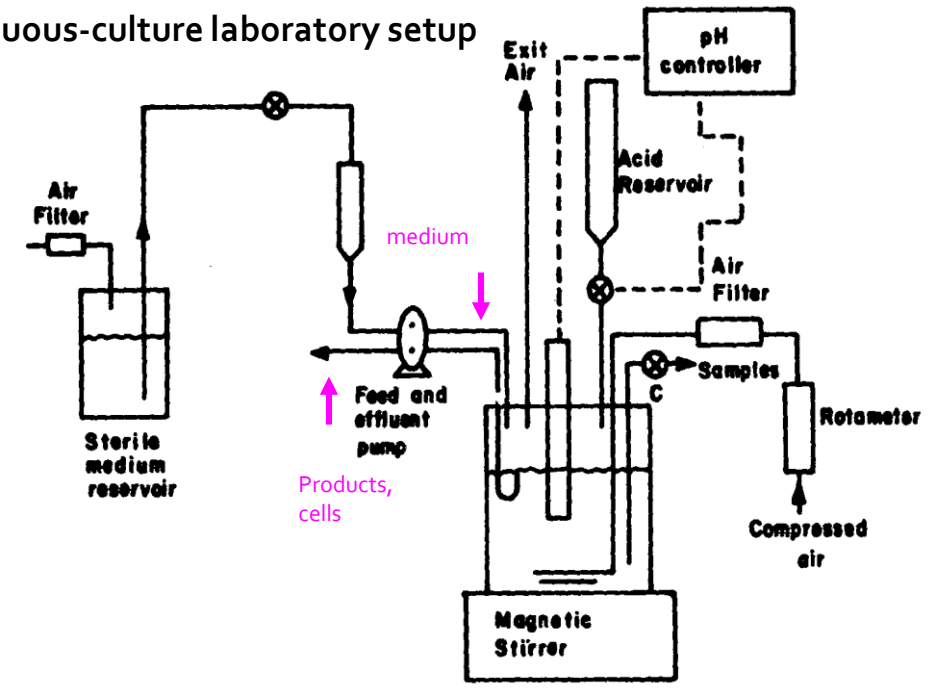
Cells Growth in Continuous Culture

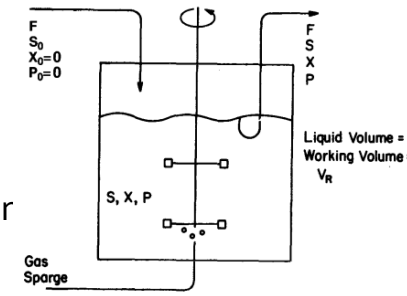
- Continuous culture: fresh nutrient medium is continually supplied to a well-stirred culture and products and cells are simultaneously withdrawn.
- At steady state, concentrations of cells, products and substrates are constant.
- In batch culture: the culture environment changes continually. Growth, product formation and substrate utilization terminate after a certain time interval.

A continuous-culture laboratory setup



Ideal Chemostat

- Same as perfectly mixed continuous-flow, stirred-tank reactor (CFSTR).
  - Control elements: pH, dissolved oxygen, temperature
  - Fresh sterile medium is fed to the completely mixed and aerated (if required) reactor.
  - Suspension is removed at the same rate.
  - Liquid volume in the reactor is kept constar

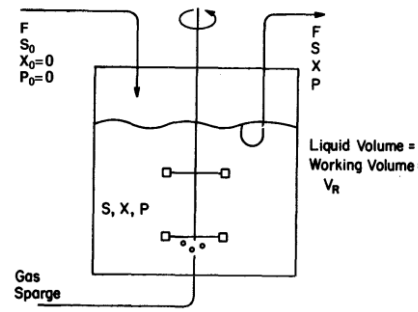


Cell Growth in Ideal Chemostat

$$FX_0 - FX + V_R\mu_g X - V_R k_d X = V_R \frac{dX}{dt}$$

F is the volumetric flowrate of nutrient solution (l/h);
V_R is the culture volume (l) (constant);
X is the cell concentration (g/l);
P is the extracellular product (g/l);
μ_g and k_d are growth rate and endogenous rate constant, respectively (h^{-1}).

Subscript o denotes the parameters at the feed medium.



Cell Growth in Ideal Chemostat

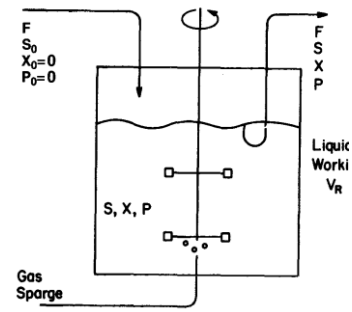
Usually, the feed media are sterile, $X_0 = 0$
If the system is at steady state, dX/dt = 0 and Monod equation applied
If the endogenous metabolism or death rate is negligible compared to the growth rate ($k_d << \mu_g$), $k_d \approx 0$

$$FX_0 - FX + V_R \mu_g X - V_R k_d X = V_R \frac{dX}{dt}$$

(FX_0, $V_R k_d X$, $V_R \frac{dX}{dt}$ → 0)

$$\mu_g = D = \frac{\mu_m S}{K_S + S}$$

Where $D = F/V_R$ = *dilution rate* = reciprocal of residence time

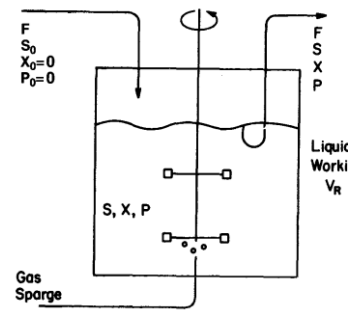


Cell Growth in Ideal Chemostat

$$\mu_g = D = \frac{\mu_m S}{K_S + S} \quad \Rightarrow \quad S = \frac{K_S D}{\mu_m - D}$$

A material balance on the limiting substrate in the absence of endogenous metabolism yields

$$FS_0 - FS - V_R \mu_g X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{p/s}} = V_R \frac{dS}{dt}$$



q_p is the specific rate of extracellular product formation (g P/g cells-h)
$Y_{P/S}$ is the product yield coefficient (g P/g S).
$Y^M_{X/S}$ is the cell yield coefficient (g cell/g S), superscript *M* <=> maximum value of the yield coefficient

Cell Growth in Ideal Chemostat

- When extracellular product formation is negligible and the system is at steady state ($dS/dt = 0$),

$$FS_0 - FS - V_R \mu_g X \frac{1}{Y^M_{X/S}} - V_R q_p X \frac{1}{Y_{p/s}} = V_R \frac{dS}{dt}$$

(0 over $q_p X$ and 0 over $\frac{dS}{dt}$)

And $\mu_g = D$ at steady state if $k_d = 0$,

$$X = Y^M_{X/S}(S_0 - S)$$

$$X = Y^M_{X/S}\left(S_0 - \frac{K_S D}{\mu_m - D}\right)$$

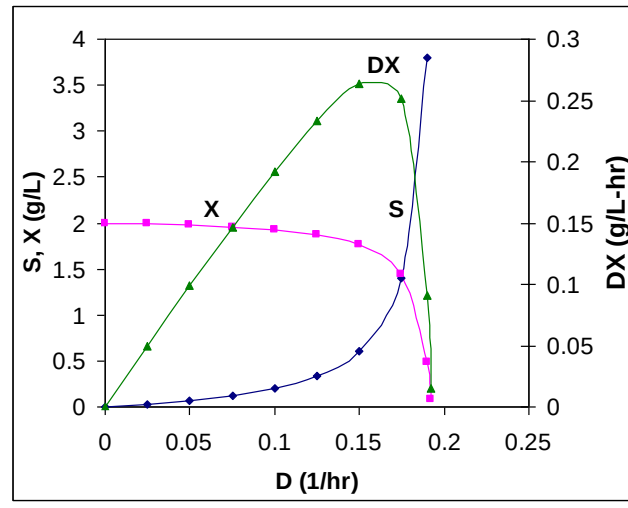
Cell Productivity = DX

Cell Growth in Ideal Chemostat

Washed out:
If D is set at a value greater than μ_m ($D > \mu_m$), the culture cannot reproduce quickly enough to maintain itself.

$$\mu_g = D = \frac{\mu_m S}{K_s + S}$$

$\mu_m = 0.2\ hr^{-1}$



Determination of Monod Parameters

- In Chemostat: μ_g=D, varying D obtains D~S

$$\mu_g = D = \frac{\mu_m S}{K_S + S} \quad \Rightarrow \quad S = \frac{K_S D}{\mu_m - D}$$

$$\frac{1}{S} = \frac{\mu_m}{K_S}\frac{1}{D} - \frac{1}{K_S} \text{ (Lineweaver - Burk)}$$

- Chemostat technique: reliable, constant environment, operation may be difficult.

Determination of Monod Parameters

- In Batch: X, S, t → lnX ~ t , get μ_m (slope) from data in exponential phase.

$$\ln\frac{X}{X_0} = \mu_{net} t \approx \mu_m t$$

$$\frac{1}{X}\frac{dX}{dt} = \mu_g = \frac{\mu_m S}{K_S + S}, \quad k_d \approx 0$$

$$\frac{1}{\mu_g} = \frac{K_S}{\mu_m}\frac{1}{S} + \frac{1}{\mu_m} \text{ (Lineweaver - Burk)}$$

$$\frac{S}{\mu_g} = \frac{K_S}{\mu_m} + \frac{S}{\mu_m} \text{ (Hanes - Woolf)}$$

Problem 6.13 (Shuler-Kargi)

- *Pseudomonas putida* with $\mu_m = 0.5\ h^{-1}$ is cultivated in a continuous culture under aerobic conditions where $D = 0.28\ h^{-1}$. The carbon and energy source in the feed is lactose with a concentration of $S_0 = 2$ g/l. The effluent lactose concentration is desired to be $S = 0.1$ g/l. If the growth rate is limited by oxygen transfer, by using the following information:

$$Y^M_{X/S} = 0.45 gX/gS, \quad Y^M_{X/O_2} = 0.25 gX/gO_2 \text{ and } C^* = 8 \text{mg/l}$$

  a. Determine the steady-state biomass concentration (X) and the specific rate of oxygen consumption (q_{O_2}).
  b. What should be the oxygen-transfer coefficient (k_La) in order to overcome oxygen transfer limitation (i.e., $C_L = 2$ mg/l)?