Mathematical model of the human colon

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UEPSD(UR910), MIA(UR341), L2S (UMR 8506)

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The Context

Thèse

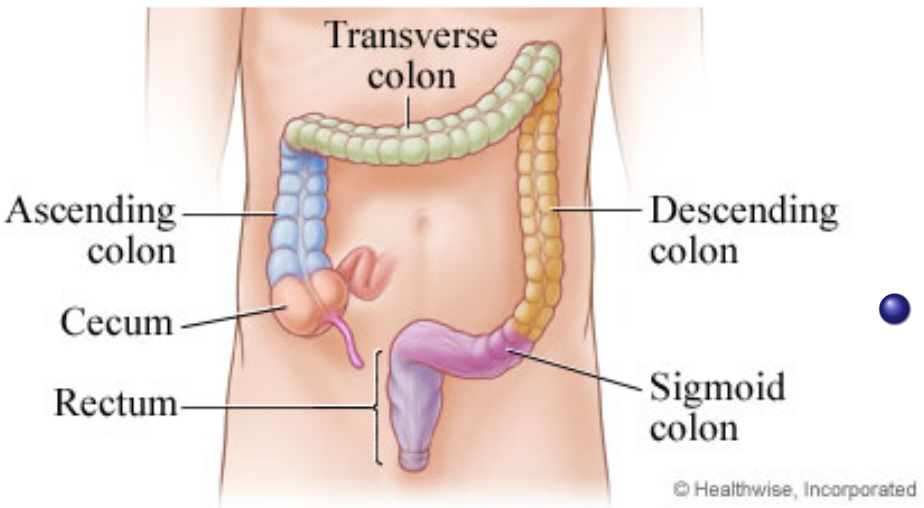
- Director: Eric Walter (L2S)
- Supervisors:
  Béatrice Laroche (L2S)
  Marion Leclerc (UEPSD-INRA Jouy en Josas)
  Kiên Kiêu (MIA-INRA Jouy en Josas)
- Partner: Jean Philippe Steyer (LBE-INRA Narbonne)
- Project: AlimIntest ANR

- Capital: Bogot*á*
- Population: 45 millions
- Language: Spanish
- Surface: 1 141 748 km^2

- My Background
  - Chemical Engineer. Universidad Nacional de Colombia. 2004
  - Master in Automatic Control.Universidad Nacional de Colombia. 2006
- Now: PhD student in Physics (2nd year. 27th november).
  Université Paris-Sud
  INRA Jouy en Josas
  L2S-Supélec

Plan

Physiology



- Function target: get energy through Anaerobic Degradation of complex carbohydrates:
  - Alimentary fibers
  - Mucus: endogenous source, secreted by epithelial cells
- Microbial community: + 800 species. Two diferents microhabitats: Lumen and Mucus

- Interactions host-bacterial: starting to be understood
- Microbiota: role on human health
- Limitations in the experimentation:
  - Uncultured bacteria
  - Samples in some cases can not be representative: Ethical considerations

An *in silico* model would be useful to:

- Improve the understanding of :
  - Carbohydrate fermentation
  - Impact of the microbiota on the stability of the digestive system
  - Role of the microbiota on IBD, Obesity
- Study the influence of dietary regimes on human gastrointestinal microbiota
- Design experiments both *in vivo* and *in vitro*

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Related works

- Microbial competition [Ballyk *et al*, 2001]
- VFA absorption [Minekus *et al*, 1999]
- Interaction Host vs Microbiota [Wilkinson, 2002]
- Fermentation *in vivo* and *in vitro* models: [Leclerc *et al*, 1997], [Macfarlane *et al*, 1998]
- Effect of transit time: [Child *et al*, 2006]

None of these models integrate the physiology, the bioreactions, the transport flux with the functional microbial diversity

Premises

- The microbiota can be functionally represented
- The colon can be defined as a high-rate system:
  - High biomass concentration: formation of aggregates in the mucus
  - Resistance to hydrodynamic forces
- In spite of its geometry, there are some factors that produce mixing:
  - Peristaltic movement
  - Shed of epithelial cells
  - Gas production
- The colon can be represented by a series of chemostats

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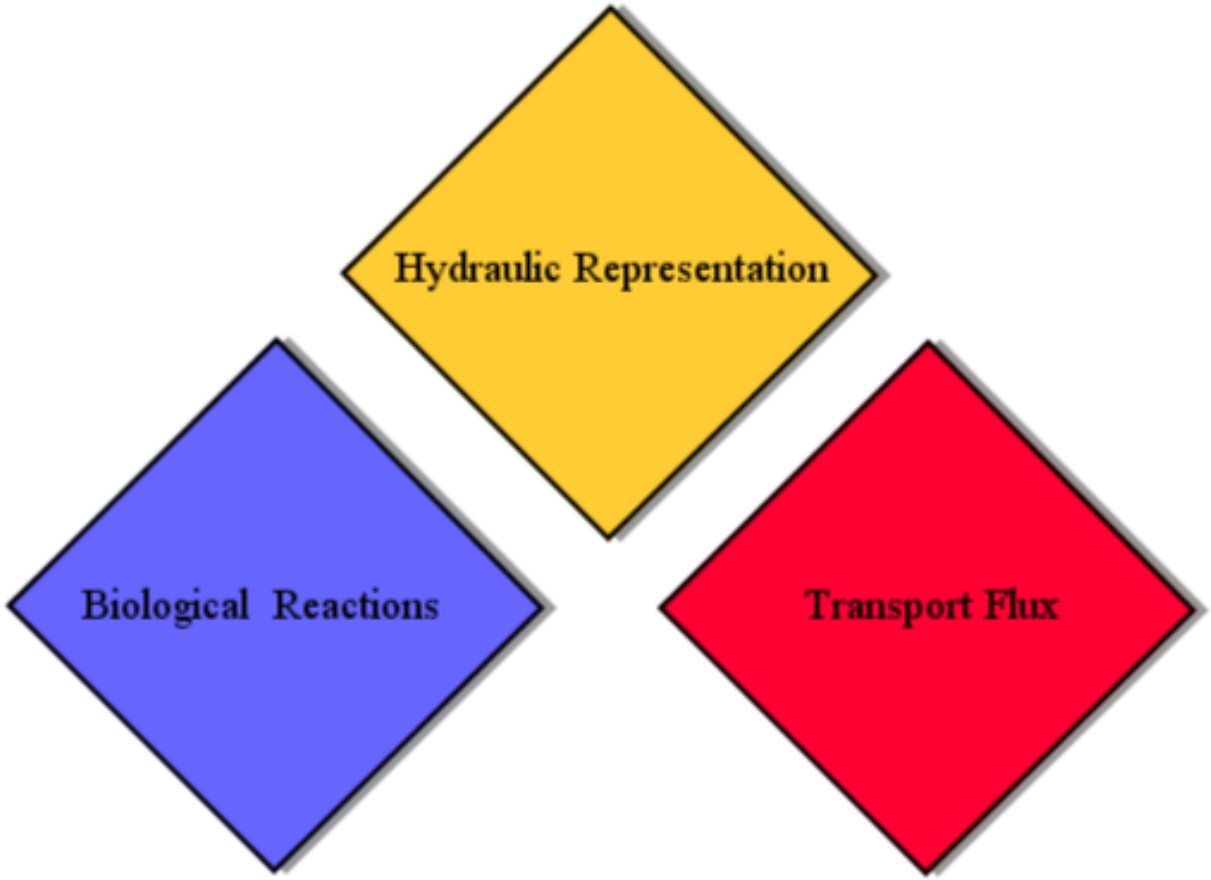
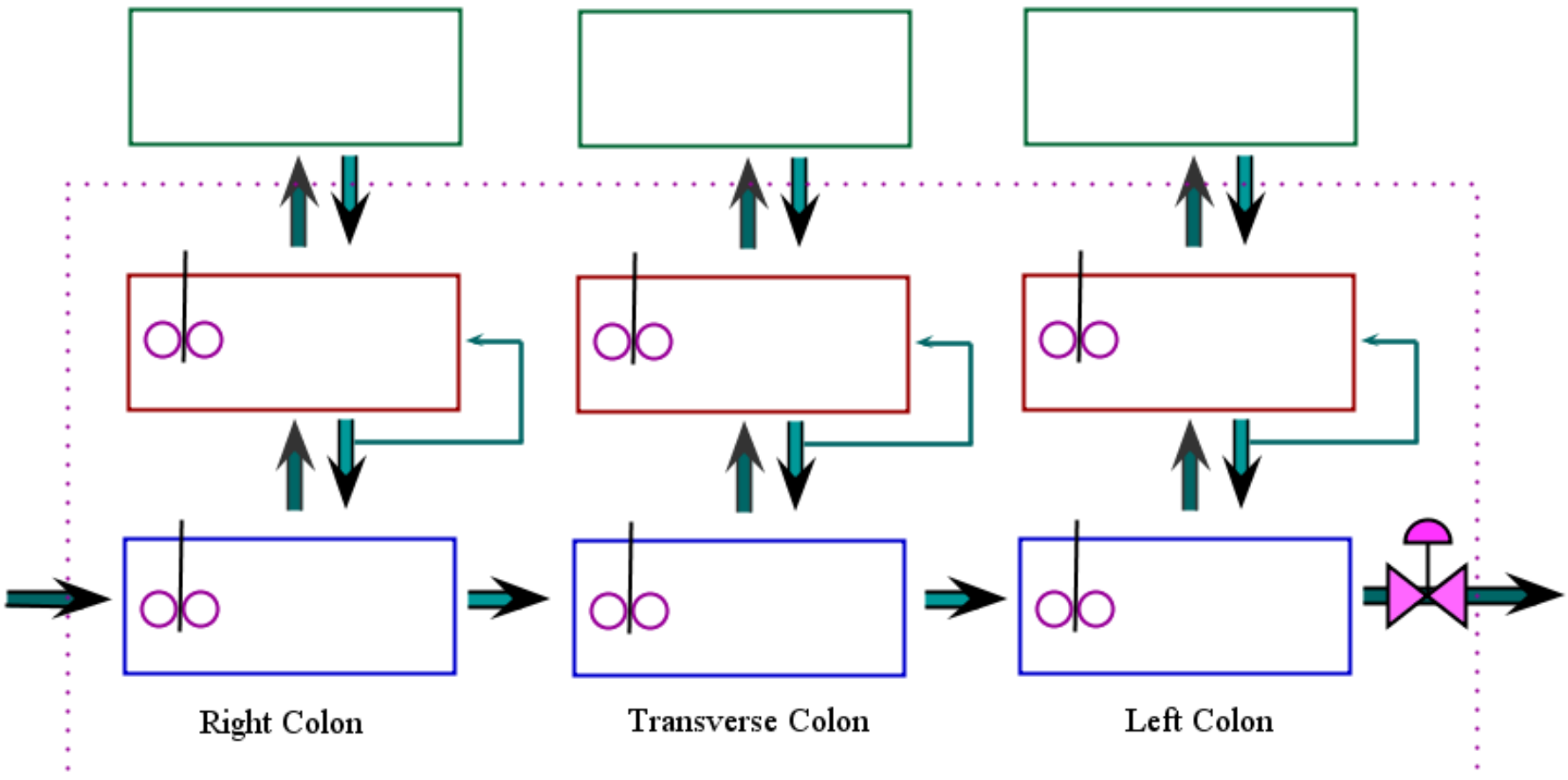
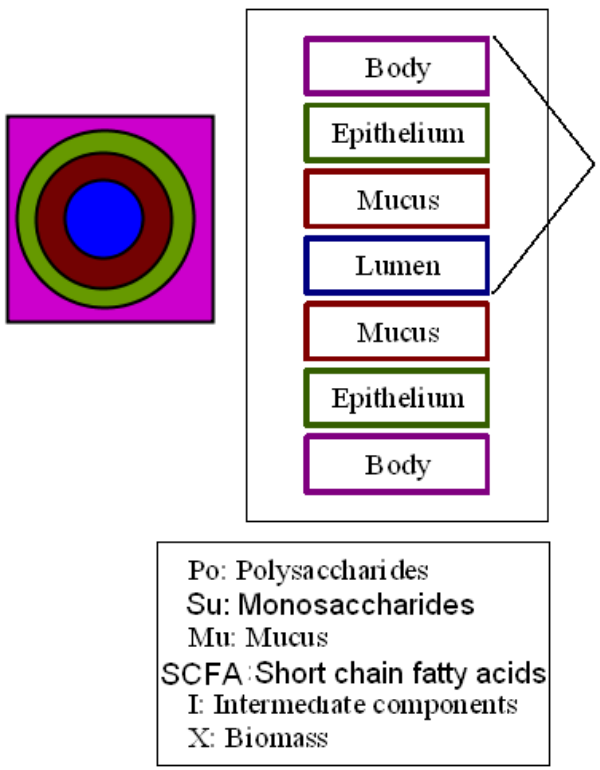
Phenomena


Hydraulic Representation
Biological Reactions
Transport Flux

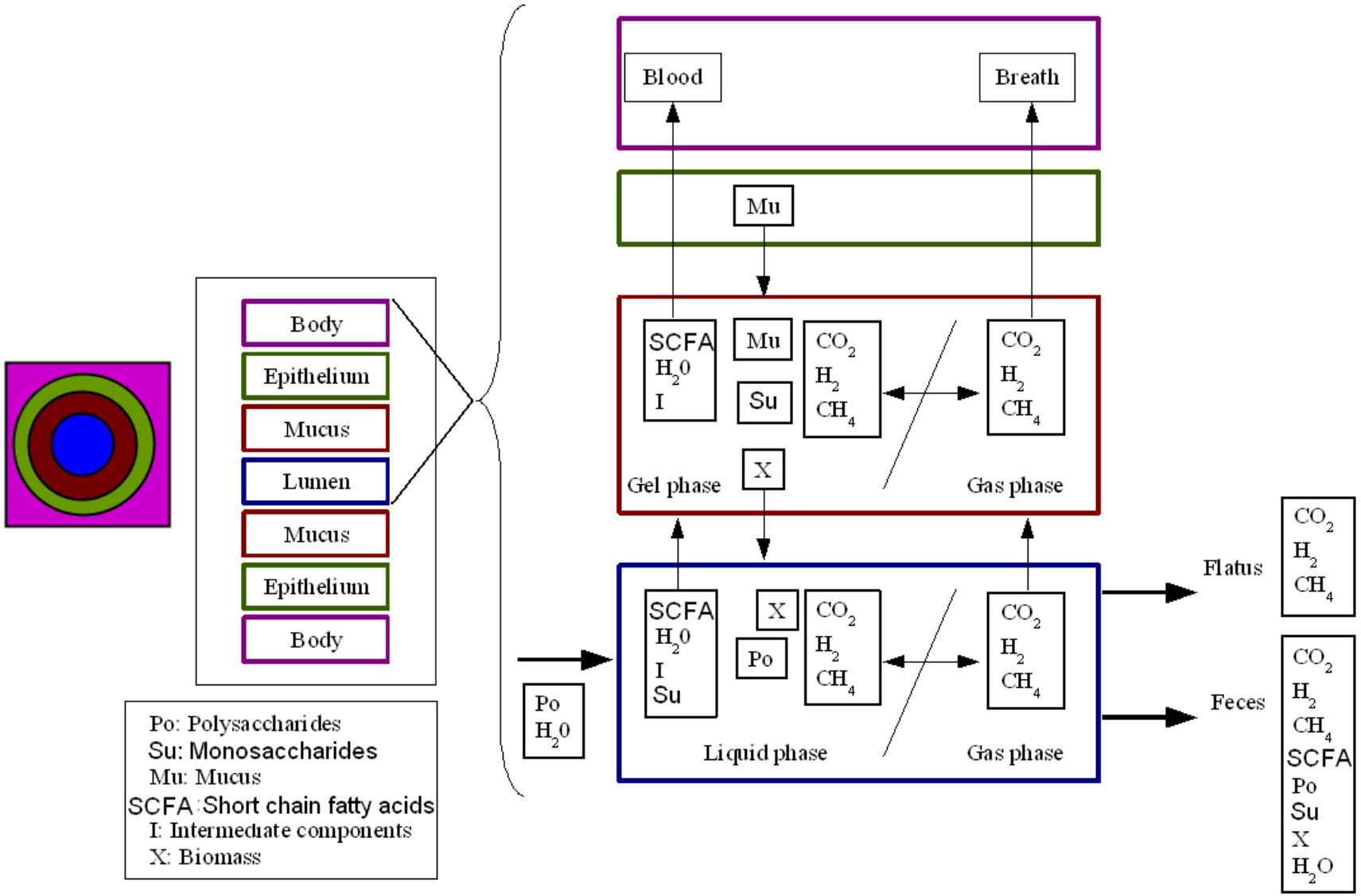
Hydraulic Representation



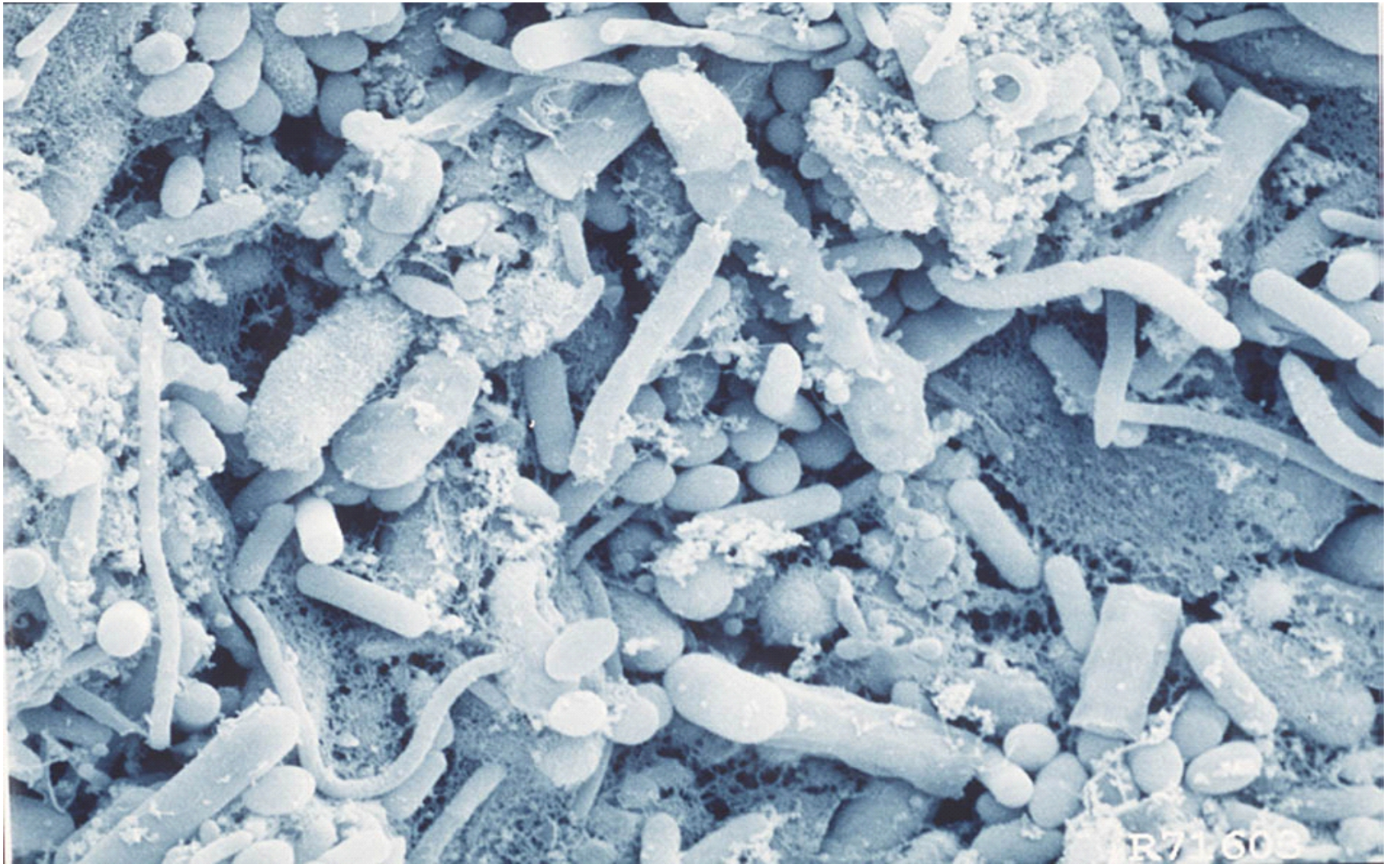
Transport Flux



Transport Flux



Biological Reactions



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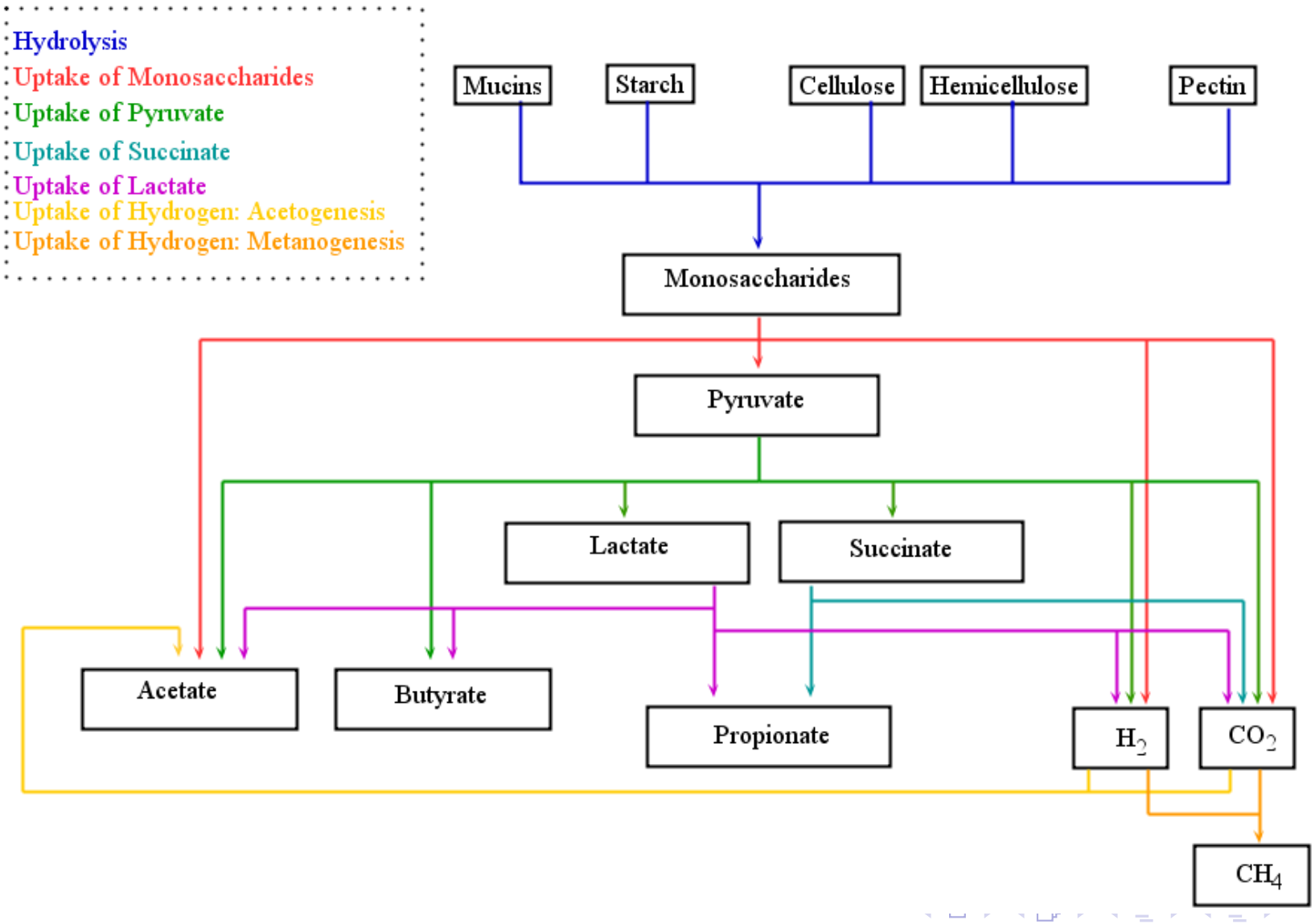

Liquid phase

- For the Lumen

$$\dot{x}_i^l = \left(\frac{q_{in}}{V_l}\right) x_{i,in}^l - \left(\frac{q_{out}}{V_l}\right) x_i^l + b_i \left(\frac{V_m}{V_l}\right) x_i^m + \sum_{j=2}^{13} \nu_{i,j}^l \rho_j^l \tag{1}$$

$$\dot{s}_i^l = \left(\frac{q_{in}}{V_l}\right) s_{i,in}^l - \left(\frac{q_{out}}{V_l}\right) s_i^l - \gamma_i^l s_i^l + \sum_{j=1}^{7} \nu_{i,j}^l \rho_j^l - Q_i^l \tag{2}$$

- For the mucus

$$\dot{x}_i^m = -b_i x_i^m + \sum_{j=2}^{13} \nu_{i,j}^m \rho_j^m \tag{3}$$

$$\dot{s}_i^m = \gamma_i^l s_i^l \left(\frac{V_l}{V_m}\right) - \gamma_i^m s_i^m + \Gamma_i + \sum_{j=1}^{7} \nu_{i,j}^m \rho_j^m - Q_i^m \tag{4}$$

Gas phase

$$\dot{s}_i^g = \left(\frac{q_{gin}}{V_g}\right) s_{i,in}^g - \left(\frac{q_{gout}}{V_g}\right) s_i^g + Q_i \left(\frac{V_{l/m}}{V_g}\right) \tag{5}$$

$$Q_i = k_L a(S_{L,i} - M_i K_{H,i} p_{gas,i}) \tag{6}$$

Kinetic rates

Process $\downarrow$ j	**Kinetic rate**
1 Hydrolysis	$\rho_1 = k_{hyd} s_1$
2 Uptake sugars	$\rho_2 = \mu_{max_2} \frac{s_2}{Ks_2 + s_2} x_2$
3 Uptake pyruvate	$\rho_3 = \mu_{max_3} \frac{s_3}{Ks_3 + s_3} x_3$
4 Uptake succinate	$\rho_4 = \mu_{max_4} \frac{s_4}{Ks_4 + s_4} x_4$
5 Uptake lactate	$\rho_5 = \mu_{max_5} \frac{s_5}{Ks_5 + s_5} x_5$
6 Uptake hydrogen: Ac	$\rho_6 = \mu_{max_6} \frac{s_6}{Ks_6 + s_6} x_6$
7 Uptake hydrogen: CH_4	$\rho_7 = \mu_{max_7} \frac{s_7}{Ks_7 + s_7} x_7$
8 Decay of x_{su}	$\rho_8 = k_{d_8} x_2$
9 Decay of x_{py}	$\rho_9 = k_{d_9} x_3$
10 Decay of x_{sc}	$\rho_{10} = k_{d_{10}} x_4$
11 Decay of x_{la}	$\rho_{11} = k_{d_{11}} x_5$
12 Decay of x_{h2-ac}	$\rho_{12} = k_{d_{12}} x_6$
13 Decay of $x_{h2_c h_4}$	$\rho_{13} = k_{d_{13}} x_7$

- Number of state variables for each subsystem (Lumen / Mucus): 20 (17 liquid phase + 3 gas phase)
- Number of state variables for each partition: 40 (2x20)
- Number of states variables for the whole system: 120 (40x3)
- Measuring variables
  - Measures: related with the last section
  - All the variables can not be measured at the same time
  - Some variables: measured once
- Parameters: 321
  - Reduction: 94. (Knowledge)
  - Prior information
  - Estimation

Obstacles and Alternatives

- Phenonema not well defined, *e.g* transport of components between the subsystems, rheology
- Difficulty of measurements
- Availability of data is limited
- Limitations for *in vitro* experiments: most of the bacteria are uncultured
- Many studies are based on the microbiota in fecal matter

- Microsensors and FISH in mucus: spatial distribution
- Studies on artificial systems
- Study of state observers
- Bayesian approach for parameter estimation
- 16SrRNA and FISH techniques
- *in vivo* models: inoculated axenic rodents, biopsy in mucus

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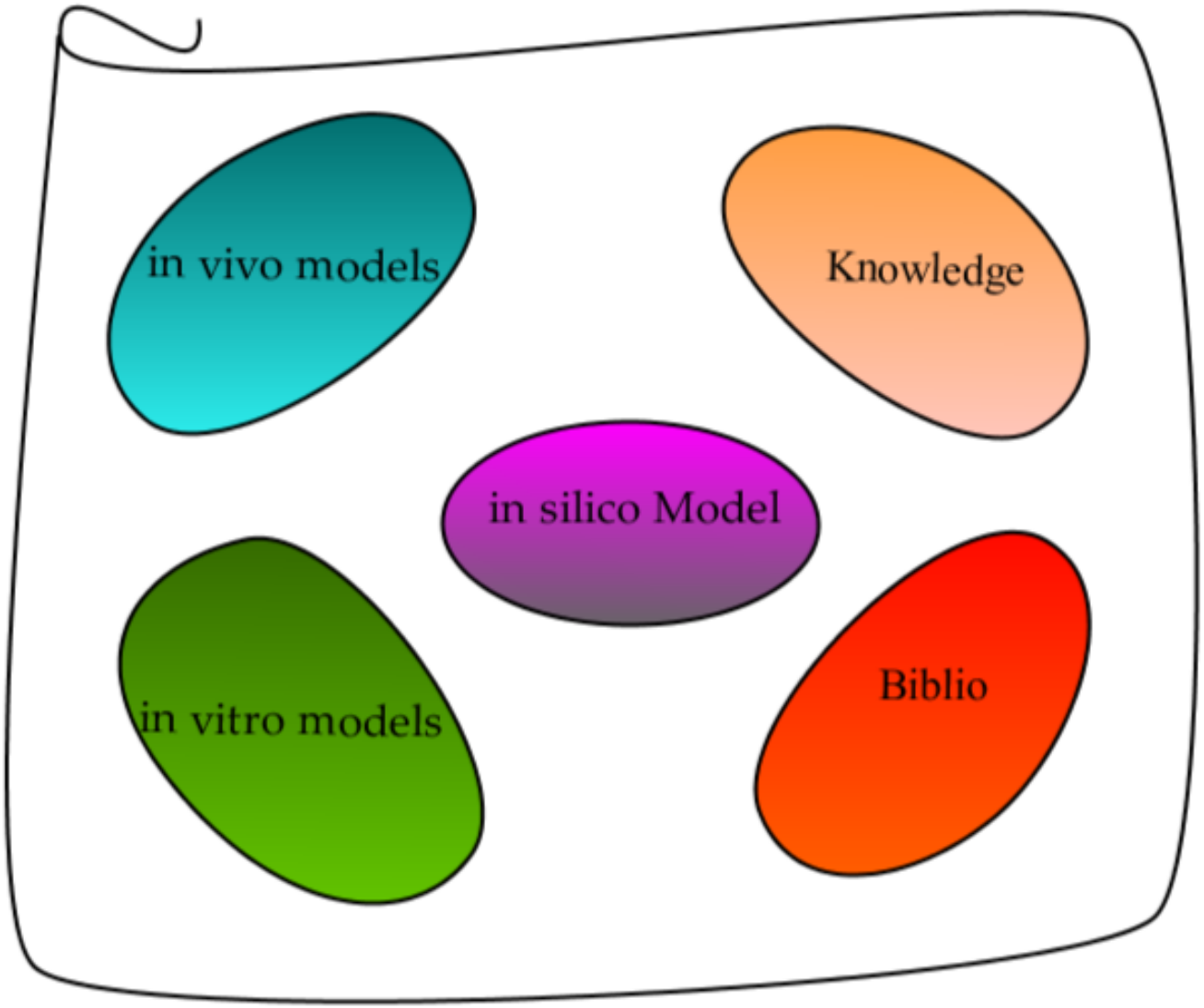
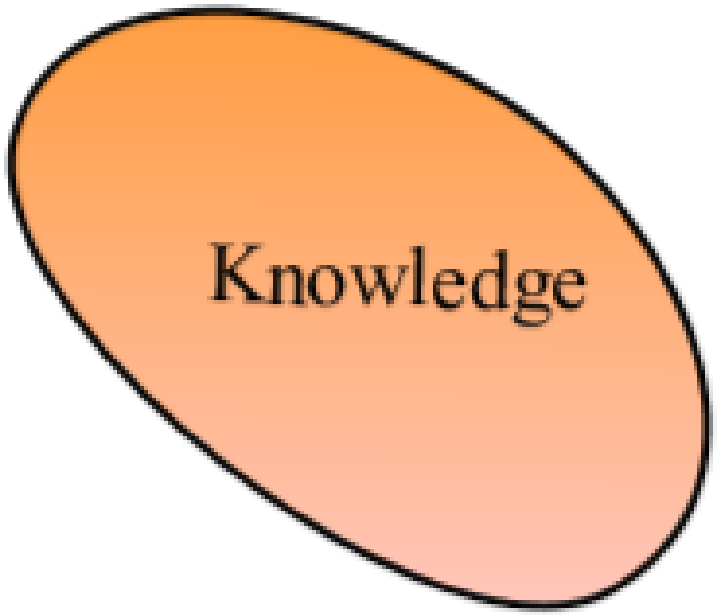
Strategy

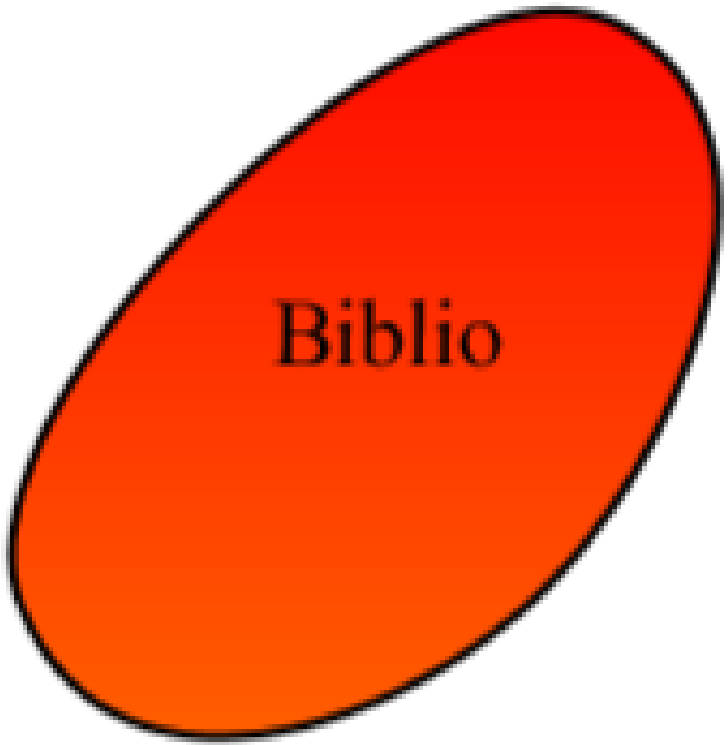

in vivo models
Knowledge
in silico Model
in vitro models
Biblio

Strategy



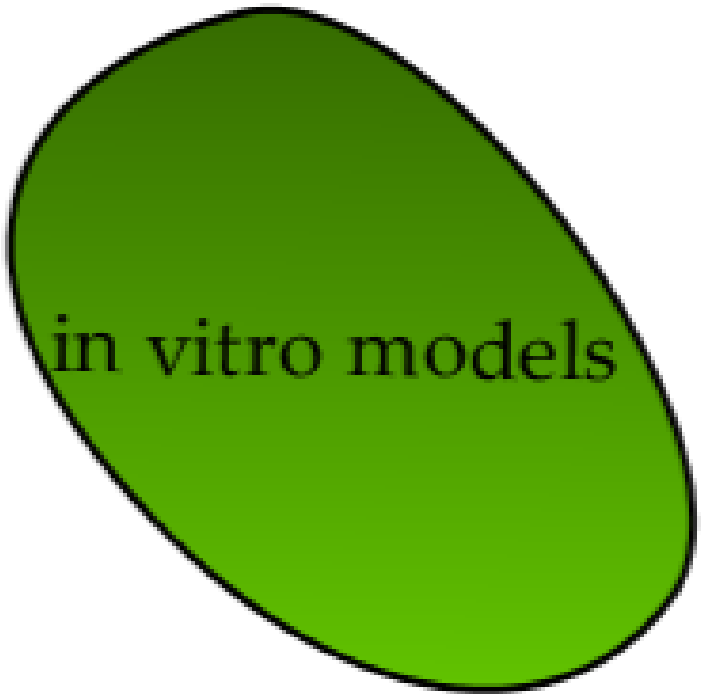
- Microbiology
- Bioprocess engineering
- Mathematics
- Control theory
- Statistics

Strategy



- Experimental data
- Prior information: Bayesian estimation

Strategy

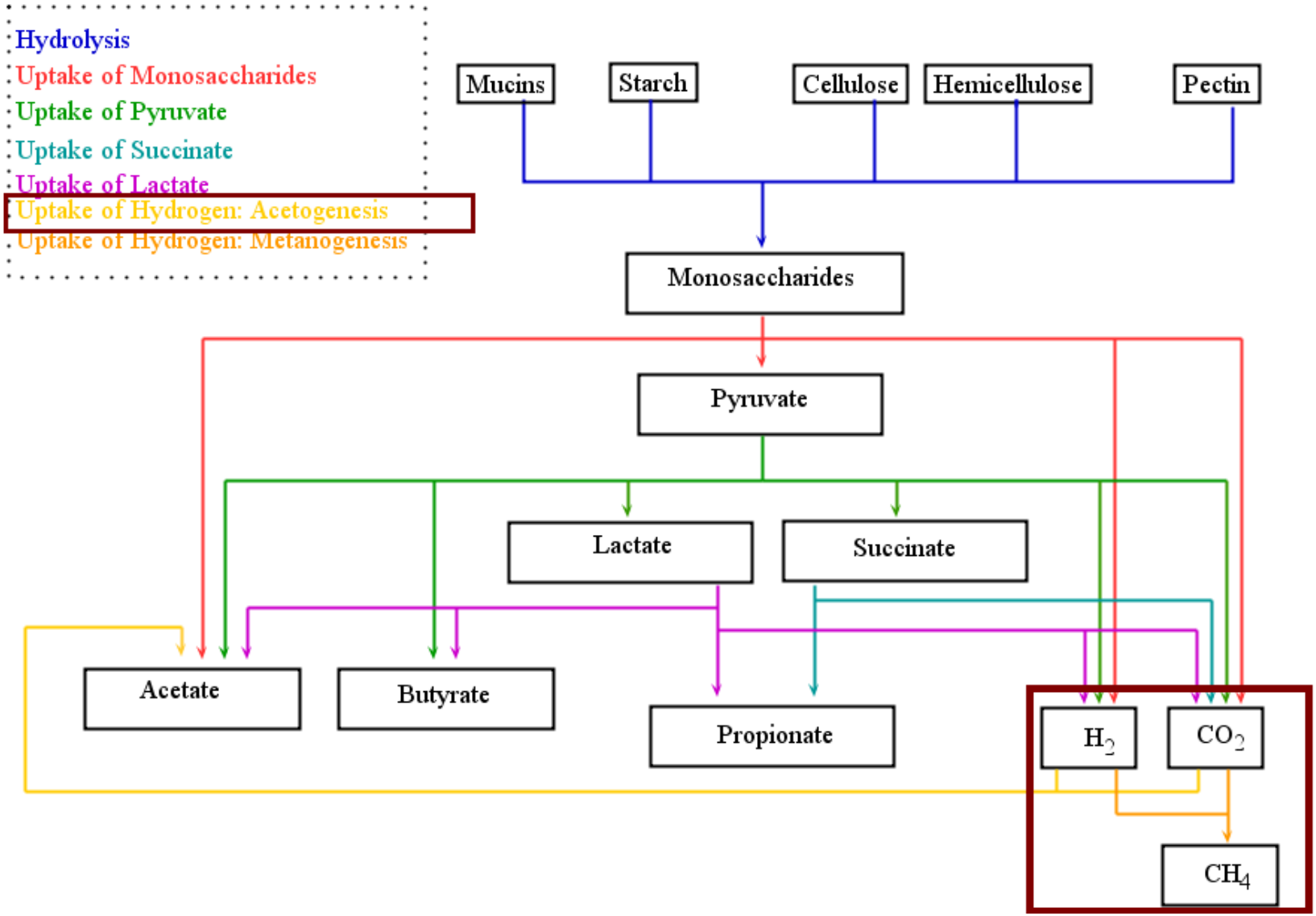


- Experimental data
- Parameter estimation

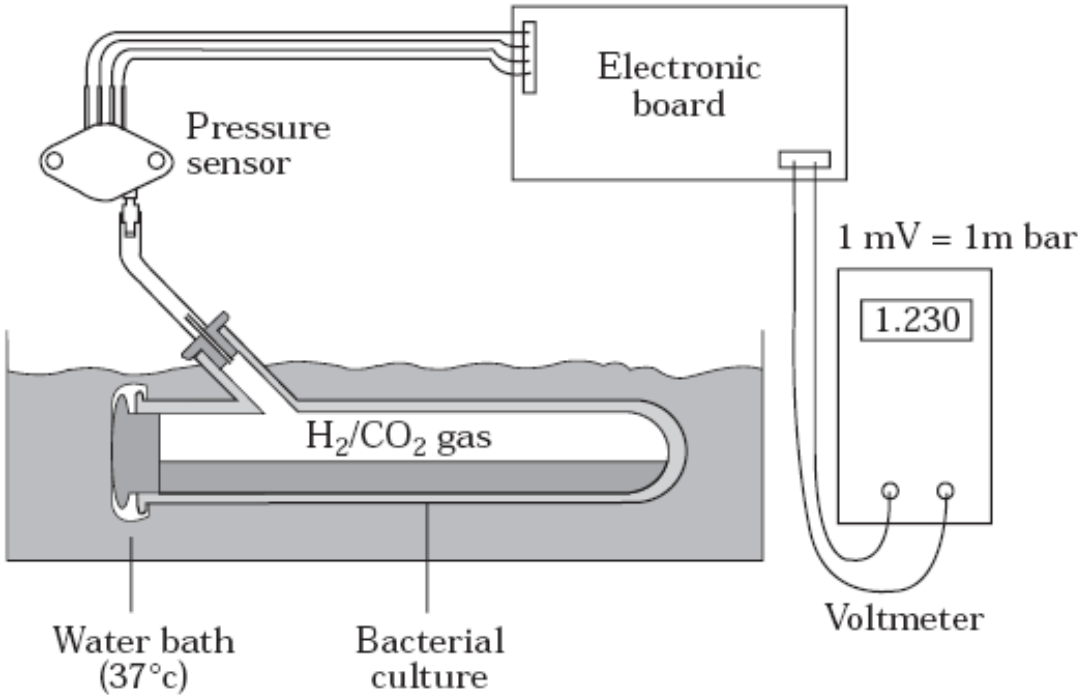
Strategy

- Axenic rats: inoculated with minimal microbiota
- Fed with different fiber diets, similar to human

Modelling of invitro homoacetogenesis



invitro model



Homoacetogenesis reaction

$$4H_2 + 2CO_2 \Rightarrow CH_3COOH + 2H_2O \quad (7)$$

A. Bernalier, A. Willems, M. Leclerc, V. Rochet V. and M.D. Collins, *Ruminococcus hydrogenotrophicus sp. nov.*, a new H_2/CO_2 - utilizing bacterium isolated from human feces, *Arch Microbial*, vol. 166, 1996, pp 176-183.

invitro model

- Measured variables:
  - Concentration of H_2 in gas phase. Manometric sensor and gas cromatography. mM
  - Concentration of Acetate. Enzymatic assay. mM
  - Optical density at 600nm. OD_{600}

Mathematical model equations

$$\dot{x} = \mu_{\max} \frac{s^l_{H_2}}{K + s^l_{H_2}} x - k_d x, \tag{8}$$

$$\dot{z} = k_d x - k_i z, \tag{9}$$

$$\dot{s}^g_{H_2} = k_L a (s^l_{H_2} - K_H RT s^g_{H_2}) \frac{V_l}{V_g}, \tag{10}$$

$$\dot{s}_{ac} = \frac{1 - Y_H}{Y_H} \mu_{\max} \frac{s^l_{H_2}}{K + s^l_{H_2}} x, \tag{11}$$

$$\dot{s}^l_{H_2} = -\frac{\mu_{\max}}{Y_H} \frac{s^l_{H_2}}{K + s^l_{H_2}} x - k_L a (s^l_{H_2} - K_H RT s^g_{H_2}). \tag{12}$$

$$-\frac{\mu_{\max}}{Y_H} \frac{s^l_{H_2}}{K + s^l_{H_2}} x - k_L a (s^l_{H_2} - K_H RT s^g_{H_2}) = 0. \tag{13}$$

$$y = (\alpha(x + z),\ s^g_{H_2},\ s_{ac})^{\mathsf{T}} \tag{14}$$

Parameters

Known Parameters:

- $kLa = 8.33\ h^{-1}$
- $K_H = 0.00078\ \frac{M_{lig}}{bar_{gas}}$
- $\alpha = 5.9472\ \frac{kgCOD/m^3}{OD_{600}}$

Unknown Parameters:

- μ_{max}
- K
- Y_h
- k_d
- k_i

Identifiability

Global identifiability

A model is said to be globally identifiable if all of its unknown parameters could be estimated uniquely from idealized (noise-free) observations.

Identifiability

Denis Vidal and Joly-Blanchard (2004). Sufficient condition for uncontrolled non linear models

The model is theorically identifiable

Identification

Vector of data collected:

$$\mathbf{y}(t_i) = \mathbf{y}_{\mathrm{m}}(t_i, \theta^*) + \varepsilon_i,\ i = 1, ..., n_{\mathrm{t}}, \quad (15)$$

$$\varepsilon_i \sim \mathbf{N}(\mathbf{0}, \Sigma). \quad (16)$$

Maximum likelihood:

$$\pi_y(\mathbf{y}^{\mathrm{s}}|\theta) \quad (17)$$

- Criterion 1: Least Squares on normalized errors (**C1**)
- Criterion 2: Σ from the experimenetal data (**C2**)
- Criterion 3: Σ unknown for synchronous data (**C3**)
- Criterion 4: Σ unknown for non-synchronous data (**C4**)

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Analysis

$$\mathbf{F}(\widehat{\theta}) = \sum_{i=1}^{n_t} \left[\frac{\partial \mathbf{y}_m(t_i,\theta)}{\partial \theta}\right]^T_{t_i,\widehat{\theta}} \Sigma^{-1} \left[\frac{\partial \mathbf{y}_m(t_i,\theta)}{\partial \theta}\right]_{t_i,\widehat{\theta}} \tag{18}$$

$$P \geq \mathbf{F}(\widehat{\theta})^{-1} \tag{19}$$

For a mathematical model in its state space representation:

$$\dot{\mathbf{x}} = \mathbf{f}(\mathbf{x},\theta),\ \mathbf{x}(0) = \mathbf{x}_0(\theta), \tag{20}$$

$$\mathbf{y}_m = \mathbf{C}\mathbf{x} \tag{21}$$

the sensitivity of $\mathbf{y}_m$ with respect to the parameter θ_i $\left(\frac{\partial \mathbf{y}_m}{\partial \theta_i}\right)$, is given by:

$$\frac{\partial \mathbf{y}_m}{\partial \theta_i} = \mathbf{C}\frac{\partial \mathbf{x}}{\partial \theta_i} \tag{22}$$

with $\frac{\partial \mathbf{x}}{\partial \theta_i}$ the sensitivity of $\mathbf{x}$ with respect to θ_i, named $\mathbf{s}_i$.

$$\dot{\mathbf{s}}_\mathbf{i} = \frac{\partial \mathbf{f}(\mathbf{x},\theta)}{\partial \mathbf{x}}\mathbf{s}_i + \frac{\partial \mathbf{f}(\mathbf{x},\theta)}{\partial \theta_i},\ \mathbf{s}_i(0) = \frac{\partial \mathbf{x}(0)}{\partial \theta_i},\ i = 1,...,n_p \tag{23}$$

Analysis

Problem of practical identifiability

K and μ_{max} are strongly correlated
Standard deviations are very high

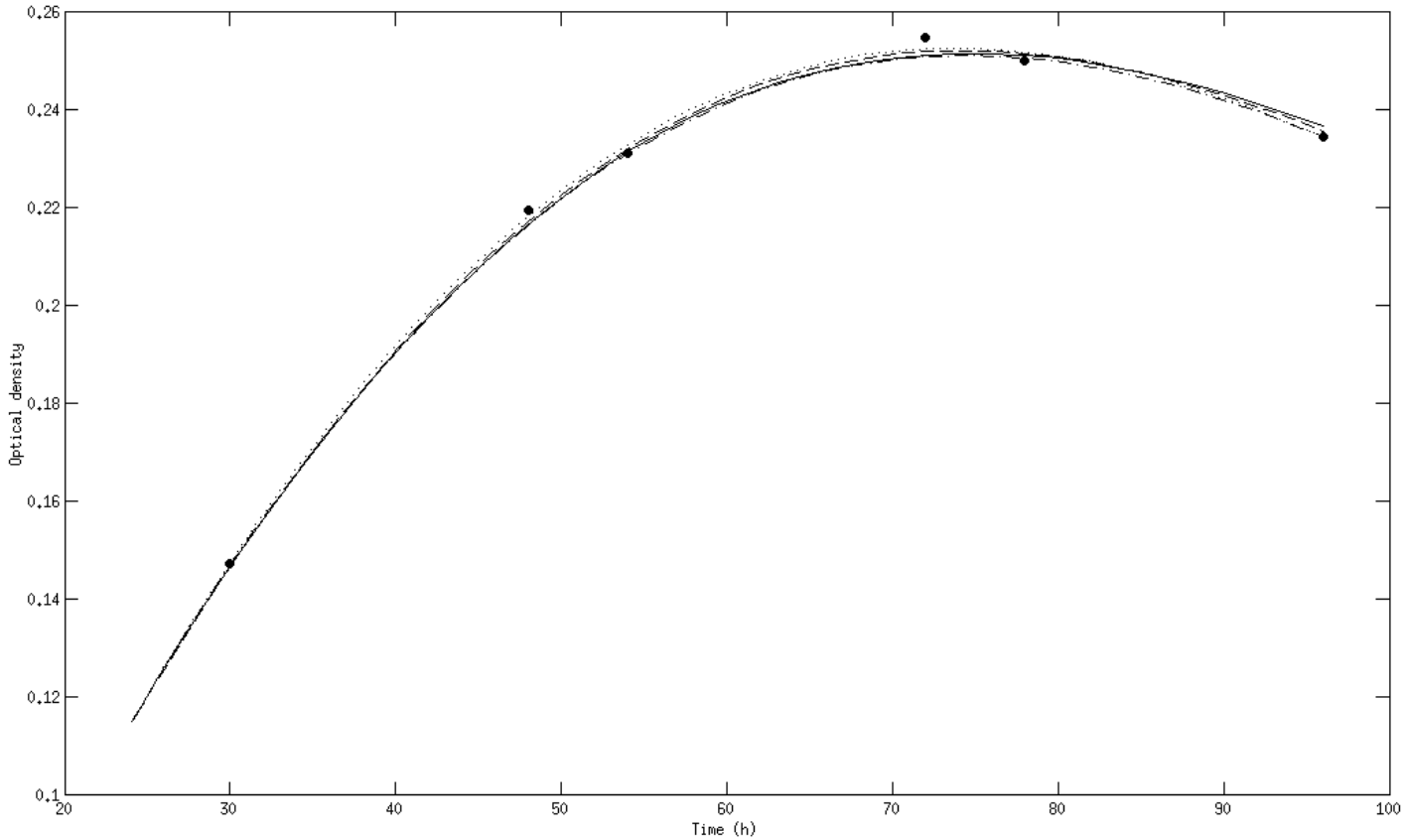
Solution

Modification on the kinetics:

$$\mu_{max} \frac{s^l_{H_2}}{K + s^l_{H_2}} x \Rightarrow k_r s^l_{H_2} x, \tag{24}$$

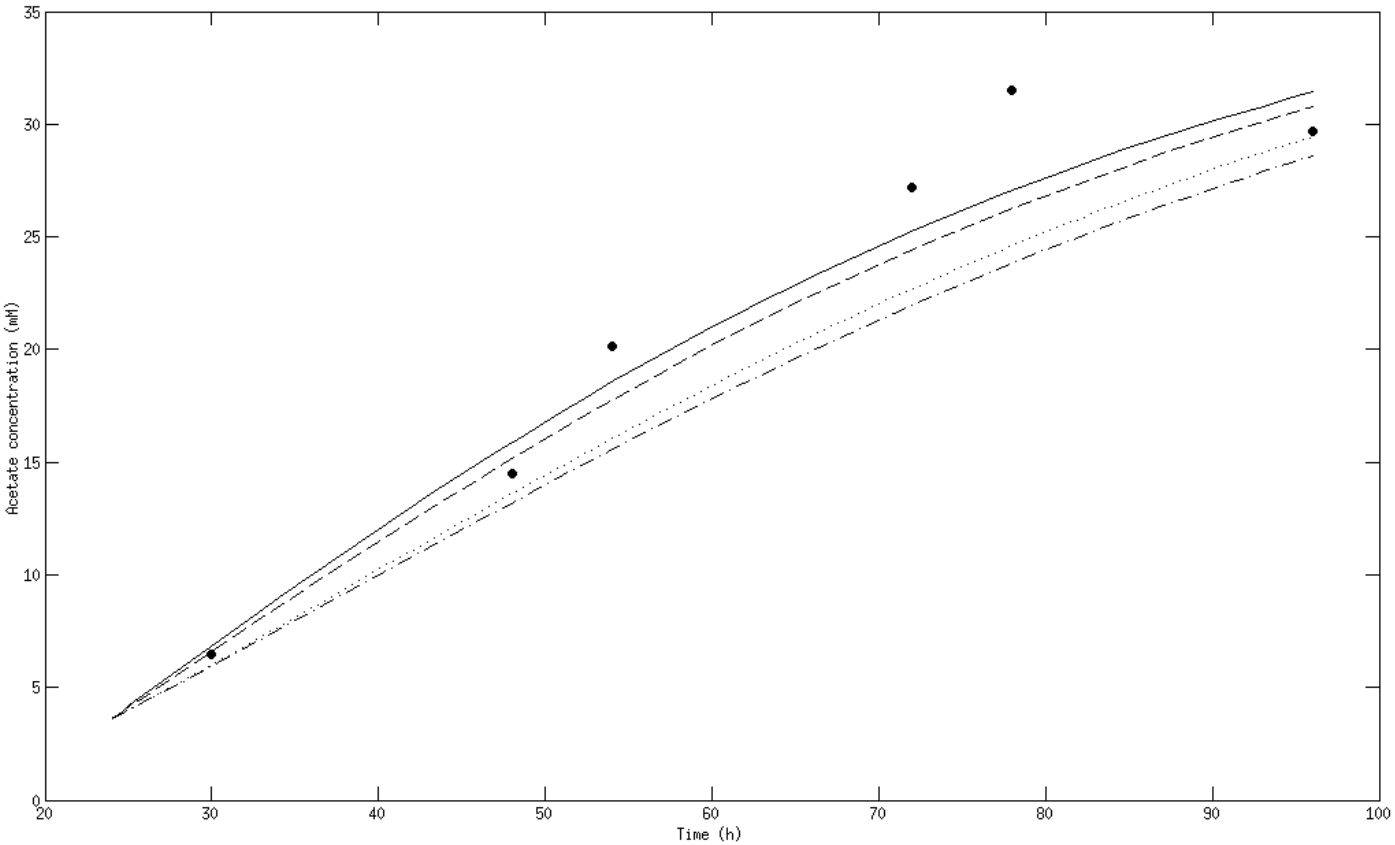
$$k_r = \frac{\mu_{max}}{K}. \tag{25}$$

Results



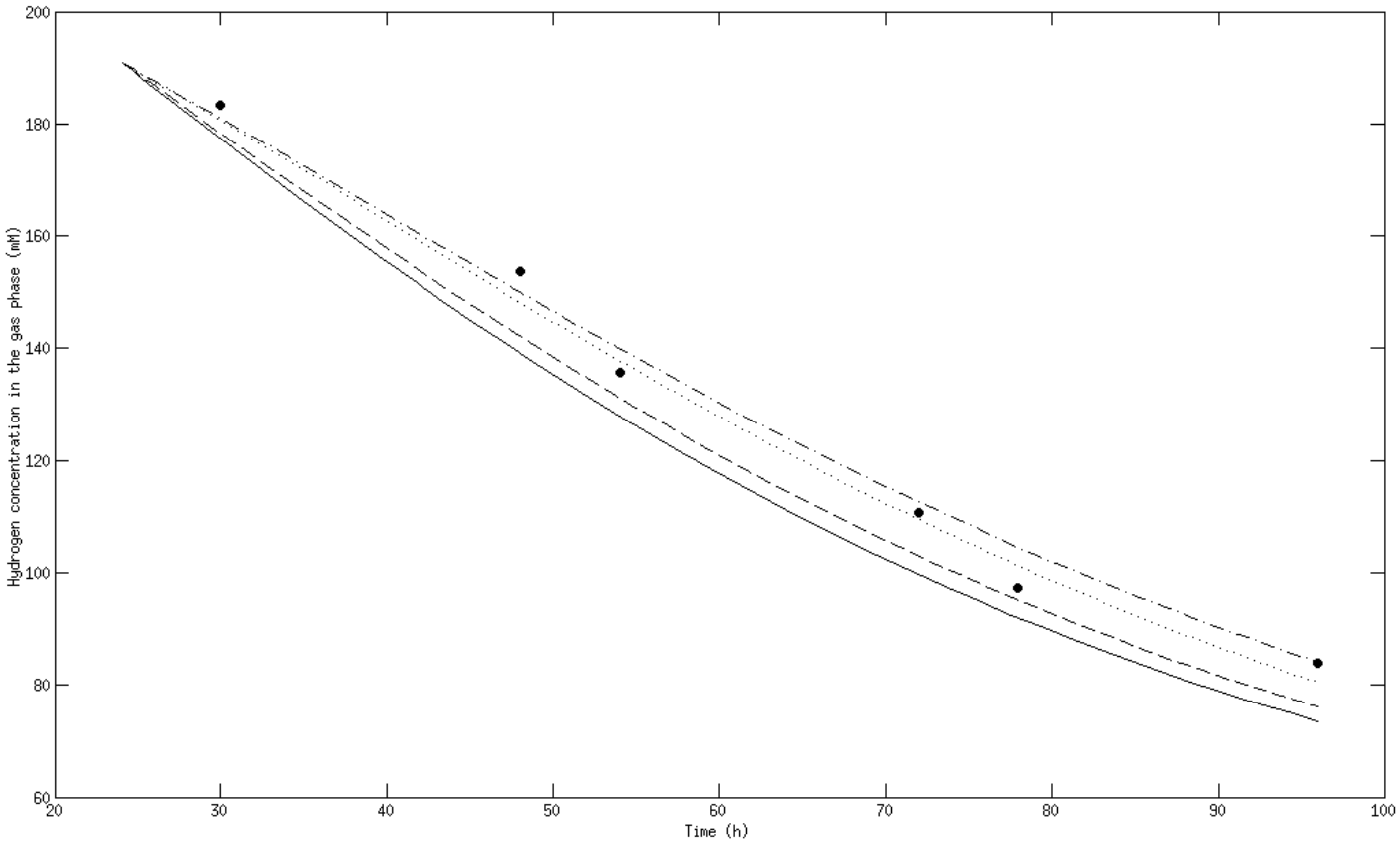
Optical density. •: experimental data, dash: **C1**, solid: **C2**, dash-dot: **C3**, dot: **C4**.

Results



Acetate concentration. •: experimental data, dash: **C1**, solid: **C2**, dash-dot: **C3**, dot: **C4**.

Results



Hydrogen concentration. •: experimental data, dash: **C1**, solid: **C2**, dash-dot: **C3**, dot: **C4**.

Results

Table: Estimated parameters

Crit.	C1	C2	C3	C4
k_r	5.50	5.71	5.10	5.41
(s.d.)	(1.34)	(1.40)	$(1.12\,10^{-1})$	$(2.13\,10^{-1})$
Y_H	$3.04\,10^{-2}$	$2.79\,10^{-2}$	$3.55\,10^{-2}$	$3.75\,10^{-2}$
(s.d.)	$(1.05\,10^{-2})$	$(7.67\,10^{-3})$	$(5.66\,10^{-3})$	$(6.02\,10^{-3})$
k_d	$2.93\,10^{-2}$	$3.16\,10^{-2}$	$2.77\,10^{-2}$	$2.71\,10^{-2}$
(s.d.)	$(1.78\,10^{-2})$	$(1.33\,10^{-2})$	$(3.74\,10^{-3})$	$(2.76\,10^{-3})$
k_i	$3.42\,10^{-2}$	$2.63\,10^{-2}$	$4.80\,10^{-2}$	$6.37\,10^{-2}$
(s.d.)	$(5.08\,10^{-2})$	$(2.33\,10^{-2})$	$(3.34\,10^{-2})$	$(4.73\,10^{-2})$

Conclusions

- A model structure of carbohydrate degradation in human colon has been proposed, including:
  - Transport phenomena
  - Reaction mechanisms: functional diversity
  - Hydraulic representation
  - Physiology
- Identification of an invitro model for the homoacetogenesis
  - The mathematical model was satisfactory
  - The best results were obtained when Σ was assumed unknown
  - The estimates are consistent with the literature and biological knowledge
  - The practical identifiability problem found led to a modification on the kinetics
  - This work can be extended to the complete model structure

Future work

- Definition of the minimal functional microbiota
- Animal experiments
- Bayesian estimation

MERCI

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