Une introduction à la théorie du chemostat

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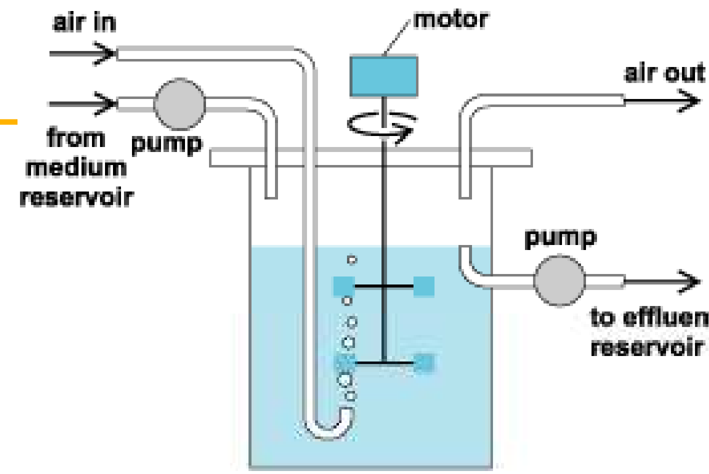
The chemostat



An apparatus for the continuous cultivation of microorganisms or plant cells. The nutrients required for cell growth are supplied continuously to the culture vessel by a pump connected to a medium reservoir. The cells in the vessel grow continuously on these nutrients. Residual nutrients and cells are removed from the vessel at the same rate by an overflow, thus maintaining the culture in the fermenter at a constant volume.

The inventors of the chemostat

- Novick A. and Szilard L. (1950), *Description of the chemostat*. Science, 112, 715-716
- Monod, J., *La technique de culture continue theorie et applications*. Ann. Inst. Pasteur, 79, 390-410, 1950

Mathematical equations

$$\left\{ \begin{array}{rcl} S'(t) & = & D[S_{in} - S(t)] - k\mu(S(t))X(t) \\ X'(t) & = & [\mu(S(t)) - D]X(t) \end{array} \right.$$

- D'où proviennent ces équations ?
- Que signifient les variables $S(t)$ et $X(t)$?
- Qui sont les paramètres k, S_{in}, et D?
- Qui est la fonction $\mu(S)$?

Principe de fonctionnement

- V : volume du réacteur (mesuré en litre, l).
- F_{in}, F_{out} ; débits d'entrée et de sortie mesurés en litre par heure (l/h).
- Concentration d'entrée S_{in}.
- $S(t)$ et $X(t)$: concentrations de substrat et de biomasse (mesurées en grammes par litre g/l)
- La réaction chimique qui transforme le substrat en biomasse est $kS \xrightarrow{r} X \qquad r = \mu X$
- k est un constant stoechiométrique sans dimension et μ est la cinétique de la réaction qui s'exprime en $1/h$.

Loi de Antoine Lavoisier

- “Rien ne se perd, rien ne se crée, tout se transforme”
- Bilan de masse entre les instants t et $t + dt$
- La masse de substrat est VS, celle des micro-organismes est VX (mesurées en grammes g).

$$
\begin{aligned}
VX\mid_{t+dt} - VX\mid_t &= & -F_{out}Xdt & \; +\mu VXdt \\
VS\mid_{t+dt} - VS\mid_t &= F_{in}S_{in}dt & -F_{out}Sdt & \; -k\mu VXdt \\
V\mid_{t+dt} - V\mid_t &= F_{in}dt & -F_{out}dt &
\end{aligned}
$$

Equations du chemostat

On divise par dt

$$\begin{aligned}
\frac{dVX}{dt} &= & & - F_{out}X &+ \mu VX \\
\frac{dVS}{dt} &= F_{in}S_{in} & & - F_{out}S &- k\mu VX \\
\frac{dV}{dt} &= F_{in} & & - F_{out} &
\end{aligned}$$

D'où

$$\begin{aligned}
X\frac{dV}{dt} + V\frac{dX}{dt} &= & & -F_{out}X & +\mu VX \\
S\frac{dV}{dt} + V\frac{dS}{dt} &= F_{in}S_{in} & & -F_{out}S & -k\mu VX \\
\frac{dV}{dt} &= F_{in} & & -F_{out} &
\end{aligned}$$

Equations du chemostat

$$
\begin{aligned}
F_{in}X - F_{out}X + V\frac{dX}{dt} &= && -F_{out}X && +\mu VX \\
F_{in}S - F_{out}S + V\frac{dS}{dt} &= F_{in}S_{in} && -F_{out}S && -k\mu VX \\
\frac{dV}{dt} &= F_{in} && -F_{out}
\end{aligned}
$$

On simplifie les termes $F_{out}X$ et $F_{out}S$ et on divise par V :

$$
\begin{aligned}
\frac{dX}{dt} &= && - \frac{F_{in}}{V}X && + \mu X \\
\frac{dS}{dt} &= \frac{F_{in}}{V}S_{in} && - \frac{F_{in}}{V}S && - k\mu X \\
\frac{dV}{dt} &= F_{in} && - F_{out}
\end{aligned}
$$

Equations du chemostat

On note

$$D = \frac{F_{in}}{V}$$

le taux de dilution, qui s'exprime en $1/h$. On obtient

$$\begin{aligned}
\frac{dX}{dt} &= -DX + \mu X \\
\frac{dS}{dt} &= D(S_{in} - S) - k\mu X \\
\frac{dV}{dt} &= F_{in} - F_{out}
\end{aligned}$$

- en “batch”: $F_{in} = 0 = F_{out}$. Donc $D = 0$
- en ”fed batch”: $F_{in} > 0$, $F_{out} = 0$
- en continu: $F_{in} = F_{out}$. Volume constant.

Exemples de cinétiques μ

- Linéaire

$$\mu(S) = \alpha S$$

- Monod

$$\mu(S) = \frac{\mu_{max} S}{K + S}$$

- Haldane

$$\mu(S) = \frac{\mu_{max} S}{K + S + S^2/K_i}$$

Batch ($D = 0$) et $\mu(S) = \alpha S$

- $X' = \mu(S)X = \alpha SX$
- $S' = -k\mu(S)X = -k\alpha SX$

$$S' + kX' = 0 \Longrightarrow S + kX = L$$

On en déduit

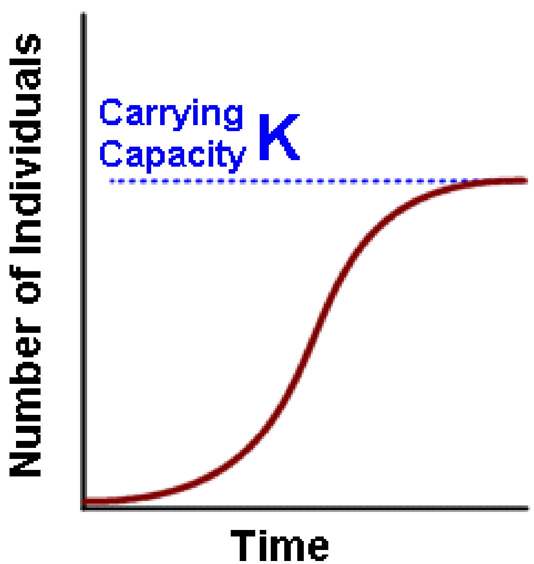
$$X' = \alpha(L - kX)X = \alpha LX(1 - kX/L)$$

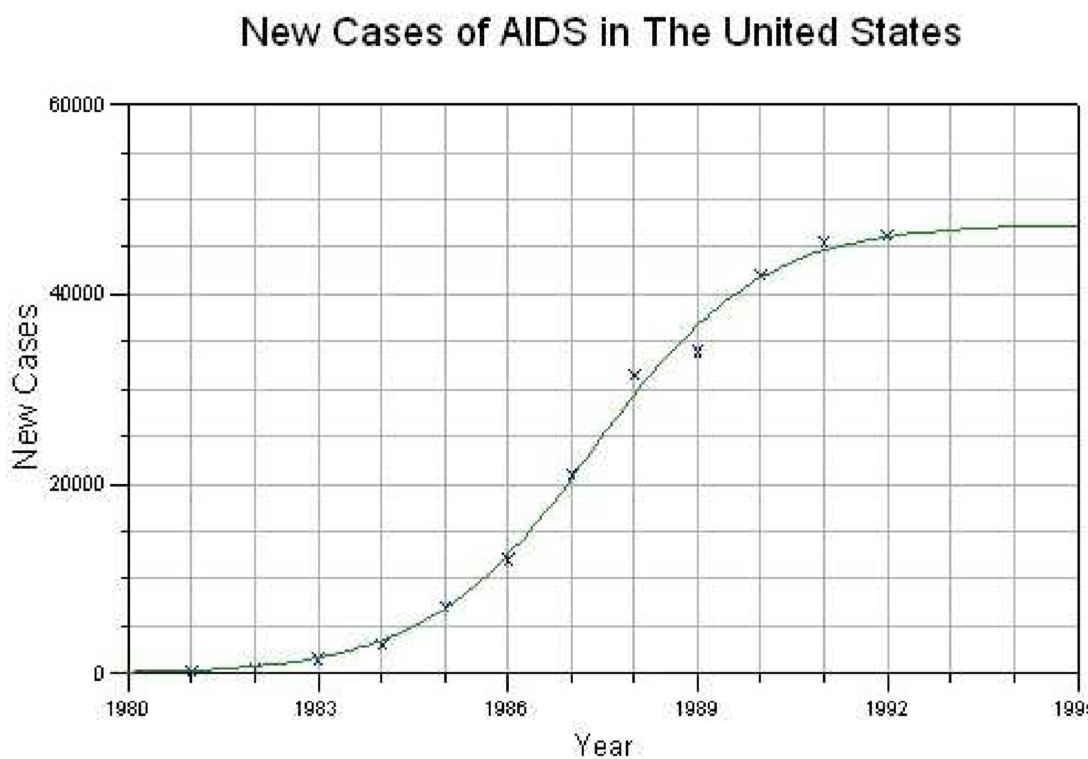
C'est l'équation logistique $X' = rX(1 - X/K)$

The Verhulst or Logistic Growth

$$\frac{x'}{x} = r\left(1 - \frac{x}{K}\right)$$

- $K > 0$ is called the carrying capacity
- $r > 0$ is called the maximal growth rate





Mathematical model of the Chemostat

$$\begin{cases} S' = D(S_{in} - S) - \frac{\mu(S)}{Y} X \\ X' = (\mu(S) - D)X \end{cases}$$

- S is the substrate density
- X is the species density
- $D = Q/V$ is the dilution rate
  $Q = F_{in} = F_{out}$ is the flow rate and V is the volume
- Y is the yield coefficient
- $\mu(S)$ is the specific growth rate of the species

The chemostat : equilibrium point

- $E_0 = (S = S_{in}, x = 0)$ (washout)
- $E^* = (S^*, x^*)$, $S^* = \mu^{-1}(D)$ and $x^* = Y(S_{in} - S^*)$
- $\mu^{-1}(D)$ is called the break-even concentration
- E^* exits and is stable if and only if $\mu(S_{in}) > D$

$$A = \begin{bmatrix} -D - \frac{\mu'(S^*)x^*}{Y} & -\frac{D}{Y} \\ \mu'(S^*)x^* & 0 \end{bmatrix}$$

$$\mathrm{tr}(A) = -D - \frac{\mu'(S^*)x^*}{Y} < 0, \quad \det(A) = D\frac{\mu'(S^*)x^*}{Y} > 0$$

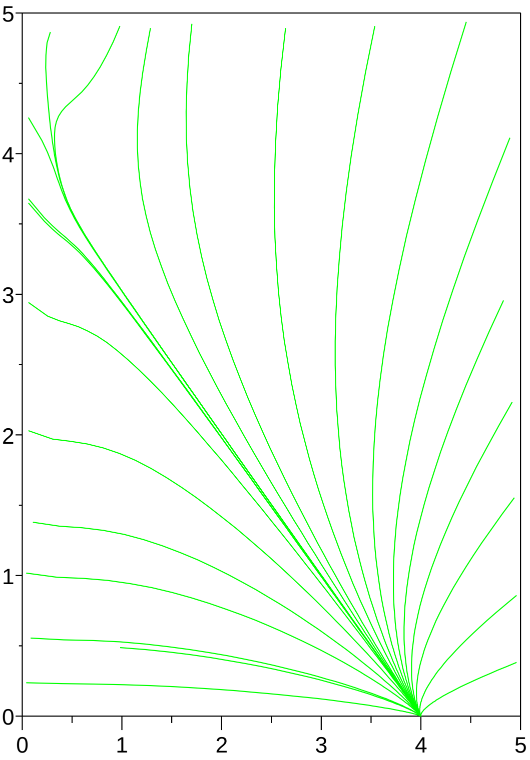
Hence the eigenvalues have negative real parts

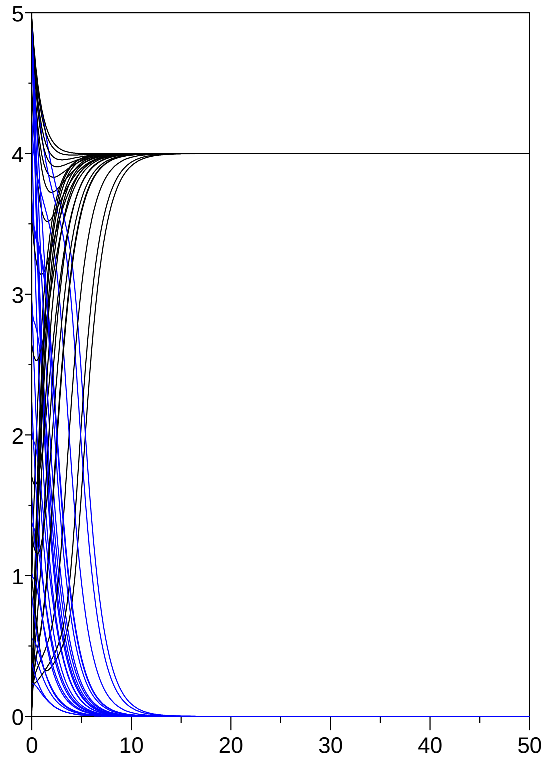
Inhibition by the substrate

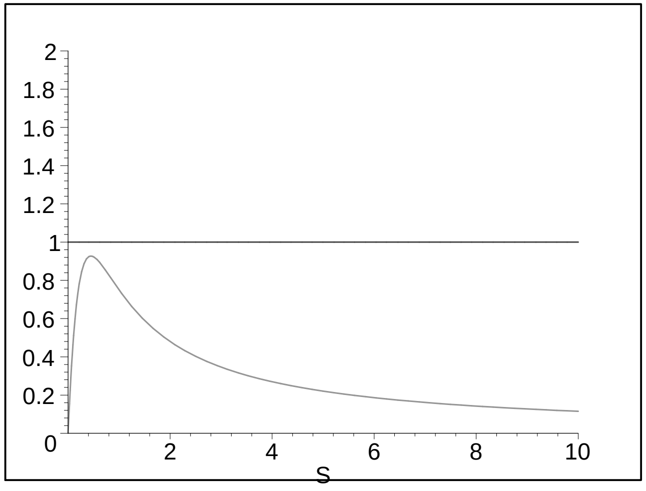
$$\left\{ \begin{array}{rcl} S' & = & D(S_{in} - S) - \frac{\mu(S)}{Y} x \\ x' & = & (\mu(S) - D)x \end{array} \right.$$

- $\mu(S) = \frac{mS}{K+S+S^2/K_i}$ is a Haldane function
- equation $\mu(S) = D$ can have two solutions $S_1^* < S_2^*$
- $E_1^* = (S_1^*, Y(S_{in} - S_1^*))$ exists if and only if $S_1^* < S_{in}$. It is stable.
- $E_2^* = (S_2^*, Y(S_{in} - S_2^*))$ exists if and only if $S_2^* < S_{in}$. It is unstable and E_0 is stable.

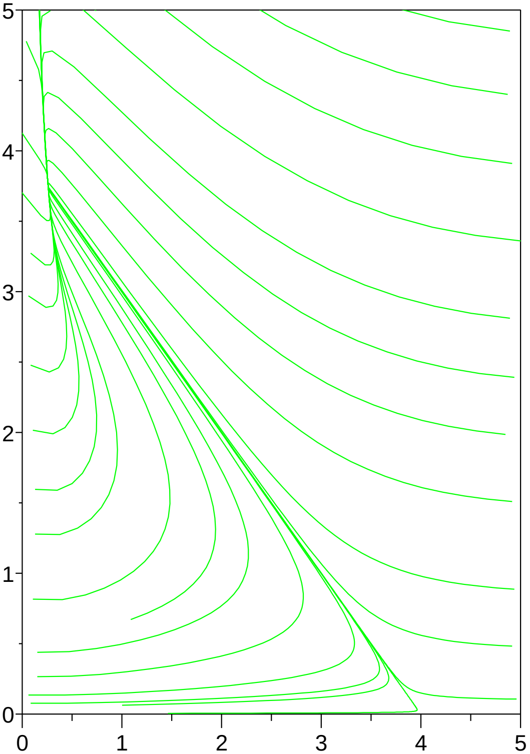
Inhibition : washout

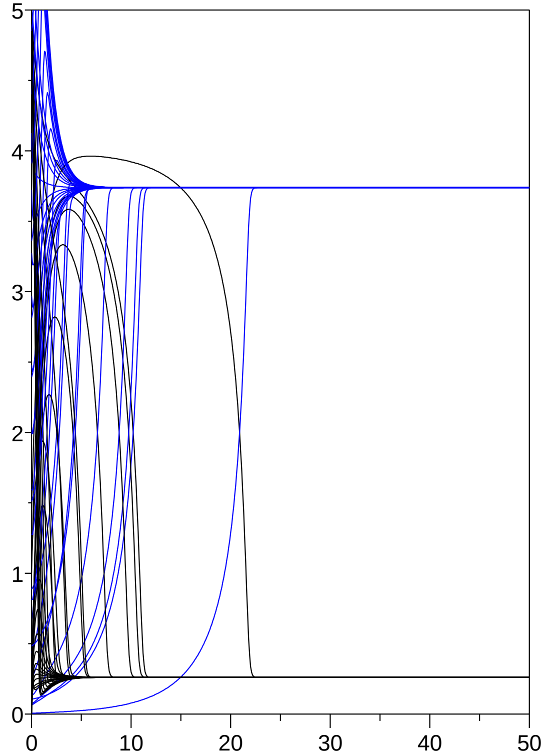


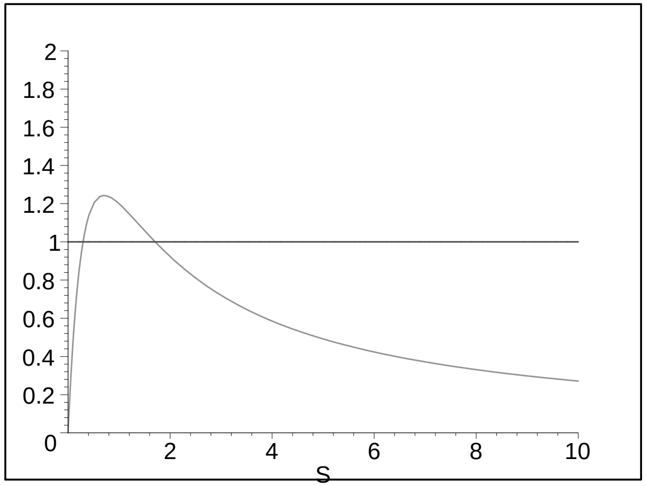




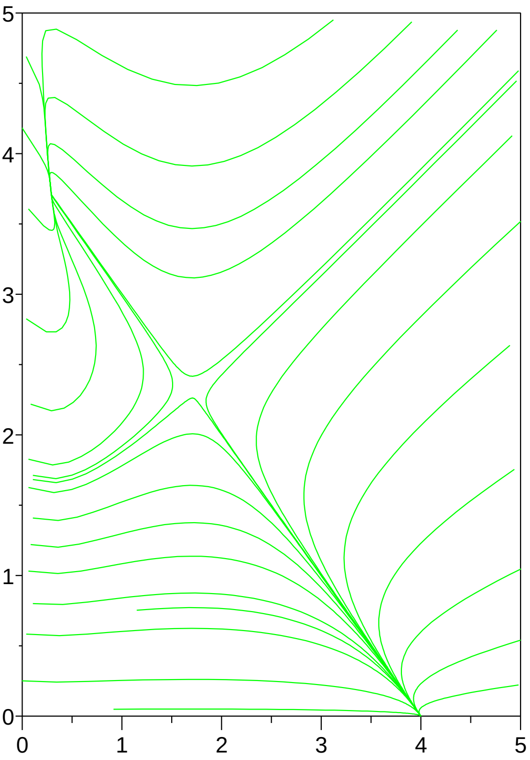
Inhibition : one equilibrium

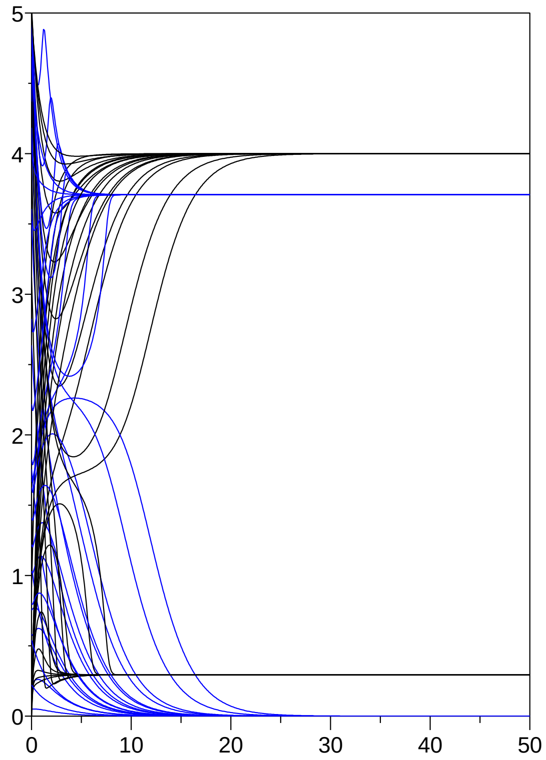


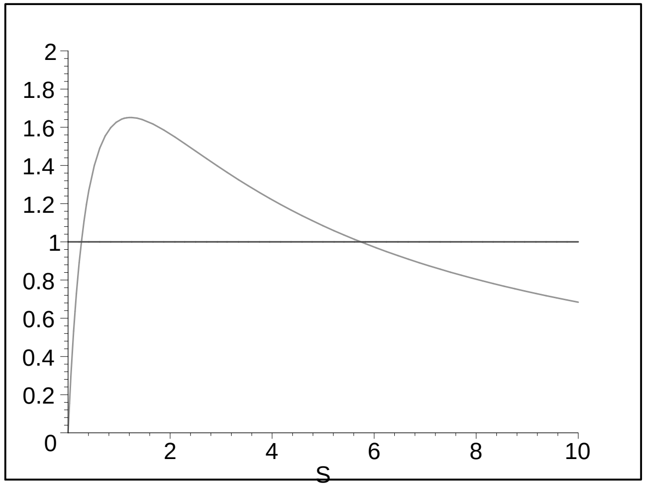




Inhibition : bistability







Competion in the chemostat

$$\begin{cases} S' &= D(S_{in} - S) - \mu_1(S)x_1 - \mu_2(S)x_2 \\ x_1' &= (\mu_1(S) - D)x_1 \\ x_2' &= (\mu_2(S) - D)x_2 \end{cases}$$

- Break-even concentrations : $\lambda_i = \mu_i^{-1}(D)$
- $E_0 = (S = S_{in}, x_1 = 0, x_2 = 0)$
- $E_1 = (S = \lambda_1, x_1 = S_{in} - \lambda_1, x_2 = 0)$
- $E_2 = (S = \lambda_2, x_1 = 0, x_2 = S_{in} - \lambda_2)$
- If $\lambda_1 < \lambda_2$ then E_1 is stable and E_0 and E_2 are unstable

The Competitive Exclusion Principle

- If $\lambda_1 < \lambda_2$ then $E_1 = (\lambda_1, S_{in} - \lambda_1, 0)$ is a globally asymptotically stable (GAS) equilibrium.
- The solutions with positive initial conditions converge to

$$S = \lambda_1, \qquad x_1 = S_{in} - \lambda_1, \qquad x_2 = 0$$

- At equilibrium E_1 the species x_2 is excluded
- the CEP is in contradiction with the observations
- The mathematical model is not good : find mechanisms that explain the coexistence

Hansen and Hubbel experiments

$$\begin{cases} S' &= D(S_0 - S) - \frac{1}{y1}\frac{\mu_1 S N_1}{K_1+S} - \frac{1}{y2}\frac{\mu_2 S N_2}{K_2+S} \\ N_1' &= \frac{\mu_1 S N_1}{K_1+S} - DN_1 \\ N_2' &= \frac{\mu_2 S N_2}{K_2+S} - DN_2 \end{cases}$$

- $S = J_i$ is defined by $\frac{\mu_i S}{K_i+S} = D$

$$J_i = K_i \frac{D}{\mu_i - D}$$

If $J_1 < J_2$ then the species N_1 wins the competition

Hansen and Hubbel experiments

Single-Nutrient Microbial Competition: Qualitative Agreement Between Experimental and Theoretically Forecast Outcomes

Abstract. *When microbial strains compete for the same limiting nutrient in continuous culture, resource-based competition theory predicts that only one strain will survive and all others will die out. The surviving strain expected from theory will be the one with the smallest subsistence or "break-even" concentration of the limiting resource, a concentration defined by the J parameter. This prediction has been confirmed in the case of auxotrophic bacterial strains competing for limiting tryptophan. Because the value of J can be measured on the strains grown alone, the theory can predict the qualitative outcomes of mixed-growth competition in advance of actual competition.*

In the past 20 years a mechanistic theory of microbial competition has been under development (*1*), an extension of the theory of single-strain growth in continuous culture formulated independently by Monod (*2*) and Novick and Szilard (*3*). This theory generates a critical parameter J which, in principle, can be used to predict the surviving strain in mixed-strain culture on a single limiting nutrient. We now report specific experimental tests that support the J criterion as a means for successfully predicting the competitive outcome when the limiting resources are known.

For two competing strains grown in mixed continuous culture, a laboratory idealization of an environment with a

ing nutrient, and D represents the influent and effluent rates of medium. For the ith organism, N_i is the concentration of cells in the culture, D is the death rate due to cell outflow, μ_i is the maximum per cell division (birth) rate, y_i is the yield (cells per unit of nutrient), and K_{s_i} is the half-saturation constant for the limiting resource (*4*).

Hsu *et al.* (*5*) have mathematically analyzed the global asymptotic behavior of Eq. 1 and its extension to an arbitrary n competing species or strains. They have proved that any system governed by the n-species generalization of Eq. 1 will approach a globally stable equilibrium, in which either (i) all competitors die out

. . . n, and $\lim S = J_1$. These results have been extended to cases of unequal death rates, in which case the D's are subscripted for each species in Eqs. 1 and 2 (*7*). The parameter J_i defines the subsistence concentration of the limiting resource for the ith species, and the steady-state concentration of the resource when ith species is grown alone.

The J criterion for competitive ability is nonobvious and requires experimental verification. It could not have been predicted from classical theories of competition (*8*). A priori it might have been expected that the winner would always be the species with the highest affinity (lowest K_s) for the nutrient, or perhaps the organism with the highest intrinsic rate of increase; in fact there are conflicting opinions on this question (*9*). However, the extended theory of Monod and of Novick and Szilard asserts that it is actually a weighted K_s value which is critical to competitive success—weighted by the ratio of the death rate to intrinsic rate of increase. Thus, a species with a higher affinity for the resource may nevertheless lose if it also has a lower intrinsic rate of increase or higher death rate. The theory also asserts that winning will be independent of the growth efficiency inuous culture formulated independently by Monod (2) and Novick and Szilard (3). This theory generates a critical parameter J which, in principle, can be used to predict the surviving strain in mixed-strain culture on a single limiting nutrient. We now report specific experimental tests that support the J criterion as a means for successfully predicting the competitive outcome when the limiting resources are known.

For two competing strains grown in mixed continuous culture, a laboratory idealization of an environment with a constant carrying capacity, the equations of growth are

$$\frac{dS}{dt} = (S_0 - S)D - \frac{\mu_1}{y_1}\frac{S \cdot N_1}{K_{s_1} + S} - \frac{\mu_2}{y_2}\frac{S \cdot N_2}{K_{s_2} + S}$$

$$\frac{dN_1}{dt} = \frac{\mu_1 S \cdot N_1}{K_{s_1} + S} - D \cdot N_1$$

$$\frac{dN_2}{dt} = \frac{\mu_2 S \cdot N_2}{K_{s_2} + S} - D \cdot N_2 \qquad (1)$$

where S is the concentration of the one limiting nutrient in the culture (all other nutrients supplied in excess of demand), S_0 is the input concentration of the limiting ... due to cell outflow, μ_i is the maximum per cell division (birth) rate, y_i is the yield (cells per unit of nutrient), and K_{s_i} is the half-saturation constant for the limiting resource (4).

Hsu *et al.* (5) have mathematically analyzed the global asymptotic behavior of Eq. 1 and its extension to an arbitrary n competing species or strains. They have proved that any system governed by the n-species generalization of Eq. 1 will approach a globally stable equilibrium, in which either (i) all competitors die out ("washout"), or else (ii) one species survives (6). Which species survives, or whether total washout occurs, depends on S_0 and on the J parameters for each species or strain. For the ith species, the J parameter is

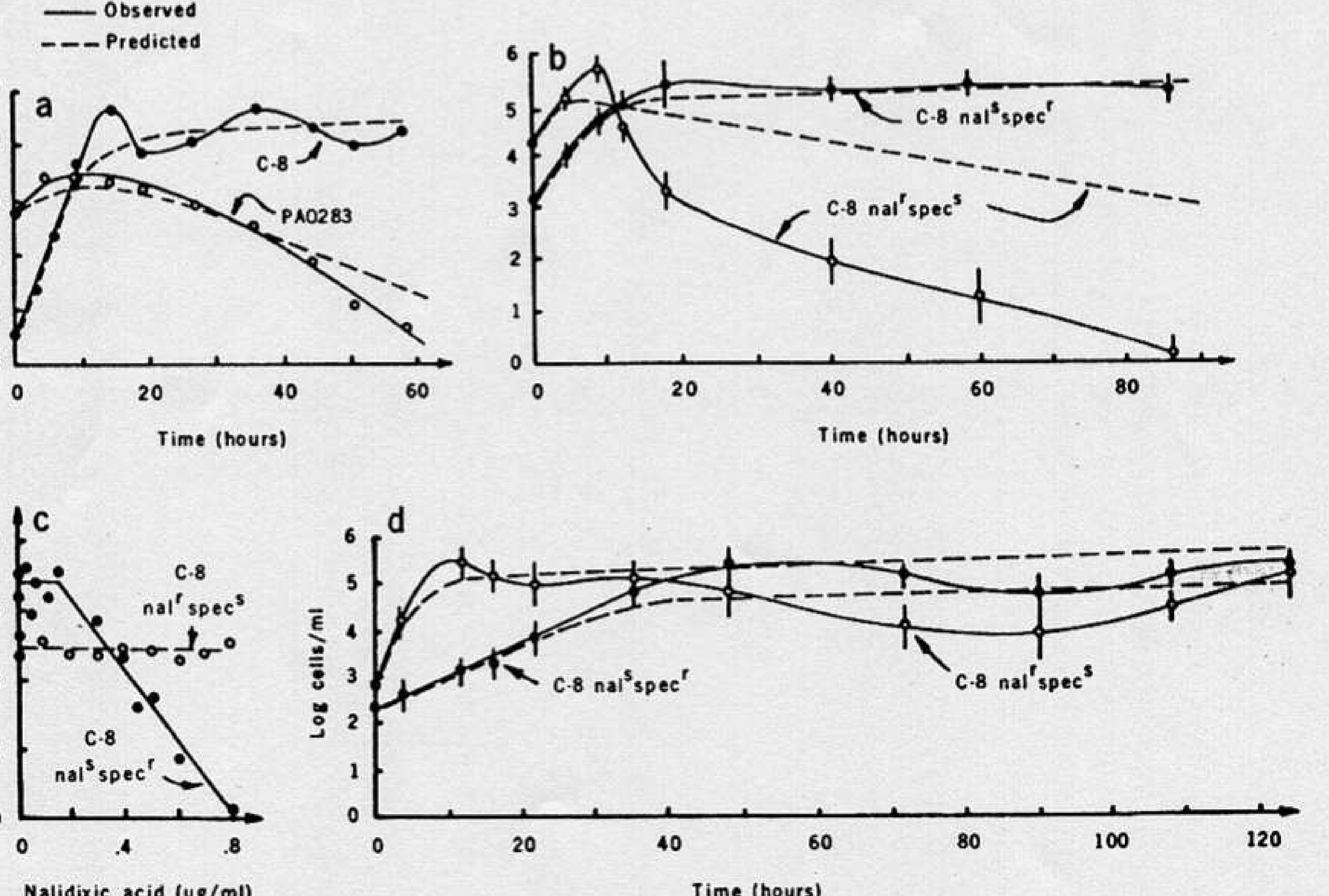
$$J_i = K_{s_i}\left(\frac{D}{r_i}\right) \qquad (2)$$

where $r_i = (\mu_i - D) > 0$, the intrinsic rate of increase of the ith species. With no loss of generality, number the species such that their J's are ordered, $J_1 < J_2 < \ldots < J_n$. Total washout occurs if $J_1 > S_0$, such that $\lim N_i = 0$, $i = 1, \ldots n$, and $\lim S = S_0$. However, if $J_1 < S_0$, then species 1 survives and outcompetes all rival species, such that $\lim N_1 = y_1(S_0 - J_1)$, $\lim N_i = 0$, $i = 2$, ... the organism with the highest intrinsic rate of increase; in fact there are conflicting opinions on this question (9). However, the extended theory of Monod and of Novick and Szilard asserts that it is actually a weighted K_s value which is critical to competitive success—weighted by the ratio of the death rate to intrinsic rate of increase. Thus, a species with a higher affinity for the resource may nevertheless lose if it also has a lower intrinsic rate or higher death rate. The theory also asserts that winning will be independent of the growth efficiency (yield) of the species grown on the limiting resource.

To make a rigorous test of the J criterion in continuous culture requires proof that (i) if two strains have equal r's and D's, the strain with the lower K_s wins; (ii) if two strains have identical K_s's and D's, the strain with the higher r wins; and (iii) if two strains have different K_s's and r's, but in spite of this still have identical J's, then the species or strains will coexist indefinitely. We have conducted all three of these tests with auxotrophic bacterial strains that require an exogenous source of tryptophan for growth. The competition experiments were conducted in two parts. First, the K_s and μ parameters were measured for each bacterial strain grown alone in batch culture

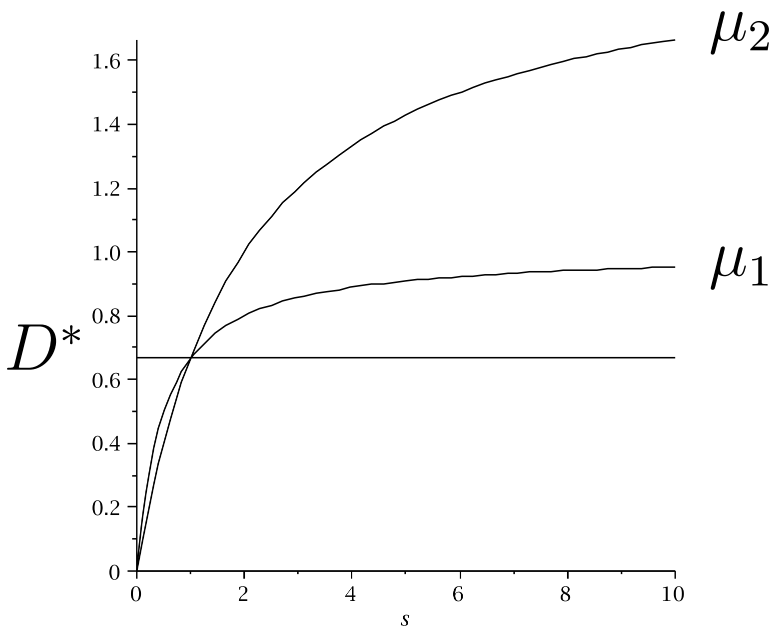
Table 1. Uptake and growth parameters for competing bacterial strains.

Auxotrophic for tryptophan



(a) Experiment 1: Strains differ principally in their half-saturation constants for tryptophan, and PAO283 loses to C-8 as predicted. (b) ment 2: Strains differ in their intrinsic rates of increase, but not in their half-saturation constants, and C-8 nal^{r}spec^{s} loses to C-8 nal^{s}spec^{r} as ed. (c) Effect of nalidixic acid on intrinsic rate of increase of strains C-8 nal^{s}spec^{r} and C-8 nal^{r}spec^{s}. (d) Experiment 3: Strains differ in the uration constants and in their intrinsic rates of increase, but nevertheless have identical J parameters, and the strains coexisted for the n of the experiment, as predicted. In each experiment, the predicted curves were obtained by numerical integration of Eq. 1. Bars around

The Competitive Exclusion Principle



- If $D < D^*$ then $\lambda_1 < \lambda_2$: the species x_1 survives and the species x_2 disappears.
- If $D > D^*$ then $\lambda_2 < \lambda_1$: the species x_2 survives and the species x_1 disappears.

Global behaviour

$$S' = D(S^0 - S) - \sum_{i=1}^{n} \frac{a_i S}{b_i + S} \frac{x_i}{Y_i}$$

$$x_i' = \left[\frac{a_i S}{b_i + S} - D_i \right] x_i, \qquad i = 1 \cdots n.$$

Assume that $\lambda_1 < \lambda_2 \leq \cdots \leq \lambda_n$. Hsu proved the global asymptotic stability of E_1^* using the function

$$V = \int_{\lambda_1}^{S} \frac{\sigma - \lambda_1}{\sigma} d\sigma + c_1 \int_{x_1^*}^{x_1} \frac{\xi - x_1^*}{\xi} d\xi + \sum_{i=2}^{n} c_i x_i,$$

where $c_i = \frac{1}{Y_i} \frac{a_i}{a_i - D_i}$, and $x_1^* = DY_1 \frac{S^0 - \lambda_1}{D_1}$.

A text book on the chemostat

Hal L. Smith, Paul Waltman (1995),
The Theory of the Chemostat
Dynamics of Microbial Competition
Cambridge Studies in Mathematical
Biology (No. 13)