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Microbial inefficient substrate use through the perspective of resource allocation models

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Abstract

Microorganisms extract energy from substrates following strategies that may seem suboptimal at first glance. Beyond the so-called yield-rate trade-off, resource allocation models, which focus on assigning different functional roles to the limited number of enzymes that a cell can support, offer a framework to interpret the inefficient substrate use by microorganisms. We review here relevant examples of substrate conversions where a significant part of the available energy is not utilized and how resource allocation models offer a mechanistic interpretation thereof, notably for open mixed cultures. Future developments are identified, in particular, the challenge of considering metabolic flexibility towards uncertain environmental changes instead of strict fixed optimality objectives, with the final goal of increasing the prediction capabilities of resource allocation models. Finally, we highlight the relevance of resource allocation to understand and enable a promising biorefinery platform revolving around lactate, which would increase the flexibility of waste-to-chemical biorefinery schemes.

Introduction

The most common interpretation of the competitive exclusion principle [1] is that microbial metabolism must tend to optimality in the use of the limiting substrates. This quest for optimality would have a different expression for pure cultures, co-cultures, open mixed-cultures, and would be subjected to spatial and temporal heterogeneity. Focusing on the prediction of intracellular fluxes for *E. coli*, Schuetz et al. [2] showed that the maximisation of biomass (or ATP) yield, the most common way to translate metabolic optimality, was indeed consistent with the experimental results in substrate (i.e. carbon source) limited conditions. Microorganisms are assumed to behave like efficient scavengers that extract as much energy as possible from the substrate. However, in batch cultures, temporarily provided with limitless substrate, the best predictions were given by maximising the ATP yield per flux unit, or equivalently, maximising the energy yield while minimising the enzyme use. Actually, how to express optimality becomes even more complex when several microorganisms are present and the observed experimental behaviour may seemingly depart further from the expected efficient metabolic paradigm. If an efficient metabolism is the one capable of extracting the most energy (i.e. ATP) from the substrate, a large number of experimental results [3–8] prove that efficiency is not a fixed condition for dominating in natural or engineered environments.

In this review, we first briefly summarise previous explanations of this inefficient use of substrate and then resource allocation modelling is proposed as the most satisfying mechanistic framework to explain optimality under different environmental conditions.

(Apparently) inefficient microbial behaviours

From the experiments, we observe that microorganisms change how efficiently they use the substrate (i.e. carbon source) depending on its availability (Fig. 1). For instance, yeasts such as *S. cerevisiae*, which rely on fermentation for growth, yield ethanol under high

substrate availability conditions, even with excess supply of oxygen, leading to a sixteenth fraction of the ATP produced under complete mineralisation [3,4], which is usually named the Crabtree effect. Some aerobic bacteria (e.g. *E. coli*) present a similar behaviour and excrete acetate when the substrate concentration is high, usually referred as acetate overflow [5]. Mammalian cells can also consume glucose inefficiently and convert it to lactate in presence of oxygen, which is named in this case as the Warburg effect. Particularly, this behaviour is usually shown by rapidly proliferating cells (e.g. cancer cells) or by highly active striated muscle cells [9]. These three examples have in common that glucose is only partially metabolised but differ in that only in acetate overflow oxygen consumption is still present to maintain the electron balance and regenerate NAD^+ for glycolysis. Other difference is that while acetate overflow and the Crabtree effect occur in response to a change in environmental conditions (i.e. substrate availability), the Warburg effect is linked to a change in regulation: cancer cells rely on glycolysis to proliferate faster and muscle cells when contraction activity requirements are high [9].

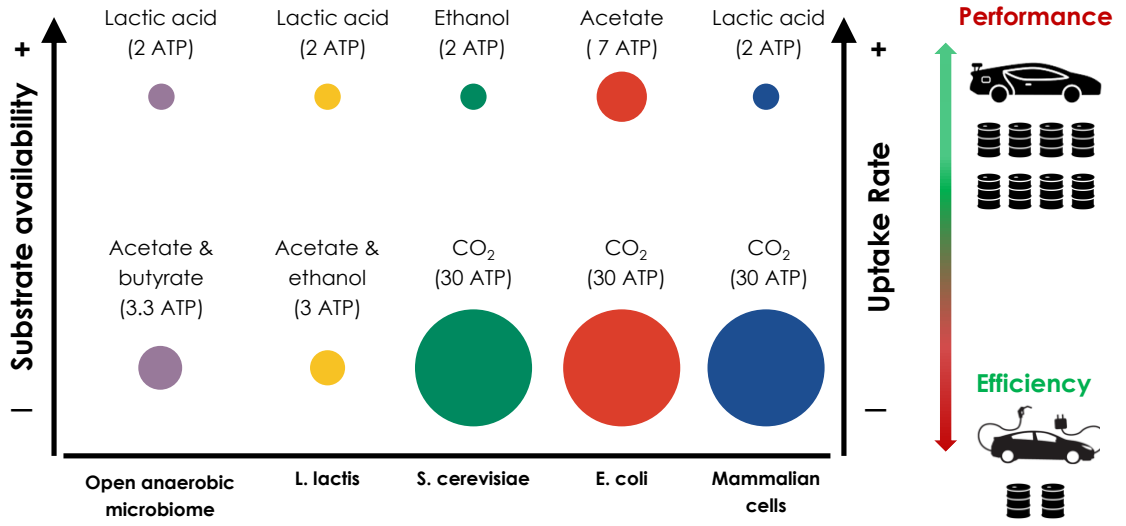

Fig. 1. Microorganisms express different phenotypes depending on substrate availability. When it is low (e.g. a continuous reactor operated at low substrate flux), an efficient metabolism is promoted that squeezes substrate ATP production potential (as a hybrid car

makes the most of a litre of fuel). On the contrary, at high substrate availabilities, microorganisms generally opt for strategies that do not optimise ATP production from the substrate but allow for a faster substrate uptake (as a sports car is designed to optimise its performance regardless of fuel consumption or efficiency). Circle areas are proportional to the ATP yield per glucose of each phenotype. In complete and partial (i.e. acetate overflow in *E. coli*) aerobic phenotypes, NADH and UQH_2 were estimated to produce 2.5 and 1.5 ATP per molecule, respectively, and the cost of transporting NADH into the mitochondria to consume 1 ATP per molecule.

In the case of anaerobic bacteria, lactic acid bacteria abandon their typical lactate production and shift towards a higher energy yielding acetate and ethanol conversion, which provides 50% more ATP per unit of substrate, at low dilution rates in a continuous reactor [6]. Anaerobic open microbiomes (i.e. mixed communities that are permeable to the entry of new strains from the surroundings), that could be considered to have a higher driving force to behave efficiently due to the fierce competition among their constituents, also may behave inefficiently (Fig. 1). It was recently shown that, in a discontinuous reactor, lactate is the main product of glucose anaerobic fermentation even though its ATP yield is the lowest of all possible products of the process [7]. These evidences have puzzled microbiologists for decades as it is striking that competitive exclusion principle selects clear inefficient behaviours that do not extract as much energy as possible from the substrate.

Hypotheses for inefficient behaviours

A recurrent hypothesis was that yeasts started to produce ethanol at a certain growth rate due to limitations in the cellular membrane for accommodating the electron transfer chain [10,11]. At a certain catalysed substrate flux, the maximum capacity is reached, and oxygen consumption could not be increased further. An anaerobic version of this limitation was

proposed by González-Cabaleiro *et al.* [12], which limits the rate of electron transport in catabolic reactions. However, experiments show that oxygen consumption rates actually decrease at increasing growth rates, indicating that the respiration capacity is not fully utilised and, therefore invalidating this hypothesis [13,14]. Another competing explanation, the chemical warfare hypothesis, states that the motivation of producing ethanol or carboxylic acids is to displace other competing species as they are likely to have a lower tolerance to their toxicity [15]. However, this hypothesis is not consistent with these chemicals being produced only during substrate abundance, i.e. when substrate availability is not the limiting growth factor.

The metabolic division of labour hypothesis affirms that in environments with high substrate fluxes (i.e. where substrate is highly available), substrate conversion is done in several steps (and performed by different microbial populations) rather than being completely converted by a single microbial species, as in low substrate flux conditions [16–18]. This hypothesis is based on the theory of optimal pathway length which states, qualitatively, that longer pathways generate a higher ATP yield and that the total enzymes concentration is limited, which result in short pathways having a higher enzyme concentration for each metabolic step [19]. Therefore, shorter pathways can attain higher substrate uptake rates, but at the expense of a lower ATP yield. In this sense, it is already suggested that in microbial systems there is a trade-off between attaining a high substrate uptake flux or using the substrate efficiently (which is also named the rate vs yield trade-off [15,20–22]). Other authors consider that the apparent trade-offs between rate and yield are not necessarily an inescapable physical constraint and that are evolved cellular properties. In replicated long-term chemostat experiments under substrate limitation, it was reported that around half of *E. coli* strains developed spontaneously cross-feeding phenotypes [23] and Meijer et al. [24] simulated evolutionary trajectories showing that, even for substrate-

limited chemostats, the emergence of metabolic labour division was an "evolutionary contingency".

The resource allocation theory

The key aspect came when thinking of cells as self-replication systems needing a certain machinery (i.e. enzymes) to function, as factories need machines to produce goods, and that models should consequently consider this factor [25–27]. From this conception the theory of resource allocation emerged, which is at the present time the most convincing theoretical framework to mechanistically explain the previously mentioned inefficient substrate use. This theory states that cells are constrained by having a limited available protein (i.e. enzymes) concentration [13]. The different cellular processes, e.g. catabolism, membrane transport, anabolism, compete for a finite protein pool that should be allocated carefully to maximise fitness, which can be defined as the success of replication of organisms competing for the same resources [15].

Models including concepts from the resource allocation theory include self-fabrication models [25,28], balance-growth models taking into account proteome allocation [29] or approaches minimizing the enzyme cost for the maximum biomass production rate in comprehensive formulations of cellular metabolism [26], but the most usual modelling approaches are Flux Balance Analysis (FBA) with additional constraints related with the limits of the protein pool [13,30–37], which is therefore the main focus of this review. These models include one or more constraints imposing an upper limit on the global protein concentration or sections of the proteome (e.g. protein concentration allocated to membrane processes), which is determined using experimentally determined values of enzymatic activities and the metabolic fluxes values determined *in silico* by the model (BOX 1). The determined cellular fluxes (i.e. the model solution) are then constrained by the concentration of the enzymes catalysing them, including the own enzyme synthesis (i.e. the

self-replicating anabolism). In this case, the models are usually referred as FBA with molecular crowding (FBAwMC) [37], as they place an upper bound on the enzyme crowdedness within cells, or Constrained Allocation FBA (CAFBA) [30], since fluxes are additionally constrained by proteome allocation.

<BOX 1 should be place approximately here>

Resource allocation modelling identifies a microbial trade-off between efficiency and flux

Resource allocation models were used successfully to explain microbial behaviours that are not correctly captured with other metabolic modelling approaches (Table 1). The results of the models have a common thread: there exists a trade-off between efficiency and flux. Assuming that the ATP requirements to form biomass are relatively constant at different environmental conditions, to maximise the specific growth rate cells have to either maximise the specific substrate uptake rate (q_S in Eq. 1) or the ATP yield on the substrate ($Y_{ATP/S}$ in Eq. 1).

$$\mu = q_s \cdot Y_{ATP/S} \cdot Y_{X/ATP} \quad (1)$$

where μ is the specific growth rate (h^{-1}), q_S is the specific substrate uptake rate ($mol_S \cdot Cmol_X^{-1} \cdot h^{-1}$), $Y_{ATP/S}$ is the ATP yield on the substrate ($mol_{ATP} \cdot mol_S^{-1}$) and $Y_{X/ATP}$ is the biomass yield on ATP ($Cmol_X \cdot mol_{ATP}^{-1}$).

Strains relevant in the biotechnological field present a broad range values of maximum substrate uptake rate and of ATP yield on the substrate, which illustrates the high phenotypic plasticity of metabolism. Values span from 0.2 $mol_S \cdot Cmol_X^{-1} \cdot h^{-1}$, shown by bacterial open microbiomes yielding butyrate [7], to values up to 0.8 $mol_S \cdot Cmol_X^{-1} \cdot h^{-1}$, displayed by *E. faecalis* [38], a lactic acid bacteria, which shows the specialisation degree of this bacterial group in consuming substrate at high rates. Eukaryotic cells present

intermediate values of around 0.35 $mol_S \cdot Cmol_X^{-1} \cdot h^{-1}$ for *E. coli* and *S. cerevisiae* [11,30,38]. Values regarding the ATP yield on the substrate are available in Fig. 1.

In the cases that there is a duality in catabolic strategies (e.g. Crabtree effect or acetate overflow), a common pattern emerges, which is related to different proteomic efficiency (i.e. proteome fraction needed to catalyse a certain flux). There is a "premium" catabolism that provides a high ATP yield but at the expense of having a low proteomic efficiency; and a "low cost" catabolism that is characterised by displaying a high proteome efficiency but with a lower ATP yield on substrate. For example, glucose fermentation to acetate provides almost 10 times less ATP than its complete oxidation to CO_2 and water, but its proteome is 50% more efficient in generated the same ATP flux [29].

Model results indicate that, at low substrate (i.e. carbon source) fluxes, the proteome does not limit cell growth as all metabolic fluxes are low and both catabolic options can sustain a similar substrate uptake rate (q_S). Therefore, the preferred option is the premium catabolism for its superior ATP yield, as it is case of low substrate flux systems such as continuous reactors [39,40] or biofilms [41,42]. Despite possible substrate availability, growth is constrained by cells struggling to capture the substrate from the medium since the limiting factor is the transport capacity, i.e. the concentration of enzymatic transporters in the membrane (Fig. 2). If the substrate is hard to obtain, it makes sense to be efficient and squeeze as much ATP from it as possible. However, at high substrate fluxes the premium catabolism can only catalyse an inferior uptake rate than the low-cost catabolism due to its low proteomic efficiency: cells cannot keep up with the substrate flux of the system. Under these conditions, growth is constrained by the capacity to transform the carbon source and the proteome located in the cytoplasm is the limiting factor (the red area of Fig. 2B starts to diminish as the proteome allocated to membrane transport is not the limiting factor). The low-cost catabolism can provide a superior uptake rate thanks to its

higher enzyme efficiency and above a certain substrate flux it can overcompensate for its characteristic low ATP yield, as observed in high rate continuous reactors [6,14,43] or discontinuous reactors [7]. Now, being fast at consuming the substrate is the winning strategy as a way of capturing as much substrate as possible and to limit the substrate availability of competitors. That these strategies "waste" part of the substrate potential to generate ATP is not a decisive factor since there is more substrate available than the cells can actually use. Leaving the substrate partially consumed has a side implication the creation of a new microbial niche for other microorganisms that can grow on finalising the metabolic conversion to the products of the premium catabolism, which is usually termed cross-feeding or division of labour [16,19,44].

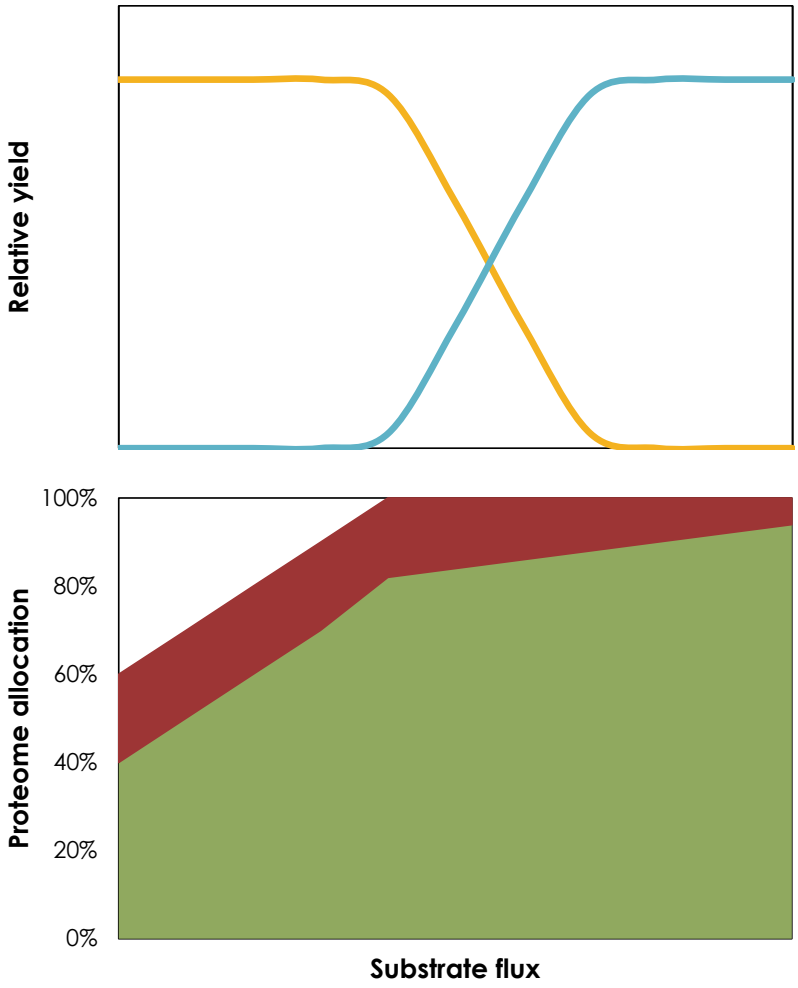

Fig. 2. A: Predicted phenotype: yields of premium (yellow) and low-cost (blue) catabolism at increasing substrate fluxes. B: Allocation of the membrane (red) and cytoplasm (green) proteome at different substrate fluxes. Proteome concentration is expressed in relation to the maximum global concentration. Membrane proteome is limited here to 20% of the maximum global proteome.

Table 1. Description of examples of resource allocation models.

Explained behaviour	Microorganisms involved	Kind of model	Outcome of the resource allocation model	Ref
Crabtree effect: Production of ethanol by yeasts in aerobic conditions	*S. cerevisiae*	FBA with molecular crowding constraint for global proteome	The model identifies that the higher protein efficiency of fermentation with respect to respiration (i.e. more ATP produced per protein mass) is responsible for the switch to ethanol production.	[13]
		FBA with molecular crowding constraints for global, external membrane and mitochondrial membrane proteome.	The aim of this model is to unravel the evolutionary trajectories that led to the Crabtree effect. It relates the appearance of the Crabtree effect to the change from a glucose-proton symport mechanism to a glucose uniporter, which does not consume energy for glucose uptake. Additionally, it proves that an increased total protein content would eliminate the appearance of Crabtree effect under any condition.	[45]
Acetate overflow by aerobic bacteria in sufficiently aerated conditions	*E. coli*	FBA with molecular crowding constraint for global proteome	The models reproduced the observed acetate excretion at high growth rate conditions. Both models reveal a trade-off between biomass yield maximisation and protein cost minimisation. Acetate production shows a lower proteomic cost and is advantageous only at higher growth rate (i.e. at high substrate fluxes)	[30,32,33]
		FBA with molecular crowding constraint for membrane proteome	This model proposed a direct link between cell morphology and physiology as a single constraint (i.e. maximum membrane occupancy) dictates the regulation of the metabolism of *E. coli*. It predicts correctly the growth rate under excess substrate conditions (i.e. batch conditions). With respect to other models that do not consider membrane-related constraints, it does correctly predict the experimentally observed oxygen consumption decrease at high substrate uptake rates.	[11]
	B. subtilis	Resource balance Analysis (self-fabrication model) with molecular crowding constraints for global and membrane proteome	This model, calibrated using genome-wide absolute protein quantification data, accurately predicts how *B. subtilis* allocates its proteome under different growth conditions. Moreover, it shows that the experimentally observed regulation patterns are consistent with the objective of growth rate maximisation, except for some processes that are not optimally regulated. In this case, it proposes that a suboptimal regulation is the result of addressing other more complex objectives (e.g. coping with stressful conditions or bet hedging).	[28]
Warburg effect: Production of ethanol by	*H. sapiens*	FBA with molecular	A mechanistic explanation is provided assisted by the model to why	[8]

mammalian cells in aerobic conditions		crowding constraint for global proteome and maximum glucose uptake rate	mammalian cells with high glucose uptake rate produce lactic acid. It states that the higher efficiency of a lactic acid catabolism in terms of the required solvent capacity is responsible for this phenotype.	
		FBA with several and varying constraints (e.g. upper bound on glucose uptake or maximum enzyme investment)	In this minimal FBA model, the authors predict different cellular phenotypes (i.e. pure respiration, pure fermentation and a mixture of both) depending on the constraints applied to each case. Pure respiration is predicted when its enzymatic cost is low; a mixture of respiration and fermentation is predicted if respiration is costly and the substrate availability is limited.	[46]
		FBA with solvent capacity constraints of metabolic enzymes and mitochondria and an upper bound on respiration rate.	The model demonstrates that the activation of aerobic glycolysis (i.e. Warburg effect) is favoured above a threshold metabolic rate in both rapidly proliferating cells (e.g. cancer, lumphocyte or hair follicle cells) or heavily contracting muscle as it provides a higher ATP yield per volume density than mitochondrial respiration.	[9]
Lactic acid bacteria (LAB) perform a high ATP yield catabolism (i.e. acetate-ethanol) at low growth rates while change its catabolism to lactate production only at high growth rates (see [6] for a detailed experimental description)	*L. lactis*	FBA with molecular crowding constraint for global proteome	The model predicts that *L. lactis* performs a high biomass yield catabolism at low growth rates (i.e. acetate-ethanol) and that only at high growth rates lactate is the main product of its catabolism due to its lower enzymatic requirements. At those conditions, attaining a higher substrate uptake rate overcomes the lower associated biomass yield of lactate yielding.	[31]
LAB show a higher maximum specific growth rate and a much higher maximum uptake rate that other anaerobic glucose consumers such as butyrate-acetate producers but are usually auxotrophic for amino acids and some vitamins and need a rich fermentation medium. Therefore, the microbial community of rich medium open microbiome discontinuous reactors is dominated by LAB [7].	LAB (*Lactobacillus* and *Lactococcus* genera) and butyrate-acetate producers of the *Clostridia* class (*Ethanoligenens* and *Clostridium* genera).	FBA with molecular crowding constraints for global and membrane proteome	This work identified that the auxotrophic anabolism of LAB represents a competitive advantage as it allows for a higher maximum specific growth rate. Auxotrophic bacteria uptake amino acids and vitamins from the medium and therefore do not need to allocate enzymes to synthesise these compounds. They feature a more efficient anabolism in terms of enzyme usage, which is advantageous at high substrate flux situations and allows them to overcome their lower ATP yield on the substrate with respect to the acetate-butyrate catabolism of their competitors.	[34]
Presence of polyphosphate accumulating organisms in anaerobic areas of aerobic activated sludge reactors.	Polyphosphate Accumulating Organisms (PAOs)	Conditional flux balance analysis (cFBA), essentially a dynamic FBA with proteome capacity constraints	The model simulates an environment in which oxygen is unavailable periodically and shows that PAOs are more competitive than other accumulating microorganisms (e.g. glycogen accumulators) in such environments.	[35]

Resource allocation models and microbial ecology and morphology

Apart from providing a plausible mechanistic explanation to behaviours of apparent inefficient substrate utilisation, resource allocation models may provide valuable information about the selective pressures acting on microorganisms under different environmental conditions (see [47] for a detailed analysis of resource allocation metabolic implications). This information can help to understand how and why pure species adapt in a changing environment or what will be the most likely outcome of microbial competition in an open microbiome reactor. The results of resource allocation models indicate that mainly two constraints limit growth depending on the substrate flux: membrane proteomic capacity at low substrate fluxes and cytoplasmatic proteomic capacity at high substrate flux conditions [11,34]. Experimental observations indeed indicate that microorganisms adapt to these varying selective pressures and modify their size [48,49]. At low growth rates, cells shrink to maximise the surface area, which allows increasing the membrane proteome capacity with respect to the global proteome capacity. On the contrary, at high growth rate cells tend to be bigger in volume as a way of lowering the area-to-volume ratio and maximising the proteome capacity in the cytoplasm, since transport is no longer the growth limiting factor.

A possible evolutionary role of auxotrophism of lactic acid bacteria (LAB) was proposed recently with a resource allocation model [34]. The results of this model indicate that the typical auxotrophy of LAB for some amino acids and vitamins allows them for attaining a higher maximum specific growth rate than other competing bacteria (e.g. butyrate-acetate producers of the *Clostridia* class or prototrophic LAB). Since LAB do not have to synthesise *de novo* these compounds, it is not necessary to allocate enzymes to these tasks and therefore their anabolism is more efficient in terms of enzymes, freeing thus proteomic capacity for other purposes (e.g. catabolism). In this case, the differential factor in the

microbial competition lies in the anabolism and not in a premium and low-cost catabolism. Given that most common LAB are natural to environments where peptides are available (e.g. milk or grass) [50], it could be reasonably hypothesised that losing the ability to build these compounds was positively selected by competitive selection, as already suggested in some studies: D'Souza *et. al* [51] showed in propagation experiments that *E. coli* rapidly developed an auxotrophic genotype for amino acids when supplementing amino acids in the cultivation media.

Limits to resource allocation models

Resource allocation models helped us explain satisfactorily some challenging microbial behaviours and predict parts of their phenotypes (i.e. their main catabolic products). However, some predictions regarding the actual proteome distribution do not match experimental observations. For example, experiments with *Lactococcus lactis* showing a switch from a catabolism yielding acetate-ethanol (premium catabolism) to solely production of lactate (low-cost catabolism) at increasing dilution rates in a continuous reactor, do not show strict proteome regulation [52]. Metabolic regulation is apparently done, in this case, using post-translational modifications as it keeps enzymes of both catabolic branches highly expressed at all conditions, which is in detriment of cellular performance and growth rate according to the resource allocation theory (BOX 1) and to experimental evidences. Goelzer *et al.* [28] compared the predicted proteome of a resource allocation model for *B. subtilis* metabolism with absolute protein quantification and detected the expression of some gratuitous enzymes related to the biosynthesis of some amino acids that were already supplemented in the cultivation media, and therefore not produced *de novo*. To test whether this overexpression was detrimental to cell performance, additional experiments were performed with mutant *B.subtilis* strains with these enzymes deleted and cells showed up to 18% higher growth rates.

We argue that the proteome regulation proposed in resource allocation models should be interpreted as the fittest phenotype possible for a given environment resulting from optimal selection through ecological competition. Studies analysing the behaviour of an isolated pure cultures, as the mentioned experiments, cannot be representative of the outcome of natural selective pressures as not regulating the proteome is not a penalising trait that could lead to outcompetition. Moreover, another possible reasoning is that expressing gratuitous proteins is the result of microorganisms having evolved mechanisms to ensure robustness and protection in the case of sudden and unforeseen environmental variations or against fluctuations in protein production [28].

Therefore strict optimality principles should account for uncertainty when describing metabolic strategies [53,54]. Actually, a versatile metabolism, understood as having the potential to address changes in the environment was demonstrated for nine wild-type bacteria [55]. It was seen by measuring ^{13}C fluxes that microorganisms actually grow at suboptimal rates making a compromise between tuning their fluxes for growth maximisation and minimising the needed flux changes to adapt to new conditions. Resource allocation models allowed us to understand mechanistically cellular behaviours that use the substrate in a seemingly inefficient way, to better comprehend how cells pursue optimality and were a significant step forward from previous modelling approaches. However, it is clear that there are still gaps in our way to unravel how cell optimality objectives are driven by evolution and shape microbial communities phenotypes.

Application in environmental biotechnology

The former paradigm of waste treatment based on substrate mineralisation (e.g. activated sludge process) is shifting towards waste-to-chemical biorefinery paradigms, as the carboxylate platform, in which waste is, in first place, anaerobically fermented to volatile fatty acids in open microbiome reactors [56,57]. The typical acids in this platform are the

products of efficient conversions in low substrate flux environments (i.e. acetate or butyrate). Waste conversion can also be driven through inefficient substrate transformations to yield lactate, creating thus an alternative and promising waste-to-chemical biorefinery scheme, the lactate platform, as this compound has diverse and established applications as feed preservative, in the production of cosmetics or as precursor of bioplastics [58]. With the mechanistic insight provided by resource allocation models, we have a deeper understanding of the factors that provoke the shift in microbial communities of the substrate use efficiency. In this sense, we can now engineer microbial communities by designing reactors with the appropriate environmental conditions leading to the production of chemicals produced at different degrees of substrate use efficiency.

Conclusions

In the past years, different explanations were proposed to reconcile experimentally observed inefficient microbial conversions with the assumed pursue of metabolic optimality. We show here that resource allocation modelling provides on most occasions the most satisfying theoretical framework for mechanistically explain what drives microorganisms to modify their substrate use efficiency at different environmental conditions in the sake of optimality. Resource allocation models identified that at low substrate flux conditions membrane transport limits growth and that an efficient use of the substrate is promoted. In environments with high substrate fluxes, the limited enzyme capacity of the cytoplasm constraints growth and inefficient partial substrates conversions with lower enzyme requirements provide a competitive advantage. The mechanistic insight provided by resource allocation models increases the flexibility of waste-to-chemicals biorefineries as we can design reactors with the appropriate environmental conditions for steering waste conversion to chemicals resulting of conversion at different degrees of efficiency.

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