

CH40001 Biochemical Engineering

Chapter 6.

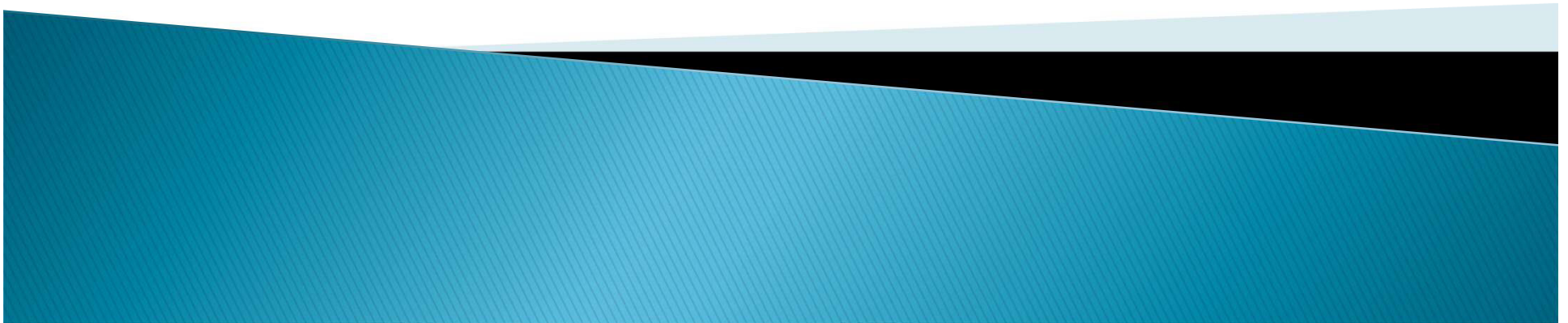
(a) Fermentation

(b) Design and Analysis of Bioreactors (Fermenters)

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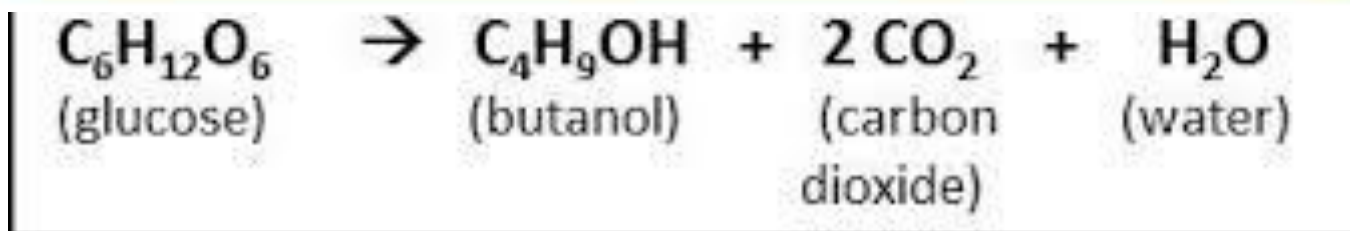
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FERMENTATION OF SUGARS

anhydrous glucose $C_6H_{12}O_6$	\longrightarrow	ethanol $2 C_2H_5OH$	+	carbon dioxide $2 CO_2$	
FW 180 1 gram		46(x2) 0.51 grams		44 (x2) 0.49 grams	
glucose monohydrate $C_6H_{12}O_6 \cdot H_2O$	\longrightarrow	ethanol $2 C_2H_5OH$	+	carbon dioxide $2 CO_2$	+ water H_2O
FW 198 1 gram		46 (x2) 0.46 grams		44 (x2) 0.44 grams	18 0.091 grams
sucrose $C_{12}H_{22}O_{11}$	+	water H_2O	\longrightarrow	ethanol $4 C_2H_5OH$	+ carbon dioxide $4 CO_2$
FW 342 1 gram		18 0.053 grams		46 (x4) 0.54 grams	44 (x4) 0.51 grams



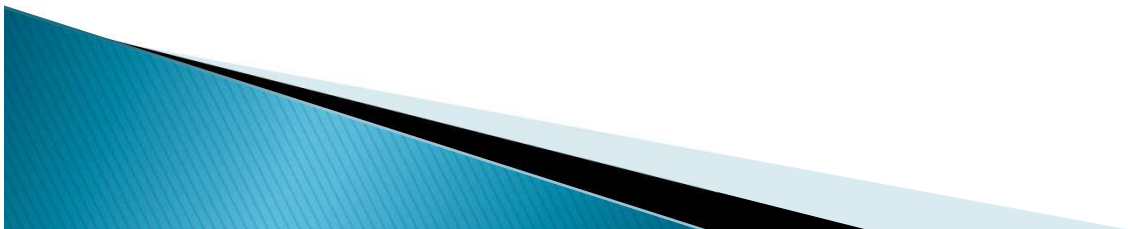
BIOLOGICAL FERMENTATION (using yeasts or bacteria)

- ▶ It is a Chemical transformation of Fermentable Sugars, especially Glucose, which gives Valuable Products such as Fructose, Ethanol, numerous Organic Acids and other by-products through Biochemical conversion by a certain microorganism such as **yeasts and bacteria**.
- ▶ The Degradation of Carbohydrates by Microorganisms is followed by Glucolytic or Embden-Myerhof-Parnas (EMP) Pathways.
- ▶ In this, Carbohydrates are reduced to Pyruvate with the aid of Nicotinamide Adenine Dinucleotide (NADH) and Ethanol is the end Product.
- ▶ Depending on the Biomass, Process Conditions, Process Economy and Cost, Fermentation can be carried out as:



► Types of Biological Fermentation

- **SHF** : In this Process, Enzyme Production, Enzymatic Hydrolysis and Fermentation Is performed Sequentially In Separate Vessels. The Process Is called Separate Hydrolysis and Fermentation.
- **DMC** : Direct Microbial Conversion combines all three major processes (Enzyme Production, Hydrolysis and Fermentation) In one step.
- **SSF** : Simultaneous Saccharification (I.e, hydrolysis) and Fermentation combines Hydrolysis of the Substrate and Fermentation In one step. It reduces the Cellulase Inhibition, which In turn Increases Sugar Production Rates, Yields and Concentrations.

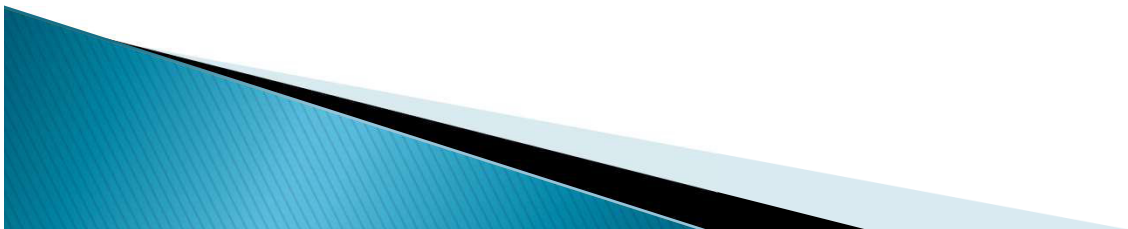


SSF Is very advantageous In comparison with other processes mentioned above.

Process Is discussed below.

SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF)

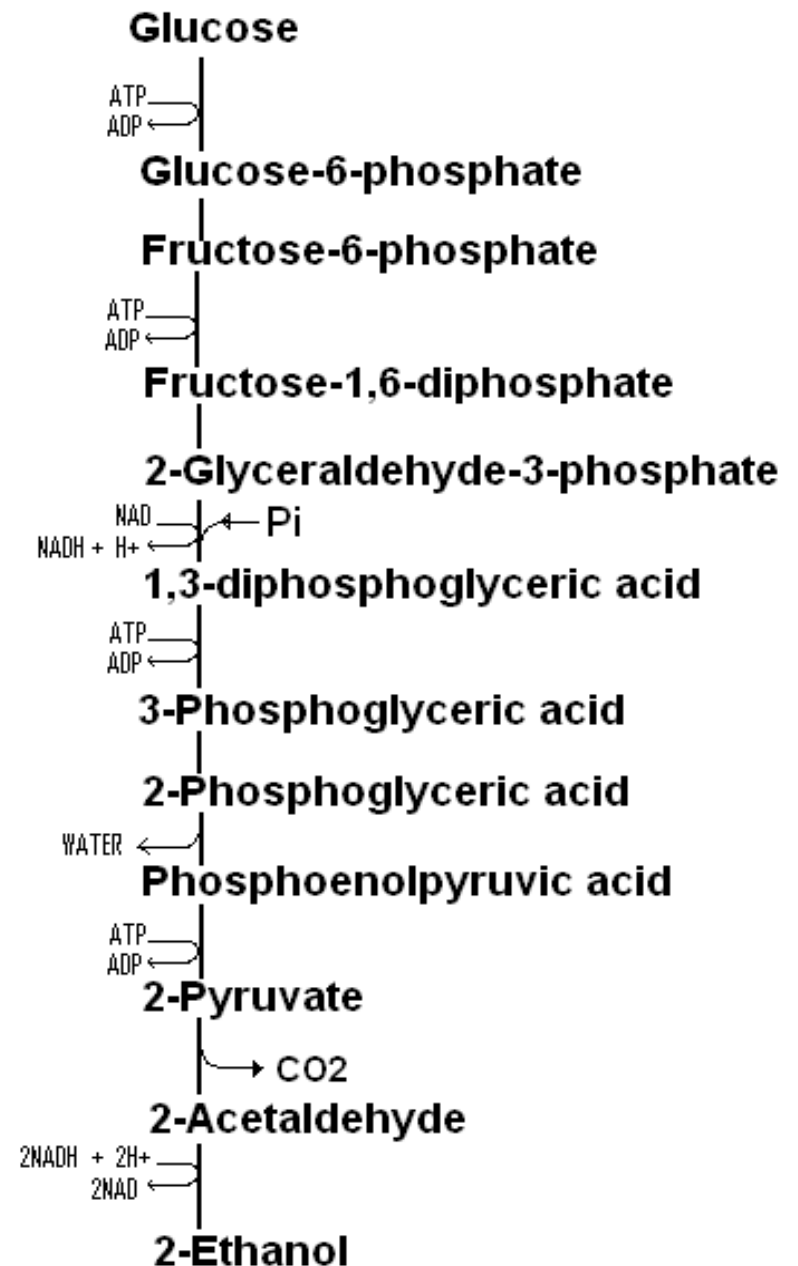
- ▶ **The process was First Introduced In 1977 and It gives High Yield and rapid Rates of Conversion.**
- ▶ **Glucose released by Cellulase Enzyme (from *T reese*) Simultaneously converted to the end product by the Microorganism.**
- ▶ **Since the Optimum Temperature for both Saccharification and Fermentation Is different, *Thermotolerant yeasts* such as *Kluyveromyces marxianus* also preferred.**



- ▶ **Most Microorganisms converts Glucose to Ethanol but unable to convert Xylose or Mannose which is mainly found in Hemicellulose.**
- ▶ **Traditional Microorganisms, *Saccharomyces cerevisiae* and *Zymomonas mobilis*, do not metabolize Pentose.**
- ▶ ***Escherichia coli* strain K011 Ferments Glucose, Xylose and Arabinose with good Ethanol Yields and Productivities and has a High Tolerance for common Hydrolysate Inhibitors, for example, Acetate.**
- ▶ **Two strains of the recombinant *E. Coli* strains are,**
 - 1. strain ATCC 11303 (pLOI297), in which the foreign genetic elements are plasmid-borne and**
 - 2. K011 in which the foreign genetic elements are integrated into the host chromosome.**
- ▶ **In metabolism; Xylose is transported across the cell membrane where it is converted to Xylulose-5-phosphate (X-5-P).**
- ▶ **It is then converted to Pyruvate by the way of pentose phosphate (PP) and Embden-Meyerhof-Parnas (EMP) or Entner-Doudoroff (ED) pathways.**



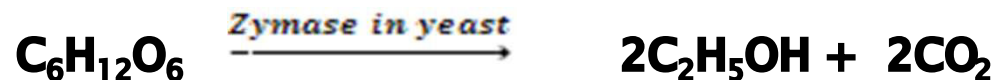
- ▶ In PP cycle, X-5-P is metabolized to Glucolytic Intermediates, (Glyceraldehyde-3-Phosphate and Fructose-6-Phosphate) which are then converted to Pyruvate via EMP or ED pathway.
- ▶ The Pyruvate is then converted to Ethanol via an Acetaldehyde Intermediate by the sequential action of Pyruvate Decarboxylase and Alcohol Dehydrogenase (ADH) Enzymes.
- ▶ In this scheme, a minimum of 3 moles of Xylose are required to produce 5 moles of Ethanol.




The Embden-Meyerhof-Parnas (EMP) Pathway

Starch Fermentation

- ▶ In Starch Fermentation α -amylase, is used as an enzyme, obtained from *Aspergillus oryzae*, *Bacillus amyloliquefaciens* and *Bacillus licheniformis*.
- ▶ The products are glucose, maltose, maltotriose, maltotetraose, maltopentose and maltohexose.
- ▶ Yeast strain *S. cerevisiae* NRRL Y132, ATCC4126 and ATCC 26603 considered as most promising for industrial ethanol production which can convert both Glucose and Maltose.
- ▶ Maltase present in the Yeast converts Maltose into Glucose. Another Enzyme Zymase present in the yeast, then converts Glucose into Ethanol and Carbon dioxide.



Zymobacter palmae T109 in co-culture fermentation with *Zymomonas mobilis* B69 can also ferment fructose, galactose, glucose, mannose, maltose, melibiose, saccharose, raffinose, mannitol and sorbitol.



Modeling and Design of Biological Fermentation

- ▶ Design of Chemostats (continuous stirred bioreactors) in which simultaneous growth of yeast/bacteria and fermentation of glucose occur
- ▶ Stability of Chemostats



Models for Growth of *Saccharomyces cerevisiae* and *Zymomonas mobilis* during Glucose Fermentation In a Batch Reactor

Monod Model (S =Glucose, X =*S. cerevisiae* or *Z. mobilis*)

$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} SX}{k_s + S}$$

$$\frac{dS}{dt} = -\frac{1}{Y_{x/s}} \frac{\mu_{\max} SX}{k_s + S}$$

expressing X in terms of S from above eqn.

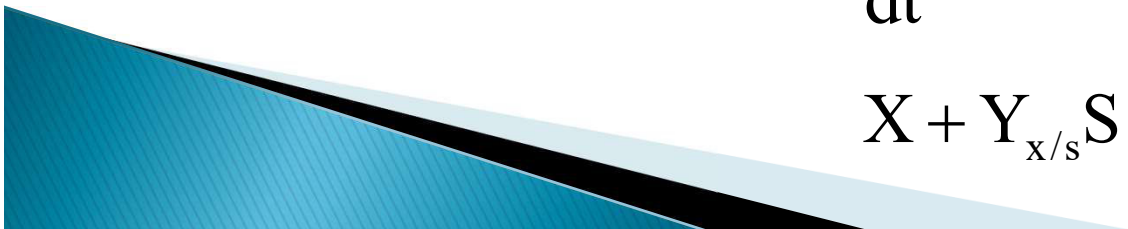
$$\frac{dS}{dt} = -\frac{\mu_{\max}}{Y_{x/s}} \frac{X_0 + Y_{x/s} \{S_0 - S\} S}{k_s + S}$$

with initial conditions: $X(0)=X_0$, $S(0)=S_0$

Using above eqns we get the invariance

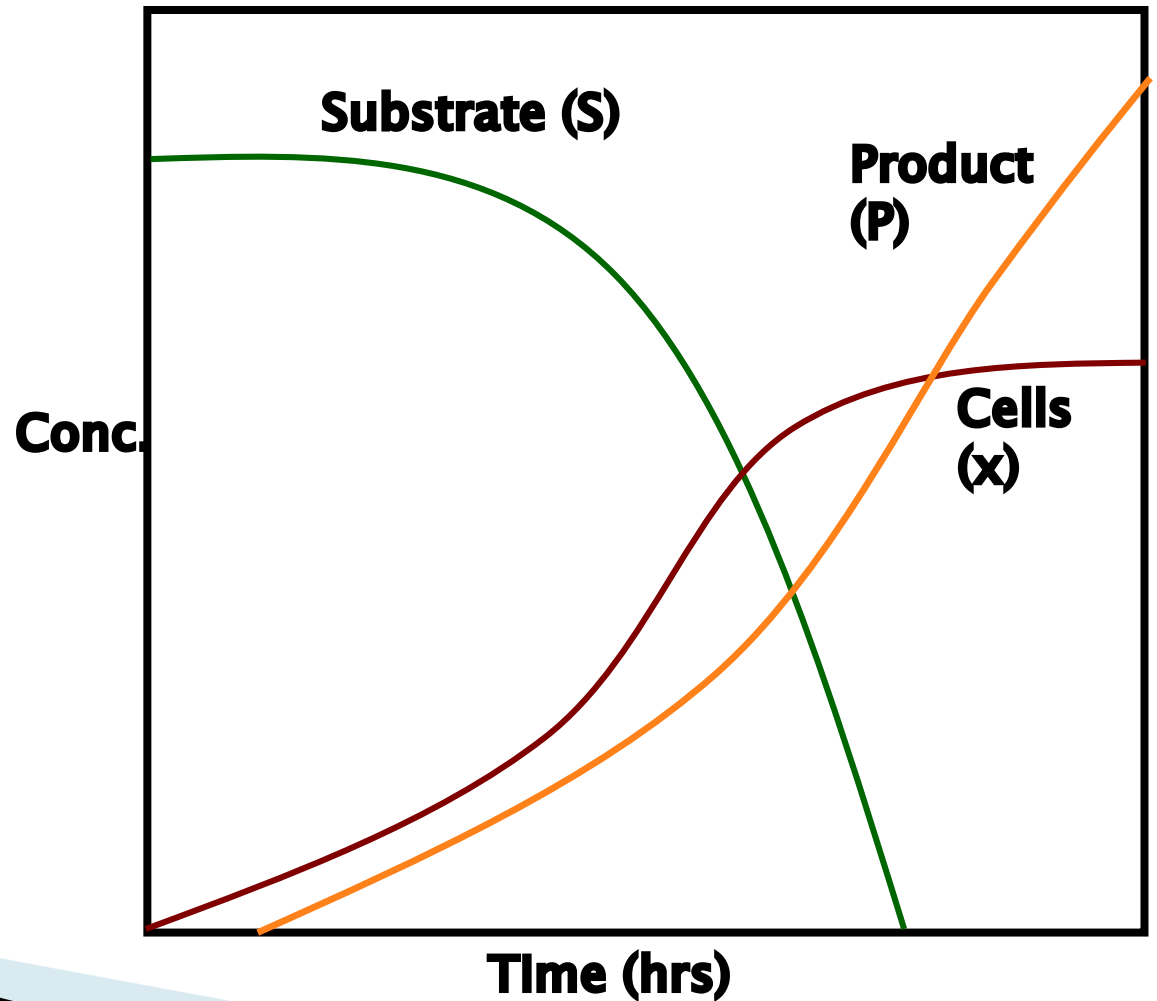
$$\frac{d}{dt} (X + Y_{x/s} S) = 0$$

$$X + Y_{x/s} S = X_0 + Y_{x/s} S_0$$



on integration , $[X_0 + Y_{x/s} \{S_0 + k_s\}] \ln\left(\frac{X_0 + Y_{x/s} \{S_0 - S\}}{X_0}\right)$

$$-k_s Y_{x/s} \ln\left(\frac{S}{S_0}\right) = \mu_{\max} (X_0 + Y_{x/s} S_0) t$$



Death of cells in batch culture:

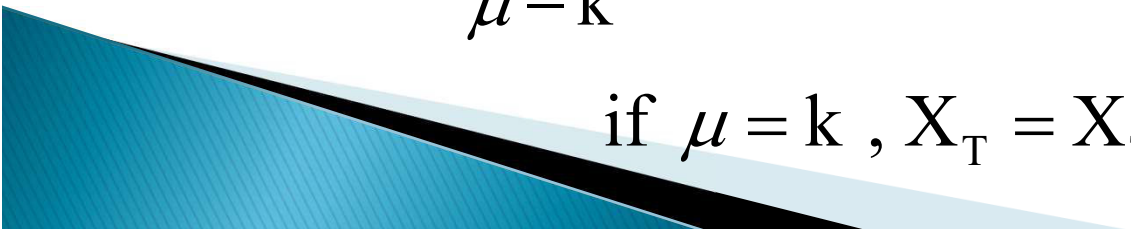
$$\frac{dX_v}{dt} = \mu X_v - kX_v$$

$$\frac{dX_d}{dt} = kX_v$$

$$\frac{dX_T}{dt} = \frac{d}{dt}(X_v + X_d) = \mu X_v$$

$$X_v = X_{v0} \exp[(\mu - k)t]$$

$$X_T = X_{T0} \left(1 + \frac{\mu}{\mu - k} \exp[(\mu - k)t]\right)$$


$$\text{if } \mu = k, X_T = X_{T0} + X_{v0}\mu t$$

Other Types of Growth Kinetics

▶ Substrate Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S + S^2 / K_I}$$

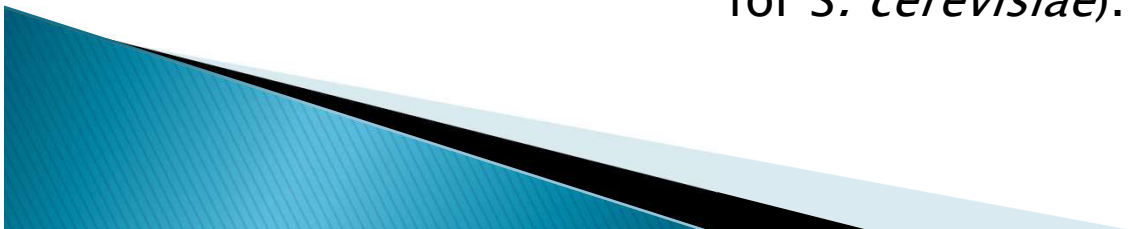
$$\frac{dX}{dt} = \mu X = \frac{\mu_{\max} SX}{K_S + S + S^2 / K_I}$$

Here, K_I is the Haldane or substrate inhibition coefficient

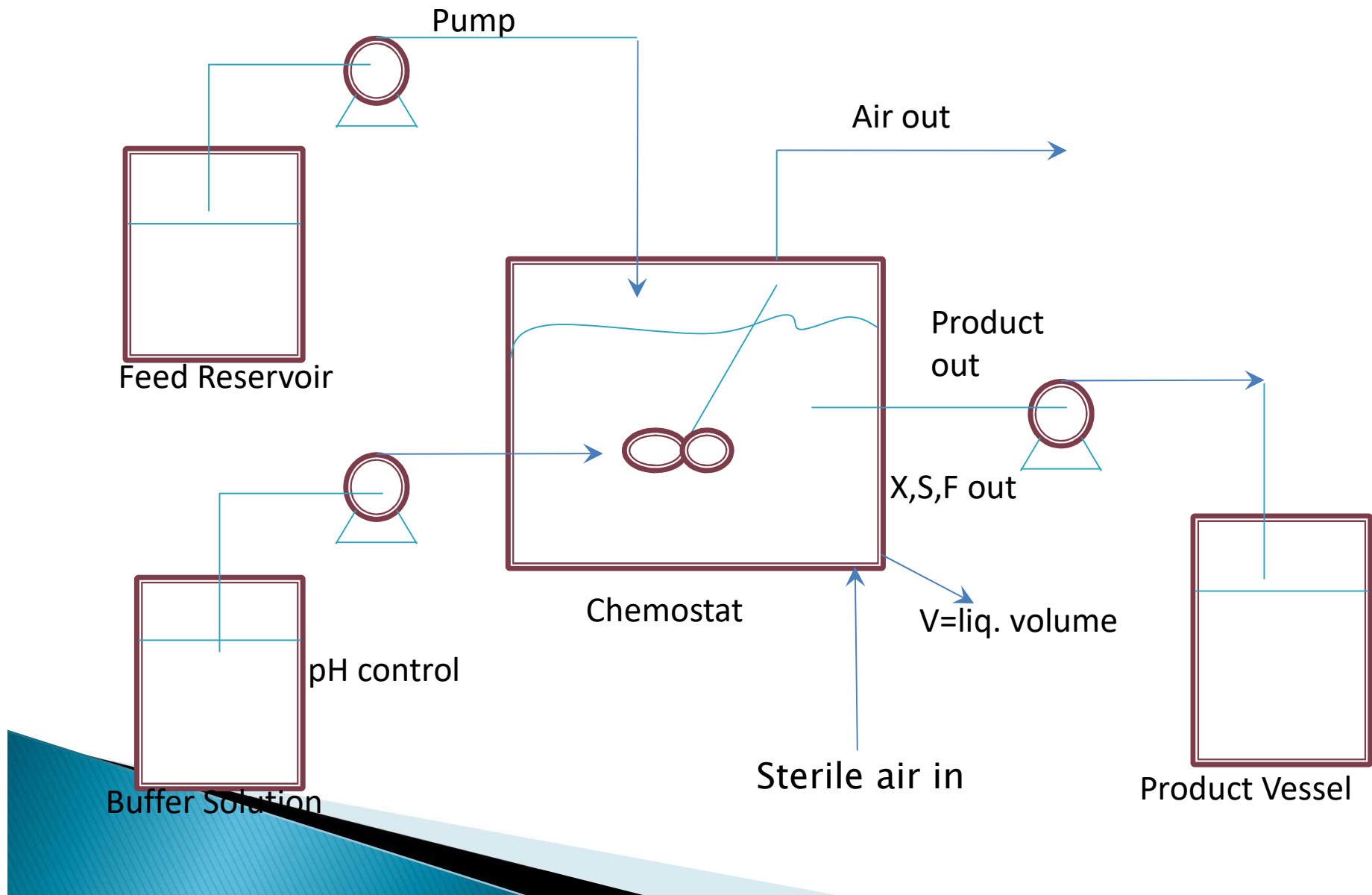
▶ Product Inhibition

$$\mu = \frac{\mu_{\max} S}{K_S + S} \left[1 - \left(\frac{[P]}{[P^*]} \right)^n \right],$$

Where $[P]$ is the ethanol conc., n is a determinable constant, $[P]^*$ is the critical ethanol conc. above which cells cease to grow ($\sim 10\text{gm/lit}$ for *S. cerevisiae*).



A Chemostat and Its accessories



Design of Chemostats (continuous stirred tank bioreactors)

$$\frac{dV}{dt} = F_{\text{in}} - F_{\text{out}}$$

Total volume balance.

$$\frac{dXV}{dt} = F_{\text{in}}X_0 - F_{\text{out}}X + \mu XV$$

Mass balance for cells

$$\frac{dSV}{dt} = F_{\text{in}}S_0 - F_{\text{out}}S - \frac{1}{Y_{x/s}}\mu XV$$

Mass balance
for substrates

if $F_{\text{in}} = F_{\text{out}} = F \Rightarrow V = \text{const.}$

$$\frac{dX}{dt} = \frac{F}{V}(X_0 - X) + \mu X$$

$$\frac{dS}{dt} = \frac{F}{V}(S_0 - S) - \frac{1}{Y_{x/s}}\mu X.$$



$$\frac{F}{V} = D(\text{dilution rate})[s^{-1}] = \frac{1}{\tau} \text{ (inverse of residence time)}$$

$$\frac{dX}{dt} = D(X_0 - X) + \mu X \quad (1)$$

$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \mu X \quad (2)$$

$$\mu = \frac{\mu_{\max} S}{k_s + S} \quad \text{:Monod Growth Kinetics.}$$

If death of microorganisms (yeast/bacteria) is included in the model, μ in eqs. (1) must be replaced by $(\mu - k)$, where k is the 1st order 'death constant' for the micro-organism.



at steady state from eq (1)

$$D X_0 = (D - \mu) X$$

for sterile feed , $X_0 = 0$ and $D = \mu$

$$\text{i.e., } \frac{\mu_{\max} S_{ss}}{k_s + S_{ss}} = D \Rightarrow S_{ss} = \frac{Dk_s}{\mu_{\max} - D}$$

provided $X_{ss} \neq 0$ and $\mu_{\max} > D$



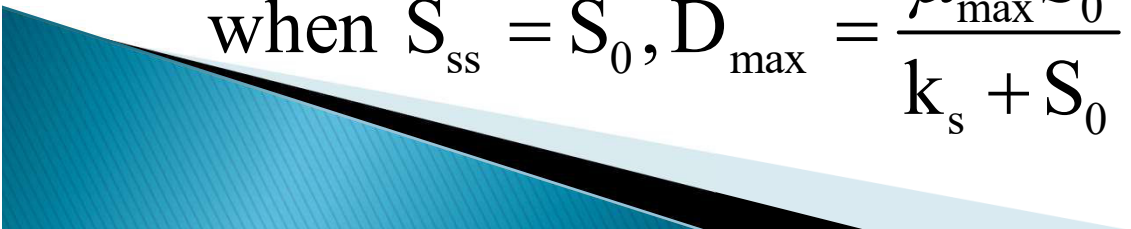
from eqn. (2)

$$D(S_0 - S_{ss}) - \frac{1}{Y_{x/s}} \mu X_{ss} = 0$$

noting $D = \mu$ for $X_0 = 0$

$$X_{ss} = Y_{x/s} \left(S_0 - \frac{Dk_s}{\mu_{\max} - D} \right)$$

when $S_{ss} = S_0$, $D_{\max} = \frac{\mu_{\max} S_0}{k_s + S_0}$



$$\text{As } D \rightarrow \mu_{\max} \quad X_{\text{ss}} \rightarrow \infty$$

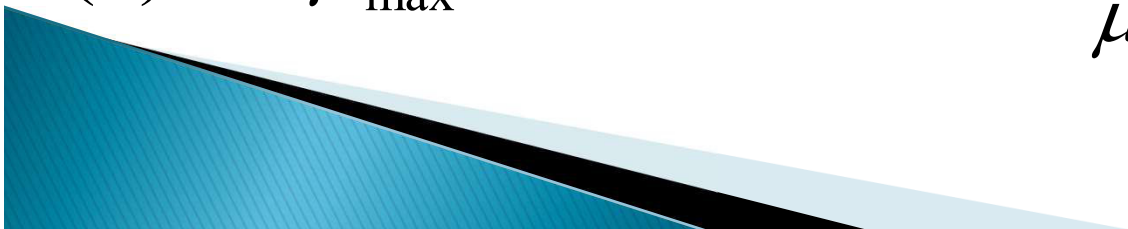
\therefore solutions are possible for $D > \mu_{\max}$ and $D < \mu_{\max}$

for $X_{\text{ss}} \neq 0$, $D = \mu \Rightarrow D < \mu_{\max}$

for $X_{\text{ss}} = 0$, $D > \mu_{\max}$

steady state solutions :

$$(1) \ D < \mu_{\max} : \quad X_{\text{ss}} = Y_{\text{x/s}} \left(S_0 - \frac{Dk_s}{\mu_{\max} - D} \right)$$



$$S_{ss} = \frac{Dk_s}{\mu_{\max} - D} \text{ provided } S_0 > \frac{Dk_s}{\mu_{\max} - D}$$

if $S_0 < \frac{Dk_s}{\mu_{\max} - D}$, X_{ss} & S_{ss} are not in the feasible domain.

(i.e they are negative)

and $S_{ss} = S_0$

$$X_{ss} = 0$$

$$(2) D < \mu_{\max} : \quad \begin{aligned} X_{ss} &= 0 \\ S_{ss} &= S_0 \end{aligned}$$



Summary of chemostat behavior

$$\frac{dX}{dt} = D(X_0 - X) + \mu X$$

$$\frac{dS}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \mu X$$

$$\mu = \frac{\mu_{\max} S}{k_s + S}$$



for sterile feed ($X_0 = 0$) :

D	$S_0 > \frac{Dk_s}{\mu_{\max} - D}$	$S_0 < \frac{Dk_s}{\mu_{\max} - D}$
$D < \mu_{\max}$	$S_{ss} = \frac{Dk_s}{\mu_{\max} - D}, X_{ss} = Y_{x/s}(S_0 - S)$	$S_{ss} = S_0, X_{ss} = 0$
$D > \mu_{\max}$	$S_{ss} = S_0, X_{ss} = 0$	$S_{ss} = S_0, X_{ss} = 0$

Stability of Chemostats during Fermentation

Liapunov method of linear stability analysis of chemical reactor
(Bilous and Amundson, 1955)

the unsteady state mass balance eqn.

For a CSTR could be written vectorially as

$$\frac{d\underline{C}}{dt} = \underline{f}(\underline{C}, \underline{p})$$

steady state solution may be obtained from:

$$\underline{f}(\underline{C}_{ss}, \underline{p}) = 0$$

\underline{x} = deviations from steady state

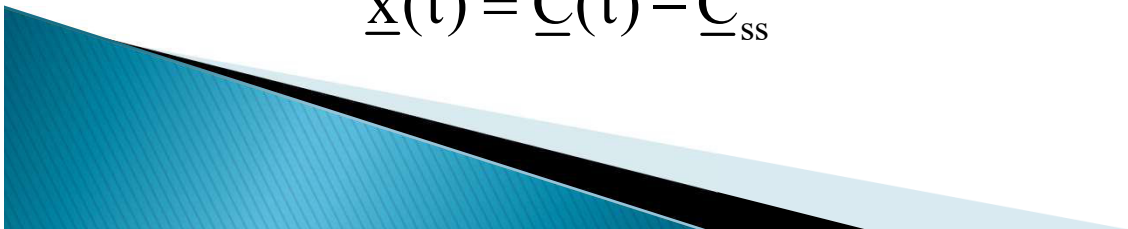
$$\underline{x}(t) = \underline{C}(t) - \underline{C}_{ss}$$

\underline{C} = conc. vector

$$= \begin{pmatrix} S \\ X \end{pmatrix}$$

\underline{p} = parameter vector

$$= \begin{pmatrix} D \\ Y_{x/s} \\ k_s \\ \mu_{\max} \\ S_0 \end{pmatrix}$$



$$\underline{\underline{C}}(t) = \underline{\underline{C}}_{ss} + \underline{\underline{x}}(t)$$

$$\frac{d\underline{\underline{x}}}{dt} = \underline{\underline{f}}(\underline{\underline{C}}_{ss} + \underline{\underline{x}}, \underline{\underline{p}})$$

If $g(\underline{\underline{C}}_{ss} + \underline{\underline{x}})$ is scalar,

Taylor series expansion of $g(\underline{\underline{C}}_{ss} + \underline{\underline{x}})$ is given as

$$g(\underline{\underline{C}}_{ss} + \underline{\underline{x}}) = g(\underline{\underline{C}}_{ss}) + \frac{\partial g}{\partial \underline{\underline{C}}_{ss}} \underline{\underline{x}} + \frac{\partial^2 g}{\partial \underline{\underline{C}}_{ss}^2} \left(\frac{\underline{\underline{x}}^2}{2!} \right) + \text{higher order terms.}$$



For reactors:

Taylor series expansion (about steady state \underline{C}_{ss})

$$\underset{n \times 1}{\underline{f}}(\underset{n \times 1}{\underline{C}_{ss}} + \underset{n \times 1}{\underline{x}}, \underset{n \times 1}{\underline{p}}) = \underset{n \times 1}{\underline{f}}(\underset{n \times 1}{\underline{C}_{ss}}, \underset{n \times 1}{\underline{p}}) + \underset{n \times n}{\underline{A}} \underset{n \times 1}{\underline{x}} + \text{h.o.t}$$

$$\underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p}) \approx \underline{A}\underline{x}$$

($\because f(\underline{C}_{ss}, \underline{p}) = 0$ & magnitude of higher order terms $\ll |\underline{A}\underline{x}|$ iff $|\underline{x}|$ is small.

n = number of species.

~~n = number of mass balance equations~~

\therefore if $|\underline{x}|$ is small i.e $|\underline{x}| \ll |\underline{C}_{ss}|$,

$$\frac{d\underline{x}}{dt} = \underline{f}(\underline{C}_{ss} + \underline{x}, \underline{p}) = \underline{A}\underline{x}$$

where a_{ij} are the elements of \underline{A} evaluated at steady state.

$$a_{ij} = \frac{\partial f_i(\underline{C}_{ss}, \underline{p})}{\partial C_j}$$

the solution of

$$\frac{d\underline{x}}{dt} = \underline{A}\underline{x} \text{ with initial conditions } \underline{x} = \underline{x}_0$$

$$\underline{x}(t) = \sum_{i=1}^n \alpha_i \underline{\beta}_i e^{\lambda_i t}$$

where $\underline{\beta}_i$ and λ_i are corresponding eigenvalues of \underline{A} .

the eigen vectors of $\underline{\underline{A}}$ are obtained by solving

$\det(\underline{\underline{A}} - \lambda \underline{\underline{I}}) = 0$ where $\underline{\underline{I}}$ is the $n \times n$ square matrix. the eigen vectors

$\underline{\underline{\beta}}_i$ is obtained by solving

$$(\underline{\underline{A}} - \lambda_i \underline{\underline{I}}) \underline{\underline{\beta}}_i = 0 \quad \text{where } i=1,2,\dots,n$$

and α_i are such that the initial condition is satisfied by solving.

$$\sum_{i=1}^n \alpha_i \underline{\underline{\beta}}_i = \underline{\underline{x}}_0$$

Note : there are neigen values for $n \times n$ non singular $\underline{\underline{A}}$ and n corresponding eigen vectors.

in general $\lambda_i = a_i \pm b_i i$.


1) if all λ_i 's have $a_i < 0$ and $b_i = 0$ for all i , then the steady state is locally stable.

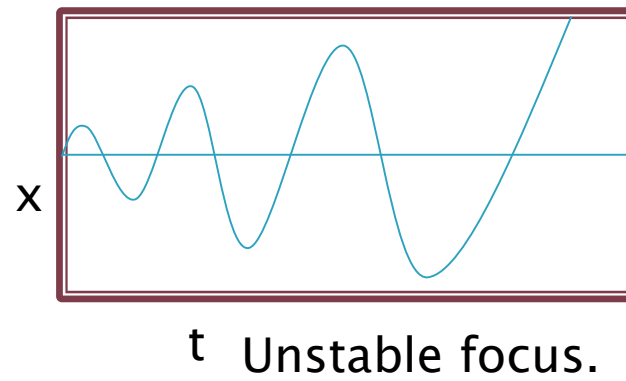
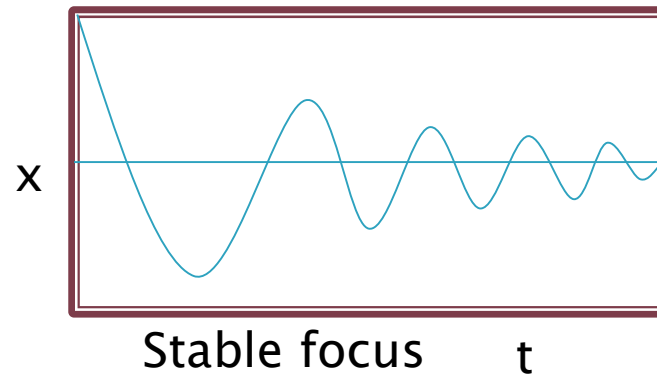
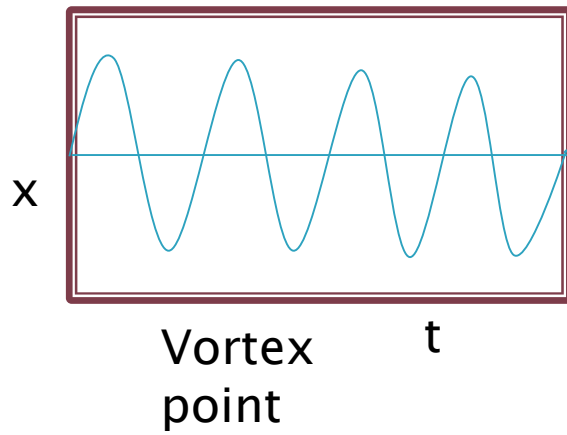
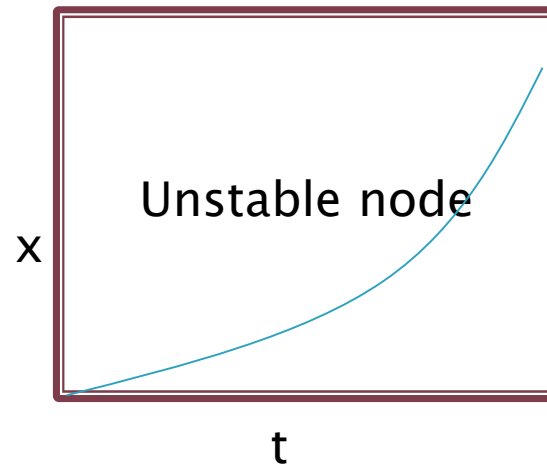
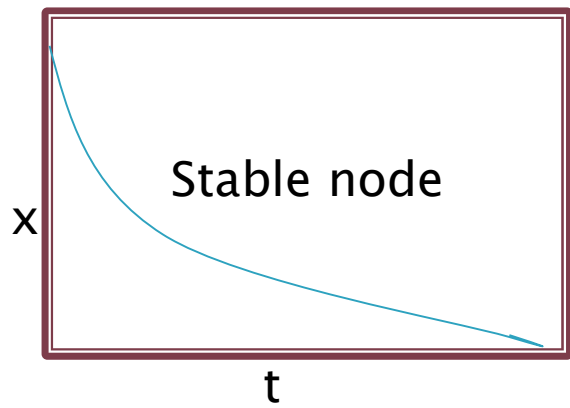
2) if even one λ_i has $a_i > 0$ and $b_i = 0$ then the steady state is unstable.

3) if $a_i = 0$ and $b_i \neq 0$, sustained oscillations.

4) if $a_i < 0$ and $b_i \neq 0$, damped oscillations.

5) if $a_i > 0$ and $b_i \neq 0$, undamped oscillation.





To check stability we need not compute all eigenvalues to determine if their real parts are positive or negative.

$$|\underline{\underline{A}} - \lambda \underline{\underline{I}}| = 0 \text{ where } \underline{\underline{A}} \text{ is } n \times n,$$

$$\lambda^n + B_1 \lambda^{n-1} + B_2 \lambda^{n-2} + \dots + B_{n-1} \lambda + B_n = 0$$

Hurwitz criteria: all roots of an eqn. will have negative real roots iff

$$B_1 > 0$$

$$\det \begin{pmatrix} B_1 & B_3 \\ 1 & B_2 \end{pmatrix} > 0$$

$$\det \begin{pmatrix} B_1 & B_3 & B_5 & \dots & 0 & \vdots & \dots & B_n \\ 1 & B_2 & B_4 & \dots & B_1 & \vdots & \dots & \\ & & B_3 & \dots & \vdots & \vdots & \dots & \\ & & \vdots & \dots & & & & \end{pmatrix} > 0$$



Stability of a chemostat :

$$\frac{dx}{dt} = D(X_0 - X) + \frac{\mu_{\max} S}{k_s + S} X$$

$$\frac{dy}{dt} = D(S_0 - S) - \frac{1}{Y_{x/s}} \frac{\mu_{\max} S}{k_s + S} X$$

$$f_1(X, S) = D(X_0 - X) + \frac{\mu_{\max} S}{k_s + S} X$$

$$f_2(X, S) = D(S_0 - S) - \frac{1}{Y_{x/s}} \frac{\mu_{\max} S}{k_s + S} X$$



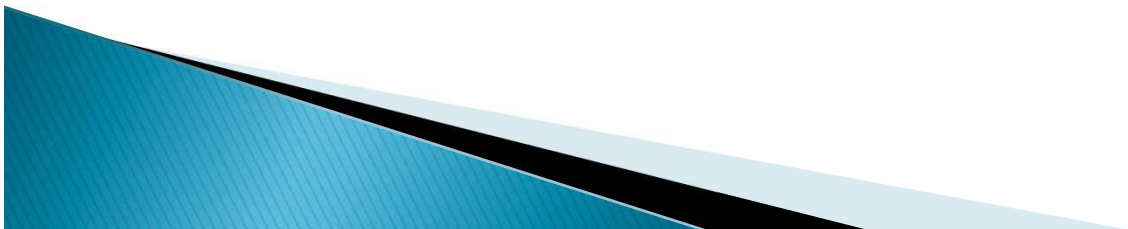
$$A = \begin{pmatrix} \frac{\partial f_1}{\partial X} & \frac{\partial f_1}{\partial S} \\ \frac{\partial f_2}{\partial X} & \frac{\partial f_2}{\partial S} \end{pmatrix} = \begin{pmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{pmatrix}$$

$$\frac{\partial f_1}{\partial X} = -D + \mu$$

$$\frac{\partial f_1}{\partial S} = X \frac{\partial \mu}{\partial S}$$

$$\frac{\partial f_2}{\partial X} = -\frac{\mu}{Y_{x/s}}$$

$$\frac{\partial f_2}{\partial S} = -D - \frac{X}{Y_{x/s}} \frac{\partial \mu}{\partial S}$$



$$|\underline{\underline{A}} - \lambda \underline{\underline{I}}| = 0$$

$$\begin{pmatrix} a_{11} - \lambda & a_{12} \\ a_{21} & a_{22} - \lambda \end{pmatrix} = 0$$

$$(a_{11} - \lambda)(a_{22} - \lambda) - a_{12}a_{21} = 0$$

$$\text{or } \lambda^2 - (a_{11} + a_{22})\lambda + (a_{11}a_{22} - a_{12}a_{21}) = 0$$

$$\therefore B_1 = -(a_{11} + a_{22})$$

$$B_2 = (a_{11}a_{22} - a_{12}a_{21})$$

$$B_3 = 0$$

\Rightarrow for both eigenvalues to have negative real parts.

$$B_1 > 0$$

$$\&\& \det \begin{pmatrix} B_1 & B_3 \\ 1 & B_2 \end{pmatrix} > 0$$

$$\text{i.e } B_1 B_2 > 0$$

$$\text{i.e } B_2 > 0$$

$$\text{criteria : } B_1 > 0 \ \&\& \ B_2 > 0$$

$$\Rightarrow (a_{11} + a_{22}) < 0 \ \&\& \ (a_{11}a_{22} - a_{12}a_{21}) > 0$$


for Monod Growth Model:


$$\mu = \frac{\mu_{\max} S}{k_s + S}$$

$$\frac{\partial \mu}{\partial S} = \frac{\mu_{\max} k_s}{(k_s + S)^2} > 0 \text{ for all } S$$

from previous analysis for $S_0 < \frac{Dk_s}{\mu_{\max} - D}$

the reaction does not take off.

so, we consider the case: $S_0 > \frac{Dk_s}{\mu_{\max} - D}$



with $X_0 = 0$ (sterile feed).

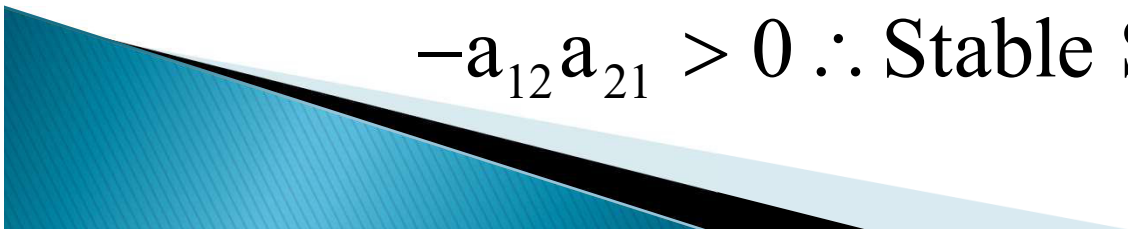
$$\Rightarrow \text{for } D < \mu_{\max} \text{ where } S_{ss} = \frac{Dk_s}{\mu_{\max} - D}$$

$$A = \begin{pmatrix} 0 & X_{ss} \left(\frac{d\mu}{ds} \right) \\ -\frac{D}{Y_{x/s}} & -\left(\frac{X_{ss}}{Y_{x/s}} \left(\frac{\partial \mu}{\partial S} \right)_{ss} + D \right) \end{pmatrix}$$

note $a_{11} = 0$, $a_{22} < 0 \therefore B_1 > 0$

$$a_{12} = X_s \left(\frac{d\mu}{ds} \right)_{ss} > 0, \quad a_{21} = \frac{-D}{\mu_{\max} - D}$$

$-a_{12}a_{21} > 0 \therefore \text{Stable Steady State}$



for $D > \mu_{\max}$ $S_{ss} = S_0$ and $X_{ss} = 0$.

$$A = \begin{pmatrix} -D + \frac{\mu_{\max} S_0}{k_s + S_0} & 0 \\ -\frac{\mu_{\max} S_0}{k_s + S_0} & -D \end{pmatrix}$$

$$a_{11} + a_{22} = -2D + \frac{\mu_{\max} S_0}{k_s + S_0} < 0 \quad (\because D > \mu_{\max})$$

$$a_{11}a_{22} - a_{12}a_{21} = D(D - \frac{\mu_{\max} S_0}{k_s + S_0}) > 0$$

\therefore both criteria satisfied \therefore both λ_i 's have a_i 's < 0

• Stable Steady State.