Review: Towards the next-generation models of the rumen microbiome for enhancing predictive power and guiding sustainable production strategies

R. Muñoz-Tamayo, M. Davoudkhani, I. Fakih, C.E. Robles-Rodriguez, F. Rubino, C.J. Creevey, Evelyne Forano

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R. Muñoz-Tamayo [a,*], M. Davoudkhani [a], I. Fakih [a,b], C.E. Robles-Rodriguez [c], F. Rubino [d], C.J. Creevey [d], E. Forano [b]

[a] Université Paris-Saclay, INRAE, AgroParisTech, UMR Modélisation Systémique Appliquée aux Ruminants, 91120 Palaiseau, France
[b] Université Clermont Auvergne, INRAE, UMR 454 MEDIS, Clermont-Ferrand, France
[c] TBI, Université de Toulouse, CNRS, INRAE, INSA, Toulouse, France
[d] Institute of Global Food Security, School of Biological Sciences, Queen's University Belfast, BT9 5DL Northern Ireland, UK

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ABSTRACT

The rumen ecosystem harbours a galaxy of microbes working in syntrophy to carry out a metabolic cascade of hydrolytic and fermentative reactions. This fermentation process allows ruminants to harvest nutrients from a wide range of feedstuff otherwise inaccessible to the host. The interconnection between the ruminant and its rumen microbiota shapes key animal phenotypes such as feed efficiency and methane emissions and suggests the potential of reducing methane emissions and enhancing feed conversion into animal products by manipulating the rumen microbiota. Whilst significant technological progress in omics techniques has increased our knowledge of the rumen microbiota and its genome (microbiome), translating omics knowledge into effective microbial manipulation strategies remains a great challenge. This challenge can be addressed by modelling approaches integrating causality principles and thus going beyond current correlation-based approaches applied to analyse rumen microbial genomic data. However, existing rumen models are not yet adapted to capitalise on microbial genomic information. This gap between the rumen microbiota available omics data and the way microbial metabolism is represented in the existing rumen models needs to be filled to enhance rumen understanding and produce better predictive models with capabilities for guiding nutritional strategies. To fill this gap, the integration of computational biology tools and mathematical modelling frameworks is needed to translate the information of the metabolic potential of the rumen microbes (inferred from their genomes) into a mathematical object. In this paper, we aim to discuss the potential use of two modelling approaches for the integration of microbial genomic information into dynamic models. The first modelling approach explores the theory of state observers to integrate microbial time series data into rumen fermentation models. The second approach is based on the genome-scale network reconstructions of rumen microbes. For a given microorganism, the network reconstruction produces a stoichiometry matrix of the metabolism. This matrix is the core of the so-called genome-scale metabolic models which can be exploited by a plethora of methods comprised within the constraint-based reconstruction and analysis approaches. We will discuss how these methods can be used to produce the next-generation models of the rumen microbiome.

Implications

Ruminants and their rumen microbiota exhibit an intimate relationship that shapes key animal phenotypes such as feed efficiency and methane emissions. Advances in omics techniques have deeply enlarged our knowledge on the rumen microbiota and its genome (microbiome). But, how can we capitalise on the large omic information to develop predictive tools that can guide the design of strategies for sustainable ruminant production? In this paper, we aim at responding partly to this question by discussing two mathematical approaches adapted to integrate microbial genomic information of the rumen microbiome into dynamic models.

* Corresponding author.
E-mail address: Rafael.munoz-tamayo@inrae.fr (R. Muñoz-Tamayo).

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Introduction

Ruminants are able to harvest nutrients from forage diets rich in fibres and transform them into human-edible products with high-quality proteins. Fibre degradation occurs predominantly in the rumen thanks to the action of a complex microbial community (microbiota) constituted by hundreds of species that include bacteria, archaea, protozoa, fungi and viruses. The rumen microbes encode a repertoire of enzymes for degrading plant cell wall carbohydrates allowing the animal host to harvest nutrients that are otherwise inaccessible. Due to its metabolic capabilities, the rumen microbiota can be viewed as an organ within the host. Ruminants and their microbiota have co-evolved in an intimate and symbiotic relationship, which makes us consider them as holobionts. The close connection between the ruminant and its rumen microbiota shapes key animal phenotypes such as feed efficiency and methane emissions (Wallace et al., 2019) and suggests the potential of reducing methane emissions and enhancing feed conversion into animal products by manipulating the rumen microbiota. However, only a few examples of direct microbial manipulation have shown beneficial outcomes (Huws et al., 2018). The design of successful manipulation strategies for sustainable ruminant production requires a better understanding of the dynamic interactions between the diet, the animal and its rumen microbiota. Disentangling this triad interplay requires to elucidate firstly central dynamic features of the rumen microbiota ecosystem such as interspecies interactions and resilience (Weimer, 2015). The significant technological progress in omics techniques has increased our knowledge of the rumen microbiota. The omic techniques applied to the rumen microbiota (summarised in Table 1) have been discussed in dedicated reviews (Firkins and Yu, 2015; McAllister et al., 2015; Denman et al., 2018; Huws et al., 2018; Wallace et al., 2017; Gruninger et al., 2019). Although important knowledge has been gained from omic studies on the rumen microbiota, a great challenge needs to be overcome for translating omics knowledge into effective microbial manipulation strategies. Most of the findings derived from metataxonomic and metagenomic studies are mainly descriptive and follow a simple correlation basis, while groups of microbes are linked by many interrelationships. Also, the data are normalised as relative abundance which does not represent an absolute quantification of taxa or genes. Furthermore, a great number of identified taxa have unknown function, and a high proportion of genes from the characterised taxa code for unknown proteins. To enhance our system-level understanding of the rumen ecosystem and translate genomic data into predictive tools for sustainable ruminant production, modelling approaches integrating causality principles that shape rumen metabolism are needed. Rumen modelling started in the seventies with empirical and mechanistic developments which can be either static or dynamic (Tedeschi et al., 2014). In the category of dynamic models, the most popular modelling structures are Molly (Baldwin et al., 1987), the Dijkstra model (Dijkstra et al., 1992) and Karoline (Danfær et al., 2006). These models have been incrementally improved over the years. Examples of extensions are Gregorini et al. (2015) for Molly, Huhtanen et al. (2015) for Karoline and van Lingen et al. (2019) for the Dijkstra model. Recent modelling efforts have been done to include the dynamics of methanogens (Muñoz-Tamayo et al., 2016; van Lingen et al., 2019), thermodynamic control and the impact of methane inhibitors on the rumen fermentation pattern and methane production (Muñoz-Tamayo et al., 2021; van Lingen et al., 2021). Modelling works have also been developed to study ecological interactions within the methanogen rumen community (Lynch et al., 2019; Muñoz-Tamayo et al., 2019). However, despite the model improvements before mentioned, existing rumen fermentation models do not integrate microbial genomic information of the rumen microbiome. This gap between the available rumen microbiota omics data and the representation of microbial metabolism in the existing rumen models needs to be filled to improve rumen understanding (Bannink et al., 2016). Filling this gap can lead to novel mathematical models with better predictive power and capabilities to guide nutritional strategies. A variety of mathematical modelling approaches have been developed to study the human gut microbiome. Kumar et al. (2019) have categorised these modelling approaches into four groups: (i) dynamic modelling that account for phenotypic traits of microorganisms, (ii) modelling based on sequence read abundance, (iii) constraint-based modelling using annotated genomes and (iv) agent-based modelling. The interested reader is referred to the review of Kumar et al. (2019) that discussed the advantages and challenges of these modelling approaches. It goes without saying that modelling approaches developed for the human gut microbiome can be applied to the rumen microbiome. In this paper, we discuss the potential use of two modelling approaches for the integration of microbial genomic information into dynamic models, namely state observers and genome-scale metabolic models (**GEMs**). A GEM is a detailed model of microbial metabolism that links the metabolites and biochemical reactions that an organism is able to perform as a result of its genetic potential. While the GEM approach has been applied to study the human gut microbiota (Heinken et al., 2023; Kumar et al., 2019), genome-based modelling of the rumen microbiota is at an infant stage. It is yet unclear how these GEMs can be integrated into whole rumen models adapted to evaluate a wide range of nutritional conditions (Bannink et al., 2020). This paper aims to foster the incorporation of genome-scale-based approaches into rumen modelling efforts.

Table 1
Meta-omics analyses used to study the rumen microbiome, and their specific contribution to our understanding of the rumen functions.

METAGENOMICS • Discovery of uncultured microbial genomes • Potential activity of microbiota • Taxa-related metabolic features • Putative interaction network	METATRANSCRIPTOMICS • Gene expression profiling • Gene expression regulation • Identification of active taxa • Identification of microbiota activity Rapid response to various factors (environmental stimuli…)
METAPROTEOMICS • Taxa-specific protein profiles • Identification of microbiota activity • Localisation of protein activity	METABOLOMICS • Metabolite profiling • Identification of metabolites associated with animal phenotype (biomarkers) or rumen microbiota profile

Modelling approaches for integrating microbial genomic knowledge

Existing dynamic models of rumen fermentation are kinetic models where microbial metabolism is represented in a simplified aggregated pathway consisting of a few macroscopic reactions defined either empirically or from dedicated literature. The dynamics of metabolism of a single rumen microbe or of the full microbial ecosystem can be described by the following differential equation resulting from applying mass balances in a stirred system

$$\frac{d\mathbf{x}}{dt} = \mathbf{S}\,\mathbf{r}(\mathbf{x},\mathbf{p}) + \mathbf{g}(\mathbf{x},\mathbf{q}) \quad (1)$$

Eq. (1) is a generic equation that can be applied to any microbial process (Bastin and Dochain, 1990). Here, $\mathbf{x}$ is the vector containing the concentrations of metabolites, which can be either intracellular ($\mathbf{x}_i$) or extracellular ($\mathbf{x}_e$). The fluxes associated to the metabolic conversions are represented by the term $\mathbf{S}\,\mathbf{r}(\mathbf{x},\mathbf{p})$, where $\mathbf{S}$ is the stoichiometric matrix and $\mathbf{r}$ is the vector of reaction rates (per unit of time). The reactions rates are expressed as mathematical functions of the concentrations $\mathbf{x}$ and the parameter vector $\mathbf{p}$. These reactions are catalysed by a proxy of microbial biomass activity and might depend on environmental conditions such as the pH. The Monod kinetic function is a typical equation used to represent the reaction rates of microbial processes. The fluxes related to mass transport phenomena (*e.g.*, passage rate, absorption, liquid–gas transfer) are represented by the vector $\mathbf{g}$, which is function of $\mathbf{x}$ and the parameter vector $\mathbf{q}$. The prediction capabilities of any rumen model will depend on how accurate is the representation of the microbial fermentation (described by $\mathbf{S},\mathbf{r},\mathbf{p}$) and the transport phenomena (described by $\mathbf{g},\mathbf{q}$). This paper focuses on the microbial fermentation. However, it should be noted that defining the structure of $\mathbf{g}$ and numerical values of $\mathbf{q}$ for the transport phenomena is also a challenging task.

In existing rumen models, $\mathbf{r}$ is a vector with few macroscopic reactions representing an aggregated pathway of the rumen microbiota. Here, the rumen microbiota is described by few major functional groups (*e.g.,* sugar utilisers, amino acid utilisers and hydrogen utilisers (Muñoz-Tamayo et al., 2016)). As previously mentioned, existing rumen models do not integrate microbial genomic information. The integration of such an information implies to translate the knowledge of the metabolic potential of the rumen microbes (inferred from their genomes) into a mathematical object. In the following sections, we will discuss two modelling approaches that allow such an integration. The first approach explores the theory of state observers to integrate microbial time series into rumen fermentation models. The second approach is based on the genome-scale network reconstruction of rumen microbes. It should be noted that these two modelling approaches follow the same generic Eq. (1).

Microbial time series and state observers

Microbial communities change over time in response to environmental changes. The analysis of microbial time series is a useful tool for monitoring and characterising the evolution of microbial community and the interactions between its members. The analysis of microbial time series can also provide insight on key dynamic properties of the ecosystem such as stability and resilience to perturbations. Analysis of rumen microbial time series has been applied to characterise rumen microbial colonisation patterns both *in vivo* (Huws et al., 2021; Piao et al., 2014) and *in vitro* (Belanche et al 2017), and the dynamic response of the methanogenic community to the supplementation of the methane inhibitor 3-nitrooxypropanol (Pitta et al., 2021).

When sufficient time points are measured, a variety of methods are available to analyse microbial time series (Faust et al., 2015). These methods include network inference reconstructions and community dynamic models, with the generalised Lotka–Volterra (**gLV**) model being one of the most widely approaches used to model microbial communities. Gonze et al. (2018) provide a detailed review on gLV approach, its applications and limitations. One of the limitations of the gLV approach is that it is not well-suited to analyse high-dimensional microbiome time series data. Another limitation is that the gLV approach does not integrate information on the concentration of fermentation metabolites. An alternative to exploit microbial time series within a mechanistic modelling framework like the one represented by Eq. (1) is the use of state observers (also called software sensors). An observer is an algorithm that uses a mathematical model and measured variables to estimate unmeasured variables of a given system. Observers have been widely applied to monitor and control biological processes including engineering anaerobic reactors (Jimenez et al., 2015). State observers for anaerobic processes are often applied to estimate the concentrations of key compounds such as volatile fatty acids (**VFAs**) concentrations from available measurements (e.g., H_2, and CO_2 gas rates) (Aceves-Lara et al., 2010). A potential application of the state observers is the estimation of functional microbial species within microbial consortia. This type of application has been rarely applied. One of these applications includes the estimation of the evolution of ammonia and nitrite-oxidising bacteria in a nitrifying chemostat (Dumont et al., 2009; Ugalde-Salas et al., 2019). A theoretical work addressed the estimation of two microbial strains in a consortium exhibiting cross-feeding interactions (dos Reis de Souza et al., 2023). The capability of the state observers to link information on microbial abundance and fermentation metabolites can be of great value for the rumen microbiota, as we discuss below.

To illustrate the concept of state observers, let us consider the following set of equations representing the concentration dynamics of a microbe (B) and a product (P) in an *in vitro* continuous reactor with dilution rate D:

$$\frac{dB}{dt} = r - D \cdot B \tag{2}$$

$$\frac{dP}{dt} = k \cdot r - D \cdot P \tag{3}$$

The growth of B follows the reaction rate r. The production of P is given by r and the stoichiometry coefficient k. Under the hypothesis that B can be measured in time and P is not measured, the goal of the observer is to estimate P from B. We will assume here that the reaction rate r is unknown while the coefficient k is known. We can then construct a new variable z defined by

$$z = k \cdot B - P \tag{4}$$

By deriving z with respect to time and using Eqs. (2)–(4), we obtain

$$\begin{aligned}\frac{dz}{dt} &= k \cdot \frac{dB}{dt} - \frac{dP}{dt} = k \cdot r - k \cdot D \cdot B - k \cdot r + D \cdot P \\ &= -D \cdot (k \cdot B - P) = -D \cdot z\end{aligned} \tag{5}$$

Let us denote $\hat{z}$ an on-line estimate of z. The dynamics of $\hat{z}$ follows

$$\frac{d\hat{z}}{dt} = -D \cdot \hat{z} \tag{6}$$

If dynamic data of B are available and given than an estimate of $\hat{z}$ is obtained from Eq. (6), we can use Eq. (4) to have the estimate $\hat{P}$ as

$$\hat{P} = k \cdot B - \hat{z} \tag{7}$$

This observer is called asymptotic observer. The great advantage of this type of observer is that it does not require knowledge on r. Indeed, defining the mathematical function of r is one of the most challenging parts in the model construction of microbiological systems. A limitation of the asymptotic observer is that the rate at which the observer estimate converges to the real value is fixed by the operating conditions of the system (*e.g.* the dilution rate). Another limitation is that the stoichiometric coefficients k should be known. These limitations can be overcome by other state observers, but more sophisticated mathematical technicalities are required for the design of such observers (Dochain, 2003).

The previous observer for P constituted by Eqs. (6) and (7) and data on biomass B can be extended to the case of consortia with n microbial species (represented for example by Operational Taxonomic Units - OTUs). Hence, the model equations of the system are

$$\frac{dB_i}{dt} = r_i - D \cdot B_i \tag{8}$$

$$\frac{dP}{dt} = \sum_{i=1}^{n} k_i \cdot r_i - D \cdot P \tag{9}$$

With k_i, r_i the stoichiometry coefficients and the reaction rates for the microbe B_i ($i = 1, 2, \cdots, n$). The variable z is thus defined by

$$z = \sum_{i=1}^{n} k_i \cdot B_i - P \tag{10}$$

If the concentrations of the different microbial species B_i can be measured on time, we can then build an observer for P following the same procedure than that used for a single species previously illustrated.

In a theoretical study, we applied this approach using the mathematical model of rumen *in vitro* fermentation developed by Muñoz-Tamayo et al. (2016) extended to account for continuous mode operation. The model considers three functional microbial groups namely sugar utilisers, amino acid utilisers and hydrogen utilisers. Fig. 1 displays the schematic representation of the fermentation pathway of our model. For our theoretical exercise applying the state observer approach, we assumed a hypothetical simulation scenario where sugar utilisers and amino acid utilisers were constituted by five microbial species with different kinetic rates (Davoudkhani et al., 2022a). Fig. 2 shows the dynamics of the five microbial species for each functional group and the simulation results of the observer for acetate, butyrate and propionate. The initial condition was set far from the "real" condition to illustrate that the estimation given by the observer converges to the real value (Davoudkhani et al., 2022a).

The approach assumes that functional assignment of the microbes is possible. However, this functional assignment is a challenging issue that can be addressed as an optimisation problem (Ugalde-Salas et al., 2019). Another strong assumption for the state observer approach is that each microbe (**OTU**) participates only in one macroscopic reaction. This assumption only holds for few microbes, such as the hydrogenotrophic methanogens. Indeed, many microbes are capable of participating in various metabolic pathways. The inability to account for the overlapping of metabolic functions is a limitation of the observer approach when applied to individual OTU data. To circumvent the obstacle of deriving a state observer on the OTU basis, an alternative is to substitute OTUs by microbial functional proxies. Following this rationale, we have recently developed an approach that exploits CowPI (Wilkinson et al., 2018) to infer, from microbial time series based on 16S data, the abundances of microbial functional proxies involved in specific processes of VFA production. Our approach was used to estimate, *via* an asymptotic observer, the dynamics of acetate, butyrate and propionate concentrations in a RUSITEC experiment carried out by Belanche et al. (2017). Our results indicated the promising application of observers and microbial time series data to investigate alternatives to connect omics data and mathematical modelling for studying the rumen microbial ecosystem (Davoudkhani et al., 2022b).

Genome-scale metabolic modelling

The core of a genome-scale metabolic model is the stoichiometry matrix **S** of the metabolism. For a genome-sequenced microorganism, the stoichiometry matrix is built on the basis of genome-scale network reconstructions following a detailed protocol

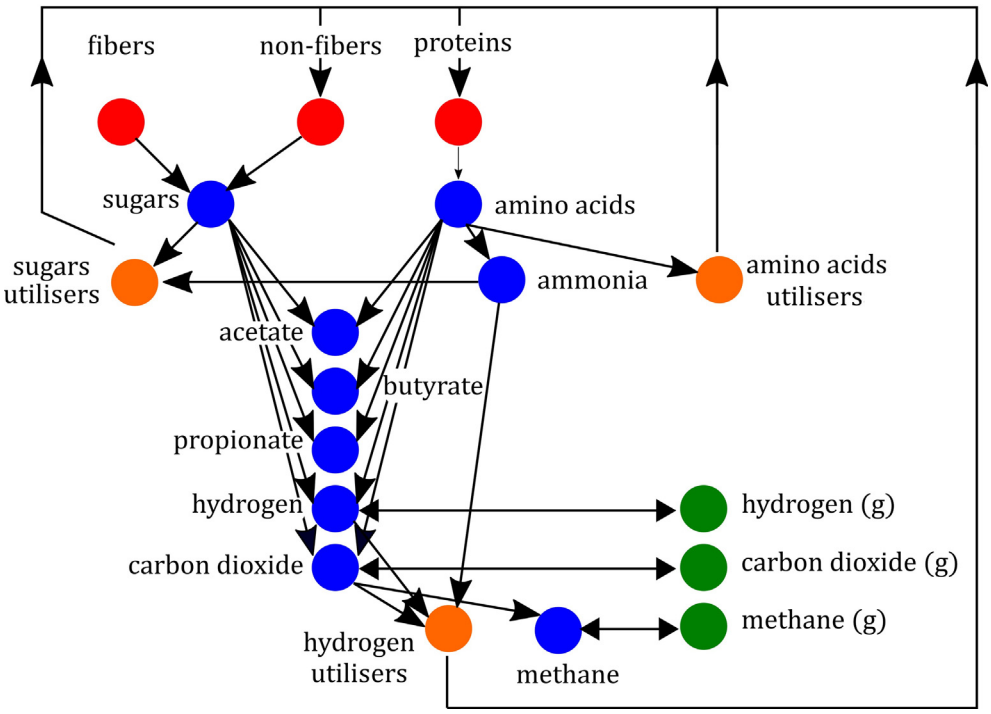

Fig. 1. Schematic representation of the rumen fermentation in the mathematical model developed by Muñoz-Tamayo et al. (2016) used to illustrate the state observer approach. Feed polymers (fibre, on-fibre carbohydrates, and proteins) are hydrolysed into sugar and amino acid monomer pools. Monomers are further metabolised by specific functional microbial groups, namely sugar utilisers and amino acid utilisers which produce acetate, butyrate, propionate, hydrogen and carbon dioxide. The microbial group of hydrogen utilisers uses hydrogen and carbon dioxide to produce methane.

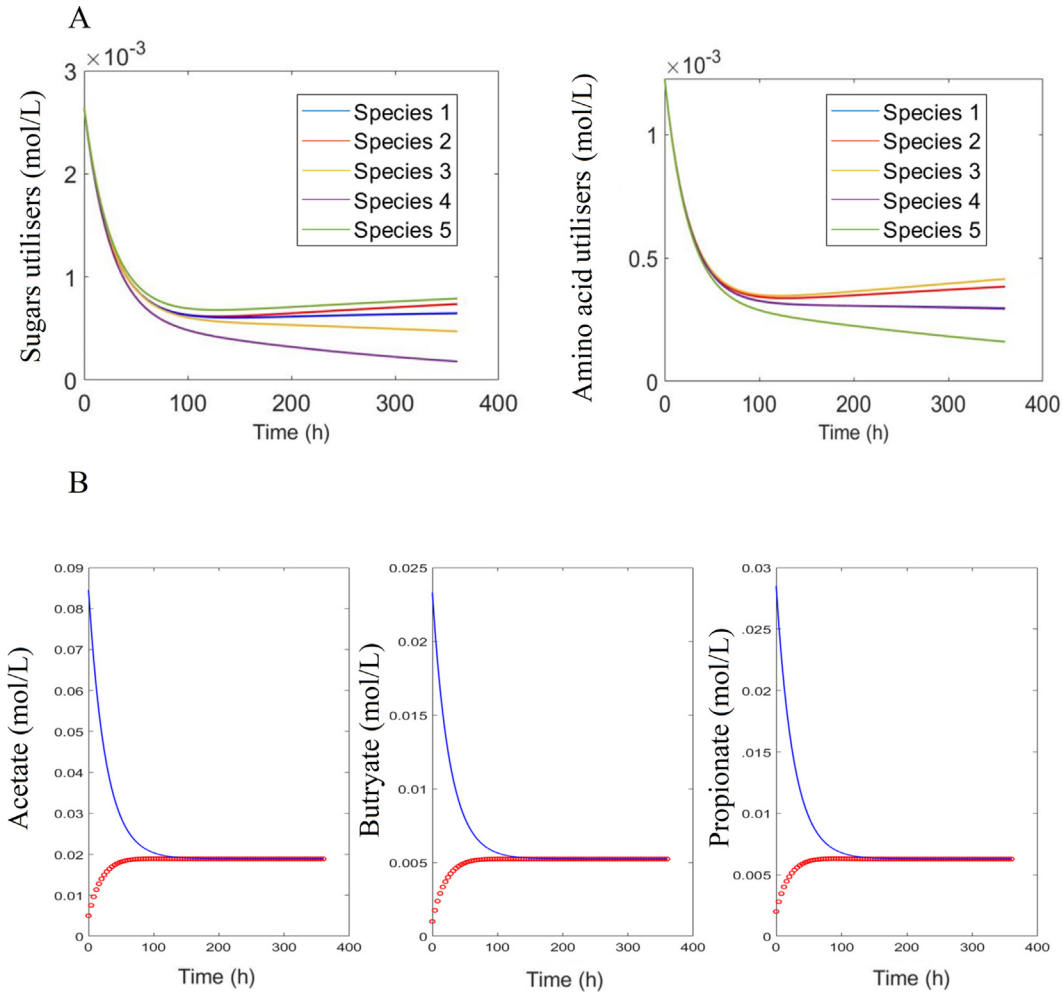

Fig. 2. Simulation study to assess the performance of an asymptotic observer applied to the mathematical model of rumen fermentation developed by Muñoz-Tamayo et al. (2016). In the original model, acetate, butyrate and propionate are produced by the action of two functional microbial groups, namely sugar utilisers and amino acid utilisers. For our theoretical exercise, we assumed a hypothetical simulation scenario where each microbial functional group (sugar utilisers and amino acid utilisers) were constituted by five microbial species with different kinetic rate parameters. The top figure (A) shows the concentration dynamics of the five species for each microbial group. The bottom figure B shows the estimated values of acetate, butyrate and propionate of the state-observer (solid line) converge to the "real" values (○).

(Thiele and Palsson, 2010) that can be briefly summarised by the next five steps:

i. Functional genome annotation. This step aims at associating genes, proteins, and reactions to a draft of metabolic reactions.
ii. Orthology: reconstruction based on the comparison with GEMs of other microorganisms.
iii. Gap-filling: process of completion of pathways.
iv. Manual curation: the network is curated on the basis of expert knowledge, experimental data and dedicated literature.
v. Translation of the reconstruction to a computational model. The final result is a detailed metabolic reaction network that can be represented mathematically in a matrix form that captures the stoichiometry of the metabolism.

Several databases and toolboxes are available to facilitate the reconstruction of GEMs including KEGG (Kanehisa and Goto, 2000), Metacyc (Caspi et al., 2016), BiGG (King et al., 2016), Pathway Tools (Karp et al., 2002), CarveMe (Machado et al., 2018), KBase (Arkin et al., 2018) and AuReMe (Aite et al., 2018). The interested reader is referred to the benchmark study by Mendoza et al. (2019) which assessed several features of seven genome-scale reconstruction tools.

The stoichiometry matrix contains a high number of rows (metabolites) and reactions (columns). From the reconstruction of draft GEMs, an average GEM of a rumen microbe can consist of 1 155 reactions and 1 422 metabolites (Belcour et al., 2020). We have reconstructed a metabolic network of the cellulolytic rumen bacterium *Fibrobacter succinogenes* S85, which comprised 1 565 reactions and 1 586 metabolites (Fakih et al., 2023). While kinetic models derive the stoichiometric matrix by prior knowledge and dedicated literature, in the GEM approach, the stoichiometric matrix is derived directly from the genome of the microbe of interest. The stoichiometry matrix can be analysed by a plethora of methods comprised within the constraint-based reconstruction and analysis (**COBRA**) approaches (see, e.g., the review by Lewis et al., 2012). The constraint-based term results from the analysis that the capabilities of the microbes are bounded by constraints that include thermodynamics and enzyme capacities.

The stoichiometric matrix **S** contains the stoichiometric matrices for intracellular ($\mathbf{S}_i$) and extracellular ($\mathbf{S}_e$) metabolites. That is:

$$\mathbf{S} = \begin{bmatrix} \mathbf{S}_i \\ \mathbf{S}_e \end{bmatrix} \tag{11}$$

COBRA approaches overcome the need of defining kinetic rates and its parameters by assuming that internal metabolism operates at steady-state condition. Consequently, genomic-scale modelling focuses mainly on the analysis of the intracellular matrix $\mathbf{S}_i$. For simplicity, let us omit the transport phenomena in Eq. (1) and focus only on the metabolism phenomena, represented by the term $\mathbf{S}\,\mathbf{r}(\boldsymbol{x}, \boldsymbol{p})$. Applying the steady-state condition for the intracellular metabolites results in

$$\frac{d\boldsymbol{x_i}}{dt} = \mathbf{S_i}\mathbf{r} = 0 \tag{12}$$

Since the number of reactions is typically higher than the number of metabolites, Eq. (12) is often underdetermined. All admissible solutions of Eq. (12) constitute the solution space, that mathematically corresponds to the null space (kernel) of the stoichiometric matrix $\mathbf{S_i}$. COBRA approaches are centred on the analysis of $\mathbf{S_i}$ and aim to predict the potential phenotypes of an organism on the basis of its genome. Flux balance analysis (**FBA**) (Varma and Palsson, 1993) and elementary flux mode analysis (**EFM**) (Schuster and Hilgetag, 1994) are the basic frameworks of COBRA. FBA and EFM have served as scaffolds for the development of a plethora of approaches that counts with more than 100 methods (Lewis et al., 2012). The principles of FBA and EFM are briefly described below.

Flux balance analysis

An infinite number of solutions exist that fulfil the steady-state Eq. (12). To reduce the solution space, FBA looks at finding the flux vector **r** by optimising a regulatory optimal condition. The most used optimisation criterion applied in FBA is the maximisation of the biomass growth rate. Other optimal criteria are for example the maximisation of production of ATP and the production of a desired by-product. FBA solves the system of linear equations (12) under defined constraints and an objective function by using linear programming. FBA is included in the collection of methods of the COBRA toolboxes (Heirendt et al., 2019) for the analysis of GEMs. Within the FBA framework, it is possible to predict the maximal growth rate of an organism and the production rates of metabolites. However, FBA does not allow the prediction of metabolite concentrations. Other applications of interest of FBA include robustness analysis that allows to assess the impact of varying a particular reaction of the network on the growth rate. For small networks, the optimal solution is often unique, while for large networks, multiple optimal solutions are frequently found. Multiple solutions are the result of the redundancy capability of the microbe, a property that is linked to metabolic robustness. Once the maximal growth rate is obtained, it is possible to perform multiple optimisations to calculate the maximum and minimum flux values of each reaction in the network to characterise the range of metabolic functions. This approach is called flux variability analysis (**FVA**) (Lewis et al., 2012). As previously mentioned, FBA is based on the steady-state assumption. However, a further extension, named as dynamic FBA (**DFBA**) (Mahadevan et al., 2002), has been developed to account for the dynamics of microbial metabolism. DFBA allows to predict the dynamics of metabolites. The DFBA approach is often applied on a reduced metabolic network. FBA applications require high-quality GEMs that result from an exhaustive reconstruction protocol based on detailed biochemical data, high level of curation and knowledge on gene functions. Nevertheless, a good portion of any genome contains genes whose functions are unknown (Zengler and Palsson, 2012). Accordingly, high-quality level reconstructions might not be feasibly reached yet for the complex rumen microbial community without a massive effort. Whereas high-quality level reconstructions of rumen microbes are not available, GEM applications for the rumen ecosystem can focus on metabolic core functionalities. It should be noted that, as presented, the construction of a GEM appears disconnected from the process of transcriptional regulation. A variety of methods have been developed to integrate gene expression into genome-based models as reviewed and assessed by Machado and Herrgård (2014). These methods should also be considered within the endeavour of constructing genome-based models of the rumen microbiome.

Elementary flux modes analysis

In contrast to FBA, EFM analysis is a non-optimisation technique. EFM analysis is intended to study the full capabilities of a given metabolic network by finding the simplest biochemical flux vectors, in terms of which all other flux vectors can be expressed (Schuster and Hilgetag, 1994). This means that the solution space can be spanned by a set of basis vectors. To find those vectors, Schuster and Hilgetag (1994) made use of concepts and tools from convex analysis. The vector that fulfils the condition in Eq. (12) – without any additional optimality constraint – are non-negative vectors contained in the null-space of the stoichiometric matrix $\mathbf{S_i}$. The space of admissible fluxes is a convex polyhedral cone. The generating vectors of the cone are called elementary flux modes. Any steady-state flux distribution can be expressed as a non-negative linear combination of the EFMs. Biochemically, in the words of Schuster et al. (2002), "EFMs (direct reaction routes) are minimal sets of enzymes that can operate at steady state, with all irreversible reactions used in the appropriate direction. They can be interpreted as component pathways of a (bio)chemical reaction network". In other words, EFMs are minimal pathways (functional building blocks) though the network (Zanghellini et al., 2013). An EFM is composed of a minimal number of reactions, where the term minimal implies that if a reaction is omitted from the reaction route, the full pathway is blocked. Zanghellini et al. (2013) used an illustrative metaphor of a subway map to explain the EFM.

Dedicated software is available for EFM computation (Klamt et al., 2007). Applications of EFMs include the assessment of yields for all independent pathways, analysis of functional redundancy of a network, and robustness of an organism subject to gene deletions and additions approaches (Lewis et al., 2012). The EFMs can be exploited to derive macroscopic kinetic models. Indeed, each EFM can be translated into a macroscopic reaction and thus be used to build a dynamic model. This type of approach has been used to model the metabolism of Chinese hamster ovary cells (Provost et al., 2006), microalgae (Baroukh et al., 2014) and yeast (Robles-Rodriguez et al., 2017). The key point is to select a minimal set of EFMs that span the metabolic capabilities of the organisms. This task can be done by using yield analysis (Song and Ramkrishna, 2009). The calculation of EFMs can become computationally expensive for large networks. Therefore, GEM reduction methods are required to provide networks with core functionalities. The Supplementary Material S1 discusses some GEM reduction methods. This model reduction follows the principle of parsimony, which states, as a general rule, that simpler theories should be preferred to more complex ones. Regarding model construction, one would like to build the less complex model that can represent adequately the system under study. Model simplification offers various advantages in terms of numerical simulation and structural properties such as parameter identifiability (Muñoz-Tamayo et al., 2018; Muñoz-Tamayo and Tedeschi, 2022), which is a key aspect that should be considered to provide reliable models without over-parameterisation.

Fig. 3 sums up the FBA and EFM approaches applied on the analysis of the solution space of Eq. (12). The capability of the EFM-based approach to translate microbial genomic knowledge into

macroscopic reactions makes the EFM framework a suited approach for modelling the rumen ecosystem. Indeed, the resulting macroscopic reactions derived from EFM analysis can be integrated into dynamic models accounting for the fluctuating rumen environment and the interaction between the rumen microbiome, the host and the diet. Fig. 4 summarises the workflow of constructing a dynamic genome-based model.

Microbial community modelling

The previous sections addressed the GEM approach applied to single microbes. The construction of GEMs of key rumen species is a key step towards the generation of a rumen microbiome model. However, to model the rumen microbiome, it is needed to address how the GEM approach should be extended to the whole microbial ecosystem. The mathematical modelling of metabolic networks of microbial consortia at genome scale is still at an early stage (Zengler and Palsson, 2012). To model microbial communities, the main challenge to be addressed relates to the question of how the species, their metabolic networks, and interspecies interactions should be represented. Tackling this challenge becomes critical when analysing high diverse ecosystems such as the rumen. The critical issue of representing the species (and their metabolic capabilities) into GEMs has been addressed by two frameworks, namely the compartmental (Stolyar et al., 2007) and the supra-organismal approaches (Klitgord and Segre, 2011). The two approaches are depicted in Fig. 5. In the compartmental approach, the metabolic network of each microbial species is treated as a separate compartment, whereas the supra-organismal approach assumes that the microbial community behaves as a single microorganism provided with all the metabolic capabilities of the individual species of the consortia. The supra-organism approach is strongly linked to the principles of whole genome sequencing.

For highly diverse ecosystems, the compartmental approach *sensu stricto* results in a highly complex model (for the rumen ecosystem, a compartmental model will imply hundreds of microbial species, and thus thousands of reactions and metabolites). On the other hand, the main weakness of the supra-organism approach is that due to its level of aggregation, it lacks a description of the connectivity principle among species which is a determining factor of the function of the whole community (Biggs et al., 2015). Thus, the supra-organism approach offers limited capabilities to study central metabolic interactions such as cross-feeding and interspecies hydrogen transfer. Following the evidence of a rumen core microbiota (Creevey et al., 2014; Henderson et al., 2015; Wallace et al., 2019), a potential alternative between the two approaches is to represent the rumen microbial community by a mini-consortium of microbes covering the rumen functional core. The selection of the members of a rumen functional core microbiome can be supported by existing literature and by the use of tools such as Metage2Metabo (Belcour et al., 2020) which uses draft GEMS to identify minimal communities and keystone species for a targeted set of compounds. The development of a rumen microbiome model will require strong integration between modelling approaches and dedicated *in vitro* experiments designed to characterise in deep rumen microbial interactions and the influence of such interactions on the fermentation profile (Popova et al., 2022). The expected data from these *in vitro* experiments should be dynamic including metabolites and microbial biomass at different sampling times to have enough information to calculate metabolic fluxes and accurate mass balance. This accuracy is critically needed to study for example the fate of hydrogen under methanogenesis inhibition (Morgavi et al., 2023). The synergy between experimentation and modelling will provide useful data for the determination of the model parameters related to microbial metabolism following the principle of parsimony previously discussed. That is, that one may not need to model all rumen species but rather a minimal set of (meta) species covering the rumen functional core.

Applications of genome-scale metabolic modelling approaches to rumen microbiome

Major potential applications of genome-based approaches for the rumen microbiota include the design of cultivation media for

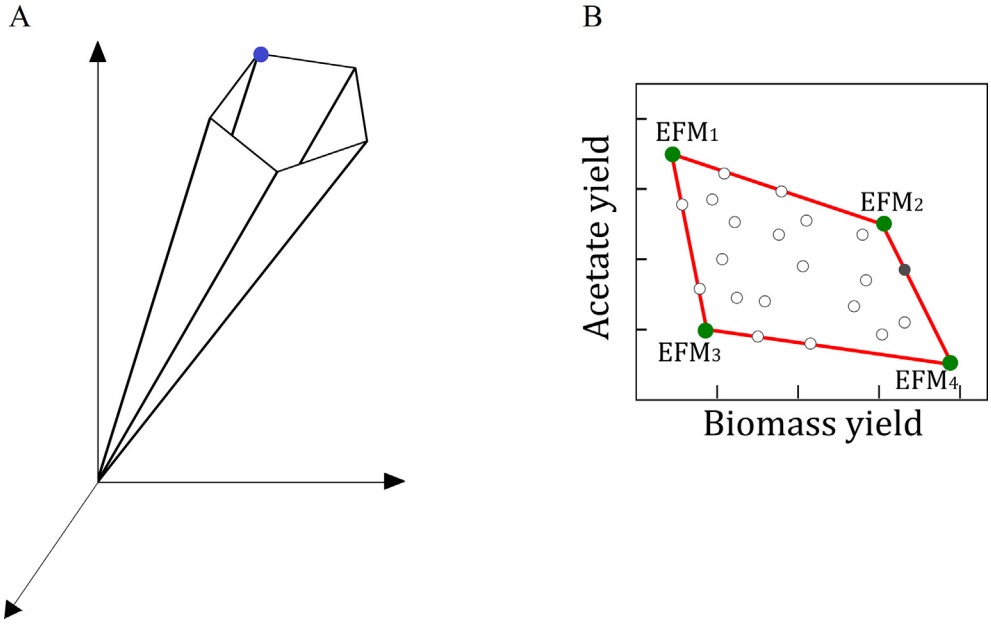

Fig. 3. COBRA approaches are based on the analysis of the allowable states of a metabolic network. These admissible states are contained in a polyhedral cone (A). The generating vectors of the cone are the elementary flux modes (EFMs). Flux balance analysis aims at finding an optimal solution in the solution space (blue circle in A). The EFMs can be projected in a yield space (B). The EFMs at the vertices of the polygon are a minimal set spanning the metabolic capabilities of the microorganism.

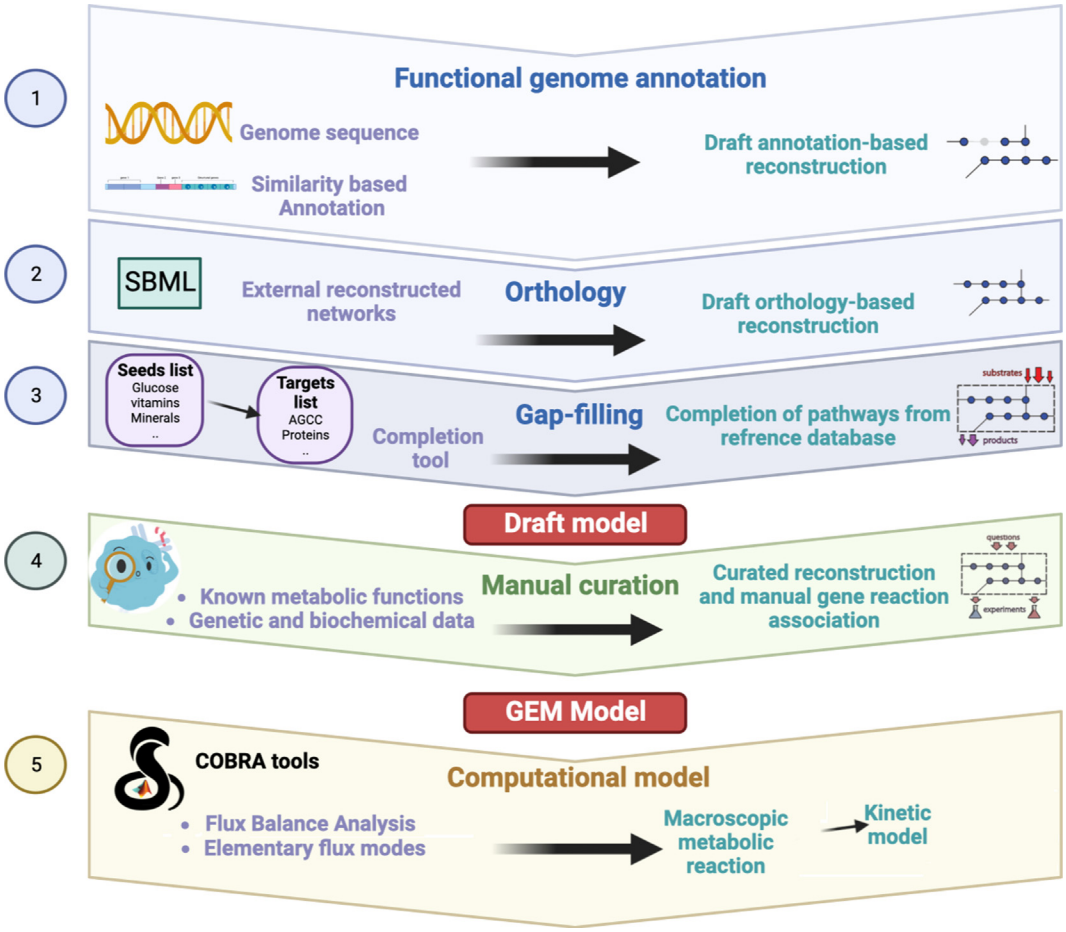

Fig. 4. The steps to build a dynamic kinetic genome-based model of microbial metabolism. SBML: Systems biology markup language; GEMs: Genome-scale metabolic models.

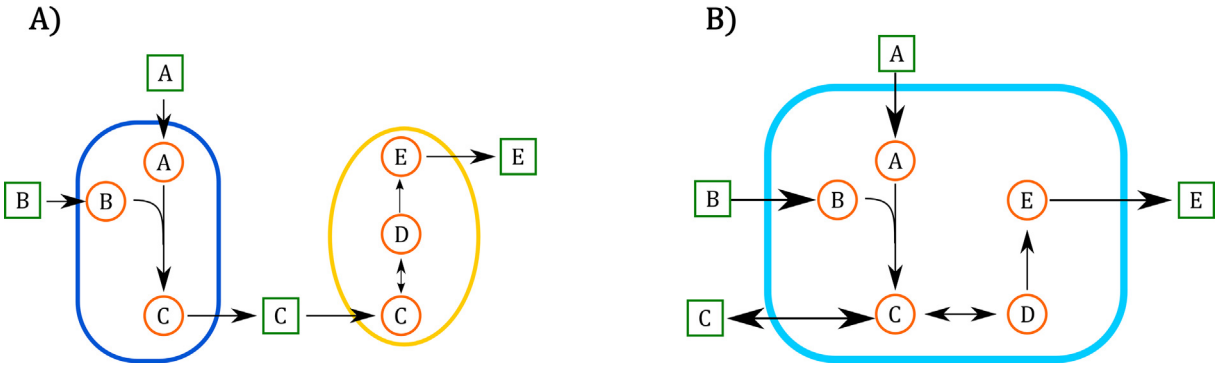

Fig. 5. Approaches for modelling microbial communities. (A) Compartmental approach; (B) Supra-organism approach. In the compartmental approach, the metabolic network of each microbial species is treated as a separate compartment. In the supra-organismal, all metabolic networks are aggregated into one supra-organism with the metabolic capabilities of all individual microbial species.

uncultured microorganisms, the identification of probiotics to enhance rumen function, the design of strategies for methane inhibition, and the exploitation of rumen microbes for the production of valuable compounds. GEMs also allow to characterise the interconnection between microbes within an ecosystem and provide insight into central ecosystem properties such as robustness, resilience and functional redundancy (Weimer, 2015) which should be considered when designing microbial manipulation strategies.

Few applications of genome-based approaches are reported for the rumen microbiota.

Within an industrial context of microbial synthesis of valuable compounds, the GEM reconstruction of *Actinobacillus succinogenes* 130Z allowed to investigate the metabolic potential of this ruminal strain for the production of succinic acid from low-cost raw materials (Pereira et al., 2018). The model was identified as a useful resource for metabolic engineering strategies aiming at improving succinic acid yields. In the same industrial-driven approach, the GEM construction of the lactate-utilising bacterium *Megasphera elsdenii* allowed the identification of pathways involved in the mechanism of metabolic production of hexanoic acid, which is an

industry-valuable product (Lee et al., 2020). We have recently reconstructed the GEM of *Fibrobacter succinogenes* S85 using the AuReMe toolbox (Aite et al., 2018). We applied further the EFM framework on the GEM to produce a dynamic model that predicts the production of acetate, succinate and formate from the metabolism of glucose, cellobiose and cellulose (Fakih et al., 2023).

At the community level, a GEM compartmental approach was applied to study a mini-consortia composed of the keystone rumen species *Ruminococcus flavefaciens, Prevotella ruminicola,* and *Methanobrevibacter gottschalkii* (Islam et al., 2019). The resulting GEM allowed to predict the metabolic yields of the community and its relative populations, but also led to the identification of 22 new inter-species interactions into this community. The authors also investigated the presence of a possible metabolic synergy between viruses and the members of the community *via* the addition of viral functionalities by local alignment. A significantly disrupted bacterial metabolism was detected, which confirmed the crucial role of viral auxiliary metabolic genes in the reprogramming of microbial metabolism.

By using the Metage2Metabo software, Belcour et al., 2020 constructed draft GEMs from the collection of 913 cow rumen MAGs (Metagenome Assembled Genomes) published in Stewart et al. (2018). Metage2Metabo allowed to identify a minimal community of 44 GEMs capable of producing the 296 metabolic end-products that the whole rumen community can potentially produce synergistically. This type of findings provides valuable information for the development of synthetic ecology strategies aiming at advancing fundamental understanding of the rumen microbiome. It should be said, however, that the computational identification of key species is a first step for the construction of minimal consortia but further experimental work is needed to verify that the fluxes of metabolic reactions are actually active and quantitatively similar to those of the whole rumen ecosystem. An iterative process should be then required for the construction of functional representative minimal consortia of the rumen.

Final remarks

The integration of microbial omics data into mathematical models of the rumen microbiome can produce novel tools with enhanced power for predicting rumen function. The potential applications of these next-generation models are broad including the design of microbial manipulation strategies to enhance feed efficiency and mitigate emissions from the ruminant sector. To reach this expected impact, a Cartesian approach build on the analysis of systems at different levels of microbial complexity (co-culture, mini-consortia and whole consortia) is needed to derive parsimonious and representative models of the rumen microbiome that can be integrated into whole rumen models that incorporate the biological levels associated with the host. This exciting challenge can only be reached *via* a strong interdisciplinary synergy between scientists with expertise in microbiology, animal physiology, computational biology, biochemistry and mathematical modelling. Such a synergy is required to provide high informative data that allow accurate mass-balance predictions of rumen microbial fluxes.

The embracement of the GEM framework into rumen modelling will require the appropriation of new skills by the rumen modelling community. In this direction, we can learn and take advantage of the developments made by the system biology community on the modelling of microbial communities such as the human gut and artificial communities. The learning process should include the enhancement of open science practices (Muñoz-Tamayo et al., 2022) to strengthen the sharing of models and resources which will result in enhanced rumen microbiome models accessible to the community.

Supplementary material

Supplementary material to this article can be found online at https://doi.org/10.1016/j.animal.2023.100984.

Ethics approval

Not applicable.

Data and model availability

Not applicable. Data or models were not deposited in an official repository. No new datasets were created.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

Author ORCIDs

RMT: https://orcid.org/0000-0002-9266-4132.
MD: https://orcid.org/0000-0001-5924-506X.
IF: https://orcid.org/0000-0001-6202-062X.
CERR: https://orcid.org/0000-0002-2436-3653.
FR: https://orcid.org/0000-0001-9090-6794.
CJC: https://orcid.org/0000-0001-7183-1555.
EF: https://orcid.org/0000-0002-7450-3466.

Author contributions

All authors contributed to conceptualisation, writing – review and editing.

Declaration of interest

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Transparency declaration

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