

# Data-driven Metabolic Network Reduction for Multiple Modes Considering Uncertain Measurements

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**Abstract:** Dynamic models of biotechnological processes form the basis of process optimization, control, and estimation. Metabolic network models are often at the core of such models. Since metabolic network models can be very large, and consequently computationally expensive, model reduction techniques can be applied. The derivation of a suitable reduced metabolic network that captures the essential metabolism is still a challenging problem. State-of-the-art network reduction algorithms utilize a priori defined phenotypes that reflect the expected behavior of the biological system. However, most bioprocesses undergo changes in the metabolism, hence, a switch in the cellular phenotype. If these phenotypes are unknown a priori, the reduced network fails to represent all observed metabolic behaviors. Contrary to these approaches, we propose a method that reduces genome-scale metabolic networks models using data from real experiments instead of relying on predefined phenotypes. Doing so, we circumvent the use of a priori information and guarantee that the network is capable to describe all observed phenotypes and can be reliably used for estimation, prediction, and optimization.

*Keywords:* Model reduction, networks, multi-mode, data-driven, measurement uncertainties, biotechnology.

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## 1. INTRODUCTION

Stoichiometric metabolic network models describe the cellular metabolism of a certain organism via a system of linear algebraic equations. Since this system is generally underdetermined, a variety of computational network analysis tools have been developed to retrieve valuable information for analyzing and optimizing biotechnological processes (Trinh et al., 2009). These methods have different objectives. Flux balance analysis can be used to identify the optimal medium formulation (Xie and Wang, 1997). Elementary flux mode analysis as well as flux balance analysis can be used to design strains tailored to specific needs or to fulfill certain tasks (Machado and Herrgård, 2015). Further, in a hybrid system representation the metabolic network can be extended with ordinary differential equations or time series (Mahadevan et al., 2002; Höffner et al., 2013; Song et al., 2009; Leighty and Antoniewicz, 2011). These hybrid models have an even broader application range as they represent the dynamics of the bioprocess and can be used for model-based process control and optimization (Chang et al., 2016; Jabarivelisdeh et al., 2018; Morabito et al., 2019).

Since these applications require a stoichiometric metabolic model, the accuracy will increase if the model has a high

descriptive power. The metabolic models with the highest descriptive power are genome-scale metabolic network models. Genome-scale models are obtained by annotating the whole genome of an organism and can consist of thousands of reactions and species. However, with the increasing complexity of genome-scale models the computational burden increases up to the point where even modern computers are not able to apply computational network analysis tools, such as elementary flux mode analysis. Nowadays, these genome-scale models are stored in online databases accessible to everyone, like BiGG or KEGG (King et al., 2016; Kanehisa and Goto, 2000), where they are maintained and kept up to date.

To be able to apply these tools but still have good descriptive power, a trade-off between the computational complexity and the descriptive power has to be made. A very efficient way of accomplishing this trade-off is the use of network reduction algorithms. These generally operate by deleting reactions that are not required to fulfill a set of constraints, which is defined by the user beforehand. The reduction process is usually executed by optimization-based techniques or methods derived from graph theory (Erdrich et al., 2015; Röhl and Bockmayr, 2017; Ataman et al., 2017). An essential aspect of the user-defined set of constraints is the phenotype of the biological system. Using a priori knowledge, expected phenotypes can be defined and considered in the network reduction. Since most biological systems go through changes during a process,

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several switches can occur in the phenotype. Usually these changes are not known a priori and, consequently, the reduced metabolic network can fail to represent all observed phenotypes.

The aim of this work is to propose a method that uses real data measured during an experiment as a replacement for a priori generated phenotypes inside the user-defined constraints. Our results show, that data driven network reduction obtains metabolic models which represent all the experimentally observed phenotypes without the need to have an in-depth knowledge about the metabolism of the considered organism.

## 2. METABOLIC NETWORK MODEL

The temporal change in the concentration  $x_i$  of the metabolite  $i = 1, \dots, m$  in an organism can be described by the following system of differential equations

$$\frac{dx_i}{dt} = \sum_{j=1}^q n_{ij} r_j(t), \quad (1)$$

or in matrix notation

$$\frac{d\mathbf{x}}{dt} = \mathbf{N}\mathbf{r}(t). \quad (2)$$

The stoichiometry matrix  $\mathbf{N} \in \mathbb{R}^{m \times q}$  represents a compact form of the metabolic network and consists of  $m$  rows, one for each participating metabolite and  $q$  columns, one for each occurring reaction. The vector  $\mathbf{r} \in \mathbb{R}^{q \times 1}$  contains the rates of each reaction  $r_j$ , which usually have a lower bound  $\alpha_j$  and an upper bound  $\beta_j$ .

Most of the network analysis tools assume that all internal metabolites of the metabolic network are in a quasi-steady-state, i.e.

$$\frac{d\mathbf{x}}{dt} = \mathbf{0} = \mathbf{N}\mathbf{r}(t). \quad (3)$$

This is a reasonable assumption, since the ratio between the reaction rates and the concentrations of the metabolites is very high, at least in the central metabolism (Stephanopoulos et al., 1998).

### 2.1 Computational Network Analysis Tools

This subsection briefly reviews the computational network analysis tools that are generally used either during the network reduction or for the analysis and validation of the reduced networks.

Flux balance analysis utilizes a linear program to find a flux distribution that minimizes or maximizes specific fluxes (Savinell and Palsson, 1992)

$$\min_{\mathbf{r}} \mathbf{c}^T \mathbf{r}, \quad (4a)$$

$$\text{s.t. Eq. (3),} \quad (4b)$$

$$\boldsymbol{\alpha} \leq \mathbf{r} \leq \boldsymbol{\beta}. \quad (4c)$$

Here the objective coefficient  $\mathbf{c}$  is usually a vector of zeros, except at the positions of the fluxes to be minimized or maximized. Flux variability analysis uses the formalism of the flux balance analysis to find a range for each flux satisfying given constraints (Mahadevan and Schilling, 2003). This is achieved by alternating the sign of the objective coefficient  $\mathbf{c}$  to find a minimal flux and a maximal flux for each reaction.

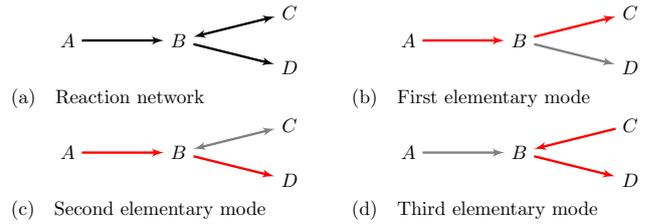


Fig. 1. Elementary flux modes in a simple network. The elementary flux modes are marked red.

Elementary flux mode analysis decomposes the network into minimal functional units, i.e. the non-decomposable steady-state pathways through the metabolic network (Schuster and Hilgetag, 1994). These pathways are also called elementary flux modes. A flux mode  $\mathbf{e}$  is called elementary flux mode, if

$$\text{supp}(\mathbf{e}) \not\subseteq \text{supp}(\mathbf{r}), \quad (5)$$

for every admissible flux mode  $\mathbf{r}$ , that meets the homogeneous constraints, where the support of a flux mode  $\mathbf{r}$  is defined as

$$\text{supp}(\mathbf{r}) = \{i \mid r_i \neq 0\}.$$

Figure 1 shows the elementary flux modes for a simple reaction network. Elementary flux modes can be used to span a yield space for the metabolic network which depicts the solution space in which the metabolic network can operate without getting infeasible (see Figure 5).

### 2.2 Network Reduction

Network reduction methods aim at identifying the most important network pathways and remove the least important. As an example of a network reduction algorithm the *NetworkReducer* (Erdrich et al., 2015) is outlined. *NetworkReducer* takes as an input from the user a set of constraints and the metabolic network model, represented by the stoichiometry matrix  $\mathbf{N}$  and the lower and upper bounds  $\boldsymbol{\alpha}$  and  $\boldsymbol{\beta}$ , respectively. The following constraints are considered

$$\mathcal{X}_{\text{prot}} = \{x_i \mid i \in [1, m]\}, \quad (6a)$$

$$\mathcal{R}_{\text{prot}} = \{r_j \mid j \in [1, q]\}, \quad (6b)$$

$$\mathbf{D}_p \mathbf{r} \leq \mathbf{d}_p, \quad p = 1, \dots, n_p, \quad (6c)$$

$$\text{dof} \geq \text{dof}_{\text{min}}, \quad (6d)$$

$$n_r \geq n_{\text{min}}. \quad (6e)$$

Where  $\mathcal{X}_{\text{prot}}$  is a set of protected metabolites,  $\mathcal{R}_{\text{prot}}$  is a set of protected reactions,  $\text{dof} = q - \text{rank}(\mathbf{N})$  is the degree of freedom, and  $n_r$  is the number of reactions in the reduced network. The linear inequalities in Equation (6c) represent the phenotypes, where  $n_p$  is the number of phenotypes which the reduced network should be able to describe.

After checking the initial feasibility of equations (6), the *NetworkReducer* will begin to iteratively remove all the reactions that will not lead to a violation of the constraints. This step is called *pruning*, and utilizes flux variability analysis to find reactions that are necessary to fulfill the constraints. Finally, the remaining reactions can be optionally lumped in an loss-free network compression step by removing redundancies in the reduced stoichiometry matrix  $\mathbf{N}_{\text{red}}$  (Gagneur and Klamt, 2004).

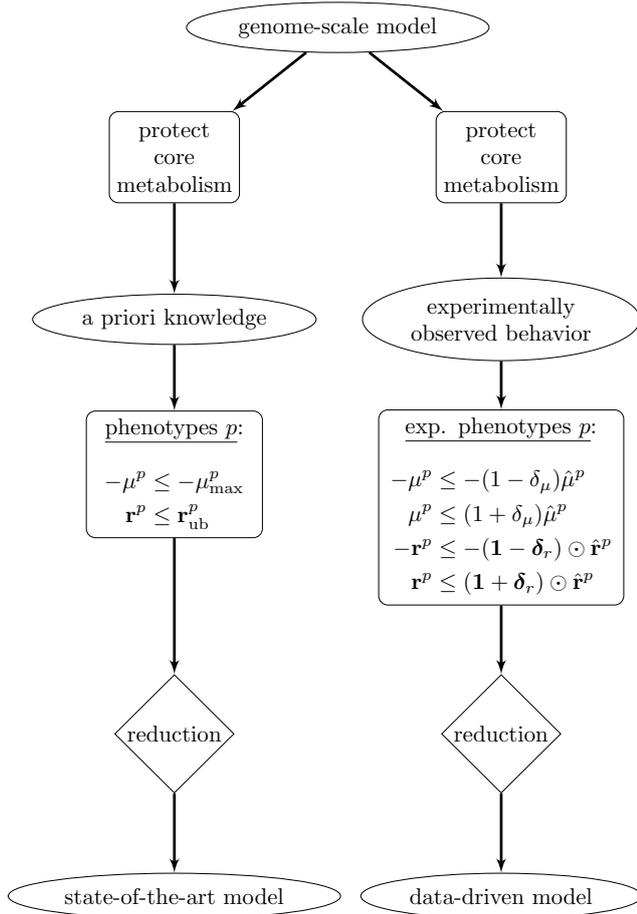


Fig. 2. Workflows of the state-of-the-art network reduction and the data-driven network reduction. The maximum growth rate for each phenotype  $\mu_{\max}^p$  in the state-of-the-art network reduction is usually generated by applying flux balance analysis, given some upper bounds on the rates  $\mathbf{r}_{\text{ub}}^p$ . The operator  $\odot$  is used to indicate the Hadamard product and  $\delta_i$  is a relaxation factor.

**Data-driven Network Reduction** Rather than using a priori knowledge to determine the phenotypes, the proposed data-driven network reduction takes experimentally measured rates into account. To prevent infeasibility of the flux variability analysis, the measured reaction rates are relaxed with relaxation factors  $\delta_r$  and  $\delta_\mu$  that define a range containing each measured rate. The ranges of the rates for each experimentally induced phenotype are used in Equation (6c) as constraints to the *NetworkReducer*. Figure 2 depicts the workflow for both reduction procedures, the state-of-the-art network reduction and the data-driven network reduction.

### 2.3 Validation of the Descriptive Power of the Network

The performance of a reduced network can be measured by its capability to represent the measured data. Usually, imposing all the measured rates to the reduced metabolic network results in an overdetermined system that, due to uncertainty in the measurements, has no feasible solution. Metabolic flux analysis can be used to find a feasible flux distribution close to the measurement data in the case of overdetermined systems by considering the measurement

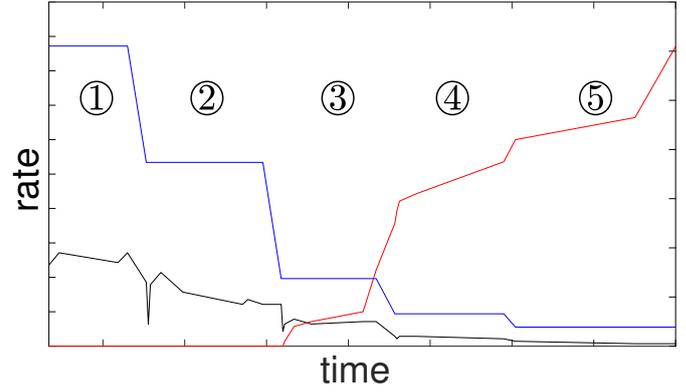


Fig. 3. The 5 experimental phases generated by different  $r_{\text{O}_2}$  set points (blue line). When the process gets more microaerobic, the cells shift from aerobic towards fermentative metabolism, which is indicated by a decreasing  $\mu$  (black line) and an increasing secretion of fermentation products (red line). The cellular behavior in each phase is referred to as a certain phenotype in the following.

uncertainties. Originally, metabolic flux analysis does not consider constraints, such as flux directions. Instead, a constraint formulation of the metabolic flux analysis is used to compare the reduced networks

$$\min_{\mathbf{r}} \frac{1}{2} \left\| \sqrt{\mathbf{P}^{-1}} (\bar{\mathbf{r}}_k - \mathbf{r}_k) \right\|_2^2, \quad (7)$$

s.t. Eq. (4b) – (4c),

where  $\mathbf{P}$  is the covariance matrix,  $\bar{\mathbf{r}}_k$  is the vector of the measured rates, and  $\mathbf{r}_k$  is the known part of the reaction rate vector  $\mathbf{r}$ .

## 3. EVALUATION OF THE DATA-DRIVEN NETWORK REDUCTION CONSIDERING ESCHERICHIA COLI

The experimental data used for the data-driven network reduction comes from an experiment with *Escherichia coli* on glycerol as carbon source, which was carried out in 5 phases. In each phase, the input to the process was changed, more specifically the oxygen uptake rate  $r_{\text{O}_2}$  was decreased. This generates multiple switches in the phenotype of the biological system, since the organism has to adapt to survive under the different degrees of oxygen-limited (microaerobic) conditions. Besides the growth rate  $\mu$ , other measured rates during this experiment are the uptake rates of oxygen ( $\text{O}_2$ ) and glycerol (Glyc) and the secretion rates of carbon dioxide ( $\text{CO}_2$ ), hydrogen ( $\text{H}_2$ ) and the fermentation products acetate (Ac), ethanol (EtOH), formate (Form) and succinate (Succ). A qualitative plot of the experimental procedure can be seen in Figure 3.

### 3.1 Network reduction

The genome-scale metabolic network model used for network reduction in this work is the reconstruction of the organism *Escherichia coli* K-12 MG1655 (Orth et al., 2011). The *NetworkReducer* by Erdrich et al. (2015) was used for the network reduction. For comparison a state-of-the-art reduced network model was used, more specifically the

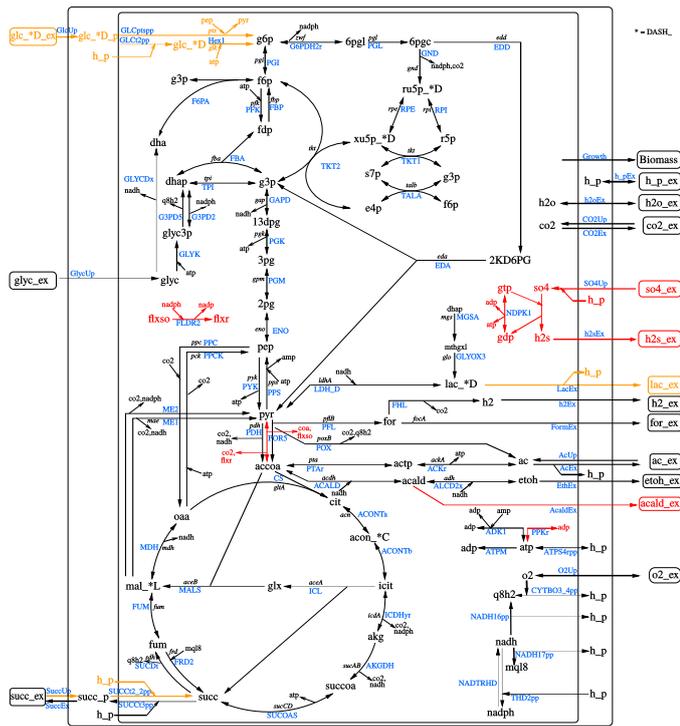


Fig. 4. Differences between both reduced networks. Pathways that are unique to *EcoliCore2* are colored in orange and pathways that are unique to the network *EcoliCoreDD* are red. Pathways in black are common to both networks and mostly contain the protected reactions of the core metabolism. The biomass composition is different for both networks. The network map was adapted from Hädicke and Klamt (2017).

reference model *EcoliCore2* (Hädicke and Klamt, 2017), which was also derived with the *NetworkReducer*. The same reactions of the core metabolism were protected for the data-driven network reduction as for *EcoliCore2*, with the exception of the glucose uptake, since the cultivation of *Escherichia coli* was performed in a glycerol medium, and not glucose. The network reduced in this work using the data-driven approach is called *EcoliCoreDD* throughout the rest of the paper. The ranges used for the data-driven network reduction are calculated by assuming an error of  $\pm 10\%$  ( $\delta_i = 0.1$ ) for each rate. This is a simplified assumption, that corresponds to smallest error observed during performed experiments. Figure 4 shows the different remaining reactions in both reduced networks. As can be seen, the network *EcoliCoreDD* has additional secretion reactions for hydrogen sulfide (H<sub>2</sub>S) and acetaldehyde (AcAld). These two alterations indicate that cells require additional reactions to balance the cellular redox potential and carbon metabolism. Literature review indicates that *Escherichia coli* indeed produces H<sub>2</sub>S during phases of high oxidative stress as a protection mechanism (Mironov et al., 2017). However, both compounds could not be measured during the experiment, since they are highly volatile. Another reason for the imbalance in the cellular redox potential and carbon metabolism could be that the error of the rates is higher than the assumed  $\pm 10\%$ .

### 3.2 Analyzing Network Performance

To analyze the descriptive power of the obtained metabolic networks the constraint metabolic flux analysis (Equation (7)) was used to determine adjusted rates. Measurement uncertainties were taken into account by assuming a standard deviation for each rate of  $\sigma_i = 0.1 \cdot \bar{r}_i$ . After calculating the least squares solution, the adjusted rates were compared to the taken measurements and an error was computed as a degree for the descriptive power of the metabolic network. The results for each phenotype are shown in Table 1 and are discussed in the following in more detail.

**Aerobic Growth Phase – Phase 1** For the aerobic growth phase, both network models are able to reflect the measurements very well. The network *EcoliCoreDD* is able to predict the measurements without any adjustments. The network *EcoliCore2* is also able to predict the measured rates quite well, since the adjustments to the measured rates are minimal, except for  $q_{O_2}$  and  $q_{CO_2}$ , which have slightly bigger errors, but still lie within the 95% confidence interval.

**Microaerobic Growth Phase – Phases 2-5** The last 4 phases from the experiment shown in Figure 3 represent microaerobic growth behavior. Overall, it can be seen, that the reference network *EcoliCore2* struggles to produce good predictions for these microaerobic phenotypes, especially in the phases 3 and 4, whereas the network *EcoliCoreDD* can reflect the measurements with only minimal adjustments. The biggest errors are produced for the carbon dioxide emission rate  $q_{CO_2}$ , with a maximum error of  $-151.65\%$  in phase 4. Here, the glycerol uptake rate  $q_{Glyc}$  also has its highest error, i.e. the network *EcoliCore2* is not able to map these two rates to the rates of the secreted fermentation products, which indicates that the carbon balance is probably not in equilibrium. This discrepancy could also be caused by the low relative standard deviations.

### 3.3 Yield Space

To further show that the network reduced using experimental data instead of user-defined phenotypes is able to better describe the behavior of a metabolic network in an experimental setup, Figure 5 depicts the yield spaces for both networks. The yield space shows a selection of elementary flux modes that are calculated via elementary flux mode analysis. Here, the elementary flux modes were calculated using the *FluxModeCalculator* (van Klinken and van Dijk, 2016). In this figure, it can be seen that for the state-of-the-art network *EcoliCore2* the experimental data is outside the solution space for the last three phases. Since the solution space of the network *EcoliCoreDD* has been adapted to the observed phenotypes, all experimental data lie within its solution space.

## 4. CONCLUSION

A key component for deriving meaningful metabolic networks from genome-scale models is the set of cellular phenotypes the derived network is expected to describe properly. State-of-the-art network reduction algorithms can be

Table 1. Comparison of the results of constraint metabolic flux analysis (Equation (7)) for both networks using experimental data and a relative standard deviation of  $\sigma_i = 0.1$  for each rate  $r_i$ . Red marks error values for rates that lie outside the  $\bar{r}_i \pm 3\sigma_i$  interval, orange marks error values for rates that lie between the  $\bar{r}_i \pm 2\sigma_i$  interval and the  $\bar{r}_i \pm 3\sigma_i$  interval, and green marks error values for rates that lie between the  $\bar{r}_i \pm \sigma_i$  interval and the  $\bar{r}_i \pm 2\sigma_i$  interval.

	$\mu$	$r_{O_2}$	$r_{CO_2}$	$r_{H_2}$	$r_{Glyc}$	$r_{Ac}$	$r_{EtOH}$	$r_{Form}$	$r_{Succ}$
Aerobic Growth Phase - Phase 1									
measured	0.3589	8.7200	8.1442	0.0000	7.8093	0.2355	0.0000	0.0000	0.0000
<i>EcoliCore2</i>	0.3601	9.9623	7.0194	0.0000	7.4244	0.2354	0.0000	0.0000	0.0000
error	+0.34 %	+12.47 %	-16.02 %	—	-5.18 %	-0.03 %	—	—	—
<i>EcoliCoreDD</i>	0.3589	8.7200	8.1442	0.0000	7.8093	0.2355	0.0000	0.0000	0.0000
error	—	—	—	—	—	—	—	—	—
Microaerobic Growth Phase - Phase 2									
measured	0.2246	5.3400	5.6400	—	5.5100	2.1100	0.0430	0.0000	0.0000
<i>EcoliCore2</i>	0.2118	6.3559	4.2507	—	5.7023	2.0383	0.0430	0.0000	0.0000
error	-6.06 %	+15.98 %	-32.68 %	—	+3.37 %	-3.52 %	$\pm 0.00$ %	—	—
<i>EcoliCoreDD</i>	0.2112	5.3400	5.4345	—	6.0985	2.0525	0.0430	0.0000	0.0000
error	-6.33 %	$\pm 0.00$ %	-3.78 %	—	+9.65 %	-2.80 %	$\pm 0.00$ %	—	—
Microaerobic Growth Phase - Phase 3									
measured	0.0917	1.9635	3.0052	0.5010	4.2380	1.9193	0.2951	0.0000	0.1358
<i>EcoliCore2</i>	0.0862	2.8020	1.9217	0.5283	3.1253	1.3876	0.3015	0.0000	0.1347
error	-6.42 %	+29.93 %	-56.38 %	+5.17 %	-35.60 %	-38.31 %	+2.11 %	—	-0.79 %
<i>EcoliCoreDD</i>	0.0870	2.1227	2.9711	0.5031	4.1953	1.9144	0.2958	0.0000	0.1358
error	-5.42 %	+7.50 %	-1.15 %	+0.42 %	-1.02 %	-0.25 %	+0.25 %	—	$\pm 0.00$ %
Microaerobic Growth Phase - Phase 4									
measured	0.0372	0.9403	1.9703	0.9657	3.5856	0.7160	0.5651	0.8823	0.3879
<i>EcoliCore2</i>	0.0369	1.2430	0.7829	1.0378	2.3909	0.7053	0.6678	0.7044	0.3559
error	-0.74 %	+24.35 %	-151.65 %	+6.95 %	-49.97 %	-1.52 %	+15.37 %	-25.26 %	-9.00 %
<i>EcoliCoreDD</i>	0.0338	1.0671	1.8486	0.9846	3.8469	0.7207	0.5716	0.8679	0.3801
error	-10.05 %	+11.88 %	-6.58 %	+1.92 %	+6.79 %	+0.65 %	+1.13 %	-1.66 %	-2.05 %
Microaerobic Growth Phase - Phase 5									
measured	0.0200	0.5539	1.3784	—	2.0300	0.5096	0.8198	0.4786	0.2063
<i>EcoliCore2</i>	0.0184	0.6853	0.9951	—	1.9974	0.5182	0.9915	0.4298	0.1981
error	-8.64 %	+19.18 %	-38.52 %	—	-1.63 %	+1.66 %	+17.31 %	-11.36 %	-4.17 %
<i>EcoliCoreDD</i>	0.0183	0.6127	1.3914	—	2.2274	0.5238	0.8198	0.4770	0.2046
error	-9.35 %	+9.59 %	+0.93 %	—	+8.86 %	+2.71 %	$\pm 0.00$ %	-0.33 %	-0.85 %

used to derive such models by introducing constraints, that represent these phenotypes.

Often, the phenotypes are defined a priori, requiring in-depth knowledge about the metabolism of the used organism. However, the organism usually switches between different phenotypes during a biological cultivation process, which makes it difficult to know all relevant phenotypes a priori. Due to these uncertainties in the phenotypes, the metabolic network may not be able to represent the experimentally observed phenotypes. Consequently, metabolic pathway analysis tools fail to analyse the experimentally obtained data sets, since the linear algebraic equation system becomes infeasible.

In this work, real data gathered during an experiment was used to define the phenotypes for the network reduction algorithm. To generate different phenotypes, the experiment was divided into 5 phases, each exploring a certain degree of oxygen limitation, resulting in different phenotypes for the used organism. The data-driven reduced network had an improved descriptive power compared to the reference network reduced with a priori knowledge. It was able to reflect the observed phenotypes with only minimal errors, whereas the reference network struggled to represent most of the observed phenotypes sufficiently, especially in the oxygen-limited phases of the experiment.

Future work will investigate the capabilities of the data-driven network reduction regarding the real time use at a biotechnological process in combination with control strategies which aim to steer the process towards a desired product yield. Furthermore, we plan to explore the impact of individual errors for each rate on the network performance and network topology.

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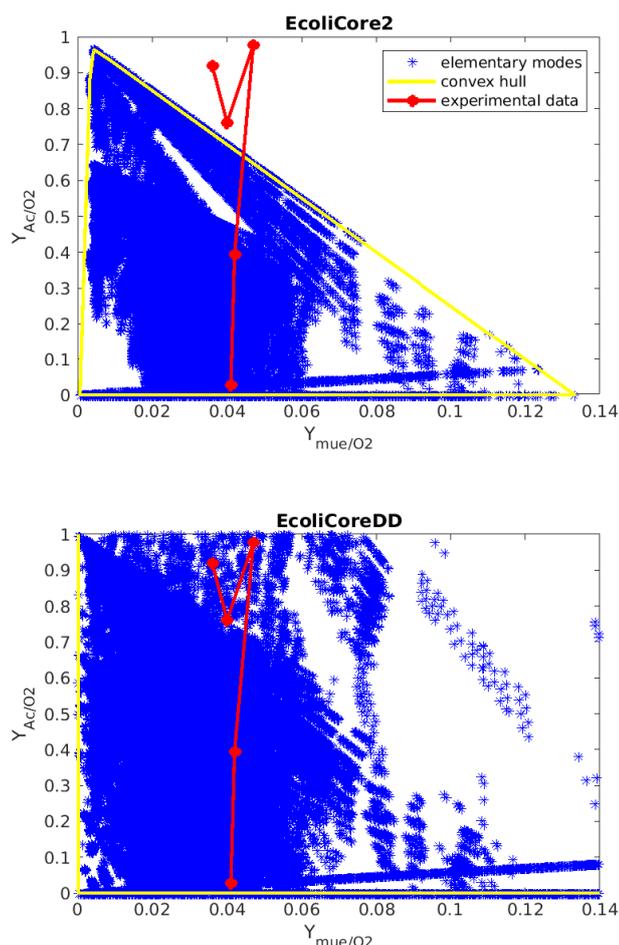


Fig. 5. Yield spaces for both networks with experimental data. Here, the yield of acetate with respect to the oxygen uptake is plotted over the biomass yield with respect to the oxygen uptake for both networks.

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