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

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Abstract
The rumen ecosystem harbours a galaxy of microbes working in synthrophy 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 basis 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, 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 review, we discuss computational biology tools to analyse the rumen microbiome and 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 (COBRA) approaches. We will discuss how these methods can be used to produce the next generation models of the rumen microbiome.

Keywords: genome-scale metabolic model, genomics, rumen fermentation, rumen microbiota, rumen modelling

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 to capitalize on the large omic information to develop predictive tools that can guide the design of strategies for sustainable ruminant production strategies? In this review, we aim at responding partly to this question by discussing mathematical approaches adapted to integrate microbial genomic information of the rumen microbiome into dynamic models.

Introduction

Forage-fed ruminants have the highest efficiency for net production of human-edible protein among livestock. This contribution is the result of the capability of ruminants to transform fibrous feedstuffs. 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 and fungi. The rumen microbes encode a repertoire of enzymes for degrading plant 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 (Huws et al., 2018) manipulating the rumen microbiota. However, only 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). Whilst significant technological progress in omics techniques has increased our knowledge of the rumen microbiota within international projects such as the Global Rumen Census (Henderson et al., 2015) and Hungate 1000 (Seshadri et al., 2018), a great challenge needs to be overcome for translating omics knowledge into effective microbial manipulation strategies. Most of the findings derived from genomic studies are mainly descriptive and follow a correlation basis. 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, three modelling structures namely Molly, Dijkstra models and Karoline have been incrementally improved over the years (Gregorini et al., 2015; Huhtanen et al., 2015; van Lingen et al., 2019). 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 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 (Bannink et al., 2016). Filling this gap can lead to novel mathematical models with better predictive power and capabilities for guiding nutritional strategies. This can be done by incorporating the framework of genome-scale metabolic models (GEMs) into rumen modelling efforts (Huws et al., 2018). 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 (Kumar et al., 2019; Magnúsdóttir et al., 2017), 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 review aims to foster the incorporation of genome-scale based approaches into rumen modelling efforts.

Brief overview of omic-based techniques and computational biology tools to study the rumen microbiome

The first application of omics to the rumen microbiota and still the most used, is metataxonomic analysis. It is based on next-generation sequencing (NGS) techniques and on single amplicon sequencing of a variable region of 16S rRNA gene, 18S rRNA gene or ITS for bacteria, protozoa and fungi, respectively. This time and cost-effective taxonomic profiling provides an accurate description of the microbial composition of the rumen microbiota and of its modulation by extrinsic or intrinsic factors such as the diet or the age of the animal for example, and has been widely used to correlate taxonomic profile and animal traits such as methane emission (Wallace et al., 2019). However, accuracy of the results relies on efficiency of DNA extraction method, hypervariable region chosen for amplification, database used for sequence affiliation and pipeline applied for alpha and beta-diversity analysis (Almeida et al., 2018). Another limitation is the difficulty to achieve species-level accuracy as well as to have access to the rare biosphere. In spite of these biases, these approaches have been widely applied because they are easy to use and at low cost. They have led to the description of a relatively robust core rumen bacterial and archaeal microbiome and of its evolution with many different biotic and abiotic factors (Henderson et al., 2015).

For metataxonomic studies, microbial genes can be analysed using a variety of pipelines, with QIIME2 (Caporaso et al., 2010) being one of the most complete and flexible. In general, metataxonomics requires filtering the sequences for quality, clustering, classification and quantification. The end result of the process is an Operational Taxonomic Unit (OTU) table that expresses the abundance of microorganisms in the samples. While the approach is limited to abundance, it can be supplemented with tools such as CowPi (Wilkinson et al., 2018) to extrapolate the functional capabilities of the microbiota examined. CowPi uses KEGG (Kanehisa and Goto, 2000), mostly in the form of pathway data, but it can be customised to allow the use of modules, which offer a finer details. Metataxonomics is a reliable and well standardised approach that results in accurate and inexpensive taxonomic assignments, but its limit lies in the high-level functional prediction that can be extrapolated.

Metagenomic analysis or shotgun sequencing, which consists of determining the whole rumen genome sequence, provides information about the microbial population and its relative (putative) function. The first rumen metagenomic study was published in 2011 (Hess et al., 2011) and since

then the number of studies has increased to dozens to analyse, for example, polysaccharide degradation and impact of diet (Li et al., 2020).

Metagenomics approaches differ in the level of computational complexity involved, but can be roughly divided into read and assembly-based analysis. Approaches based on reads can be used for taxonomic assignment using Kraken2 (Wood et al., 2019) among others. Assembly-based approaches use assemblers such as MEGAHIT (Li et al., 2016) to reconstruct contigous fragments of the genomes from sequencing reads. The resulting assembly can then be further refined in a variety of ways, and one such approach is binning the assembled contigs with Metabat 2 (Kang et al., 2019), among others, into MAGs. These MAGs can then be taxonomically assigned with PhyloPhlAn (Asnicar et al., 2020). While genome binning may offer extraordinary insights into the rumen microbiota, quantity and quality of sequenced data needs to be taken into consideration, since each step will involve discarding data that cannot be reliably used with this approach. However, with more MAGs collections publicly available (Xie et al., 2021), it may be possible to use these as reference, along with available cultured genomes. Functional annotation of MAGs and assembled sequences uses similarity search tools (Altschul, 2014; Buchfink et al., 2021) against a variety of databases (Bateman et al., 2021; Huerta-Cepas et al., 2019) of known function proteins. In general, gene calling is first performed to extrapolate Open Reading Frames (ORFs) from sequences using Prodigal (Hyatt et al., 2010) and passing the resulting ORFs to a variety of tools such as eggNOG-mapper (Cantalapiedra et al., 2021) that will provide a function assignment and a variety of links to specialised databases such as CAZy (Drula et al., 2022). A certain level of expertise and high-cost machinery are necessary to process the huge amount of data generated by these approaches, but up to now, nearly 5000 putative bovine MAGs, including previously unknown rumen bacterial species, have been assembled (Stewart et al., 2019).

Metatranscriptomics allows to sequence the rumen transcriptome using NGS (RNA-Seq) and to obtain the gene expression profile getting access to functional activity. The main limitations are extraction of sufficient high-quality mRNA and removal of the rRNA, which represents the major part of the extracted RNA. Another difficulty for rumen studies is to obtain, on a same sample, RNA from prokaryotic and eukaryotic origins. As for metagenomics, the obtained reads must be aligned to reference databases. A limited number of rumen metatranscriptomic studies have been published until now. They have targeted, for example, methane emission (Kamke et al., 2016) and fibre-degrading function (Comtet-Marre et al., 2017). These studies identified the most expressed glycosyl-hydrolase genes under specific diet, which varied according to the studies, and enlightened the role of protozoa in fibre degradation (Comtet-Marre et al., 2017; Williams et al., 2020). However, the expression of a gene does not reflect the synthesis of the corresponding protein, and metaproteomic analysis is required to overcome this limitation.

Metaproteomics and metabolomics are the most informative approaches to access the rumen microbiome activity and function. These analytical techniques are based on mass spectrometry to provide differential analysis of protein expression (metaproteomics) and identification and quantification of small molecules (sugars, amino acids, SCFA, etc.) present in the rumen fluid (metabolomics). Metabolomics analysis can also be performed using NMR, or combining both MS and NMR (Zhang et al., 2019). For both metaproteomics and metabolomics, the results rely on the existence of a reference database, and for metaproteomics, another difficulty resides in efficient protein extraction from the rumen microbiota (Andersen et al., 2021). The few applications of metaproteomics to rumen microbiota (reviewed in Andersen et al., 2021) have detected important rumen functions and enlightened specific cross-feedings (Solden et al., 2018) but did not give a complete view of the rumen metabolic networks yet.

Finally, the multi-omics approach combines several (or all) of these methodologies. It is still a challenge, due to technical issues about the analysis methods which need a lot of computing space, post-analytical processing and integration of data, but also to the sample preparation for the different analyses. Nevertheless, several studies have used a multi-omics approach, combining metagenomics, metatranscriptomics and metabolomics, to study *in vivo* fibrolytic bacteria competition (Yeoman et al., 2021), bacterial species and metabolic markers of feed efficiency (Xue et al., 2022) and microbiome and activities of low methane emitters (Kamke et al., 2016). Due to the cost of such multiple meta-omics, these studies used a low number of animals, and large sample size analyses in the future will be necessary to increase our comprehension of the rumen functioning and allow identification of new strategies to improve rumen efficiency while lowering the environmental impact of ruminant production. The application of these meta-omics approaches to many rumen samples as well as longitudinal studies will allow to identify microbial species, metabolic pathways and metabolites to build wide metabolic networks and connect them with phenotypic traits in ruminant production (Table 1).

Table 1. Use of different meta-omics analyses 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 • Localization 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 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 generic differential equation resulting from applying mass balances

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

Where $\mathbf{x}$ is the vector containing the concentrations of metabolites, which can be either intracellular ($\mathbf{x}_i$) or extracellular ($\mathbf{x}_e$). The vector $\mathbf{r}$ represents the reaction rates, which are function of the concentrations $\boldsymbol{x}$ and the parameter vector $\mathbf{p}$. These reactions are catalysed by a proxy of microbial

biomass activity. Phenomena related to mass transport (input and output flows) are represented by the vector $\mathbf{g}$, which is function of $\mathbf{x}$ and the parameter vector $\mathbf{q}$. The matrix $\mathbf{S}$ is termed as the stoichiometric matrix. To simulate the model described by equation (1), the kinetic rates $\mathbf{r}(\boldsymbol{x}, \boldsymbol{p})$ need to be defined. 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.,* sugars utilizers, amino acids utilizers and hydrogen utilizers (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, 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 reconstructions of rumen microbes. It should be noted that these two modelling approaches follow the same generic equation (1).

Microbial time series and state observers

Microbial communities change over time in response to environmental changes. The analysis of microbial time series is an useful tool for monitoring and characterizing 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 have been applied to characterize rumen microbial colonization 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 times (Faust et al., 2015). These methods include network inference reconstructions and community dynamic models, being the generalized Lotka–Volterra (gLV) model one of the most widely approaches used to model microbial communities. (Gonze et al., 2018) provides a detailed review on gLV approach, its applications and limitations. One of the limitations of the gLV approach is that they are not well-suited to analyse high-dimensional microbiome time series data. Another limitation is that the gLV approach does not integrate information on concentration of fermentation metabolites. An alternative to exploit microbial time series within a mechanistic modelling framework like the one represented by equation (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. One of these applications includes the estimation of the evolution of ammonia oxidizer bacteria and nitrite oxidizer bacteria in a nitrifying chemostat (Ugalde-Salas et al., 2019). 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:

$$\frac{dB}{dt} = r - D \cdot B \quad (2)$$

$$\frac{dP}{dt} = k \cdot r - D \cdot P \quad (3)$$

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

$$z = k \cdot B - P \quad (4)$$

By deriving with respect to time, we obtain

$$\frac{dz}{dt} = k \cdot r - k \cdot D \cdot B - k \cdot r + D \cdot P = -D \cdot z \quad (5)$$

If dynamic data of B are available and an estimate of $\hat{z}$ is obtained from equation (5), we can have the estimate $\hat{P}$ as

$$\hat{P} = k \cdot B - \hat{z} \quad (6)$$

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. For deeper discussion on observers, the interested reader is referred to dedicated reviews *e.g.* (Dochain, 2003).

The previously developed observer can be extended to include n microbial species, that is the dynamics of the product will depend on the action of different microbes following

$$\frac{dP}{dt} = \sum_{i=1}^{n} k_i \cdot r_i - D \cdot P \quad (7)$$

with k_i, r_i the stoichiometry coefficients and the reaction rates for the microbe i. 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 sugars utilizers, amino acids utilizers and hydrogen utilizers. We assumed a hypothetical simulation scenario where sugar utilizers and amino acids were constituted by 5 microbial species with different kinetic rates (Davoudkhani et al., 2022a). Figure 1 shows the dynamics of the 5 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 in time the estimation given by the observer converges to the real value (Davoudkhani et al., 2022a).

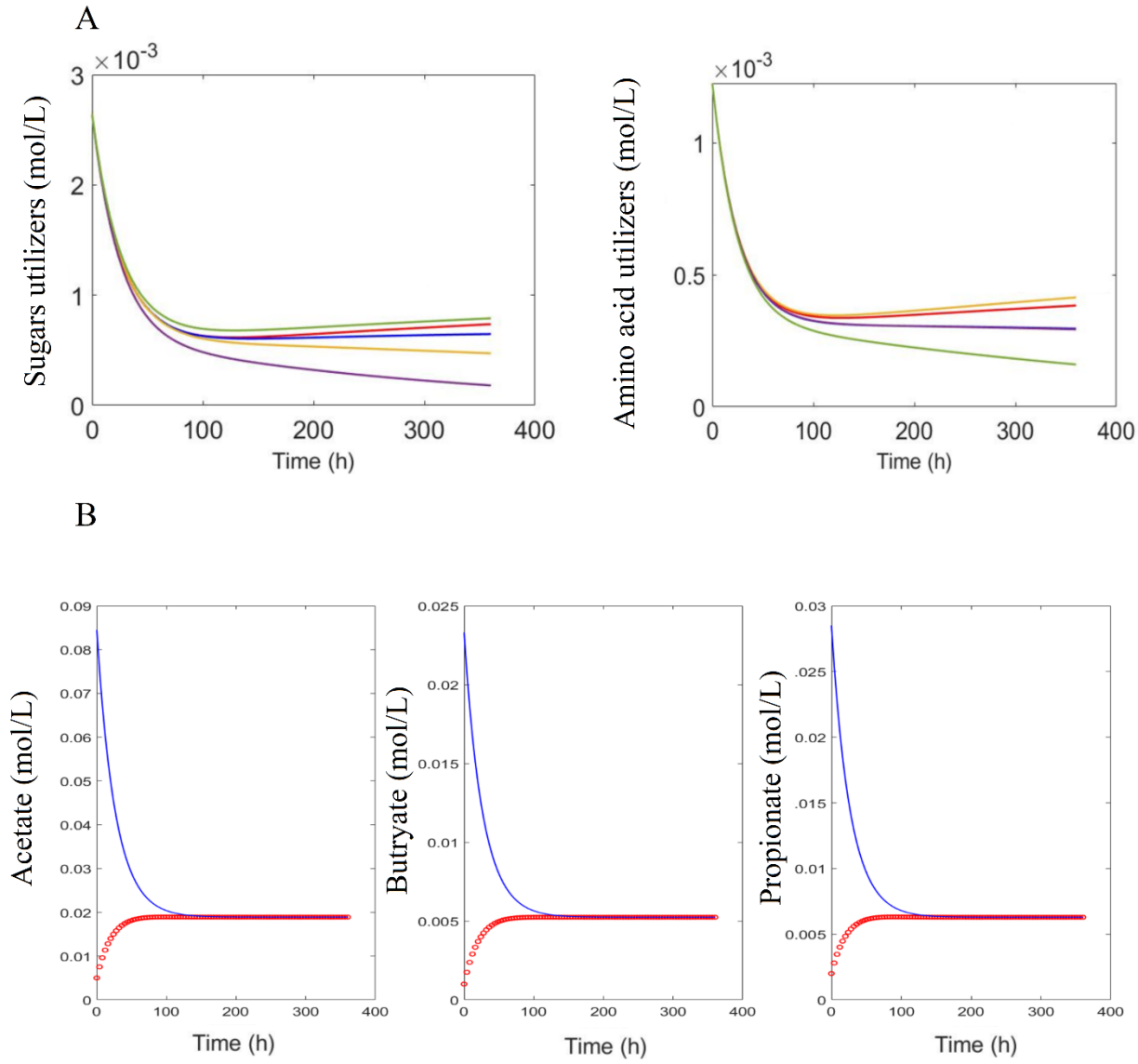

Fig. 1. Simulation study to assess the performance of an asymptotic observer applied to the mathematical model of rumen fermentation developed by (Muñoz-Tamayo et al., 2016). In this hypothetical study, we assumed that 5 species constituted the microbial groups of sugars utilizers and amino acids utilizers (A). Each species has different kinetic parameters. B. The estimated values of acetate, butyrate and propionate of the state-observer (solid line) converge to the real values.

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 optimization problem (Ugalde-Salas et al., 2019). Recently, we used the asymptotic observer approach to estimate the dynamics of the fermentation profile of a rusitec experiment carried out by (Belanche et al., 2017). We used the capabilities of CowPI (Wilkinson et al., 2018) to infer the microbial function of microbial time series based on 16S data. Our results indicated the promising application of observers and microbial time series data to investigate alternatives to connect omic 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 $\mathbf{S}$ 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 (Thiele and Palsson, 2010) that can be briefly summarized 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 1155 reactions and 1422 metabolites (Belcour et al., 2020). 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 $\mathbf{S}$ contains the stoichiometric matrices for intracellular ($\mathbf{S}_i$) and extracellular ($\mathbf{S}_e$) metabolites. 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's omit the transport phenomena in equation (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

$$\frac{d\boldsymbol{x_i}}{dt} = \mathbf{S_i}\,\mathbf{r} = 0 \qquad (8)$$

Since the number of reactions is typically higher than the number of metabolites, equation (8) is often underdetermined. All admissible solutions of equation (8) 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 equation (8). To reduce the solution space, FBA looks at finding the flux vector $\mathbf{r}$ by optimizing a regulatory optimal condition. The most used optimization criterion applied in FBA is the maximization of the biomass growth rate. Other optimal criteria are for example the maximization of production of ATP and the production of a desired by-product. FBA solves the system of linear equations (8) 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 includes 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 optimizations to calculate the maximum and minimum flux values of each reaction in the network to characterize 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, GEMs applications for the rumen ecosystem can focus on metabolic core functionalities.

Elementary flux modes analysis

In contrast to FBA, EFM analysis is a non-optimization 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 equation (8) - without any additional optimality constraint - are non-negative vectors contained in the null-space of the stoichiometric matrix $\mathbf{S}_{\boldsymbol{i}}$. 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, the EFMs represent the minimal set of enzymes of the metabolic network that can operate at steady state. 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). 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.
Figure 2 sum up the FBA and EFM approaches applied on the analysis of the solution space of equation (8).

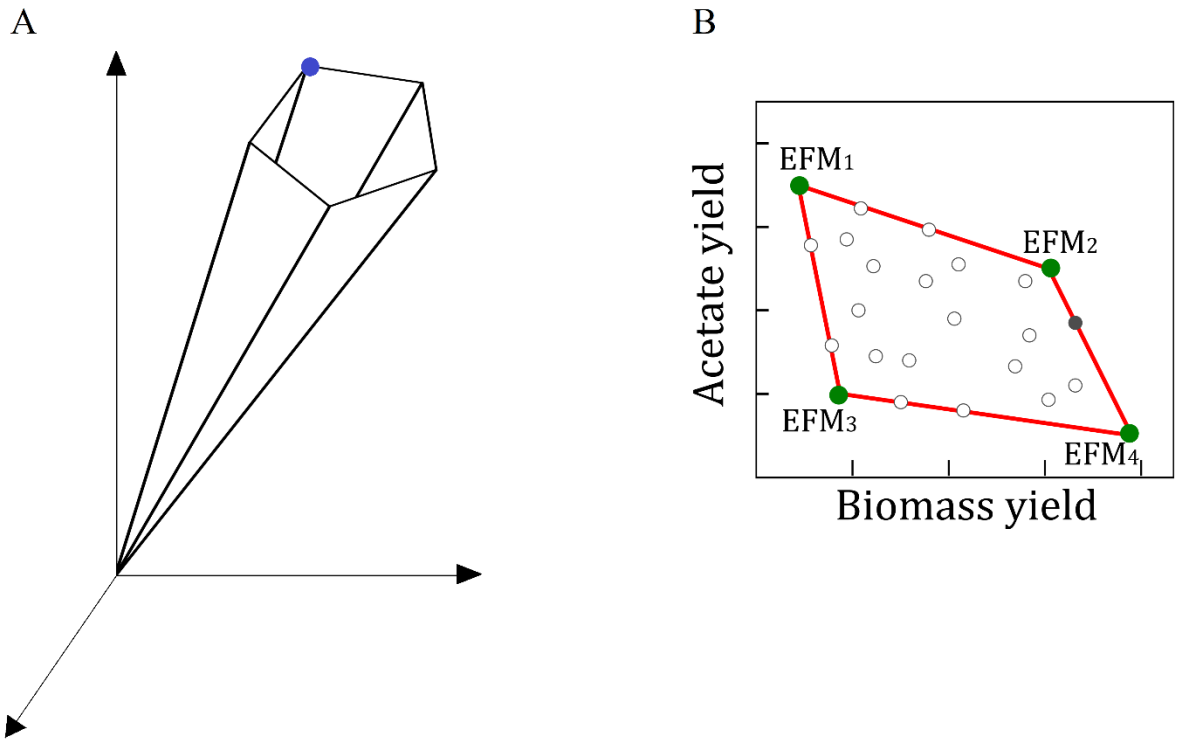

Fig. 2. COBRA approaches are based on the analysis of the allowable states of a metabolic network. These admissible states are contained in a polyhedral cone (A). The generating vectors of the cone are the elementary flux modes (EFMs). Flux balance analysis aims at finding an optimal solution in the 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.

The capability of the EFM-based approach of translating 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 by the fluctuating rumen environment and the interaction between the rumen microbiome, the host and the diet. Figure 3 summarizes the workflow of constructing a dynamic genome-based model. To provide parsimonious and reliable models, the property of parameter identifiability should be considered in the model construction to avoid over-parameterised models (Muñoz-Tamayo et al., 2018).

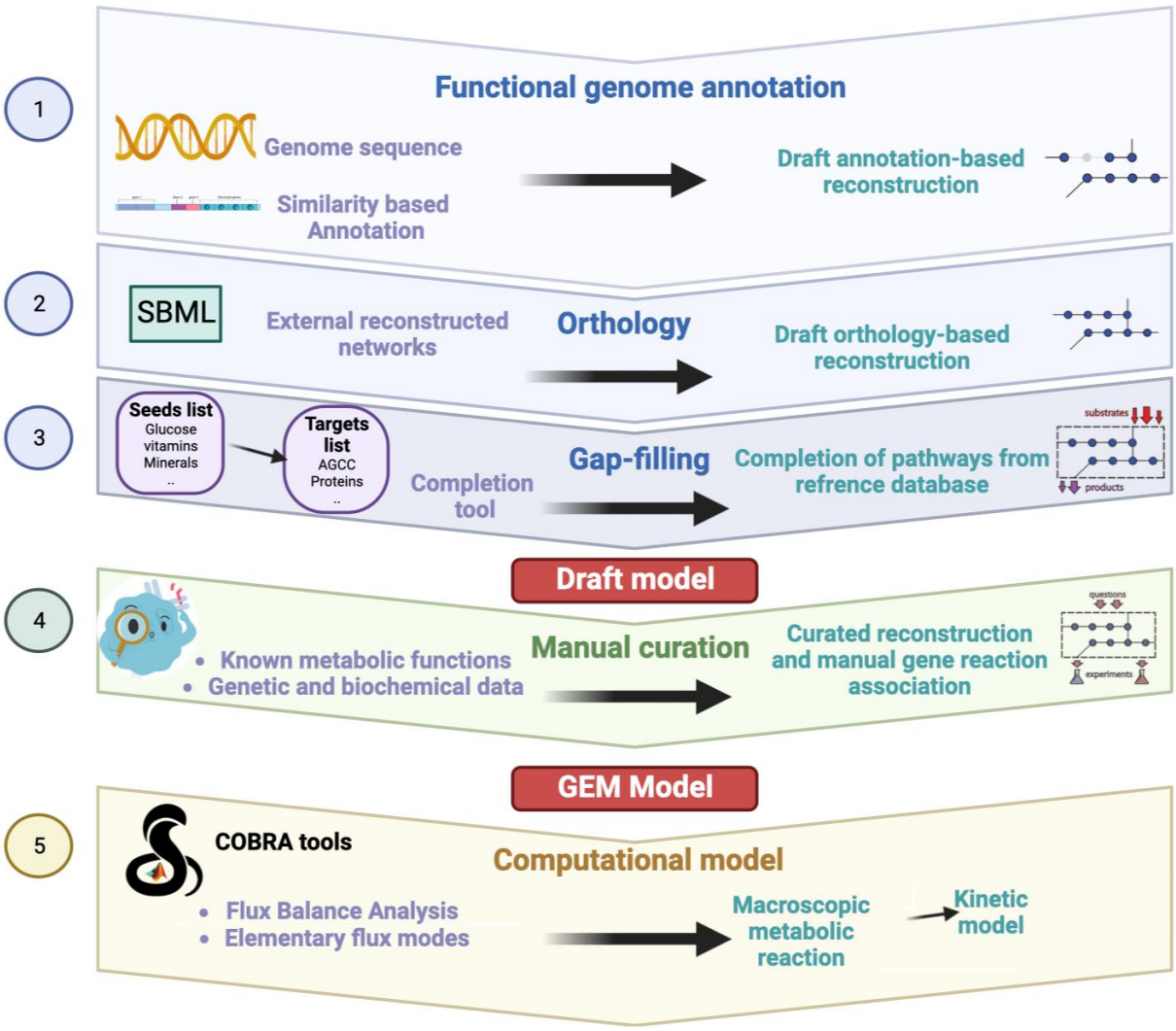

Fig. 3. The steps to build a dynamic kinetic genome-based model of microbial metabolism.

Microbial community modelling

The previously 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. GEMs of microbial communities is still at an early stage. In 2012, Zengler and Palsson (2012) argued that the status of knowledge on the function of microbial communities was comparable to the knowledge of the systems biology of single species ten years ago. 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. 4. 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 with the principles of whole genome sequencing.

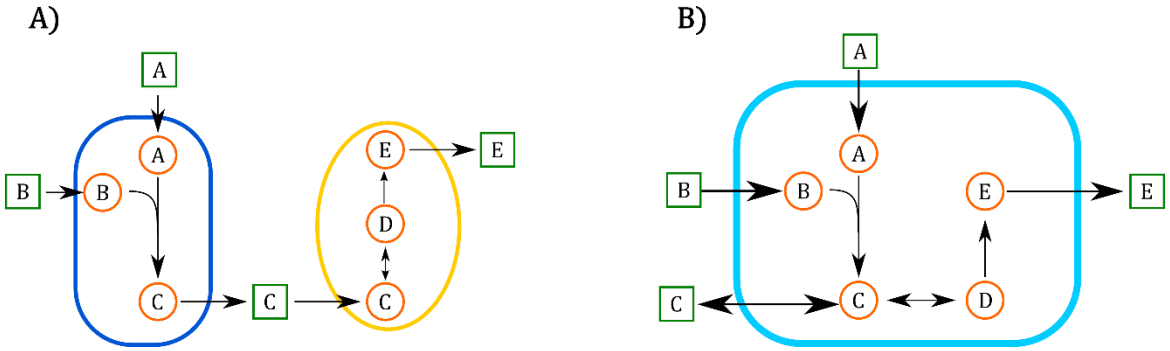

Fig. 4. Approaches for modelling microbial communities. A) Compartmental approach; B) Supra-organism approach.

For highly diverse ecosystems, the compartmental approach in *sensu stricto* results in a model that is difficultly tractable (for the rumen ecosystem, a compartmental model will imply hundreds of microbial species). 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 characterize in deep rumen microbial interactions and the influence of such interactions on the fermentation profile (Popova et al., 2022).

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 uncultured microorganisms, the identification of probiotics to enhance rumen function and the design of strategies for methanogen inhibition, and the exploitation of rumen microbes for the production of valuable compounds. GEMs also allow to characterize 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

utilizing 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., 2022).

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 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 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.

Final remarks

The integration of microbial omic data into mathematical models of the rumen microbiome can produce novel tools with enhance power for predicting rumen function. The potential applications of this 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. 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.

Ethics approval

Not applicable

Data and model availability

Not applicable

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Declaration of interest

None

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