Bioreactor Monitoring & Control

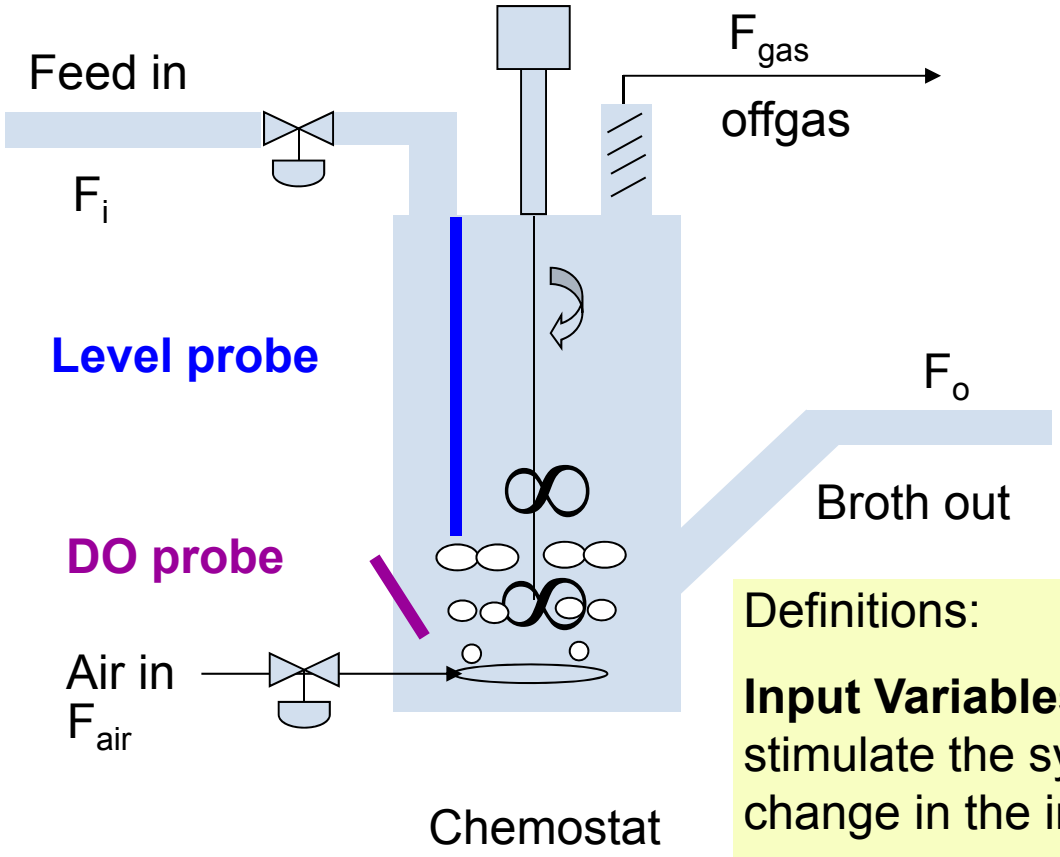
Bioreactor Monitoring & Control

- Basic principles of process control
- Fermentation monitoring
  - Dissolved oxygen
  - pH
  - Temperature
  - Offgas monitoring
  - Substrate (glucose)

Useful references

- Ogunnaike, B.A., and Ray, W.H., **Process Dynamics, Modeling, and Control**, Oxford University Press, 1994.
- Vogel, H.C., Todaro, C.L., eds., **Fermentation & Biochemical Engineering Handbook 2nd Ed**., Elsevier 1996.
- Shuler, M.L. and Kargi, F., **Bioprocess Engineering: Basic Concepts 2nd Ed**., Prentice Hall, 2001.
- Van Impe, J.F.M., Vanrollegham P.A.,and Iserentant, D.I., eds., Advanced Instrumentation, Data Interpretation, and Control of Biotechnological Processes, Kluwer Academic Publishers, 1998.

Process Control Basics: The concept of process control



Presentation of problem:

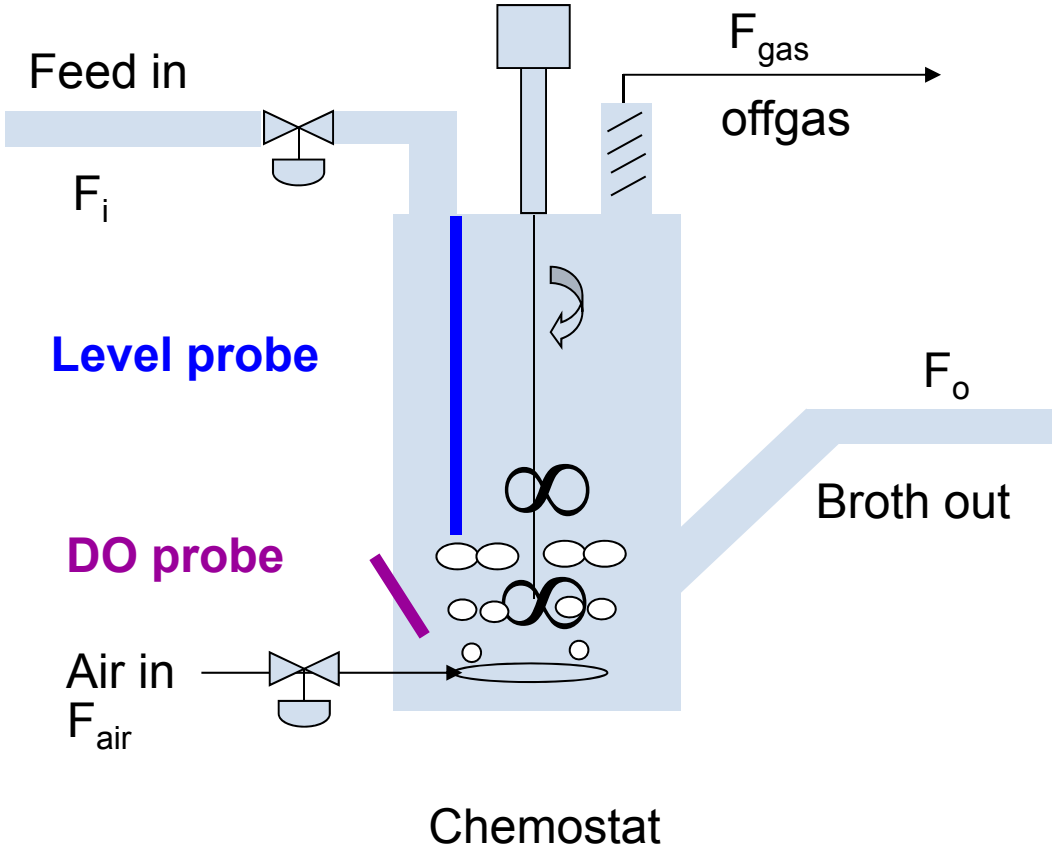
1. Control level of liquid
2. Control concentration of O_2 in fermentor

Definitions:

Input Variables are those that independently stimulate the system and can thereby induce change in the internal conditions of the process

Output Variables are those by which one obtains information about the internal state of the system

Process Control Basics: The concept of process control



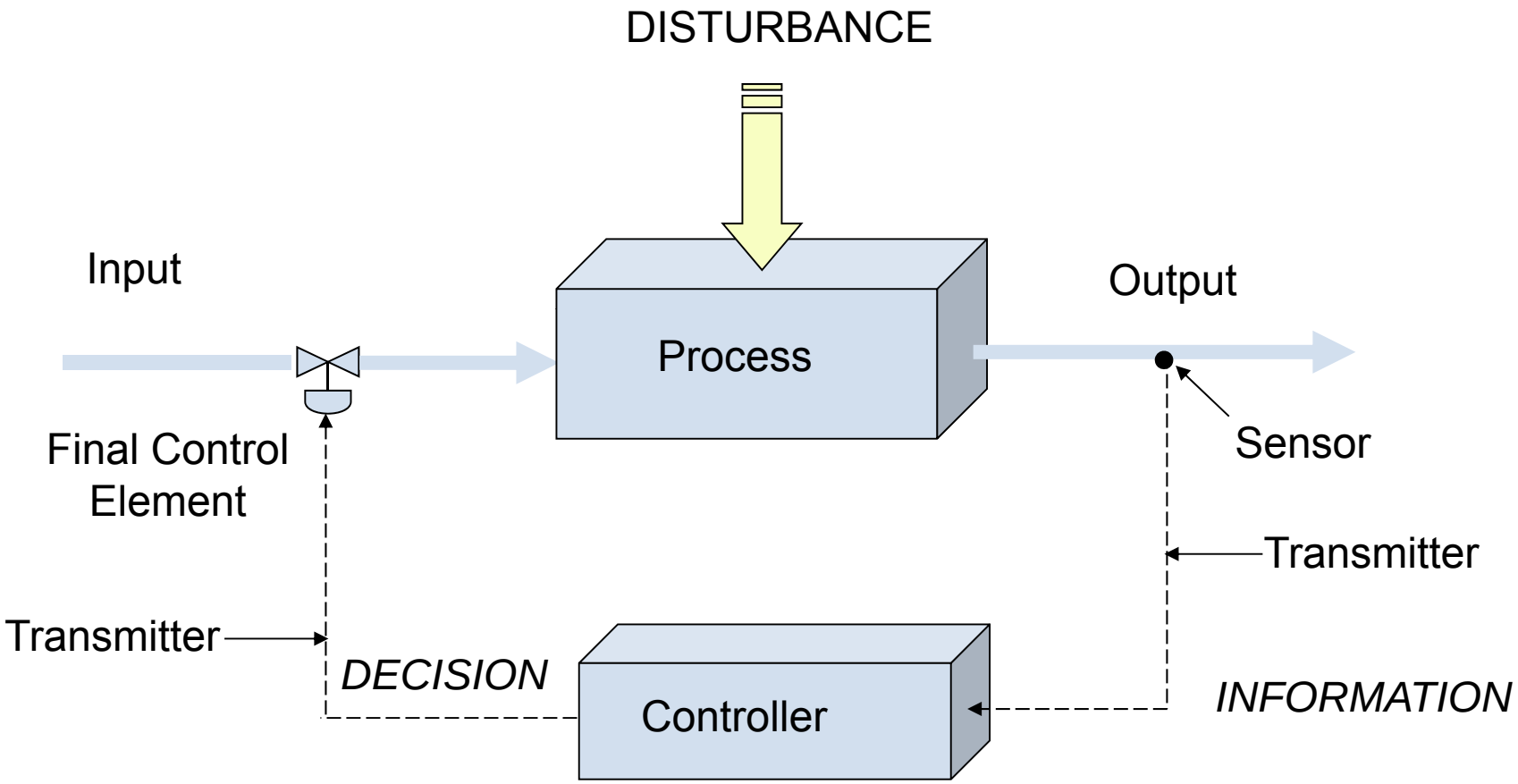
Two types of input variables

Those we can *manipulate* are control variables

Those we *cannot control* are disturbance variables

We can separately identify variables that we can measure.

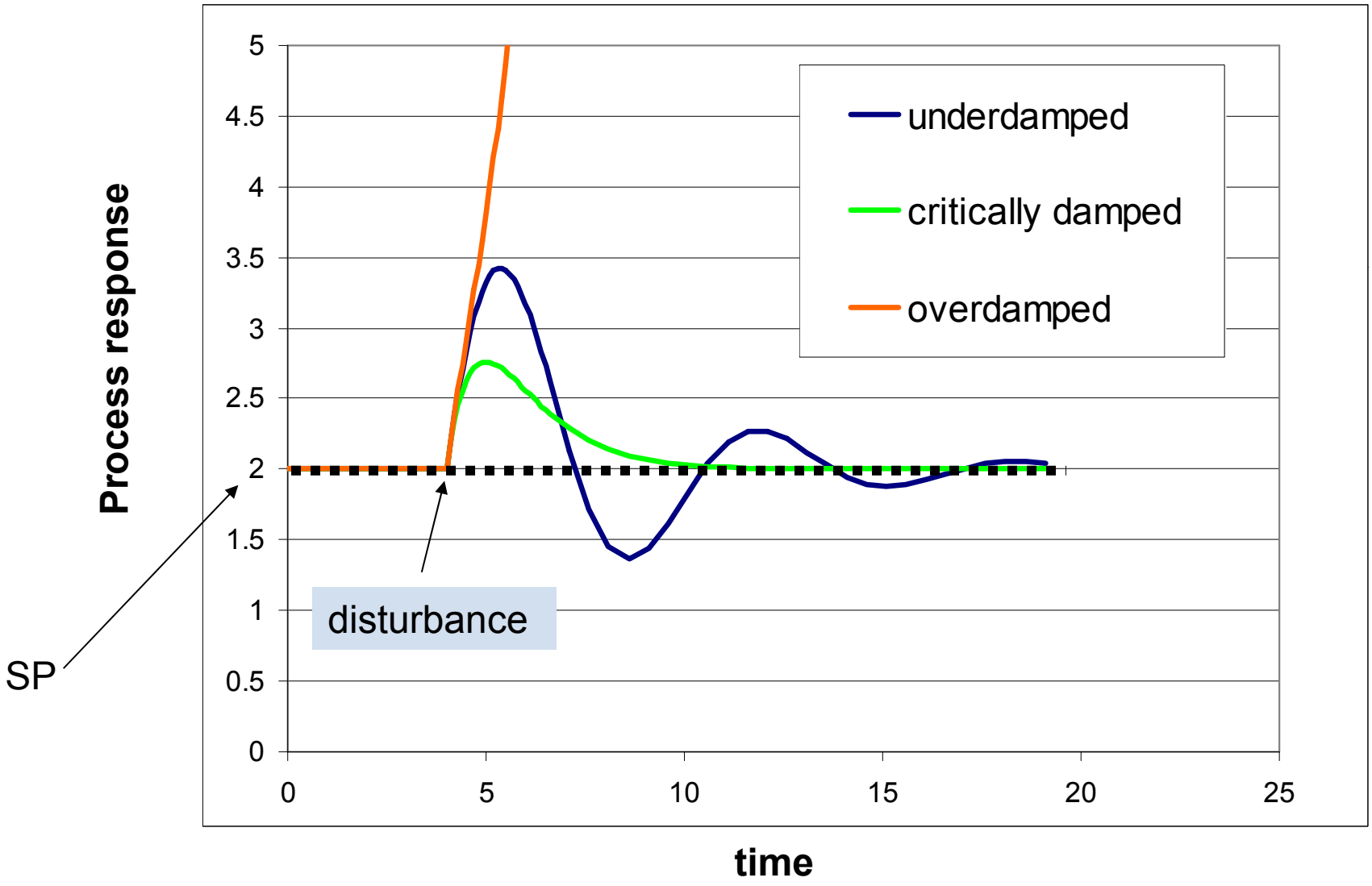
Feedback Control Configuration



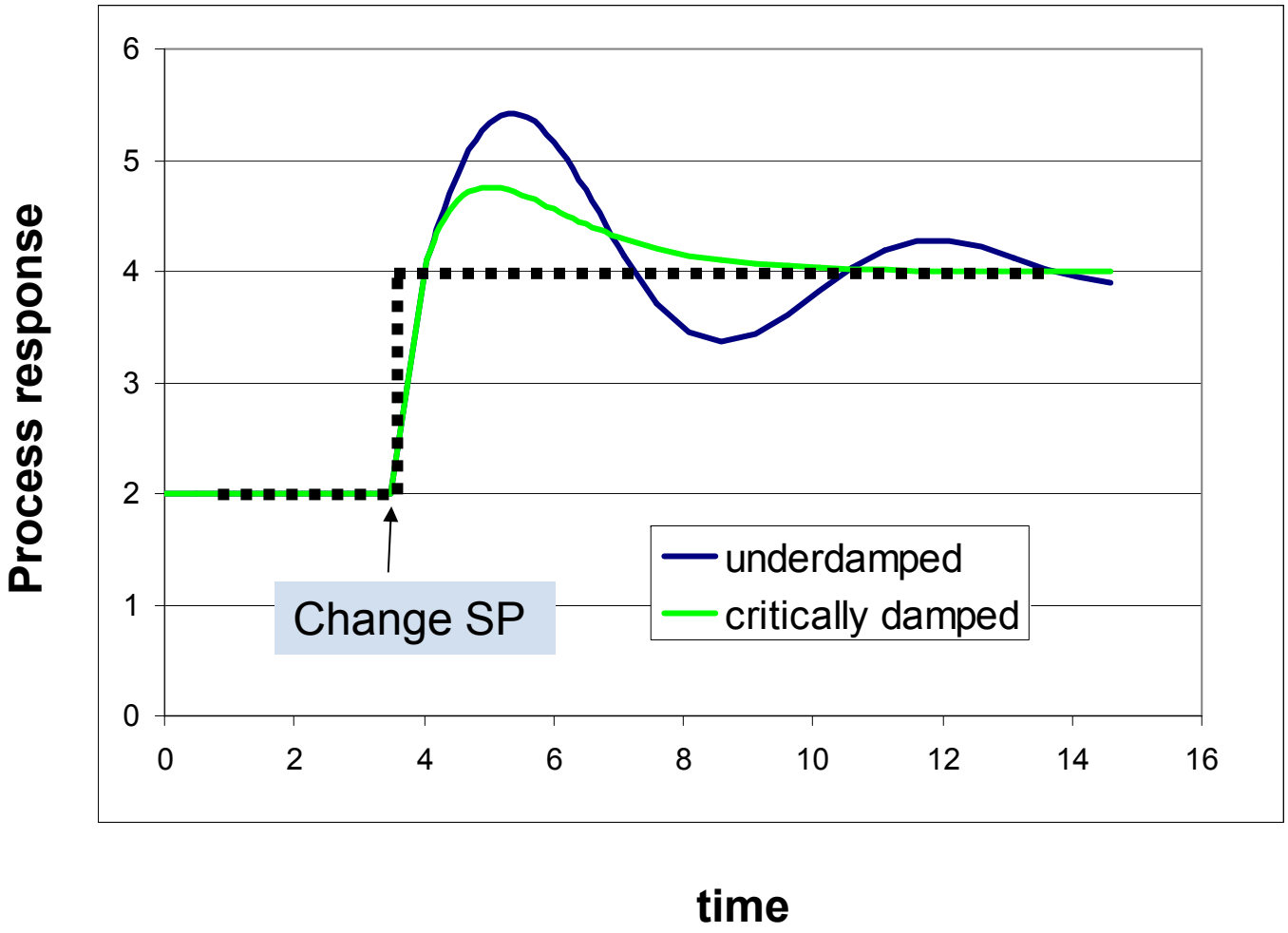
Control *action* is taken based on *information* that there is a process *upset*

Upset occurs when the process output variable deviates from the *setpoint.*

Feedback Control: Regulatory Control

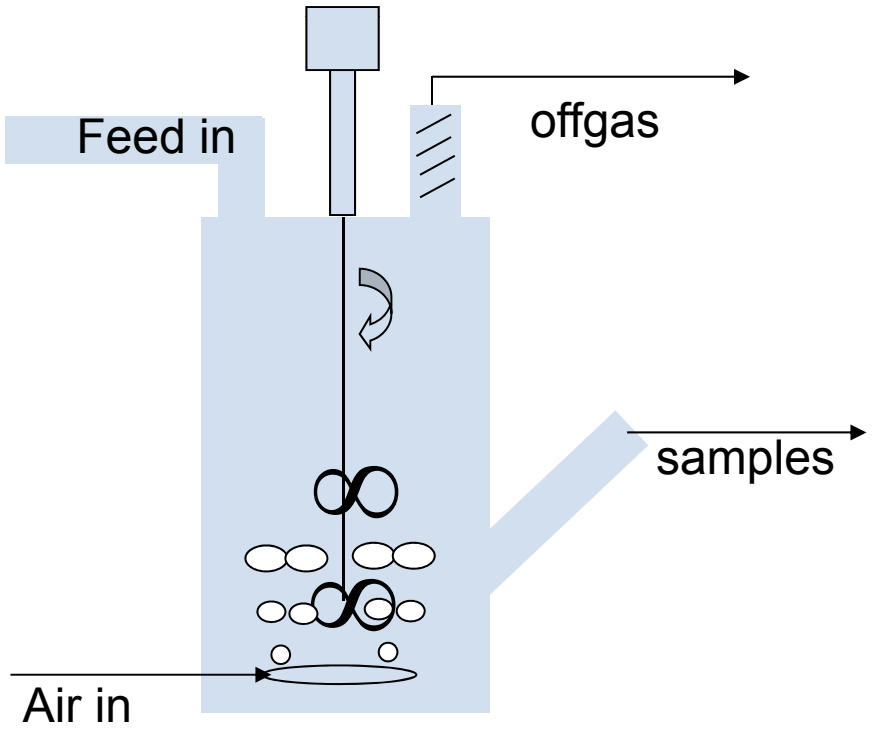


Feedback Control: Regulatory Control



Standard Feedback Controllers

- Proportional Controller (P)
- Proportional + Integral Controller (PI)
- Proportional + Integral + Derivative Controller (PID)



Bioreactor systems generally come set up so that you don't need to know which type of control strategy has been employed – they just do it…

Standard:

- temperature
- pH
- dissolved oxygen

Process Monitoring: Hardware Sensors

Hardware sensors

- Dissolved oxygen probe
- pH probe
- Temperature probe
- Offgas monitoring (IR + flow sensor; mass spec)
- ~~Dissolved CO_2 probe~~
- ~~Substrate (glucose) flow sensor~~
- Fermentor volume (via load cell)

Bioreactor Control: Nonlinear Process

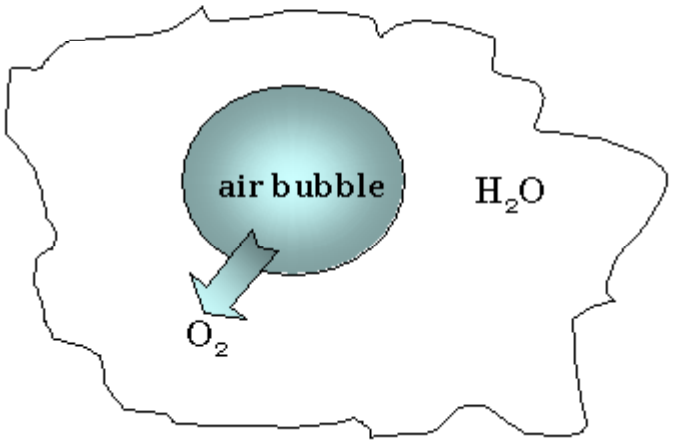
- PI or PID control strategies can be used for simply managing process variables such as temperature and pressure.
- pH is somewhat more sophisticated to avoid oscillation and offset
- DO is managed by *cascade control*
- Critical variables, such as nutrient feed rate(s) may not have simple hardware sensors available (glucose monitoring)

We will begin with oxygen

- Oxygen is needed for respiration for aerobic cultures.
- Strict aerobes will die without sufficient O_2
- Facultative aerobes will not die in the absence of O_2 but will produce byproducts due to the need to transfer electrons to metabolites when O_2 is not present to do the job.
- Oxygen is *monitored* by a dissolved oxygen probe
- Oxygen is fed as a gas but can only be utilized in the liquid phase

The E. coli we use in the 194 lab is a facultative aerobe and therefore grows best with oxygen but can grow also without it. In the absence of O_2, however, it will produce byproducts that inhibit the growth. Thus, we aim to avoid an oxygen-limited situation in the process.

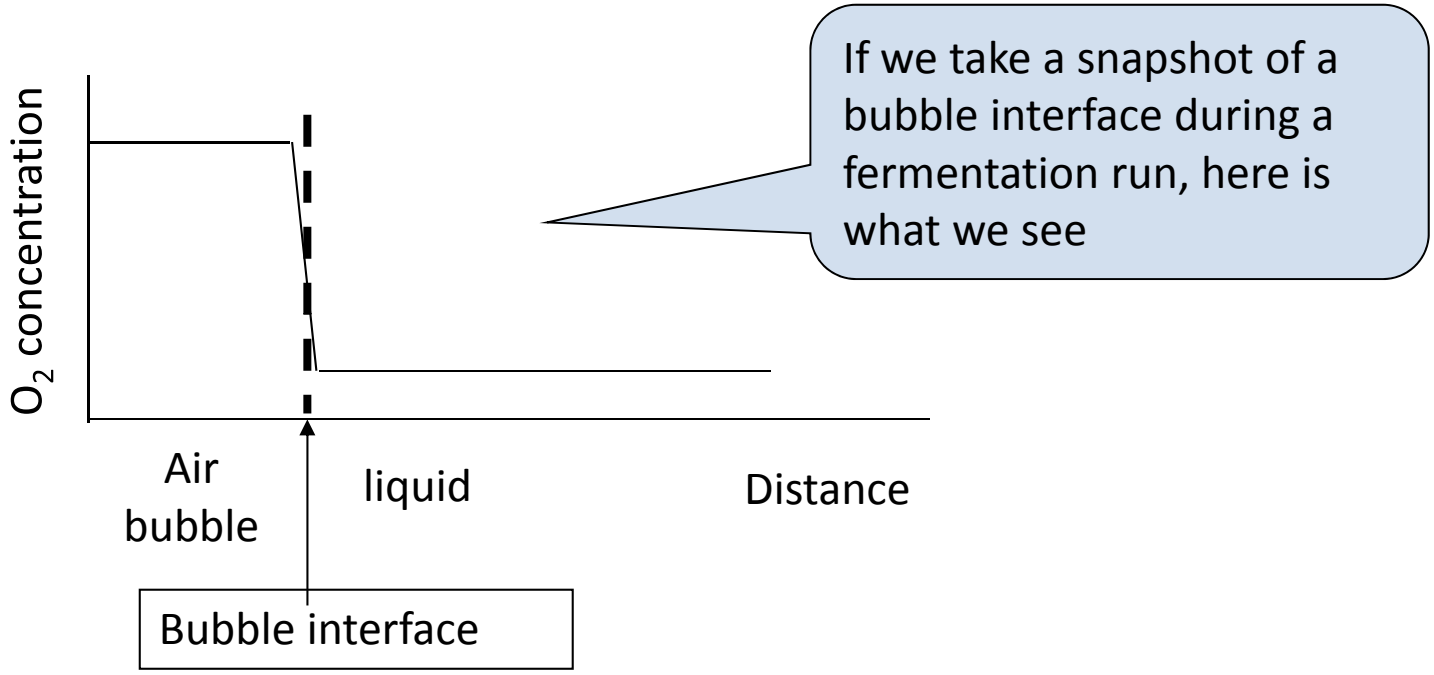
Challenges with DO control



- Oxygen will dissolve in water up to $[DO]_{sat'd}$ ~ 7 ppm (= 7 mg/L) at 30ºC.
- This concentration is sufficient for bugs to grow, what is limiting is the *rate* of transfer from the air bubble to the liquid.

T ^{0}C	x (mol O_2/mol H_2O)	[O_2] mg O_2/l H_2O
10	0.0000064	11.377
20	0.0000052	9.277
30	0.0000044	7.832
40	0.0000039	6.954
50	0.0000036	6.327

Oxygen transfer engineering style



Assumptions: air bubble and liquid are two well mixed systems

Oxygen transfer - engineering style

The oxygen concentrations vary both with distance and with time.

The actual mechanism of oxygen transfer is complex, so engineers use an empirical method to describe the process.

$$\text{Oxygen transfer rate (OTR)} = k_L a(C^* - C_L)$$

- C^* is the saturation concentration of O_2 in the liquid
- C_L is the actual liquid concentration (measured by DO probe)
- "$k_L a$" is the "Liquid side mass transfer coefficient".

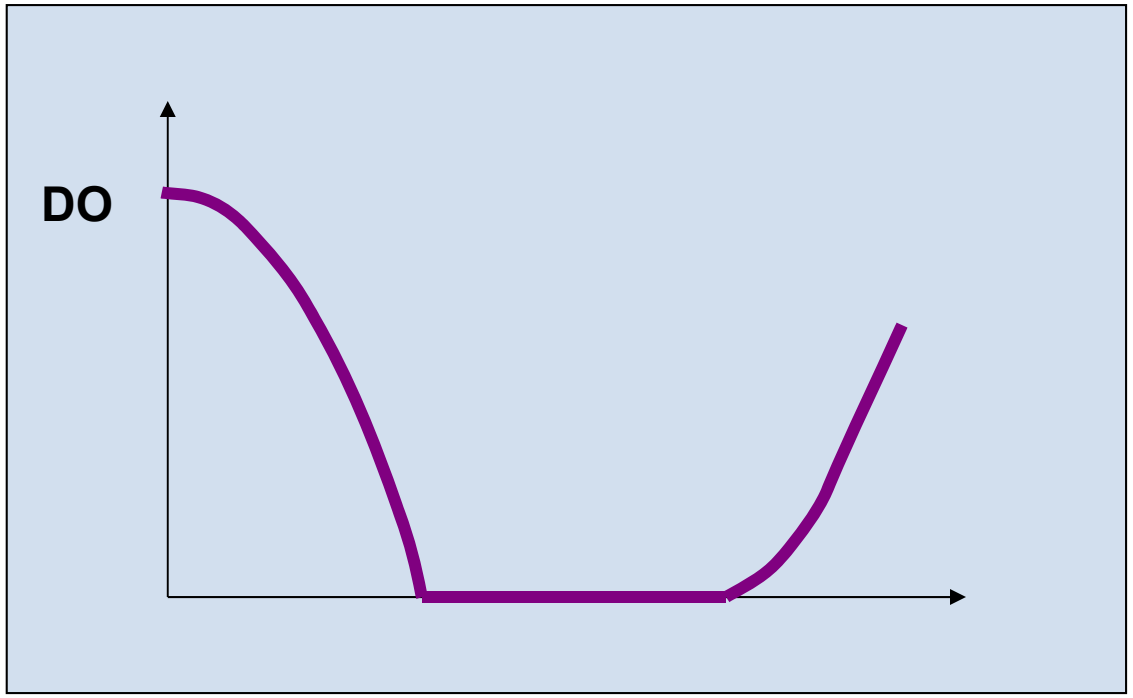
Transferring the O_2 from the bubble interface into the liquid is the rate-limiting step to get the air into the liquid.

Oxygen supply vs. demand

Oxygen *supply* is what is transferred from the bubble into the liquid

Oxygen transfer rate (OTR) = $k_L a(C^*-C_L)$ $\left(\frac{mM}{h}\right)$ or $\left(\frac{mg \cdot O_2}{l \bullet h}\right)$

If we have a bacteria culture that consumes a lot of oxygen, then the measured concentration of O_2 in the liquid can drop to zero.



How can we *increase* the oxygen supply??

Oxygen supply vs. demand

What cells consume - or the *demand*

The rate of O_2 consumption is the ***oxygen uptake rate***.

The units are the same as for OTR => $\left(\frac{mM}{h}\right)$ or $\left(\frac{mg \cdot O_2}{l \bullet h}\right)$

OUR is a *rate* OUR = $\frac{dO_2}{dt}$ mols O_2 consumed/time

if O_2 is the limiting substrate, and the O_2 limitation comes into play, then

OTR = OUR

or *the demand = supply* ==> can the demand be greater than the supply?

The amount of oxygen required by cells depends on their growth rate and on the total number of cells.

Even cells that are not growing need oxygen, and this is called the *maintenance requirement.*

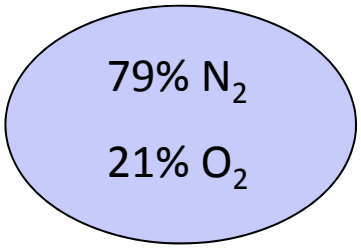
Oxygen supply vs. demand

How can we *increase* supply??

Oxygen transfer rate (OTR) = $k_L a(C^*-C_L)$

We can either increase $k_L a$ or we can increase C*

How can we get oxygen to move across the bubble interface faster?



Air bubble

$$k_L a = 0.1\left(\frac{P(w)}{M}\right)^{0.4} \upsilon_{gs}^{\ 0.6}$$

M is the mass of the fluid.

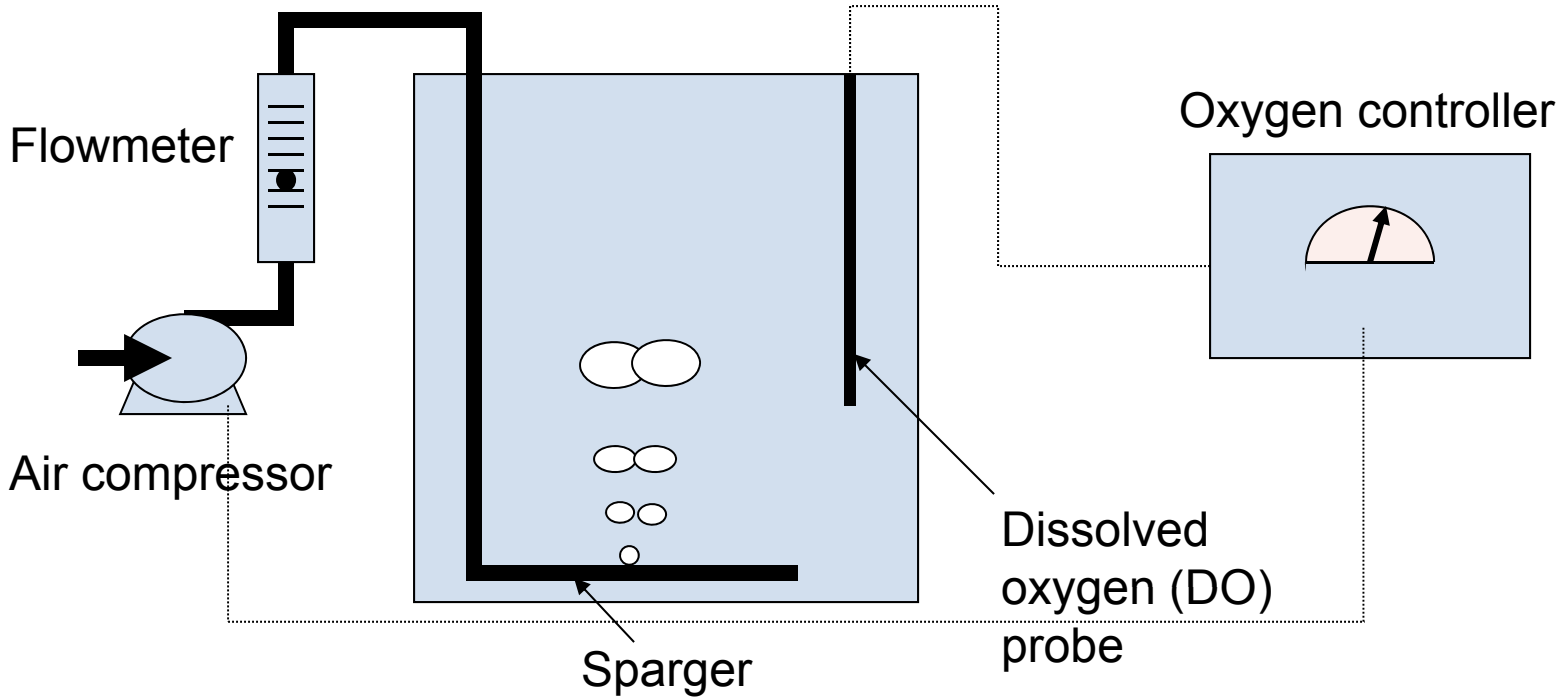
0.1 is experimentally determined (should be determined for each culture broth).

υ_{gs} is superficial gas velocity.

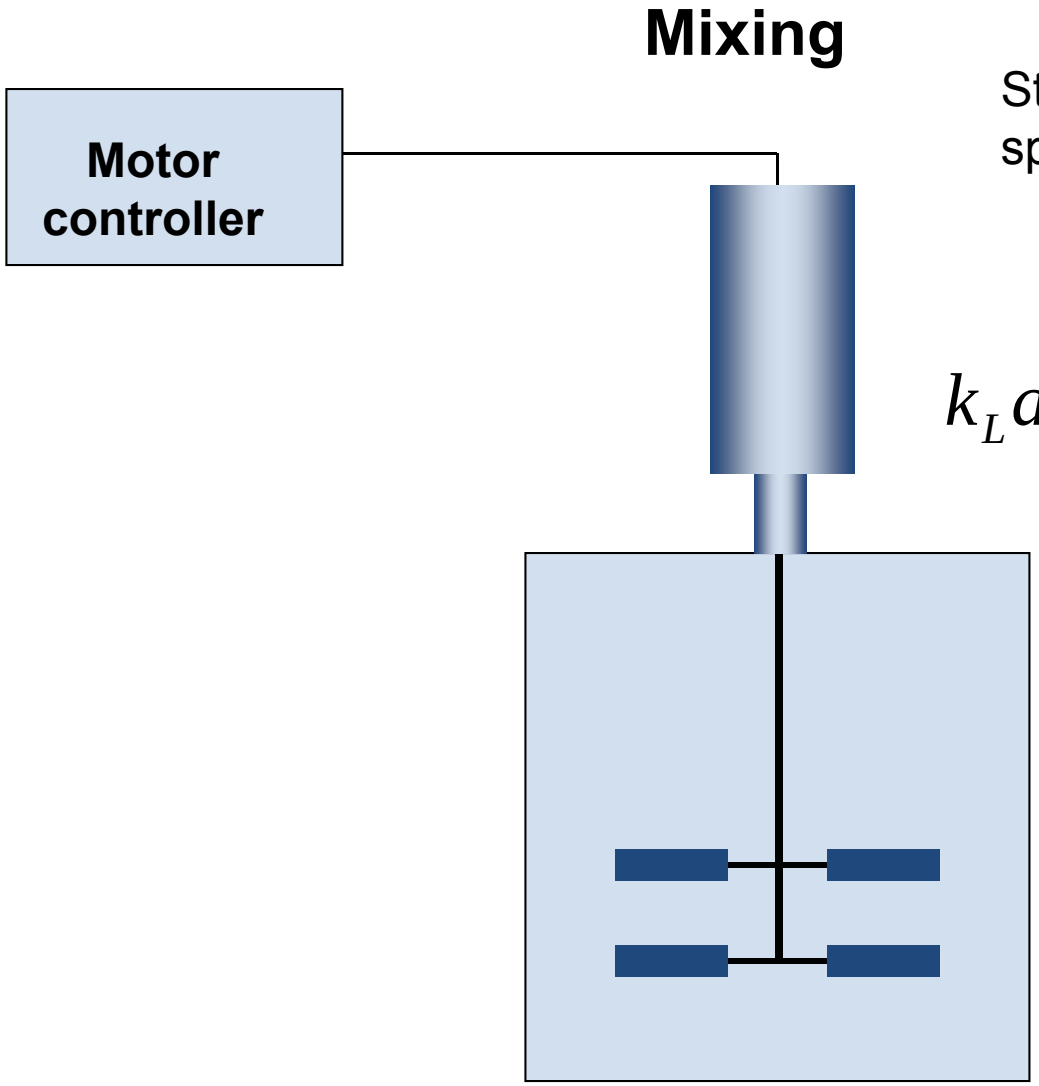
Increase O_2 Supply to Bioreactor

Strategy 1: Increase flow of gas into bioreactor

$$k_L a = 0.1\left(\frac{P(w)}{M}\right)^{0.4} \upsilon_{gs}^{0.6}$$



Increase O_2 Supply to Bioreactor



Strategy 2: Increase mixer speed.

$$k_L a = 0.1\left(\frac{P(w)}{M}\right)^{0.4} \upsilon_{gs}^{0.6}$$

Increase O_2 Supply to Bioreactor

How can we increase C*?

Oxygen transfer rate (OTR) = $k_L a(C^*-C_L)$

C* is the saturation concentration of O_2 in the liquid phase…

We can increase it by increasing the concentration of O_2 in the GAS phase

Feed pure O_2 or a mixture of O_2 + air

If this strategy can be avoided, it should be

- added cost to purchase purified O_2
- safety issues associated with handling pure O_2

Increase O_2 Supply to Bioreactor

DO Control involves a combination of both -- called *Cascade Control*

- increasing stir speed
- increasing air flow rate

- There is a maximum possible stir rate
- Air flow rate cannot be increased indefinitely

> *Flooding* is the phenomena where there is so much air blown into the reactor that you create a pocket of air in the middle of the reactor around the impellers – just beating the air and not the liquid

When flooding occurs, there is a notable drop in the power supply to the mixer.

pH

What is pH

Pure water dissociates to yield 10^{-7} moles/L of H^+ at 25^0C:

H_2O <----> H^+ + OH^-

Since water dissociates to produce one OH^- ion for each H^+ ion, it is obvious that

10^{-7} OH^- ions are produced simultaneously.

The product of [H+] and [OH-] always remains constant. When the value for one of the species changes the other also changes accordingly.

$[H^+] \times [OH^-] = 10^{-14}$

The concentration of H^+ ions can be increased when compounds are added which release H+ ions such as H_2SO_4:

H_2SO_4 <----> $2H^+ + SO_4^{2-}$

Control of pH in Bioreactor

Calculate the concentration of hydrogen ions from the pH

[H+] (molar concentration) = 10^{-pH}

eg for pH = 7.0 and 7.2

[H+] = 10^{-7} molar or 0.0000001

[H+] = $10^{-7.2}$ molar or 0.0000000631

notice that increasing the pH by 0.2 decreases the [H+] by 37%

Control of pH in Bioreactor

Most bacteria can grow over a wide range of pH, although many enzymes upon which microbial growth depends function only within a narrower range of pH.

The bacteria then must maintain their internal pH near a fixed optimal value.

Bacteria (E. coli) that grow at neutral pH (6.0-8.0) are called neutrophiles.

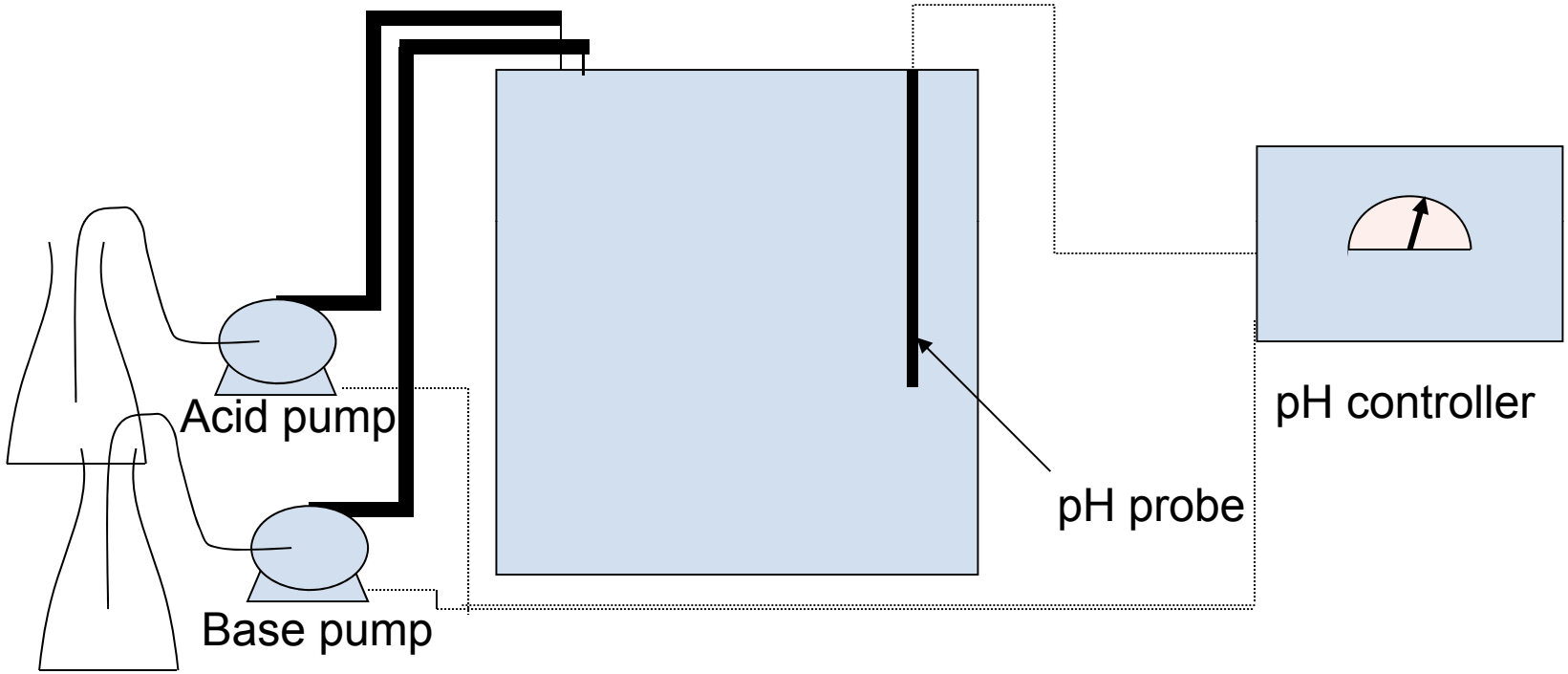
Regardless of the external pH, the internal pH is maintained at ~7.6.

pH is maintained by ion pumps on the membrane of the bacteria.

- Operation of the pumps requires energy input
- Effort put into maintaining the pH will be at the expense of other cellular functions
- Bugs tend to grow more slowly when the pH is not at the optimum.

Any processes that involve interaction with the external medium, such as uptake of nutrients, secretion of proteins, etc. will be directly affected by the external pH.

pH control



What causes the pH to change?

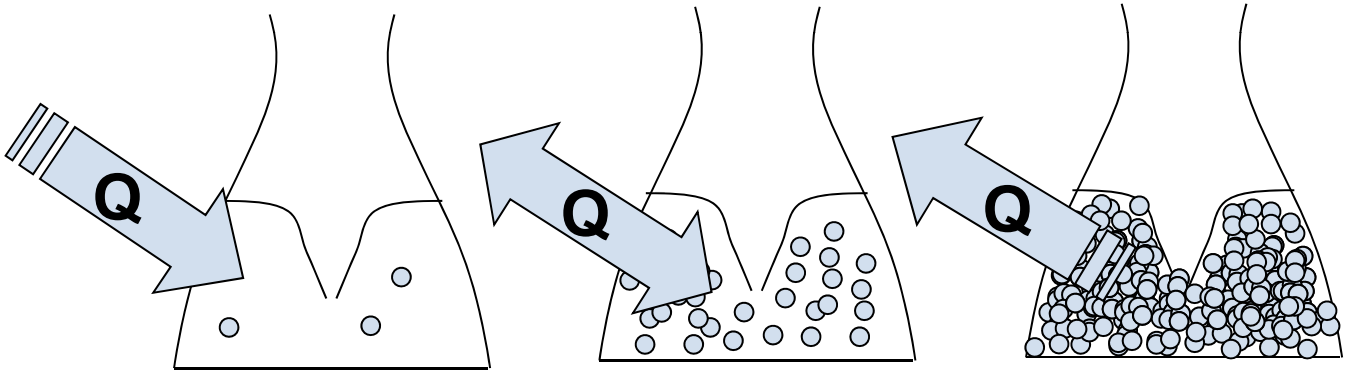
- Overfeeding substrate can cause the cells to produce organic acids, such as acetate – pH drops
- Lack of carbohydrate substrate causes the cells to consume protein in the media – producing NH_3 → NH_4OH – pH rises
- When producing a protein product, cells consume ammonia from the media from the cellular demand for more nitrogen – causes the release of a proton – pH drops.

pH control in CHE 194 process

- We use NH_4OH for base to control the pH.
- We don't need any acid because the process control is designed to avoid overshooting.
- We wait for a pH spike (production of base) and then we turn on the glucose feed pump. A very slow glucose feed is used to enable growth of bacteria and avoid oxygen limitation. (explained later in this presentation)

Temperature Control

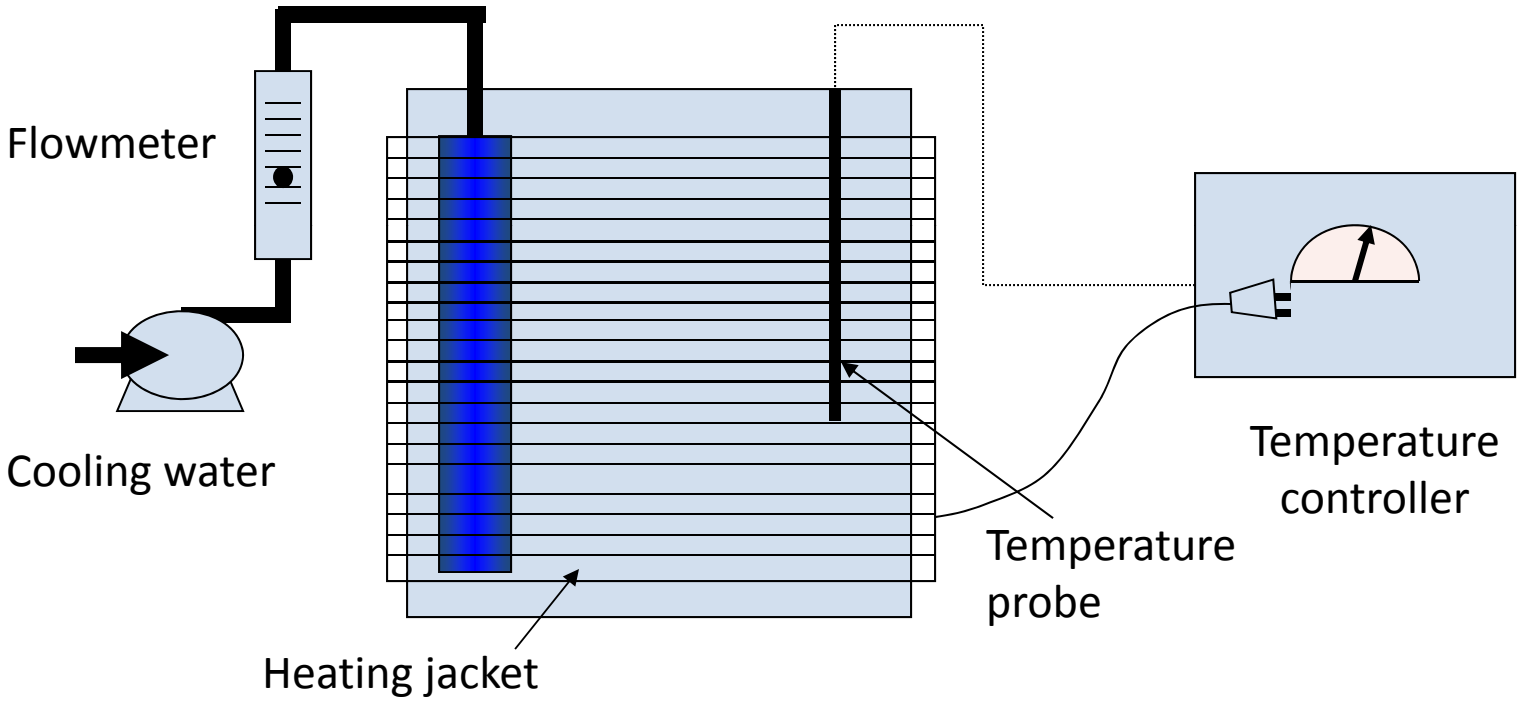
Heat management is another engineering task



The amount of heat (Q) to be added or removed depends on the density of the culture, the volume of the culture, and the growth rate of the culture.

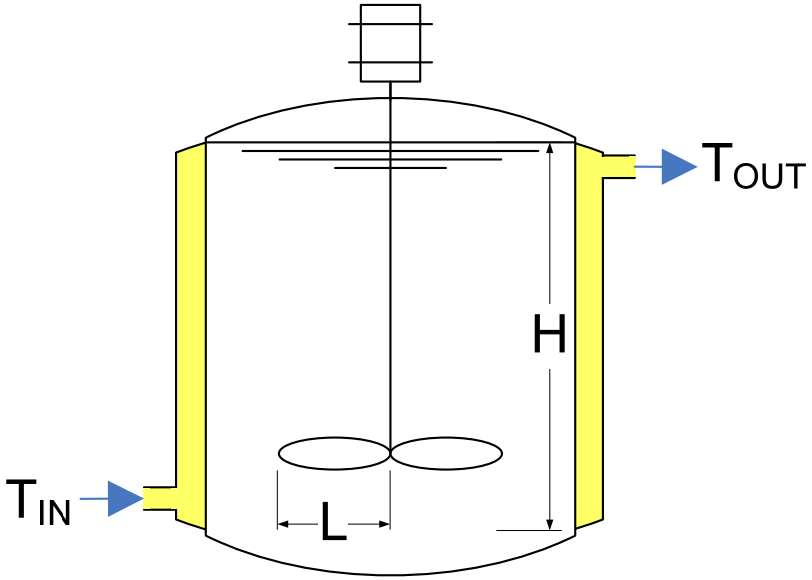
$$Q_{GR} = V_L \mu X Y_H$$

Heat management in a laboratory bioreactor is analogous to that at a large scale



At large scale, heating jacket is replaced with a steam jacket

Heating Jacket on Fermentor



Our (lab 109) heating jacket is electrical, not using hot water or steam. We use it to heat the bioreactor and to help maintain temperature. We use cooling water in a little heat exchanger to cool the system. The combination of heating and cooling inputs carefully maintains the temperature.

Metabolic Heat Generation

40-50% of energy produced by substrate catabolism is converted to ATP, the rest is released as heat.

Glucose + NH_3 + O_2 -------> CO_2 + H_2O + cells

Heat of Combustion (ΔH_C) of cells 20 - 25 kJ/kg

Y_H is the metabolic heat evolved per gram of cell produced

Y_H depends on the degree of oxidation of the substrate

- Y_H ~ 2.4 kcal/g on glucose
- Y_H ~ 5.6 kcal/g on ethanol
- Y_H ~ 8.3 kcal/g on methanol
- Y_H ~ 16.4 kcal/g on CH_4

Total heat evolved (Q_{GR}) depends on

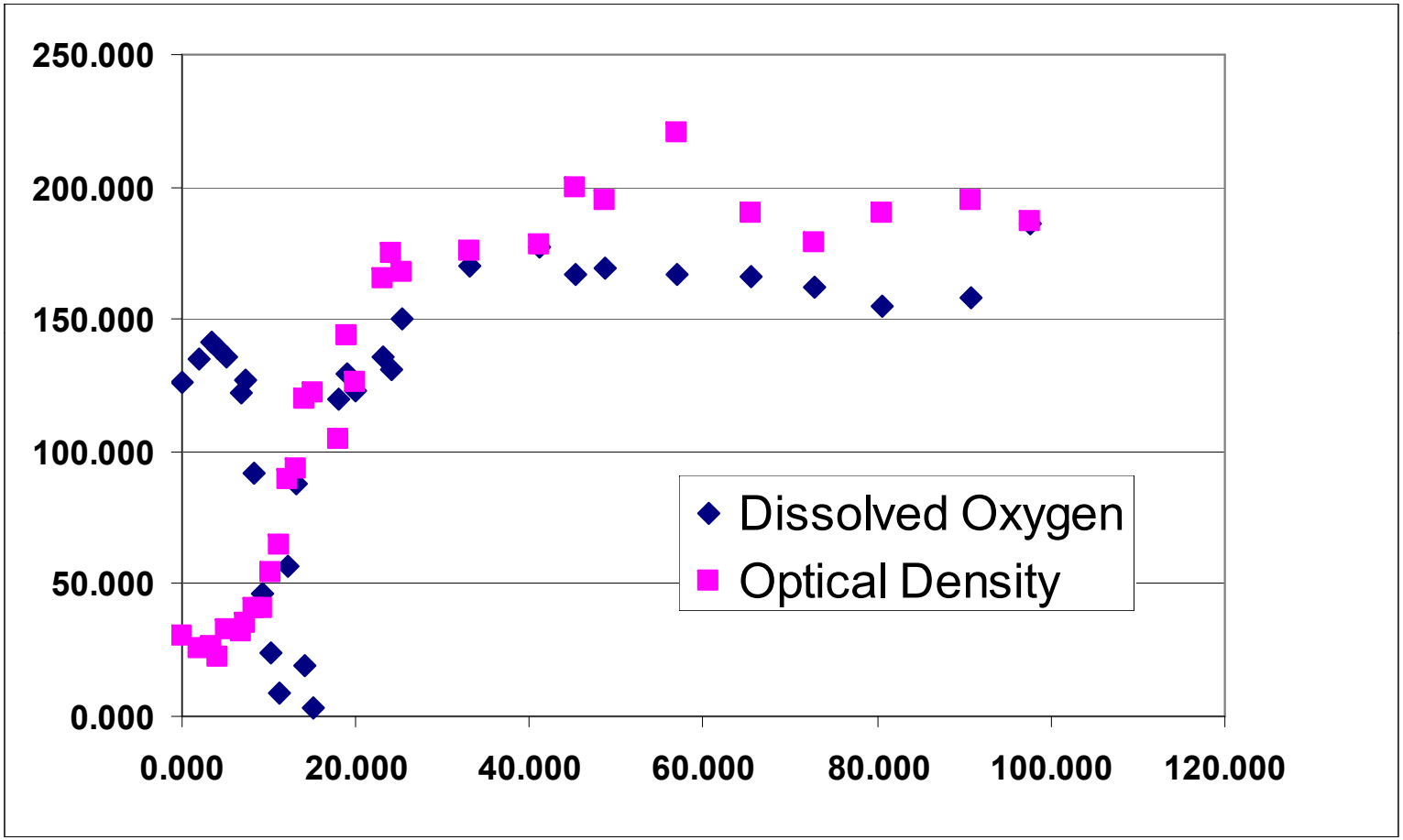
$$Q_{GR} = V_L \mu X Y_H$$ Units kcal/hr

Oxygen uptake rate

For *aerobic* fermentations,

$$Q_{GR} \cong 0.12 Q_{O_2}$$ Units kcal/lit·hr

Where is the heat production the greatest?



$$Q_{GR} = V_L \mu X Y_H$$

Cooling water addition

$$Q = Q_{GR} + Q_S$$

Total heat to be removed by cooling water is due to metabolic heat generation plus heat added from the stirring

$$Q = UA_C\Delta T$$

Heat is removed by running cooling water through the cooling coils in the reactor

U is the overall heat transfer coefficient of the coils

A_C is the total area of the coils in contact with the broth

ΔT is the "log-mean temperature difference" between the broth and the cooling water

$$\Delta T = \frac{\left(T - T_{in}\right) - \left(T - T_{out}\right)}{\ln\left[\left(T - T_{in}\right)/\left(T - T_{out}\right)\right]}$$

Offgas Analysis

Offgas composition is an indicator of culture activity

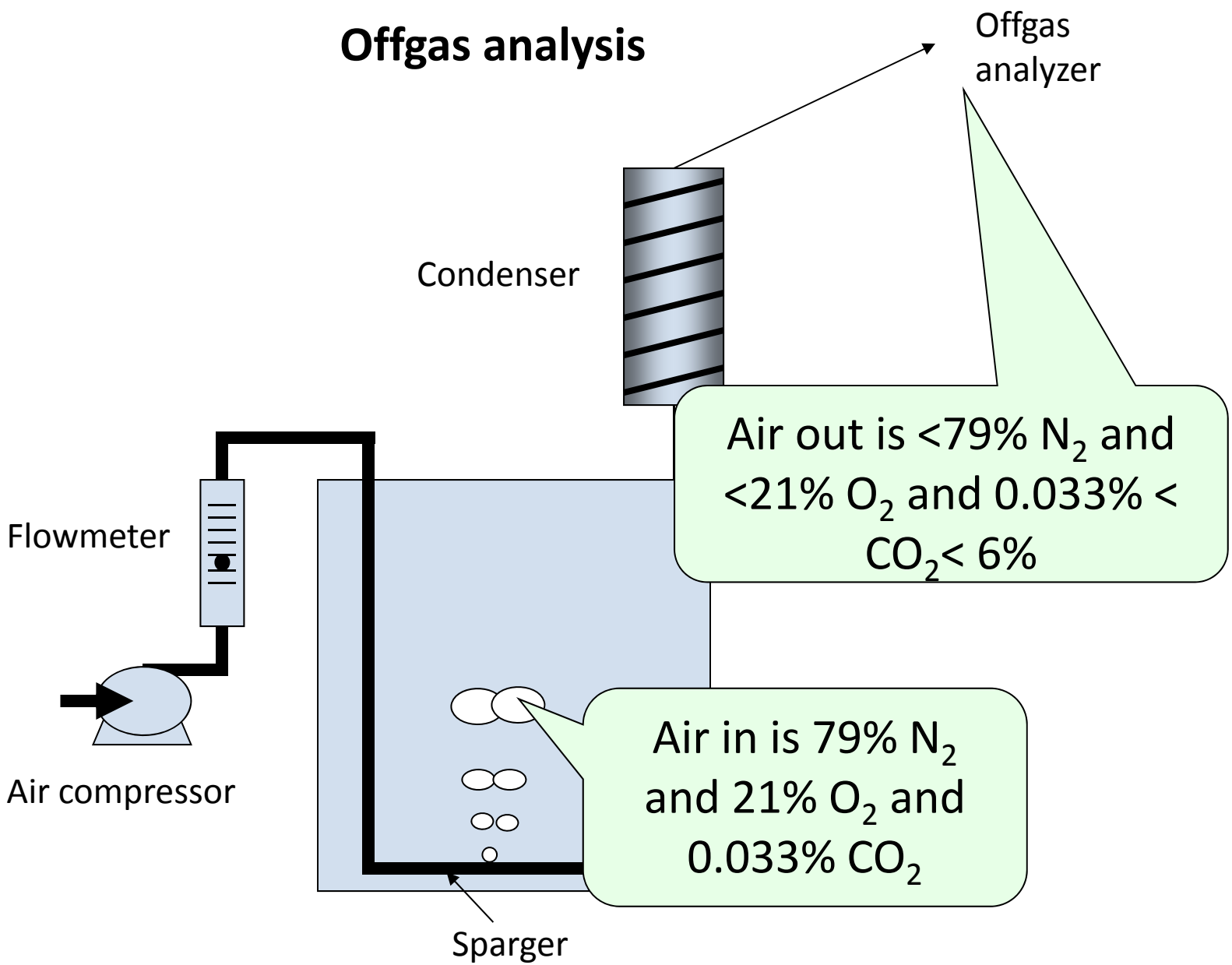
Bug: $\text{glucose} + NH_3 + 2O_2 \longrightarrow C_4H_7O_2N\ (\text{bug}) + 4H_2O + 2CO_2$

Respiration:

$\text{glucose} + 36\ Pi + 36\ ADP + 6\ O_2 \longrightarrow 6\ CO_2 + 6\ H_2O + 36\ ATP$

Based on this simple description of the chemistry, we can see that production of CO_2 is an indicator of living cells. A high rate of CO_2 production coupled to O_2 consumption is an indicator of growth.

Offgas analysis



Offgas composition is an indicator of culture activity

Carbon dioxide **E**volution **R**ate (CER) is the rate of production of CO_2.

$$\frac{CO_{2,out} - CO_{2,in}}{\text{Time interval}}$$ Is the CER

Since we know the concentration of CO_2 in the air is 0.033%, by measuring the $CO_{2,out}$ we can determine CER.

Note that this is measured for the whole culture volume, so we can divide out the culture volume for a more meaningful value.

Offgas composition is an indicator of culture activity

Likewise, $\frac{O_{2,out} - O_{2,in}}{\text{Time interval}}$ Is the OUR

We can measure the CER and OUR on-line using a mass spectrometer.

The mass spectrometer measures concentrations, and we need to know total amounts.

By comparing the O_2 and CO_2 concentrations with the N_2 we can determine what the flow rate out is, since N_2 is neither produced or consumed by the bugs.

Calculation of OUR & CER

- Handout – example problem

Best practice: design your feeding strategy such that O_2 is not the limiting nutrient…

Depending on your product, you may choose Carbon or Nitrogen to be your limiting nutrient, and you can choose different limiting nutrients during different phases of the run.

When the cells are growing, the feed rate can be determined from:

$$F_S = \frac{\mu}{Y_{X/S}} X(t)$$

F_S is the substrate feed rate,

μ is the specific growth rate of the organisms

$Y_{X/S}$ is the yield coefficient (rate of cell production/rate of substrate uptake)

X(t) is the time dependent concentration of organisms in the bioreactor.

Can we estimate the cell growth rate and cell concentration from on-line measurements?

Best practice: design your feeding strategy such that O_2 is not the limiting nutrient, cont'd

Since we know (we can measure) the yield of cells on oxygen, we can estimate the cell concentration by

$$\frac{dX(t)}{dt} = Y_{X/O} OUR(t)$$

OUR can be measured on-line

Together with an estimate of X(t) we can estimate μ from…

$$\mu = \frac{1}{X(t)} \frac{dX}{dt}$$

$$\mu(t) = \frac{OUR(t)}{\frac{X(t_0)}{Y_{X/O}} + \int_0^t OUR(t)dt}$$

IOUR - can be measured on-line

Best practice: design your feeding strategy such that O_2 is not the limiting nutrient, cont'd

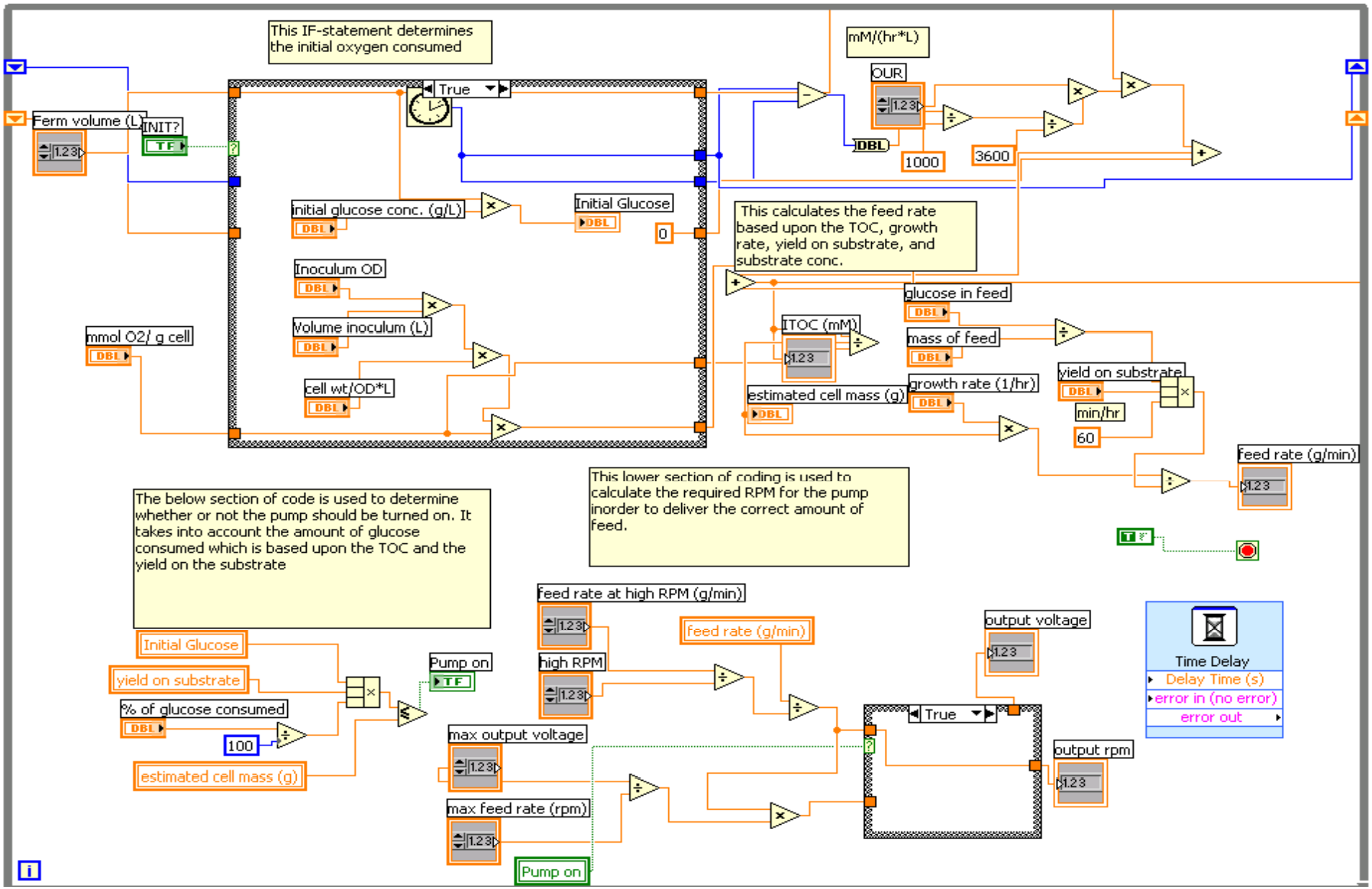
Putting everything back into our Feed rate calculation, we have

$$F_S = \frac{\mu}{Y_{X/S}} X(t)$$

$$F_S = \frac{1}{Y_{X/S}} \cdot \frac{OUR(t)}{\frac{X(t_0)}{Y_{X/O}} + IOUR} \cdot \left[Y_{X/O} \cdot IOUR + X(t_0) \right]$$

If the cells have everything they need (balanced growth), the μ you are estimating may be close to μ_{max}. If your feed rate should exceed the amount needed for the growth of the cells, you will accumulate substrate and it will result in the formation of byproducts.

It solves the problem to choose some fraction of μ_{max}, such as **$\underline{0.7\mu_{max}}$**


This IF-statement determines the initial oxygen consumed
Ferm volume (L)
INIT?
TF
True
initial glucose conc. (g/L)
Initial Glucose
0
Inoculum OD
Volume inoculum (L)
cell wt/OD*L
mmol O2/ g cell
mM/(hr*L)
OUR
DBL
1000
3600
This calculates the feed rate based upon the TOC, growth rate, yield on substrate, and substrate conc.
glucose in feed
TOC (mM)
mass of feed
estimated cell mass (g)
growth rate (1/hr)
yield on substrate
min/hr
60
feed rate (g/min)
The below section of code is used to determine whether or not the pump should be turned on. It takes into account the amount of glucose consumed which is based upon the TOC and the yield on the substrate
This lower section of coding is used to calculate the required RPM for the pump inorder to deliver the correct amount of feed.
Initial Glucose
yield on substrate
% of glucose consumed
100
estimated cell mass (g)
Pump on
feed rate at high RPM (g/min)
high RPM
feed rate (g/min)
max output voltage
max feed rate (rpm)
Pump on
output voltage
output rpm
Time Delay
Delay Time (s)
error in (no error)
error out

Off-line analysis methods used for fermentation

Off-line analysis

- Advantages:
  - No interfacing required
  - Flexibility
  - Low cost
  - Small sample volumes
- Disadvantages:
  - Requires removing samples
  - Requires tracking the sampling time
  - Requires operator interaction, potential for bias error from operator

Offline Analysis Methods

Glucose

- Glucose concentration is measured by a glucose analyzer. For E. coli fermentations, glucose should be kept above about 5 g/liter and below about 20 g/liter for optimum growth.
- The Yellow Springs Instruments (YSI) Bioanalyzer is a standard instrument for fermentation analysis. It can measure other sugars, ethanol and methanol, as well.
- A small sample from the fermentor is centrifuged to settle the bacteria and the supernatant is fed through a sipper tube into the analyzer. It is automatically calibrated.

High Performance Liquid Chromatography (HPLC)

- HPLC is useful for measuring fermentation by-products, such as ethanol, organic acids – acetic acid, succinic acid, lactate, etc., and specific amino acids and virtually all small molecules and proteins.
- Each molecule should have a specific method to be analyzed.

Offline Analysis Methods

Determining cell mass concentration

Direct methods:

•Dry Weight

→ Solids-free medium.

•Packed Cell Volume

•Optical Density Method

→ Spectrophotometer.

→ 550 or 600 nm wavelengths.

→ light absorbance cell mass/volume

→ OD ≤ 0.3 → need to measure dry-weight of the cells.

Typical Off-line analyses

- Optical density (OD) analysis of cell concentration
- Glucose can be measured off-line
- NH_3 can be measured by chemical analysis
- Phosphate and other macro-nutrients
- Protein activity assays
- HPLC – everything else…