Building microbial kinetic models for environmental application: a theoretical perspective

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ABSTRACT

Kinetic modeling of microbial reactions is a common tool for addressing the central environmental questions of our time, from contaminant remediation to the global carbon cycle. This review presents an overview of trait-based frameworks for modeling the kinetics of microbial reactions, with an emphasis on environmental application. I first highlight two key model assumptions: the simplification of microbial communities as ensembles of microbial functional groups and the description of microbial metabolism at a coarse-grained level with three metabolic reactions – catabolic reaction, biomass synthesis, and maintenance. Next, I aim to establish a connection between microbial rate laws and the mechanisms of metabolic reactions. For metabolic reactions limited by single substrates, the widely used rate law is the Monod equation. However, in cases where substrates are solids or nonaqueous phase liquids (NAPLs), the Contois equation and the Best equation may offer better alternatives. In microbial metabolisms limited by multiple nutrients simultaneously, two competing rate laws exist: the multiplicative rate law and Liebig's law of the minimum. Then I present two strategies for extending the modeling framework developed for laboratory cultures to natural environments. One strategy follows the multiplicative rate law and incorporates dimensionless functions to account for pH, temperature, salinity, cell density, and other environmental conditions. The other strategy focuses on the physiology of natural microbes, explicitly considering dormancy, biomass decay, and physiological acclimation. After that, I highlight recent improvements enabled by the application of molecular biology tools, ranging from functional gene-based models to metabolic models. Finally, I discuss the inherent limitations of trait-based modeling frameworks and their implications for model development and evaluation, including the gap between functional groups represented *in silico* and microbial communities found in natural environments, as well as the requirement of internal consistency in microbial parameter sets.

1. INTRODUCTION

Microbial kinetics is a subfield of microbiology that studies the rates of microbial metabolisms – including the rates of chemical reactions catalyzed by microbes and microbial growth (Jannasch and Egli, 1993; Jin et al., 2013; Kovárová-Kovar and Egli, 1998; Panikov, 1995). It has become a backbone of conceptual and modeling frameworks across disciplines, from food and nutrition science (Whiting, 1995), to water quality (Reichert et al., 2001), wastewater treatment (Wanner, 2021), and to contaminant remediation and biogeochemical element cycling (Fennel et al., 2022; Li, 2019; Seigneur et al., 2019). As a research topic of microbiology, microbial kinetics had a humble beginning, developed by a handful of scientists fascinated with patterns of microbial growth. Among the pioneers, the most influential is Jacques Monod, a French biochemist who discovered that microbial growth rates vary hyperbolically with the concentrations of limiting nutrients (Monod, 1942, 1949). He captured the hyperbolic relationship with a rate law akin to the adsorption isotherm equation or the Michaelis-Menten equation. This rate law, named after him, made possible for the first time the prediction of microbial population dynamics without prior knowledge of population sizes (Panikov, 1995). Monod also left behind an often-cited but now obsolete statement that "*the study of the growth of bacterial cultures does not constitute a specialized subject or branch of research: it is the basic method of Microbiology*" (Monod, 1949).

Accompanying the great expansion of microbial kinetics is a paradigm shift in our view of microbes and the technological revolution in microbiological research. Before World War II, most microbiologists were preoccupied with obtaining pure cultures and solving problems related to human life – curing for human diseases and improving food production and processing (Bonnet et al., 2020). At their dispense included optical microscopes and classical culture-dependent tools, such as liquid culturing media and agar plates (Vitorino and Bessa, 2017). After World War II, microbiological tools have gone through a series of innovations and upgrades, including the invention of polymerase-chain reaction (PCR) and16S rDNA clone library construction in the 1970s and, more recently, high-throughput next generation sequencing methods (Garner et al., 2021; Vitorino and Bessa, 2017). These culture-independent tools opened the opportunity of directly interrogating microorganisms *in situ*, including their genes and expressions, and metabolic activities and ecological functions. Without the need for pure culture, they avoided the bias of culture-dependent methods, and revolutionized and broadened the scope of both pure and applied microbiology, including environmental microbiology and geomicrobiology that study microbes and their activities in natural environments. Today, we accept that microbes are the Earth's hardest-working chemical engineers and a key driver of global element cycling (Falkowski et al., 2008; Finlay et al., 2020). They are widespread in the environment, from the atmosphere to deep crust, and their biomass constitutes approximately one fifth of the total biomass (Bar-On et al., 2018; Flemming and Wuertz, 2019). They display vast genomic and trophic diversities, and their metabolisms shape the chemistry of the environment by catalyzing redox reactions, mineral precipitation and dissolution, and other chemical reactions (Soong et al., 2020; Zinger et al., 2012).

Understanding and forecasting the geochemical and environmental significance of microorganisms require quantitative tools, such as kinetic models of microbial reactions. These models simulate not only the chemical fluxes driven by microbial metabolisms but also the sizes of microbial populations. They lay their foundation on the kinetic modeling framework of chemical reactions, which itself builds on the fundamental principles of mass conservation, thermodynamics, and chemical kinetics (Bebernes and Eberly, 2013; Bethke, 2022; Higham, 2008). For a reacting mixture of chemical compounds, its kinetic model is formulated as a mathematical problem of a group of ordinary differential equations (ODEs), constrained by the equation of state and other thermodynamic relationships. Each ODE uses time as the independent variable to describe the concentration balance of a chemical compound, and is constructed on the basis of the stoichiometric equations and the rate laws of chemical reactions. Stoichiometric equations express reaction products as the proportional combinations of reactants, while rate laws are analytic expressions that relate reaction rates to temperature, chemical concentrations, and other macroscopic parameters.

Modeling microbial reactions in natural environments has been the subject of significant interest among geochemists, biologists, and environmental engineers, and a number of review articles, book chapters, and special issues of journals have appeared over the last decade or so (Arndt et al., 2013; Bethke, 2022; Calderer et al., 2010; Chambon et al., 2013; Fennel et al., 2022; Geng et al., 2022; Jeong et al., 2019; Meile and Scheibe, 2019; Paraska et al., 2014; Perez-Garcia et al., 2016; Song et al., 2014; Sookhak et al., 2019; Tan et al., 2021; Thullner and Regnier, 2019). Here our intention is to present the theory of microbial reaction modeling in a systematic and coherent way. We first start with the concept of microbial functional groups and the framework of microbial reaction modeling, clarifying the necessary assumptions and simplifications of microbial communities and metabolisms in natural environments. Next, we focus on microbial rate laws and demonstrate the mechanistic link between microbial rate laws and metabolic reactions. Then we discuss the strategies that work to extend the modeling framework for laboratory cultures to natural environments. Afterwards, we highlight recent model improvements enabled by molecular biology tools. Finally, we emphasize the biases of microbial kinetic modeling and the challenges and limitations associated with its application to natural environments.

While the framework for modeling microbial kinetics is general, we illustrate the framework by focusing on chemotrophs – microbes that utilize chemical compounds as their energy sources. Also, given the large number of microbial rate laws, we limit the rate laws and modifications to those that capture microbial physiology and the growth conditions of the environment (see table 1), rather than providing an exhaustive historical chronology. By taking this approach, we acknowledge that certain important models and applications might be overlooked. Nonetheless, our aim is to underscore the key assumptions and limitations of microbial kinetic models, as well as showcase modeling strategies that have proved effective in natural systems. These topics and discussions hold relevance to today's endeavors that seek to improve microbial reaction modeling by incorporating insights from cutting-edge tools of chemical analysis and molecular biology.

2. MODELING FRAMEWORK

Microbial kinetic models are a special type of chemical reaction models that treat microorganisms as autocatalysts – catalysts that reproduce themselves by catalyzing chemical reactions. They are constructed on the basis of the kinetic modeling framework for abiotic multicomponent reacting mixtures (Bebernes and Eberly, 2013; Bethke, 2022; Higham, 2008), and by applying simplifications and assumptions related to microbial communities and their metabolisms.

2.1. Microbial Functional Group

Models are simplifications of reality. Compared to other microbiological models, such as stoichiometric and kinetic metabolic models that focus on enzymes and agent-based models that focus on individual cells (Foster et al., 2021; Nagarajan et al., 2022; Orth et al., 2010), microbial kinetic models for environmental applications adopt two unique simplifications – one addresses microbial diversity and the other is about microbial metabolism (fig 1). The two simplifications strike a balance between the complexity arising from microbial diversity and the practical applicability enabled by a relatively simple modeling framework and its straightforward deployment.

The first simplification is the application of functional groups as the basic unit of microorganisms. From the lens of functional ecology, a functional group is a cohort of microbial cells defined by their functional traits – microbial characteristics that affect the growth and survival of microorganisms (Flynn et al., 2015; Nock et al., 2016; Violle et al., 2007). Accordingly, a microbial community can be simplified as a collection of different functional groups. By default, microbial kinetic models do not explicitly consider biochemical, structural, or morphological traits, but only focus on functional traits that directly modulate the rates of microbial reactions – for example, metabolic rates at very high and very low nutrient concentrations, and the efficiency of microbial metabolism. In addition, microbial kinetic models neglect trait differences between microbial cells of the same group, or trophic relationships and interactions within the same group. Instead, cells of the same functional group share the same functional trait values, respond in similar fashions to environmental perturbations, and play similar roles in ecosystem functioning and biogeochemical processes (Gitay and Noble, 1997). With these assumptions, a functional group can be quantified with a single state variable – the biomass concentration C_X ($g \cdot kg^{-1}$), the mass of *living* cells belonging to a functional group per kg water (Bachmann et al., 2016; Bölter, 1994; Murphy et al., 2007).

Microbial communities have also been analyzed with identity-based approaches, such as 16S rRNA-based phylogenetic characterization and taxonomic classification (Nkongolo and Narendrula-Kotha, 2020). The focus on functional traits, not phylogeny or taxonomy, is supported by the consensus that the composition of functional groups serves as a mechanistic link between microbial diversity and ecosystem functioning (Crowther et al., 2019; McGill et al., 2006). This focus has led to the widespread use of the term "trait-based microbial models" to highlight the central role of functional traits in formulating microbial kinetic models.

The second simplification pertains to the description of microbial metabolism. Microbial kinetic models treat each functional group as a single reacting component, and describe its metabolism at a coarse-grained level without accounting for the details of metabolic pathways or regulations. Specifically, microbial kinetic models concentrate on three fundamental metabolic reactions – catabolic reaction, biomass synthesis, and maintenance (fig 2A). Catabolic reactions harvest the energy available in the environment by making ATPs, biomass synthesis reactions utilize ATPs to produce new biomass from essential nutrients, such as carbon, nitrogen, phosphorus, and trace elements, in the environments, and biomass maintenance reactions consume ATPs to sustain the integrity and functionality of cellular components as well as to ensure the viability and survival of microbial cells (Hoehler and Jørgensen, 2013; van Bodegom, 2007). Other metabolic processes, such as co-metabolism and detoxification, are not explicitly considered. The three metabolic reactions are governed by the principle of energy balance,

$$J_{\mathrm{P,C}} = J_{\mathrm{P,X}} + J_{\mathrm{P,M}}\,, \tag{1}$$

that is, the flux $J_{\mathrm{P,C}}$ of ATP production by catabolism is balanced by the fluxes of ATP consumption by biomass synthesis ($J_{\mathrm{P,X}}$) and by maintenance ($J_{\mathrm{P,X}}$) (Pirt, 1965). This principle ensures the overall consistency and reliability of microbial metabolic rates within the model.

Following the standard framework of chemical reaction modeling, the description of a metabolic reaction splits into three prongs: the stoichiometric equation and the rate law of the metabolic reaction, and the functional traits (or microbial parameters) required by the rate law. The three prongs describe metabolic reactions from different perspectives and with different emphases:

- Stoichiometric equations list the substrates and products of metabolic reactions, and make possible the modeling of the chemical interactions between microbes and the environment – the environment supports microbes by supplying nutrients (e.g., energy and element sources) and, in return, microbes alter ambient environments by consuming nutrients and releasing waste products. These equations also define the ratios of the nutrient and product fluxes driven by microbial metabolisms, and ensure that microbial kinetic models honor the principles of mass conservation and charge balance.

- Microbial rate laws express metabolic reaction rates in terms of macroscopic parameters, such as pH, temperature, and the concentrations of biomass, nutrients, metabolic products, and chemical inhibitors. They provide a theoretical basis to compute chemical fluxes through microbial biomass and the rates of microbial growth and how the chemical fluxes and growth rates respond to the changes in the physicochemical conditions of the environment.
- Functional traits are represented in microbiological models as rate law parameters, including stoichiometric and kinetic parameters. Stoichiometric parameters quantify the efficiency of microbial metabolism, and include the yields of ATPs and biomass per unit nutrient. Kinetic parameters define microbial reaction rates at extreme nutrient concentrations, such as very high and very low concentrations, and include rate constants, half-saturation constants, and nutrient affinities (i.e., the ratios of rate constants to half-saturation constants) (Healey, 1980; Kovárová-Kovar and Egli, 1998; Law and Button, 1977).

The three prongs enable us to describe microbial functional groups as auto-catalysts with a minimum set of stoichiometric equations and rate laws, thereby keeping modeling tasks in check. In this way, microbial kinetic models are robust enough to capture the essential features of microbial metabolisms yet generic enough to be applicable to diverse microorganisms in both engineered systems and natural environments.

2.2. Stoichiometric Equation and Reaction Rate

In line with standard practices in chemical kinetics, metabolic reactions and their rates are defined in terms of stoichiometric reaction equations. Catabolic reactions synthesize ATPs by consuming extracellular energy sources. Their stoichiometric equations can be constructed according to the principles of mass balance and charge balance. For example, respiration reactions couple redox reactions in the environment to ATP synthesis in the cytoplasm and can be represented as

$$\sum_{\mathrm{D}} \nu_{\mathrm{D}}\mathrm{D} + \sum_{\mathrm{A}} \nu_{\mathrm{A}}\mathrm{D} \rightleftarrows \sum_{\mathrm{D}^+} \nu_{\mathrm{D}^+}\mathrm{D}^+ + \sum_{\mathrm{A}^-} \nu_{\mathrm{A}^-}\mathrm{A}^- , \qquad (2)$$

where D and D^+ are the electron donor and its oxidized form, respectively, and A and A^- are the electron acceptor and its reduced form, respectively, and ν_{D} and others are stoichiometric coefficients. Following the common practice in chemical kinetics, catabolic reaction rate r_C (mol·kg^{-1}·s^{-1}) is defined as

$$r_C = -\frac{1}{\nu_D}\frac{dC_D}{dt} = -\frac{1}{\nu_A}\frac{dC_A}{dt} = \frac{1}{\nu_{D^+}}\frac{dC_{D+}}{dt} = \frac{1}{\nu_{A^-}}\frac{dC_{A^-}}{dt}. \tag{3}$$

Here C_D and others are molal (M) concentrations. The double arrow in equation 2 emphasizes that chemical reactions proceed forward and backward at the same time. For this reason, catabolic rates

$$r_C = r_{C+} - r_{C-} \tag{4}$$

represent the differences between the forward (r_{C+}) and backward rates (r_{C-}) of the reactions. For reactions far away from thermodynamic equilibrium, such as aerobic respiration and nitrate reduction, their backward rates can be safely neglected and their net rates can be approximated as the forward rates.

Biomass synthesis produces biomass from extracellular sources of carbon, nitrogen, and other elements. Constructing a stoichiometric equation for biomass synthesis can be challenging, particularly when the elemental composition of biomass is unknown, or when more than one chemical compound serves as the source of carbon, nitrogen, or other elements. In these cases, as an approximation, we can apply a generic chemical formula of biomass, for example, $C_5H_7O_2N$ (Hoover and Porges, 1952), and assume that acetate (CH_3COO^-) and ammonium (NH_4^+) serve as the sources of carbon and nitrogen, respectively. Under these assumptions, the stoichiometric equation of biomass synthesis is

$$2.5CH_3COO^- + NH_4^+ + 1.5H^+ \rightarrow C_5H_7O_2N + 3H_2O. \tag{5}$$

Where other sources of carbon and nitrogen are available, or if a different biomass chemical formula is assumed, results similar to equation 5 can be derived by following the principles of mass and charge balance (Geider and Roche, 2002; Jin and Bethke, 2007; Popovic, 2019). The rate r_X (g·kg^{-1}·s^{-1}) of biomass synthesis is the rate at which biomass synthesis raises biomass concentration C_X,

$$r_X = \frac{dC_X}{dt}. \tag{6}$$

Accordingly, the rate at which nutrient N is consumed by biomass synthesis is

$$\frac{dC_{\mathrm{N}}}{dt} = -\frac{\nu_{\mathrm{N}}}{W_{\mathrm{M}}} r_{\mathrm{X}} . \tag{7}$$

Here C_{N} is nutrient concentration (M), ν_{N} is the stoichiometric coefficient of nutrient N in equation 5, and W_{M} is the molecular weight calculated from the biomass chemical formula.

Maintenance summarizes the metabolic pathways that preserve the integrity and functions of cellular components and structures. No stoichiometric equation is available to describe biomass maintenance in terms of extracellular nutrients and metabolic products. The rate r_{M} ($\mathrm{g\cdot kg^{-1}\cdot s^{-1}}$) of biomass maintenance is expressed as the rate at which biomass concentration is decreased by the maintenance pathways,

$$r_{\mathrm{M}} = -\frac{dC_{\mathrm{X}}}{dt} . \tag{8}$$

This definition is consistent with the view that maintenance metabolism consumes ATPs, but does not yield new biomass – if maintenance did not occur, its ATP flux would be available to biomass synthesis, increasing biomass concentrations.

In addition to maintenance metabolisms, other processes, such as transition to dormancy, cell lysis and death, and predation by higher microorganisms, also contribute to lowering biomass concentrations (Moger-Reischer and Lennon, 2019; Pérez et al., 2016). However, the contributions of the individual processes to biomass loss are technically difficult to pinpoint. For this reason, *biomass decay* has been used as a collective term to summarize all the processes that work together to lower biomass concentrations (Van Loosdrecht and Henze, 1999). Biomass decay rate r_{D}

$$r_{\mathrm{D}} = \sum_{\mathrm{i}} r_{\mathrm{D,i}} , \tag{9}$$

is the sum of the rates of biomass maintenance, cell lysis, predation, and others (r_{Di}).

Related to biomass synthesis and decay is microbial growth, a macroscopic phenomenon defined as the apparent increase in microbial biomass or cell number over time. In microbiology, growth is commonly characterized with specific growth rate μ ($\mathrm{s^{-1}}$), the instantaneous relative rate of biomass increase,

$$\mu = \frac{1}{C_{\mathrm{X}}} \frac{dC_{\mathrm{X}}}{dt}. \quad (10)$$

Following the principle of mass conservation,

$$\mu = \frac{1}{C_{\mathrm{X}}} \left(r_{\mathrm{X}} - r_{\mathrm{D}} \right). \quad (11)$$

In other words, specific growth rate reflects the balance between the rates of biomass synthesis and decay per unit biomass (fig 3A).

2.3. Mass Conservation Equation

For a system of *m* number of functional groups and *n* number of chemical compounds, its state is defined by the concentrations of chemical compounds and functional groups. For an isothermal system, its state is captured by applying the principle of mass conservation to each chemical compound and functional group and by accounting for the rates of catabolic reactions, biomass synthesis, and biomass decay of functional groups (eqs 3, 6, and 9). The results are a system of coupled equations, commonly summarized with a matrix equation and a vector equation. The matrix equation accounts for the chemical fluxes driven by metabolisms,

$$\mathbf{J}_{\mathrm{A}} = \mathbf{S}_{\mathrm{C}} \boldsymbol{\times} \mathbf{R}_{\mathrm{C}} + \frac{1}{W_{\mathrm{M}}} \mathbf{S}_{\mathrm{X}} \boldsymbol{\times} \mathbf{R}_{\mathrm{X}}, \quad (12)$$

where $\mathbf{J}_{\mathrm{A}}$ is a column vector of size *n*, its element J_{Ai} is the production flux of chemical $\mathrm{A_i}$, the net rate at which $\mathrm{A_i}$ is produced (or consumed, if negative) per kg water ($\mathrm{M \cdot s^{-1}}$), $\mathbf{R}_{\mathrm{C}}$ and $\mathbf{R}_{\mathrm{X}}$ are two vectors of size *m*, their elements $r_{\mathrm{C,j}}$ and $r_{\mathrm{X,j}}$ are the rates of catabolic reaction ($\mathrm{M \cdot s^{-1}}$) and biomass synthesis ($\mathrm{g \cdot kg^{-1} \cdot s^{-1}}$) of group j, respectively, $\mathbf{S}_{\mathrm{C}}$ and $\mathbf{S}_{\mathrm{X}}$ are the stoichiometric matrices of size $m \times n$, their element $S_{\mathrm{C,ij}}$ and $S_{\mathrm{X,ij}}$ are the stoichiometric coefficient of $\mathrm{A_i}$ in the catabolic reaction and biomass synthesis reaction of group j, respectively, and can be constructed from the stoichiometric equations for catabolic and biomass synthesis reactions, respectively.

The vector equation describes microbial growth,

$$\mathbf{J}_{\mathrm{X}} = \mathbf{R}_{\mathrm{X}} - \mathbf{R}_{\mathrm{D}}. \quad (13)$$

Here $\mathbf{J}_{\mathrm{X}}$ is a column vector of size *m* and its element $J_{\mathrm{X,j}}$ is the growth rate of group j, $\mathbf{R}_{\mathrm{X}}$ is a vector whose element $r_{\mathrm{X,j}}$ is the rate of biomass synthesis of group j, and $\mathbf{R}_{\mathrm{D}}$ is a vector whose element $r_{\mathrm{D,j}}$ is the decay rate of group j (see equation 11). For a non-isothermal system, an additional equation for temperature (or energy) is required.

2.4. Frameworks in Common Use

The trait-based modeling framework uses biomass concentration C_X as a state variable, and describes microbial metabolism at the coarse-grained level with catabolic reaction, biomass synthesis, and maintenance. The framework presented above is general and simplifies to the various frameworks in common use today:

First, the biomass-explicit modeling framework takes a simplified form by assuming that biomass synthesis and catabolism are tightly coupled (Fang et al., 2009; Li et al., 2009; Yabusaki et al., 2007). Under this assumption, biomass yield per unit of energy substrate stays constant, the entire metabolism can be described with a single stoichiometric equation that combines equation 2 and 5. With this unified equation, a single rate expression is sufficient to calculate the chemical fluxes and growth rates of microorganisms.

The general modeling framework reduces to the biomass-explicit catabolism-focused framework by assuming that biomass synthesis and catabolism are tightly coupled and by neglecting the nutrient consumption by biomass synthesis (Jin et al., 2013; Rittmann et al., 1980; Schäfer et al., 1998; Sykes et al., 1982; Talin et al., 2003; Thullner et al., 2005; Yabusaki et al., 2011). In this case, microbial growth rate is calculated according to equation 13, but the nutrient consumption by biomass synthesis is not considered (i.e., the second term on the right side of equation 12 is set to 0). Where a nutrient participates in both catabolism and biomass synthesis, such simplification tends to underestimate the flux of nutrient consumption.

The modeling framework further reduces to the biomass-implicit catabolism-focused framework by assuming that biomass concentrations remain constant. In this case, biomass synthesis and decay are neglected (i.e., eq 13 is not considered), and microbes are no longer described as autocatalysts. This framework has found wide application in sediment diagenesis modeling (Berner, 1980; Boudreau, 1996; Dale et al., 2008; Soetaert et al., 1996; Wang and Van Cappellen, 1996), as well as to some extent in groundwater reactive transport modeling, where biomass concentration or other growth-related information is not a primary concern (Hunter et al., 1998).

3. RATE LAWS FOR LABORATORY APPLICATIONS

Rate laws are the workhorses of chemical kinetics, providing robust tools not only for computing reaction rates but also for dissecting reaction mechanisms (Masel, 2001). In microbial kinetics, most rate law applications have focused on rate calculations. Here we illustrate the link between microbial rate laws and metabolic mechanisms by first focusing on the rate laws for microbial metabolisms limited by a single nutrient and then the rate laws for metabolisms limited by more than one nutrient.

3.1. Rate Laws of Single Limiting Nutrients

Monod (1942, 1949) developed the first microbial rate law, an empirical equation for microbial growth limited by a nutrient dissolved in solution. Other rate expressions have also been proposed, but they rarely gained much traction (Panikov, 1995). However, at least two of these rate expressions should deserve more attention. One is the Contois equation that describes an inverse relationship between specific growth rate and biomass concentration originally observed in a glucose-fed *Aerobacter aerogenes* culture (Contois, 1959). This equation has found widespread applications in microbial degradation of particulate organic matter (POC) (Chen and Hashimoto, 1980; Hemsi et al., 2010). Its alternative forms have also been applied to microbial reduction of ferric minerals (Hacherl et al., 2003; Roden, 2006).

The other rate expression is the Best equation (Best, 1955). This equation has been derived in theory for a special type of interaction between microbial cells and substrates of poor solubility or strong hydrophobicity, such as minerals or nonaqueous phase liquids (NAPLs). In these cases, microbes do not directly react with solids or NAPLs, but only utilize their dissolved forms. Taking as an example polycyclic aromatic hydrocarbons (PAHs), microbes can utilize naphthalene and phenanthrene dissolved in water, but cannot directly consume PAHs (Volkering et al., 1992). To sustain metabolism, the dissolution of PAHs into bulk solution is required. Interestingly, both the Contois and the Best equations relate specific growth rate to the concentration ratio of substrate to biomass, and despite their apparent difference, the Best equation can be approximated with the Contois equation.

3.1.1. Monod equation

The original Monod equation consists of a single-term hyperbolic function of nutrient concentration (Monod, 1942, 1949), and was later amended to account for biomass maintenance (Herbert, 1958; Pirt, 1965). The revised Monod equation in its commonly used form is

$$\mu = \mu_{\max} \cdot F_{\mathrm{C}} - k_{\mathrm{D}}, \tag{14}$$

where $\mu_{\max}$ is the maximum specific growth rate, F_{C} is a dimensionless factor of nutrient concentration, and k_{D} is the specific rate of biomass decay (s^{-1}). The revised equation assumes that the rate of biomass decay is proportional to biomass concentration, with the proportionality constant denoted as k_{D}. From the perspective of mass conservation (see eq 11), the first term in equation 14 gives the specific rate of biomass synthesis.

The concentration factor F_{C},

$$F_{\mathrm{C}} = \frac{C_{\mathrm{N}}}{C_{\mathrm{N}} + K_{\mathrm{M}}}, \tag{15}$$

is a relatively simple hyperbolic function that describes saturation-type kinetics. Here C_{N} is the concentration of nutrient N, and K_{M} is the half-saturation constant. Concentration factor F_{C} quantifies the limitation by nutrient N – the extent to which the nutrient lowers growth rate below its maximum value (fig 4A). Where the factor is close to 0, specific growth rate μ stays near 0, and growth is limited significantly by the nutrient. Where the factor is close to unity, variations in nutrient concentration do not change the rate much, and the nutrient limitation can be safely neglected.

Monod (1942, 1949) also discovered that the biomass yield $Y_{\mathrm{X/N}}$ per unit of nutrient (N) can be treated as a constant ($\mathrm{g \cdot mol^{-1}}$). This yield coefficient relates the rate r_{X} of biomass synthesis to the rate r_{N} of nutrient consumption, i.e.,

$$Y_{\mathrm{X/N}} = \frac{r_{\mathrm{X}}}{r_{\mathrm{N}}}. \tag{16}$$

It provides a basis to recast the Monod equation in terms of nutrient consumption. A special case occurs where microbial metabolism is limited by a substrate consumed by catabolism (or an energy source) and biomass synthesis rate is limited by the supply flux of ATP. Under these conditions, combining equation 14 and 16 gives the rate law for catabolic reaction, i.e.,

$$r_C = k_{C,max} C_X F_N \,. \tag{17}$$

Here $k_{C,max}$ is the rate constant (mol·g^{-1}·s^{-1}) or the maximum rate of catabolic reaction per unit biomass,

$$k_{C,max} = \frac{\mu_{max}}{\nu_S Y_{X/S}} \,. \tag{18}$$

Here ν_S is the stoichiometric coefficient of the energy substrate in catabolic reaction (eq 2), and $Y_{X/S}$ is the biomass yield per unit energy substrate.

Despite its empirical nature and relatively simple form, the Monod equation does bear a limited connection with metabolic mechanism (Jin et al., 2022). A metabolic reaction consumes nutrients dissolved in solution in a series of steps (fig 5A). First, nutrients diffuse from the bulk solution to the cell surface, and are then taken up into the cytoplasm and consumed by enzymes along metabolic pathways. For microbial cells suspended in solution, nutrient diffusion does not limit the progress of overall metabolism (Smith et al., 2014). Instead, metabolic rates are determined by one or more enzymes. Therefore, metabolic reactions can be described as *cell-controlled metabolic reactions* to highlight that it is the enzymes within cells that control metabolic rates (table 2). However, the extents of the rate limitation by different enzymes are not fixed, but dependent on nutrient concentrations in the bulk solution. Where nutrient concentrations are relatively small, the enzymes that participate in nutrient uptake dominate the rate control, and can be considered as rate-limiting enzymes. At relatively large nutrient concentrations, other enzymes become rate limiting. The Monod equation accounts for the rate-limiting enzymes at very low nutrient concentrations with the ratio of μ_{max} to K_M or the ratio of $k_{C,max}$ to K_M,

$$k_{aff} = \frac{k_{C,max}}{K_M} \,. \tag{19}$$

This parameter ratio is commonly termed as nutrient affinity or affinity constant k_{aff} (Healey, 1980; Law and Button, 1977). The Monod equation also accounts for the rate-limiting enzymes at very high nutrient concentrations with $k_{C,max}$ (or μ_{max}). These results support previous laboratory observations that the Monod equation provides reasonable approximations to microbial growth. The results also suggest that typical hyperbolic relationships between growth rates and nutrient concentrations reflect the shift in rate-limiting enzyme between very high and very low nutrient concentrations, not the rate-substrate relationships of individual enzymes.

A related equation, the Michaelis-Menten equation, has also been widely applied to microbial reaction modeling (Dugdale, 1967; Dugdale, 2018). While both equations use the same hyperbolic function, they differ in their physical significance and the specific systems they are applied to. The Michaelis-Menten equation was derived in theory for biochemical reactions catalyzed by enzymes; its mechanistic basis involves the formation of an enzyme-substrate complex and its subsequent conversion to products (Cornish-Bowden, 2013). The Monod equation was constructed empirically for microbial growth and reflects the emergent property of the entire network of metabolic enzymes. Therefore, to clearly specify the subject of the modeling and the underlying principles, the hyperbolic equation applied to enzyme-catalyzed reactions, such as the enzymatic uptake of nutrients from the environment into the cytoplasm and the extracellular hydrolysis of organic matter, should be referred to as the Michaelis-Menten equation. Conversely, for metabolic reactions catalyzed by a series of enzymes, such as respiration and fermentation, the equation should be referred to as the Monod equation.

3.1.2. Contois equation

The Contois equation has been applied to microbial oxidation of particulate organic matter and reduction of ferric minerals. In both cases, microbes interact with solid-phase substrates via various mechanisms, including direct contact, ligands, and electron carriers (fig 5B). For example, to utilize particulate organic matter, microbes first synthesize and release extracellular enzymes (or exoenzymes) (Wang and Li, 2014; Wu et al., 2021). The exoenzymes attack the surface of particulate organic carbon (POC) and hydrolyze organic carbon to oligomers and monomers of amino acids, nucleic acids, sugars, and lipids, which are then taken up by microbial cells. To reduce ferric minerals, microbes transfer electrons from the cell surface to the mineral surface via electrically conductive filamentous pili or surface proteins, by dissolving ferric minerals with ligands and then reducing aqueous ligand-Fe(III) complex, and by employing electron carriers, such as quinone-bearing humic substances (Shi et al., 2016; Weber et al., 2006). To differentiate these reactions from cell-controlled metabolic reactions (table 2), we follow the common practice in chemical kinetics and describe metabolic reactions involving solid surfaces as surface-controlled reactions (Masel, 2001).

Organic matter degradation. The kinetics of organic matter degradation has been studied under two extreme conditions by two disciplines, highly concentrated bioreactors by wastewater engineers and sediments of low abundance by biogeochemists. From wastewater treatment studies (Bhattacharya and Khai, 1987; Chen and Hashimoto, 1980; Ghaly and Echiegu, 1993; Hemsi et al., 2010; Vavilin et al., 1996; Vavilin et al., 1997), an emerging consensus is that specific growth rate varies with the concentration ratio $Q_{\mathrm{N/X}}$ of organic matter to biomass,

$$Q_{\mathrm{N/X}} = \frac{C_{\mathrm{N}}}{C_{\mathrm{X}}}, \tag{20}$$

and that the variation follows the Contois equation, an equation that uses the same hyperbolic function in the Monod equation (Contois, 1959). Here C_{N} is the concentration of organic matter. The Contois equation calculates specific growth rate according to equation 14 and with the concentration function F_{C},

$$F_{\mathrm{C}} = \frac{Q_{\mathrm{N/X}}}{Q_{\mathrm{N/X}} + K'_{\mathrm{M}}}, \tag{21}$$

where the K'_{M} is the biomass-specific half-saturation constant. Figure 4B takes microbial degradation of fragmented walnut shells as an example and illustrates how the concentration factor varies with the concentration ratio $Q_{\mathrm{N/X}}$.

In comparison, the kinetic studies of sedimentary organic matter suggest that the rate r_{OM} of organic matter degradation follows the G model,

$$r_{\mathrm{OM}} = k_{\mathrm{OM}} \cdot C_{\mathrm{N}}, \tag{22}$$

where k_{OM} is the rate constant (s^{-1}) and is believed to reflect the reactivity of organic matter (Berner, 1964). The G model represents a special case of the Contois equation, where the concentration ratio $Q_{\mathrm{N/X}}$ is far less than the specific half-saturation constant, i.e., $Q_{\mathrm{N/X}} \ll K'_{\mathrm{M}}$. Under this condition, the specific rate constant k_{OM} becomes the affinity constant for organic matter, or the ratio of the rate constant to the biomass-specific half-saturation constant,

$$k_{\mathrm{OM}} = \frac{k_{\mathrm{C,max}}}{K'_{\mathrm{M}}}. \tag{23}$$

Equation 23 suggests that the rate constant k_{OM} reflects not only the reactivity of organic matter but also the kinetic properties of microorganisms. In many applications, the multi-G model is required (Westrich and Berner, 1984). This model separates organic matter into different groups according to their specific rate constants, which can be indicative of the different affinity constants exhibited by microbes utilizing these organic matter groups.

Ferric mineral reduction. Ferric iron respiring microbes utilize ferric minerals as electron acceptors. Hacherl et al. (2003) recognized that during microbial iron reduction, electrons are transferred to the reactive surface sites of ferric minerals, and suggested to use the concentrations of bioavailable surface sites, instead of ferric minerals, to evaluate the rates of iron reduction. They also proposed a rate expression to relate iron reduction rate to the concentration ratio of biomass to surface sites. The same equation was used by Roden (2006) to describe the kinetics of iron reduction, and applied in modeling microbial iron reduction in natural environments (Bethke et al., 2008; Jin and Roden, 2011; Johannesson et al., 2019). This rate expression can be recast as the Contois equation (eqs 17 and 21). Figure 4C takes nanocrystalline goethite as an example and shows how the factor (eq 21) varies with concentration ratio $Q_{\mathrm{SS/X}}$ of bioavailable surface sites to biomass.

The Contois equation differs from the Monod equation in that the hyperbolic function is expressed in terms of the concentration ratio $Q_{\mathrm{N/X}}$ of nutrient to biomass. This difference arises from the fact that solid-phase compounds cannot be directly taken up by microbes from the environment into the cytoplasm, and their utilization requires metabolic strategies different from those for aqueous substrate consumption. Wang and Li (2014) noted that during the degradation of POC, colonies develop on the surface of POC, and derived the Contois equation by accounting for the coverage of POC's surface by microbial cells, and by assuming that microbes attached to the surface of POC grow exponentially and that the growth of suspended microbes can be neglected. Likewise, according to the mechanisms of ferric iron reduction (Shi et al., 2016; Weber et al., 2006), we can derive the Contois equation by assuming that the electron transfer between cell and mineral surfaces are at quasi-steady state (see Supplementary Information). These derivations suggest a trade-off between biomass concentration and the surface area of solid-phase substrates. At a given concentration of solid-phase substrates, increases in biomass concentration increase the rate, but diminish the solid-phase substrate available to individual cells, which in turn decreases the rate.

3.1.3. Best equation

The Best equation has been derived in theory by considering two reaction steps, the dissolution of minerals (or NAPLs) followed by microbial uptake and consumption (fig 5C) (Bosma et al., 1997; Koch, 2005; Sanford and Crawford, 2000; Wick et al., 2001). By assuming that the two steps are at quasi-steady state, e.g., the dissolution flux and the consumption flux equal each other (Bosma et al., 1997), the rate of metabolic reaction follows the Best equation (Best, 1955). Alternatively, a kinetic model can be built to explicitly simulate the two processes according to the rate laws of mineral dissolution and the Monod equation. However, where the concentrations of dissolved nutrients are too low or technically difficult to analyze, it might be desirable to directly relate metabolic rates to the concentrations of solids (or NAPLs). To highlight the requirement of the dissolution reaction, we follow the classification of chemical reactions and describe these metabolic reactions as mass transfer-controlled reactions (table 2).

Similar to the Contois equation, the Best equation also recognizes that the determinant of specific growth rate is not simply nutrient concentration C_{N}, but the concentration ratio $Q_{\mathrm{N/X}}$ of nutrient to biomass (eq 20) (see Supplementary Information). It replaces the concentration factor in equation 14 and 17 with

$$F_{\mathrm{C}} = \frac{1+(1+\alpha)\beta\cdot Q_{\mathrm{N/X}}}{2}\left\{1-\sqrt{1-\frac{4\alpha\beta\cdot Q_{\mathrm{N/X}}}{\left[1+(1+\alpha)\beta\cdot Q_{\mathrm{N/X}}\right]^2}}\right\}. \tag{24}$$

Here α and β are two parameters. α is the ratio of the equilibrium concentration C_{eq} – the concentration at which the nutrient dissolution reaction is at equilibrium – to the half-saturation constant K_{M}, i.e., $\alpha = C_{\mathrm{eq}}/K_{\mathrm{M}}$. Parameter β is the ratio of the kinetic coefficient of dissolution to the affinity constant of metabolism (eq 19),

$$\beta = \frac{k_{\mathrm{diss}}}{k_{\mathrm{aff}}}. \tag{25}$$

Here k_{diss} is the dissolution rate per unit solid or NAPL (see Supplementary Information). Factor α measures the relative strength of a solid (or NAPL) in supporting metabolism, whereas factor β compares the kinetic constants of the dissolution reaction to those of microbial metabolism.

Similar to the Contois equation, the Best equation (eqs 14 and 24) also predicts a hyperbolic relationship. Figure 4D takes as an example microbial degradation of *α*-hexachlorocyclohexane (*α*-HCH) and shows, according to the Best equation, how the rate varies with the concentration ratio $Q_{\mathrm{N/X}}$ weighted with factor β. In evaluating equation 24, we take the equilibrium concentration C_{eq} of *α*-HCH at 25 ºC at 7.3 $\mathrm{mg{\cdot}L^{-1}}$ (Richardson and Miller, 1960), and the half-saturation constant K_{M} at 8.5 $\mathrm{mg{\cdot}L^{-1}}$ (Bachmann et al., 1988). Where the weighted concentration ratios are relatively large, metabolic rates are limited by microbial metabolism. Under this condition, the concentration factor approaches a maximum value ($F_{\mathrm{C,max}}$) that is dependent on the equilibrium concentration and the half-saturation constant (see Supplementary Information),

$$F_{\mathrm{C,max}} = \frac{C_{\mathrm{eq}}}{C_{\mathrm{eq}} + K_{\mathrm{M}}}\,. \tag{26}$$

Substituting the above values of C_{eq} and K_{M}, the maximum concentration factor is 0.46. This example $F_{\mathrm{C,max}}$ value highlights the limitation of mass transfer on microbial kinetics – if *α*-HCH completely dissolves in growth media, the $F_{\mathrm{C,max}}$ value should be unity.

Where the weighted concentration ratios approach 0, metabolic rates are determined by the dissolution rates of minerals and NAPLs. Under this condition, the concentration factor becomes a linear function of the concentration ratio,

$$F_{\mathrm{C,min}} = \frac{k_{\mathrm{diss}} C_{\mathrm{eq}}}{k_{\mathrm{C}}} \cdot Q_{\mathrm{N/C}}\,, \tag{27}$$

and metabolic rates vary linearly with nutrient concentrations,

$$r_{\mathrm{C}} = k_{\mathrm{dis}} C_{\mathrm{eq}} C_{\mathrm{N}}\,. \tag{28}$$

The hyperbolic relationships defined by the Best equation (eqs 14 and 24) and the Contois equation (eqs 14 and 21) resemble each other. To illustrate this point, we approximate the concentration factor F_{C} defined by the Best equation with the concentration factor defined by the Contois equation,

$$F_{\mathrm{C}} \approx F_{\mathrm{C,max}} \frac{\beta \cdot Q_{\mathrm{N/X}}}{\beta \cdot Q_{\mathrm{N/X}} + K'_{\mathrm{M}}}\,. \tag{29}$$

In the example of microbial degradation of α-HCH, the biomass-specific half-saturation constant is about 0.4, and the difference between the two equations are too close to show in figure 4D.

3.2. Rate Laws of Multi Limiting Nutrients

Where metabolic reactions are controlled by more than one nutrient, two strategies have been applied to compute reaction rates. One strategy is based on the multiplicative rate law, and the other is according to Liebig's law of the minimum (Zinn et al., 2004).

3.2.1. Respiration

A respiration reaction synthesizes ATPs by transferring electrons from an electron donor to an acceptor. The most common rate law for respiration rate r_R is the multiplicative or the dual Monod equation (Bungay III, 1968; Humphrey, 1974). This equation accounts for the concentrations of the electron donor and acceptor simultaneously,

$$r_{\mathrm{R}} = k_{\max} C_{\mathrm{X}} F_{\mathrm{D}} F_{\mathrm{A}}, \tag{30}$$

where F_D and F_A are the concentration factors of the electron donor and acceptor, respectively. In addition, Liebig's law of the minimum has also been applied,

$$r_{\mathrm{R}} = k_{\max} C_{\mathrm{X}} \cdot \min\left(F_{\mathrm{D}}, F_{\mathrm{A}}\right). \tag{31}$$

This equation postulates that respiration rate is determined by the concentration of either the electron donor or the acceptor, whichever places a stronger limitation (Ryder and Sinclair, 1972; Williamson and McCarty, 1976).

3.2.2. Growth

Both the multiplicative rate law and Liebig's law of the minimum have also been applied to microbial growth. MeGee et al. (1972) constructed the first multiplicative rate law to simulate the growth of *Lactobacillus casei* limited by glucose and riboflavin. According to their rate law, specific growth rate of *L. casei* is

$$\mu = \mu_{\max} F_{\mathrm{S}} F_{\mathrm{N}} - k_{\mathrm{D}}. \tag{32}$$

where F_S and F_N are the concentration factors for glucose and riboflavin, respectively. Alternatively, the specific growth rate may follow Liebig's law of the minimum,

$$\mu = \mu_{\max} \min\left(F_{\mathrm{S}}, F_{\mathrm{N}}\right) - k_{\mathrm{D}}. \tag{33}$$

This rate law assumes that growth rate is determined either by an energy substrate (i.e., glucose) or by an essential nutrient (i.e., riboflavin) (Bader, 1978; Droop, 1974; Sykes, 1973).

3.2.3. Chemical inhibition

Chemical inhibition is a common phenomenon of microbial metabolism. A typical example includes nutrients of high concentrations, which act as a double-edged sword. Taking methanogens as an example, their metabolism is supported by acetate, ammonium, methanol, sulfide, and other nutrients. On one hand, relatively high nutrient concentrations raise growth rates. On the other hand, high concentrations also inhibit the growth, and the extent of the inhibition depends on both methanogen species and physicochemical conditions, such as pH and temperature (Chen et al., 2014; Fukuzaki et al., 1990). Methanogen metabolism is also inhibited by other chemical inhibitors, including structural analogs of coenzyme M, short- to long-chain fatty acids, ethylene and acetylene, halogenated aliphatic hydrocarbons (e.g., chloroform, fluoroacetate, and methyl fluoride), and by the presence of alternative electron acceptors (Liu et al., 2011).

To account for chemical inhibition, numerous empirical functions have been proposed to amend microbial rate laws (Mulchandani and Luong, 1989). One of the earliest examples came from Boon and Laudelout (1962). They assumed that the nitrite inhibition on aerobic nitrite oxidation of *Nitrobacter winogradskyi* follows a pattern similar to non-competitive inhibition observed in enzyme reactions, and quantified the significance of the inhibition by using a dimensionless inhibition factor F_{I},

$$F_{\mathrm{I}} = \frac{K_{\mathrm{I}}}{K_{\mathrm{I}} + C_{\mathrm{I}}}. \tag{34}$$

Here K_{I} is the inhibition constant (M), and C_{I} is the concentration of inhibitor (fig 6A).

3.3. Thermodynamic Consistency

Rigorous rate laws must honor the principles of thermodynamics – a requirement so called “thermodynamic consistency” (Boudart, 1976). This requirement can be met by amending rate laws with the thermodynamic potential factor F_{T},

$$F_{\mathrm{T}} = 1 - \exp\left(-\frac{f}{\chi RT}\right) \tag{35}$$

where f is the thermodynamic drive ($\mathrm{J \cdot mol^{-1}}$), χ is the average stoichiometric number, and can be approximated as the number of times the rate-determining step occurs per metabolic reaction, R is the gas constant ($8.3145\ \mathrm{J \cdot mol^{-1} \cdot K^{-1}}$), and T_k is temperature in Kelvin.

Thermodynamic drives for metabolic reactions depend on whether ATPs are synthesized or consumed. For example, catabolic reactions couple redox reactions to ATP synthesis, and their thermodynamic drives are the differences between the energies available in the environment and the energies saved by ATP synthesis,

$$f = \Delta G_A - Y_P \cdot \Delta G_P \tag{36}$$

Here available energies ΔG_A are calculated as the negative of Gibbs free energies of chemical reactions, ΔG_P is the phosphorylation energy – the energy consumed by ATP synthesis from ADP and phosphate in the cytoplasm, about 45 $\mathrm{kJ \cdot (mol\ ATP)^{-1}}$, Y_P is the number of ATPs synthesized per respiration or fermentation reaction (Jin and Bethke, 2003; 2005). Figure 6B shows, according to equation 35, how the thermodynamic factor varies with the thermodynamic drive.

The thermodynamic potential factor in equation 35 and 36 represents a general description of the relationship between the thermodynamics and kinetics of microbial reactions, and can be simplified to other models of the thermodynamic control under different conditions (Jin and Bethke, 2005). For example, the model of Hoh and Cord-Ruwisch (1996) is best applied to microbial reactions that do not conserve energy. The models of Fennell and Gossett (1998) and Liu et al. (2001) are developed primarily for metabolic reactions with a stoichiometric number χ_{rd} of 1 per reaction. The model of LaRowe et al. (2012) draws an analogy from Fermi-Dirac statistics in quantum systems, and predicts that, where chemical reactions are at thermodynamic equilibrium, reaction rates are not zero, but instead have finite and positive values. This prediction contradicts the principle of detailed balance, where at equilibrium, the forward and backward rates precisely compensate for each other, resulting in net rates of zero.

3.4. Generic Rate Laws

Rate laws of microbial metabolisms have been summarized in two generic equations (Jin et al., 2013; Zwietering et al., 1992). One is the rate law for microbial respiration,

$$r_{\mathrm{R}} = k_{\mathrm{C,max}} \cdot C_{\mathrm{X}} \cdot F_{\mathrm{I}} \cdot F_{\mathrm{T}} \cdot f\left(F_{\mathrm{D}}, F_{\mathrm{A}}\right), \tag{37}$$

where *f*( ) is the multiplicative (e.g., eq 30) or the minimum function (e.g., eq 31). The other is for biomass synthesis, and is either the multiplicative rate law (e.g., eq 32),

$$r_{\mathrm{X}} = r_{\mathrm{X,P}} \cdot \prod_{\mathrm{i}} F_{\mathrm{N_i}} , \tag{38}$$

or Liebig's law of the minimum (e.g., eq 33),

$$r_{\mathrm{X}} = \min\left(r_{\mathrm{X,P}},\, \mu_{\max} C_{\mathrm{X}} F_{\mathrm{N_1}},\; \mu_{\max} C_{\mathrm{X}} F_{\mathrm{N_2}}, \cdots\right). \tag{39}$$

Here $r_{\mathrm{X,P}}$ is the rate of ATP-dependent biomass synthesis, or the rate where biomass synthesis is limited by the availability of energy sources only, and $F_{\mathrm{N_1}}$ and $F_{\mathrm{N_2}}$ are the concentration factors of nutrient $\mathrm{N_1}$ and $\mathrm{N_2}$, respectively. According to equation 16,

$$r_{\mathrm{X,P}} = \nu_{\mathrm{S}} Y_{\mathrm{X/S}} r_{\mathrm{R}} . \tag{40}$$

The concentration factors, i.e., F_{D}, F_{A}, $F_{\mathrm{N_1}}$ and others, may take different forms, depending on whether nutrients are dissolved in solution (eq 15) or occur as solids and NAPLs (eqs. 21 and 24). From the rate of biomass synthesis, specific growth rate is calculated according to equation 11. Where microbial metabolism is limited either by electron donor D or by acceptor A, equation 37 reduces to equation 17 and, at the same time, equation 38 and 39 converge and specific growth rate is calculated according to equation 14.

The generic rate laws (eqs 37, 38, and 39) have been applied with two distinct approaches. One approach centers around respiration, and first determines respiration rate by evaluating the rate law of respiration (eq 37). The respiration rate is then applied to assess the rate law of biomass synthesis (eq 38 or 39). The other approach emphasizes biomass synthesis, and first calculates the rate of ATP-dependent biomass synthesis by combining equation 37 with 40. The rate $r_{\mathrm{X,P}}$ is then applied to evaluate the rate of microbial growth (eq 11 and 38 or eq 11 and 39) and to calculate respiration rate by rearranging equation 40. Both approaches yield identical results.

4. EXTENDING TO NATURAL ENVIRONMENTS

Applying the trait-based modeling framework to natural environments necessitates modifications to the fundamental model assumptions related to microbial communities, metabolic reactions, and the rate laws of microbial kinetics (figs 2 and 3, and table 1). Natural environments are home to microbial communities that experience a broad range of growth conditions, including variations in pH, temperature, and nutrient concentrations. Additionally, in most natural environments, microbial activities are generally low, and a substantial portion of microbial cells remain dormant (Wang et al., 2014; Wörmer et al., 2019). In contrast, the trait-based framework was initially developed from the observations of laboratory experiments. These experiments typically take place in controlled environments that are uniform and rich in nutrients, and optimized for microbial growth in terms of physicochemical conditions. Furthermore, laboratory studies often focus on the exponential growth phase, during which most microbial cells display high metabolic activity. Consequently, modeling microbial processes in natural environments should account for the physiology of natural microbes as well as the specific growth conditions of the environment. Here we focus on the modifications that account for dormancy, biomass decay, physiological acclimation, and environmental conditions.

Microbial kinetics in natural environments is also subject to a multitude of additional factors, including microbial interactions and the intricate nature of the physicochemical conditions. Microbes interact with both other microbes and their surroundings via various mechanisms, such as physiochemical alterations, exchange of metabolites, signaling, biofilm formation, and chemotaxis, among others (Dong et al., 2022; Hibbing et al., 2010). The complexity of the ambient environment is exemplified by spatial heterogeneity and temporal fluctuations in physicochemical conditions (Nguyen et al., 2020; Nunan et al., 2020). In principle, incorporating these factors can be achieved by assembling relevant functional groups into a microbial reaction model and by coupling microbial reaction modeling to geochemical modeling and to reactive transport modeling (Bethke, 2022; Gharasoo et al., 2012). However, delving into a detailed exploration of this topic extends beyond the scope of the current discussion.

4.1. Dormancy

To apply the trait-based modeling framework to natural environments, it is essential to divide a functional group into subgroups in order to explicitly consider the different physiological states of microbial cells, including actively proliferating cells and the different stages of dormancy (Hunt, 1977; Stevenson, 1977). This treatment allows a functional group to have two types of biomass – one for actively growing cells and the other for dormant cells (fig 3B). This treatment is supported by two observations. Firstly, dormant cells constitute a significant proportion of microbial cells in diverse environments, such as soils, marine sediments, and other natural settings (Wang et al., 2014; Wörmer et al., 2019). Secondly, while dormant cells do not actively grow, it is important to note that dormancy does not imply inactivity (fig 2C). Dormant cells may engage in limited metabolic processes, albeit at rates significantly lower than those of actively growing cells (Hoehler and Jørgensen, 2013; Lever et al., 2015; Price and Sowers, 2004; Reeve et al., 1984).

In theory, to accurately simulate the three physiological states of natural microbes, at least three subgroups are required. These subgroups encompass an actively growing subgroup, a dormant subgroup primarily focused on biomass maintenance, and another dormant subgroup dedicated to the repair of damaged cellular components and structures (Price and Sowers, 2004). Transitions between the subgroups occur, with the transition rates assumed to be proportional to biomass concentrations. Several factors influence these transition rates, including physicochemical conditions and physiological states. The latter can be quantified by the difference in energy flux between catabolism and maintenance or the growth rate relative to the maximum growth rate (Ayati, 2012; Bradley et al., 2019; Konopka, 1999; Wang et al., 2014).

4.2. Biomass Decay

Modeling microbial metabolisms in natural environments requires the consideration of chemical fluxes driven by biomass decay, including those of biomass maintenance and cell lysis (Bradley et al., 2018; Hoehler and Jørgensen, 2013; Liang et al., 2019). Although these fluxes in laboratory nutrient-enriched bioreactors may not reach the same magnitude as those of catabolic reactions and biomass synthesis, they hold significant importance in natural environments.

For instance, in natural environments, cell death and subsequent lysis and fragmentation of microbial cells release cellular components into the surroundings, producing so-called necromass (fig 3) (Kästner et al., 2021; Liang et al., 2019). Microbial necromass represents a distinct pool of natural organic matter, accounting for up to 80% of organic carbon in soils. It serves as a unique exogenous nutrient for microbial metabolism, contributing to cryptic growth –

the growth enabled by utilizing cellular components released through cell lysis (Banks and Bryers, 1990; Koch, 1959; Ryan, 1959). Microbial necromass differs from other forms of organic matter, such as plant residuals, in terms of degradation kinetics and interactions with minerals (Cotrufo et al., 2013; Fan et al., 2021; Wang et al., 2020a).

Maintenance pathways consume ATPs, which are supplied by catabolism that utilizes two different types of energy sources, exogenous (or external) and endogenous substrates (figs 2B and C) (Beeftink et al., 1990; Marr et al., 1963; Schulze and Lipe, 1964). Endogenous substrates encompass various cellular components, such as poly-β-hydroxybutyrate and other energy-storage compounds, proteins, lipids, RNA, and more (Dawes and Ribbons, 1964; Herbert, 1958; Porges et al., 1953). The consumption of endogenous substrates for ATP production can be viewed as a special reaction – endogenous catabolic reaction. This particular reaction decreases biomass concentrations and produces CO_2, and other waste products (Roslev and King, 1995). To emphasize the sources of energy substrates, catabolic reactions using extracellular substrates can be referred to as exogenous catabolic reactions. While microbial maintenance in nutrient-rich bioreactors is powered primarily by exogenous substrates, the consumption of endogenous substrates becomes significant in oligotrophic environments, such as pristine aquifers, marine sediments, and other (Kjelleberg et al., 1987).

Simulating endogenous catabolic reaction and microbial necromass production requires amending trait-based microbial reaction models with respective stoichiometric reaction equations and rate expressions. When formulating the rate expressions for these fluxes, a common assumption is that they are directly proportional to biomass concentrations (Fan et al., 2021). Other factors, such as temperature, pH, salinity, and viral abundance, also influence these processes (Shimoda et al., 2002; Wang et al., 2020b). These fluxes are governed by the principle of energy balance (fig 2B and C). In particular, the energy fluxes of actively growing subgroups must honor

$$J_{\mathrm{P,ex}} + J_{\mathrm{P,in}} = J_{\mathrm{P,X}} + J_{\mathrm{P,M}} . \qquad (41)$$

Here $J_{\mathrm{P,ex}}$ and $J_{\mathrm{P,in}}$ are the ATP fluxes of exogenous and endogenous catabolic reactions, respectively. For dormant subgroups, equation 41simplifies to

$$J_{\mathrm{P,ex}} + J_{\mathrm{P,in}} = J_{\mathrm{P,M}} , \qquad (42)$$

because the energy flux of biomass synthesis reduces to 0.

4.3. Environmental Conditions

To account for the physicochemical and biological conditions present in natural environments, an intuitive strategy is to follow the multiplicative rate expression (eq 30) by amending the rate laws for laboratory cultures (eqs 37 and 38) with additional dimensionless functions. These functions are designed to capture the specific environmental conditions of interest (Zwietering et al., 1992), and examples include those for temperature, pH, water activity, and biomass concentrations.

4.3.1. pH and temperature

Microbial catabolism and biomass synthesis respond to pH and temperature variations by following bell- or triangle-shaped curves characterized by a set of three parameters, the minimum, optimal, and maximum pHs and temperatures. At pHs and temperatures below the minimum or above the maximum values, microbial metabolism pauses. Between the minimum and optimal values, temperature and pH increases raise microbial catabolic and growth rates. Between the optimal and maximum values, the increases lower microbial rates. A commonly-used model to describe the bell-shaped temperature response of catabolism and biomass synthesis is the empirical cardinal temperature model (Rosso et al., 1993). According to this model, the dimensionless factor F_{TMP} for temperature is

$$F_{\mathrm{TMP}} = \max\left\{0,\ \frac{(T-T_{\max})(T-T_{\min})^2}{(T_{\mathrm{opt}}-T_{\min})\left[(T_{\mathrm{opt}}-T_{\min})(T-T_{\mathrm{opt}})-(T_{\mathrm{opt}}-T_{\max})(T_{\mathrm{opt}}+T_{\min}-2T)\right]}\right\}. \qquad (43)$$

Here $T_{\min}$, T_{opt}, and $T_{\max}$ are the minimum, optimal, and maximum temperatures of microbial reactions. Likewise, the cardinal pH model is,

$$F_{\mathrm{pH}} = \max\left\{0,\ \frac{(\mathrm{pH}-\mathrm{pH}_{\max})(\mathrm{pH}-\mathrm{pH}_{\min})}{(\mathrm{pH}-\mathrm{pH}_{\min})(\mathrm{pH}-\mathrm{pH}_{\max})-(\mathrm{pH}-\mathrm{pH}_{\mathrm{opt}})^2}\right\}, \qquad (44)$$

where $\mathrm{pH}_{\min}$, $\mathrm{pH}_{\mathrm{opt}}$, and $\mathrm{pH}_{\max}$ are the minimum, optimal, and maximum pHs (Rosso et al., 1995). Figure 7A and B show, according to the cardinal models, how the temperature factor F_{TMP} of mesophilic microbes varies with temperature and how the pH factor F_{pH} of neutrophiles varies with pH.

In comparison, current experimental evidence suggests that the temperature response of biomass maintenance might be monotonic, and can be described with the Arrhenius equation (Price and Sowers, 2004; Tijhuis et al., 1993). Specific maintenance rate k_M is calculated according to

$$k_{\mathrm{M}} = A_{\mathrm{M}} \cdot \exp\left(\frac{E_{\mathrm{a,M}}}{RT_{\mathrm{K}}}\right), \tag{45}$$

where A_M is the pre-exponential factor, and $E_{a,M}$ is the apparent activation energy. Figure 8 shows, according to equation 45 and on the basis of the data compiled by Price and Sowers (2004), how specific maintenance rate varies with temperature. For every 10 ºC increase in temperature, the specific maintenance rate increases about an order of magnitude, which confirms a rule-of-thumb view about the different specific maintenance rates in laboratory bioreactors and natural environments (Schmidt, 1992).

4.3.2. Salinity and water activity

Salinity, the total ion concentration in a solution (Williams and Sherwood, 1994), is a primary factor that controls the diversity, composition, and function of natural microbial communities (Yang et al., 2016; Zhang et al., 2021). Salinity determines turgor pressure, the difference in hydrostatic pressure between the cytoplasm and the ambient environment, which in turn determines microbial growth rates (Rojas and Huang, 2018). In the food and pharmaceutical industry, the effect of salinity has been approached within the context of water activity (a_w), a solution property that quantifies the amount of "free" unbound water molecules available for chemical reactions and microbial metabolisms (Daniel et al., 2004; Troller and Christian, 1978). Water activity is measured experimentally as the ratio between the fugacity of water vapor of a solution and the fugacity of pure water at the same temperature and pressure. In theory, water activity is calculated from molar water concentration, stoichiometric ionic strength, and the osmotic coefficient and can be taken as the effective water content expressed in terms of water mole fraction (Cazier and Gekas, 2001; Stokes and Robinson, 1948).

Similar to microbial kinetic responses to pH and temperature, microbial response to salinity follows bell- or triangle-shaped curves (Stevenson et al., 2015). A cardinal water activity model can also be constructed with three cardinal parameters, the minimum, optimal, and maximum water activities (Peleg, 2022). For non-halophiles and many halotolerant microbes, their optimal water activity stays close to unity, and their growth rates appear to decrease linearly with decreasing water activity (Emborg and Dalgaard, 2008; McMeekin et al., 1987). In these cases, the factor F_W of water activity can be described with a simple linear function (McMeekin et al., 1987; Zwietering et al., 1992),

$$F_W = \max\left\{0,\ \frac{a_w - a_{w,\min}}{1 - a_{w,\min}}\right\}, \tag{46}$$

where $a_{w,\min}$ is the minimum water activity required by microbial metabolism. This parameter is not a constant, but varies with both microbial strains and the physiochemical conditions of the environment, including temperature (Santos et al., 1994). Figure 7C shows how the factor of *Staphylococcus xylosus* varies with water activity (McMeekin et al., 1987).

4.3.3. Biomass concentration

The Monod equation assumes that biomass-specific rates of catabolism and growth are independent of biomass concentrations. This assumption assumes unlimited resources and does not account for the limitations on the total biomass that can be sustained by the environment. Also, the assumption contradicts quorum sensing – cell-to-cell communication via the production and release of signal molecules (Fuqua et al., 1994). Quorum sensing enables microbes to regulate their gene expression and to adjust their phenotypic traits in accordance with the biomass concentration in the ambient environment (Abisado et al., 2018; Swift et al., 2001).

Following the classical Verhulst-Pearl logistic equation (Peleg and Corradini, 2011), a simple approach to relate cell-specific rate to biomass concentration is to amend microbial rate laws with the following biomass capacity function (Wu et al., 2022),

$$F_X = 1 - \frac{C_X}{C_{X,\max}}. \tag{47}$$

Here $C_{X,\max}$ is carrying capacity, the maximum biomass concentration that can be supported by an environment (Chapman and Byron, 2018). According to this factor (fig 7D), relatively low biomass concentrations have negligible effect on microbial kinetics, but increases in biomass concentrations slow down microbial reactions. Other approaches, such as the inhibition function, have also been proposed to account for biomass concentration (Hilau et al., 2022).

Carrying capacity is determined by a suite of environmental and biological factors, and therefore its inclusion in the biomass factor brings additional benefits. For example, by including the biomass factor, we can constrain microbial growth with environmental factors, such as space and surface area, and hydrodynamics. Furthermore, the biomass factor keeps simulated biomass concentrations in check (Wu et al., 2022). This is especially important for simulating the metabolic reactions of a functional group within a community. Without this factor, the biomass concentration of a functional group is determined by nutrient fluxes into the environment, and therefore may exceed the carrying capacity of the environment.

4.4. Physiological Acclimation

Applying the trait-based modeling framework to natural environments requires the kinetic and stoichiometric parameters of natural microbes. These parameters have been analyzed for laboratory cultures, but only to a very limited extent in natural environments (Jin et al., 2013; Pallud and Van Cappellen, 2006). For example, Wu et al. (2022) compiled the trait parameters for acetoclastic methanogenesis – a process responsible for ~70% of methane bioproduction in terrestrial environments (Conrad, 1999). Despite the global significance of the process, only two studies have directly determined half-saturation constants in natural systems, and the analysis of rate constants and biomass yield coefficients still needs to be carried out.

Most microbial parameters obtained with laboratory experiments are not directly applicable to natural environments. Current available data suggest that microbial parameters, including rate constants, half-saturation constants, and biomass yield coefficients, are not constants, but dependent on culture history and physicochemical conditions of the environment (Ferenci, 1999; Grady et al., 1996; Kovárová-Kovar and Egli, 1998). The variation in trait values, or phenotypic plasticity, highlights a fundamental difference between regular catalysts and microorganisms. While the catalysts in chemical processes are fairly stable, microorganisms are capable of acclimating to their ambient environment (Aksnes and Cao, 2011; Flynn et al., 2015; Merchant and Helmann, 2012). Here acclimation refers to reversible modification of phenotypic traits, including microbial kinetic and stoichiometric parameters, in response to changes in temperature, pH, resource availability, and other environmental conditions.

Physiological acclimation provides a theoretical basis to extrapolate laboratory-determined trait values to natural environments. The extrapolation is based on the optimality assumption that through natural selection only organisms whose physiological traits are optimally adjusted according to the conditions of the ambient environment could survive and reproduce in continual competition for resources (Smith et al., 2011). By building optimization-based models of acclimation, we can constrain the plasticity of microbial parameters as a function of environmental conditions (fig 9). The optimization models focus on two functional traits and assume that the two traits are determined by the allocation of limited cellular resources, such as proteins, ribosomes, and other macromolecules. Allocating more resources to one trait limits the resources available to the other trait, leading to the trade-off between the two traits. These models have been built for the kinetics of nutrient uptake and microbial catabolism, and the thermodynamic efficiency of microbial metabolism.

4.4.1. Nutrient uptake

Pahlow (2005) and Smith and Yamanaka (2007) developed a model of optimal nutrient uptake. In their model, nutrient uptake flux J_{N} is maximized by taking nutrient affinity and maximum uptake flux as control variables. Nutrient affinity defines the slope at which uptake fluxes increase with very low nutrient concentrations, and maximum uptake flux defines the flux where nutrient concentrations are very large. According to their results (Smith et al., 2015), by acclimating to nutrient concentration C_{N} in the ambient environment, microbes change their Michaelis constant $K_{\mathrm{N,a}}$ of nutrient uptake according to

$$K_{\mathrm{N,a}} = \frac{J_{\mathrm{max,N}}}{\alpha_{\mathrm{max,N}}} + 2\sqrt{\frac{J_{\mathrm{max,N}}}{\alpha_{\mathrm{max,N}}} C_{\mathrm{N}}} \ . \tag{48}$$

Here $J_{\mathrm{max,N}}$ and $\alpha_{\mathrm{max,N}}$ are the potential maximum values of maximum uptake flux and nutrient affinity, respectively.

From the acclimation-dependent Michaelis constant $K_{\mathrm{N,a}}$, nutrient uptake flux J_{N} can be calculated from the Michaelis-Menten equation, i.e.,

$$J_{\mathrm{N}} = J_{\mathrm{max,N}} \frac{C_{\mathrm{N}}}{C_{\mathrm{N}} + K_{\mathrm{N,a}}} . \tag{49}$$

By assuming that biomass synthesis rate is determined by nutrient uptake flux, we can calculate specific growth rate according to equation 11, that is

$$\mu = Y_{\mathrm{X/N}} J_{\mathrm{N}} - k_{\mathrm{D}} . \tag{50}$$

Figure 10 applies the optimal nutrient uptake model (eq 48 to 50) to nitrate consumption by phytoplankton, and shows how the Michaelis constant, nitrate uptake flux, and specific growth rate vary with nitrate concentration in the ambient environment.

The optimal uptake model (eq 48 to 50) is most effective in environments with low nutrient concentrations. This limitation arises from the assumption that nutrient uptake is the rate-determining step. While this assumption is generally valid when nutrient concentrations are close to 0, the rate-determining steps of microbial metabolisms are not fixed, but shift to other enzymes in environments with relatively large nutrient concentrations (Jin et al., 2022).

4.4.2. Catabolic reaction

Wu et al. (2022) constructed an acclimation model for microbial catabolism limited by a single energy substrate. Their model assumes that catabolic rates are limited by substrate uptake at very low substrate concentrations and by a different reaction at very high concentrations. Their model further assumes that microbes maximize their catabolic rates by optimizing the partition of cellular resources between the two rate-determining steps.

The model of Wu et al. (2022) relates the kinetic parameters of a microbe in natural environments to the parameter values of laboratory cultures. Specifically, the half-saturation constant $K_{\mathrm{M,a}}$ of natural microbes depends on the half-saturation constant $K_{\mathrm{M,o}}$ of laboratory cultures according to

$$K_{\mathrm{M,a}} = \sqrt{\beta} \cdot K_{\mathrm{M,o}} . \tag{51}$$

Here β is the ratio of substrate concentration $C_{\mathrm{S,a}}$ in the environment to the concentration $C_{\mathrm{S,o}}$ in laboratory bioreactors,

$$\beta = \frac{C_{\mathrm{S,a}}}{C_{\mathrm{S,o}}} . \tag{52}$$

The rate constant k_{a} of natural microbes is

$$k_{\mathrm{a}} = k_{\mathrm{o}} \frac{C_{\mathrm{S,o}} + K_{\mathrm{M,o}}}{C_{\mathrm{S,o}} + K_{\mathrm{M,a}}} , \tag{53}$$

where k_{o} is the rate constant of laboratory cultures.

Wu et al. (2022) applied the model and explored how ambient acetate concentrations may affect the kinetic parameters of acetoclastic methanogenesis. According to their results, compared to those of laboratory cultures, the rate constants and the half-saturation constants of methanogens acclimating to the environment of a few μM acetate can be one order of magnitude smaller (fig 11A and B). These results help explain the dominance of *Methanosaeta* over *Methanosarcina* at <0.1 mM acetate. Without acclimation, the methanogenesis rates of *Methanosarcina*, calculated with the Monod equation, would stay close to those of *Methanosaeta* at < 1 mM acetate (fig 11C). Accordingly, *Methanosarcina* and *Methanosaeta* would co-exist in environments of <1 mM acetate, a prediction that contradicts field observations that *Methanosaeta* dominates low-acetate environments. By accounting for acclimation, *Methanosaeta* obtains larger methanogenesis rates at <0.1 mM acetate, which confers to competitive advantage against *Methanosarcina*.

4.4.3. Metabolic efficiency

Wu et al. (2022) developed a model of maximum ATP flux to predict the thermodynamic efficiency of microbial catabolism. This model maximizes the flux of ATP synthesis by trading-off the rate against the ATP yield of catabolism. At a given available energy, increases in the ATP yield raise the rate of ATP production, but lower the thermodynamic drive and hence the catabolic rate, which in turn lowers the rate of ATP production. The optimal yield of ATP, expressed in mol ATP synthesized per mol nutrient, varies with the energy ΔG_{A} available in the environment (see eq 36), and the variation can be approximated according to

$$Y_{\mathrm{P,op}} = \alpha \cdot \Delta G_{\mathrm{A}}^{\beta}\,. \tag{54}$$

For microbial reactions that have an average stoichiometric number of 2 per reaction, exponent β is estimated at 1.1 and coefficient α is about 4.2×10^{-6} $\mathrm{mol}^{1.1}\cdot\mathrm{J}^{-1.1}$ (fig 12A). From the optimized ATP yield, we can calculate the optimal biomass yield $Y_{\mathrm{X,op}}$ as the product of the ATP yield and biomass yield per ATP,

$$Y_{\mathrm{X,op}} = \alpha \cdot Y_{\mathrm{X/P}} \cdot \Delta G_{\mathrm{A}}^{\beta}\,, \tag{55}$$

where $Y_{\mathrm{X/P}}$ is the biomass yield per ATP, which is about 5 $\mathrm{g}\cdot\mathrm{mol}^{-1}$ for anaerobic respiration (Jin, 2012).

The maximum ATP flux model predicts that ATP and biomass yields increase with available energy (fig 12A and B), which is consistent with the paradigm that where more energy is available, more energy is conserved (Jin, 2012). The efficiency of microbial energy conservation can be calculated as the percentage of saved energy per available energy (Roels, 1983). Where available energy is low, the thermodynamic efficiency is also small, close to 50%. Increases in available energy raise the efficiency. For aerobic respiration, the efficiency can reach ~85% (fig 12C).

The relationship between biomass yield and the available energy has been a long-standing question in microbiology and biotechnology. Various methods have been proposed to estimate biomass yield, including the ATP-based method (Kleerebezem and Van Loosdrecht, 2010), Gibbs energy dissipation method (Heijnen and Dijken, 1992), thermodynamic electron equivalents model (McCarty, 2007), and Gibbs Energy Dynamic Yield Method (Smeaton and Van Cappellen, 2018). Compared to these methods, the maximum ATP flux method accounts for the tradeoff between the rate and yield of microbial catabolism, requires minimum information about metabolic pathways, and provides a straightforward estimation directly from a commonly analyzed chemical parameter – the energy available in the environment.

5. MOLECULAR BIOLOGY-ENABLED MODEL IMPROVEMENTS

Recent advancements in molecular biology have made available a wide range of low-cost high-throughput cultivation-independent tools and techniques for studying natural microbes. These tools have been applied to characterize and quantify various biological molecules, from phylogenetic markers (or ribosomal rRNA genes), functional genes, and their products (ribosomal RNA, mRNA transcripts, and proteins), to the complete sets of DNA (or genome), mRNA, proteins, and metabolites in a microbial cell or population (i.e., genomics, transcriptomics, proteomics, and metabolomics, respectively) and in a microbial community (i.e., meta-omics). Their applications informed the construction and applications of microbial kinetic models, and inspired a multitude of novel strategies that integrate molecular data into the simulation of microbial reactions, including functional gene-based modeling, pathway-specific kinetic modeling, and genome-scale stoichiometric metabolic modeling (Störiko et al., 2021).

5.1. Functional Gene-based Model

A functional gene is a portion of DNA that codes for a polypeptide chain or other gene product. Functional genes in natural environments can be directly detected and quantified with gene and metagenomic sequencing. The first functional gene-based model came from Reed et al. (2014). They replaced functional groups with functional genes and built a model of nitrogen cycling in the Arabian Sea oxygen minimum zone (OMZ) with eight functional genes, including the ammonia monooxygenase gene and the hydrazine oxidoreductase gene. This approach links microbes *in silico* to those in the environment, and makes possible model validation by comparing gene abundances to field observations.

Using functional genes as surrogates for microbial functional groups builds on the following considerations. First, microbes are grouped into functional groups according to their metabolic reactions. Metabolic reactions are catalyzed with enzymes coded by functional genes. Therefore, microbes can also be grouped according to their functional genes. Taking the functional group of methanogens as an example, this group is conventionally constructed with microbes capable of producing methane. Because all methanogens carry the methyl-coenzyme M reductase (or *mcr*) gene, this group can also be represented with the *mcr* gene. Second, DNA is a stable biomolecule and its nucleotide sequence does not change significantly in response to short-term external environmental changes. By linking gene productions to catabolic reactions and by applying the principle of mass balance, the net rate of gene production can be calculated on the basis of concentration balance principle according to

$$\frac{1}{C_{\mathrm{G}}} \cdot \frac{dC_{\mathrm{G}}}{dt} = v_{\mathrm{S}} Y_{\mathrm{G/S}} \frac{r_{\mathrm{C}}}{C_{\mathrm{G}}} - k_{\mathrm{D,G}} . \tag{56}$$

where C_{G} is the gene copy number per kg water, $Y_{\mathrm{G/S}}$ is the yield of gene copy number per energy substrate S, and $k_{\mathrm{D,G}}$ is the specific rate of gene decay. Combining equation 37 with 56 gives the framework of the functional gene-based model that replaces biomass concentration C_{X} with functional gene concentration C_{G} as a state variable.

Louca et al. (2016) expanded the functional gene approach by numerically tracking the concentrations of mRNA and enzymes, and simulated microbial reactions associated with the cycling of nitrogen and sulfur in an oceanic oxygen minimum zone. They expressed the production rates of mRNAs and enzymes in terms of the rates of catabolic reactions, and simulated the concentrations of the two macromolecules on the basis of concentration balance. By doing so, their modeling framework integrates metagenomic, metatranscriptomic and metaproteomic datasets into the simulation.

The functional gene approach uses a single gene to represent microbes, and hence requires that a gene be unequivocally associated with only one chemical reaction. This requirement is problematic. First, functional genes may be involved in more than one reaction. For example, the dissimilatory sulfite reductase gene (*dsr*) has been applied as a diagnostic tool for microbial sulfate reduction, but this gene also participates in microbial sulfur oxidation (Ghosh and Dam, 2009). Likewise, the *mcr* gene is required for both methanogenesis and anaerobic methane oxidation. In these cases, the functional-gene approach is best applied to environments where biogeochemical cycling is truncated. For example, where methanogens are producing methane, anaerobic methane oxidation is negligible.

Second, more than one gene is required to describe microbial respiration. A typical example would be methanogenesis. By using the *mcr* gene alone, we would not be able to simulate hydrogenotrophic and acetoclastic methanogenesis at the same time. We would also fail in accounting for the competition between *Methanosarcina* and *Methanosaeta*, the two genera capable of acetoclastic methanogenesis. Therefore, the functional-gene approach is best applied to environments where microbial respiration of an electron acceptor is powered primarily by a single electron donor and driven by a single functional group.

Finally, the functional gene approach is not applicable to the fermentation of organic matter to H_2, short-chain fatty acids, alcohols, and other simple organic compounds, dissimilatory reduction of ferric iron, or other microbial processes, whose functional genes have yet to be identified. To simulate organic matter degradation to carbon dioxide and methane with the functional gene approach, previous studies combined organic matter fermentation with the respiration of sulfate and other electron acceptors into single reactions, and simulated the progress of the reactions on the basis of the functional genes of respiration pathways (Louca et al., 2016; Reed et al., 2014). While this approach of lumping together organic matter fermentation and respiration is popular among many biogeochemists, it assumes that the rates of organic matter degradation are limited by the reduction of electron acceptors, which contradicts the consensus that organic matter degradation is limited by the step of organic matter hydrolysis or fermentation (Wu et al., 2021). To avoid this assumption, we propose to simulate organic matter degradation to inorganic carbon by combining the trait-based framework for organic matter fermentation with the functional gene framework for respiration.

5.2. Metabolic Models

Metabolic models are mathematical representations of individual biochemical pathways or at genome scale the entire metabolic pathways of organisms (Dahal et al., 2020; Embree et al., 2015). They are commonly simulated with stoichiometric and kinetic methods. Stoichiometric modeling searches for the chemical fluxes through metabolic networks on the basis of the mass balance principle and the optimality principle. Kinetic modeling traces the progress of individual biochemical reactions within a metabolic network and therefore network fluxes by combining the principle of mass balance with enzyme kinetics.

5.2.1. Genome-scale stoichiometric model

Genome-scale stoichiometric metabolic models are built by applying the mass balance principle to metabolic fluxes. For a metabolic network of m number of reactions and n number of metabolites, its metabolic fluxes are related to the rates of biochemical reactions according to

$$\mathbf{J} = \mathbf{S} \times \mathbf{R}, \tag{57}$$

where $\mathbf{J}$ is a column vector of size n, its element J_i is the flux of metabolite, net rate at which metabolite $\mathrm{A_i}$ is produced (or consumed, if negative) per unit biomass, $\mathbf{R}$ is a vector of size m, its element r_j is the rate of biochemical reaction j per unit biomass, $\mathbf{S}$ is a stoichiometric matrix of size $m \times n$, its element S_ij is the stoichiometric coefficient of $\mathrm{A_i}$ in the reaction of enzyme j, and can be constructed from genomic information and related literature data (Orth et al., 2010). To link the metabolic network to biomass synthesis, a hypothetical reaction is included in the network to describe the production of one gram of biomass dry weight from a stoichiometric combination of nucleic acids, proteins, carbohydrates, and other macromolecules.

Stoichiometric modeling solves rate vector $\mathbf{R}$ by assuming that microbial metabolism is at steady state. Under this assumption, metabolic fluxes, except those of nutrient uptake, waste product excretion, and biomass synthesis, stay at zero. Because there are more metabolites (hence metabolite fluxes) than there are reactions (i.e., $m < n$), the solution for the rate vector is underdetermined.

A common approach to uniquely determine the vector **R** is flux balance analysis (FBA) (Feist and Palsson, 2008; Heirendt et al., 2019). FBA formulates the underdetermined vector **R** as an optimization problem by imposing an objective upon metabolic networks, and by accounting for maximum and minimum values on metabolite fluxes **J** and enzyme rates **R**. An example objective is to maximize one of the biochemical reactions – the hypothetical biomass synthesis reaction (Price et al., 2004; Schuetz et al., 2007; Schuster et al., 2008). FBA solves the rate vector **R** that meets the objective by using linear programming (or linear optimization). Because FBA uses nutrient uptake fluxes as input, and predicts the fluxes of waste product excretion and the rates of growth, it is essentially a yield coefficient estimator, or stoichiometric coefficient estimator (Senger et al., 2014).

Stoichiometric metabolic modeling enables a direct integration of high-throughput genomic data into microbial reaction modeling, and offers a powerful tool for exploring microbial metabolism at the system level and in unprecedented detail (Henry et al., 2010). For example, stoichiometric metabolic models have been combined with the enzyme kinetics of nutrient uptake to predict microbial chemical fluxes (Mahadevan et al., 2002). The parameters for substrate uptake are assigned either by fitting the simulation outcome to laboratory observations (Scheibe et al., 2009b), or by directly using the values determined for relevant enzymes (Zhuang et al., 2010). Scheibe et al. (2009b) applied this approach to the genome-scale metabolic model of *Geobacter sulfurreducens*, and simulated the progress of uranium bioremediation in an aquifer. Their predictions had to be scaled down by a factor of 10 in order to be applicable to the environment (Fang et al., 2011; Scheibe et al., 2009a). This *ad hoc* adjustment likely reflects the high sensitivity of the modeling results to the kinetic parameters of enzymes (Klier, 2012). As an alternative, the flux of nutrient uptake can be calculated according to the Monod equation (Shapiro et al., 2018).

Stoichiometric metabolic modeling has also being applied to solve the stoichiometric equations of metabolic reactions. Shapiro et al. (2018) updated the genome-scale metabolic model of *M. barkeri*, performed FBA, and determined the stoichiometric equation for the biomass synthesis of *M. barkeri* growing on acetate and ammonium:

$$\begin{gathered}1.73\mathrm{CH_3COO^-} + 0.95\mathrm{NH_4^+} + 0.08\mathrm{HPO_4^{2-}} + 0.05\mathrm{C_3H_7NO_2S} + 0.88\mathrm{H^+} \\ \rightarrow \mathrm{X} + 0.08\mathrm{CO_2} + 0.02\mathrm{CH_3SH} + 2.39\mathrm{H_2O}.\end{gathered} \tag{58}$$

Here X represents the biomass with a chemical formula of $C_{3.52}H_{5.42}O_{1.33}NP_{0.08}S_{0.03}$, and was determined from the macromolecules composition of *M. barkeri* biomass. Compared to the generic equation for biomass synthesis (eq 5), equation 58 improves the accuracy and expands the capability of the kinetic model of *M. barkeri*. Specifically, equation 58 captures cysteine ($C_3H_7NO_2S$) as an essential nutrient and methanethiol (CH_3SH) as an unconventional metabolic product of *M. barkeri* metabolism. Also, this equation predicts that the biomass synthesis of *M. barkeri* requires that acetate and ammonium are supplied a flux ratio of 1.8, smaller than the ratio of 2.5 given by the generic equation of biomass synthesis. These results have been applied to assess the extent of nitrogen limitation on the metabolisms of aquifer methanogens (Shapiro et al., 2018).

The limitations of stoichiometric metabolic modeling have been discussed previously, including the incomplete knowledge of genome sequences and the lack of a unified standard in model construction (Bernstein et al., 2021; Ebrahim et al., 2015; Ravikrishnan and Raman, 2015). In applying stoichiometric metabolic modeling to natural environments, we should also note that most models are validated against laboratory observations, which bear limited relevance to natural environments. In addition, while the objective of maximizing growth yield has worked well in laboratory bioreactors, this objective may not reflect the strategies employed by microbes in natural environments.

5.2.2. Pathway-specific kinetic model

Kinetic metabolic models are constructed by combining the principle of mass balance with the rate equations of biochemical reactions. A kinetic metabolic model consists of (1) a matrix equation that describes the mass balance of metabolites (eq 57), (2) a set of equations that relate the rates of biochemical reactions to enzyme turnover, saturation, allosteric regulation, and reaction thermodynamics,

$$r_{\mathrm{j}} = f(C_{\mathrm{Ej}}, C_{\mathrm{Mi}}, k_{\mathrm{j}}, K_{\mathrm{j}}, \ldots), \tag{59}$$

and (3) the initial state of the metabolic system (i.e., metabolite and enzyme concentrations at time 0) (Saa and Nielsen, 2017). Here C_{Ej}, is the concentration of enzyme j expressed in terms of cell volume, C_{Mi} is the cellular concentration of metabolite i, and k_{j} and K_{j} represent the kinetic and thermodynamic parameters of the biochemical reaction catalyzed by enzyme j, respectively. Parameter k_{j} and K_{j} can be sourced from online databases, borrowed from homologous enzymes from other organisms, estimated with optimization, or by fitting to metabolomics and fluxomic datasets (Foster et al., 2021). Kinetic metabolic models are solved numerically from a given initial state by integrating metabolic fluxes forward over time. Their results describe the temporal variation in metabolite concentrations and reaction fluxes.

Constructing kinetic metabolic models requires kinetic parameters of biochemical reactions and regulatory metabolic interactions, which are not available for most enzymes of biomass synthesis. As a result, previous efforts have focused primarily on catabolic pathways, including those of geochemical significance. Example models include the acetate consumption model by *Geobacter sulfurreducens* (King et al., 2009), nitrate reduction model (Li et al., 2017), dissimilatory sulfate reduction model (Wing and Halevy, 2014), and the models of hydrogenotrophic, acetoclastic, and methylotrophic methanogenesis (Gropp et al., 2022; Jin et al., 2022; Peterson et al., 2014; Rhim and Ono, 2022). Some example model applications include:

- Metabolic kinetic models are applied as plug-in modules to compute microbial reaction rates in biogeochemical reaction models and reactive transport models (King et al., 2009). Metabolic kinetic models predict how metabolite concentrations and biochemical reaction rates vary with time, which can be applied to compute the overall rates of metabolic reactions and how the rates respond to changes in environmental conditions.
- Kinetic metabolic modeling is applied to estimate the rate constant, the half-saturation constant, and other microbial parameters (Jin et al., 2022). Kinetic metabolic models predict microbial reaction rates from enzyme kinetic and thermodynamic parameters, without the need for trait parameters or the properties of overall metabolic reactions. These models can be applied to estimate the kinetic parameters of microbial catabolism.
- Kinetic metabolic modeling is applied to understand the mechanistic underpinning of microbial physiology (Jin et al., 2022). By tracking chemical fluxes through metabolic reactions and their responses to environmental conditions, kinetic metabolic modeling simulate physiological properties as emergent properties from underlying biochemical reactions and their interactions.
- In addition, kinetic metabolic modeling is applied to decipher isotope fractionations associated with microbial metabolisms and how the fractionations depend on environmental and microbiological factors (Gropp et al., 2022; Wing and Halevy, 2014).

These examples illustrate the potential of kinetic metabolic models in improving the modeling of microorganisms by moving trait-based models beyond the coarse-grained level description of metabolic reactions.

6. CHALLENGES AND LIMITATIONS

The application of trait-based microbial models to natural environments requires the abstraction, simplification, and idealization of microbial communities and their metabolisms, which inevitably introduces biases and limitations. Acknowledging these biases and limitations is critical for a comprehensive understanding of the boundaries and uncertainties associated with model outcomes. It enables us to make informed interpretations and decisions based on simulation results. For example, these biases and limitations must be considered during model construction and application, particularly when evaluating whether the trait-based modeling framework addresses the questions and issues of interest and, if so, whether our current knowledge and available data are adequate for building quality trait-based models. Also, in interpreting and applying simulation results, the model biases and limitations provide a baseline and help ensure that our efforts align with both model assumptions and underlying biological principles.

6.1. Model Features and Limitations

Trait-based kinetic models adopt two simplifications to simulate the metabolisms of microbial communities. One simplification is the treatment of microbial communities as ensembles of microbial functional groups, and the separation of functional groups to actively growing and dormant subgroups (figs 1 to 3). This simplification gives rise to two model features. First, trait-based models are unstructured in that they do not account for the internal state of microbial cells (i.e., cellular chemical composition and physical structure) or their variation with the microbial life cycle (Esener et al., 1983; Şimşek and Kim, 2018). By assuming that the internal state remains fixed, we also assume that microbial metabolism is at steady state, and microbial growth is balanced – the growth of cellular components occurs at the same rate per unit biomass. In addition, balanced growth implies that microbial growth is exponential (Koch, 1993; Painter and Marr, 1968); the steady state assumption dictates that the rates of biomass production and cell number increase converge (Fishov et al., 1995).

Second, trait-based models are unsegregated because we assume that individual cells within a group (or subgroup) share the same functional traits (Campbell, 1957; Oldewurtel et al., 2021). Trait-based models do not consider morphological traits or cellular chemical composition. In this way, trait-based models simulate the changes in population sizes of functional groups and subgroups, not the development and reproduction of individual cells.

The other simplification is the description of microbial metabolism at the coarse-grained level with three reactions – catabolic reaction, biomass synthesis, and maintenance (figs 1 and 2). This simplification leads to an underappreciated feature of trait-based models – they are mechanistic in nature, as they account for the fluxes of mass and energy through microbial biomass (figs 2 and 3). First, the fluxes of a chemical compound is described by the ODEs for concentration conservation, and by accounting for the rates of metabolic reactions. The contributions of the metabolic reactions to the overall flux depend on the coupling between catabolism and anabolism, or the efficiency of microbial metabolism. Second, the rates of the metabolic reactions are constrained by the principle of energy balance (eqs 1, 41, and 42). This principle ensures that the rates of these reactions are consistent with the overall energy requirements and ATP production within the microbial system.

The two simplifications have important implications in model development and application. First, a major criticism towards using functional groups (or subgroups) as a basic unit of biology is the elusive relationship between functional groups *in silico* and microbes in natural environments. Natural microbes have been analyzed with both culture-dependent and independent approaches. Culture-dependent approaches, such as the most probable number (MPN) method, select microbes on the basis of nutrient requirement, and therefore provide a potential quantification of functional groups. However, the MPN method is strongly biased, and tends to underestimate cell abundances by orders of magnitude (Vester and Ingvorsen, 1998; Woomer, 1994). Culture-independent methods include the molecular biology tools based on marker genes or functional genes. While marker genes, such as 16S rRNA for prokaryotes, are highly conservative and thus provide a faithful description of microbial phylogeny, their link to microbial functional traits is not necessarily unique or conclusive, due to the widespread redundancy of metabolic functions in microbial communities (Louca et al., 2018b). On the other hand, the functional gene-based tools directly target at the metabolic potentials of microbial communities, and therefore have been applied to probe the functional diversity of microbial communities. However, the relationship between functional genes and microbial functional groups may not be unique or unequivocal (see 5.1. Functional gene-based model), and the quantitative relationship between cell counts and gene copy numbers (or the abundances of gene products) remains unclear (Louca et al., 2018a; Morton et al., 2019). Because of the disconnect between functional group and natural microbes, the biomass concentration of functional group should be treated as a pseudo-variable. The disconnect also questions the application of the trait-based framework to the simulation of the sizes of microbial populations. Moreover, trait-based models cannot be independently validated, and simulation results cannot be verified by experimentally analyzing the abundances of natural microbes.

Second, the separation of microbial biomass into two types, actively growing and dormant, is not sufficient to accurately reproduce the different phases of microbial growth (fig 3). Trait-based models track the size of actively growing biomass from the rate difference between biomass synthesis and decay. Depending on the relative rates of the two processes, growth is at the exponential, stationary, or death phase. Missing from the model outcome is the lag phase, a period before the onset of exponential growth where microbes adjust to new environmental conditions (Bertrand and Margolin, 2019). To simulate the lag phase, rate expressions must be amended. Nonetheless, due to their unstructured nature, trait-based models overlook the dramatic physiological and metabolic differences among the various growth phases (Bergkessel et al., 2016; Jaishankar and Srivastava, 2017). This simplified perspective on growth phases limits the applicability of trait-based models, especially in surface or near-surface natural systems, where microbial growth frequently shifts between different growth phases in response to temporal variations in nutrient supplies and other growth conditions.

Finally, model revisions are required to simulate co-metabolism, detoxification, and other metabolic processes of biogeochemical significance. Co-metabolism transforms chemical compounds without supporting microbial growth (Nzila, 2013). It takes place because many enzymes and cofactors are promiscuous. For example, cytochromes found in sulfate-reducing and iron-reducing microbes can reduce soluble hexavalent uranium U(VI) to insoluble tetravalent uranium U(IV), such as uraninite (Majumder and Wall, 2017). Methane-mono-oxygenase (MMO) from methanotrophs oxidizes not only its natural substate – methane – but also >300 different organic compounds (Nzila, 2013). Detoxification refers to microbial transformation of toxic chemicals to nontoxic or inert forms. Organic pollutants can be degraded to CO_2 and other harmless compounds. Metals and metalloids, such as mercury and arsenic, can be detoxified via redox reactions and methylation (Maguffin et al., 2015; Yan et al., 2019). Simulating these processes requires additional functions that relate process rates to the kinetics of catabolism, biomass synthesis, or maintenance.

6.2. Limited Knowledge of Microbial Kinetics

Biogeochemical modelers face a significant and often overlooked dilemma in their pursuit of accurately reproducing experimental observations or forecasting future biogeochemical changes. This dilemma stems from the inherent inadequacy of microbial kinetic theory in precisely describing microbial metabolic rates. Microbial rate laws, such as the Monod equation, only provide approximations, not accurate descriptions, of the relationship between microbial rates and their controlling factors. Microbial kinetics draws inspiration from chemical kinetics and adopted its basic tools, including rate laws. While rate laws have proven effective in describing abiotic chemical reactions and catalysts, their application to microbial metabolisms falls short due to the gap between microbial rate laws and metabolic mechanisms.

Numerous studies have highlighted the discrepancy between the predictions of microbial rate laws and the experimental observations of microbial kinetics (Panikov, 1995). These observations are not surprising – microbial rate laws appear too simplistic to fully capture the complexity inherent in microbial metabolism. Most microbial rate laws follow the same structure as rate laws for abiotic chemical reactions, characterized by single-term analytical expressions with a limited number of parameters. In comparison, microbes employ intricate networks consisting of tens to hundreds of enzymes to drive catabolism and biomass synthesis, while also regulating the expression and activities of these enzymes according to ambient environmental conditions.

The dilemma of microbial kinetic modeling has been addressed through the use of kinetic metabolic modeling. Jin et al. (2022) simulated the growth of *Methanosarcina barkeri* in laboratory bioreactors, specifically focusing on the limitation imposed by methanol. They found that the growth rate of *M. barkeri* is determined by two different enzymes at methanol concentrations near 0 and >10 mM, respectively. At intermediate concentrations, all enzymes in the methanogenesis network contribute to controlling the growth rate, albeit to varying extents. However, the Monod equation only incorporates two parameters, $\mu_{\max}$ and K_{M} (or the ratio of $\mu_{\max}$ to K_{M}). These parameters account for the rate-determining enzymes at very high and very low methanol concentrations. No additional parameter is available to account for the contribution from the remaining enzymes in the methanogenesis network. In essence, microbial rate laws do not sufficiently account for how microbial reaction rates are controlled by enzymes. For this reason, classical rate laws fail in accurately reproducing the relationship between microbial rates and various controlling factors. In the case of methanol methanogenesis, the application of the Monod equation leads to a relative error of up to 42% in rate predictions.

The gap between rate law and metabolism is also supported by the debate about which law, the multiplicative rate law or Liebig's law of the minimum (i.e., eq 30 or 31, respectively), provides a better description of microbial respiration. In practice, respiration rates have been widely predicted with the dual-Monod equation (eq 30). However, the two rate laws might reflect the different catalytic mechanisms of respiration reactions. Figure 13A shows a model of a hypothetical respiration reaction: an electron donor and acceptor are taken up from the environment into the cytoplasm, where the electron transfer between them is catalyzed by a redox enzyme. If the electron-transfer step is the rate determining step, respiration rate depends on the ambient concentrations of electron donors and acceptors at the same time. As a result, respiration rate follows the dual-Monod equation (Jin and Bethke, 2002). On the other hand, if the rate-determining step is the uptake of the electron donor or acceptor, respiration rate would follow Liebig's law of the minimum.

Likewise, we are still arguing about the applications of the multiplicative rate law and Liebig's law of the minimum to biomass synthesis (Bader, 1978; Droop, 1974; Egli, 2013; Zinn et al., 2004). The two rate laws ultimately converge to the Monod equation (eq 14) where microbial metabolism is limited by an energy source. Under this condition, biomass synthesis rate increases linearly with catabolic rate (eq 16), the slope of the increase corresponds to the maximum biomass yield per unit of energy substrate, and catabolism and biomass synthesis are considered as being tightly coupled (Russell and Cook, 1995).

Where biomass synthesis is limited not only by energy sources, but also by one or more element sources, the multiplicative rate law and Liebig's law of the minimum make contrasting predictions. For example, where the limitation by nitrogen (or phosphorus) sources is more pronounced than the limitation by energy substrates, Liebig's law of the minimum predicts that biomass synthesis rate is solely determined by the availability of nitrogen sources, and biomass synthesis becomes decoupled from catabolism. This prediction is consistent with laboratory observations that where ammonium or phosphate is limiting, the biomass synthesis by *M. barkeri* is decoupled from methanogenesis (Archer, 1985; Kenealy et al., 1982). In comparison, the multiplicative Monod equation predicts that biomass synthesis remains coupled to catabolism, but at a decreased efficiency – the slope of the increase in growth rate with catabolic rate is the product of the biomass yield per unit of energy substrate and the nutrient factor, i.e., $Y_{X/S} \cdot F_N$. Similar to respiration, biomass synthesis may follow the multiplicative rate law, Liebig's law of the minimum, or other rate expressions, depending on the configuration of metabolic networks and the position of rate-determining steps (fig 13B).

Perhaps, the worst case is the calculation of biomass maintenance rates. While biomass maintenance has long been recognized as a critical factor for slow microbial growth (Pirt, 1965), we are still arguing about the definition of maintenance metabolism and its physiological contributors (Hoehler and Jørgensen, 2013; Kempes et al., 2017; Lahtvee et al., 2014; Wang and Post, 2012). Furthermore, despite the mounting evidence on the variations of specific maintenance rate k_M with pH, temperature, growth rate, and other environmental and physiological factors (Biselli et al., 2020; Price and Sowers, 2004; van Bodegom, 2007), most of us continue to treat this parameter as a constant.

The incomplete understanding of microbial kinetics becomes more evident when considering the demand for microbial parameters in constructing trait-based models compared to their limited availability. Microbial kinetic parameters can be determined through laboratory incubation experiments (Kovárová-Kovar and Egli, 1998). By incubating laboratory cultures of known density, we can determine the maximum rate or uptake affinity from catabolic rates at relatively large or low nutrient concentrations, respectively. Determining the half-saturation constant may require incubations across a range of nutrient concentrations (Owens and Legan, 1987). Alternatively, multiple parameter values can also be obtained simultaneously by fitting microbial activity measurements, such as the concentrations of nutrients, metabolites, biomarkers, and biomass over time. This method is limited by parameter identifiability and uncertainty. In essence, only combinations of parameters, not individual parameters, can be uniquely estimated (Holmberg, 1982; Petersen et al., 2003). Moreover, the monitoring of chemical and microbial variables often occurs with different frequencies and levels of accuracy, further contributing to parameter uncertainty.

As of today, experimental analyses of microbial kinetics have largely focused on common laboratory cultures and the parameters related to their exponential growth, such as rate constants, half-saturation constants, and biomass yields. This emphasis on laboratory cultures is an inevitable outcome of the 'great plate count anomaly', which postulates that the number of bacterial cells that can be cultivated under laboratory conditions represents <1% of the number of bacterial cells in an environmental sample (Pande and Kost, 2017; Staley and Konopka, 1985). The preference for studying exponential growth parameters is largely due to the inconvenience associated with investigating slow or non-growing microbial populations. While parameters related to exponential growth define the competitive fitness of microbes, the parameters of biomass decay, such as those related to cell lysis and biomass maintenance, are essential in forecasting the occurrence and fate of microbes in natural environments. In practice, the assignment of microbial parameters with no or little experimental support is often done on an *ad hoc* basis. Although this practice may align simulation results with experimental observations or empirical expectations, it is analogous to driving a brand-new car with a leaking tire – reports can be drafted, but confidence in reaching the intended goal may be jeopardized. The disparity between rate laws and metabolism, coupled with the limited availability of microbial parameters, poses significant challenges in trait-based microbial modeling, from model construction and sensitivity analysis to deployment, result interpretation, and application. Advancements in experimental approaches and a more comprehensive understanding of microbial kinetics are crucial for overcoming these limitations and improving the applicability of trait-based microbial models.

6.3. Internal Consistency of Microbial Parameters

Modeling microbial metabolisms in natural environments requires a set of internally consistent values of trait parameters. Internal consistency means that all the parameter values of functional groups agree with each other according to the same set of physical, chemical, and biological principles. The requirement of internal consistency has been widely appreciated in building thermodynamic databases and geochemical modeling (Helgeson, 1978; Nordstrom et al., 2014). Applying inconsistent parameter datasets is akin to building a house on sand. Without internal consistency, microbial parameter datasets overlook the potential relationships between microbial parameters and the assumptions imbedded in microbial kinetic models, introduce errors in simulation results, and limit our confidence in model applications.

Microbial parameters measure the kinetic and stoichiometric traits of a functional group and reflect how microbes adapt and acclimate to the ambient environment under the constraints of physical, chemical, and biological principles. To align with the underlying mechanisms of microbial metabolisms, the values of these parameters should be obtained from microorganisms of same metabolic and regulatory pathways, similar culturing history, and with experiments under the same set of physiochemical conditions. However, rarely did a single experimental study determine a complete set of microbial parameters at the growth conditions of interest. Instead, trait parameters have been determined with different experimental setups (e.g., batch, fed-batch, and chemostat reactors), different growth media (e.g., chemically defined media made from chemical compounds of know composition and complex media prepared with organic compounds of unknown composition), and with microbes at different physiological states (i.e., resting vs growing) at different pH, temperature, and other physicochemical conditions. In addition, parameter values have also been obtained by fitting simulation results to experimental observations, and by applying *ad hoc* assumptions and estimating theoretically. By pooling parameter values from heterogenous sources, we introduce inconsistency into microbial parameter datasets.

Previous experimental studies have uncovered a series of relationships between microbial parameters, which provide a strong support for the requirement of internal consistency in microbial parameter sets. Some example relationships include:

- Rate constants correlate positively with half-saturation constants (Litchman et al., 2015). Alternatively, rate constants correlate negatively with substrate affinities, the ratios of rate constants to the half-saturation constants. These relationships have been accounted for by physiological acclimation to different nutrient concentrations.
- Where more energy is available in the environment, more energy is conserved as ATP, and more biomass is synthesized (Jin, 2012). The increase in ATP yield with increasing available

energy is supported by the ATP yields of aerobic respiration, microbial ferric mineral reduction, sulfate reduction, and methanogenesis. In addition, the pathways of biomass synthesis in different microbes shares similar efficiency, i.e., similar biomass yields per ATP (Stouthamer and Van Verseveld, 1985). Therefore, where more ATPs are synthesized, more biomass is produced.

- Specific maintenance rate k_M increases with increasing specific growth rate μ. Experimental observations support that specific maintenance rate is not a constant, but varies with specific rate of biomass synthesis (Neijssel and Tempest, 1976). These observations are further supported by the positive correlations between specific maintenance rate and maximum specific growth rate μ_{max} (van Bodegom, 2007).

On the basis of the common practice in geochemical modeling, a quality microbial parameter set should reflect the current state of knowledge, and account for all available information about the metabolism of interest. Parameter values in such a dataset should be internally consistent, and accurate over relevant physiochemical conditions. To meet these requirements, the following quality criteria should be considered in building microbial parameter sets:

- All the data of a functional group is derived from clearly defined reference conditions, including pH, temperature, pressure, ionic strength, nutrient concentrations, and with a single group of physiological constants. These physiological constants include cell size and shape, biomass chemical formula and molecular weight, the phosphorylation potential, the weight percentage of protein in cell dry weight, and cell dry weight per wet weight.
- To determine the value of a parameter, all relevant original experimental results and knowledge-based information are compiled and considered simultaneously. Conflicts and inconsistencies are documented and resolved.
- Appropriate mathematical functions (e.g., section 4.2) are applied to reflect microbial acclimation to ambient environment and to relate the data at the reference state to the state of different physicochemical conditions.
- All the data is compatible with basic principles of metabolism and physiology.
- Any inconsistency should be documented and minimized to the extent possible.

These criteria help ensure the reliability and accuracy of parameter values and represent a first step towards building internally-consistent dataset of microbial kinetics. They should be adopted in constructing microbial kinetic models, a critical step towards improving the environmental applications of microbial kinetic modeling.

7. CONCLUDING COMMENTS

Trait-based microbial reaction modeling simulates the kinetics of chemical reactions catalyzed by microbial metabolisms by treating microbes as autocatalysts. It builds on the modeling framework for abiotic reacting mixtures (Bebernes and Eberly, 2013; Bethke, 2022; Higham, 2008), and adopts two assumptions on microbial communities and metabolisms, including simplifying microbial communities as ensembles of functional groups (or subgroups) and describing metabolisms at the coarse-grained level with three metabolic reactions – catabolic reaction, biomass synthesis, and maintenance. Trait-based kinetic models are mechanistic in that they implicitly link the three metabolic reactions via ATP production and consumption (eqs 1, 41, and 42), and explicitly account for chemical fluxes driven by both catabolism and biomass synthesis. Trait-based models use microbial functional groups as the basic units of microorganisms, and hence are unstructured and unsegregated – they neglect the internal state of microbial cells and assume that cells within a functional group are the same in terms of their metabolism and functional traits. The focus on the three metabolic reactions balances the complexity inherent to microbial metabolism and the efficacy in capturing essential metabolic features. The application of functional groups is supported by the consensus that functional diversity, not phylogenetic or taxonomic diversity, serves as a mechanistic link between microbes and biogeochemical processes (Crowther et al., 2019; McGill et al., 2006). However, biomass concentrations of functional groups should be treated as *pseudo-variables* because of the gap between functional groups in trait-based models and microbial communities in the environment. Due to metabolic diversity of individual microbes and metabolic redundancy in most microbial communities, microbial groups defined solely with metabolic reactions may not be directly linked to microbial cells in natural environments. This gap has significantly hindered the validation and application of microbial kinetic models. Potential improvements, such as replacing functional groups with functional genes or gene products, have appeared from the recent application of molecular biology tools (Louca et al., 2016; Reed et al., 2014).

Building trait-based models requires stoichiometric equations of metabolic reactions. These equations are relatively straightforward to construct for catabolic reactions, provided that energy sources and reaction products have been determined. Writing the equations for biomass synthesis can be challenging, especially where neither nutrient nor the elemental composition of biomass has been analyzed. In these situations, current solutions include the application of generical chemical formulars for biomass and the assumption that microbes use common carbon and nitrogen sources in the environment of interest. Recently, the stoichiometric equation of biomass synthesis has also been solved with genome-scale metabolic models. Example applications show that in addition to improve the accuracy of reaction stoichiometry, genome-scale metabolic modeling also expands the capabilities of trait-based models by uncovering essential nutrients and unconventional metabolic products (Wu et al., 2022).

Building trait-based models for environmental applications requires the account of environmental conditions in microbial rate laws. Most microbial rate laws were originally developed for laboratory applications and fall into three types, depending on metabolic mechanisms. The first type is the rate laws for cell-controlled metabolic reactions that directly consume nutrients dissolved in solution, and the most popular example is the Monod equation (Monod, 1942, 1949). The second type are the rate laws, such as the Contois equation (Bhattacharya and Khai, 1987), for surface-controlled metabolic reactions, including microbial oxidation of particulate organic matter and reduction of ferric minerals. The Best equation represents the third type that applies to mass transfer-controlled metabolic reactions, where metabolic reactions require the dissolution of solids or NAPLs (Bosma et al., 1997). While the Monod equation treats nutrient concentrations as the sole determinant of specific growth rates, the Contois equation and the Best equation relate specific growth rates to the concentration ratios of nutrient to biomass. Despite the many rate laws currently available for laboratory cultures, our knowledge of microbial kinetics still remains far from complete – these rate laws only provide approximations, not accurate descriptions of microbial reaction rates (Jin et al., 2022). In addition, where more than one nutrient limits microbial metabolism, which rate law, the multiplicate law, Liebig’s law of the minimum, or some other expressions, should we apply still remains an open question.

Applying trait-based modeling frameworks developed for laboratory cultures to natural environments requires modifications that consider both microbial physiology and growth conditions in the environment. Growth conditions, such as temperature, pH, and water activity, are accommodated by amending microbial rate laws with dimensionless factors. Modifications related to microbial physiology involves subdividing functional groups into subgroups to encompass actively growing and dormant cells, and explicitly considering biomass maintenance, cell death and lysis, and predation to simulate the chemical fluxes of biomass decay. Additionally, optimization-based models of physiological acclimation have been built to extrapolate microbial parameters determined in the lab to natural systems. Acclimation models directly address the challenges that the trait parameters of laboratory cultures are not directly applicable to natural environments, and have been developed for nutrient uptake, catabolism, and thermodynamic efficiency of microbial metabolism (Smith et al., 2011; Wu et al., 2022). While the significance of growth conditions has gained widespread recognition, the potential of accounting for microbial physiology is yet to be fully appreciated by the modeling community.

Building quality trait-based models also demands internal consistency in microbial parameter sets. The requirement of internal consistency stems from the plasticity and interdependence of microbial parameters: most microbial parameters do not have fixed values, but co-vary with environmental conditions (Ferenci, 1999; Grady et al., 1996; Kovárová-Kovar and Egli, 1998). As a first step towards building parameter dataset of internal consistency, we put forward a series of guidelines, including the rigorous standards for deriving microbial parameters from laboratory observations and the parameter accuracy across the environmental conditions of interest. By focusing on the modeling frameworks and model assumptions, the limitations of microbial rate laws, and the necessity of internal consistency in microbial parameter sets, we hope to make clear the capabilities and limitations of trait-based microbial kinetic modeling frameworks, and to build and apply trait-based microbial reaction models in a transparent, objective, and reproducible manner.

ACKNOWLEDGMENTS

This research was funded by the National Science Foundation under Award EAR-1636815 and 1753436 and by the National Aeronautics and Space Administration under Grant NNX16AJ59G. Comments from two reviewers improved the quality of this manuscript.

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Table 1. Example models of microbial kinetics and their assumptions.

Model	Equation	Assumption	Reference
<u>Microbial metabolism</u>			
Monod equation	14, 15, 17	Microbial rate varies hyperbolically with the concentration of a limiting nutrient.	Monod (1942, 1949)
Contois equation	14, 20, 21	Microbial rate varies hyperbolically with the concentration ratio of nutrient to biomass.	Contois (1959)
Best equation	14, 24	Microbial consumption of a solid nutrient requires an initial step of nutrient dissolution.	Best (1955)
Multiplicative rate law	30, 32	Microbial rate is controlled by multiple nutrients.	Humphrey (1974); MeGee et al. (1972)
Liebig's law of the minimum	31, 33	Microbial rate is determined by the most limiting nutrient.	Ryder and Sinclair (1972); Williamson and McCarty (1976)
Non-competitive inhibition model $^{(a)}$	34	Chemical inhibition is analogous to non-competitive enzyme inhibition.	Boon and Laudelout (1962)
Thermodynamic factor	35, 36	Microbial reactions honor the consistency between thermodynamics and kinetics.	Jin and Bethke (2003); (2005)
Biomass capacity factor	47	Growth rate per capita decreases as biomass approaches a maximum imposed by limited resources in the environment.	Wu et al. (2022)
Cardinal response model	43, 44	Microbial rates respond to the changes in temperature, pH, and water activity by following bell-shaped curves.	Rosso et al. (1995); Rosso et al. (1993)

Biomass decay model	45	Biomass decay rate increases linearly with biomass concentration and the slope of the increase varies with temperature according to the Arrhenius equation.	Herbert (1958); Pirt (1965); Tijhuis et al. (1993)
Physiological acclimation			
Optimal nutrient uptake model	48 to 50	Microbes trade-off nutrient affinity against maximum uptake flux to maximize the flux of nutrient uptake.	Smith et al. (2015)
Optimal catabolic rate model	51 to 53	Microbes trade-off nutrient affinity against maximum catabolic rate to maximize the rate of catabolic reaction.	Wu et al. (2022)
Optimal metabolic efficiency model	54, 55	Microbes trade-off the rate and ATP yield of catabolic reaction to maximize the rate of ATP production.	Wu et al. (2022)
Molecular biology-enabled improvement			
Functional gene-based model	56	Microbial reaction rates are proportional to the abundances of related functional genes.	Louca et al. (2016); Reed et al. (2014)
Genome-scale stoichiometric metabolic model	57	The fluxes through a metabolic network are determined by the principle of mass balance.	Orth et al. (2010)
Pathway-specific kinetic metabolic model	57, 59	The fluxes through a metabolic network are determined by the principle of mass balance and the rate laws of biochemical reactions.	Jin et al. (2022); King et al. (2009)

Note: (a) For other inhibition models, see Mulchandani and Luong (1989).

Table 2. Metabolic reactions of different mechanisms require different rate laws.

Reaction type	Example	Rate law	Reference
Cell-controlled	Oxidation of acetate and H_2; reduction of O_2, nitrate, and sulfate.	Monod equation (eqs 14 and 15)	Monod (1942, 1949)
Surface-controlled	Organic matter fermentation; ferric mineral reduction	Contois equation (eqs 14, 20, and 21)	Chen and Hashimoto (1980); Hacherl et al. (2003); Roden (2006)
Mass transfer-controlled	Degradation of polycyclic aromatic hydrocarbons	Best equation (eqs 14 and 24)	Volkering et al. (1992); Wick et al. (2001)

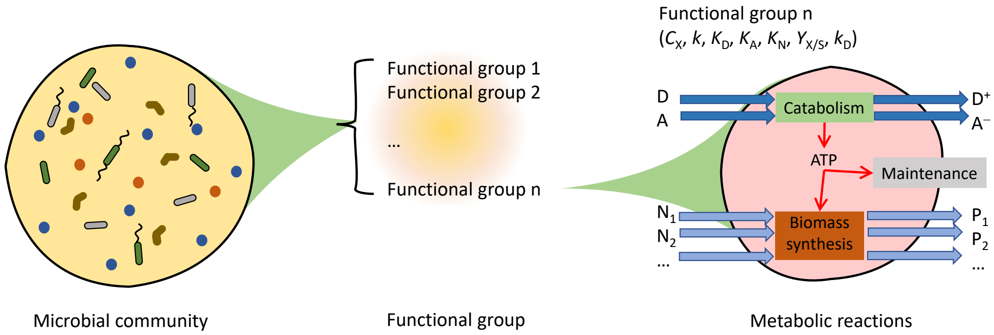
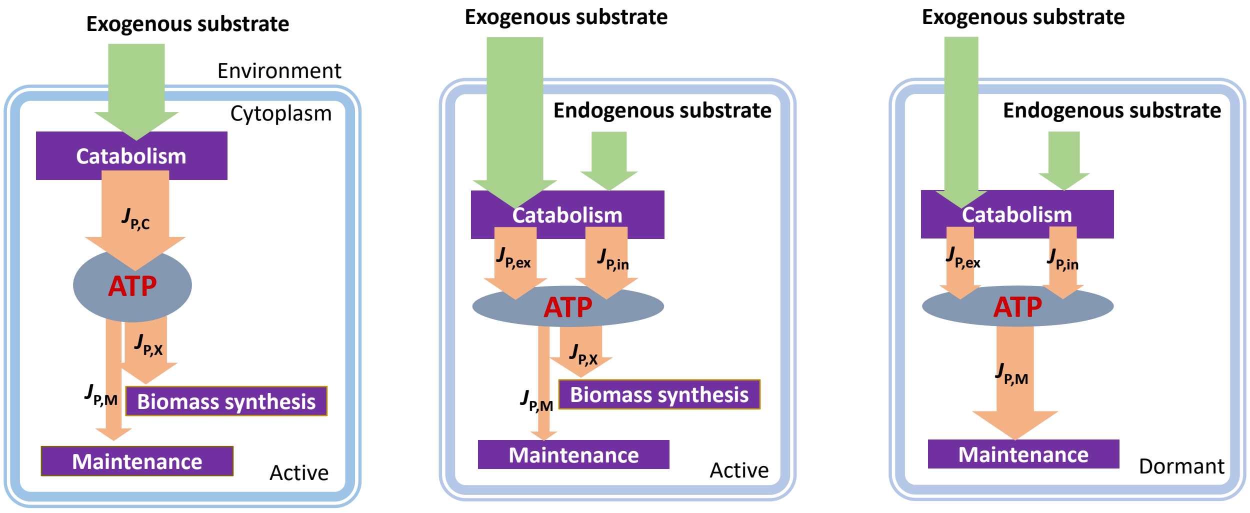
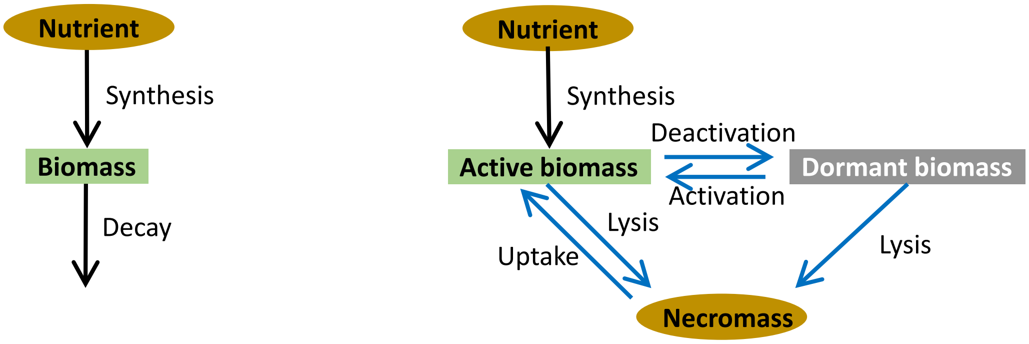


Figure 1. Microbial kinetic models describe the metabolisms of a microbial community with two assumptions. One assumption treats the community as an ensemble of different microbial groups whose functional traits have different values; the other treats the metabolism of a functional group at a coarse-grained level with three metabolic reactions, catabolic reaction, biomass synthesis, and maintenance. Each functional group is defined with a set of kinetic and stoichiometric parameters, including the rate constant (k), the half-saturation constants for electron donors (K_D), acceptors (K_A), and nutrients (K_N), the biomass yield ($Y_{X/S}$), and the specific decay constant (k_D), and measured with biomass concentration (C_X). Catabolic reaction makes ATP by catalyzing the redox reaction between electron donor D and acceptor A, and by producing oxidized electron donor D$^+$ and reduced electron acceptor A$^-$. Biomass synthesis reaction consumes ATPs to produce new biomass and metabolic products (P_1, P_2, …) from nutrients (N_1, N_2, …) in the environment.



(A) (B) (C)

Figure 2. Microbial kinetic models describe metabolism with three metabolic reactions, catabolic reaction, biomass synthesis, and maintenance. Models for laboratory applications focus on the catabolic reaction that uses exogenous energy substrates, and the ATP fluxes driven by the three reactions are balanced (eq 1, A). Models for environmental applications consider two distinct energy sources – exogenous and endogenous, and two types of microbial cells – active growing (B) and dormant (C); the ATP fluxes for actively growing (B) and dormant microbes (C) are governed by equation 41 and 42, respectively.



(A)

(B)

Figure 3. Microbial kinetic models use biomass concentration as a state variable to track the sizes of microbial functional groups. Laboratory-focused models consider the balance between biomass synthesis from essential nutrients (or element sources) in the environment and biomass decay (A), while models for environmental application differentiate actively-growing biomass from dormant biomass (B). The two types of biomass transit between each other via activation and deactivation, and both types undergo lysis to form necromass. Necromass, a component of natural organic matter, serves as a nutrients source, contributing to cryptic growth.

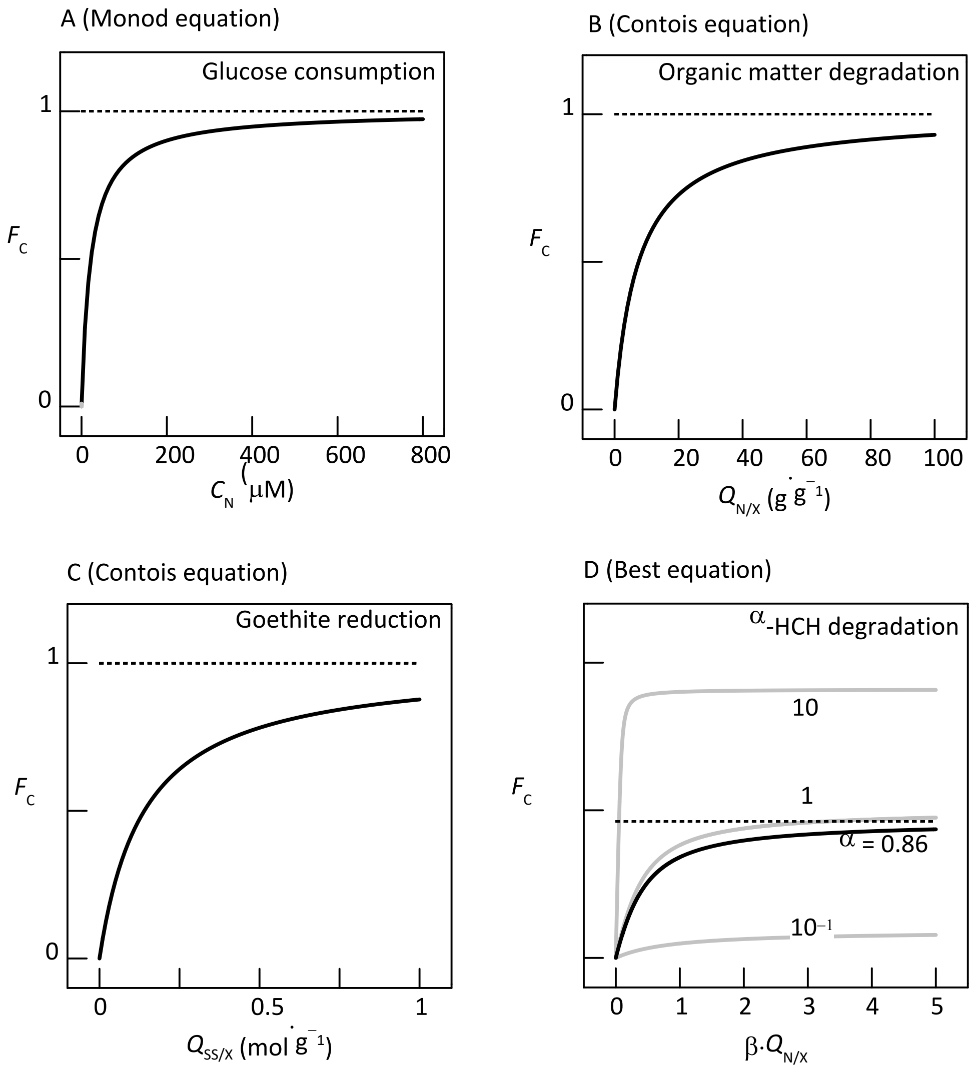
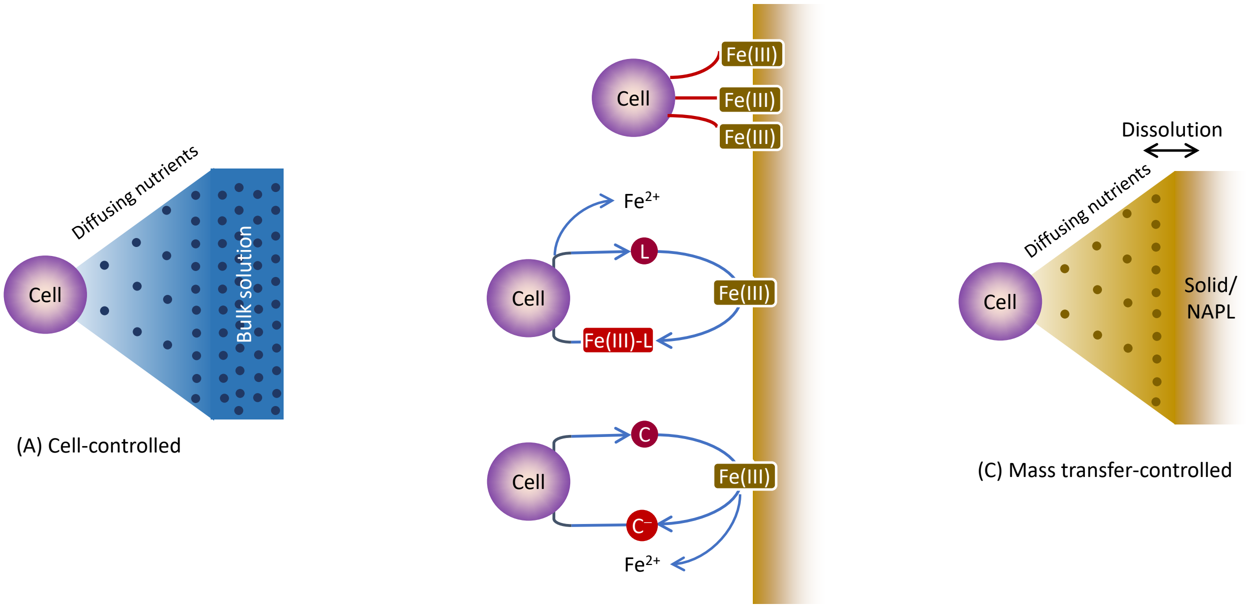


Figure 4. Concentration factors F_C in the Monod equation (A), the Contois equation (B and C) and the Best equation (D) quantify the effect of limiting nutrients on cell-, surface-, and mass transfer-controlled metabolic reactions, respectively. The concentration factor of cell-controlled reaction (A) is calculated at different nutrient concentrations C_N according to equation 15 for the growth of *Escherichia coli* on glucose, by taking the half-saturation constant K_M of glucose at 22 μM (Monod, 1949). The concentration factor for surface-controlled metabolic reaction is calculated according to equation 21 by taking the biomass-specific half-saturation constant of organic matter at 7.5 g·g^{-1} (B) (Hemsi et al., 2010) and the biomass-specific half-saturation constant of bioavailable surface sites at 0.14 mol·g^{-1} (C) for microbial reduction of nano-crystalline goethite (Jin and Roden, 2011); $Q_{N/X}$ is the concentration ratio of organic matter to biomass; $Q_{SS/X}$ is the concentration ratio of bioavailable surface site to biomass; to calculate the abundance of surface sites from goethite concentrations, one mole nano-crystalline goethite is assumed to have 5.3×10^{-3} mol surface site. The concentration factor for mass transfer-controlled metabolic reaction (D) is calculated at different parameter β-weighted concentration ratios for microbial degradation of *α*-hexachlorocyclohexane (*α*-HCH, dark line with parameter *α* of 0.86) according to equation 24; grey lines are the concentration factors for *α* values of 0.1, 1, and 10. Dashed lines in panel A to D represent the maximum values of the concentration factors ($F_{C,max}$)



(A) Cell-controlled

(B) Surface-controlled

(C) Mass transfer-controlled

Figure 5. Metabolic reactions separate into three types, cell-, surface-, and mass transfer-controlled reactions. (A) Cell-controlled metabolic reactions consume nutrients dissolved in solution, and include nutrient diffusion from the bulk solution to the cell surface and the uptake and consumption of dissolved nutrients by microbial cells. (B) Surface-controlled reactions, such as microbial reduction of ferric iron minerals, are metabolic reactions that interact with the surface of solids via direct contact, ligands (L), and electron carriers (Shi et al., 2016; Weber et al., 2006); rectangles labelled with Fe(III) represent the bioavailable reactive sites on the surface of minerals, Fe(III)-L is an aqueous ferric iron and ligand complex species, and C and C^- are the oxidized and reduced forms of electron carriers, respectively. (C) Mass transfer-controlled reactions are metabolic reactions that include the dissolution of solids or nonaqueous phase liquids (NAPLs), nutrient diffusion from the bulk solution to the cell surface, and the uptake and consumption of dissolved nutrients by microbial cells.

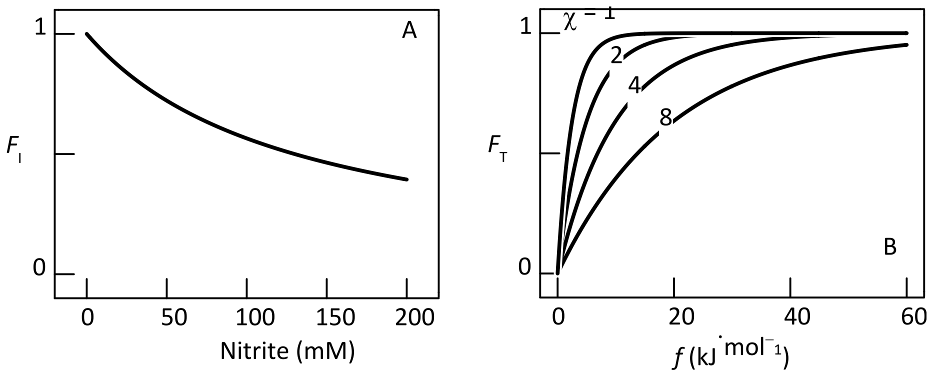


Figure 6. Inhibition factor F_I (A) and thermodynamic potential factor F_T (B) quantify the rate limitation by inhibiting compound and thermodynamic drive f, respectively. Inhibition factor (eq 34) is calculated for the inhibition of nitrite on aerobic nitrite oxidation by *Nitrobacter winogradskyi* with an inhibition constant of 130 mM (Boon and Laudelout, 1962). The thermodynamic potential factor is calculated according to equation 35 with the average stoichiometric number χ of 1, 2, 4, and 8.

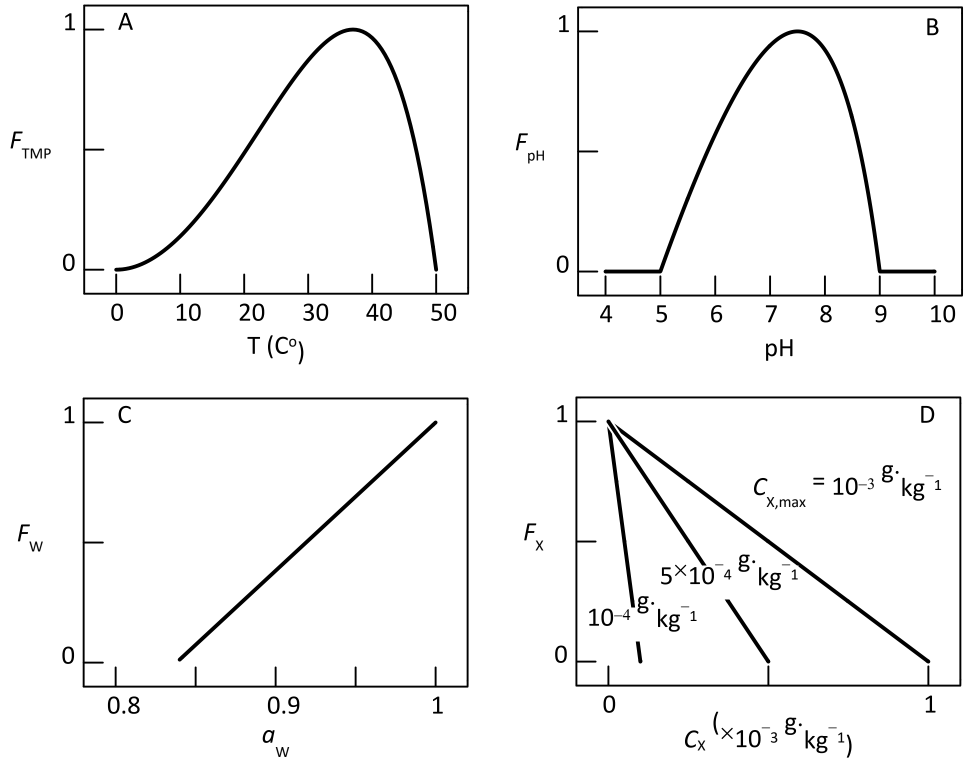


Figure 7. Dimensionless factors account for the kinetic effects of temperature T (F_{TMP}, A), pH (F_{pH}, B), water activity a_{W} (F_{W}, C), and biomass concentration C_{X} (F_{X}, D). Temperature factor F_{TMP} is calculated according to equation 43 and by taking mesophilic microbes as an example with their minimum, optimal, and maximum temperature at 0, 37 and 50 ºC, respectively. pH factor F_{pH} is calculated according to equation 44 and by taking neutrophilic microbes as an example with their minimum, optimal, and maximum pH at 5, 7.5, and 9, respectively (Jin and Kirk, 2018). Water activity factor F_{W} is calculated according to equation 46 and by taking the minimum water activity at 0.84 for *Staphylococcus xylosus* (McMeekin et al., 1987). Biomass factor F_{X} is calculated according to equation 47 with different maximum biomass concentrations $C_{\mathrm{X,max}}$.

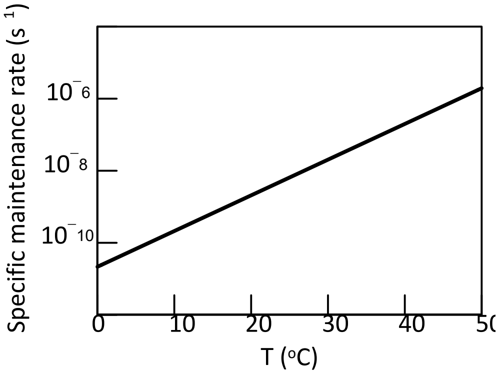


Figure 8. Variations with temperature T in specific maintenance rate. Line is calculated according to equation 45 by taking A_M at 2.5×10^{10} s^{-1} and $E_{a,M}$ at 1.02×10^5 $J\cdot mol^{-1}$ (Wu et al., 2021).

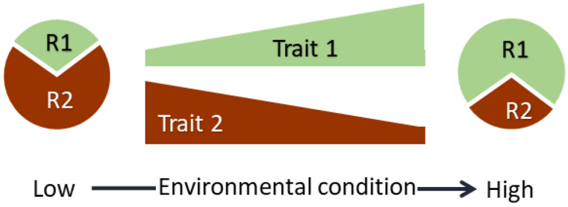


Figure 9. Optimization-based models of physiological acclimation relate microbial parameters to environmental conditions via cellular resource allocation (Wu et al., 2022). These models assume that growth rates are determined by two functional traits (trait 1 and 2) and trait values depend linearly on the cellular resources allocated to them (R1 and R2, respectively). Microbes maximize their growth rate by adjusting the partition of the limited resources between the two traits in accordance with environmental conditions, such as nutrient concentration or available energy, leading to the trait-off between the two traits.

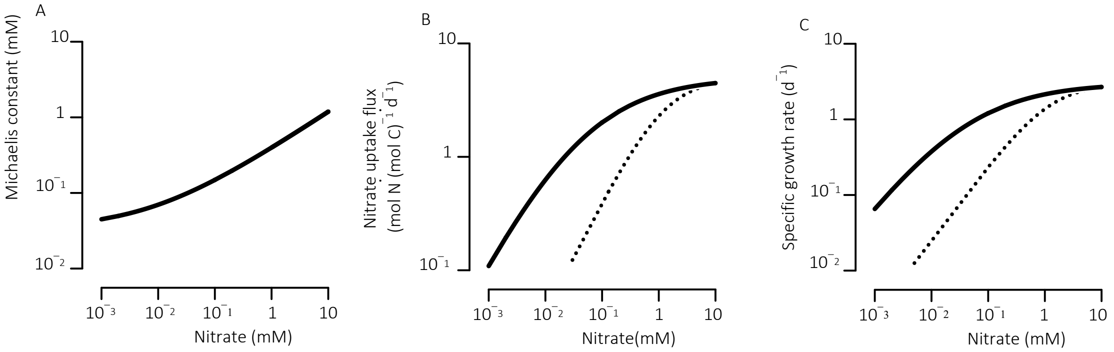


Figure 10. The model of optimal nutrient uptake accounts for the acclimation of phytoplankton to ambient nitrate concentrations by relating the Michaelis constant of nitrate uptake to nitrate concentrations in the environment. (A) The Michaelis constant is calculated according to equation 48. (B) Nitrate uptake flux is calculated according to equation 49. (C) Specific growth rate is calculated according to equation 50. In the calculations, the potential maximum nitrate uptake flux is set at 5.0 mol·(mol C)$^{-1}$·d^{-1}, the potential maximum nitrate affinity at 0.15 m^3·(mmol C)$^{-1}$·d^{-1}, the biomass yield $Y_{X/N}$ at 0.6 mol C·(mol N)$^{-1}$, and the specific decay constant at 0 (Smith et al., 2015). Dotted lines are calculated with the half-saturation constant at 10 mM, without accounting for acclimation.

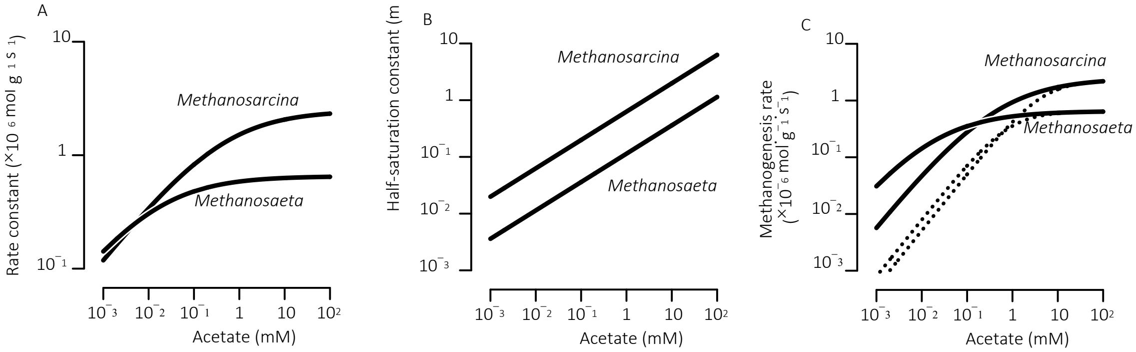


Figure 11. The model of maximum catabolic flux accounts for the acclimation of acetoclastic methanogens, *Methanosarcina* and *Methanosaeta*, by relating their rate constant and half-saturation constant to ambient acetate concentrations (Wu et al., 2022). The rate constant (A) and the half-saturation constant (B) are calculated according to equation 53 and 51, respectively; the specific rate of methanogenesis (C) is calculated according to equation 17. In these calculations, the acetate concentration used for routine laboratory culturing is 50 mM, the rate constants of laboratory *Methanosarcina* and *Methanosaeta* are at 2.3×10^{-6} and 6.4×10^{-7} $\mathrm{mol\cdot g^{-1}\cdot s^{-1}}$, respectively; the half-saturation constants of the two methanogens are at 4.44 and 0.81 mM, respectively (Wu et al., 2022, their supplementary table 1). Dotted lines in panel C are calculated according to equation 17, without accounting for acclimation.

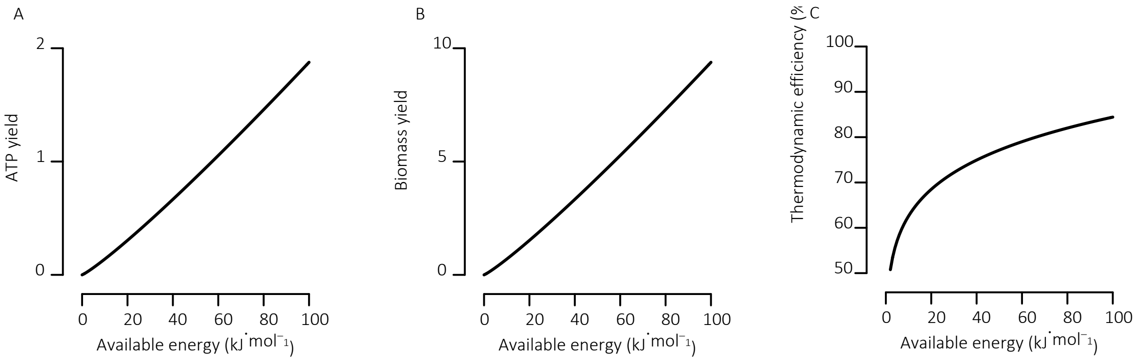


Figure 12. The model of maximum ATP flux accounts for microbial acclimation to thermodynamic conditions of the environment by relating ATP yield (A), and therefore biomass yield (B) and thermodynamic efficiency (C), to the energy available in the environment (Wu et al., 2022). The ATP yield and biomass yield are calculated according to equation 54 and 55, respectively. The thermodynamic efficiency is calculated as the ratio of the energy saved as ATP to the energy available in the environment; the saved energy is the product of the ATP yield and the phosphorylation energy – the energy consumed by ATP synthesis from ADP and phosphate in the cytoplasm and its value is about 45 kJ·(mol ATP)$^{-1}$ (Jin, 2012).

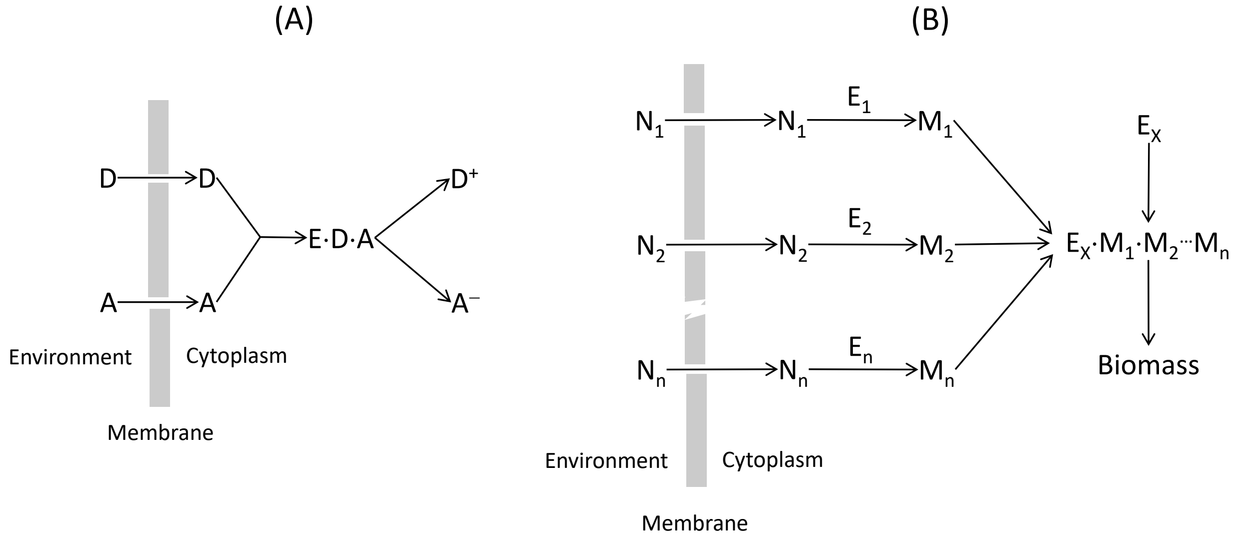


Figure 13. The dual-Monod equation and Liebig's law of the minimum reflect the different rate-determining steps of microbial respiration (A) and biomass synthesis (B). In a hypothetical case of respiration, electron donors (D) and acceptors (A) are first transported from the environment to the cytoplasm, where they combine with enzymes to form enzyme-substrate complexes (E·D·A) and react to oxidized electron donors (D^+) and reduced electron acceptors (A^-). If the uptake of electron donors or acceptors is the rate-determining step, respiration rates follow Liebig's law of the minimum; if the rate-determining step is the formation of enzyme-substrate complexes or the electron transfer between electron donors and acceptors, respiration rates follow the dual-Monod equation. In a hypothetical case of biomass synthesis, nutrients (N_1, N_2, …, N_n) are first transported from the environment to the cytoplasm, where they are converted by enzymes (E_1, E_2, …, E_n) to metabolites (M_1, M_2, …, M_n). The metabolites then combine with another enzyme (E_X) to form biomass. If nutrient uptake or metabolite formation is the rate-determining step, biomass synthesis rates follow Liebig's law of the minimum; if the rate-determining step is the assemblage of metabolites to biomass, biomass synthesis rates follow the multiplicative rate law.