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Predictive capabilities of a dynamic mechanistic model of *in vitro* fermentation by rumen microbiota

Rafael Muñoz-Tamayo

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The Team



UNIVERSITÀ
CATTOLICA
del Sacro Cuore

Antonio Gallo
Francesco Masoero



uniss
UNIVERSITÀ DEGLI STUDI DI SASSARI

Alberto S. Atzori
Antonello Cannas



INRA
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Sylvie Giger-Reverdin
Rafael Muñoz-Tamayo

Daniel Sauvant

Rumen modelling: a longstanding research activity with room for improvement

Baldwin et al., 1987; Dijkstra et al., 1992; Lescoat and Sauvant, 1995; Bannink et al., 2006; Mills et al., 2014; Vetharaniam et al., 2015; van Lingen et al., 2019

Still some limitations for accurate predictions of the fermentation pattern (volatile fatty acid, CH₄) (Offner and Sauvant, 2004; Alemu et al., 2011).

$$\frac{d}{dt} \left[\text{cow} \right]$$

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Features for improvement (Ellis et al., 2008; Janssen 2010):

- Better representation of the microbiota
- Fermentation stoichiometry
- Hydrogen dynamics
- Description of pH

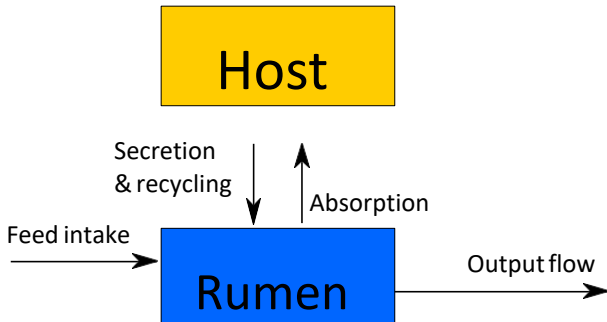
$$\frac{d}{dt} \left[\text{cow} \right]$$

Our long-term goal: to develop a model that

- Enhances the mechanistic description of rumen fermentation
- Can be used as tool for guiding sustainable feeding strategies

$$\frac{d}{dt} \left[\text{cow} \right]$$

Strategy: a stepwise approach



$$\frac{d}{dt} \left[\text{[Rumen]} \right]$$

Strategy: a stepwise approach

Rumen

$\frac{d}{dt}$ []

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Mechanistic modelling of *in vitro* fermentation and methane production by rumen microbiota

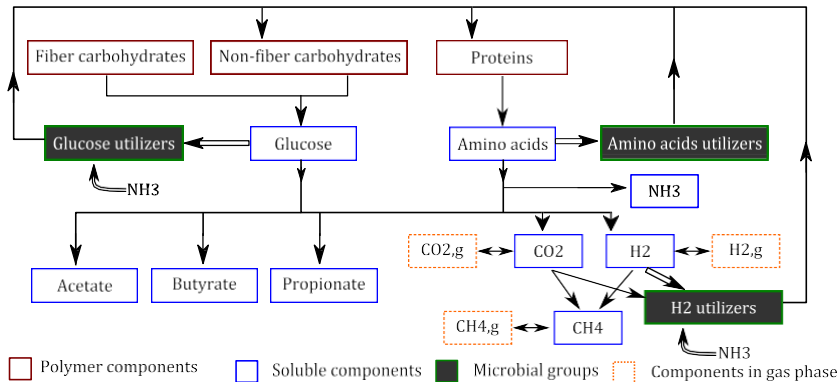


Rafael Muñoz-Tamayo*, Sylvie Giger-Reverdin, Daniel Sauvant

UMR Modélisation Systémique Appliquée aux Ruminants, INRA, AgroParisTech, Université Paris-Saclay, 75005 Paris, France



The model in a picture: satisfactory representation of *in vitro* fermentation using rumen inocula from goats



- Complexity: 18 state variables (compartments), 30 parameters
- Structure: based on models for engineering reactors (Batstone et al., 2002) and human colonic fermentation (Muñoz-Tamayo et al., 2010)
- Implementation: Matlab, R implementation by Kettle et al., 2018

Objective of this work

- Evaluate the model for its ability to predict VFA and CH₄ dynamics using an independent data set (cows)
- Assess whether the hydrolysis rate constants of diet polymers determined enzymatically could be integrated into the model and provide satisfactory predictions

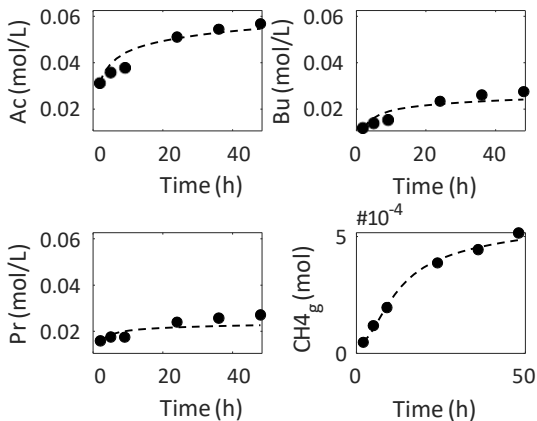
$$\frac{d}{dt} \left[\text{cow} \right]$$

In vitro experimental case study: rumen inocula from two cows

- Gas production adapted from Pirondini et al., 2012; Serment et al., 2016
- Five total mixed rations (TMR) collected from different dairy farms: NDF, starch, CP
- Degradability rates determined enzymatically (Gallo et al., 2017. J Anim Sci; Gallo et al., 2018. J Agric Sci; Gallo et al., 2019. J Dairy Sci)
 - Hemicellulase from *Aspergillus niger*
 - Amylase from *Aspergillus oryzae*, amyloglucosidase from *Aspergillus niger*
 - Cellulase from *Trichoderma viride* and Viscozyme L enzymes
 - Xylanase from *Thermomyces lanuginosus*
 - Protease from *Streptomyces griseus*
- Measurements: acetate, butyrate, propionate, CH₄

$$\frac{d}{dt} \left[\text{cow} \right]$$

Model performance: example for one *in vitro* fermentation

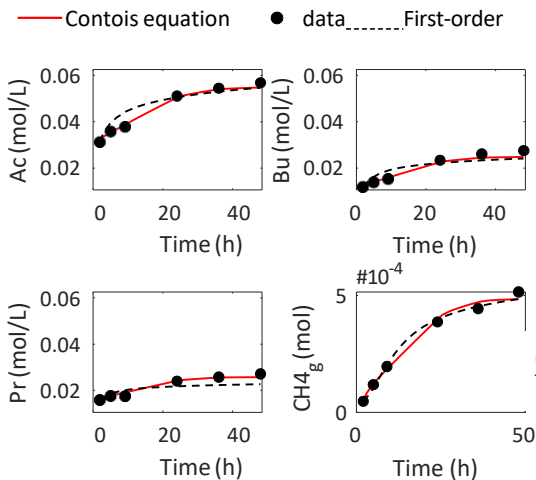


First-order hydrolysis rate: adequate predictions but VFA profile exhibits a delay pattern wrt model response

$$\frac{dz_{\text{ndf}}}{dt} = -k_{\text{hyd,ndf}} \cdot z_{\text{ndf}} \quad (1)$$

$k_{\text{hyd,ndf}}$: determined enzymatically

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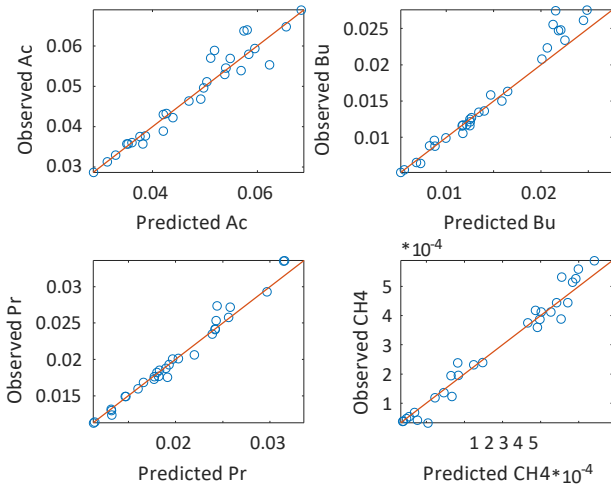
$k_{\text{hyd,ndf}}$: determined enzymatically

Contois hydrolysis rate (widely used for solid degradation in reactors) (Vavilin et al 2008)

$$\frac{dz_{\text{ndf}}}{dt} = -k_{\text{c,ndf}} \cdot z_{\text{ndf}} \cdot \frac{x_{\text{su}}}{K_{\text{hyd,ndf}} \cdot x_{\text{su}} + z_{\text{ndf}}} \quad (2)$$

CCC	Acetate	Butyrate	Propionate	CH ₄
First-order	0.94	0.88	0.71	0.99
Contois	0.99	0.98	0.98	0.99

Model performance using Contois kinetics: 5 fermentations



	Acetate	Butyrate	Propionate	CH ₄
CCC	0.96	0.96	0.99	0.98
R ²	0.93	0.94	0.97	0.97

Take home message

- Our model represented effectively the profile of individuals VFA and methane produced during *in vitro* rumen fermentation
- Contois kinetics appears more adequate than first-order kinetics to represent hydrolysis of diet polymers
- Enzymatic determination of hydrolysis rates is a promising approach: adaptation to Contois model required

$$\frac{d}{dt} \left[\text{cow} \right]$$

Take home message

- Our model represented effectively the profile of individuals VFA and methane produced during *in vitro* rumen fermentation
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Perspectives for model extensions to tackle methane mitigation strategies

- Lipid metabolism, additives, modulation factors (H_2 , pH)
- *In vivo* conditions

$$\frac{d}{dt} \left[\text{cow} \right]$$



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Diet composition

	NDF	Crude Protein	Starch	Fatty acids
1	29.04	17.53	23.82	4.09
2	31.74	17.08	23.69	3.81
3	29.49	17.25	25.14	4.89
4	36.22	14.44	20.11	3.28
5	33.81	14.58	22.04	3.52

$\frac{d}{dt}$ []