the broth through a dosing pump, which the chapter ranges from 0 to 40 L/h, and the pH through a split between a carbonate base and carbon dioxide in the sparge gas. Base and gas are the two legs of AIC-102. The agitator drive and the gas train carry the oxygen demand in sequence, and the jacket supply carries the temperature shift at 1 K/h.

TECHNOLOGY Final Element: A Dosing Pump and a Split Gas

Two final elements carry the glucose and the pH of a culture vessel: a dosing pump on the stock bottle and a split between carbon dioxide in the sparge gas and a carbonate base. In one reported set of cultures the pH was held inside a deadband of 0.1 by sparging carbon dioxide and adding sodium carbonate, and the glucose pump started 38 and 39 times in fourteen days under a spectroscopic feedback strategy against 13 times under a daily bolus by hand [22]. Base is the harder of the two at scale, since base entering from the top leaves zones of high pH and low carbon dioxide behind it, while carbon dioxide that accumulates lowers growth and productivity [23].

Where the Controller Runs

The platform is the vessel's own control system, with the spectroscopic analyzer and its calibration models on a separate computer that exchanges values with it over a standard machine-to-machine protocol. In the reported installation the analyzer delivered a new prediction every 12.5 min and the feed decision followed each one, against the once-a-minute scan of the four loops in this chapter and the hourly plan of its predictive controller [22]. The supervisor of Section 8.5 is the recipe layer above those loops, and in a plant it is the layer that holds the batch record.

IN PRACTICE What Makes a Probe Part of the Control Strategy

A measurement becomes part of manufacturing through process analytical technology, which the United States regulator describes as a system for designing, analyzing and controlling manufacturing through timely measurements of critical quality and performance attributes, with the goal of ensuring final product quality. Within that framework a process end point is not a fixed time but the achievement of the desired material attribute [24]. Automatic equipment used in manufacture is routinely calibrated, inspected or checked under a written program whose records are kept (21 CFR 211.68) [25]. Engineers who have put such a loop into service report that a fallback to sampling by hand is needed for the hours when the link between analyzer and controller fails [22].

What Is Modernizing It

Three developments bear on this feed. The first is the closed analyzer loop itself. A spectroscopic strategy that held glucose at a setpoint instead of topping the culture up once a day raised the product titer by up to 35 % and lowered glycation of the product by up to 27 % [22]. The second is making a calibration survive a change of scale or medium. A 2026 benchmark of four unsupervised transfer learning methods on Raman data found two of them useful where differences between the source and the target dominate, and found no benefit where the variability inside one domain is large [26]. The third is a feed-forward taken from a model rather than from the last interval. Regression of nutrient

consumption on viable cell density held several amino acids and glucose at their targets in fed-batch and perfusion cultures [27], which is the model-based feed of Section 8.5 in a plant.

8.8 Limitations

The broth is one well-mixed volume with unstructured kinetics, a declared switch and a shift that acts at once, and every constant is a teaching value, so no number here predicts a cell line, a titer or a harvest day. The real values come from a line's own characterization and from measured responses to temperature, carbon dioxide and osmolality [3, 10, 12, 15]. The pH buffer is bicarbonate alone, the gas phase has no head and no foam, and the capacitance probe is a cube law on one mean diameter. The 1 to 6 g/L band, the 400 mOsm/kg comfort line, the harvest limits of 70 and 65 % and the challenge's targets are teaching values, while a plant takes its limits from its own process characterization and its regulatory filing. Product quality is not modeled, and the scorecard credits titer and constraints rather than the glycans and impurities a purification has to remove.

> ⚠ **OPEN CHALLENGE** **Telling How Many Cells From How Large**
>
> A capacitance probe answers with one number that mixes how many cells there are with how large they are, which is why this chapter's probe reads high as the cells swell. A sensitivity study of such probes on cell cultures found the permittivity increment sensitive to both cell size and concentration, so it suits biovolume while the cells grow, and found the single population assumption behind the usual reading invalid once a culture enters its stationary and death phases, where cells of another size and other dielectric properties appear. Membrane permittivity and internal conductivity cannot be recovered from the measured spectrum at all [18]. Progress would be a reading that separates count from size late in a culture.

The control scope is four loops on one shared law, four feeding strategies and a rule-based supervisor. The model-based feed is a feed-forward on a platform yield relation, and the MPC is a receding-horizon optimization of the kind that has been applied to glucose control [17], but it optimizes only the feed profile, and nothing in the application optimizes the shift day or the harvest. The estimator corrects the probe and the glucose from the samples but does not estimate the switch, the cell size or the true stock. The off-gas rates are logged and unused, although an on-line oxygen uptake carried the feeding of a hybridoma fed-batch as early as 1995 [4].

The MPC's model carries the probe's growth rate forward with no decline of its own, so its day-7 titer is a quarter high, and it omits starvation, the amino acids, pH, DO, carbon dioxide and cell size. Its far horizon is rough, its estimator is optimistic after the review, and its objective prices neither product quality nor medium nor time in the vessel.

8.9 Conclusion

This chapter studied a CHO fed-batch for a monoclonal antibody as a simulator in which the culture's appetite, its one sparge and its osmolality are logged beside everything its controller reads. Fed by hand, the culture runs out of glucose on day 4.67 and makes 3.28 g/L. Fed a daily bolus to 4 g/L it spends 34.8 h outside the band, and held warm until day 8 it eats up to 5.93 g/L in a day, wider than the 5 g/L band, so no bolus can hold it. Fed continuously on the capacitance probe it overfeeds the cooled culture to 6.09 g/L and makes 3.43 g/L, while the Raman trim holds 1.74 to 2.55 g/L, brings the switch on day 6.32 and makes 5.03 g/L. A target of 4.5 g/L prevents the switch and reaches 519 mOsm/kg, gas before oxygen saves base and makes 5.28 g/L against 5.04, and a probe drifting 0.02 a day leaves the broth at 7.280. The challenge improves from 4.25 g/L at 430 mOsm/kg to 5.21 g/L at 381 mOsm/kg. A model predictive controller planning to the harvest keeps 5.01 g/L when the Raman is lost, against 3.12 for the continuous feed, but a model-based feed on the same estimate makes 5.06, so the plan's own measurable gain is the earliest switch of the shipped structures and half their pump travel.

The kinetics are teaching values in one well-mixed broth. A fuller model would add a structured switch, a compartment model of mixing, foam, product quality and an optimized shift day and harvest. The cases show that the feed of a growing culture is decided by what the controller can measure, and when.

KEY POINTS

- A culture that doubles its cells doubles its appetite, so a feed sized from this morning's sample is already too small tomorrow.
- Once the glucose the cells take between two reports passes the 5 g/L width of the band, no bolus target can hold the band.
- A feed-forward on the uptake measured before a temperature shift overfeeds the cooled culture to 6.09 g/L, where an hourly direct reading holds 1.74 to 2.55 g/L.
- Every correction leaves something behind: a gram per liter of lactate held at the pH setpoint costs 22 mOsm/kg, and the sodium never leaves the vessel.

Where else this applies. A feed-forward on an estimate that a day-old laboratory result corrects returns in Chapter 10 and Volume 5, Chapter 10, and a band held on a sampled analyzer in Volume 5, Chapter 4.

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8.10 Exercises

8.1 ○ Concept check · 2 min

A culture is fed by a daily bolus that brings the glucose of each morning sample up to a target when its result arrives four hours after the draw. The glucose must stay between 1 and 6 g/L. When can no choice of bolus target keep the glucose inside that band?

(a) When the glucose of a sample is already below the band's floor of 1 g/L at its draw

(b) When the stock pump cannot deliver a full bolus within the four hours of the analysis

(c) When the glucose the cells take between two reports is more than the band's width of 5 g/L

(d) When a sample's result lies more than 5 g/L below the target the bolus restores

8.2 ○ Concept check · 2 min

In case 3 the glucose is fed continuously on the capacitance probe and on the per-cell uptake measured between the last two samples, with no Raman. The culture is cooled on day 5, and by day 6.17 its true glucose has climbed to 6.09 g/L while the controller's estimate reads its 2 g/L target. What causes the climb?

(a) The capacitance probe stops responding once the cells are cooled, so the feed's cell count freezes

(b) The colder jacket slows the glucose pump, so the stock delivered falls short of what is commanded

(c) The PI trim on the glucose estimate raises the pump, because the estimate falls below its target

(d) The feed keeps the uptake measured before the shift while the cooled cells take less than half of it

8.3 Concept check · 2 min

The agitator of the culture runs at its full 140 rpm in the 1300 L of inoculation, and the bubbles leave the broth 87.5 % saturated with carbon dioxide. Which actuator of the DO sequence can still raise the carbon dioxide the sparge removes?

(a) The gas flow, because the removal is bounded by the moles of gas that pass through the broth

(b) The oxygen enrichment, because a richer gas leaves more room in each bubble for carbon dioxide

(c) The agitator, because a higher transfer coefficient brings the bubbles still closer to saturation

(d) None of them, because the pH loop sets the carbon dioxide removed through its own leg

8.4 Theory and calculation · 6 min

Fed continuously so that it eats what it wants, the default culture takes 0.53, 1.02, 1.83, 2.97 and 4.27 g/L of glucose in its first five days. Kept at 37 °C past the planned shift, it takes 5.24, 5.84 and 5.93 g/L in days 5 to 6, 6 to 7 and 7 to 8. The band is 1 to 6 g/L. Use the values as given, to two decimals.

(a) Compute the ratio of each of the first five days' uptake to the day before, and state the range as a percentage increase per day. *Checkpoint:* 1.44 to 1.92, so 44 to 92 % more each day.

(b) Apply the band condition of Equation (8.15) to the three warm days. By how much does each exceed the band's width? *Checkpoint:* By 0.24, 0.84 and 0.93 g/L: all three fail.

(c) Had the warm culture kept the smallest ratio of part (a) after day 5, what would it take in day 5 to 6? Compare with the measured value and name the terms of Equation (8.3) that explain the difference. *Checkpoint:* 6.14 g/L against 5.24 g/L measured.

8.5 Theory and calculation · 6 min

Lactate (90 g/mol) made by the cells spends bicarbonate, and the pH loop restores it with sodium carbonate while base is its active leg.

(a) Each millimole of lactate held at the pH setpoint adds itself and one sodium to the osmolality. Taking 11.1 mM per gram per liter of lactate, find the osmolality cost of 1 g/L of lactate. *Checkpoint:* About 22 mOsm/kg.

(b) Over a whole culture the base is half the lactate made, less half the ammonium made, plus half the change in the broth's bicarbonate. The default culture makes 49.3 mol of lactate net and 21.6 mol of ammonium, and its bicarbonate grows from 23.4 to 50.9 mol. Find the base. *Checkpoint:* 27.6 mol.

(c) Case 7, held at a 4.5 g/L glucose target, makes 178.1 mol of lactate and 12.6 mol of ammonium and ends with 11.2 mol of bicarbonate from the same start. Find its base, and say why the osmolality it causes stays after the cells take lactate back. *Checkpoint:* About 76.7 mol.

8.6 Theory and calculation · 7 min

Use Equations (8.9) and (8.10) with $k_L a_{ref} = 8\,\mathrm{h}^{-1}$, $\alpha_N = 1.8$, $\beta_Q = 0.5$, $k_{CO_2} = 0.89$, $\alpha = 0.0307\,\mathrm{mM/mmHg}$, $P = 760\,\mathrm{mmHg}$ and the 1300 L of inoculation.

(a) Compute the sparge k_La at 40 rpm and 15 L/min, and at 140 rpm and 15 L/min. *Checkpoint:* 0.325 and 3.10 h^{-1}.

(b) At 140 rpm and 15 L/min, compute $\dot{n}_g$, NTU and ε. *Checkpoint:* 0.669 mol/min, 2.08 and 0.875.

(c) Compute the coefficient $60\,000\,\dot{n}_g$ of the removal bound at 15 and 100 L/min, and state what it implies for the DO sequence. *Checkpoint:* About 40 200 and 268 000 mmol/h, a ratio of 6.67.

8.7 App challenge · 8 min

Open scenario 2, Daily bolus from the offline sample, at the default seed. Set the speed to 48 h/s, so a fourteen-day culture takes about 7 s. Each run stops by itself at the harvest. Read the tiles Glucose after day 2 (true), Titer at harvest (true) and Highest osmolality (true). Give hours within 0.5 h, titers within 0.02 g/L and osmolality within 2 mOsm/kg.

(a) Run as shipped, with the Glucose target at 4 g/L. *Checkpoint:* 34.8 h out, 4.14 g/L, 435 mOsm/kg.

(b) Press the reload button (Ⓡ) to restore the scenario, set the Glucose target on the FIC-201 card to 6.0 g/L before Run, and run to the harvest. *Checkpoint:* 0.7 h out, 2.73 g/L, 511 mOsm/kg.

(c) Explain why the higher target nearly clears the glucose tile and still loses a third of the antibody. *Checkpoint:* The glucose stays above the 2.5 g/L switch threshold.

8.8 App challenge · 8 min

Open scenario 4, The Raman trim, at the default seed and 48 h/s. For each run read the tiles Lactate switch (true), Titer at harvest (true), Base added and Highest osmolality (true). Give titers within 0.02 g/L, the switch day within 0.02, the base within 0.2 mol and osmolality within 2 mOsm/kg.

(a) Run as shipped, at the 2.0 g/L Glucose target, to the harvest. *Checkpoint:* Switch on day 6.32, 5.03 g/L, 27.6 mol, 393 mOsm/kg.

(b) Reload the scenario, set the Glucose target to 1.5 g/L before Run, and run to the harvest. *Checkpoint:* Day 5.10, 5.07 g/L, 26.7 mol, 390 mOsm/kg.

(c) Reload, set the Glucose target to 2.5 g/L, and run to the harvest. *Checkpoint:* No switch, 3.66 g/L, 55.9 mol, 467 mOsm/kg.

8.9 App challenge · 7 min

Open scenario 6, The temperature shift, early and late, at the default seed and 48 h/s. Read the tiles Titer at harvest (true), Viability at harvest (true) and Base added after each run. Give titers within 0.02 g/L, viability within 0.2 % and base within 0.2 mol.

(a) Run as shipped, with the Shift day on the Supervisor card at 5. *Checkpoint:* 5.03 g/L, 80.9 %, 27.6 mol.

(b) Reload, set the Shift day to 3 before Run, and run to the harvest. Then reload, set it to 8, and run to the harvest. *Checkpoint:* 4.33 g/L, 87.4 %, 3.1 mol; then 3.57 g/L, 68.2 %, 45.6 mol.

(c) Explain why the optimum lies in the middle, using the base and the viability you read. *Checkpoint:* Too few cells early, lactate, base and dying cells late.

8.10 Application area · 4 min

Case 9 harvests when a daily viability sample, drawn once a day and reported four hours later, reads below 65 %. The culture is 70.2 % viable when the day-17 sample is drawn and is harvested on day 18.17 at 54.8 % true viability with 7.40 g/L. A 75 % limit harvests on day 17.17 at 67.7 %. Why does each rule harvest well below its own limit, why does a plant care, and what would you change in the harvest rule?

8.11 Application area · 4 min

Case 5, DO and pH through one sparge, ships with the DO sequence set to Gas before O_2: agitation, then sparge flow, then oxygen enrichment. Against the order O_2 before gas, its culture reaches a highest osmolality of 382 against 393 mOsm/kg, uses 24.0 against 27.7 mol of base and makes 5.28 against 5.04 g/L (Table 8.5). Explain why the order of the DO sequence changes the osmolality. Then name two costs a plant pays for a higher sparge flow that this model leaves out, and one cost of the other order.

8.12 Review the agent · 5 min

An AI assistant reviews the trends of scenario 8, A pH probe drift seen only offline, run as shipped at the default seed. It reports: “The pH loop held its measurement at 7.00 for all fourteen days, and the culture used 27.2 mol of base against 27.6 mol for the reference culture of case 4, so the pH control is healthy. The titer of 4.50 g/L against 5.03 g/L and the cell peak of 12.22 against 14.78 million cells/mL therefore point to the seed or the medium. The next batch should start from a new vial.” Find the flaw in the reasoning, say what in the app shows it, and say how you would confirm the cause.