Emergent sub-population behavior uncovered with a community dynamic metabolic model of *Escherichia coli* diauxic growth

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Abstract

Microbes have adapted to greatly variable environments in order to survive both short-term perturbations and permanent changes. A classical, yet still actively studied example of adaptation to dynamical environments is the diauxic shift of *Escherichia coli*, in which cells grow on glucose until its exhaustion, and then transition to using previously secreted acetate. In this work we tested different hypotheses about the nature of this transition with dynamic metabolic modeling. Towards this goal, we developed an open source modeling framework integrating dynamic models (ordinary differential equation systems) with structural models (metabolic networks), which can take into account the behavior of multiple subpopulations, and smooth flux transitions between different time points. We used this framework to model the diauxic shift, first with a single *E. coli* model whose metabolic state represents the overall population average, and then with a community of two subpopulations each growing exclusively on one carbon source (glucose or acetate). After introducing an environment-dependent transition function that determines the balance between different subpopulations, our model generates predictions that are in strong agreement with published data. We thus confirm recent experimental evidence that, rather than a coordinated metabolic shift, diauxie would be the emergent pattern from individual cells differentiating for optimal growth on different substrates. This work offers a new perspective on how to use dynamic metabolic modeling to investigate population heterogeneity dynamics. The proposed approach can be easily applied to other biological systems composed of metabolically distinct, interconverting sub-populations, and could be extended to include single-cell level stochasticity.

Importance *Escherichia coli* diauxie is a fundamental example of metabolic adaptation that has not yet been completely understood. Further insight into this process can be achieved by integrating experimental and computational modeling methods. We present a dynamic metabolic modeling approach that captures diauxie as an emergent property of sub-population dynamics in *E. coli* monocultures. Without fine tuning of the parameters of *E. coli* core metabolic model we obtain good agreement with published data. Our results suggest that single organism metabolic models can only to a certain approximation represent the average population metabolic state, therefore offering a new perspective on how such modeling approaches could be used. The open source modeling framework we provide can be used to model general sub-population systems in more complex environments and extended to include single-cell level stochasticity.

Keywords: Metabolic network modeling, Microbial communities, Diauxic growth.

1 Introduction

In natural environments microorganisms are exposed to high fluctuations of nutrient and micronutrient availability and have therefore developed adaptation strategies, both short-term to respond to temporary perturbations and long-term to increase evolutionary fitness [1]. We still miss a sound theoretical understanding of the mechanisms driving such strategies, but the recent technological advances in high-throughput experimental techniques pave the way to novel approaches integrating experimental and theoretical biology [2]. Theoretical ecology has developed over the last centuries as a solid discipline that describes ecosystems in mathematical terms as dynamic organism-environment interactions [3]. Indeed, as in statistical physics, individual behaviors in an *ensemble* result in observable emergent patterns that can be modeled with mathematical equations [4]. This is the case for the earliest models of population dynamics by Verhulst [5], Lotka [6] and Volterra [7] and for the pioneering works by Jaques Monod in modeling microbial growth [8]. With the rising academic and industrial interest in the "microbiome", systems biology approaches are becoming a new standard [9] and more methods for mathematical modeling of microbial communities are being developed [10, 11].

In constraint-based stoichiometric modeling the metabolic network model of an organism is reconstructed from its annotated genome and described mathematically as a stoichiometric matrix $\mathbf{S}$. After imposing the steady-state assumption and introducing thermodynamic and biological boundaries for the metabolic fluxes $\vec{v}$, flux balance analysis (FBA) [12, 13] defines an optimization problem in order to identify one particular flux distribution in the solution space. As long as the objective function (which introduces further biological assumptions on the system) is linear in the fluxes, the optimization problem can be solved with linear programming (LP). FBA returns a unique solution for the objective function, but the metabolic flux distribution is in general not unique, especially in genome-scale metabolic network models (GEMs). Under the hypothesis that metabolism has evolved to make efficient use of resources and minimize waste, two specific methods were developed to extend FBA: parsimonious FBA (pFBA) [14] and minimization of metabolic adjustment (MOMA) [15]. In pFBA a second LP is defined where the value of the objective function is set to the FBA solution and the new objective is the minimization of the overall fluxes. MOMA was developed to simulate the response to the perturbation introduced by gene deletion and is based on the principle that the organism will readjust its metabolism to a minimally different configuration with respect to the wild type optima. Another extension of FBA, dynamic FBA (dFBA) [16], allows to partly recover the dynamic information lost under the steady-state assumption. In the static optimization approach (SOA) that underlies dFBA, time is divided in discrete intervals and a new FBA problem is solved at time i after updating the external conditions according to the FBA solution at time $i-1$. Approaches to model microbial communities with GEMs are reviewed *e.g.* in [17].

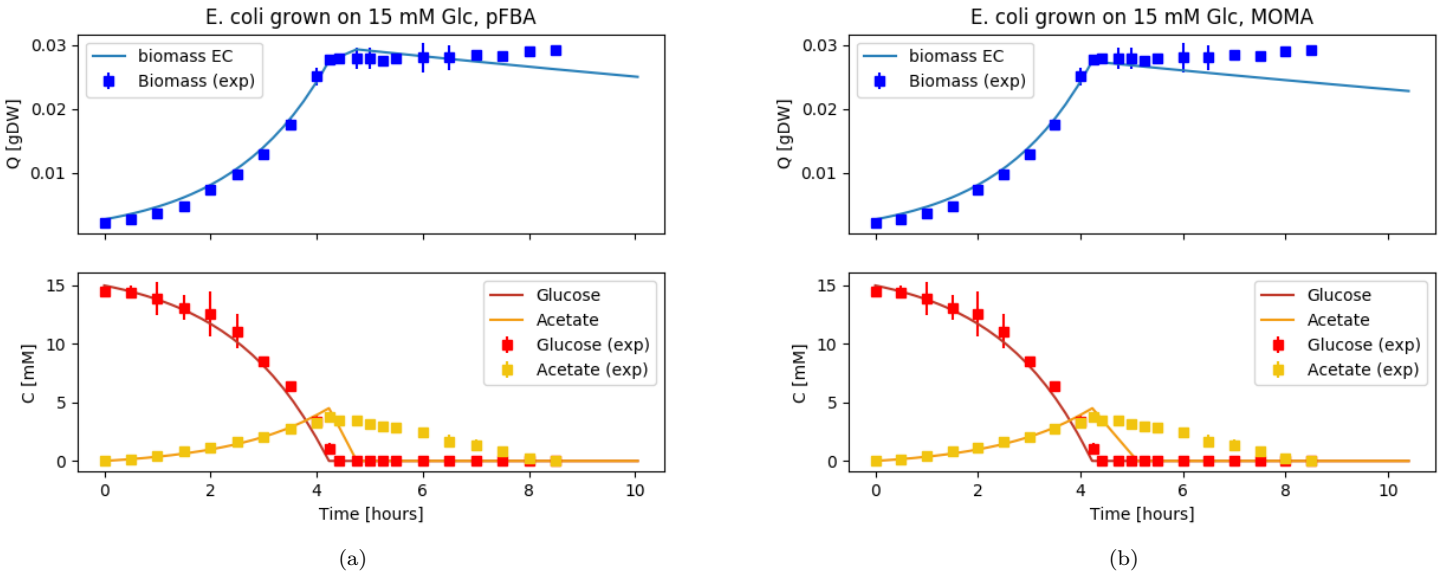
FBA and dFBA have been applied to study one of the most basic examples of metabolic transitions: diauxie [18, 16, 19]. Discovered in the model organism *Escherichia coli* in 1941 by Monod [20, 8], diauxie is still today a topic of active research [21, 22, 23]. In aerobic conditions with glucose as sole carbon source (also generally the preferred one), *E. coli* grows secreting acetate, which it then consumes after glucose is exhausted. The molecular mechanisms driving this transition are still not completely understood, but over the last few years the fundamental role of stochasticity and population heterogeneity has been demonstrated experimentally [24], often with the support of mathematical models. Indeed, in unpredictable natural environments with fluctuating nutrient availability and variable fitness landscapes, homogeneous populations have higher chances to face extinction and bet hedging becomes a selective advantage [25]. Single cell studies have suggested that the observed biphasic growth is possibly the effect of stochastic gene expression [21], eventually co-regulated by memory mechanisms [26]. Kotte *et al.* [27] have systematically investigated bistability in a clonal *E. coli* population. After ruling out responsive switching as homogeneous adaptation, their results strongly suggested that the heterogeneous adaptation that results in two co-exhisting phenotypes was driven by responsive diversification (with a single phenotype diversify upon environmental changes) rather than stochastic switching (where the two phenotypes would co-exhist from the beginning). While stochastic mathematical models have been proposed to support those findings, metabolic modeling approaches have been so far considered suitable only to describe homogeneous systems, with single organism GEMs representing the average population metabolic state.

Varma and Palsson [18] performed the first dFBA analysis on *E. coli*, with a single GEM growing aerobically first on glucose and then on the secreted acetate. In this work we present a study of *E. coli* diauxic growth on these two carbon sources, with the bacterial population being modeled either as having an average, unique metabolic state (standard FBA and dFBA approach) or as being the combination of two *E. coli* populations adapted to one of the two carbon sources. We use a modeling approach that integrates ordinary differential equation (ODE) models with dFBA, extending methods typically applied to study the dynamics of multi-species communities to the investigation of emergent patterns from individual behavior in monocultures. We implement three approaches: first we model a homogeneous, yet smooth shift, with a single *E. coli* GEM, adapting the MOMA algorithm (i), then we introduce the hypothesis of sub-populations growing on specific carbon sources and model population transition as a purely stochastic mechanism (ii) or introducing an environment-driven response (iii). Our results suggest that rather than as a coordinated metabolic shift, diauxie can be modeled as the emergent pattern resulting from sub-populations optimizing growth on different substrates in response to environmental changes. This is much in agreement with experimental evidence from *e.g.* Kotte *et al.* [27], and offers a new perspective on how to use dynamic metabolic modeling to investigate population dynamics. The proposed approach can be easily transfered to studies of generic sub-populations or communities and also, eventually, expanded to investigate single cell dynamics.

2 Results

We run simulations with an open source modeling framework developed to model ecosystem dynamics. The models are ODE systems solved with integrating routines that at each integration step solve an FBA problem. We first validate the *E. coli* GEM on the data from Varma and Palsson [18] (which reported the first dFBA analysis of the glucose-acetate shift in 1994) and then use the calibrated model to reproduce the independent sets of experiments from Enjalbert *et al.* [22] (who published in 2015 results from *E. coli* grown in aerobic batch systems with different concentrations of glucose and acetate). In the standard dFBA approach, a population is modeled with a unique GEM and fluxes instantaneously change to adapt to new environmental conditions. In reality, however, transcriptional changes and flux rerouting may display delays, which are not currently captured by existing algorithms. Furthermore, dFBA might predict metabolic states in which more carbon sources are simultaneously utilized, and it is not obvious that such approach will correctly capture the complexity of a population diversifying into metabolically distinct subpopulations. Therefore, we modified the dFBA algorithm taking advantage of optimization strategies previously developed for different biological questions and implementing novel concepts as well. In particular, we use either pFBA [14] or an adaptation of MOMA [15] to solve the FBA problem at each time step, respectively replicating the standard dFBA approach or implementing a homogeneous, yet smooth shift. The MOMA algorithm is integrated into the dFBA routine by imposing that the solution of the FBA problem at time i is minimally different from the solution at time $i-1$. We test three different hypotheses: (i) homogeneous, smooth population shift; (ii) stochastic-driven and (iii) environment-driven sub-population differentiation. After we observe that dFBA with both pFBA and MOMA predicts abrupt transitions from acetate catabolism to acetate anabolism, making it impossible to capture lag phases and thus contradicting our hypothesis (i), we model two *E. coli* sub-populations growing exclusively on glucose or acetate. For this we extend the standard dFBA approach to include processes like cell death and population shifts. We test whether purely stochastic switches (ii) or rather a responsive diversification (iii) can capture the diauxic behavior by modeling the populations transitions either with constant rates (ii) or with a heuristic function dependent on carbon source concentrations (iii). As we did not find significant improvements in using MOMA rather than pFBA within the same metabolic state, the simpler pFBA implementation is used in the sub-populations simulations where each model is fixed into one metabolic configuration. Further details on the modeling approach are provided in Materials and Methods.

***E. coli* diauxie modeled with a uniform population.** A single GEM is used to model the average *E. coli* metabolic state and we compare the simulation results with the original data from Varma and Palsson [18] (Text S1, Fig. S1). The parameters for the simulations are reported in Tab. 1 and 2, and the only flux constraints that we calibrate to the data are the oxygen uptake rate and the maximal acetate secretion rate. With this dataset, a lower absolute flux variation at each simulation time step is observed with the MOMA implementation (Fig. S2). We use the same GEM model to reproduce the results from Enjalbert *et al.* [22], changing only the initial values of biomass, glucose and acetate (Fig. 1). While the pFBA simulation (Fig. 1(a)) shows a brief shift to growth on acetate at the time of glucose exhaustion ($\sim$ 4 hr), the MOMA simulation predicts a complete growth arrest already at that point, with a minimal acetate consumption to satisfy the ATP maintenance requirement implemented in the GEM (Fig. 1(b)). Both simulations well capture glucose consumption and acetate secretion, but neither of them is able to reproduce the slow acetate consumption observed experimentally. Additionally, both pFBA and MOMA simulations show an abrupt change in the flux distribution upon shift from glucose to acetate consumption (Fig. S3). We evaluate the agreement between experiment and simulation with the R^2 distance between *in vivo* and *in silico* data for the biomass (pFBA $R^2 = 0.989$; MOMA $R^2 = 0.982$) glucose (pFBA $R^2 = 0.993$; MOMA $R^2 = 0.993$) and acetate (pFBA $R^2 = 0.277$; MOMA $R^2 = 0.409$). In Fig. 2 we compare the flux distributions from simulation results to the experimental results from Enjalbert *et al.* [22] for over/under-expression of key genes associated with glucose and acetate metabolism (represented graphically in the top panels). First, we compute the flux solutions for *E. coli* growing either on glucose or on acetate exponentially (data not shown) and compare the fluxes through the relevant reactions in *E. coli* growing on acetate with the ones in *E. coli* growing on glucose. Fig. 2(a) shows the absolute values for the flux results in the two simulations, normalized between 0 and 1 for direct comparison with the qualitative representation of the gene expression data (with 0 for non expressed and 1 for expressed genes respectively). The simulation results are consistent with the experiments, with active reactions (dark green) related to acetate consumption and anabolism (ACKr, PPCK, FBP, ICL, MALS) and inactive reactions (white) related to glycolysis (PFK, PYK) during growth on acetate, and vice-versa during growth on glucose, while PPS does not carry flux in neither simulation. We then use the simulation results presented in Fig. 1 to compare the metabolic fluxes before and after glucose exhaustion (GE), *i.e.* before and after the single *E. coli* model shifts from growth on glucose to growth

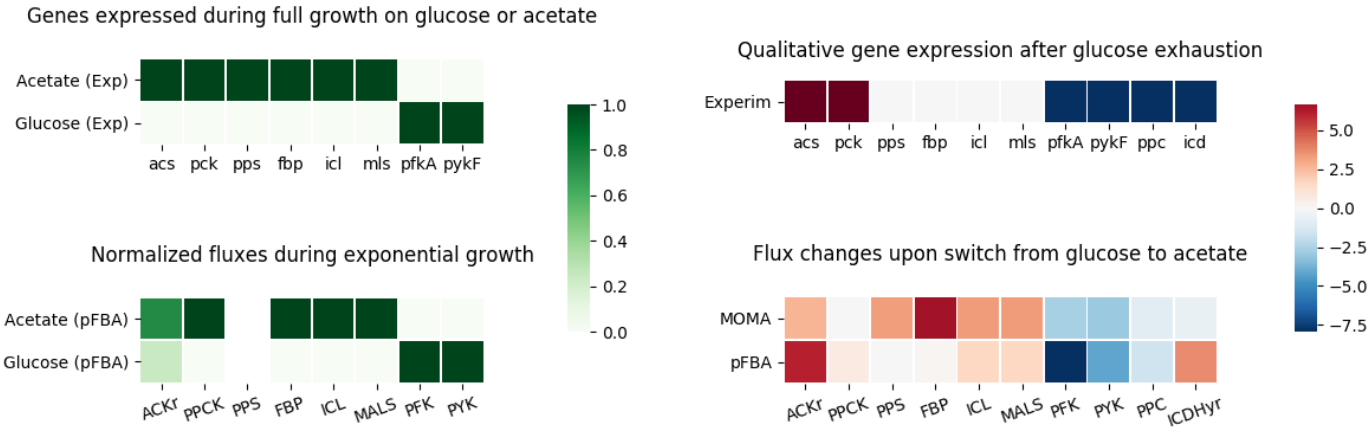


(a) (b)

Figure 1: Diauxic growth of *E. coli* modeled as a uniform population in batch conditions. Simulation (lines) is compared to data (squares) from Enjalbert *et al.* [22] as a function of time. Biomass (blue, top sub-plots), glucose and acetate (red and yellow, bottom sub-plots) are shown. The flux distribution at each time step is obtained with pFBA (a) or MOMA (b).

on acetate. Enjalbert *et al.* [22] compared the gene expression levels between samples taken at time (GE + 30 minutes) and (GE - 115 minutes). However Fig. 1 shows that according to the simulation, growth has already stopped after 30 minutes from the GE point. Indeed comparing the absolute values of fluxes taken at time (GE + 30 minutes) and (GE - 115 minutes) (Fig. S4), we observe that both pFBA and MOMA simulations qualitatively capture the down regulation trends, while neither reproduces the observed up-regulation. Fig. 2(b) shows the difference of the absolute values of fluxes taken at time (GE + 18 minutes) and (GE - 115 minutes), where in pFBA simulations growth is still observed. In this case, both simulations qualitatively capture most of the up/down regulation trends. Fig. S5 shows the metabolic network (modified from the Escher [28] map for the *E. coli* core model) with reactions of Fig. S4 highlighted and color-coded according to the gene expression data, and Fig. S6 presents the same picture but with respect to the results of Fig. 2(b).

***E. coli* diauxie modeled with a mixed population.** We use two GEMs (same parameter values as before) to model *E. coli* monoculture as a mixture of two populations, one adapted to grow on glucose and one adapted to grow on acetate. The two models EC_{Glc} and EC_{Ac} are hence constrained to uptake exclusively the respective carbon source, and a cell death rate is introduced as in [19]. Two transition functions, dependent on acetate or glucose concentrations, are introduced to model cellular differentiation and shift from one population to the other (see Materials and Methods for details). We run simulations to compare different scenarios investigated experimentally by Enjalbert *et al.* [22]. Initial values of biomass, glucose and acetate are adjusted to the respective datasets. The transition rates, as well as the initial population ratios, were chosen following the assumption, supported by a simple mathematical model, that in constant environments the populations will converge to a constant ratio (see Text S2 for details). Fig. 3 shows simulations for the same condition as Fig. 1(a), with same absolute initial biomass, distributed in this case as 95% EC_{Glc} and 5% EC_{Ac}. This initial ratio was chosen considering the range of steady state values for the population ratio (reported in Table S2) as well as considering that it is reasonable to assume that a higher number of cells will be adapted to grow on glucose, which is the carbon source on which laboratory cultures are usually maintained. Fig. 3(a) shows the simulation results for a scenario without transitions between the two states, whereas the results of Fig. 3(b) are obtained with active transition functions, defined here by constant transition rates as reported in Tab. 2. While both Fig. 3(a) and Fig. 3(b) well capture the biomass ($R^2 = 0.987$ and $R^2 = 0.990$, respectively) and glucose concentrations ($R^2 = 0.996$ and $R^2 = 0.997$, respectively), only the simulation including population transition can realistically reproduce the acetate consumption ($R^2 = 0.336$ and $R^2 = 0.951$ respectively), as well as a lag phase before culture crash. Neither of the two simulation however captures the eventual recovery of growth hinted by the last data points. We reproduce two other results (where

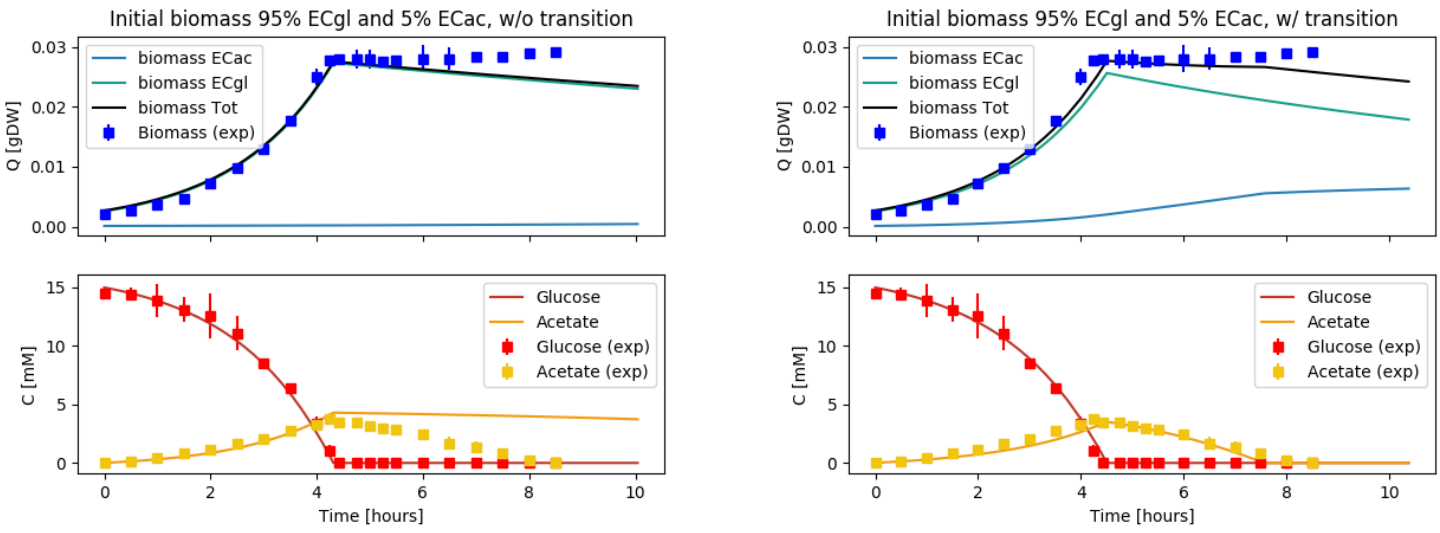


(a) (b)

Figure 2: Comparison of experimental information on gene expression levels with simulated flux distributions. The top plots represent qualitatively the gene expression data from Enjalbert *et al.* [22]. Flux solutions in the simulations for the reactions associated to the reported key genes are compared (a) between two independent simulations with *E. coli* exponentially growing either on acetate or on glucose; (b) within the same simulation (Fig. 1, growth on glucose simulated with MOMA or pFBA) before and after the point of glucose exhaustion.

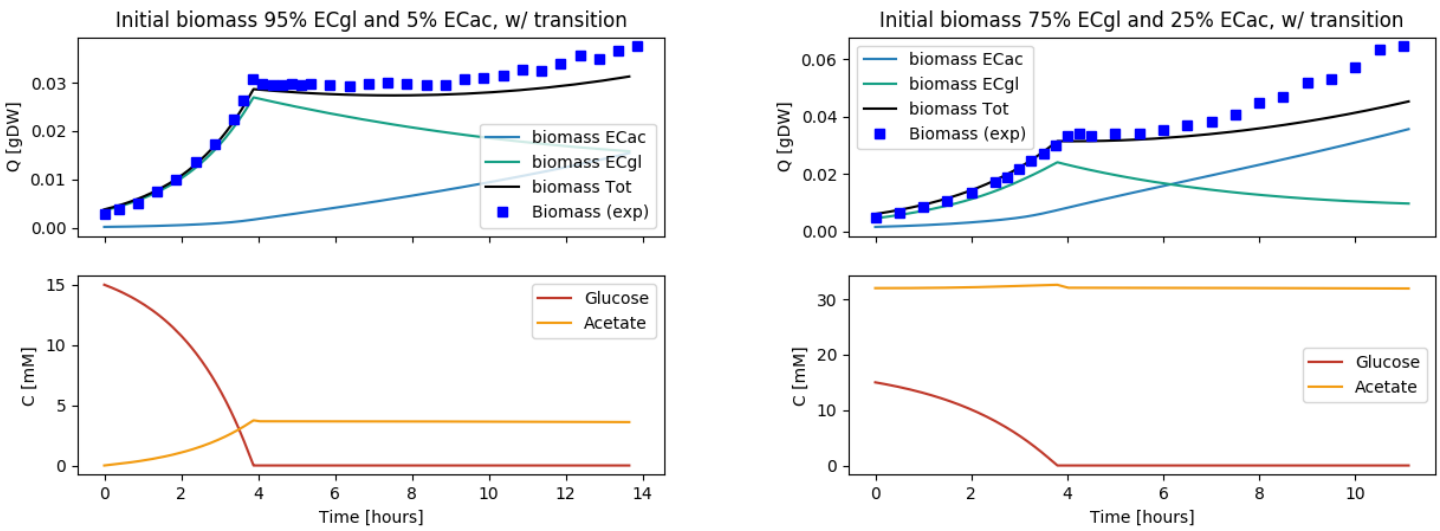
only biomass measurements are available) from Enjalbert *et al.* [22], again using the same GEMs and changing only the initial conditions (biomass quantity and distribution among EC_{Glc} and EC_{Ac}) and experimental setup accordingly (see also Text S3). By modeling the population transition with the same constant rate, we are able to explain the biomass profile in the case where *E. coli* is grown on 15 mM glucose and after glucose exhaustion the acetate concentration is maintained at around 4 mM (Fig. S11(a), $R^2 = 0.986$), but not in the case where *E. coli* is grown on 15 mM glucose and 32 mM acetate, and after glucose exhaustion the acetate concentration is maintained at the same high level (Fig. S12(a), $R^2 = 0.727$). We therefore introduce a dependency of the transition functions on the substrate concentration (see Materials and Methods for details) that well captures all the experimental scenarios (Fig. S10, S11, S12). Fig. 4(a) shows that an *E. coli* population starting with 95% EC_{Glc} and 5% EC_{Ac} well describes the biomass dynamics ($R^2 = 0.985$) and the glucose exhaustion point after around 4 h when acetate is maintained at 4 mM. Again, without fine-tuning of the GEM simulation parameters, Fig. 4(b) shows that an *E. coli* population starting with 75% EC_{Glc} and 25% EC_{Ac} reproduces the biomass measurements ($R^2 = 0.940$) and the glucose exhaustion point after around 4 h also in the experimental setup with acetate maintained at 32 mM. This initial population ratio is again consistent with the range for steady state values found in the study of Text S2 for the population ratio in mixed carbon conditions (Table S2), and is justified by a difference in the experimental initial values for the biomass quantity (see Text S4 and Fig. S15). The effect of varying the initial biomass ratios in the different experimental conditions is reported in Text S4. Overall, simulations starting with 95% EC_{Glc} and 5% EC_{Ac} or 75% EC_{Glc} and 25% EC_{Ac} do not show strong differences, while decreasing further the percentage of EC_{Glc} (and leaving the range of steady state values of Table S2) shows drastic changes in the shape of the growth curves.

Lag time for growth on acetate explained with population distribution. Enjalbert *et al.* [22] showed different trends in *E. coli* cultures lag time to achieve maximal growth after glucose exhaustion (GE). In their switch experiments, they sampled at different time points "mother cultures" of *E. coli* cells growing in batch conditions on 15 mM of glucose alone (M9G condition) or on 15 mM of glucose and 32mM of acetate (M9GA condition), and re-inoculated the sampled cells as "daughter cultures" into fresh media containing exclusively glucose (M9G condition) or acetate (M9A condition). We replicate this experiment *in silico* by running first simulations in the M9G and M9GA conditions. We use as M9G mother culture the simulation of the mixed EC_{Glc} and EC_{Ac} population shown in Fig. 3(b), as the experimental conditions are the same. For the M9GA mother culture we do not have an experimental dataset to refer to and we simulate a new scenario similar to the one of Fig. 4(b), with the same initial



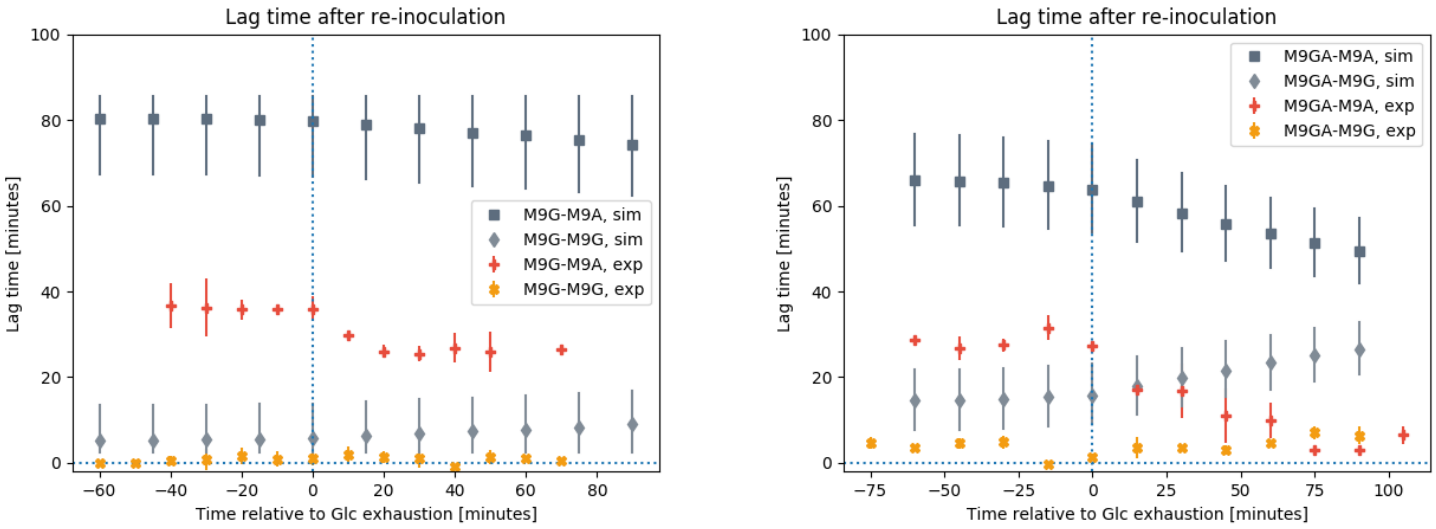
(a) (b)

Figure 3: Diauxic growth of *E. coli* modeled as a mixture of two *E. coli* populations EC_{Glc} and EC_{Ac} growing exclusively on glucose or acetate, respectively, and without (a) or with (b) possibility to shift from one population to the other. Simulation (lines) is compared to data (squares) from Enjalbert *et al.* [22] as a function of time. The upper plots show simulation results (using pFBA) for EC_{Glc} and EC_{Ac} biomasses (light blue and aqua, respectively) and the observable *E. coli* biomass (black line simulation, blue dots data). The bottom plots show glucose and acetate (red and yellow respectively).



(a) (b)

Figure 4: Diauxic growth of *E. coli* modeled as a mixture of two *E. coli* populations EC_{Glc} and EC_{Ac} growing exclusively on glucose or acetate, respectively, with possibility to shift from one population to the other. (a) *E. coli* grows on 15 mM glucose and after glucose is exhausted, acetate concentration is kept at about 4 mM. (b) *E. coli* grows on 15 mM glucose and 32 mM acetate and after glucose is exhausted, acetate concentration is maintained at around that concentration. The upper plots show simulation results (using pFBA) for EC_{Glc} and EC_{Ac} biomasses (light blue and aqua lines, respectively) and the observable *E. coli* biomass (black line simulation, blue dots data from Enjalbert *et al.* [22]). The bottom plots show simulation results for glucose and acetate (red and yellow lines respectively).



(a) (b)

Figure 5: Simulation (dark and light gray points) and experimental (orange and yellow points, data from Enjalbert *et al.* [22]) results for the delay of daughter cultures before reaching maximal growth after media switch. Mother cultures are grown either on 15 mM of glucose (M9G, (a)) or 15 mM of glucose and 32 mM of acetate (M9GA, (b)). Daughter cultures are re-inoculated into fresh media with either 15 mM of glucose (M9G, square and plus markers) or 45 mM of acetate (M9A, diamond and cross markers). The simulation error bars are obtained by varying the initial population ratios (obtained from sampling the simulated mother cultures) by $\pm 15\%$.

population composed of 75% EC_{Glc} and 25% EC_{Ac}, but without the feeding of additional acetate. The biomass, glucose and acetate profiles resulting from this simulation are shown in Fig. S16. The GE time point is about 4.6 hours for M9G and 3.9 hours for M9GA, consistent with the observations of Enjalbert *et al.* [22]. The *in silico* mother cultures are sampled at regular time intervals to obtain the initial biomass distribution of EC_{Glc} and EC_{Ac} for the daughter cultures (reported in Tab. 3) and the lag time of each daughter culture is computed (see Materials and Methods for details). Fig. 5 shows the simulation results compared with the experimental data from Enjalbert *et al.* [22]. The error bars on the simulated lag time is obtained by varying the initial biomass ratio of the daughter cultures by $\pm 15\%$. Whereas a quantitative agreement between simulation and experimental results is only achieved in the M9G-M9G switch experiment (Fig. 5(a)) with the correct prediction of almost zero lag time for the daughter cells, the trend for the delay to reach maximal growth is in general qualitatively reproduced also for the other scenarios. Cultures switched from M9G to M9A (Fig. 5(a)) need, according to the simulations, about 1.5 hours before reaching maximal growth, which is more than twice the time observed experimentally. For cultures pre-grown in M9GA (Fig. 5(b)) we observe both in simulations and experiment a decreasing (increasing) lag time for daughter cultures sampled after GE for M9GA-M9A (M9GA-M9G) switches. Additional studies are reported in Text S5, in particular Fig. S17 displays the dependence of the lag time in the daughter cultures on the maximal transition values and Fig. S18 shows the same dependence, including also the distribution of the biomass ratio in the mother cultures, for a limited set of parameters.

3 Discussion

In this work we investigated a fundamental example of metabolic adaptation, namely diauxic growth of *E. coli* on glucose and acetate, with the aim of testing whether a dynamic metabolic modeling approach could capture diauxie in monocultures of *E. coli* as the observable emergent result of individual (sub-populations) behavior. To this end, we first developed a modeling framework to integrate dynamic models (ODE systems) with structural models (metabolic networks) and then performed simulations to reproduce *in silico* published experimental results.

Avoiding fine-tuning of model parameters. One recurrent criticism of stoichiometric and constraint-based modeling approaches, such as FBA, is that they can be easily adjusted to reproduce experimental results by *ad hoc* changes of flux constraints. In this work we chose to take the FBA parameters mostly from experimental measurements (Tab. 1). In the case of oxygen uptake and acetate

secretion, we calibrated the constraints using the results from Varma and Palsson, 1994 [18], where *E. coli* diauxic shift from glucose to acetate was first simulated with a genome scale model. Afterwards, the FBA parameters were not changed to reproduce the independent experiments from Enjalbert *et al.*, 2015 [22]. This approach to use an independent set of data for calibration of the FBA model parameters is a possible way to strengthen the confidence in subsequent results. Further model parameters of the ODE system have been chosen following reasonable hypotheses and slightly adjusted to obtain fair agreement with the experimental results, consistently among all the simulations. The initial conditions are finally specific to the experiment to reproduce.

Standard dFBA allows for abrupt metabolic readjustments. The flux distributions obtained from FBA solutions represent an average picture of the metabolic state of a population, which is in general modeled with a single genome scale model. Therefore, standard dFBA implementations where the FBA constraints evolve according to the updated external conditions, will reproduce the average change in metabolic state of the population in response to the external variations. This is equivalent to assuming that a population undergoes a coordinated, uniform metabolic shift under changing environmental conditions. Furthermore, with dFBA such transitions are, in general, abrupt. We tested two alternative approaches to simulate the diauxic shift in uniform *E. coli* monocultures, solving the FBA problems either with pFBA (mostly equivalent to the usual dFBA implementations) or with an adaptation of the MOMA algorithm. In the latter case, instead of minimizing the difference in flux distribution between a "wild type" GEM and a modified one (original MOMA implementation), we used the same concept to impose that, while integrating the dFBA system, at time t_i the flux solution is minimally different from that at time t_{i-1}, where the time steps are set by the integrating routine. Contrary to our expectations however, this approach does not allow for smoother metabolic adjustments in the system in response to the changing external conditions. Both implementations indeed resulted in abrupt changes in the flux distributions upon the shift from glucose to acetate metabolism (Fig. S3), therefore justifying the need of a different modeling approach.

Monocultures can be modeled as multi-sub-population systems to capture individual heterogeneity. With the introduction of two basic assumptions (first, there are two distinct metabolic states consuming either glucose or acetate; second, transition from one state to the other is driven by glucose and acetate concentrations) we were able to capture all the experimental trends published by Enjalbert *et al.* [22]. The transitions were modeled as Hill functions of the respective substrate concentrations with a noise offset. This follows the hypothesis that there is a constant, small noise component in cell regulation, while at high concentrations of certain substrates a coordinated transition occurs, *e.g.*, if acetate is highly abundant, more cells in the glucose-consumption state will shift to the acetate-consumption state in response to the changed environment. The introduction of a transition efficiency term is motivated by the observation that cells can get "lost in transition", an effect that was estimated in *Saccharomyces cerevisiae* to account for about 7% of the cells unable to start up glycolysis upon shift to high glucose levels [29]. Using a simple mathematical model we identified ranges for the parameters of the transition functions and selected reasonable values that would return good agreement between simulations and experiments. Both values for the constant transition rate (4% hr^{-1}) and for the maximal transition rate (20% hr^{-1}) are found in good agreement with measured average protein turnover rates in *E. coli* cultures from the literature [30, 31, 32]. Simulation results were mostly in very good agreement with the experimental data and our results strongly further support the idea, suggested over the last few years by independent experimental works on different organisms [21, 25, 33], that monocultures are an ensemble of sub-populations in different metabolic states, partially regulated by the environmental conditions. Under conditions where acetate concentrations were too low to support growth it was sufficient to model the transition as a constant random process. In contrast, in order to reproduce the data under conditions with high acetate concentrations, we had to introduce an active transition rate dependent on substrate concentrations. Interestingly, this assumption alone is sufficient to model the experimentally observed growth rate, without further fine tuning of model parameters. The introduction of substrate-dependent transition functions is also consistent with the experimental observations by Kotte *et al.* [27], supporting the hypothesis that a monoculture udergoes responsive diversification upon environmental changes.

The lag phase between growth on different substrates can be explained by population distributions. With standard dFBA simulations, the metabolic transition during the shift from one carbon source to another is abrupt. This is however rarely observed and, in particular, the duration of the lag phase between the exhaustion of the favored carbon source and the resumption of optimal growth on the alternate carbon source is highly variable for different environmental conditions. This observation can be easily explained as an emergent property of sub-population dynamics. Our simulations are consistent with the explanation that the delay to resume full growth actually depends on the ratio of the two sub-populations. Although the simulation results did not reproduce the experimental data quantitatively,

all qualitative trends were fully explained. A number of reasons may be responsible for the observed discrepancies. For example, no experimental control of the mother cultures regarding biomass, glucose and acetate dynamics were available, to which the modeled initial population could be compared. This could lead to a bias in the later sampling and determination of sub-population ratio and therefore strongly influence the quantification of the lag time. Indeed, it was shown by Solopova *et al.* [25] on *Lactococcus lactis* that population density (translating in practice to the rate at which the primary carbon source is consumed) played a significant role in determining the fraction of cells successfully transitioning to growth on the secondary carbon source. The strong connection between lag time and subpopulation distribution (discussed also in Text S5) could in principle be exploited to estimate initial population distributions from lag time measurements. However, with the currently available data it is difficult to assess how robust and reliable such predictions would be, and further investigation, including devoted experiments, are required. An additional source of discrepancy between our quantitative results and experimental measurements could be the experimental procedure itself. For example, abrupt changes of conditions such as the re-inoculation of daughter cultures into a different media in the switch experiments might select for additional adaptation strategies. Interestingly, we observed a dramatic improvement in the quantitative agreement between experiment and simulation by relaxing the condition imposing no growth for populations inoculated on the "wrong" carbon source (Tab. S5 and Fig. S19). By allowing the glucose-consumer population sampled from glucose mother cultures to have a reduced growth on acetate, we are mimicking a situation where cells stored resources and are able to survive a bit longer (Fig. S19(a)). On the other hand, allowing a reduced growth on acetate (glucose) for the glucose-consumer (acetate-consumer) population that was exposed to both carbon sources in the mixed mother cultures, could be a proxy for a memory effect. It was indeed observed that bacterial cells show memory effects upon changes in environmental conditions [26]. To explore this potential explanation further, however, would require more systematic experiments to carefully and reproducibly determine the lag times as functions of external parameters. Finally, a recent stochastic model of the regulatory network of diauxic growth in *E. coli* suggests that the limitations of biological sensors are responsible for the lag phase [34]. From their results we can infer that in our model the transition functions, currently depending on the absolute concentration of one carbon source at a time, might not be able to capture the fine details of population shifts. A possible extension would be to introduce more complex transition mechanisms dependent on relative concentrations of primary and secondary carbon sources, a process that would need dedicated experiments for construction and validation of the new transition functions.

Sub-populations in the dynamic metabolic modeling approach. In this work, we developed a modeling framework to perform FBA simulations embedded in a system of ODE. Building on previous methods and approaches like [19, 35], we further extended the standard dFBA implementation and introduced novel concepts. In particular, standard dFBA approaches assume that fluxes can instantaneously change to adapt to new environmental conditions, and flux solutions at subsequent time steps might significantly differ. This is an obvious limitation when aiming at capturing diauxic shift, where lag phases, highly dependent on the environmental conditions, are typically observed. We implemented the MOMA algorithm (originally developed to model the response to genetic perturbations in static FBA) in dFBA to minimize the metabolic adjustment between different time points. Furthermore, we integrated in dFBA dynamic mechanisms that cannot be included in metabolic models, such as population transitions. Indeed, while the use of dFBA to model subpopulations bears some similarities to other platforms for the simulation of microbial communities, a notable difference in our formulation is the capacity of subpopulations to interconvert with each other. The current study relied on the *a priori* knowledge that only two carbon sources would be available to *E. coli*, thus motivating a two sub-populations community, but in principle an arbitrary number of sub-populations can be defined, and more generic transition functions introduced. Further experiments, in particular single cell studies could be designed to define and parameterize these transition functions. Thanks to the object oriented design of the framework, it is relatively easy to introduce other functions regulating the constraints on specific reaction fluxes in the FBA problem. In this way, different hypotheses can be further extensively tested to better understand how to capture regulatory dynamics in dFBA. It is worth mentioning that methods developed in this framework to study population heterogeneity could then be transfered to other platforms more specific for microbial community modeling where different features are implemented (e.g. spatial structure [19] or community-level objectives [36]). Finally, the framework could also be further developed to include stochastic mechanisms, such as mutations in metabolic genes altering their function. Indeed, our implementation of the dFBA algorithm is able to call different methods at each time steps to e.g. update the flux rates, and a regulatory function with random components could be in principle defined.

Outlook. There is extensive experimental evidence that bacteria differentiate into sub-populations as a result of survival strategies [25, 27]. Simulations based on standard dFBA model the dynamics of cells by predicting the putative average behavior of a whole population. For example, if a population of

cells globally utilizes a combination of two carbon sources, dFBA would predict metabolic states in which both carbon sources are simutaneously utilized. Our model assumes that cells are either in the glucose or acetate consuming state, with an instantaneous transition between these two subpopulations that follows a simplistic rule which cannot capture intermediate states. This simplification is both practical and plausible when we observe population dynamics as the emergent properties of individual behavior and works well in dynamically changing environments with a continuous transient. However, rather than having a well defined metabolic state, especially during the transition between states, cells might exhibit a mixed state, which could be described as a superposition of 'pure' states, much in analogy to the state vectors in quantum physics. Furthermore, our approach suggests a fundamental difference in how metabolic fluxes in heterogeneous populations are to be accounted for, as the average fluxes in a uniform population might differ from the cumulative average fluxes of sub-populations. We think that further investigations of this novel concept of superimposed metabolic states might provide a promising new approach to study the principles of metabolic regulation.

4 Materials and Methods

FBA methods used. In stoichiometric models the stoichiometric matrix $\mathbf{S}(m \times n)$ is defined with the row and column dimensions corresponding to the numbers of metabolites m and reactions n respectively, the elements s_{ij} being the stoichiometric coefficients of metabolite i taking part in reaction j. FBA defines and solves the following LP problem:

$$\text{maximize} \quad \vec{z}, \tag{1}$$

$$\text{subject to :}$$

$$\mathbf{S}\vec{v} = 0, \tag{2}$$

$$l.b._j \leq v_j \leq u.b._j. \tag{3}$$

The steady-state assumption (Eq. 2) gives a system of equations that is under-determined and has an infinite number of solutions. Constraints on the fluxes (Eq. 3) allow to restrict the solutions to a convex solution space, however still resulting in an infinite number of solutions. The definition of an objective (Eq. 1) selects one solution, which however is in general still not unique for large (genome-scale) metabolic networks.

We consider in the following two extensions to the FBA problem definition, namely pFBA [14] and MOMA [15]. We then use these two methods to solve the FBA problem in an approach similar to dFBA [16]. Assuming that metabolism is evolved towards an efficient use of resources, pFBA finds the minimal flux distribution that returns the same objective defined by the FBA problem. We use the pFBA implementation from COBRApy [37] with maximal flux through the biomass reaction as objective function. Considering that metabolism has to respond quickly to perturbations, MOMA implements a quadratic algorithm to find the FBA solution after gene deletion that is most similar to the optimal wild type configuration. In our case we do not introduce modifications to the metabolic network but rather require that the MOMA solution obtained at time t_{i-1} is used to compute the MOMA solution at time t_i as the minimally different solution that satisfies the objective function. Also here the objective function is maximal flux through the biomass reaction. We use the MOMA implementation from COBRApy [37] in the linear approximation, with a slight modification to allow the reset of the LP problem in an iterative way, necessary to run MOMA within the dFBA approach.

Modeling framework integrating ODE and FBA. In the SOA of dFBA the boundary conditions in Eq. 3 are updated at discrete time steps according to the solution of the FBA problem in the previous time interval. Assuming quasi-steady-state conditions, *i.e.* that metabolism readjustments are faster than external environmental changes, dFBA can approximate the dynamic response of a GEM to a changing environment. Our approach is an extension of dFBA. The model is built as a system of ODEs, whose dimension depends on the dynamics to be modeled. Each ODE describes the variation in time of either an organism's biomass, metabolites, or other regulatory/dynamic processes. The biomasses and the metabolites can, but don't have to, correspond to the respective variables in a GEM. Their ODEs vary according to a function that can then depend on the flux solutions $\vec{v}$

$$\frac{dq_i}{dt} = \mathcal{F}(\vec{p}; \vec{v}, \vec{q}, t). \tag{4}$$

The ODE system is then solved with integrating routines with automated choice of time step. Each integration step solves the FBA problem (or pFBA, or MOMA) to obtain the current reaction rates for Eq 4, updates the metabolite quantities according to the FBA solution, re-computes the flux boundaries

of Eq. 3 according to specific reaction kinetics (typically Michaelis-Menten enzyme kinetics), and redefines the FBA problems with the new boundaries and/or other regulatory mechanisms defined by the user.

The modeling framework is written in Python (Python Software Foundation, https://www.python.org/) following the object-oriented programming (OOP) paradigm for efficiency and flexibility of use. The framework uses functionality from the following third-party packages: numpy [38], scipy [39], matplotlib [40], COBRApy [37], pandas [41]. In particular, we use COBRApy methods to solve the FBA problems and python integrators from the `scipy.integrate` method `ode` to solve the system of ordinary differential equations.

***E. coli* uniform population model.** The GEM of *E. coli* used is the core model from [42] downloaded from http://bigg.ucsd.edu/models/e_coli_core. The *E. coli* model $\mathrm{EC_{any}}$ is constrained on the consumption of "any" carbon sources (glucose, Gl, or acetate, Ac) solely by the environmental conditions, and the lower bound of the exchange reactions (EX_Glc_e and EX_Ac_e respectively) follows two simple Michaelis-Menten kinetics

$$l.b._{\mathrm{EX_Glc_e}}^{\mathrm{EC_{any}}} = -V_M^{\mathrm{Gl}} \frac{[\mathrm{Gl}]}{[\mathrm{Gl}] + K_M^{\mathrm{Gl}}}, \tag{5}$$

$$l.b._{\mathrm{EX_Ac_e}}^{\mathrm{EC_{any}}} = -V_M^{\mathrm{Ac}} \frac{[\mathrm{Ac}]}{[\mathrm{Ac}] + K_M^{\mathrm{Ac}}}. \tag{6}$$

The ODE system is defined as

$$\frac{d\mathrm{BM_{EC_{any}}}}{dt} = v_\mu^{\mathrm{EC_{any}}} \cdot \mathrm{BM_{EC_{any}}} - \delta \mathrm{BM_{EC_{any}}}, \tag{7}$$

$$\frac{d\mathrm{Gl}}{dt} = v_{\mathrm{EX_Glc_e}}^{\mathrm{EC_{any}}} \cdot \mathrm{BM_{EC_{any}}} + \xi_{\text{fed-batch}}, \tag{8}$$

$$\frac{d\mathrm{Ac}}{dt} = v_{\mathrm{EX_Ac_e}}^{\mathrm{EC_{any}}} \cdot \mathrm{BM_{EC_{any}}}, \tag{9}$$

where v_μ is the reaction rate of the biomass function (proxy for growth rate) in the FBA model, δ is the cell death rate and $\xi_{\text{fed-batch}}$ is a positive rate in fed-batch conditions and zero in batch conditions. Parameters and initial conditions are reported in Tab. 2. Either pFBA or MOMA are used to solve the FBA problem.

***E. coli* mixed population model.** Two *E. coli* core models are loaded and defined as either Glucose consumer ($\mathrm{EC_{Glc}}$) or Acetate consumer ($\mathrm{EC_{Ac}}$) by switching off uptake of the other carbon source

$$l.b._{\mathrm{EX_Glc_e}}^{\mathrm{EC_{Glc}}} = -V_M^{\mathrm{Gl}} \frac{[\mathrm{Gl}]}{[\mathrm{Gl}] + K_M^{\mathrm{Gl}}}, \tag{10}$$

$$l.b._{\mathrm{EX_Glc_e}}^{\mathrm{EC_{Ac}}} = 0, \tag{11}$$

$$l.b._{\mathrm{EX_Ac_e}}^{\mathrm{EC_{Glc}}} = 0, \tag{12}$$

$$l.b._{\mathrm{EX_Ac_e}}^{\mathrm{EC_{Ac}}} = -V_M^{\mathrm{Ac}} \frac{[\mathrm{Ac}]}{[\mathrm{Ac}] + K_M^{\mathrm{Ac}}}. \tag{13}$$

The ODE system is defined as

$$\frac{d\mathrm{BM_{EC_{Glc}}}}{dt} = (v_\mu^{\mathrm{EC_{Glc}}} - \psi - \delta) \cdot \mathrm{BM_{EC_{Glc}}} + \varepsilon\phi \mathrm{BM_{EC_{Ac}}}, \tag{14}$$

$$\frac{d\mathrm{BM_{EC_{Ac}}}}{dt} = (v_\mu^{\mathrm{EC_{Ac}}} - \phi - \delta) \cdot \mathrm{BM_{EC_{Ac}}} + \varepsilon\psi \mathrm{BM_{EC_{Glc}}}, \tag{15}$$

$$\frac{d\mathrm{Gl}}{dt} = v_{\mathrm{EX_Glc_e}}^{\mathrm{EC_{Glc}}} \cdot \mathrm{BM_{EC_{Glc}}} + v_{\mathrm{EX_Glc_e}}^{\mathrm{EC_{Ac}}} \cdot \mathrm{BM_{EC_{Ac}}} + \xi_{\text{fed-batch}}, \tag{16}$$

$$\frac{d\mathrm{Ac}}{dt} = v_{\mathrm{EX_Ac_e}}^{\mathrm{EC_{Glc}}} \cdot \mathrm{BM_{EC_{Glc}}} + (v_{\mathrm{EX_Glc_e}}^{\mathrm{EC_{Ac}}} + \zeta \cdot H(t - t_x)) \cdot \mathrm{BM_{EC_{Ac}}}, \tag{17}$$

where $\zeta \cdot H(t - t_x)$ is a Heaviside function activated at the time t_x of glucose exhaustion in order to keep acetate constant, and ψ and ϕ are functions that model the cellular shift from $\mathrm{EC_{Glc}}$ to $\mathrm{EC_{Ac}}$ and $\mathrm{EC_{Ac}}$ to $\mathrm{EC_{Glc}}$, respectively, and $0 < \varepsilon < 1$ is a positive factor representing the transition efficiency. The functions ψ and ϕ are modeled as Hill functions with a noise offset

$$\psi([\mathrm{Ac}]) = \psi_0 + V_M^\psi \frac{[\mathrm{Ac}]^n}{[\mathrm{Ac}]^n + {K_M^\psi}^n}, \tag{18}$$

$$\phi([\mathrm{Gl}]) = \phi_0 + V_M^\phi \frac{[\mathrm{Gl}]^n}{[\mathrm{Gl}]^n + {K_M^\phi}^n}, \tag{19}$$

Table 1: Fixed parameters for all simulations. The death rate δ is computed assuming a cell death of 1% per generation (Saint-Ruf *et al.*, 2004 [43]) and a generation time of 20 minutes. The Michaelis-Menten parameters for substrate uptake are taken from Gosset, 2005 [44]. These parameters are also used in previously published dFBA implementations [45].

	L.B. EX_O2 $\frac{\text{mmol}}{\text{gDW hr}}$	$\boldsymbol{\delta}$ hr^{-1}	$\mathbf{V_M^{Glc}}$ $\frac{\text{mmol}}{\text{gDW hr}}$	$\mathbf{K_M^{Glc}}$ mM	$\mathbf{V_M^{Ac}}$ $\frac{\text{mmol}}{\text{gDW hr}}$	$\mathbf{K_M^{Ac}}$ mM
Value	-10.0	0.03	10.0	0.01	10.0	0.01

Table 2: Parameters of the simulations.

	BM(0) 10^{-3} gDW	$\left.\frac{\mathbf{EC_{Glc}}}{\mathbf{EC_{Ac}}}\right\|_0$	**Glc(0)** mmol	**Ac(0)** mmol	$\boldsymbol{\xi}$ $\frac{\text{mmol}}{\text{hr}}$	$\boldsymbol{\zeta}$ $\frac{\text{mmol}}{\text{hr}}$	t_x hr	ψ_0 hr^{-1}	$\mathbf{V_M^{\psi}}$ hr^{-1}	$\mathbf{K_M^{\psi}}$ mM	ϕ_0 hr^{-1}	$\mathbf{V_M^{\phi}}$ hr^{-1}	$\mathbf{K_M^{\phi}}$ mM	$\boldsymbol{\varepsilon}$
Fig. S1(a), S1(c)	0.3	NA	10.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fig. S1(b), S1(d)	0.24	NA	0.82	0.1	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fig. 1(a), 1(b)	2.7	NA	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fig. 3(a)	2.7	$\frac{0.95}{0.05}$	15.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fig. 3(b), S10(a)	2.7	$\frac{0.95}{0.05}$	15.0	0.0	0.0	0.0	0.0	0.04	0.0	0.0	0.04	0.0	0.0	0.9
Fig. S10(b)	2.7	$\frac{0.95}{0.05}$	15.0	0.0	0.0	0.0	0.0	0.04	0.2	30.0	0.04	0.2	5.0	0.9
Fig. S11(a)	3.8	$\frac{0.75}{0.25}$	15.0	0.0	0.0	9.1	4.0	0.04	0.0	0.0	0.04	0.0	0.0	0.9
Fig. 4(a), S11(b)	3.8	$\frac{0.75}{0.25}$	15.0	0.0	0.0	9.1	4.0	0.04	0.2	30.0	0.04	0.2	5.0	0.9
Fig. S12(a)	6.0	$\frac{0.75}{0.25}$	15.0	32.0	0.0	9.1	4.0	0.04	0.0	0.0	0.04	0.0	0.0	0.9
Fig. 4(b), S12(b)	6.0	$\frac{0.75}{0.25}$	15.0	32.0	0.0	9.1	4.0	0.04	0.2	30.0	0.04	0.2	5.0	0.9
M9G (m.c.)	2.7	$\frac{0.95}{0.05}$	15.0	0.0	0.0	0.0	0.0	0.04	0.2	30.0	0.04	0.2	5.0	0.9
M9GA (m.c.)	6.0	$\frac{0.75}{0.25}$	15.0	32.0	0.0	0.0	0.0	0.04	0.2	30.0	0.04	0.2	5.0	0.9

and for $V_M^{\phi} = V_M^{\psi} = 0$ they are constant transition rates. For the simulations presented in this work, we used a Hill coefficient $n = 5$. Indeed, simulations seem to work best for a transition function with a high degree of cooperativity, and the results are robust with respect to small deviations relative to this value. The other parameters and initial conditions, specific to the different simulations, are reported in Tab. 2. For mixed population simulations, pFBA is used to solve the FBA problem.

Switch experiment simulations. Two *E. coli* mixed population model simulations are run as "mother cultures" as shown in Tab. 2 for "M9G" and "M9GA" conditions (glucose and glucose plus acetate, respectively). From each mother culture we sample 11 time points between -1 and +1.5 hours from the respective GE time (4.6 hours for M9G and 3.9 hours M9GA) to obtain the biomass ratio between EC_{Glc} and EC_{Ac} to be used as initial condition for the re-inoculation simulations. The percentage of EC_{Glc} biomass at these time points is reported in Tab. 3. The "daughter cultures" are then grown either in "M9G" or "M9A" conditions (glucose and acetate only, respectively; see Tab. 2). A total of 44 simplified simulations (see Text S5) are hence run, 11 for each of the following 4 switch experiments: M9G to M9G; M9G to M9A; M9GA to M9G; M9GA to M9A. For each simulation, the lag time is computed according to Enjalbert *et al.* [22]:

$$t_{\text{lag}} = t_1 - \frac{\ln(X_1/X_0)}{\mu_{\max}} \tag{20}$$

Table 3: Percentage of EC_{Glc} biomass in M9G and M9GA conditions at time points relative to glucose exhaustion, in hours.

	-1.0	**-0.75**	**-0.5**	**-0.25**	**0**	**0.25**	**0.5**	**0.75**	**1.0**	**1.25**	**1.5**
M9G	95.1	95.0	94.8	94.5	94.3	93.2	92.3	91.4	90.6	89.4	88.2
M9GA	78.7	78.6	78.1	77.3	76.5	73.2	70.1	67.2	64.5	62.0	59.7

where X_0 is the total initial *E. coli* biomass, X_1 is the total *E. coli* biomass value at time t_1 (chosen as 1.5 hours as in [22]), and $\mu_{\max}$ are taken as the values reported in Enjalbert *et al.* [22].

Published experimental data. Experimental data (values with standard deviations, when available) from the publication [22] were kindly provided by Prof. Enjalbert. The data from [18] were extracted from the publication's figures using WebPlotDigitizer [46].

Availability of data and materials. The version v1.0 of the modeling framework used to obtain the results presented in this manuscript is publicly available with instructions to install and run simulations at `https://github.com/QTB-HHU/dfba-ode-framework_ecoli-diauxie`. The development version is hosted on `https://gitlab.com/asuccurro/dfba-ode-framework` and people interested in contributing can request access by contacting the corresponding author (A.S.).

5 Supplemental Material

Supplemental material for this article may be found at `xxx`

TEXT S1, FIGURE S1, S2, S3, PDF file, 994 KB.
FIGURE S4 PNG file, 17 KB.
FIGURE S5 EPS file, 2.2 MB.
FIGURE S6 EPS file, 2.2 MB.
TEXT S2, FIGURE S7, S8, S9, TABLE S1, S2, PDF file, 557 KB.
TEXT S3, FIGURE S10, S11, S12, TABLE S3, PDF file, 625 KB.
TEXT S4, FIGURE S13, S14, S15, PDF file, 389 KB.
TEXT S5, FIGURE S16, S17, S18, S19, TABLE S4, S5, PDF file, 554 KB.

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