OPTIMIZATION AND CONTROL OF BIO-CONVERSION OF POLYMERIC SUBSTRATE IN THE CHEMOSTAT

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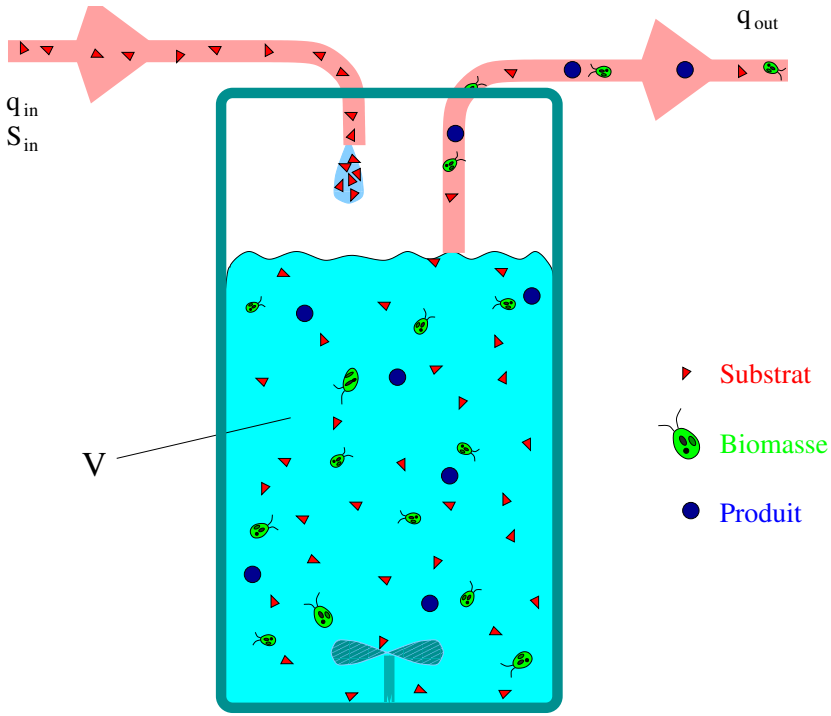
Outline

- Introduction
- The process
- The model
- Dynamics and optimization
- Control for optimization
- Conclusion

Introduction

- Bioreactor model

$$\begin{cases} \dot{s} = D(s_{in} - s) - k\mu(s)x \\ \dot{x} = (\mu(s) - D)x \end{cases} \tag{1}$$



Introduction

$$\begin{cases} \dot{s} = D(s_{in} - s) - k\mu(s)x \\ \dot{x} = (\mu(s) - D)x \end{cases}$$

- V volume, q throughput, constant volume
  $D(t) = q/V$ dilution rate CONTROL
- s substrate, x biomass VARIABLES
- s_{in} input concentration for substrate, μ growth rate, k yield
- $\mu(s)$ may be increasing (Monod model, $\mu(s) = a\frac{s}{K+s}$) or have a maximum (Haldane model)

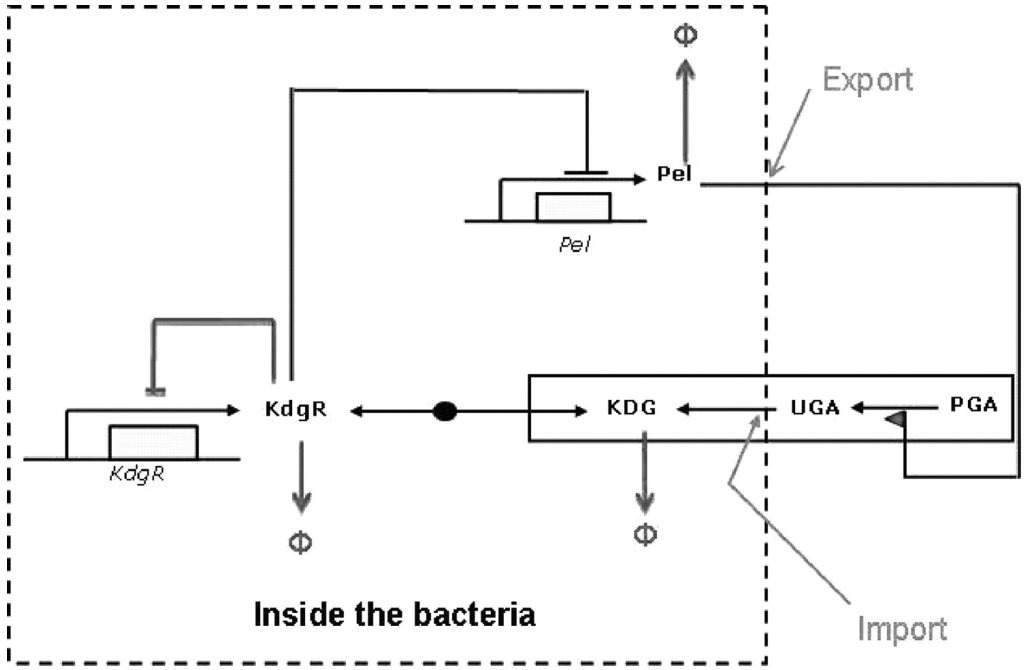
Introduction

in a chemostat
polymer input
cells cleaves polymers into small monomers
optimize the output of small monomers
near this optimum, the model is unstable (washout)
control to obtain a robust optimal equilibrium

The process

in a bioreactor (CSTR)
plant pathogenic bacterium *Dickeya dadantii*
releases plant cell wall-degrading enzymes such as pectate lyases (Pels)
these enzymes cleave pectin polymers (polygalacturonate (PGA)) into small unsaturated oligogalacturonates (UGA)
UGA is of interest because it is a precursor of biofuels
optimize the output of UGA (also used for growth)

The process



Kepseu, W. D., Sepulchre, J. A., Reverchon, S., Nasser, W. (2010). Toward a quantitative modeling of the synthesis of the pectate lyases, essential virulence factors in Dickeya dadantii. Journal of Biological Chemistry

The process

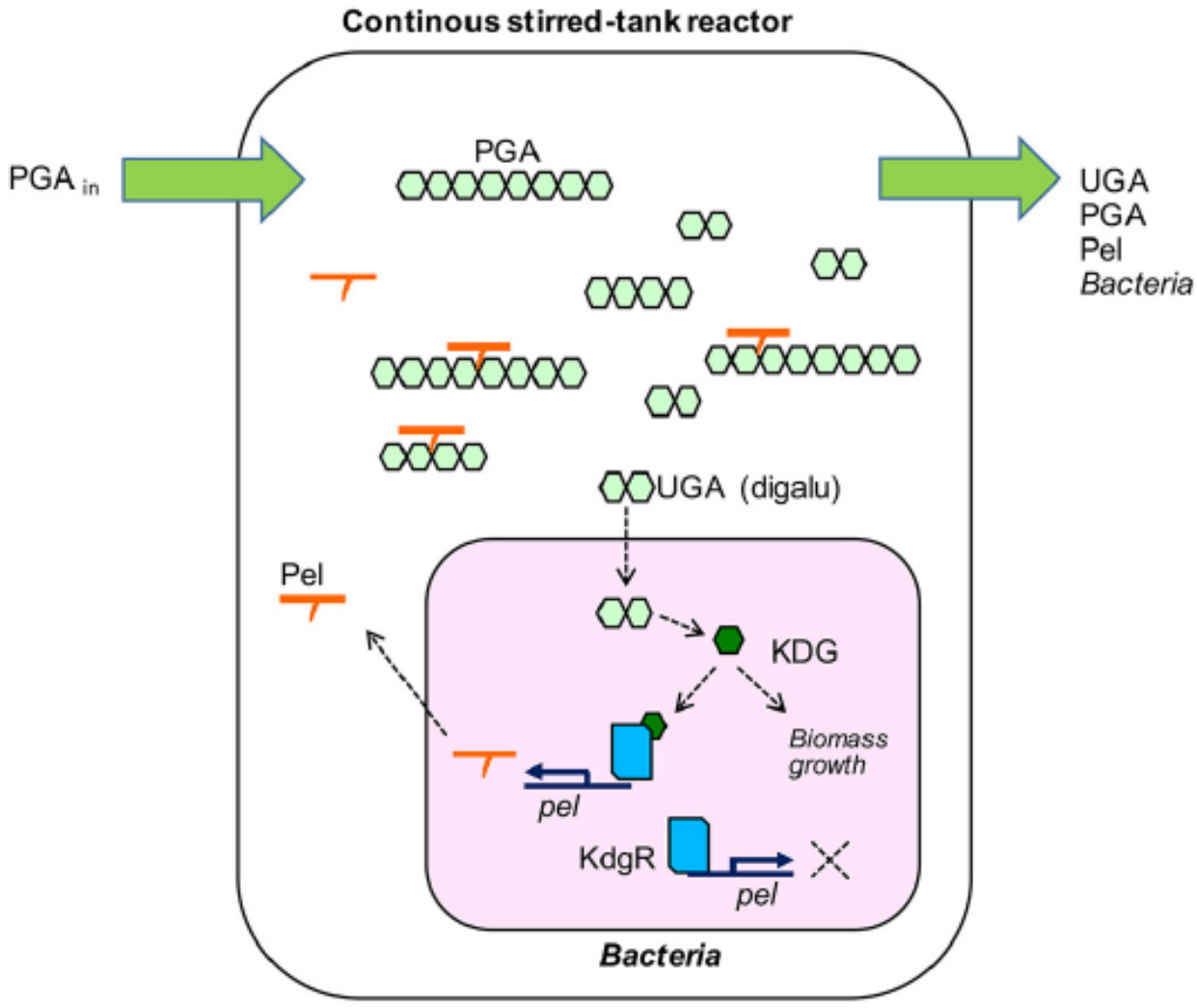
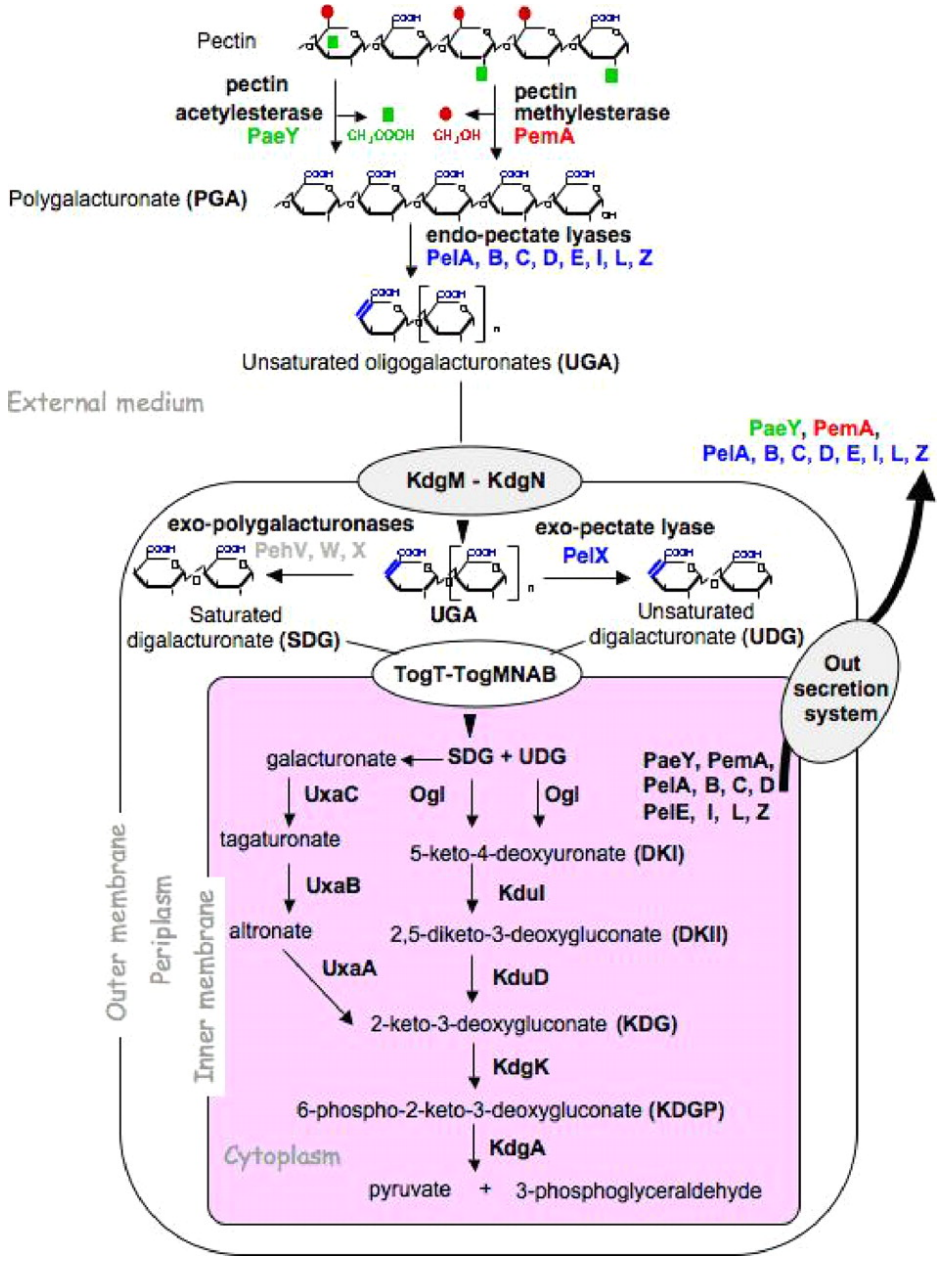


Figure 1. A *schematic representation* of the continuous reactor system.

The process


Pectin
pectin acetylesterase
PaeY
pectin methylesterase
PemA
Polygalacturonate (PGA)
endo-pectate lyases
PelA, B, C, D, E, I, L, Z
Unsaturated oligogalacturonates (UGA)
External medium
PaeY, PemA,
PelA, B, C, D, E, I, L, Z
KdgM - KdgN
exo-polygalacturonases
PehV, W, X
exo-pectate lyase
PelX
Saturated digalacturonate (SDG)
UGA
Unsaturated digalacturonate (UDG)
TogT-TogMNAB
Out secretion system
galacturonate
SDG + UDG
UxaC
OgI
OgI
PaeY, PemA,
PelA, B, C, D
PelE, I, L, Z
tagaturonate
5-keto-4-deoxyuronate (DKI)
UxaB
KduI
altronate
2,5-diketo-3-deoxygluconate (DKII)
UxaA
KduD
2-keto-3-deoxygluconate (KDG)
KdgK
6-phospho-2-keto-3-deoxygluconate (KDGP)
KdgA
Cytoplasm
pyruvate + 3-phosphoglyceraldehyde
Outer membrane
Periplasm
Inner membrane

The model

Variables s PGA, z UGA, ρ bacterial biomass
Input s_i PGA flux, D controlled dilution

$$\begin{aligned} \frac{ds}{dt} &= -\alpha(s)\rho + D(s_i - s) \\ \frac{dz}{dt} &= 2\alpha(s)\rho - \gamma\mu(z)\rho - Dz \\ \frac{d\rho}{dt} &= (\mu(z) - D)\rho \end{aligned} \tag{2}$$

$$\alpha(s) = \bar{\alpha}\frac{s}{K_m + s} \quad \text{and} \quad \mu(z) = \bar{\mu}\frac{z}{K_z + z}.$$

$\alpha(s)\rho$ represents the conversion of PGA s into UGA z, catalyzed by the biomass ρ
$\mu(z)\rho$: the cell grows on the z substrate
The number 2 means that PGA is cleaved into **two** UGA (for simplicity)

The model

this model has three variables
it is nonlinear, with Michaelis-Menten (Monod) functions
the variables are nonnegative
global study could be difficult...

The model

J. Math. Biol. (1992) 31: 1–23

Journal of
Mathematical
Biology

Mathematical models of microbial growth and competition in the chemostat regulated by cell-bound extracellular enzymes

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The model

$$\begin{aligned}
\frac{dS}{dT} &= S^0 D - SD - \frac{1}{\gamma} XF(S), \\
\frac{dP}{dT} &= -PD + XF(S) - \frac{1}{\eta} XQ(P), \\
\frac{dX}{dT} &= -XD + XQ(P), \\
S(0) \geqslant 0, &\qquad P(0) \geqslant 0, \qquad X(0) > 0,
\end{aligned} \tag{2.1}$$

where

$S(T)$ = concentration of nutrient (in the chemostat) at time T,
$P(T)$ = concentration of intermediate product at time T,
$X(T)$ = concentration of microorganisms at time T,
$F(S)$ = per-capita production rate of intermediate product as a function of the concentration of the nutrient,
$Q(P)$ = per-capita growth rate of microorganisms as a function of the concentration of the intermediate product,
γ = yield constant for conversion from nutrient to intermediate product,
η = yield constant for consumption of intermediate product,
S^0 = concentration of nutrient supply in the feed bottle of the chemostat,
D = dilution rate. (Species specific death rates are assumed to be negligible compared to the dilution rate.)

Dynamical study

Washout equilibrium E_0 for $\rho = 0$ and $s = s_i, z = 0$
depends on dilution rate D ($\mu(z) = D$ last equation)
E_0 is always locally asymptotically stable, and is globally stable if it is the only equilibrium
It may exist another unstable equilibrium E_u and a stable one E_s, with some basin of attraction. the separatrix is the stable manifold of E_u
Bifurcation diagram wrt. D

Equilibria

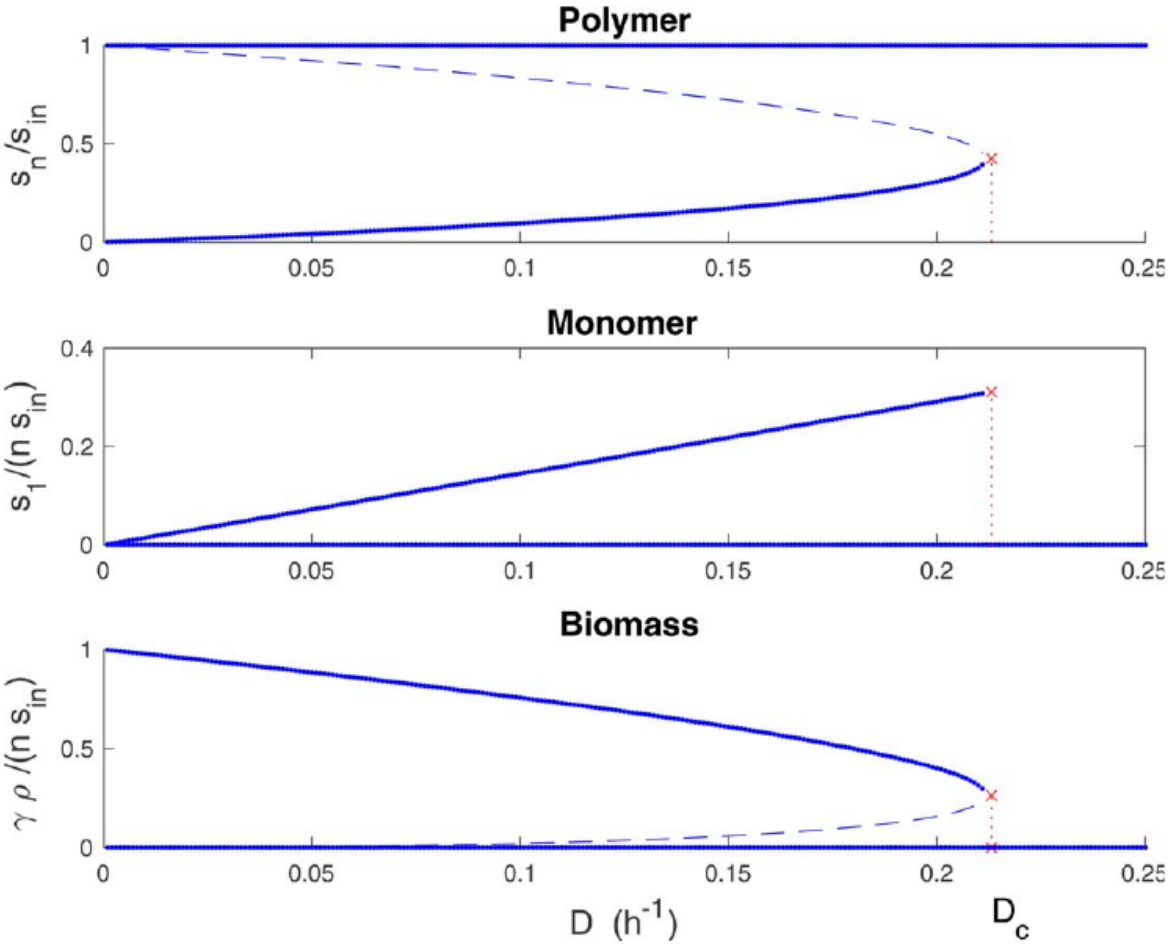


Figure 1. The bifurcation diagrams of the chemostat steady states show the existence of a limit point.

The dashed curves are unstable equilibria, whereas the plain curves are stable equilibria. For the monomer variable, the non-zero stable and unstable equilibria are superposed on the same curve. The limit value D_c is computed from the approximation (5) and the corresponding limit points are drawn with symbols "x." Parameters used in Eqs. 1 are partly taken from a study on the polygalacturonate decomposition by *Dickeya dadantii*[10] and are the following: $\bar{\alpha}=12\ h^{-1}$, $\bar{\mu}=17.1\ h^{-1}$, $\gamma=7.9$ M, $s_{in}=0.003$ M, $K_M=6.8;10^{-3}$ M, $K_1=0.14$ M, $n=2$. [Color figure can be viewed at wileyonlinelibrary.com]

Dynamical study

reduction to two dimensions
$w = z + 2s + \gamma\rho$
then $\dot{w} = 2Ds_i - Dw$
so w converges toward $2s_i$
we can reduce the system in two dimensions
(there could be some proofs with the help of theory of asymptotically autonomous systems, Thieme...)

Dynamical study

with new variables $w = z + 2s + \gamma\rho$ and $b = z + \gamma\rho$
3 variables b, ρ, w
the system is cooperative (the Jacobian matrix is off diagonal positive)
the partial order of the flow is conserved
strong properties of convergence (n dimensions)
no stable periodic orbits
global study

Dynamical study

same model in Tang Wolkowicz J Math Biol 1992

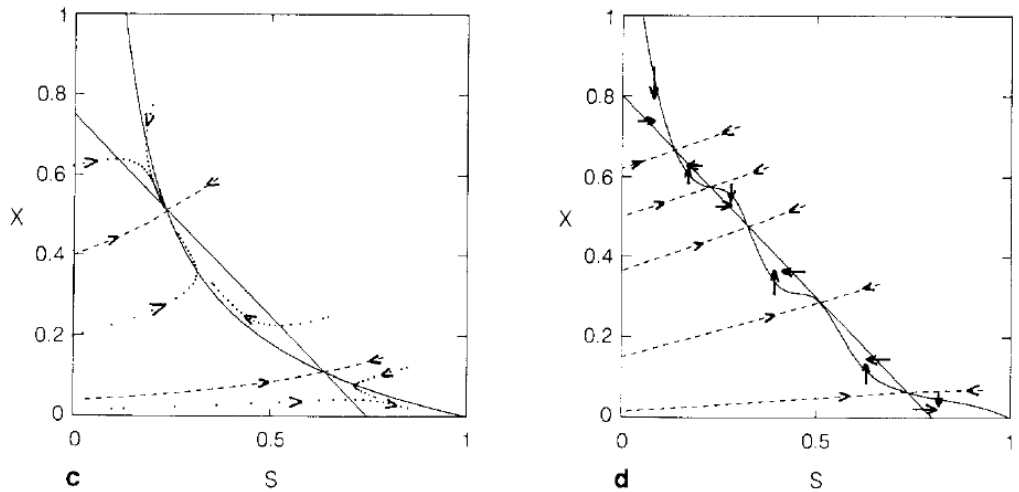
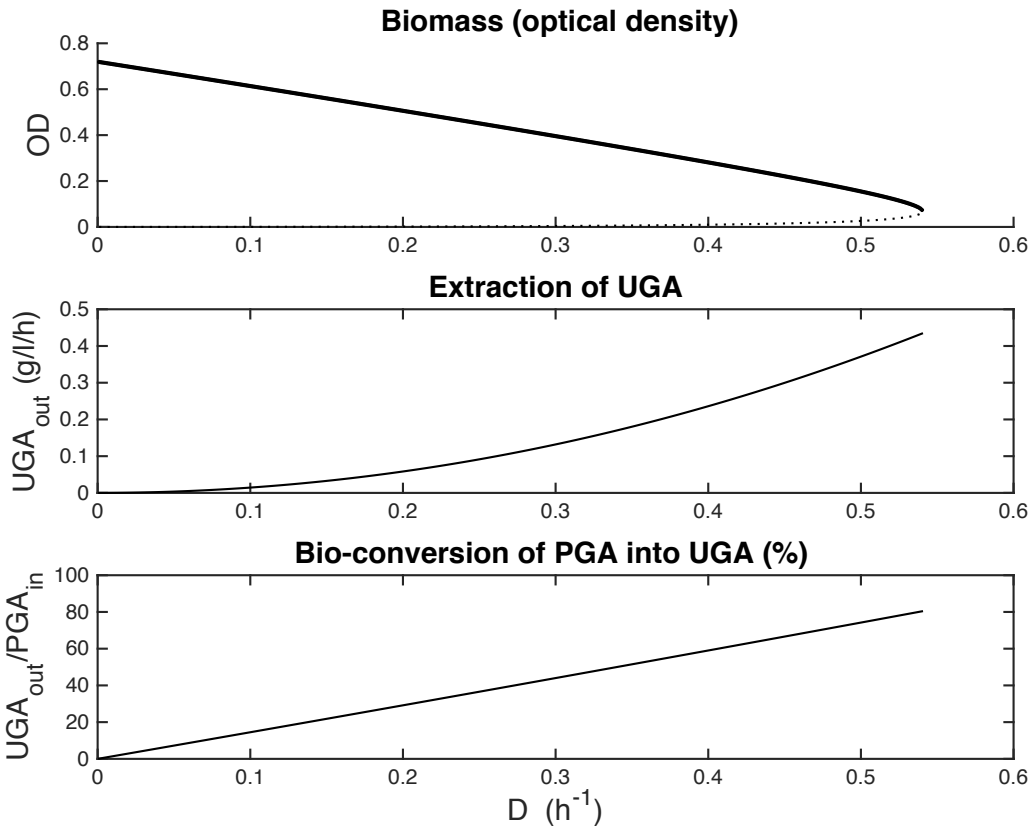


Fig. 3.2. Phase plane analysis of (3.9) for different intersections of $g(s)$ and $h(s)$. ——: isoclines $g(s)$ and $h(s)$, – – –: the stable manifold of each equilibrium transverse to the line $g(s)$, · · ·: sample trajectories. **a** $g(s)$ and $h(s)$ do not intersect, E_0 is the global attractor. **b** $g(s)$ and $h(s)$ intersect exactly once. The stable manifold of E_1 forms the separatrix. All trajectories above the separatrix converge to E_1, and those below converge to E_0. **c** $g(s)$ and $h(s)$ intersect twice, E_0 and E_2 are locally asymptotically stable and E_1 is a saddle. The stable manifold of E_1 forms the separatrix. All trajectories above (below) the separatrix converge to E_2 (E_0). **d** $g(s)$ and $h(s)$ intersect many times at $E_i = (\bar{s}_i, \bar{x}_i)$. Local stability of each equilibrium point is given by (3.10). The stable manifold of the saddle points and of the semi-stable equilibria partition the space

Optimization

output to optimize: *Dz* with control *D*



optimal point near instability (washout)
control is needed for robust stability

Control

control is needed for robust stability
The control is D
but we also need measurements for this control (feedback) for robustness
the measure is biomass $\rho(t)$
we have to design a law of control depending on ρ

Control

$$D(t) = \delta\rho(t), \tag{3}$$

where δ is a positive fixed design parameter. The new equilibrium is:

$$\begin{aligned} -\alpha(s) + \delta(s_i - s) &= 0, \\ 2\alpha(s) - \gamma\mu(z) - \delta z &= 0, \\ \mu(z) - \delta\rho &= 0. \end{aligned}$$

Theorem

Control law (3) *globally stabilizes System* (2) *towards the non-trivial equilibrium* $E^*(\delta) := (z^*(\delta), s^*(\delta), \rho^*(\delta))$.

Control

new system

$$\begin{aligned} \frac{ds}{dt} &= \rho\left[-\alpha(s) + \delta(s_i - s)\right] \\ \frac{dz}{dt} &= \rho\left[2\alpha(s) - \gamma\mu(z) - \delta z\right] \\ \frac{d\rho}{dt} &= \rho\left[(\mu(z) - \delta\rho)\right] \end{aligned} \tag{4}$$

time change will simplify ρ...
if you prove that ρ is lower bounded by a positive number... not difficult...

Control

$$\begin{aligned} \frac{ds}{dt} &= [-\alpha(s) + \delta(s_i - s)] \\ \frac{dz}{dt} &= [2\alpha(s) - \gamma\mu(z) - \delta z] \\ \frac{d\rho}{dt} &= [(\mu(z) - \delta\rho)] \end{aligned} \tag{5}$$

we obtain a system with triangular structure which is globally stable around his equilibrium (with good justifications)
δ is chosen so that $E^*(\delta)$ corresponds to optimal yield (it is possible)

Choice of δ

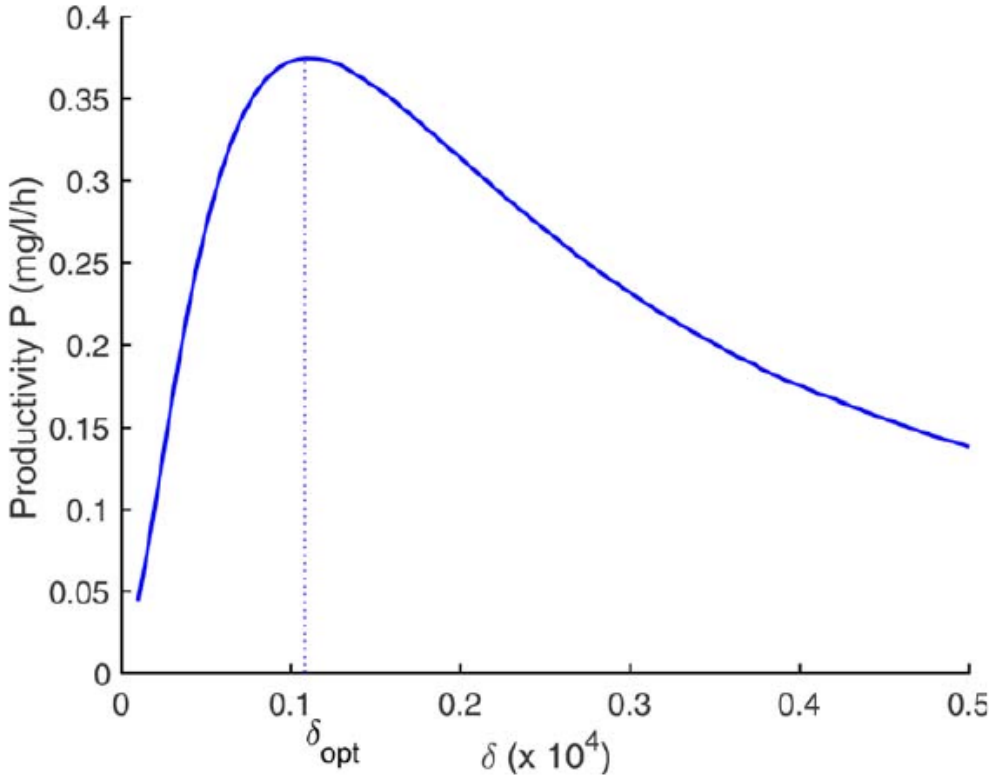


Figure 2. Productivities of the closed-loop controlled system (8), in function of control parameter δ.

The optimal parameter δ_{opt} is the abscissa corresponding to the maximal productivity. System parameters used in equations are the same as for Figure 1. [Color figure can be viewed at wileyonlinelibrary.com]

Simulation of control

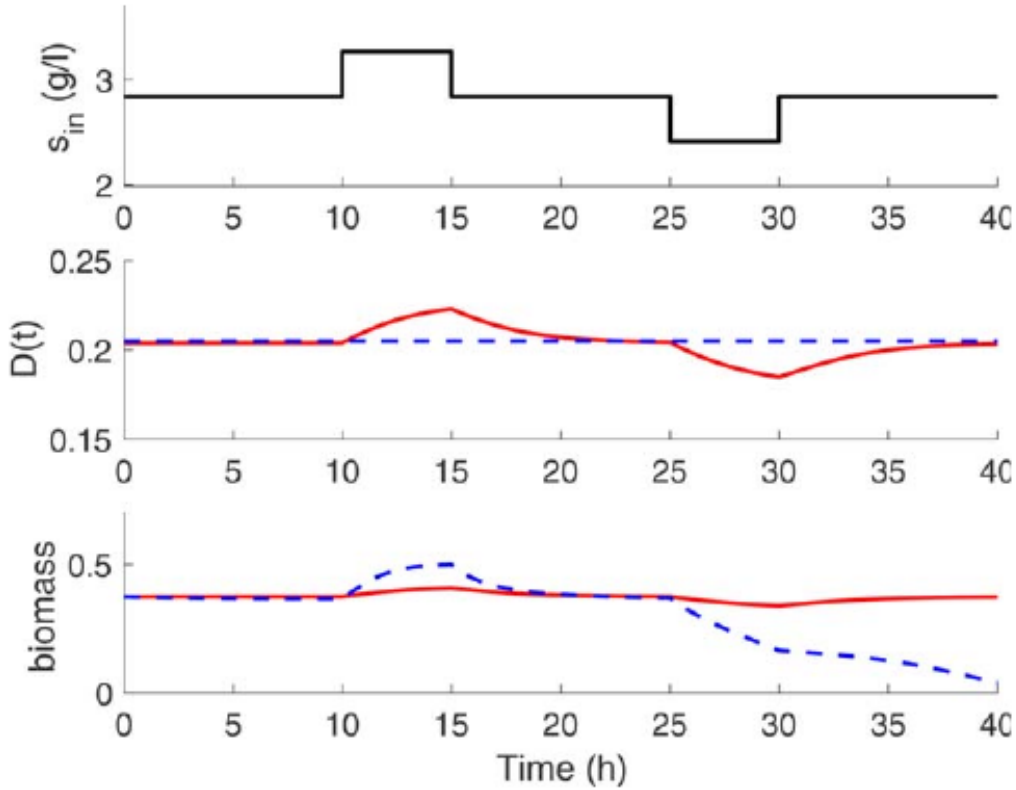


Figure 3. Comparison of the controlled (continuous red lines) and uncontrolled system (dashed blue lines) when it works at its optimal point in presence of a fluctuating input s_{in} of the chemostat.

A fragmentation model

enzymatic decomposition could have several steps

$$S_l + E \underset{d_{ml}}{\overset{a_{ml}}{\rightleftharpoons}} C_{ml} \xrightarrow{k_{ml}} S_{l-m} + S_m + E$$

more complex model

A fragmentation model

similar treatment

equations

$$\frac{ds_n}{dt} = D(s_{in} - s_n) - \frac{\bar{\alpha}\rho}{K_M + \sum_{j=2}^{n}(j-1)s_j}(n-1)s_n$$

$$\frac{ds_l}{dt} = -Ds_l + \frac{\bar{\alpha}\rho}{K_M + \sum_{j=2}^{n}(j-1)s_j}$$

$$\left[\sum_{j=l+1}^{n} 2s_j - (l-1)s_l\right], \; l = n-1, \ldots, 2$$

$$\frac{ds_1}{dt} = -Ds_1 + \frac{\bar{\alpha}\rho}{K_M + \sum_{j=2}^{n}(j-1)s_j}\left[\sum_{j=2}^{n} 2s_j\right] - \gamma\mu(s_1)\rho$$

$$\frac{d\rho}{dt} = (\mu(s_1) - D)\rho$$

A fragmentation model

similar results for a control $D = \delta\rho(t)$

equations

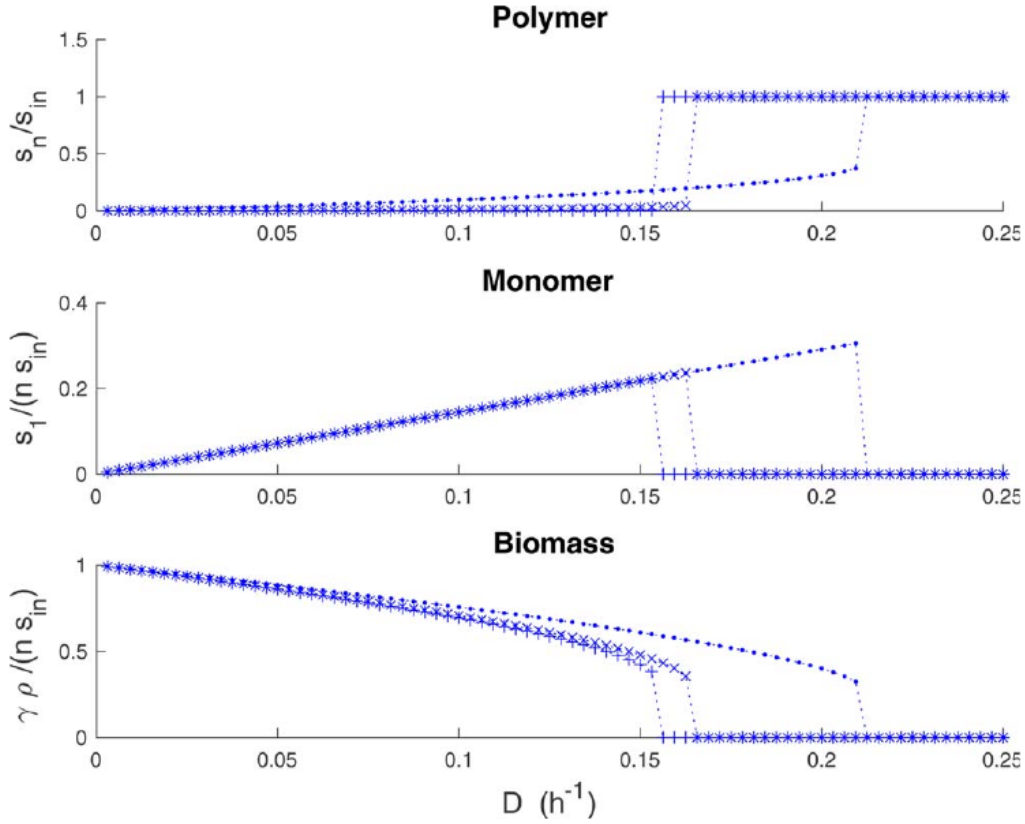


Figure 4. Bifurcation diagrams of the nontrivial stable steady states of the concentrations of *n*-polymers, of monomers and biomass in the chemostat, for different values of *n*.

The symbols represent respectively (•) $n = 2$, (x) $n = 10$, (+) $n = 100$. Parameter values as in Figure 1. [Color figure can be viewed at wileyonlinelibrary.com]

Conclusions

- control is needed for optimization of the output for optimal yield in a bioreactor
- we are able to obtain global stability in the whole space
- very robust optimal point (with control)
- extension with a second substrate (glucose)
- extension with a more detailed model
- robustness
- real implementation?
- adaptive control is possible...

paper

Optimization and control of bio-conversion of polymeric substrate in the chemostat

Jacques-Alexandre Sepulchre, Francis Mairet, Jean-Luc Gouzé

AIChE Journal, Wiley, 2017, 63 (11), pp.4738-4747.

Thank you!

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