Action and Power Efficiency in Self-Organization: The Case for Growth Efficiency as a Cellular Objective in *Escherichia coli*

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1 Introduction

Flux balance analysis (FBA; (Fell and Small 1986; Varma and Palsson 1994)) has been successfully applied to genome-scale models of microorganisms in order to characterize their metabolic capabilities (Price et al. 2004). FBA makes it possible to simulate different growth phenotypes attained under different environmental conditions and genetic modifications. The analysis can be performed without kinetic parameters in biochemical reactions equations, but using only stoichiometric and thermodynamic constraints. Typically, an objective function, representing the true cellular objective, also needs to be determined in flux balance analysis.

FBA is one of the constraint-based modeling methods that are based on the steady-state assumption. The assumption states that the concentrations of

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metabolites not freely exchangeable with the environment are in a steady state. Given a metabolic network of m metabolites and n reactions, the network structure and the stoichiometric coefficients in reactions can be expressed in an $m \times n$ stoichiometric matrix S. Using S and the steady-state assumption, it is possible to form the following set of equations to comprehensively characterize all the feasible metabolic flux distributions:

$$\begin{aligned} &\frac{dc}{dt} = Sv = 0 \\ &v_i^{lb} \leq v_i \leq v_i^{ub} \; i = 1, \ldots, n \end{aligned} \tag{1}$$

In Eq. (1), c is a vector of concentrations of non-exchangeable metabolites, and v is a vector of reaction rates. The lower and upper bounds of reaction rates are defined by v_i^{lb} and v_i^{lb}, respectively. The bounds for reaction rates can be used to constrain specific reactions to be irreversible and to constrain the substrate uptake rate that usually is an important parameter in constraint-based metabolic modeling. In FBA it is often assumed that microorganisms aim to maximize their growth rate (Feist and Palsson 2010). Therefore, a specific reaction is implemented to describe the generation of new biomass. In FBA the maximum rate of this reaction is determined using linear programming under the constraints of Eq. (1). Currently, the maximal growth rate has established usage as an objective function, but the rationale for cells always pursuing at maximal growth remains debatable (Feist and Palsson 2010). Therefore, the research for other possible objective functions continues active. Other suggested functions include the maximization of ATP yield (Ramakrishna et al. 2001; van Gulik and Heijnen 1995), the minimization of the overall intracellular flux (Blank et al. 2005; Bonarius et al. 1996), the maximization of ATP yield per flux unit (Dauner and Sauer 2001), the maximization of biomass yield per flux unit (Schuetz et al. 2007), the minimization of glucose consumption (Oliveira et al. 2005), the minimization of reaction steps needed to produce biomass (Meléndez-Hevia and Isidoro 1985), the maximization of ATP yield per reaction step (Schuetz et al. 2007), the minimization of redox potential (Knorr et al. 2007), the minimization of ATP producing fluxes (Knorr et al. 2007), and the maximization of ATP producing fluxes (Ebenhoh and Heinrich 2001; Heinrich et al. 1997; Knorr et al. 2007). Similarly to these studies, FBA provides the methodological framework also for our study of growth efficiency.

In the present work, we define the concept of growth efficiency and hypothesize that *Escherichia coli* uses it as the cellular objective. Maximal growth efficiency as the cellular objective would allow bacteria to utilize substrates efficiently to the production of new biomass while producing only little amount of waste, heat, or other side products. In this work we explore the properties of growth efficiency using a genome-wide metabolic model for *Escherichia coli* (Feist et al. 2007) and study whether the growth efficiency could be considered a plausible cellular objective in phenotypic simulations.

In physics, all the laws of motion of particles are obtained from the principle of least action (Goldstein 1980; de Maupertuis 1750). Action is defined as the integral

of the kinetic minus the potential energy over time. It has the units of energy times time (Goldstein 1980). This principle states that for a process to occur, the product of time and energy for it is least, as compared to all neighboring paths (Goldstein 1980). A restatement of this principle is that all motions and processes in nature occur with the least expenditure of action or in the most action-efficient way. In the processes of self-organization in complex systems, the principle is for least unit action connects to a principle for most total action (Georgiev 2012, 2016; Georgiev and Georgiev 2002; Georgiev et al. 2012, 2015, 2016a, b). The least action principle does not act in isolation, but is connected in a positive feedback loop to the maximum total action and the size of the system (Georgiev 2012, 2016; Georgiev et al. 2012, 2015, 2016a, b). This is because to do work to structure a system so the processes inside occur in the most action-efficient way, more energy and time are required. Therefore, the total amount of action is connected with increased action efficiency. Action efficiency allows growth and growth allows action efficiency. Action efficiency can be converted to power efficiency, as shown below. In *E. coli* the growth objective is efficiency, but another implicit objective is the growth itself. So the total growth is maximized when the unit action is minimized, i.e., when its action efficiency is maximized. In previous studies of CPUs, as the unit action for one event (computation) decreases, the total amount of action in the CPU increases. The two are correlated as it has been noticed through the Complexity-Size rule (Georgiev 2012, 2016; Georgiev and Georgiev 2002; Georgiev et al. 2012, 2015, 2016a, b). There is no increase of action efficiency without increase of total action. Correlated to that, the total amount of elements, their density, and the flow of events increase proportionally as well. All those increases are correlated and feed on each other. They are interdependent functions of each other, and they can be termed interfunctions. This study shows that the growth efficiency of *E. coli* obeys the action efficiency principle for self-organization and that the action efficiency principle, and the least action principle for physical systems, expresses itself at biological level in the case of growth efficiency of *E. coli* as power efficiency.

2 The Definition and Calculation of Growth Efficiency

We define the growth efficiency η as the growth rate v_{bm} (i.e., biomass production rate) divided by the substrate uptake rate v_s ($\eta = v_{bm} / v_s$). Because v_{bm} is largely determined by v_s, and in the following analysis we specifically focus on the effects of v_s to η, we now define the so-called growth efficiency function as $\eta = \mathrm{H}(v_s)$ and explore its properties. This simplification ignores specific other factors affecting η via v_{bm}, but the sensitivity of η to these factors will also be examined. The key assumption in our approach is that under specific conditions, bacteria actively work to tune the substrate uptake rate such that the growth efficiency η will be maximized. That is, the bacteria aim at substrate uptake rate v_s^* that is optimal in the sense of $v_s^* = \underset{v_s}{\arg\max}\, \mathrm{H}(v_s)$

In order to characterize the properties of growth efficiency and to study its use as a cellular objective function, we apply the constraints of Eq. (1) and set maximal η as the objective in flux balance analysis. The problem is a linear-fractional problem where η, that is the ratio of biomass production rate to the substrate uptake rate, is maximized:

$$\begin{gathered} \max \ \eta \\ s.t. \ Sv = 0 \\ v_{irr} \geq 0 \\ v_i^{lb} \leq v_i \leq v_i^{ub} \ \forall i \end{gathered} \tag{2}$$

We used the metabolic model iAF1260 for *Escherichia coli* (Becker et al. 2007) to study growth efficiency. Different substrates in cultivation media were modeled by changing the uptake bounds of the corresponding substrates. Different gene knockouts were modeled by setting the lower and upper bounds of the respective enzymatic reactions to zero. We examined substrate uptake rates v_s between 0 and 50 mmol/h/grams of cell dry weight (mmol/h/gDW). The linear-fractional optimization problem in Eq. (2) was solved by sampling the allowed values v_s (60 samples in equal distances between 0 and 50), maximizing v_{bm} in each case, and selecting the value for v_s that maximizes η. The analysis was performed using COBRA Toolbox (Becker et al. 2007), and the linear programming problems were solved using glpk (http://www.gnu.org/software/glpk/).

2.1 Deriving the Growth Efficiency from the Principle of Least Action

A growth efficiency objective function can be justified from broader self-organization and complexity perspective. The rise of complexity in nature includes physical constraints, which must be taken into account. In physics, all motions occur in accordance with the principle of least action (Goldstein 1980; de Maupertuis 1750). All branches of physics, from quantum mechanics to relativity, from classical mechanics to electromagnetism and optics, are derived from it. As mentioned above, action broadly defined is the product of time and energy for a motion. Expressed through power, it is the product of the power and the square of time. For all trajectories in nature, it is true that the motions of objects along them occur with the least amount of physical action as defined above. Any other trajectory has a higher amount of action, and therefore it does not occur. Recently the principle of least action has been expanded to complex systems (Georgiev 2012, 2016; Georgiev and Georgiev 2002; Georgiev et al. 2012, 2015, 2016a, b; Chatterjee 2012, 2013; Annila 2010; Annila and Salthe 2010). According to this principle, all processes in complex systems occur with the least amount of physical action in the given set of constraints, and in flow networks, they do work on the constraints to reduce

the constraints to motion and therefore approach the least action state of motion, i.e., the product of time and energy consumed by a process, or the product of power and time squared. Anila expands this to natural selection for least action (Annila 2010; Annila and Salthe 2010). This model has been tested in the case of CPUs (Georgiev 2012, 2016; Georgiev et al. 2012, 2015, 2016a, b). Starting from this most fundamental physical principle, we propose that metabolic chemical reactions evolve to lower their energy barrier and to occur for the shortest time, obeying the principle of least action. In this case the product of the energy barrier and the time for the reaction is the amount of action consumed by it. In this vein of thought, Stuart Kauffman defines self-organization as the evolution of those autocatalytic cycles (Kauffman 1993). In his model, each of the reactions catalyzes the other in a positive feedback loop. If the metabolic reactions occur with the most action efficiency obeying physical laws, then the whole metabolisms must obey the same objective function as a natural self-organizing system, as it is built on them. Therefore, action and power efficiency in general translates to metabolic efficiency for living organisms. Thus, the metabolic efficiency can be connected to the most fundamental principle in physics, the principle of least action. The role of physics and specifically the principle of least action in biological structure and function and top-down control in complex living systems have been illuminated and furthered by Michael Levin (Giovanni and Levin 2016).

When modified for a complex system, the principle of least action is that the variation of the average actions per one is zero in the most organized state (Georgiev 2012, 2016; Georgiev and Georgiev 2002; Georgiev et al. 2012, 2015, 2016a, b). This means that the process occurs in the most action-efficient way.

$$\delta \frac{\sum_{ij}^{nm} I_{ij}}{nm} = 0$$

where I_{ij} is the action of an element, i is to cross an edge, m is per unit time, and $\sum_{ij} I_{ij}$ is the total amount of action in the system per unit time. n is the number of elements.

When the variation is zero, it means that the function has a minimum, which is the least action state.

2.2 A Connection Between Action Efficiency and Power Efficiency

Here we connect action efficiency to power efficiency. Action defined as the product of energy and time can be converted to a product of power and time squared: Action = Energy*time = Power*time^2 = Pt^2. The last term has the same units as the quantity of action, namely, [Joules*seconds] = [Js]. In this case, action efficiency

is defined as the ratio of all events occurring in a complex system, i.e., the number of metabolic reactions, which translates in the biomass production, divided by the total amount of action consumed by them,

$$Action\ efficiency = \frac{biomass\ production}{Energy^{*}time} = \frac{number\ of\ reactions}{\sum_{ij} I_{ij}}.$$

It can be converted to power efficiency by changing the denominator to the equal of action:

$$Power\ efficiency = \frac{biomass\ production}{Power^{*}time^{*}time}.$$

If the time is fixed to 1 sec, Action Efficiency per unit time = Power Efficiency.

The flow of events is the biomass production or the number of metabolic reactions.

In our work the total amount of action for all events in a system which is proportional to its total power consumption is also maximized in self-organization proportionally to the action efficiency. When considered per unit time, this becomes a maximum power principle. The power efficiency is connected to the power consumption by a power law function which determines a proportionality between the two at each value. As power efficiency increases, the system can absorb more power, and as the power increases, it helps increase the power efficiency of the system, which is in agreement with data that we obtained for CPUs as a complex system (Georgiev 2012, 2016; Georgiev et al. 2012, 2015, 2016a, b).

For a metabolic cycle, the (nm) term is the number of metabolic reactions occurring per unit time, and $\sum_{ij} I_{ij}$ is the total amount of energy and time spent for those reactions, which is the rate of energy consumption by the metabolic cycle.

When the unit action for one metabolic reaction decreases, the system becomes more efficient, obeying the principle of least action, i.e., it self-organizes, as in the example of autocatalytic cycles:

$$\delta \frac{\sum_{ij}^{nm} I_{ij}}{nm} < 0$$

This leads to a measure for organization, α, inversely proportional to the average number of quanta of action per event (Georgiev 2012, 2016; Georgiev et al. 2012, 2015, 2016a, b):

$$\alpha = \frac{\mathrm{h}\, nm}{\sum_{ij} I_{ij}}.$$

where h is Planck's constant, n is the total number of elements in the system, and m is the number of edge crossings of one element per unit time.

Therefore, *nm* is the total number of events in the system, metabolic reactions in this case, the flow ϕ of events per unit time in the flow network, $\phi = nm$. $Q = \frac{\sum_{ij} I_{ij}}{h}$ is the total number of quanta in the system in certain interval of time, the sum of all products of time and energy for all metabolic reactions divided by Planck's constant. We can rewrite the expression for α in the following way:

$$\alpha = \frac{\phi}{Q}.$$

Therefore, the average action efficiency per one event in complex systems is their growth objective, derived from the principle of least action in physics. The growth efficiency as a cellular objective for *Escherichia coli* is one example of this general physics principle. In the growth efficiency of *Escherichia coli*, the growth rate is proportional to the number of metabolic reactions, which gives the biomass production rate, and the substrate uptake rate is proportional to the energy and the power consumed by the system for its biomass production. Therefore α, in the above equation, becomes proportional to η. The growth efficiency, which is defined as v_{bm} – the biomass production rate – is proportional to the total number of metabolic reactions, ϕ. It is divided by v_s _ the substrate uptake rate –which is proportional to the total action and power consumption, Q. Substituting those in the above expression for α, we obtain $\eta = v_{bm}/v_s$. The analogy between α and η is up to a constant.

3 Properties of Growth Efficiency

3.1 Maximum of Growth Efficiency

The growth efficiency function H(v_s) obtains its maximum at a finite substrate uptake rate v_s. This is illustrated in Fig. 1 which shows the predicted growth rate and growth efficiency for a wild-type *E. coli* strain in glucose minimal media assuming varying glucose uptake rates. While the growth rate increases monotonically as the function of the glucose uptake rate, the growth efficiency has a maximum at $v_s^* = 9.2$ mmol/h/gDW. If the uptake rate is greater than v_s^*, the cell starts to secrete increasing amounts of excess metabolites like acetate.

3.2 Sensitivity of Growth Efficiency to Model Uncertainties

Metabolic network models are based on a well-known and validated information on stoichiometric coefficients in biochemical reactions. However, the models also include specific uncertainties. We examined the robustness of the growth efficiency

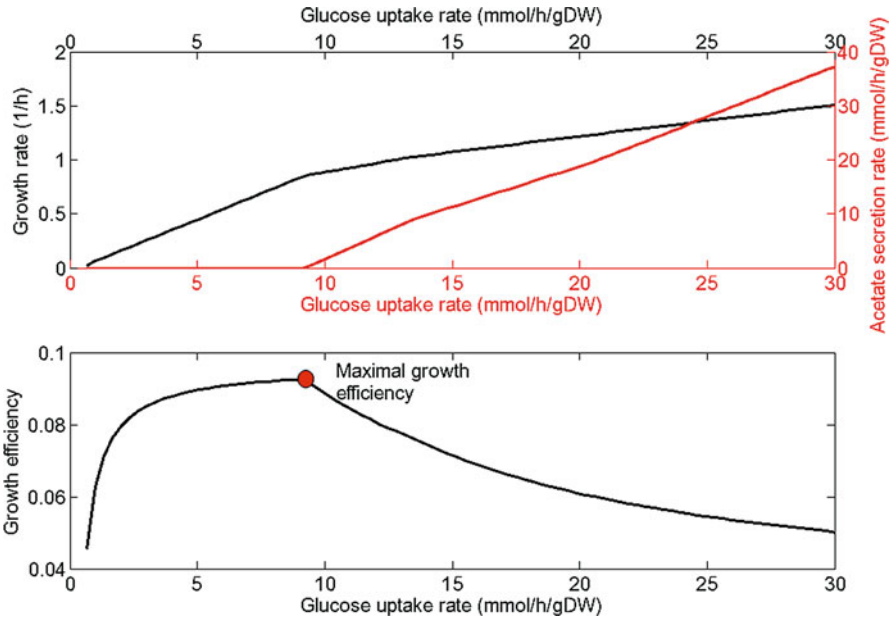

Fig. 1 Maximal growth efficiency is obtained at a finite substrate uptake rate. The upper panel depicts the growth rate and the acetate secretion rate as the function of glucose uptake rate. The growth rate is a monotonically increasing function without a maximum. The lower panel shows the growth efficiency as the function of glucose uptake rate, i.e., the growth efficiency function $H(v_s)$

function against four model parameters: (1) the maximal oxygen uptake rate, (2) ATP requirement for growth-associated maintenance (GAM), (3) ATP requirement for nongrowth-associated maintenance (NGAM), and (4) the phosphorus-to-oxygen (P/O) ratio that reflects the efficiency of ATP synthesis in the electron transfer chain. These parameters have been identified most critical to the behavior of the iAF1260 model (Feist et al. 2007). We first examined the form of the growth efficiency function while varying the maximal oxygen uptake rate between 0 and 50 mmol/h/gDW (the original value being 18.5 mmol/h/gDW). Second, GAM and NGAM were varied for $\pm 50\%$ of their original values (59.81 and 8.39 mmol/h/gDW, respectively) by constraining the respective reaction rates. Finally, P/O ratios 0.5, 1.0, 1.75, and 2.67 were tested by modifying the stoichiometric coefficients in the electron transfer chain and constraining specific reactions of the electron transfer chain (similarly as described in Feist et al. (2007)). Figure 2 shows that all four parameters affect the growth efficiency function. Oxygen uptake rate has the most drastic effect which shifts v_s^*, i.e., the glucose uptake rate at which the maximum of growth efficiency is obtained. GAM and NGAM have similar effects of shifting v_s^*, but the effect is more moderate. The increased P/O ratio increases the maximum growth efficiency without notable effects to v_s^*.

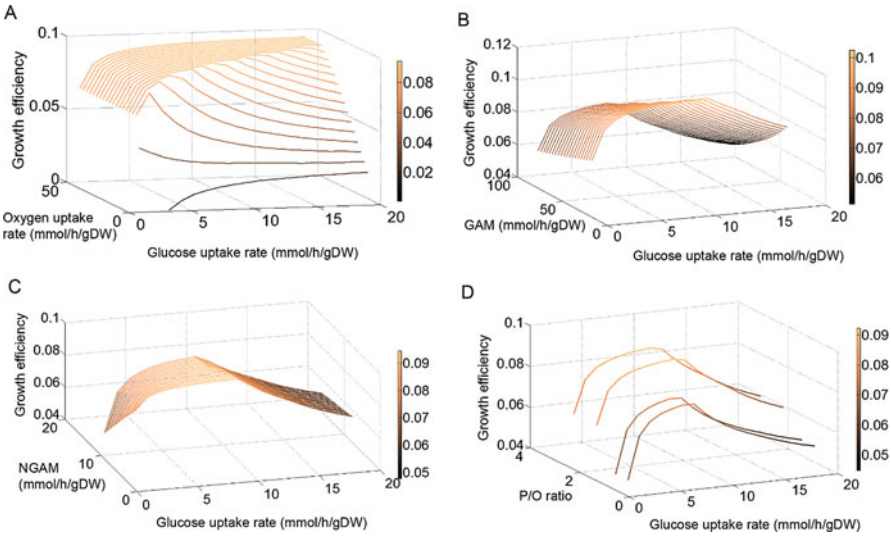

Fig. 2 The robustness of the growth efficiency function against four uncertain model parameters. (**a**) Oxygen uptake rate, (**b**) growth-associated maintenance, (**c**) nongrowth-associated maintenance, (**d**) P/O ratio

3.3 Substrate Uptake Rate Distributions

Because we assume a bacterium to self-regulate the substrate uptake rate to the maximum η at a finite v_s^*, we are able to set the substrate uptake rate unconstrained. Usually in FBA it is crucial to constrain the substrate uptake rate properly. Otherwise, as shown in the upper panel of Fig. 1, the growth rate simply increases monotonically with increasing substrate input rate. In the following analysis, we simulated 10 different cultivations and 1261 genetic conditions to study the distribution of v_s^*. Figure 3 shows the results for phenotypes that are predicted to be viable (i.e., the growth rate is greater than 0.1 h^{-1}). For them, the substrate uptake rate v_s^* always remains at a finite range. Typical values for v_s^* are from 5 to 20 mmol/h/gDW. The largest v_s^* is obtained under the knockouts of components of ATP synthase, in particular in pyruvate cultivation. The blockage of ATP synthase requires that the needed ATP is synthesized by other mechanisms, such as glycolysis and the citric acid cycle, which requires a large substrate uptake rate.

3.4 The Relation of Growth Efficiency and Overflow Metabolism

In situations where the maximal growth is achieved, a bacterium may not be able to transform all the substrate efficiently to new biomass, but an increasing amount

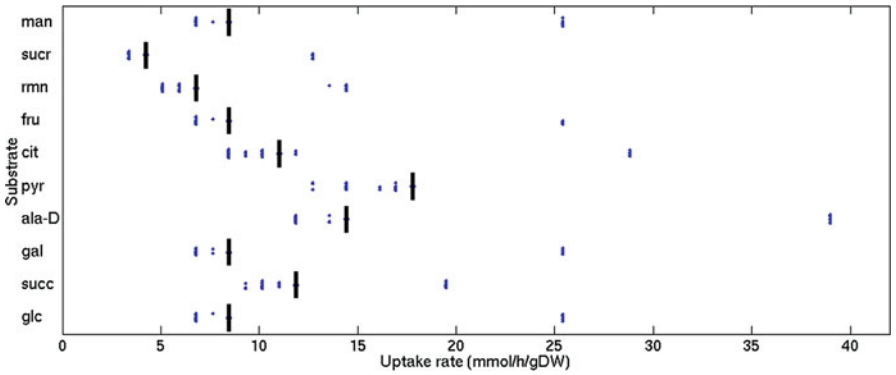

Fig. 3 Distributions of substrate uptake rates under the maximal growth efficiency criterion. The uptake rate is calculated using both criteria for 1261 gene knockout strains in 10 carbon sources (glc, glucose; succ, succinate; gal, galactose; ala-D, D-alanine; pyr, pyruvate; cit, citrate; fru, fructose; rmn, rhamnose; sucr, sucrose; man, mannose). All the distributions are presented as boxplots. The distributions are so concentrated that their data points without outliers appear together as a black bar. Dots represent outliers. Eight data points representing the largest substrate uptake rate (224 mmol/h/gDW for deletions of ATP synthase components in pyruvate cultivation) are not shown

of material is directed to waste. This phenomenon of overflow metabolism has been extensively studied as it is detrimental in industrial applications. In the case of *E. coli*, overflow metabolism directs valuable carbon to acetate production instead of biomass generation. This inhibits growth and it may also disturb product synthesis (Valgepea et al. 2010).

We studied the relationship between the maximal growth efficiency and overflow metabolism by simulating all single-gene knockouts in the iAF1260 model under varying carbon sources. We found that usually the substrate uptake rate at the maximal growth efficiency (v_s^*) equals to the substrate uptake rate at the start of overflow metabolism (i.e., the start of acetate production). There are few exceptions to this rule, for example, when the knockout is directed to specific genes of ATP synthase, pyruvate dehydrogenase, or succinate dehydrogenase. Thus, we reason that the maximal growth efficiency is a concept of its own, and it cannot be directly interpreted as the substrate uptake rate threshold above which overflow metabolism starts.

The use of the maximal growth criterion in growth phenotype simulation may easily produce estimates that are suboptimal in growth efficiency and likely to express overflow metabolism. In order to illustrate the suboptimality under the maximal growth criterion, we calculated the loss of growth efficiency using the abovementioned set of 1261 genetic and 10 environmental conditions. In simulations with the maximal growth criterion, the maximal substrate uptake rate was constrained to 10 mmol/h/gDW. The relative loss in growth efficiency was determined as the growth efficiency under the maximal growth criterion divided by the maximal achievable growth efficiency. Figure 4 summarizes the calculated loss

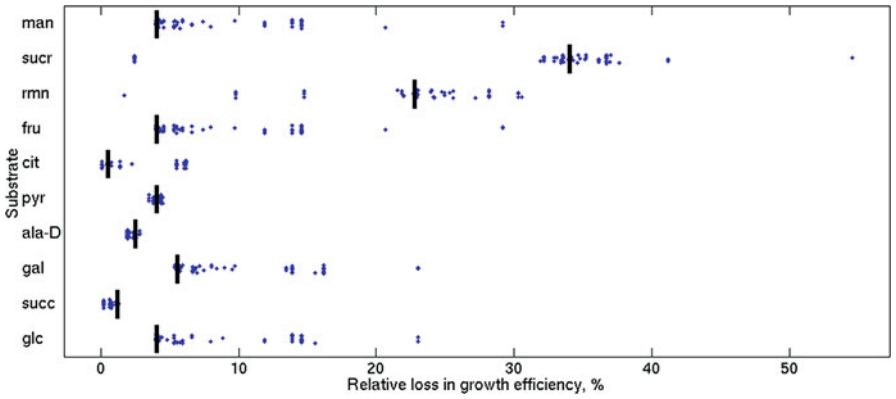

Fig. 4 Suboptimality of growth efficiency under the maximal growth criterion. The relative loss in growth efficiency under the maximal growth rate criterion is depicted for 1261 gene knockout strains in 10 cultivation conditions. The bar represents the median of the 1261 mutants. Almost all the mutants are very close to the median, so they cluster under the bar. The distribution is right skewed, and the points are at least 2.7 S.D. above or occasionally below the median. The median in almost all culture conditions shows a loss of growth efficiency under the maximal growth rate criterion

ratios. In the figure, the loss of growth efficiency at maximal growth demonstrates that maximal growth wastes input substrate energy.

4 Maximal Growth Efficiency as a Cellular Objective

We examined whether the maximal growth efficiency is a plausible cellular objective for *E. coli* cultivated in a small-scale batch process. Therefore, we used the metabolic model iAF1260 to predict the cellular growth rate assuming *E. coli* maximizes the growth efficiency. The growth predictions were compared to two experimental data sets as follows.

First, we predicted the viability for mutant strains carrying single-gene deletions. The predictions were produced for 1117 mutant strains cultivated under glucose minimal media (Baba et al. 2006). Each mutant strain in the data set has been experimentally determined to be either viable or inviable. For 982 viable mutants, the viability was correctly predicted (i.e., true positive rate was 97%), and for 76 inviable mutants, the inviability was correctly predicted (true negative rate was 72%). The results are identical with the prediction results obtained using the maximal growth criterion.

Second, we predicted the growth rate for 5096 growth conditions, consisting of 91 single-gene knockout strains cultivated under 56 different media conditions. The optical density (OD) of *E. coli* grown in these conditions has been measured in a high-throughput experimental screen using the Biolog platform

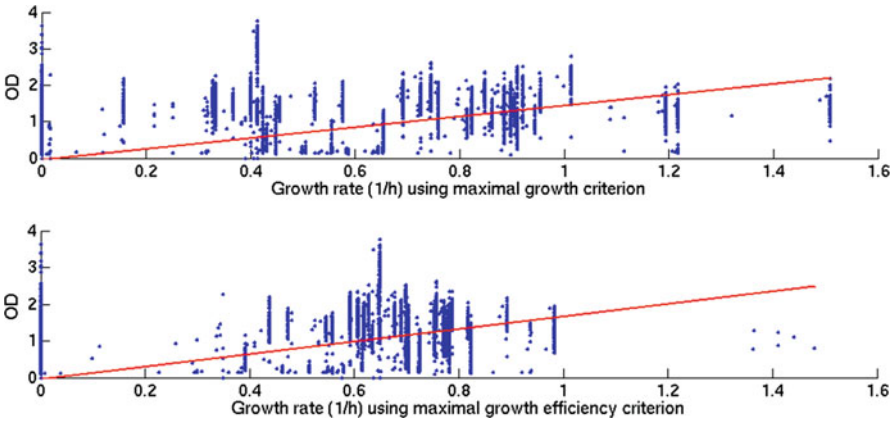

Fig. 5 The correspondence of predicted growth rates and experimentally observed optical density. OD values are presented as the function of the predicted growth rates. The upper and the lower panels present the predictions under the maximal growth criterion and the maximal growth efficiency criterion, respectively. A linear model is fitted to both data. In simulations with the maximal growth criterion, the maximal substrate uptake rate was constrained to 10 mmol/h/gDW

(http://www.biolog.com), and the data has been set available through the ASAP database (Glasner et al. 2003). Figure 5 shows the growth predictions for each of the growth conditions versus the corresponding OD value. Under the maximal growth criterion, the Spearman correlation between the predicted growth rates and the experimental OD values was 0.19, while under the maximal growth efficiency criterion, the Spearman correlation was 0.24. We also fitted linear models to both data in order to further compare the phenotype prediction performance of the two criteria. Under the maximal growth criterion, the linear model had residual standard error of 0.83 and the adjusted R^2 was 0.59. Under the maximal growth efficiency criterion, the linear model had residual standard error of 0.76 and the adjusted R^2 was 0.65. The better fit in the case of the maximal growth efficiency was confirmed using Akaike's information criterion (12,537.83 for maximal growth criterion versus 11,639.86 for maximal growth efficiency criterion).

5 Conclusions

The identification of cellular survival strategies and their simulation by realistic objective functions have fundamental importance on phenotype prediction in metabolic analysis. It is probable that there is no single survival strategy that is optimal in all situations but the strategy is likely to depend on growth conditions of a microorganism (Feist and Palsson 2010). Feist and Palsson discuss three qualitatively different environments: nutritionally rich, nutritionally scarce, and elementally limited (Feist and Palsson 2010). Nutritionally rich laboratory-like

conditions are probably very rare in the nature, and thus, maximal growth is probably an unrealistic objective function in most of the situations. In a study by Schuetz et al. (2007), it was found that under nutrient scarcity in continuous cultivations, the best prediction accuracy was achieved using linear maximization of ATP or biomass yields. On the other hand, in unlimited growth on glucose in oxygen or nitrogen respiring batch cultures, the best prediction accuracy was achieved by nonlinear maximization of the ATP yield per flux unit.

In this work we introduced a concept called growth efficiency and characterized its properties. The study was performed using the metabolic model iAF1260 for *Escherichia coli*. As a result we found that the growth efficiency function has its maximum within a finite substrate uptake rate. According to our predictions, the substrate uptake rate at which the maximal growth efficiency is obtained (v_s^*) varies typically from 5 to 20 mmol/h/gDW. Our simulations with several different cultivation media and a set of single-gene knockouts demonstrated that the optimal rate v_s^* depends on the cultivation and genetic conditions. For example, with sucrose the median uptake rate of v_s^* was 4.5 mmol/h/gDW, while with pyruvate the median rate was 17 mmol/h/gDW. We also found that the growth efficiency function is affected by specific parameters that usually remain unsure in metabolic network models. In particular, oxygen uptake and ATP requirement for growth-associated maintenance affect v_s^*, and increasing P/O ratio in electron transfer chain increases growth efficiency while maintaining the form of the growth efficiency function.

A straightforward application of growth efficiency is to use it as an optimization criterion (i.e., objective function) for predictions of cellular growth. We explored this possibility and validated our computational predictions using two sets of experimental data. We found that maximal growth efficiency can be considered a feasible optimization criterion in metabolic modeling. The criterion predicted the given experimental data slightly better than the commonly applied maximal growth rate criterion.

In this study we used data from batch cultivations to validate the feasibility of growth efficiency as an objective function. However, based on the work by Schuetz et al. (2007), we hypothesize that the growth efficiency criterion could perform better in situations where cells are under nutrient scarcity, i.e., they are cultivated in nutrient-limiting chemostats. Such chemostat data was not available in this study, and the hypothesis should be validated in a future study.

Considering maximal growth efficiency as a cellular objective suggests that cells can save nutrients in the benefit of other cells or to be used to themselves at a later moment. This raises the question about the mechanisms, e.g., quorum sensing, which bacteria growing in colonies may use to tune their growth rate in each growth situation. As a further point, we note that if it proves true that bacterial cells maximize the growth efficiency per unit food or energy uptake, this picks out an optimal rate of energy utilization, hence an optimal displacement from chemical equilibrium for nonequilibrium living cells. We note that we lack the theory of an optimal displacement from equilibrium for living, nonequilibrium, cells. Jacques Monod, in Chance and Necessity, notes that optimally growing bacteria give off little heat (Monod 1971). This may be consonant with maximal growth efficiency, so

that the maximal amount of energy coming into cells goes into biomass production and minimizes waste heat.

We have shown that this growth efficiency objective function can be derived from a fundamental physics principle, the principle of least action, from which all equations of motion in each branch of physics are derived and describes all processes that occur naturally. Expanding this principle for a complex self-organizing system, it predicts that the processes in that system will occur with least expenditure of the product of energy and time per one event, or the product of power and time squared, such as a metabolic reaction in the case of *E. coli*. Therefore, using an objective function from complexity, for increase of action and power efficiency in self-organization, we derive the expression for growth efficiency, used to describe the metabolic processes of *E. coli*. In this way we justify it from first principles in physics using the new objective function defined in this paper, and we find a case where this principle yields fruitful results. This connection between physics and biology illuminates the common principles behind all processes in nature and gives us a tool to explain more of the phenomena in biology using fundamental first principles. It also expands the validity, applicability, and importance of those physics principles.

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