Ecole CIMPA Vert numérique, Septembre 2022

Optimizing Bacterial Resource Allocation: Metabolite Production in Continuous Bioreactors*

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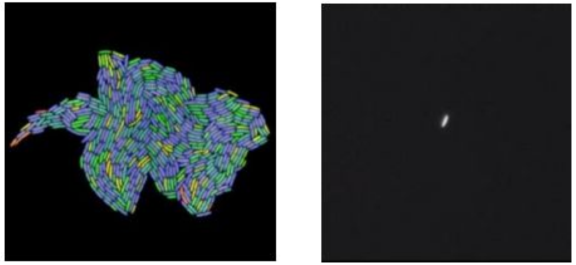
*This work was partially supported by ANR project Maximic (ANR-17-CE40-0024-01), Inria IPL Cosy and Labex SIGNALIFE (ANR-11-LABX-0028-01).

Bacterial growth

- Bacteria are unicellular organisms naturally geared towards growth.
- For biotechnological applications, one would like to change the natural resource allocation strategies of the cell.

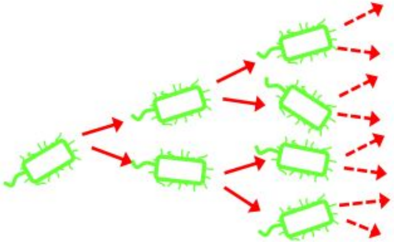
de Jong et al. (2017), Trends Microbiol., 25(6):480-93



Stewart *et al.* (2005), *PLoS Biol.*, 3(2): e45

Tools used:

- Simple self-replicator model of resource allocation
- Dynamical systems analysis
- Optimization, (Optimal control theory)

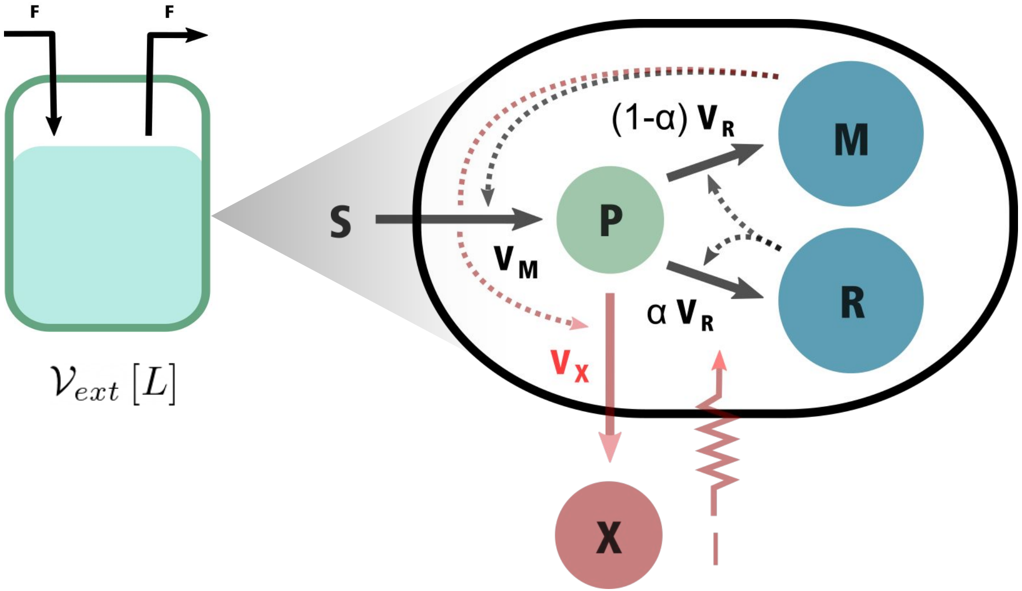


Model definition

Metabolite production in continuous bioreactors

The system is described by the following reactions:

$$S \xrightarrow{V_M} P,$$
$$P \xrightarrow{V_R} \alpha R + (1\text{-}\alpha)\, M,$$
$$P \xrightarrow{V_X} X.$$



S: Incoming nutrient
P: Precursor metabolites
R: Gene expression machinery
M: Metabolic machinery
X: Metabolite of interest

Giordano, Nils, et al. **Dynamical allocation of cellular resources as an optimal control problem: novel insights into microbial growth strategies.** PLoS computational biology 12.3 (2016).

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Model definition

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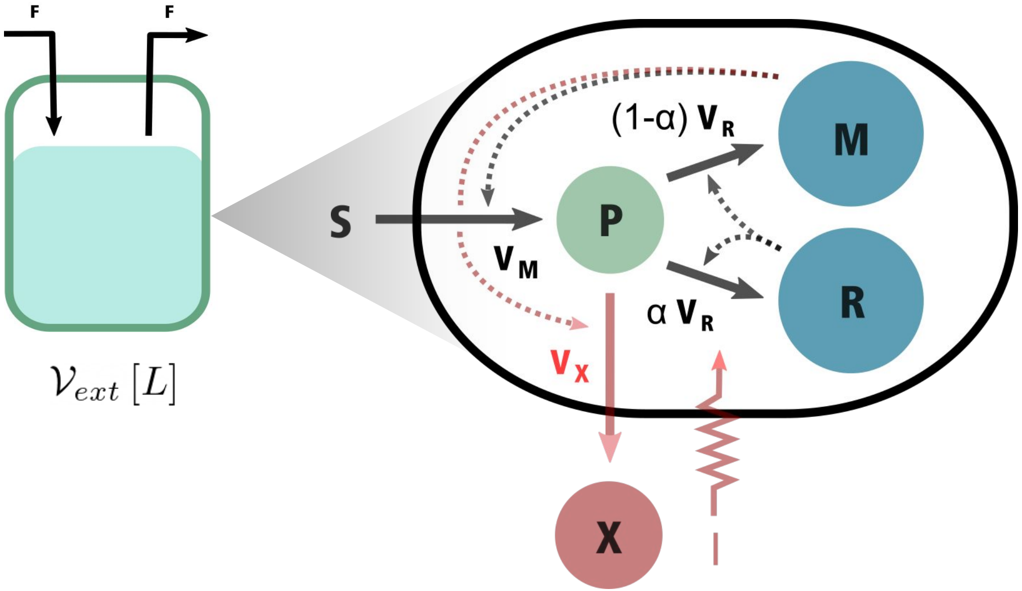
$$
\begin{cases}
\dot{S} = V_{S_{in}} - V_M - V_{S_{out}}, \\
\dot{P} = V_M - V_R - V_X - V_{P_{out}}, \\
\dot{M} = (1-u)\,V_R - V_{M_{out}}, \\
\dot{R} = uV_R - V_{R_{out}}, \\
\dot{X} = V_X - V_{X_{out}},
\end{cases}
$$



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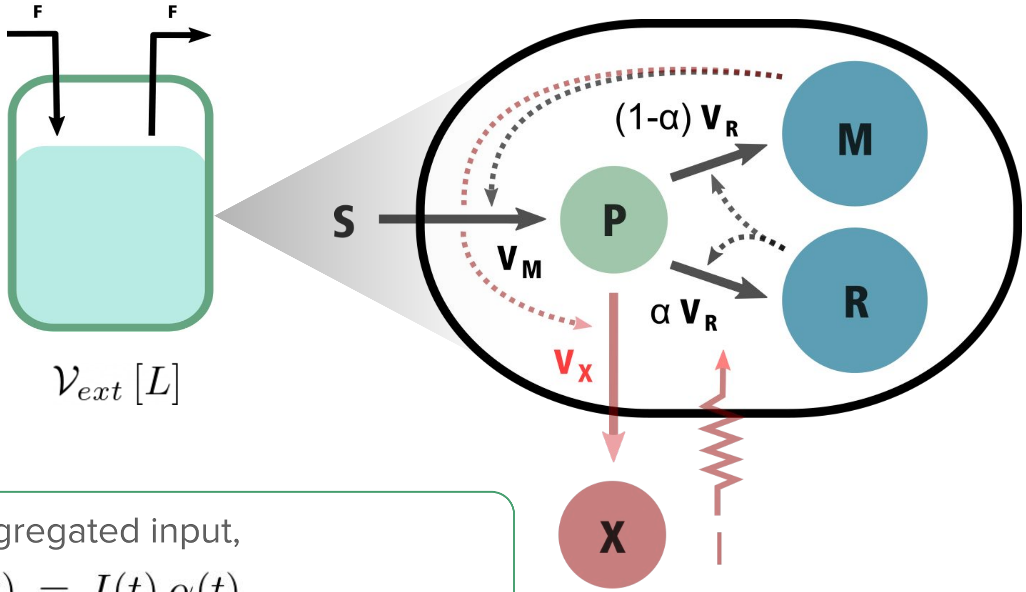

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Aggregated input,

$$u(t) \;=\; I(t)\,\alpha(t),$$

$\alpha(t)$: natural allocation strategy
$I(t)$: external control

Model definition

Express in terms of concentrations

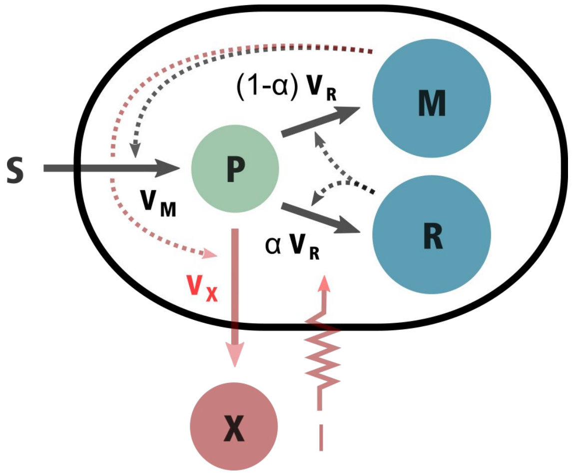
Cytoplasmic density of the cells in the population is constant, with $\beta > 0 \; [\mathrm{L\ g^{-1}}]$ as the inverse density.

$$\mathcal{V} \doteq \beta(M + R) \qquad\qquad \mu \doteq \left.\frac{\dot{\mathcal{V}}}{\mathcal{V}}\right|_{F=0}$$

which allows to express mass quantities per unit population volume,

$$p \doteq \frac{P}{\mathcal{V}}, \quad r \doteq \frac{R}{\mathcal{V}}, \quad m \doteq \frac{M}{\mathcal{V}}, \quad s \doteq \frac{S}{\mathcal{V}_{ext}}, \quad x \doteq \frac{X}{\mathcal{V}_{ext}}, \qquad [gL^{-1}]$$

$$v_M(s, m) \doteq \frac{V_M}{\mathcal{V}}, \quad v_R(p, r)\cdot \doteq \frac{V_R}{\mathcal{V}}, \quad v_X(p, m) \doteq \frac{V_X}{\mathcal{V}}, \qquad [gL^{-1}h^{-1}]$$



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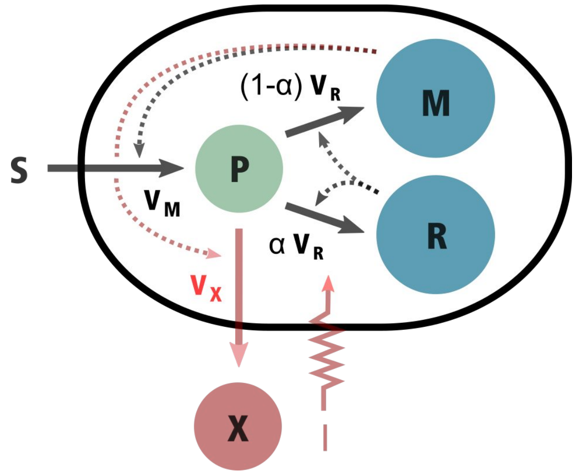
Metabolite production in continuous bioreactors

$$S: \begin{cases} \dot{s} = D(s_{in} - s) - v_M(s,m)\frac{\mathcal{V}}{\mathcal{V}_{ext}}, \\ \dot{p} = v_M(s,m) - v_R(p,r) - v_X(p,m) - \mu(p,r)p, \\ \dot{r} = u\, v_R(p,r) - \mu(p,r)r, \\ \dot{m} = (1-u)\, v_R(p,r) - \mu(p,r)m, \\ \dot{x} = v_X(p,m)\frac{\mathcal{V}}{\mathcal{V}_{ext}} - Dx, \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\,\mathcal{V}, \end{cases}$$

where the dilution rate is $D = \frac{F}{\mathcal{V}_{ext}}\,[h^{-1}]$ and $\mu(p,r) = \beta v_R(p,r)$.



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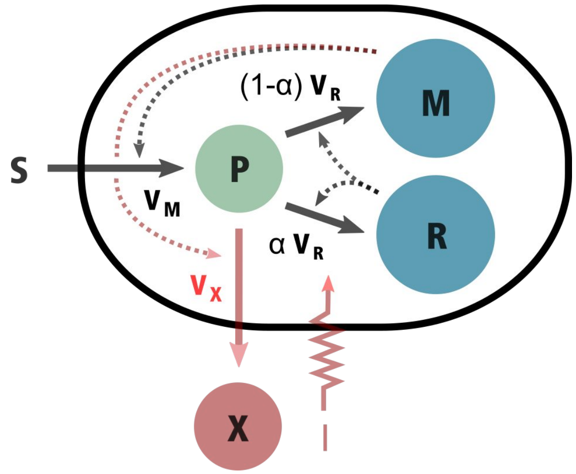
Model definition

Metabolite production in continuous bioreactors

$$v_M(s,m) \doteq k_M\, m \frac{s}{K_M + s} = k_M\,(1-r)\frac{s}{K_M+s},$$
$$v_R(p,r) \doteq k_R\, r \frac{p}{K_R + p},$$
$$v_X(p,m) \doteq k_X\, m \frac{p}{K_X + p} = k_X\,(1-r)\frac{p}{K_X+p},$$



$$\boxed{m + r = \frac{1}{\beta}}$$

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Metabolite production in continuous bioreactors

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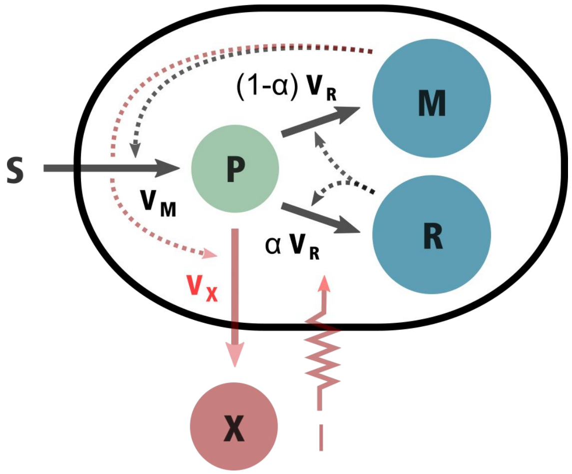

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Asymptotic behavior

Mass conservation laws

Rearranging the system,

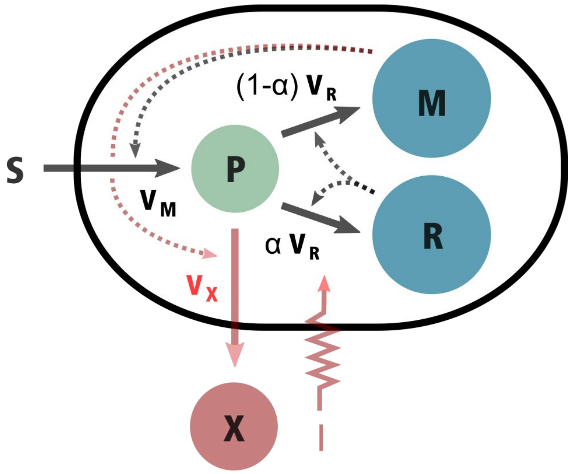
$$\begin{cases} \dot{\varphi} = N\boldsymbol{v_i} - \boldsymbol{v_\mu}\,\mu(p,r) + D\left(s_{in}\boldsymbol{v_{in}} - \boldsymbol{v_{out}}\right), \\ \dot{\mathcal{V}} = (\mu(p,r) - D)\mathcal{V}, \end{cases}$$

with

$$N \doteq \begin{bmatrix} -\mathcal{V} & 0 & 0 \\ 1 & -1 & -1 \\ 0 & u & 0 \\ 0 & 1-u & 0 \\ 0 & 0 & \mathcal{V} \end{bmatrix}, \qquad \boldsymbol{v_i} \doteq \begin{bmatrix} v_M(s, 1-r) \\ v_R(p,r) \\ v_X(p, 1-r) \end{bmatrix},$$

$$\boldsymbol{v_\mu} \doteq \operatorname{diag}(\varphi)\,[0,1,1,1,0]^T,$$

$$\boldsymbol{v_{in}} \doteq [1,0,0,0,0]^T, \qquad \boldsymbol{v_{out}} \doteq \operatorname{diag}(\varphi)\,[1,0,0,0,1]^T.$$



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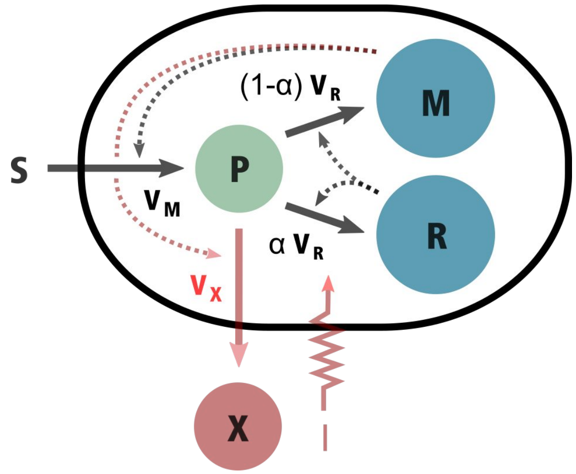
Lemma 3.4. *The ω-limit set of any solution of system S_1 lies in the hyperplanes*

$$\Omega_1 \doteq \left\{(s,p,r,x,\mathcal{V}) \in \mathbb{R}^5 \;:\; s + (p+1)\,\mathcal{V} + x = s_{in}\right\},$$

$$\Omega_2 \doteq \left\{(s,p,r,x,\mathcal{V}) \in \mathbb{R}^5 \;:\; s + \left(p + \frac{r}{\bar{u}}\right)\mathcal{V} + x = s_{in}\right\}.$$



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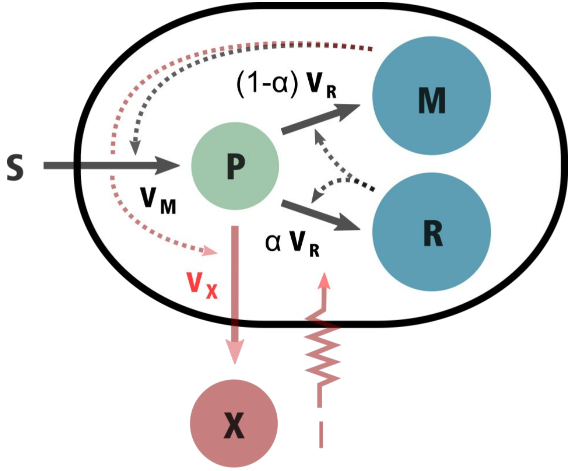

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$$r \Rightarrow \bar{u}$$

$$x \Rightarrow s_{in} - s - (p+1)\mathcal{V}$$

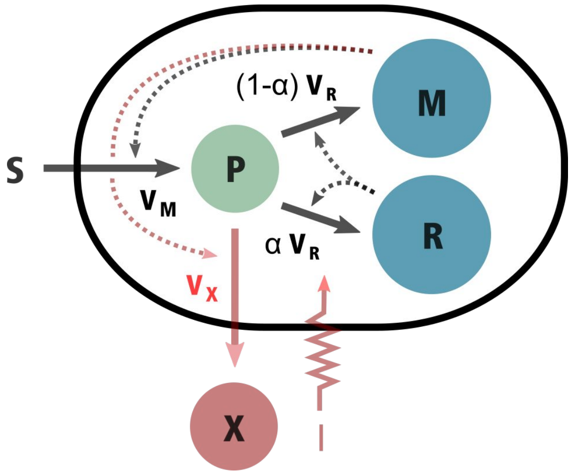
Asymptotic behavior

Limiting system

Which reduces the model to

$$S_1 \ : \begin{cases} \dot{s} = D(s_{in} - s) - \bar{v}_M(s)\mathcal{V}, \\ \dot{p} = \bar{v}_M(s) - \bar{v}_X(p) - \bar{\mu}(p)(p+1), \\ \dot{\mathcal{V}} = (\bar{\mu}(p) - D)\,\mathcal{V}, \end{cases}$$

(where the bars on top of the functions denote new functions that do not depend on r and m)



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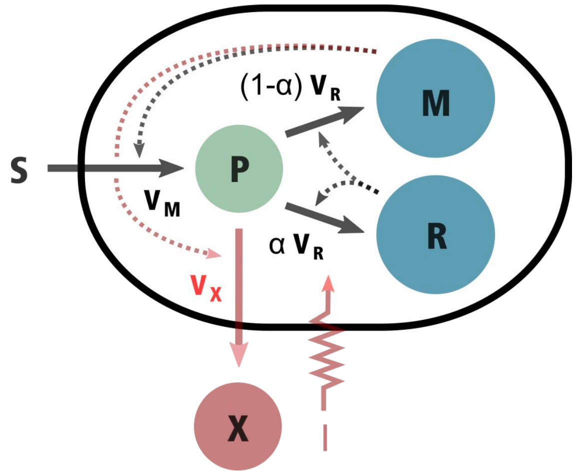

(where the bars on top of the functions denote new functions that do not depend on r and m)

Assumption 2.2. *For $r \in (0,1)$, the metabolite production rate $v_X(p, 1-r)$ can be expressed in terms of macromolecule synthesis rate $v_R(p,r)$ as,*

$$
v_X(p, 1-r) = c(r) v_R(p,r),
$$

being $c(r) : (0,1) \to \mathbb{R}^+$ a positive continuously differentiable function.

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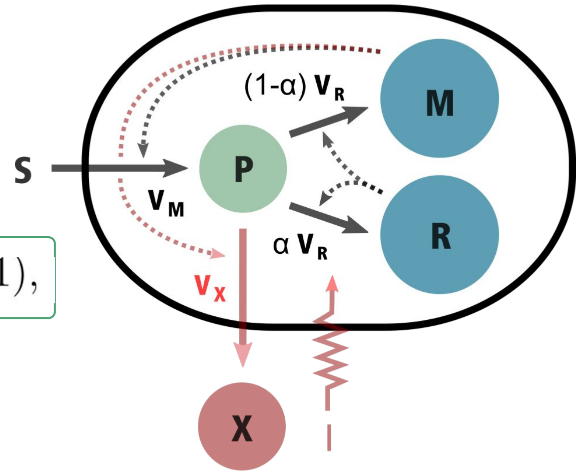
$$\boxed{\dot{p} = \bar{v}_M(s) - \bar{\mu}(p)(p + \bar{c} + 1),}$$



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Asymptotic behavior

Local stability

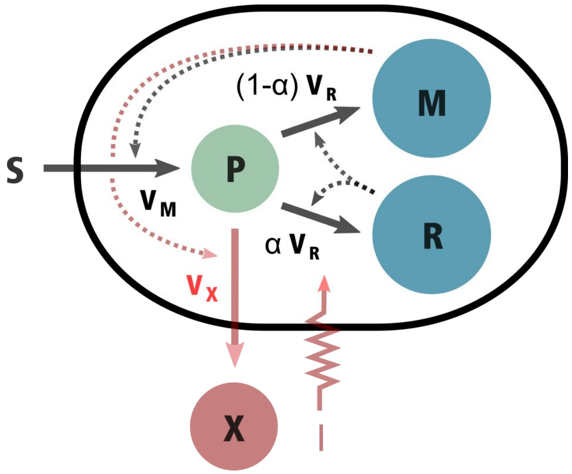
- The interior equilibrium $E_i \doteq (s_i, p_i, \mathcal{V}_i)$, with

$$p_i : \{p \in \mathbb{R}^+ \ : \ \bar{\mu}(p) = D\},$$
$$s_i : \left\{s \in \mathbb{R}^+ \ : \ \bar{v}_M(s) = \bar{f}(p_i)\right\},$$
$$\mathcal{V}_i \doteq \frac{D(s_{in} - s_i)}{\bar{v}_M(s_i)}.$$

with $\bar{f}(p) \doteq \bar{v}_R(p) + \bar{v}_X(p) + \bar{\mu}(p)p$

- The washout equilibrium $E_w \doteq (s_{in}, p_w, 0)$, with

$$p_w : \left\{p \in \mathbb{R}^+ \ : \ \bar{f}(p) = \bar{v}_M(s_{in})\right\}.$$



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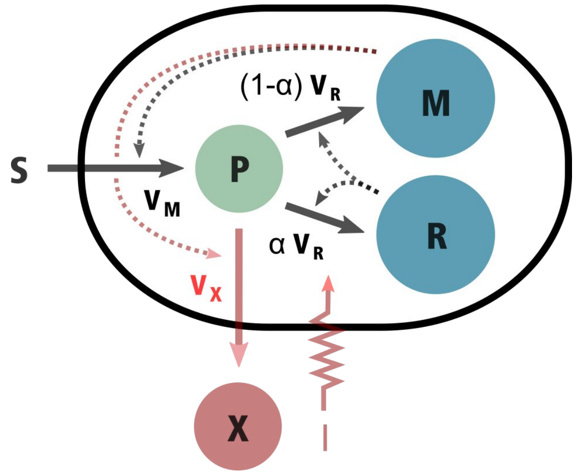

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$$p_w : \left\{p \in \mathbb{R}^+ \ : \ \bar{f}(p) = \bar{v}_M(s_{in})\right\}.$$

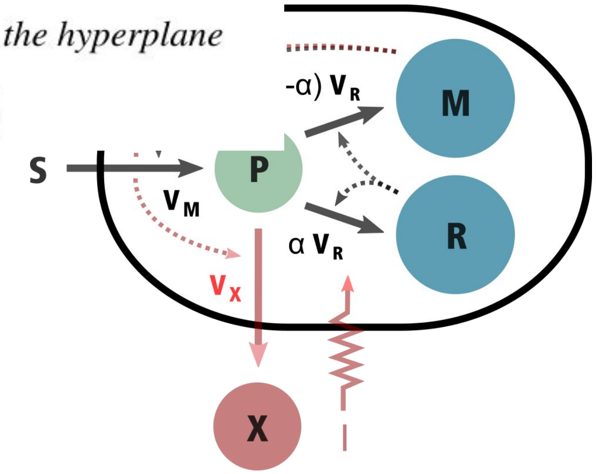
- If $\bar{\mu}(p_w) \geq D$:
  - E_i exists and is locally stable
  - E_w exists and is locally unstable
- If $\bar{\mu}(p_w) < D$:
  - E_i does not exist
  - E_w exists and is locally stable

Asymptotic behavior

Global stability

Lemma 3. *The ω-limit set of any solution of the limiting system S'_1 lies in the hyperplane*

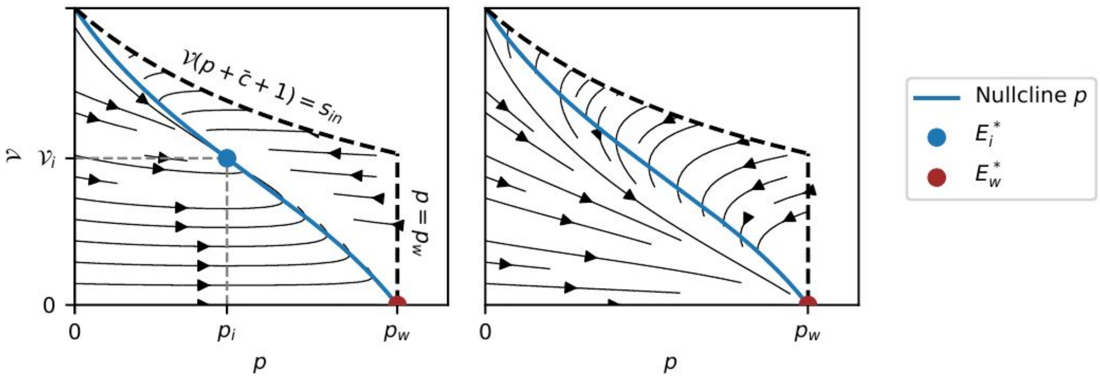
$$\Omega_3 \doteq \left\{(s, p, \mathcal{V}) \in \mathbb{R}^3 \;:\; s + (p + \bar{c} + 1)\mathcal{V} = s_{in}\right\}$$

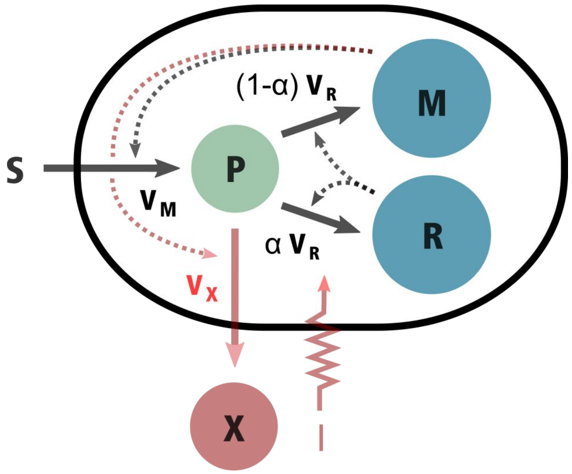


Limiting system in two dimensions

$$S''_1 \;:\; \begin{cases} \dot{p} = \bar{v}_M\,(s(\cdot)) - \bar{\mu}(p)(p + \bar{c} + 1), \\ \dot{\mathcal{V}} = (\bar{\mu}(p) - D)\mathcal{V}. \end{cases}$$

Global stability





$$S_1'' : \begin{cases} \dot{p} = \bar{v}_M(s(\cdot)) - \bar{\mu}(p)(p + \bar{c} + 1), \\ \dot{\mathcal{V}} = (\bar{\mu}(p) - D)\mathcal{V}. \end{cases}$$

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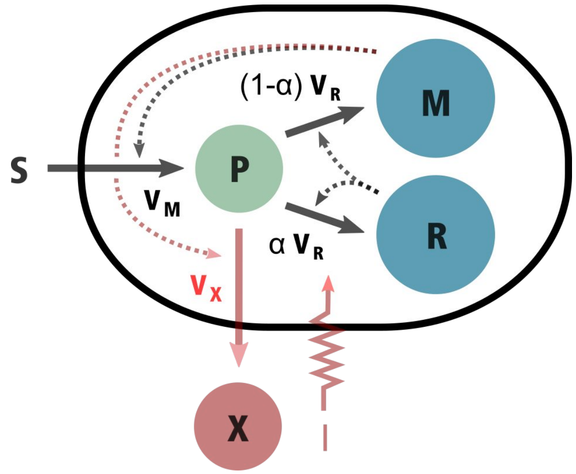


Result on the full system with asymptotically autonomous systems theory

Theorem 2. *Every solution of system S_1 with initial conditions (2.9) converges to,*

- *The extended interior equilibrium $\hat{E}_i \doteq (s_i, p_i, \bar{u}, x_i, \mathcal{V}_i)$ if it exists, being $x_i \doteq \bar{c}\mathcal{V}_i$.*
- *The extended washout equilibrium $\hat{E}_w \doteq (s_{in}, p_w, \bar{u}, 0, 0)$ if $\hat{E}_i$ does not exist.*

where the condition for the existence of the extended interior equilibrium is $\bar{\mu}(p_w) \geq D$.

Static maximization problem

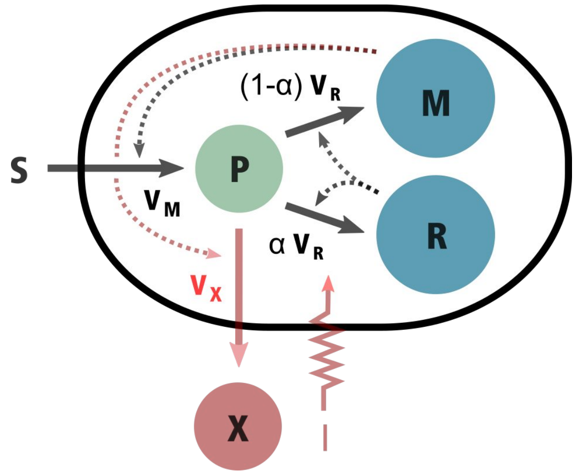
Biomass and metabolite production

The static biomass maximization problem (BMP) can be written as

$$(BMP) : \begin{cases} maximize \quad J_{\mathcal{V}}(\bar{u}, D) \doteq D\mathcal{V} \\ subject\ to\ \ dynamics\ at\ equilibrium \\ \qquad and \quad 0 \leq \bar{u} \leq 1. \end{cases}$$

Analogously, the product maximization problem can be defined as

$$(PMP) : \begin{cases} maximize \quad J_{x}(\bar{u}, D) \doteq Dx \\ subject\ to\ \ dynamics\ at\ equilibrium \\ \qquad and \quad 0 \leq \bar{u} \leq 1. \end{cases}$$



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Static maximization problem

Optimization results

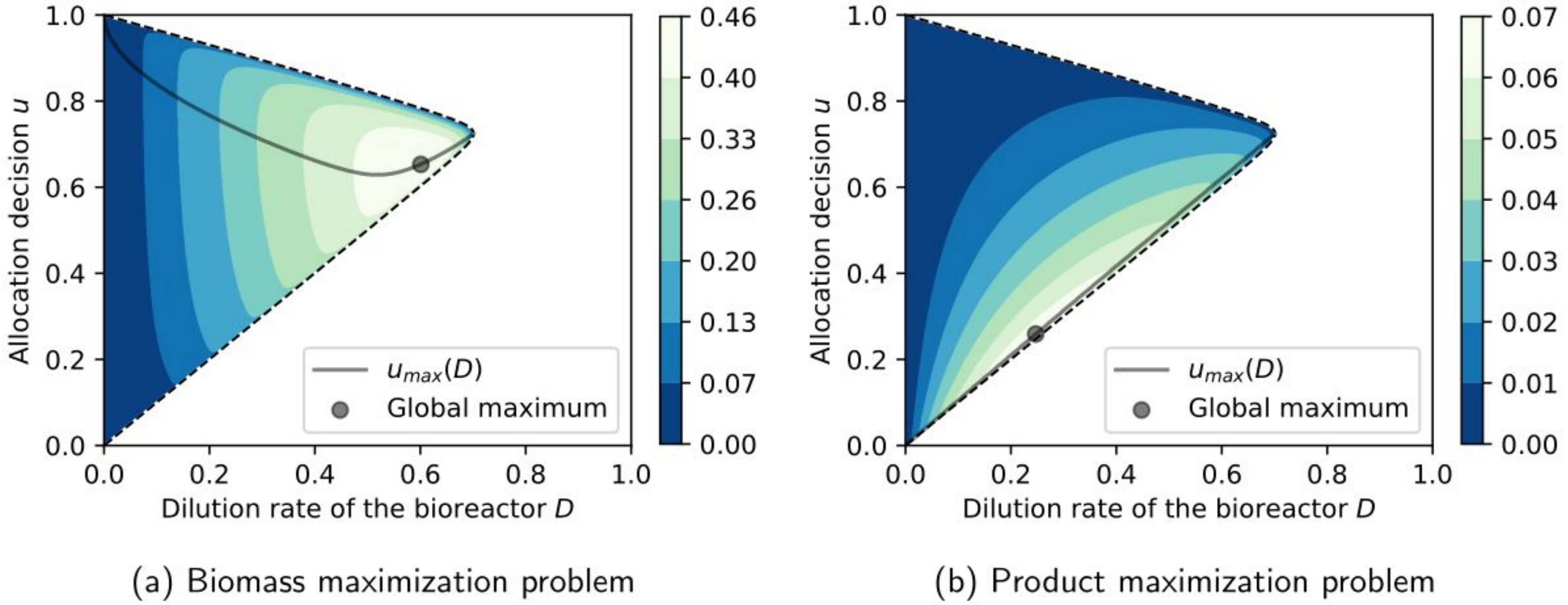
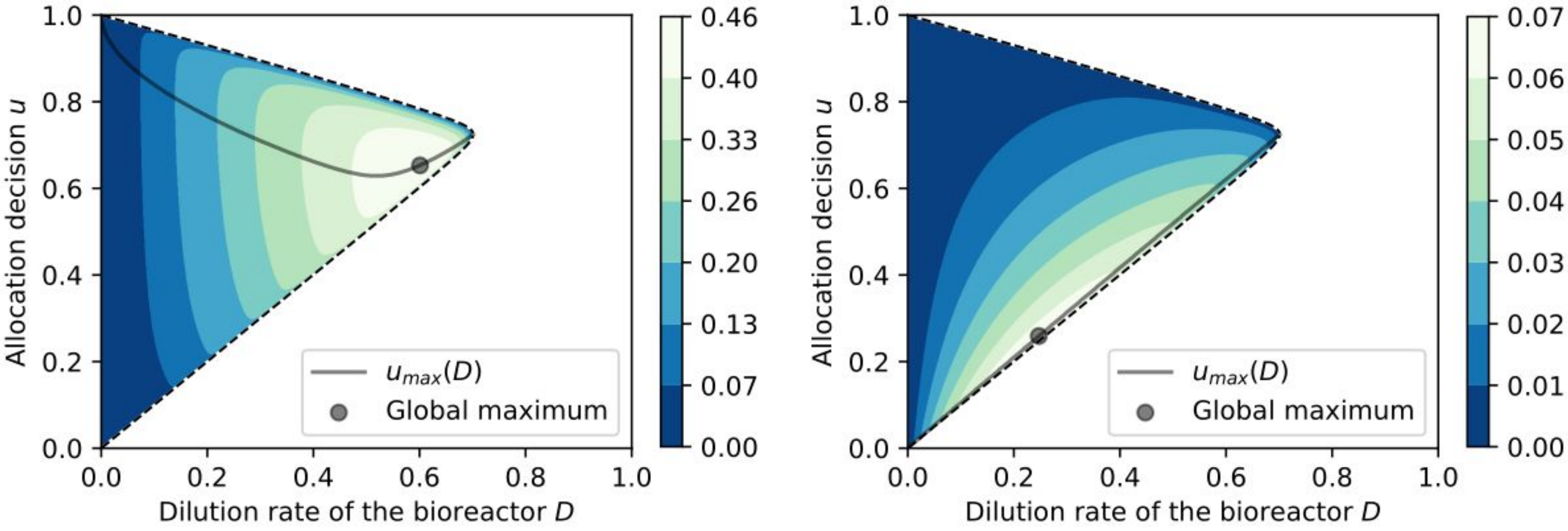


Figure 3: Numerical results for the steady-state production problems. The values for the objective functions are represented through a qualitative colormap. Curves $u_{opt}(D)$ show the optimal allocation u in terms of the dilution rate D.

Static maximization problem

Optimization results



(a) Biomass maximization problem

(b) Product maximization problem

Figure 3: Numerical results for the steady-state production problems. The values for the objective functions are represented through a qualitative colormap. Curves $u_{opt}(D)$ show the optimal allocation u in terms of the dilution rate D.

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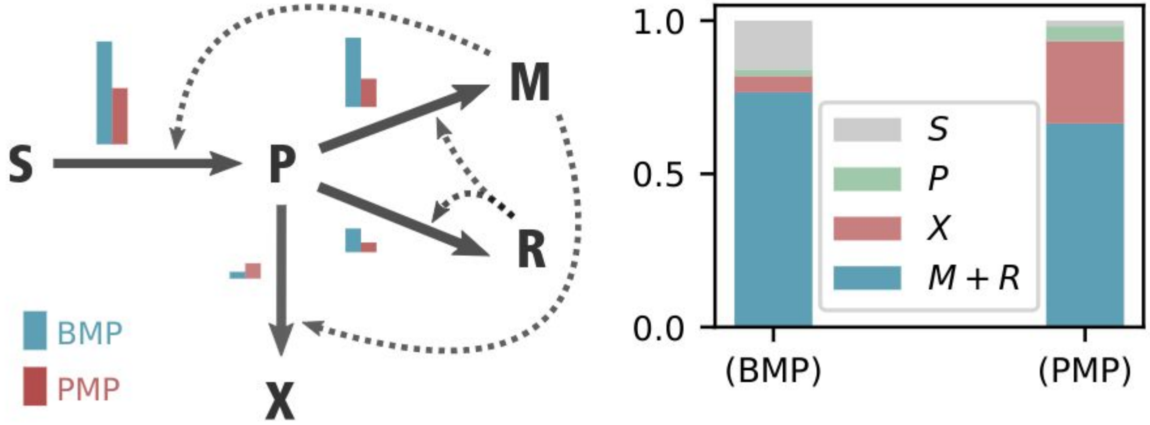


(a) Synthesis rates magnitudes (b) Percentage mass quantities

Fig. 4. Numerical results for both static problems.

Optimal control

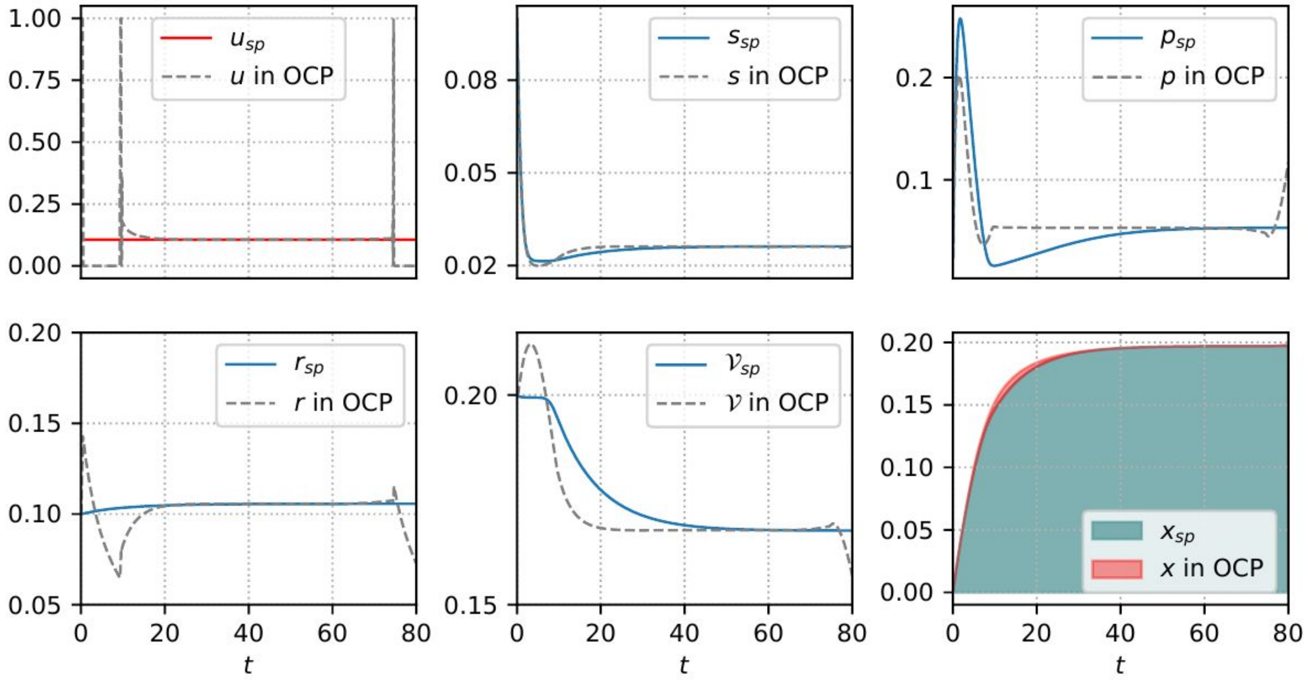
$$
(OCP)\ :\ \begin{cases} \textit{maximize} & J_x(u) = D\,\mathcal{V}_{\text{ext}} \displaystyle\int_0^T x(t)\,\mathrm{d}t \\ \textit{subject to} & \textit{dynamics of } S_1, \\ & u(\cdot) \in \mathcal{U}. \end{cases}
$$

see the paper

YABO, Agustín Gabriel, CAILLAU, Jean-Baptiste, et GOUZÉ, Jean-Luc. Optimal bacterial resource allocation: metabolite production in continuous bioreactors. *Mathematical Biosciences and Engineering*, 2020, vol. 17, no 6, p. 7074-7100.

Dynamic maximization problem

Optimal control



Conclusion

- Studied reallocation of cellular resources via external control in continuous bioreactors.
- Compared steady-state optimal strategies for:
    - Natural biomass maximization
    - Artificial metabolite production maximization
- Explored the underlying trade-offs involved in synthesizing metabolites.
- optimal control...

https://team.inria.fr/biocore/fr/ https://team.inria.fr/mctao/ https://project.inria.fr/maximic/

- Agustín Yabo, Jean-Baptiste Caillau, and Jean-Luc Gouzé. **Optimal bacterial resource allocation: metabolite production in continuous bioreactors**. In Mathematical Biosciences and Engineering (2020).
- Yegorov, Ivan, et al. **Optimal control of bacterial growth for the maximization of metabolite production**. *Journal of mathematical biology* 78.4 (2019).
- Giordano, Nils, et al. **Dynamical allocation of cellular resources as an optimal control problem: novel insights into microbial growth strategies.** PLoS computational biology 12.3 (2016).
- Izard, Jérôme, et al. **A synthetic growth switch based on controlled expression of RNA polymerase.** Molecular systems biology 11.11 (2015).

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