Annual Review of Marine Science

Metabolic Flux Modeling in Marine Ecosystems

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Annu. Rev. Mar. Sci. 2025. 17:593–620

First published as a Review in Advance on September 11, 2024

The *Annual Review of Marine Science* is online at marine.annualreviews.org

https://doi.org/10.1146/annurev-marine-032123-033718

Keywords

metabolic network, flux balance analysis, stoichiometric modeling, microbial communities, marine ecosystems, systems biology

Abstract

Ocean metabolism constitutes a complex, multiscale ensemble of biochemical reaction networks harbored within and between the boundaries of a myriad of organisms. Gaining a quantitative understanding of how these networks operate requires mathematical tools capable of solving in silico the resource allocation problem each cell faces in real life. Toward this goal, stoichiometric modeling of metabolism, such as flux balance analysis, has emerged as a powerful computational tool for unraveling the intricacies of metabolic processes in microbes, microbial communities, and multicellular organisms. Here, we provide an overview of this approach and its applications, future prospects, and practical considerations in the context of marine sciences. We explore how flux balance analysis has been employed to study marine organisms, help elucidate nutrient cycling, and predict metabolic capabilities within diverse marine environments, and highlight future prospects for this field in advancing our knowledge of marine ecosystems and their sustainability.

TOWARD MECHANISTIC PREDICTIONS OF METABOLIC FLUXES IN MARINE ENVIRONMENTS

Metabolic flux: the rate of a biochemical reaction, in units of millimoles of metabolite per gram of dry biomass per hour

Metabolic fluxes describe the transformation of molecules along reactions and pathways. They capture the dynamic nature of living systems and the flow of energy and nutrients necessary to maintain life at all scales (Falkowski et al. 2008, Goldenfeld & Woese 2011, Morowitz 1979, Nielsen 2003). In marine science, metabolic fluxes are of interest in a number of contexts, ranging from metabolic engineering to biogeochemical cycles (**Figure 1**).

Metabolism is typically considered a cellular or organismal phenomenon, as every organism needs to harness energy and produce building blocks (Inomura et al. 2020, Maarleveld et al. 2013, Nielsen 2003). Yet the exchange of metabolites across cells and organisms connects individual metabolic networks with each other (Du et al. 2022, Giannari et al. 2021, Goldford et al. 2018, Lipsman et al. 2024, Nadell et al. 2016, Pacheco et al. 2019, Roth-Rosenberg et al. 2020, van Hoek & Merks 2017). For example, when microbes exchange or compete for metabolites, metabolic fluxes orchestrate ecological interactions that shape community dynamics and functions (Harcombe et al. 2014, Khandelwal et al. 2013), with potential implications at the planetary scale (e.g., biogeochemical cycles).

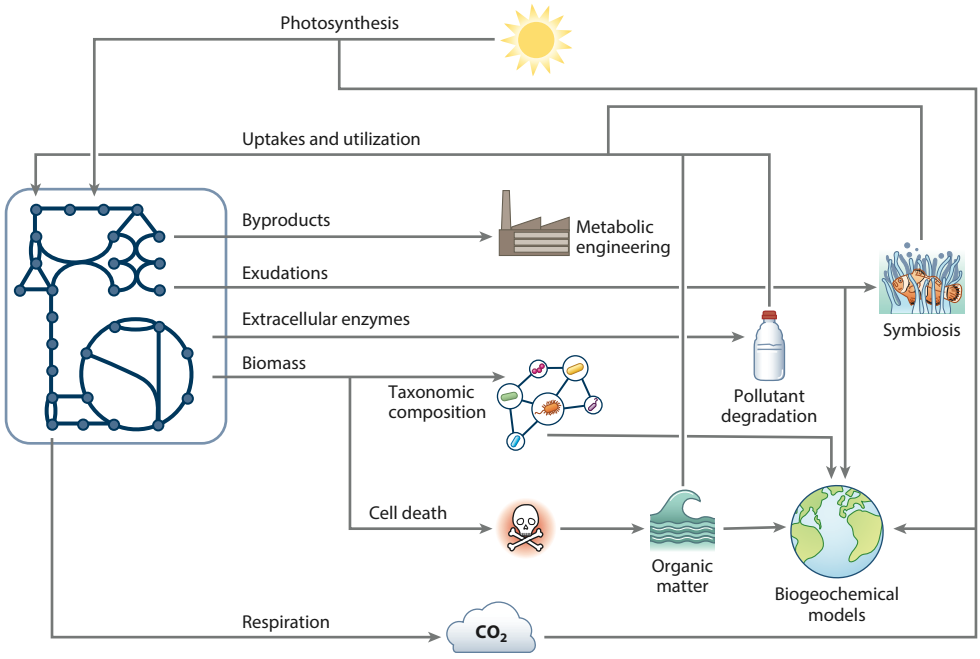


Figure 1

Schematic visualization depicting how the study of cellular metabolic fluxes can help address different questions in ocean science. The generic cell shown here (which could represent an autotrophic or heterotrophic organism) contains a metabolic network, whose intracellular fluxes are coupled with several exchange fluxes that are relevant for different applications. Fluxes of CO_2 production (respiration) or absorption (photosynthesis) can have important consequences for atmospheric carbon balance. The flux representing biomass production (i.e., growth) can be used to understand community (e.g., microbiome) assembly and dynamics. Exudation and cell death release metabolites that can affect other organisms and mediate symbiosis. Extracellular enzymes can degrade macromolecules, including plastic. Modified metabolic networks can lead to organisms with increased fluxes for the production of specific byproducts, which have applications in metabolic engineering.

Given the complexity of metabolic networks and the multiscale nature of metabolism, addressing questions about marine metabolism requires mathematical representations and computational models. Data-driven approaches, including artificial intelligence (AI) and machine learning (ML), could be used to generate predictions of metabolic phenotypes (Ma et al. 2018, Zhang et al. 2020). However, mechanistic models are still uniquely suited to provide a fundamental understanding of metabolic processes and their causal relationships. For example, systems of ordinary differential equations based on chemical kinetics have been used to model specific pathways (Kotte et al. 2010) or even whole cells (Agmon et al. 2022, Karr et al. 2012). These models generally require many empirically derived kinetic parameters. Alternative approaches limit the number of parameters needed by coarse-graining biological processes, e.g., by focusing on macromolecular pools (Inomura et al. 2020); representing organisms in terms of their input–output relationship, as in consumer–resource models (Marsland et al. 2020); or capturing multiple complex biological processes in a reduced number of variables and parameters, as in biogeochemical models (Follows et al. 2007, Kim et al. 2023). An alternative way of limiting parameters without sacrificing completeness is to make simplifying assumptions about intracellular metabolic dynamics and focus entirely on predicting fluxes based on the stoichiometry of the metabolic reactions. This approach is at the core of flux balance analysis (FBA), which we use here as an umbrella term equivalent to constraint-based modeling, constraint-based reconstruction and analysis (COBRA), stoichiometric modeling, or genome-scale modeling. Many of these modeling techniques can, in principle, be applied to populations of cells or to individual cells, as in agent- or individual-based models (Bauer et al. 2017, Borer et al. 2019, Hellweger et al. 2016).

FBA was initially developed for metabolic engineering (Papoutsakis 1984, Varma et al. 1993) and has since been used in multiple fields, including evolutionary biology (Harcombe et al. 2013, Ibarra et al. 2002, Papp et al. 2004), ecology (Henry et al. 2016, Klitgord & Segrè 2010a, Zomorrodi & Maranas 2012), and synthetic biology (Wang et al. 2017). FBA modeling requires two distinct but synergistic tasks: generating a formal representation of the metabolic network for the organism (metabolic network reconstruction) and predicting the fluxes through the metabolic network under certain conditions (phenotype prediction). In this article, we review FBA and its previous applications to ocean science, and we highlight current limitations and promising future directions. Note that in addition to marine organisms, we occasionally refer to freshwater phytoplankton as valuable examples.

Flux balance analysis (FBA): an algorithm for predicting fluxes of a metabolic network at steady state

Genome-scale metabolic model (GEM): a mathematical representation of known metabolic reactions for an organism, including their stoichiometries and genes

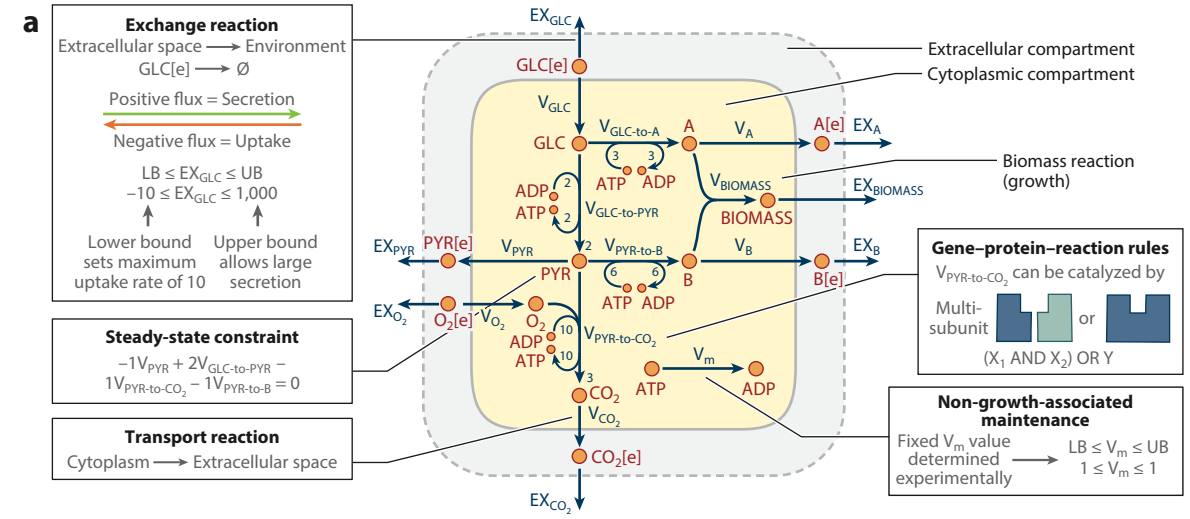
Stoichiometric matrix: a matrix whose element (i,j) encodes the stoichiometric coefficient of metabolite i in reaction j

GENOME-SCALE METABOLIC MODELS ARE FORMAL REPRESENTATIONS OF ORGANISMAL METABOLISM

Metabolic network reconstruction is the process of creating a formal representation of metabolism for the goal of computationally generating phenotype predictions (Thiele & Palsson 2010). When this process is completed for the entire known metabolic network of an organism, the reconstruction results in a genome-scale metabolic model (GEM).

The list of metabolic reactions to be included in a GEM is derived from the set of genes annotated as metabolic enzymes in the organism's genome (Aziz et al. 2008, Feist et al. 2009, Jing et al. 2014, Labena et al. 2018, Seemann 2014, Wang et al. 2017). Using standard nomenclature (e.g., Enzyme Commission numbers; McDonald et al. 2009), databases such as the Kyoto Encyclopedia of Genes and Genomes (Kanehisa et al. 2016), MetaCyc (Caspi et al. 2020), BiGG (Schellenberger et al. 2010), and ModelSEED (Arkin et al. 2018, Seaver et al. 2021) can help extract the detailed stoichiometry for each reaction to form the stoichiometric matrix (**Figure 2*c***).

The relationship between genes and reactions can be quite complex. A given enzyme may catalyze distinct reactions (multifunctional enzyme) (Bekiaris & Klamt 2020), while a given



b

Maximize $Z = \mathbf{c}^T\mathbf{v}$
Subject to $\mathbf{Sv} = 0$
And $LB_i \le \mathbf{v}_i \le UB_i$

c

	EX_{GLC}	EX_{PYR}	EX_{O_2}	EX_{CO_2}	EX_A	EX_B	$EX_{BIOMASS}$	V_{GLC}	V_{PYR}	V_{O_2}	V_{CO_2}	V_A	V_B	$V_{GLC\text{-}to\text{-}A}$	$V_{GLC\text{-}to\text{-}PYR}$	$V_{PYR\text{-}to\text{-}CO_2}$	$V_{PYR\text{-}to\text{-}B}$	V_m	$V_{BIOMASS}$		
GLC	0	0	0	0	0	0	0	1	0	0	0	0	0	−1	−1	0	0	0	0		
GLC[e]	−1	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0		
PYR	0	0	0	0	0	0	0	0	−1	0	0	0	0	0	2	−1	−1	0	0		
PYR[e]	0	−1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0		
CO_2	0	0	0	0	0	0	0	0	0	−1	0	0	0	0	0	3	0	0	0		
CO_2[e]	0	0	0	−1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0		
O_2	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	−1	0	0	0		
O_2[e]	0	0	−1	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0		
ATP	0	0	0	0	0	0	0	0	0	0	0	0	0	−3	2	10	−6	−1	0		
ADP	0	0	0	0	0	0	0	0	0	0	0	0	0	3	−2	−10	6	1	0		
A	0	0	0	0	0	0	0	0	0	0	0	−1	0	1	0	0	0	0	−1		
A[e]	0	0	0	0	−1	0	0	0	0	0	0	1	0	0	0	0	0	0	0		
B	0	0	0	0	0	0	0	0	0	0	0	0	−1	0	0	0	1	0	−1		
B[e]	0	0	0	0	0	−1	0	0	0	0	0	0	1	0	0	0	0	0	0		
BIOMASS	0	0	0	0	0	0	−1	0	0	0	0	0	0	0	0	0	0	0	1		

× $\begin{bmatrix} EX_{GLC} \\ EX_{PYR} \\ EX_{O_2} \\ EX_{CO_2} \\ EX_A \\ EX_B \\ EX_{BIOMASS} \\ V_{GLC} \\ V_{PYR} \\ V_{O_2} \\ V_{CO_2} \\ V_A \\ V_B \\ V_{GLC\text{-}to\text{-}A} \\ V_{GLC\text{-}to\text{-}PYR} \\ V_{PYR\text{-}to\text{-}CO_2} \\ V_{PYR\text{-}to\text{-}B} \\ V_m \\ V_{BIOMASS} \end{bmatrix}$ = **0**

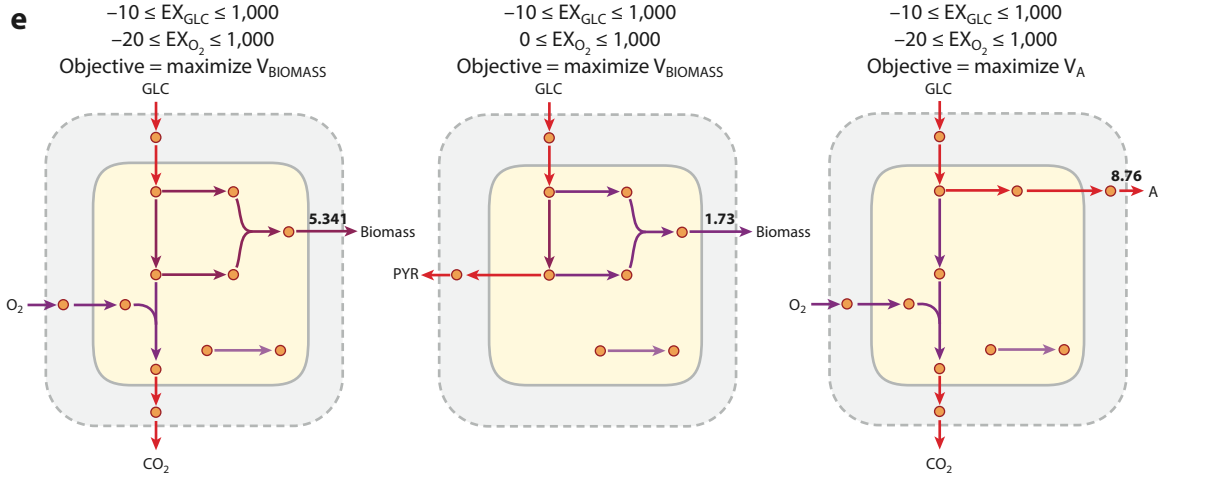
d

$$\mathbf{c} = \begin{bmatrix} 0 \\ \dots \\ 1 \end{bmatrix}$$ Objective coefficients

$$\mathbf{c}^T\mathbf{v} = \begin{bmatrix} 0 \dots 1 \end{bmatrix} \times \begin{bmatrix} EX_{GLC} \\ \dots \\ V_{BIOMASS} \end{bmatrix}$$

$= 0EX_{GLC} + \dots + 1V_{BIOMASS}$
$= V_{BIOMASS}$

e



(*Caption appears on following page*)

Figure 2 (*Figure appears on preceding page*)

Formulation of FBA for a toy model of a cell that captures some realistic features of metabolism. (*a*) A map of the metabolic network, annotated with details necessary for performing FBA calculations. The network includes some realistic metabolites (e.g., glucose as GLC and pyruvate as PYR) and some fictitious ones (A and B) meant to represent building blocks (e.g., amino acids) to be used for the production of biomass (whose flux is the growth rate). Some reactions in the network are coarse-grained representations of real metabolic pathways (e.g., $V_{GLC\text{-}to\text{-}PYR}$ for glycolysis and $V_{PYR\text{-}to\text{-}CO_2}$ for the citric acid cycle). The exchange reaction EX_{GLC} is highlighted to illustrate an increasingly standard convention for how to define and use exchange reactions to enable balancing of uptake/secretion of metabolites across the cell boundary. This reaction is defined in the direction of metabolite secretion, implying that metabolite uptake is associated with a negative flux. The $V_{PYR\text{-}to\text{-}CO_2}$ reaction is highlighted to exemplify a gene–protein–reaction rule. For example, this fictional reaction can be catalyzed either by a complex of proteins X_1 and X_2 or by a single enzyme Y, and the gene–protein rule would be the Boolean expression (G[X_1] AND G[X_2]) OR G[Y], where G[P] is a Boolean variable indicating the presence of the gene coding for a protein P. (*b*) The mathematical formulation of FBA, using matrix and vector notations that make it easy to solve the problem using existing tools, borrowed from the field of linear programming. The main idea is that the space of possible solutions is gradually restricted by imposing linear constraints, followed by an optimization step that identifies a set of fluxes that maximize (or minimize) a given objective function. Here, Z is the objective function, $\mathbf{c}$ is the vector of objective function coefficients, $\mathbf{v}$ is the vector of fluxes, $\mathbf{S}$ is the stoichiometric matrix, and LB_i and UB_i are the lower and upper bounds, respectively, for a given flux, v_i. (*c*) The steady-state constraint of FBA, $\mathbf{Sv} = 0$, displayed in detailed matrix form. Each row in $\mathbf{S}$ is associated with a metabolite, and each column corresponds to a reaction. Matrix element $\mathbf{S}_{ij}$ represents the stoichiometric coefficient of metabolite i in reaction j. Setting $\mathbf{Sv} = 0$ is equivalent to requiring a mass balance (or flux balance) equation for each metabolite. This can be verified by multiplying each row of $\mathbf{S}$ by the flux vector. (*d*) The objective function (Z). The objective function is defined by a vector of coefficients $\mathbf{c}$, which define the weight of each reaction in the objective function, i.e., $Z = \mathbf{c}^T\mathbf{v}$. A common objective function for FBA is the maximization of biomass production flux. This is represented by coefficients of 0 for every reaction other than the biomass reaction, which has a coefficient of 1. (*e*) Visualization of FBA solutions for this network under three different environmental conditions and objective functions. Solid arrows visualize the actual direction of each reaction, with color reflecting the intensity of the flux, from low (*purple*) to high (*red*). The left simulation sets lower bounds for the glucose and oxygen exchange reactions to represent aerobic growth with glucose as the sole carbon source and optimizes for growth. Under these conditions, the biomass flux is 5.341. The center simulation only changes the bounds for EX_{O_2}, leading to anaerobic growth. Under these conditions, PYR is secreted (fermentation), and the biomass flux decreases to 1.73. The right simulation represents a metabolic engineering strategy where a cell is grown aerobically on glucose to produce molecule A. Maximization of the production of A provides the theoretical maximum production flux of A, which is 8.76 mmol gDW^{-1} h^{-1}. The flux visualization was done using Escher (King et al. 2015). A full description of this model and the corresponding Python code are available in the **Supplemental Material** and at **https://github.com/segrelab/learn-fba**. Abbreviations: FBA, flux balance analysis; gDW, grams of dry weight; LB, lower bound; UB upper bound.

reaction may be catalyzed by multiple enzymes (isoenzymes) (Bernstein et al. 2023) or multimeric proteins. This mapping, encoded in the form of Boolean functions called gene–protein–reaction rules (Thiele & Palsson 2010) (**Figure 2*a***, right), represents a fundamental bridge between biochemistry and genomics and is particularly important when models are used to predict the effects of genetic modifications (e.g., deletions), as one needs to know what reaction would be impacted by the modulation or loss of that gene.

Supplemental Material >

Reactions of high importance to ocean modeling include photon fluxes and light harvesting for photosynthesis, which are part of GEMs for phototrophs (Baroukh et al. 2015, Chang et al. 2011). Photons of different wavelengths can be explicitly encoded (Broddrick et al. 2016), enabling simulations of different spectral distributions of incoming light and cellular absorbance.

In addition to enzymatic reactions, GEMs include the production of biomass from precursors (including amino acids, nucleotides, lipids, and cofactors) in known proportions. This reaction represents cellular growth. Accurate organism-specific biomass compositions are difficult to obtain but can contribute to more complete and predictive models. Marine organisms may include unique biomass components, such as osmolytes, that provide resistance to high salinity (Iffland-Stettner et al. 2023). The biomass compositions for some marine organisms have been determined experimentally, including for cyanobacteria (Ahmad et al. 2020a, Casey et al. 2016, Gardner & Boyle 2017, Saha et al. 2012, Vu et al. 2012), microalgae (Levering et al. 2016), heterotrophic bacteria (Iffland-Stettner et al. 2023), and sponges (Watson et al. 2014).

Biomass: the amount (in grams) of a given organism, composed of a set of building blocks in precise proportions

GEMs generally also include a reaction that accounts for non-growth-associated maintenance (Maarleveld et al. 2013, Pramanik & Keasling 1997, Varma et al. 1993), i.e., expenditure of ATP that relates to nonmetabolic processes, such as protein turnover and flagellar motility. Some maintenance fluxes have been measured for marine bacteria (e.g., Iffland-Stettner et al. 2023), but the systematic measurement of maintenance across species and conditions remains an important and challenging task.

Gap filling: the process of identifying missing reactions in a GEM, generally by testing incapacity of growth under a given condition

In spite of all efforts to carefully construct and test GEMs from genomes, incomplete or incorrect annotations pose significant challenges (Bernstein et al. 2021). Missing or erroneous annotations of reactions and transporters can strongly impact a model's predictive capabilities (Ren et al. 2004). This problem is increasingly addressed by automated gap-filling algorithms (Machado & Herrgård 2014, Seaver et al. 2021, Wang et al. 2018), which leverage metabolic knowledge across the tree of life and experimental growth phenotype data to identify critical gaps that prevent a metabolic network from producing all biomass components (Orth & Palsson 2010). The products of these automated workflows may still require laborious manual curation for reliable performance (Seif & Palsson 2021). GEMs are now available for many marine organisms, including prokaryotic phytoplankton (Ahmad et al. 2020a, Casey et al. 2016), eukaryotic phytoplankton (Ahmad et al. 2020b, J. Kim et al. 2016, Lavoie et al. 2020, Levering et al. 2016, Shah et al. 2017), heterotrophic bacteria (Du et al. 2022, Fondi et al. 2015), archaea (Du et al. 2022, Li et al. 2018, Vailionis et al. 2023), protists (Shene et al. 2020), and multicellular eukaryotes (Gao et al. 2021, Zakhartsev et al. 2022).

FLUX BALANCE ANALYSIS IS A MATHEMATICAL APPROACH FOR PREDICTING METABOLIC PHENOTYPES

FBA is a mathematical approach to generate phenotypic predictions from a metabolic network reconstruction (e.g., a GEM) (O'Brien et al. 2015, Orth & Palsson 2010) (**Figure 2**). Key input variables to FBA include the metabolic network stoichiometry (the **S** matrix; **Figure 2*c***) and the lower and upper bounds to each flux. These bounds are used to define the extracellular environment (e.g., the availability of carbon sources, nutrients, and light). The main output is a set of putative rates, or fluxes, for all reactions in the network (**Figure 2*c*,*e***), including uptake and secretion, as well as the biomass production flux, representing the growth rate (in units h^{-1}). Fluxes are expressed per unit of biomass, i.e., millimoles of transformed metabolite per gram of dry weight of biomass per hour (mmol gDW^{-1} h^{-1}).

A simplifying assumption at the core of FBA's capabilities is that the metabolic network is at steady state (**Figure 2*a–c***). This assumption is equivalent to requiring that the fluxes producing each metabolite be balanced by the fluxes utilizing that metabolite so that the concentration of the metabolite does not change. Fluxes thus become the main variables, related to each other through linear relationships. These linear relationships have a geometrical interpretation that, in low-dimensional cases, can be directly visualized (**Figure 3**; for an interactive graph, see the **Supplemental Material**). The steady-state assumption is reasonable for cell populations observed over a certain period of time, under slowly changing environments. One should be cautious, though, when approaching single-cell levels or fast processes.

Supplemental Material >

The second assumption often used in FBA is that metabolism operates close to a mathematically predictable optimum, represented through an objective function (**Figure 2*d***). A common objective function is maximization of the growth flux, reflecting evolutionary adaptation for efficient use of resources toward growth (Ibarra et al. 2002, Segrè et al. 2002). However, different objectives could be explored and hypothesized, including for different tissues in multicellular organisms (Mori et al. 2019, Schuetz et al. 2012, Segrè et al. 2002). The linearity of the flux

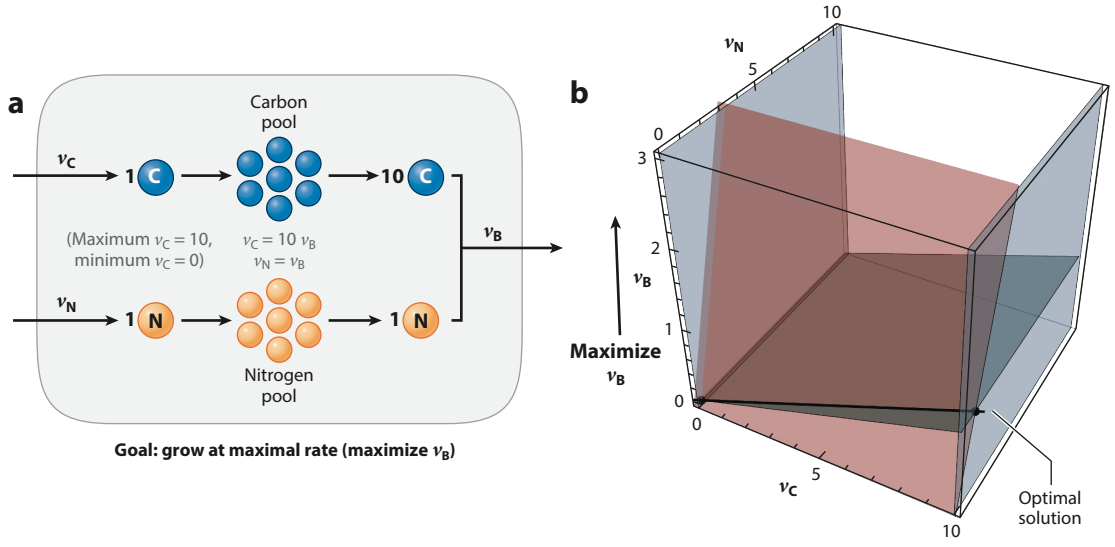


Figure 3

Geometrical interpretation of FBA using an ultrasimplified metabolic network with only three reactions. (*a*) The network, which has three reactions: v_C, the transport of carbon into the cell; v_N, the transport of nitrogen into the cell; and v_B, the biomass-producing reaction that combines 10 carbons and 1 nitrogen into one unit of biomass, which is removed from the system. There are bounds on the flux of v_C, so the flux must be between 0 and 10. The objective of this system is to grow as much as possible, which is equivalent to maximizing the value of v_B. The schematic also shows the mass balance equations for each metabolite. (*b*) A graphical representation of the FBA procedure to predict flux. The four planes represent the four constraints on fluxes (shown as equations in panel *a*). The two parallel vertical planes represent the bounds on the value of v_C. The dark blue diagonal plane represents the mass balance constraint on carbon, and the orange plane represents the same for nitrogen. The intersection of the two mass balance planes (*black line*) represents solutions that satisfy both mass balance constraints. The solutions along this black line and between the two vertical v_C-bound planes are feasible solutions to the FBA problem. The feasible solution with the highest v_B is the optimal solution. A full description of this model and the corresponding Python code are available in the **Supplemental Material** and at **https://github.com/segrelab/learn-fba**. Abbreviation: FBA, flux balance analysis.

constraints and of the objective function makes it possible to solve basic FBA problems in a fraction of a second, using efficient linear programming libraries.

Supplemental Material >

Dedicated tools [e.g., COBRA Toolbox for MATLAB (Heirendt et al. 2019), COBRApy (Ebrahim et al. 2013), KBase (Arkin et al. 2018), and Escher-FBA (Rowe et al. 2018)] can be used to easily perform FBA calculations. However, it is useful to remember (and valuable for educational purposes) that FBA can be coded from scratch, as shown in two simple examples associated with **Figures 2** and **3** (see the **Supplemental Material** and **https://github.com/segrelab/learn-fba**).

Testing and Validation of Flux Balance Analysis

Predictions of fluxes and growth phenotypes generated with FBA can be tested through comparison with corresponding experimental measurements. Mismatches can be used to refine models and their assumptions. For example, FBA-predicted growth capabilities can be compared with experimentally measured phenotypes (growth/no growth) on different carbon sources or light conditions (Coppens et al. 2023, Ong et al. 2014, Tec-Campos et al. 2023) or with measured growth rates (Broddrick et al. 2019, Coppens et al. 2023, Iffland-Stettner et al. 2023, Pinchuk et al. 2010). In some cases, mismatches can be reconciled by adding missing transport or exchange reactions to a GEM (Pinchuk et al. 2010). Comparisons between experimental and in silico gene knockout phenotypes have also been explored extensively, yielding relatively high accuracy for manually

Linear programming: a mathematical technique to optimize a linear function of a set of variables given linear constraints between them

curated model organisms (Bernstein et al. 2023) and variable outcomes for marine or freshwater organisms (Broddrick et al. 2016, Coppens et al. 2023, Santos-Merino et al. 2023). Disagreements between predicted and expected gene essentiality provide valuable insights into the functioning of metabolic networks. For example, in cyanobacteria, disagreements in gene essentiality predictions revealed modifications to the canonical tricarboxylic acid (TCA) cycle (Broddrick et al. 2016, Chapman et al. 2015).

Deeper testing of FBA predictions can be obtained by measuring uptake/secretion fluxes or intracellular ones. Uptake and secretion fluxes can be assessed experimentally by probing extracellular metabolites (exometabolites; e.g., Brisson et al. 2021) at multiple time points. Measurements of intracellular fluxes require the much more laborious handling of isotopically labeled metabolites, e.g., as performed in ^{13}C-metabolic flux analysis (Harcombe et al. 2013, Kaste & Shachar-Hill 2024, Long & Antoniewicz 2019, Qian et al. 2017, Schuetz et al. 2012, Schulze et al. 2022). Similar techniques with isotopes of nitrogen and hydrogen, in addition to carbon, have been used to study fluxes in marine or freshwater cyanobacteria (Roth-Rosenberg et al. 2020, 2021; Schulze et al. 2022) and may help validate FBA-predicted fluxes (Broddrick et al. 2019, Coppens et al. 2023, Qian et al. 2017).

It is important to note that true validation of the predictive power of a GEM requires comparing predictions with data that were not used during the network reconstruction process. For example, one should test model predictions on carbon sources that were not used for gap filling, as was done recently for *Vibrio splendidus* 1A101 (Iffland-Stettner et al. 2023).

Limitations of Flux Balance Analysis

A key consequence of the steady-state assumption and of the way FBA is formulated is that fluxes become the only variables in the model, while concentrations are absent. A major implication of this is that standard FBA cannot predict the effects of allosteric regulation of enzymes or metabolite-mediated transcriptional regulation. Note that flux through a metabolite cannot be used as a proxy for the concentration of that metabolite in the cell, as there is no reason for the two to correlate (e.g., flow through a highly abundant metabolite may be very slow). In addition to affecting metabolic engineering applications (where an intermediate metabolite could allosterically block a flux), the lack of explicit concentrations in FBA may impact predictions of functions in marine ecosystems (e.g., by failing to account for carbon-concentrating mechanisms in carbon fixation in diatoms; Roberts et al. 2007).

While in FBA it is usually convenient to use a single objective function to predict fluxes, true biological objectives may be hard to define or may change under different conditions (Mahadevan & Schilling 2003, Schuetz et al. 2012, Shoval et al. 2012). Even when biomass maximization is a reasonable fixed objective, the composition of biomass itself may be condition dependent (Pramanik & Keasling 1997), as has been explored for cyanobacteria at different light intensities (Qian et al. 2017, Saha et al. 2012). An elegant alternative to the use of an objective function is sampling the space of possible flux solutions (Herrmann et al. 2019, Ofaim et al. 2021, Santos-Merino et al. 2023, Vailionis et al. 2023), giving rise to distributions of flux states instead of individual values. This approach may help explicitly incorporate uncertainty (Bernstein et al. 2021) and could shed light on phenotypic diversification in clonal populations.

Incorporating Gene Expression Data in Flux Balance Analysis

One goal that the FBA modeling community has long struggled with is the integration of FBA-based flux predictions with gene expression data. Transcriptomic and proteomic data can point to highly expressed enzyme genes and associated reaction fluxes. In the study of multicellular

organisms (e.g., fish; Molversmyr et al. 2023), gene expression data have been used to generate tissue-specific models, where silenced genes translate into zero flux through the corresponding reactions (de Oliveira Dal'Molin et al. 2010, Li et al. 2010, Martins Conde et al. 2016). A similar approach has been applied to marine microbes that display discrete phenotypic states (Gardner & Boyle 2017).

Yield: the ratio of the flux producing a given metabolite to the flux of consumption of a specific resource

More broadly, researchers have used gene expression–based constraints on fluxes in several ways to try to improve flux predictions, often encountering significant challenges (Machado & Herrgård 2014). Any approach for the integration of gene expression in FBA should be approached with caution: Posttranscriptional regulation, posttranslational modifications, allosteric regulation, enzyme inhibition, and lack of substrate can drastically modulate the flux through a reaction, even if the enzyme is highly expressed (Blazier & Papin 2012). It is likely that substantially new ways of posing the problem (e.g., integration between mechanistic and AI approaches) will be necessary for a leap in this direction.

APPLICATIONS OF FLUX BALANCE ANALYSIS TO ADDRESS QUESTIONS IN MARINE BIOLOGY

Some fundamental questions about marine metabolism can be addressed using FBA and its extensions and variants. For simplicity, we organize these questions into four categories: metabolic efficiency, environmental dependence, genetic perturbations, and microbial communities and multicellular systems.

Metabolic Efficiency

Metabolic efficiency, often defined by the yield of biomass or a specific product of interest, can depend on two intertwined but distinct properties of metabolism. The first is the stoichiometry itself. For example, stoichiometry may dictate how many of the carbon atoms from a sugar end up in the biomass versus in secreted CO_2 or byproducts, or how a cell could manage mismatches between biomass and environmental carbon/nitrogen ratios (Pacheco et al. 2019). The second property that affects efficiency is the investment needed to produce and maintain the enzymes necessary for operating a given metabolic pathway (proteome allocation) (Hui et al. 2015, Mori et al. 2016). A pathway stoichiometrically capable of producing more molecules of ATP per glucose may be overall less efficient if it requires a lot of building blocks and ATP investment for enzyme production (Flamholz et al. 2013). Standard FBA only includes the stoichiometry of metabolism, but there are extensions of FBA that can partially incorporate the protein cost.

FBA solutions can be used to estimate different metabolic efficiency metrics, equivalent to yield coefficients in metabolic engineering (Stephanopoulos et al. 1998). A yield coefficient can be calculated as the ratio between the flux producing a given metabolite of interest and the flux of production or consumption of a specific resource, often the carbon source or biomass (e.g., moles of lysine produced per mole of glucose consumed, or grams of lysine produced per gram of biomass produced). In some metabolic engineering efforts focused on marine or freshwater microalgae (e.g., *Chlamydomonas reinhardtii*), FBA has been used to guide strain design by predicting the yield of interest in silico before moving to laboratory experiments (Imam et al. 2015, Japhalekar et al. 2022, Vu et al. 2013, Yoshikawa et al. 2017). Building upon this type of calculation, several advanced optimization methods have been constructed to suggest specific genetic modifications and up- or downregulation strategies likely to increase desired yields (Burgard et al. 2003, Pharkya et al. 2004, Ranganathan et al. 2010, Zomorrodi & Maranas 2012), which have possible applications in metabolic engineering of phytoplankton.

A specific yield that is important in microbial ecology is the growth yield, defined as the amount of biomass produced relative to the amount of substrate consumed (e.g., grams of

biomass produced per gram of glucose consumed) (Stephanopoulos et al. 1998, Westerhoff et al. 1983, Wilken et al. 2021). A long-standing question in microbiology and microbial ecology is whether fundamental trade-offs exist between rate and yield (Mori et al. 2019, Novak et al. 2006, Pfeiffer et al. 2001, Westerhoff et al. 1983, Wilken et al. 2021). FBA-predicted growth yields for marine organisms have been used to study how these trade-offs enable a bacterium to thrive in extremely saline environments where growth occurs in blooms (Gonzalez et al. 2008) and uncovered the optimality behind seemingly wasteful processes, such as photorespiration (Knoop et al. 2010). Studies about growth yield have also guided the industrial cultivation of phytoplankton (Boyle & Morgan 2009, He et al. 2015). While the use of FBA to estimate efficiency is generally focused on microbes, metabolic models of salmon (Zakhartsev et al. 2022) and shrimp (Gao et al. 2021) have been used to evaluate growth yield on different substrates to evaluate the best feeds to be used in aquaculture.

Carbon use efficiency (CUE): the ratio of carbon remaining in a system to the carbon that enters that system

Parsimonious FBA: an FBA variant in which, among fluxes maximizing biomass, the one with minimal overall net flux is chosen

In the ecological context, the ratio of carbon remaining in a system to the carbon that enters that system, known as carbon use efficiency (CUE), is particularly relevant for the study of carbon storage and the carbon cycle, in both terrestrial and marine environments (Domeignoz-Horta et al. 2020, Manzoni et al. 2018, Saifuddin et al. 2019). CUE has been calculated from FBA solutions for soil bacteria (Saifuddin et al. 2019), and the same could be done for marine organisms and extended to predict the CUE of communities and ecosystems. FBA-predicted CUE could be used to predict the effects of environmental change on carbon storage in the ocean, including through incorporation in ocean biogeochemical models.

As mentioned above, the efficiency of a given metabolic process depends not only on the stoichiometry itself but also on the cost of operating the corresponding set of enzymes. This cost, which is related mainly to protein production, can play a significant role in physiological and evolutionary processes (Dekel & Alon 2005). In FBA, a broadly used strategy to incorporate an approximate notion of limited internal resources (including proteins) is to impose an upper bound on the total metabolic flux an organism can support. For example, an upper bound on a weighted sum of all fluxes (on top of standard FBA constraints) has been used to recapitulate proteome allocation, induced by limited cellular space (Beg et al. 2007).

A more recent approach, constrained allocation FBA (Mori et al. 2016), specifically addresses the proteome allocation by separately evaluating the cost of different functional categories, or sectors (ribosomal, transport, biosynthetic, and housekeeping) (Hui et al. 2015). As before, an additional constraint is imposed on the weighted sum of all fluxes, but in this case, the proportion of the proteome allocated to different sectors changes as a function of the growth rate, reflecting the fact that a higher growth rate will require a larger fraction of ribosomal proteins.

A related but simpler notion of frugal use of resources is at the core of parsimonious FBA (Lewis et al. 2010). This method, now broadly used, including in salmon (Molversmyr et al. 2023) and freshwater cyanobacteria (Broddrick et al. 2019), performs a secondary optimization to identify, among flux solutions with equivalent growth rates, those that minimize total flux.

A completely different avenue for taking into account protein cost in FBA is through the incorporation of thermodynamic constraints (Beard et al. 2002, Flamholz et al. 2013, Pandey et al. 2019). This strategy is based on the fundamental notion that a metabolic flux can only proceed in the direction that dissipates free energy and that reactions close to equilibrium will require increased amounts of protein to support higher flux (Beard et al. 2002). Thermodynamic FBA has been used successfully (Pandey et al. 2019, Salvy et al. 2019) but has not been broadly adopted due to the challenge of obtaining free energy differences for each metabolic reaction and to the elaborate technical implementation. However, it is an exciting area for future development and a promising strategy for the integration of proteomic and metabolomic data in stoichiometric models.

In summary, metabolic efficiency is hugely important to marine metabolism. Practically, efficiency determines yields of biotechnological and aquaculture products, and ecologically, it is one determinant of carbon storage in the ocean. We anticipate that FBA will continue to be a valuable tool to advance the understanding of metabolic efficiency.

Environmental Dependence

Many experiments on metabolic processes are conducted in the laboratory under relatively simple conditions. Natural environments, however, are molecularly complex, variable in time, and spatially structured. Bridging the gap between these two scenarios constitutes an interesting challenge and opportunity for flux balance modeling. A key question in marine ecosystems is how an organism will respond to changes in its environment, including changes in resource availability. Studying marine organisms' responses to environmental perturbations is crucial for making more realistic predictions about the ecological effects of changing ocean conditions (Boyd et al. 2018, Cavicchioli et al. 2019, Moran et al. 2022, Nguyen et al. 2022). FBA simulations can be used to see how metabolic fluxes are rewired in response to key environmental parameters such as the sources and concentrations of inorganic and organic carbon, oxygen, and light.

One notable aspect of CO_2 and light as environmental sources of energy and carbon is that phototrophs may not be able to easily control their influx, setting them apart from other environmental inputs (e.g., sugars). To address this, Ofaim et al. (2021) imposed specific uptake fluxes (pushed fluxes) and explored the consequences of these forced environmental inputs for carbon storage and exudation strategies. These pushed fluxes recapitulated overflow metabolism better than standard FBA.

For marine organisms, several studies have used FBA to compare growth rates on different organic carbon sources (e.g., Dufault-Thompson et al. 2017), nitrogen sources (e.g., Goyal et al. 2014), oxygen concentrations (e.g., Shene et al. 2020), and light intensities (e.g., Qian et al. 2017, Vu et al. 2012). Such studies have been used to address biological questions with relevance to ecological processes. For example, Dufault-Thompson et al. (2017) used growth rates on different carbon sources to investigate the metabolic flexibility of the heterotrophic bacterium *Shewanella piezotolerans* and highlight the bacterium's adaptation to the fluctuating organic carbon availability in the deep sea.

FBA-predicted responses to environmental change can also have ocean-scale implications. For example, Casey et al. (2022) used variants of FBA to accurately predict metabolic variations in *Prochlorococcus* ecotypes along an oceanic transect. They represented each individual strain with a unique GEM and acclimated the strains to the environmental conditions (temperature, nutrient concentration, and light spectra) at each transect location by adjusting the cell size, nutrient transporters, pigments, biochemical composition, and metabolic fluxes. The growth rates of the strains predicted by the GEMs were correlated with the observed abundances of the different strains along the transect, showing a link from metabolic processes to global-scale processes.

Since conditions are dynamic in natural environments, metabolism must be constantly rewired and fluxes redirected as needed. One highly studied example of a dynamic environment for marine organisms is the diel cycle of light availability and its effect on phytoplankton. Saha et al. (2012) were able to model the diel cycle of *Cyanothece* 51142 by separately modeling steady states during light and dark phases. Knies et al. (2015) used a similar approach, handled through optimization over the entire day–night cycle, to model the transfer of storage metabolites in *Emiliania huxleyi*. Sarkar et al. (2019) obtained a finer resolution of this cycle by curating 12 time-point-specific models of the freshwater cyanobacterium *Synechocystis* PCC 6803 and allowing for the flow of metabolites across time points, and Reimers et al. (2017) were able to create models along

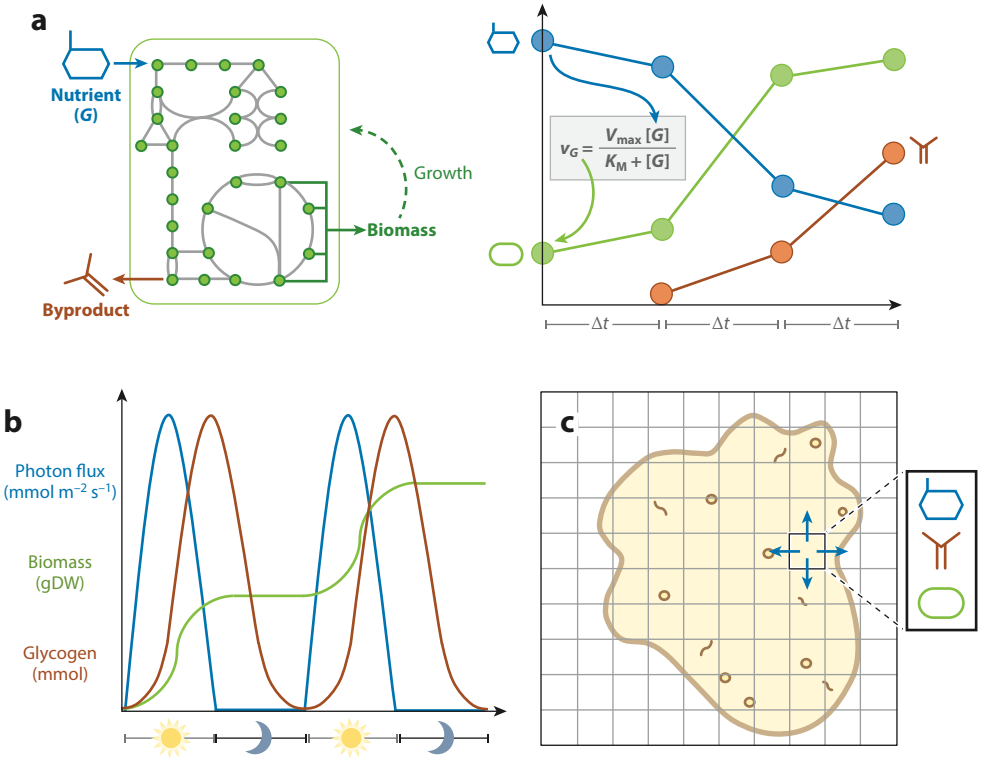


Figure 4

Extensions of FBA capable of modeling dynamic and heterogeneous environments. (*a*) dFBA extends FBA to estimate environmental changes in biomass and metabolite abundances, while still assuming intracellular steady state. At every time step, bounds for all uptake fluxes are recalculated using Michaelis–Menten equations (applied to the current extracellular metabolite concentrations). Using these bounds, an FBA calculation is performed to predict metabolite uptake/secretion and biomass fluxes, which in turn are used to estimate the next expected variation in extracellular molecular concentration. (*b*) dFBA has been used to model the diel cycle of phytoplankton growth, by implementing periodic boundary conditions for light availability and dynamically tunable storage of glycogen. (*c*) Spatiotemporal FBA can simulate a heterogeneous environment by discretizing the environment into small grid cells that are treated as having a homogeneous environment inside of them. The concentrations of biomass and metabolites within each grid cell are calculated using dFBA, and metabolites and biomass can move between grid cells due to diffusion. Spatiotemporal FBA (e.g., implemented through the COMETS framework) could be used for spatially explicit simulations of multiple bacteria engaging in cross-feeding (e.g., for microbial communities on a particle). Abbreviations: COMETS, Computation of Microbial Ecosystems in Time and Space; dFBA, dynamic flux balance analysis; FBA, flux balance analysis; gDW, grams of dry weight.

Dynamic FBA (dFBA): an FBA variant that iterates the analysis over multiple small time steps, updating the concentrations of external metabolites at each time point

a continuous diurnal cycle by explicitly modeling the capacity for catalytic macromolecules (e.g., enzymes and ribosomes).

To perform de novo simulations of dynamic environments, one can use an extension of FBA called dynamic FBA (dFBA) (Mahadevan et al. 2002). In dFBA, FBA is solved over small time steps, updating environmental metabolites dynamically based on uptake/secretion fluxes (**Figure 4*a***). This approach is also at the core of Computation of Microbial Ecosystems in Time and Space (COMETS) (Harcombe et al. 2014), discussed in more detail below. Several studies (Baroukh et al. 2016, Ofaim et al. 2021, Smith et al. 2019, van Tol & Armbrust 2021) have used dFBA to model phytoplankton metabolism over the diel cycle by modulating the flow of incoming photons over

time (**Figure 4*b***). One should note that in general, dFBA requires knowledge of kinetic parameters for uptake reactions in order to translate extracellular concentrations into rates (**Figure 4*a***).

The dynamics of metabolism can often depend on the spatial structure of the environment and modify it (Chacón et al. 2018, Lipsman et al. 2024, Nadell et al. 2016, Pfeiffer et al. 2001). Some extensions of dFBA have incorporated spatial structure through a discretized space where dFBA updates the concentrations of metabolites and biomass in each location, and numerical solutions of partial differential equations are used to model diffusion (Harcombe et al. 2014). Variants of this approach have been used to study location-dependent interspecies interactions in synthetic communities (Harcombe et al. 2014), spatial structure of microbial biofilms (Phalak et al. 2016), and microbial diversity in the human gut (van Hoek & Merks 2017). Since its inception as a framework for spatially structured dFBA, COMETS has continued growing as a collaborative project that integrates multiple modules, including complex boundary conditions, evolutionary dynamics, and nonlinear diffusivity (Dukovski et al. 2021). Other approaches to spatially structured FBA focus instead on agent-based frameworks (Bauer et al. 2017, Borer et al. 2019, Hellweger et al. 2016), offering the opportunity to connect to single-cell experiments but at the same time limiting the scalability to large populations.

Computation of Microbial Ecosystems in Time and Space (COMETS): software combining dFBA and diffusion to predict spatially structured metabolic activity

While spatiotemporal FBA has not been explored in much detail for marine organisms, we envisage that it could have valuable applications in studying particle-associated communities (**Figure 4*c***) as well as gradients of nutrients in the water column. In the future, the application of FBA and its extensions in marine ecosystems is poised to tackle more complex scenarios involving nutrient mixes and spatial structures.

Genetic Perturbations

Genetic perturbations are frequently used to understand how living systems work. Because GEMs typically include information that ties reactions in the metabolic network to genes, FBA can be used to study how an organism responds to genetic perturbations. In metabolic engineering, gene deletions can be prioritized based on FBA phenotype predictions (e.g., OptKnock; Burgard et al. 2003) to help increase yield by rerouting flux toward the production pathways. In phytoplankton (mostly freshwater species), similar efforts have focused mainly on the production of biodiesel from microalgae triacylglycerols and bioethanol from cyanobacteria carbohydrates. For example, Yoshikawa et al. (2015) identified gene deletion targets to maximize the production of ethanol in *Arthrospira platensis* NIES-39, and Santos-Merino et al. (2023) did the same for the production of α-linolenic acid in a freshwater *Synechococcus* strain. In silico gene knockouts are also a valuable tool for answering basic research questions about the role of specific genes (Ofaim et al. 2021). FBA makes it computationally feasible to perform all single-gene deletions in a given organism. Ahmad et al. (2020a) looked at the distribution of essential genes across the different pathways of *Synechococcus* and found that when the bacteria were growing phototrophically, close to half of all reactions were essential. Klanchui et al. (2012) predicted that TCA cycle genes are essential in *Arthrospira* only during phototrophic growth.

When the knockouts across the genome are looked at as a whole, they can also be used to quantify an organism's robustness and ask questions about evolutionary adaptation. Casey et al. (2016) used the lethality of all single-gene deletions to determine that *Prochlorococcus marinus* MED4 has an extremely high proportion of essential metabolic genes, reinforcing the adaptive gene loss hypothesis for *Prochlorococcus*. Lavoie et al. (2020) computed all gene knockouts using a variant of FBA specifically meant to capture the imperfect response to gene deletions [minimization of the metabolic adjustment (MOMA); Segrè et al. 2002] and showed that a polar diatom was highly robust to genetic perturbations, likely contributing to the organism's success in polar environments.

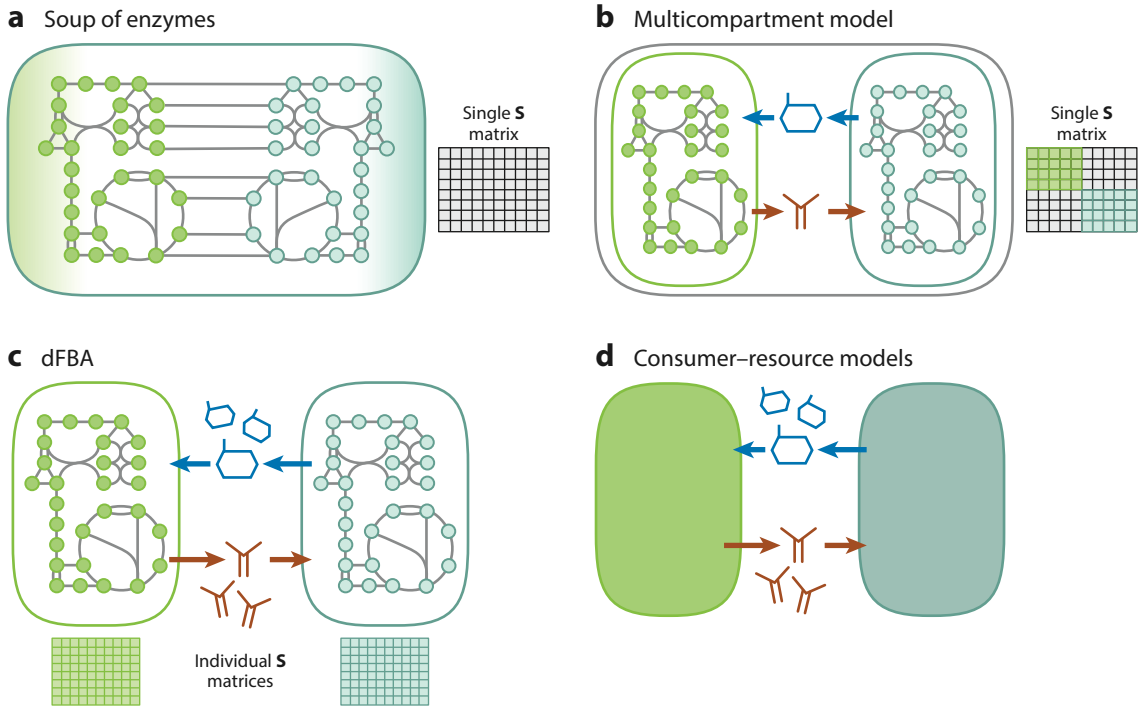


Figure 5

Different approaches for modeling multicellular communities. (*a*) The soup-of-enzymes approach combines all community members into a single model, with no distinction of which reaction is happening in which cell. (*b*) Multicompartment models create a single model for the community, but the organisms are contained in individual compartments, sharing metabolites via pools outside their compartments. (*c*) dFBA models the external metabolite concentrations, which allows multiple organisms to exchange metabolites via the shared external environment. In dFBA, each organism is a separate model with its own dynamics. (*d*) Consumer–resource models use a similar logic of shared environments as dFBA but do not model the mechanistic detail of metabolism and instead use parameterized input–output relationships. Abbreviation: dFBA, dynamic flux balance analysis.

Microbial Communities and Multicellular Systems

Metabolism does not end at the cellular membrane but extends to connect each cell to other members of a community. FBA approaches have been extended to predict metabolism in a community, enabling research about community composition and function (**Figure 5**).

To scale from modeling single microbial species to a microbial community, FBA can be modified in several ways. The soup-of-enzymes approach combines the metabolic networks for all community members into a single model, treating the community as a supraorganism (Frioux et al. 2020, Henry et al. 2016, Klitgord & Segrè 2010b) (**Figure 5*a***). This approach can be useful for analyzing environmental interactions and can scale up to complex communities, but it can obscure individual contributions, and decompartmentalization may affect certain metabolic functions (Klitgord & Segrè 2010b). To preserve the identity of each species in a consortium, one can alternatively combine multiple metabolic networks into a multicompartment model (Henry et al. 2016, Klitgord & Segrè 2010b, Rajala et al. 2022, Stolyar et al. 2007, Zuñiga et al. 2020) (**Figure 5*b***). Initial applications of this approach included a model of archaeal–bacterial symbiosis that mediates the anaerobic oxidation of methane (Stolyar et al. 2007) and a systematic analysis of many pairs of microbes demonstrating how nutrients could induce and modulate cross-feeding (Klitgord & Segrè 2010a). A detailed comparison of single- and multicompartment approaches

has been done using a simple marine community with a cyanobacterium and a heterotrophic bacterium (Henry et al. 2016). While standard multicompartment approaches do not lend themselves to predicting taxonomic abundances and ecological dynamics, variants have been proposed to effectively address this limitation, e.g., by using multilevel optimization (e.g., OptCom) (Zomorrodi & Maranas 2012) or nonlinear optimization (community FBA) (Khandelwal et al. 2013).

dFBA (**Figure 5*c***) offers an opportunity to simultaneously address the issues of abundance prediction and ecosystem-level objectives in a straightforward and elegant way. Because dFBA updates the external metabolite concentrations, it allows for multiple organisms to exchange metabolites via a shared external environment, and it provides insight into the specific molecules expected to mediate competition or cross-feeding (Harcombe et al. 2014). Moreover, in a dFBA simulation of a community, one can assume that multiple species are all concurrently pursuing their own objective function, and ecosystem-level behavior is an emergent property of the multiple individual strategies pursued by individual players. Previous studies have highlighted the ability of dFBA to study community dynamics for artificial (Harcombe et al. 2014) and environmental communities (Zhuang et al. 2011). This approach has also been applied in marine communities to predict the relative populations of diazotrophic and phototrophic subtypes of *Trichodesmium erythraeum* within a population (Gardner & Boyle 2017). The fact that dFBA can be embedded in three-dimensional physical space also paves the way for dynamical models of multispecies metabolism in complex structured environments.

Another ecological question where community-level FBA simulations have the potential to provide valuable insight is unculturability (Vartoukian et al. 2010). It is important to mention that, especially when it comes to uncultivated organisms with limited genomic information and poorly annotated genomes, FBA predictions should be interpreted with extra caution. An interesting emerging frontier is the reconstruction of GEMs for communities, where gap filling is achieved for a multicompartment consortium model (Giannari et al. 2021). The mathematical framework of stoichiometry also offers avenues to address questions related to how community metabolism supports organisms that cannot survive in axenic cultures, including, e.g., the producibility of different metabolites from imperfect (non-gap-filled) metabolic reconstructions (including previously uncultivated TM7 taxa) (Bernstein et al. 2019), which can be extended to marine bacteria. An optimization algorithm designed to identify possible strategies for division of labor (Thommes et al. 2019) could be used to generate hypotheses about the pathway modifications that cause obligate mutualism. Using this algorithm to limit the number of allowed reactions (a form of proteome cost constraint) in *Escherichia coli* resulted in a prediction that multiple interacting substrains may emerge. In some solutions, the resulting metabolic networks displayed incomplete TCA cycles, similar to what is observed in some marine microbes, notably cyanobacteria (Zhang & Bryant 2011). The interplay between ecology and evolution in shaping cross-feeding can also be addressed by integrating FBA with game theory, e.g., to ask what rates of exudation may support evolutionarily stable states (Zomorrodi & Segrè 2017).

Just like a microbial community, the individual cells of a multicellular organism can have different metabolic capabilities and interact to support one another. FBA has been abundantly employed to study metabolism in multicellular eukaryotes, with significant challenges but also exciting opportunities for discovery. While in microbial communities it is often assumed that all community members have an individual objective to grow efficiently, the aim of a cell type or tissue within a larger organism is not uncontrolled growth (unless it is in a proliferating state). Martins Conde et al. (2016) reviewed the modeling techniques used for multicellular organisms; for marine organisms, there are generic or tissue-specific models for cod (Hanna et al. 2020), salmon (Molversmyr et al. 2023, Zakhartsev et al. 2022), and shrimp (Gao et al. 2021), and there has been progress on a model of a sponge (Watson et al. 2014). These models have been used to optimize feeding

for aquaculture (Gao et al. 2021, Zakhartsev et al. 2022), and models of organisms such as corals, sponges, or mangroves and their associated microbiomes could aid ecosystem restoration efforts.

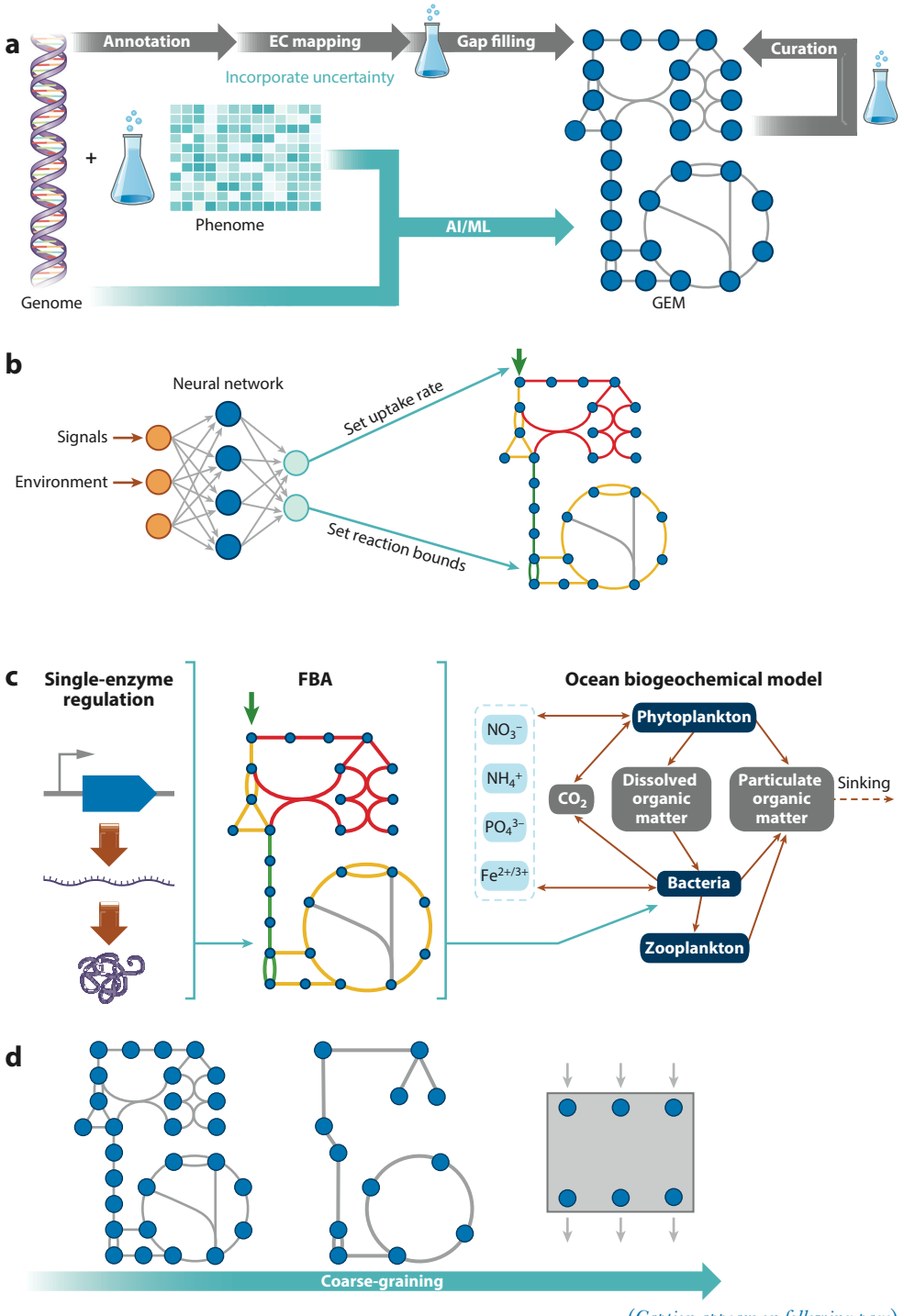
THE FUTURE OF FLUX BALANCE ANALYSIS AND HOW IT WILL IMPACT OCEAN SCIENCE

FBA and related methods for metabolic network reconstruction and flux prediction constitute a rich and constantly expanding field. Genome-scale reconstructions of metabolic networks will likely continue playing an important role as a mathematically tractable formalization of organism-specific biochemical knowledge, and novel phenotype prediction methods are bound to be developed. It is unclear exactly what the future of FBA modeling may look like, as new algorithms and techniques may come from unexpected directions and research fields, including physics, economics, and data science.

An obvious question moving forward is what role mechanistic models like FBA will play in a world that is increasingly swept by data-driven approaches, such as AI/ML. We expect that both will be important for modeling metabolism and that the two paradigms will display increased synergy in the future. In certain circumstances, data-driven statistical models may be extremely powerful and practically useful. For example, harmful algal blooms were successfully predicted using ML models based on water temperature, nitrogen, phosphorus, nutrients, or ocean color as input features (Hill et al. 2020). However, it is not obvious how such statistical models would perform when the conditions are significantly different from the data that were used to train the model, including upon extreme environmental perturbations or newly arising pollutants and invading species. Conversely, mechanistic models use biologically interpretable mathematical formulations of the understood cause-and-effect relationships, enabling extrapolation to new conditions. For example, mechanistic (FBA) models of harmful algal bloom metabolism could help us understand interactions between community members and predict the effects of possible intervention strategies. Because of these advantages, we expect that mechanistic models of metabolism, like FBA, will continue to be useful for many applications.

However, it is also likely that exciting opportunities will arise from combinations of FBA with AI/ML approaches, which may take several shapes and be advantageous in different research areas. One part of the FBA modeling pipeline where AI/ML approaches will likely have a large effect in the near future is in the creation and curation of GEMs. Metabolic network reconstruction is currently a laborious process, with many steps, each of which loses information. Potential future advances in network reconstruction may include the use of Bayesian approaches able to explicitly incorporate uncertainty into each of these steps (Bernstein et al. 2021), increased usage of high-throughput phenotype measurements in parallel to the genome, or the use of AI approaches to infer metabolic networks from genomes in a single step (**Figure 6*a***). Until these stages are reached, however, manual curation is still likely to play an important role, especially in efforts where high accuracy of an individual model is required.

AI/ML approaches may also be used to augment the phenotype prediction capabilities of FBA-related algorithms. Recent work has started to explore different avenues to integrate FBA and ML (reviewed in Sahu et al. 2021). For example, ML could be used to translate regulatory data or environmental cues (e.g., through a neural network) into lower and upper bounds to exchange or intracellular fluxes as an input to FBA (M. Kim et al. 2016) (**Figure 6*b***), to help explore large spaces of environmental conditions and organism assortments for microbial consortium engineering (Pacheco & Segrè 2021), or to extract features from FBA simulation results (Vijayakumar et al. 2020). It is conceivable that increased availability of flux measurements across different microbial species will gradually enable the rise of hybrid models that can learn patterns of metabolic activities



(Caption appears on following page)

Figure 6 (*Figure appears on preceding page*)

Possible future directions for the field of FBA modeling. (*a*) Metabolic network reconstruction is currently a multistep process (*gray arrows*). In the future, network reconstruction could be further developed by incorporating uncertainty into each step of the process, resulting in ensembles of GEMs and flux predictions as well as better integration of high-throughput phenotype data. This integration could include the use of AI techniques (such as large language models) to generate a GEM from a genome and phenotype data or from the genome alone. (*b*) FBA can be integrated with AI/ML techniques to create hybrid models (e.g., using neural networks to set reaction bounds). (*c*) FBA is integrated with other mechanistic models to create multiscale models. FBA may be connected to processes occurring at a smaller scale (e.g., detailed models of single reactions and their regulation) or at a larger scale (e.g., ocean biogeochemical models, where FBA could replace the parameterizations of microbial behavior). (*d*) While FBA models are generally built at genome-scale detail, ongoing efforts to apply the models to larger communities may require the creation of coarse-grained networks that only capture fundamental effective fluxes, sacrificing complete mechanistic details for feasibility. With their simple input–output relationship (*right subpanel*) consumer–resource models can be viewed as drastically coarse-grained but highly scalable versions of microbial metabolic dynamics. Abbreviations: AI, artificial intelligence; EC, Enzyme Commission; FBA, flux balance analysis; GEM, genome-scale metabolic model; ML, machine learning.

under different conditions, while taking into account the unavoidable constraints of stoichiometry (Faure et al. 2023).

We anticipate that FBA will also continue to evolve to address more complex and ecologically relevant questions in marine environments. Toward this goal, it will be beneficial to connect FBA to other modeling techniques, creating multiscale models that can extend the scope of simulations to either finer details or larger scales (**Figure 6*c***). This type of integration would require methods to interface the inputs and outputs of the different modeling techniques to allow them to communicate with each other (Agmon et al. 2022). For ocean scientists, future multiscale ocean biogeochemical models could call specific mechanistic models to obtain relevant microbial metabolic rates rather than using parameterizations of microbial function.

Multiscale modeling will be particularly important for understanding the role of microbial metabolism in large ecosystems. For these applications, it may become necessary to coarse-grain metabolic models of microbial communities to effectively capture metabolic diversity without having to know and simulate all details of each individual species (**Figure 6*d***). Finding the correct level of coarse-graining will be important to balance model accuracy with model feasibility (Lennon et al. 2024). Some potential strategies for coarse-graining would be to group related taxa or capture only the metabolic processes that are essential for larger scale cycling. An interesting avenue might be to explore the continuum of detail between dFBA and consumer–resource models (**Figure 5*d***). Conceptually, consumer–resource models are very similar to dFBA, except that different species are defined by fixed input–output relationships encoded in a matrix rather than by full metabolic networks (Goldford et al. 2018, Marsland et al. 2020). Possible intermediate levels between FBA/dFBA and consumer–resource models, such as mechanistic microbial ecosystem models (Mayerhofer et al. 2021), have barely been explored and may serve as valuable effective models for future environmental applications.

The field of metabolic network modeling and many of the variants and extensions of FBA are rapidly evolving and finding applications in multiple research areas, including marine biology and ecology. Some of the tools and examples mentioned above demonstrate the applicability of metabolic modeling for understanding and responding to challenges in sustainable ocean management. As shown in **Table 1**, FBA techniques can be directly relevant to the critical ocean management challenges defined by the United Nations Decade of Ocean Science for Sustainable Development for 2021–2030 (UNESCO-IOC 2021). The practical usability of models for accurate forecasts and informed decision-making at the ecosystem level will require increased

Table 1 Published and potential uses of FBA relevant to the United Nations Decade of Ocean Science for Sustainable Development Challenges

#	Challenge	Published and potential uses of FBA
1	Understand and beat marine pollution	■ Guide metabolic engineering efforts in microbes for plastic degradation (Lewis et al. 2020) and bioplastic production (Pardelha et al. 2012, Testa et al. 2021) ■ Assess the effectiveness of bioremediation strategies on pollutants accumulated in higher-trophic-level organisms by coupling FBA of a bioremediating microorganism with a food web bioaccumulation model (Taffi et al. 2014)
2	Protect and restore ecosystems and biodiversity	■ Profile the metabolic interactions of microbes in mangrove sediments (Du et al. 2022) ■ Characterize the effects of microbial communities and their biophysical microenvironments on the attachment and metamorphosis of coral larvae ■ Develop bioremediation techniques that can prevent or ameliorate dead zones
3	Sustainably feed the global population	■ Determine sustainable commercial feeds for salmon aquaculture that supplement amino acids in plant- and insect-based feeds (Zakhartsev et al. 2022) ■ Discover vaccine targets for *Piscirickettsia salmonis* (a pathogen affecting farmed salmon) (Fuentealba et al. 2017) ■ Analyze the nutritional requirements of commercial shrimp varieties and suggest modifications to feed to increase growth yield (Gao et al. 2021) ■ Guide cost-effective drug targeting and discovery against *Vibrio vulnificus* (a human pathogen causing skin infections and foodborne illness) (Kim et al. 2011)
4	Develop a sustainable and equitable ocean economy	■ Guide metabolic engineering efforts for the production of native and heterologous products in marine and freshwater phytoplankton (Ahmad et al. 2020a; He et al. 2015; Japhalekar et al. 2022; Vu et al. 2013; Yoshikawa et al. 2015, 2017) ■ Model microbially induced corrosion of steel in the deep sea (Rajala et al. 2022) ■ Elucidate the metabolic pathways of microorganisms that contribute to biocorrosion and rationally engineer protective biofilms that can prevent corrosion ■ Predict how mining might disrupt essential metabolic processes of microbial communities present at deep-sea mining sites
5	Unlock ocean-based solutions to climate change	■ Probe the carbon storage capabilities and ecological effects of macroalgae cultivation and deep ocean sinking ■ Probe the carbon storage capabilities and ecological effects of iron fertilization
6	Increase community resilience to ocean hazards	■ Develop better harmful algal bloom forecasts and mitigation strategies based on a mechanistic understanding of bloom-causing organisms
7	Expand the Global Ocean Observing System	■ Identify critical metabolic pathways for monitoring ■ Use flux predictions across large spatial and temporal scales to guide path planning and adaptive sampling of biological observations
8	Create a digital representation of the ocean	■ Analyze how gradients of nutritional constraints modulate metabolic reactions for phytoplankton across the global ocean (Regimbeau et al. 2023) ■ Enhance the representation of biological processes in ocean and Earth system models by using FBA to predict key biological rates within a multiscale model
9	Skills, knowledge, and technology for all	■ Ensure that core tools for metabolic modeling are free and open source ■ Ensure that previously reconstructed and curated GEMs are findable and accessible via databases (e.g., BiGG and KBase) ■ Develop more free and accessible materials to train those who are interested in FBA
10	Change humanity's relationship with the ocean	■ Use metabolic modeling to help scientists, policymakers, and citizens appreciate the role of cellular metabolism and quantitative science in restoring and maintaining ocean health

Abbreviations: FBA, flux balance analysis; GEM, genome-scale metabolic model.

efforts toward the advancement of new frontiers, including hybrid mechanistic/statistical learning approaches and integrated multiscale simulations, catalyzed by continued collaborations between experimental and theoretical scientists.

FUTURE ISSUES

1. Improvements are needed in automated curation and testing of genome-scale metabolic models based on phenotypic data under diverse environments and perturbations. This will benefit from improved standardization and accessibility of datasets.
2. Multiscale models should be generated that assume steady state, as in flux balance analysis (FBA), but incorporate elements of kinetics, e.g., when regulation of specific enzymes is essential for accurate prediction or when cell numbers are small and environmental fluctuations matter.
3. FBA models should be connected to larger-scale models, particularly Earth system models. Multiscale models, in this case, could solve ordinary differential equations for large-scale processes and call specific FBA models to obtain relevant microbial metabolic rates.
4. FBA and dynamic FBA models should be augmented with specific metabolic processes that happen extracellularly, including microbial death and degradation of biomass, extracellular enzyme activity, generation, decay, and metabolization of organic matter.
5. Variants of FBA should be explored that are able to incorporate the effects of environmental conditions such as temperature and pH on metabolic flux.
6. Artificial intelligence and machine learning, as well as available datasets, should be used to complement knowledge missing from FBA. This knowledge could range from regulatory interactions to protein structure and sensing/signaling networks.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We are grateful to members of the Segrè lab, Mary Ann Moran, and Daniel Sher for helpful feedback on the manuscript, and to many members of the National Science Foundation (NSF) Center for Chemical Currencies of a Microbial Planet (C-CoMP) for helpful conversations about applications of FBA to ocean microbes. We acknowledge support from the NSF C-CoMP Science and Technology Center (publication #043), the NSF–US-Israel Binational Science Foundation (BSF) (grant 2246707), and the Human Frontiers Science Program (grants RGP0020/2016 and RGP0060/2021). H.S. was also supported by the Boston University Kilachand Multicellular Design Program and by National Institutes of Health predoctoral training grant T32GM100842.

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