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A framework based on fundamental biochemical principles to engineer microbial community dynamics

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Simple rules to tame complexity in microbial communities

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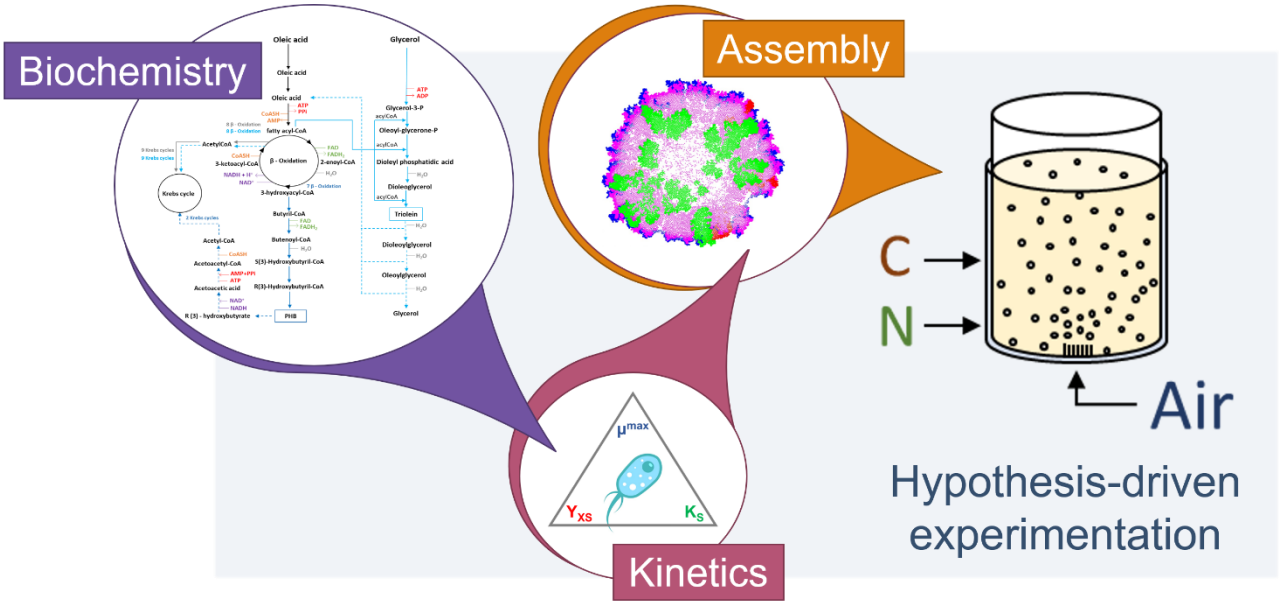
Highlights *(3 – 5, each 85 characters max.)*

- Individual microbial cell growth can be classified as a function of its metabolic biochemistry
- The spatial structure of a community is predictable and shaped by microbial dependencies
- Mechanistic models can be fitted to experimental 'omics' data
- Selective pressures can be exploited to shape microbial dependencies and community structure

Abstract *(100 – 120 words max.)*

Microbial communities are complex but there are basic principles we can apply to constrain the assumed stochasticity of their activity. By understanding the simple trade-offs behind the kinetic parameters that define microbial growth, we can explain how local interspecies dependencies arise and shape the emerging properties of a community. If we integrate these theoretical descriptions with experimental 'omics' data and bioenergetics analysis of specific environmental conditions, predictions on activity, assembly and spatial structure can be obtained reducing the *a priori* unpredictable complexity of microbial communities. This information can be used to define the appropriate selective pressures to engineer bioprocesses and propose new hypotheses which can drive experimental research to accelerate innovation in biotechnology.

Graphical abstract



Biotechnology needs to understand microbial communities

As the activity of complex microbial communities is defined by its robustness, vast diversity [1], fast evolution [2], and, in some cases, large metabolic flexibility [3], it is assumed that rational approaches to engineer these communities will always remain challenging. However, universal ecological rules that confine the stochasticity and heterogeneity of microbial communities is proving that in many cases, complex communities may not be as complex as we perceive them. Similar traits and assemblies are repeatedly observed across different systems, which are a consequence of specific interspecies dependencies [4]. Thus, better understanding of microbial dependencies can increase our capacity to engineer communities proposing more predictable, efficient, and malleable biosystems. In this perspective, we explore theoretical descriptions of microbial growth sustained by simple fundamental principles of chemistry and physics. We propose that equipped with this understanding, there is potential to go beyond models that are constrained by the availability of empirical measurements of kinetic parameters, to predict microbial dependencies and community assembly. By adapting this conceptual framework to specific cases [5], it can be potentially integrated with molecular approaches to explain and/or support their findings, overall increasing our predictive capacity [6]. We, of course, realise that dependencies within a microbial community are far more complex and nuanced than presented here, with predator-prey or/and parasite-host relationships, dependencies on metals, vitamins, light levels, and others not accounted for, at play [7]. However, we show that simplified microscale descriptions can be very useful to predict microbial emerging properties which can be subsequently used to design hypothesis-driven experimental research. We believe that these approaches have the potential for creating a platform to rationally direct biotechnological design, accelerating the process of proposing novel engineering systems capable of harnessing microbial activity to direct, control and predict it in a more efficient way.

I. Microbial growth: identifiable trade-offs between kinetic parameters

The development of theories of biochemical resource allocation have enabled the design of generic cell models that capture the essence of key physiological activities describing microbial growth [8]. In these models, maximising an individual growth rate (or minimising metabolic energy dissipation) is the assumed objective for each individual [9,10]. Although there are arguments that support the development of these highly complex models [11], resource allocation models require deeper understanding of specific cell functions which, inevitably, will subject them to complex calibration which can only be possible if full reconstructions of metabolic networks, quantitative proteomics and/or fluxomic data is available. Therefore, their application to complex microbial communities and environmental biotechnology although possible, is limited.

The development of advanced Monod-like approaches, have the potential to describe kinetic trade-offs that mechanistically constrain microbial growth within a community without requiring intracellular information [12]. In these approaches, a Monod curve is associated to each metabolic function within the community and characterised by a microbial growth yield (Y_{XS}, eq. 1 where α, β and γ refer to the stoichiometry of catabolism, anabolism and metabolism respectively and X to biomass concentration), a specific maximum growth rate (μ^{max}), and a half saturation constant (K_S) (eq. 2 where S refers to a specific limiting substrate). This can then be used to predict substrate consumption (eq. 3), product yield and biomass production (eq. 4) under specific conditions.

$$\begin{bmatrix} -\gamma_i \\ -\gamma_j \\ \vdots \\ \gamma_k \\ 1_x \end{bmatrix}_{met} = \begin{bmatrix} -\alpha_i \\ -\alpha_j \\ \vdots \\ \alpha_n \end{bmatrix}_{cat} \cdot \frac{1}{Y_{XS}} + \begin{bmatrix} -\beta_i \\ \vdots \\ 1_x \end{bmatrix}_{ana} \quad [1]$$

$$\mu = \mu^{max} \frac{S}{K_S + S} \quad [2]$$

$$\frac{dS_i}{dt} = -\gamma_i \cdot \mu \cdot S_i \quad [3]$$

$$\frac{dX}{dt} = \mu \cdot X \quad [4]$$

At cellular level, these kinetic parameters are not mechanistically describing specific cell activities as resource allocation models do. Yet, in many microbial systems relevant to environmental biotechnology, an estimation of Monod kinetics function of specific metabolic functionalities and their biochemistry, can provide the guidelines to understand survival and dominance within a microbial community under specific conditions. Quantitative values might require experimental data; however, in the following we show how it is possible to realistically estimate qualitative differences between kinetic parameters of specific metabolic activities by only analysing their biochemistry, which could serve as the first insight to describe microbial dependencies [13,14].

In order to simplify the following analysis, we will assume that all microbial species in the community utilise a similar anabolic process, requiring the same adenosine triphosphate (ATP) consumption per mole of biomass produced (ATP/X_{new}) and a similar anabolic rate under non-limiting conditions of energy (ATP) and carbon source. This analysis focusses on differences in catabolic pathways, but similar conclusions are obtained when the main differences are observed in the characteristics of the anabolic ones [15,16], or in cellular investments to increase resource acquisition [17].

Bioenergetics to predict maximum growth yield: The maximum growth yield (Y_{XS}^{max}) for any metabolic activity can be predicted as moles of ATP harvested per mole of substrate consumed (eq. 5) [18]. This can be calculated if the biochemistry of the specific metabolic activity is defined [18] but also estimated if it is not. All microbial catabolic pathways are shaped through thermodynamic constrains [19], aiming to maximise ATP production which occurs only via two methods: substrate level phosphorylation and generation of proton

motive force. Estimating that around 50 kJ are needed per mole of ATP produced [20], and knowing that membrane potential defines the energy required to translocate a proton, thermodynamics calculations can predict the potential ATP production associated to any catabolic activity.

$$Y_{XS}{}^{max} \equiv \frac{X_{new}}{S_{S\rightarrow P}} \cdot \left(\frac{ATP}{X_{new}}\right)_{cte} \equiv \frac{ATP}{S_{S\rightarrow P}} \qquad [5]$$

Pathway length to predict maximum specific growth rate: If the ATP requirement to produce each new mole of biomass is assumed to be constant, growth rates can be defined as ATP produced per unit of biomass and time (eq. 6). The maximum specific growth rate is defined under conditions of non-limiting substrate, and in these conditions and assuming a fixed anabolic rate, only differences in the catabolic rate define different μ^{max}. Although kinetic bottlenecks can arise from thermodynamic limitations in specific catabolic reactions [20,21], it has been postulated [22], and observed [23-26], that longer catabolic pathways will have on average a reduced maximum specific growth rate, as more reactions need to be performed per mole of substrate uptake. They will also tend to be more efficient, as if more reactions are performed per mole of substrate consumed, there are more opportunities for energy harvesting (Box.1) [27].

$$\mu^{max} \equiv \frac{X_{new}}{X \cdot t} \cdot \left(\frac{ATP}{X_{new}}\right)_{cte} \equiv \frac{ATP}{X \cdot t} \qquad [6]$$

Half saturation constants are linked to metabolic efficiency: The Monod-half saturation constant is defined as the substrate concentration at which the actual growth rate of the microorganism is half of its maximum specific growth rate. Following the rational exposed for predicting μ^{max}, substrate availability will be a more important limitation in the growth rate of microbial activities defined by shorter catabolic pathways with lower efficiency (Box.1). A lower substrate concentration surrounding the cell will imply a reduced rate of substrate diffusion over the membrane. If the catabolic pathway is short, substrate diffusion will

become the rate limiting process even under not-so-low substrate concentrations. For longer pathways with higher yields and an overall lower catabolic rate, less substrate is needed to produce more ATP, and substrate concentrations must be lower to make diffusion the limiting-rate process. If active transport occurs, shorter pathways would have to invest more energy to maintain their growth rate than efficient metabolisms [7]. Therefore for longer and more efficient pathways, higher affinities are expected [24]. However, broad ranges of half saturation constants for specific functionalities are found in literature. This might be explained by the fact that half saturation constant measurements are directly affected by the experimental conditions limiting the substrates' transport [28,29].

The rational to predict the values for fundamental kinetic parameters describing microbial growth, illustrates essential trade-offs between different organisms (Box.1). Under microbial competition, a specific organism cannot be the fastest (highest μ^{max}) and the most efficient (highest Y_{XS}) in all conditions. Differences in efficiencies and rates create dependencies between species catalysing different functionalities, allowing metabolic diversity to flourish (specially under thermodynamic constrains [30]). This defines the survival of specific activities, the assembly of the community and the stoichiometry of the overall bioprocess [31]. Efficient metabolic activities (superior growth yields and lower growth rates and half saturation constants), will tend to dominate in oligotrophic environments [13]. In contrary, faster growers, will tend to share resources (division of labour) [27]. The observation of these trade-offs between kinetic parameters in microbial populations (Box.1) might indicate that kinetics are not as flexible as was once thought [32], but importantly constrained by the specific metabolic activities microorganisms are catalysing.

Box 1. Metabolic characteristics can explain observed differences between the kinetic parameters that define the microbial growth of aerobic nitrifiers

Metabolic characteristic		Clade	Experimental values	Kinetic trade-off
Catabolic pathway length in the competition for ammonia	Ammonia oxidation to nitrite	Ammonia oxidizing bacteria	μ^{max} = 0.50–1.50d^{-1} [33] Y_{XS} = 0.020 C_{mol}/N_{mol} [24]	Faster and less efficient
		Ammonia oxidizing archaea	μ^{max} = 0.20–0.26 d^{-1} Y_{XS} = 0.025 C_{mol}/N_{mol} [24]	
	Ammonia oxidation to nitrate	Complete ammonia oxidizing bacteria	μ^{max} = 0.05 d^{-1} Y_{XS} = 0.031 C_{mol}/N_{mol} [24]	Slower and more efficient
Different terminal oxidises	Cytochrome aa_3-type	*Nitrobacter*	$k_{O2,aa3}$ = 4.3 µM [34] K_{O2} = 24.5–272 µM [35]	Lower half saturation constant if the terminal oxidise has higher affinity
	Cytochrome *bd*-like	*Nitrospira*	$k_{O2,bd}$ = 3.2 µM [34] K_{O2} = 3.0-3.5 µM [35]	
	Cytochrome cbb_3-type	*Nitrospina*	$k_{O2,cbb3}$ = 0.23 µM [34] K_{O2} = 0.8 µM [36]	
Different nitrite oxidoreductase (NXR)	Cytoplasmic NXR	*Nitrobacter*	K_{NO2} = 49–544µM [35]	Lower half saturation constant correlates with the presence of a periplasmic NXR
	Periplasmic NXR	*Nitrospira*	K_{NO2} = 9–27µM [35]	
Different anabolic pathways	HP/HB cycle	Ammonia oxidizing archaea	Y_{XS} = 0.025 C_{mol}/N_{mol} [24]	HP/HB cycle consumes less ATP than CBB cycle per mole of biomass produced [37]
	CBB cycle	Ammonia oxidizing bacteria	Y_{XS} = 0.020 C_{mol}/N_{mol} [24]	
Different anabolic pathways for nitrite oxidisers	O_2-tolerant rTCA	Nitrite oxidising bacteria (*Nitrospira*)	Y_{XS} = 0.008 C_{mol}/N_{mol} [35]	O_2-tolerant rTCA consumes less ATP than CBB cycle per mole of biomass produced [38]
	CBB cycle	Nitrite oxidising bacteria (*Nitrobacter*)	Y_{XS} = 0.005 C_{mol}/N_{mol} [35]	

II. Microbial community assembly: dependencies between species and the environment

The microbial activity of the individuals, it is responsible for creating dependencies between different species within the community meanwhile modifying the local environment. This can be beneficial or detrimental for theirs and others survival [39]. As microbial dependencies occur at the microscale level and function of the local environmental conditions surrounding each cell, the emerging properties of the community must be predicted at the microscale level. However, microscale models are limited by the availability of parameters and our inability to upscale them [40]. No mathematical model can account for a comprehensive description of all the processes occurring at all the scales within a microbial community [5,41]. However, by applying the above outlined guidelines to describe the kinetics of microbial growth, probable microbial dependencies can be defined which give insights of the overall community assembly. To further extend this analysis to the whole community while maintaining its relative simplicity, we propose to integrate it with information regarding: i) the characterisation of the metabolic potential of the community; ii) the bioenergetics of the environment; and iii) the prediction of the spatial distribution of the microbial species within the community.

Characterisation of the metabolic potential of the community: The rapid development and now widespread application of '*omics*' approaches is generating enormous amounts of data and shifting the bottleneck from their application that was expensive in the past, towards their downstream processing and analysis [6]. While molecular approaches can characterise the metabolic potential of complex communities [42,43], and can, when complemented with time-series and/or experimental approaches, reveal information on changes in community function associated to specific environmental pressures [44], they are generally constrained from providing the mechanistic understanding for these changes [45]. In contrast, mechanistic models describe environmental selection in a more meaningful

way, which in turn could then be validated by molecular approaches [46]. However, mathematical models cannot accurately characterise an inoculum, or predict stochastic aspects of spatial distributions of populations (e.g. along a filter for drinking water treatment [47]), or the migration of new species entering with a feeding stream [48]. We argue that the integration of '*omics*' data in the construction of microscale mechanistic models can generate a powerful computational framework for understanding communities. The experimental molecular data can fit the models to specific experimental set-ups, meanwhile models could also contribute to process the molecular data integrating mechanistical understanding to direct their meta-analysis, increasing the overall predictive capacity.

Characterization of the environmental energy of a cell's local surroundings: As described above, bioenergetics analysis of catabolic pathways can predict the maximum ATP that can be produced from a specific mole of substrate. However, the actual energy harvested by a cell is defined by environmental conditions. Therefore, environmental energetics' analysis can be used to assess the limits these conditions impose on the activity of the community. When applied to the local conditions surrounding the cells belonging to a community, this type of analysis can be used to assess the dominance of specific metabolic activities over others [49-51], relative abundances [52], potential diversities [30], metabolic flexibility of specific microorganisms [3,53], and opportunities for microbial evolution [54]. This will also serve in some cases to predict the existence of not-yet-reported metabolic functionalities [13]. If reconstruction of biochemical pathways can be developed through molecular information, bioenergetics analysis can help predict the direction of these pathways [20], and their dominance over others within a community of any given system.

Characterization of the spatial structure of a community: The metabolic dependencies established between microbial species and nutrient gradients at microscale combine to shape the spatial structure of a community [55-57]. In bioengineered systems, communities will form microbial aggregates such as granules, biofilms or flocs or indeed a mixture of all

of these [58]. These spatial structures are defined by microbial dependencies and in some cases, space will also act as another selective pressure, favouring or limiting specific activities [31]. Short metabolic pathways will favour metabolic division of labour that generates identifiable spatial assortments (Figure 1). In these assortments, microorganisms carrying out the sequential metabolic steps of a given process, are the slower growers as they depend on the activity of others. Although faster individuals will push them taking up the available space and common substrates (e.g. oxygen), they can survive competition by forming individual clusters (Figure 1.a) (or microcolonies) [59]. This spatial advantage could explain in some cases the difficulty to remove their activity from specific systems, even when imposing unfavourable operational conditions for their growth [60].

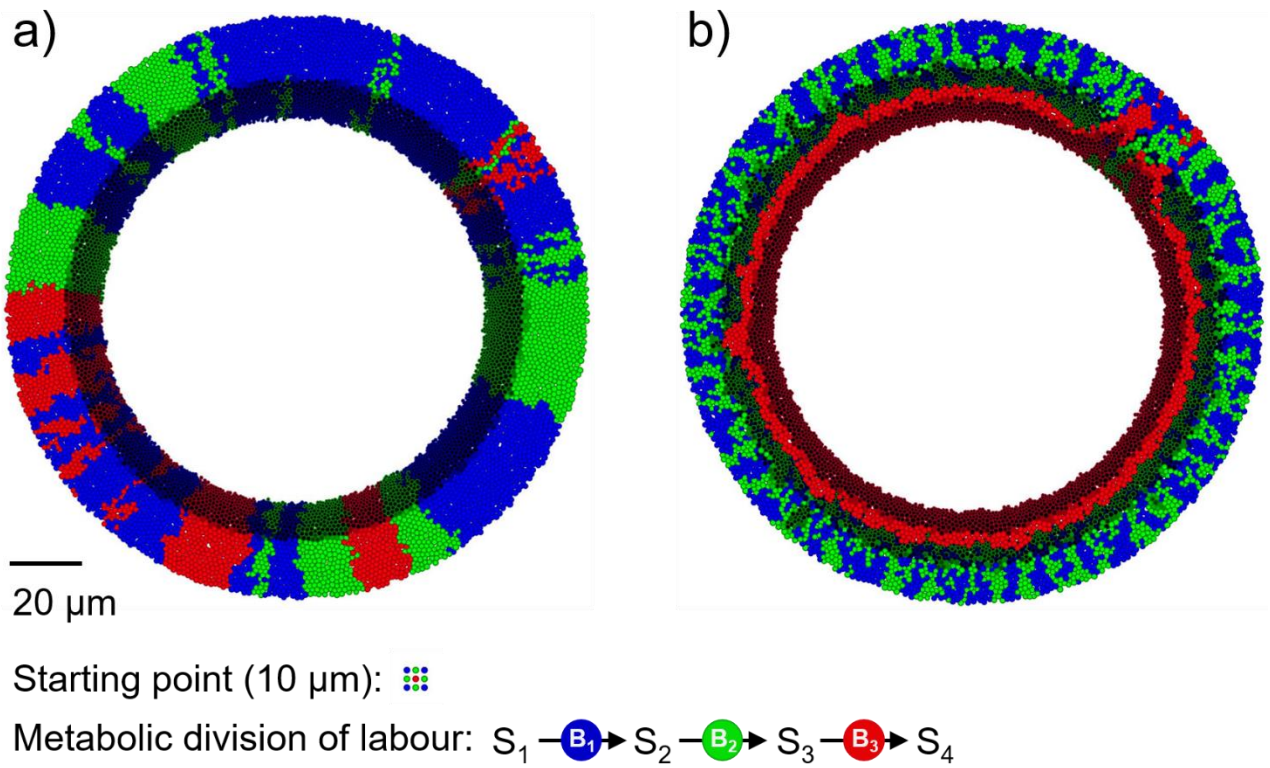


Figure 1: Simulations of the spatial structures of a granule growing in a continuous reactor in which S_1 (substrate 1) is fed maintaining a constant concentration of 1 mM in the liquid bulk of the reactor (hydraulic retention time adjusted). a) aerobic system (each metabolic activity consumes one mole of O_2); b) anaerobic system. For all the metabolic activities μ^{max} = 1 day^{-1}; maintenance = 0.4 day^{-1}; K_S = 10 µM; K_{O2} = 1 µM; Y_{XS} = 0.1 C_{mol}/S_{mol} for

the aerobic system and Y_{XS} = 0.01 C_{mol}/S_{mol} for the anaerobic one. Simulations use the equations presented in [40,49].

Community dynamics can be predicted in case it is understood how specific metabolic activities define growth kinetics of the involved microorganisms, and with it, the microbial dependencies that shape community assembly creating identifiable structural patterns under specific environmental conditions [61]. This information can be used to generate hypotheses that will enable us to direct and engineer microbial communities in a predictable manner.

III. Knowledge for application: imposing the right selective pressures to engineer the community

By choosing the correct selective pressures it is possible to change the community assembly and direct its specific metabolic stoichiometry (Figure 2) [62-64]. In biotechnology, unless the objective is to oxidise easily degradable organic matter, we will want to favour metabolic activities that store energy in the targeted product (e.g. methane, biofuels, polyhydroxyalkanoates, production), or grow using energy-limited substrates that minimise biomass yield (e.g. pollutants, nutrients). This implies that the majority of these bioprocesses are energy limited systems, catalysed by microbial networks [27,65] that (with limits [66]) maximise productivity [67]. Traditionally, chemostats are used in biochemistry ensuring the most favourable conditions to produce the compound of interest. This could imply that in some cases, increasing substrate influx or maintaining higher temperatures, we are favouring shorter pathways over efficient ones [27,68]. Therefore, interesting and efficient metabolic activities, might be not reported yet, and remain hidden in natural environments with lower substrate influx and longer residence times, and are missed in many bioprocesses as they have not being engineered for their dominance [69].

Under anaerobic conditions, environmental biotechnologies use microbial communities defined by division of labour where microorganisms catalyse short steps (e.g. anaerobic digestion, fermentations). In these metabolic chains, microorganisms catalysing the later steps grow slower, creating (if the environmental conditions allow) stratified biofilms and granules (Figure 1.b). In this conditions, longer retention times, larger granules' diameters, or biofilms' thicknesses can favour the elongation of the overall metabolic activity of the community [70]. Contrary, when division of labour occurs under aerobic conditions (e.g. nitrification), the selection of specific microbial activities might be more complicated. This is because, although microorganisms catalysing the later steps grow slower, they all compete for O_2, which reduces growth rate differences protecting overall metabolic pathway elongation (Figure 1.a) [60].

Other environmental variables can be used to exert selective pressures over the community. In many cases, pH affects specific substrate availability (e.g. microorganisms might preferable consume the protonated instead the deprotonated form of a substrate), which can play a role in selecting faster growers versus efficient ones or reduce/promote the elongation of the overall metabolic activity of the community (reducing or increasing the access to substrate of a specific population) [62]. pH can also affect differently the physiology of each individual [71] and its capacity to harvest energy for growth, which influences their survival and the overall community assembly. A similar analysis can be done for other environmental variables such as salinity, light, presence of toxic compounds, etc. In many cases, the effect of these variables on microbial growth could be directly classified function of their capacity to limit microbial growth rate (to be fast, e.g. nutrient availability), or energy harvesting for growth (to be efficient, enduring unfavourable conditions will cost ATP), allowing us to analyse their effect on the microbial dependencies observed in the community using the simple analyses exposed above. A specific example of how these metabolic analyses can direct experimentation is presented in Figure 2.

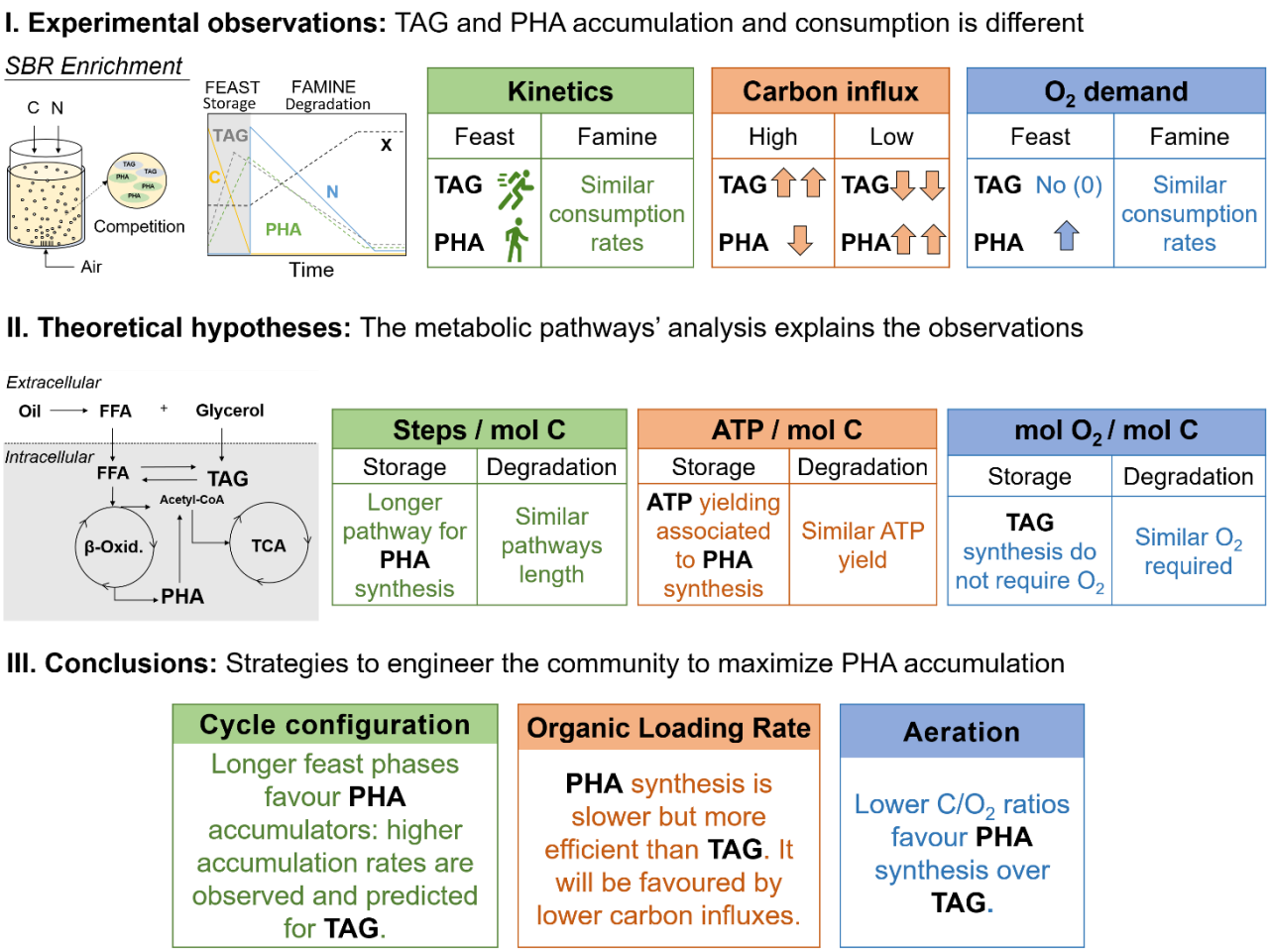


Figure 2: Formulating hypothesis driven experiments to optimise polyhydroxyalkanoates (PHA) accumulation when competing with triacylglycerides (TAG) accumulation in a sequential batch reactor (SBR). References for experimental observations: faster TAG accumulation than PHA [72]; higher TAG accumulation at higher carbon influx, and higher PHA accumulation at lower carbon influx [73,74]; oxygen consumption on PHA accumulation [75]. Abbreviations: ATP (adenosine triphosphate), carbon (C), nitrogen (N), free fatty acids (FFA), active biomass (X).

Conclusion: a rational platform for directing biotechnological experimentation

Even if microbial communities hold large heterogeneity, applying a first principles approach to understand the ecological patterns that control microbial interspecies dependencies and community assembly under specific conditions, we can define the limits of their activity. A potential computational platform for rationally directing experimentation supported by 'omics' data, bioenergetics analysis of the environment, and microscale descriptions of the community spatial distribution, could contribute to the acceleration of innovation in biotechnology directing experimentation in a meaningful way.

Acknowledgments

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