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1. INTRODUCTION

1.1. Metabolic Networks

1.1.1. Mathematical Modeling of Cellular Metabolism

Mapping the network of the chemical reactions in the cells is another field of synthetic biology. Organism specific reconstructed metabolic networks allow researchers to design experiments, and even to predict outcomes before the experimental works. These networks may also be used to build mathematical models which can simulate metabolic fluxes reflecting the reality (Thiele I, Palsson BO., 2010).

One of the most extensively studied metabolic networks is the model of S. cerevisiae. More than 25 models of metabolic network for the yeast have been published since 2003 (B.D. Heavner, N. D. Price, 2015), and the number is increasing. Researchers have combined the experimental data into models to simulate phenotypes in different environmental conditions (Snitkin et al., 2008).

Indeed, the ultimate goal of many fields of the life sciences is to understand cell physiology and hence the flux distribution, which characterizes the metabolic state of a cell. Based on this information, it will be possible to design strategies, for example, to avoid certain phenotypes (applications in infection biology and cancer research) or to redirect fluxes (applications in industrial biotechnology to increase rate, yield and titre of a product of interest). [1]

1.1.2. Steady State Assumption

A rather frequently used assumption for metabolic network modelling is that the production and consumption of internal metabolites must balance (steady-state assumption). [2]

1.1.3. Genome Scale Metabolic Models

1.1.4. Constraint-Based Models

1.1.5. Flux Balance Analysis

1.2. *Saccharomyces cerevisiae*

1.2.1. Industrial importance of *S. cerevisiae*

Saccharomyces cerevisiae is one of the main microorganisms used in the biochemical industry such as alcohol fermentations, baking processes and bio-ethanol production

1.2.2. Metabolic Models of *S. cerevisiae*

1.2.3. Applications of *S. cerevisiae* GSMMs

Citation example is [**?**].

2. MATERIALS AND METHODS

2.1. Experimental Data Preparation

2.1.1. Data Acquisition

Extracellular metabolomics data is obtained from Cakar's Lab [3]. Briefly, ethyl methane sulfonate (EMS) mutagenesis was performed on the prototrophic *Saccharomyces cerevisiae* strain CEN.PK 113-7D (MATa, MAL2-8c, SUC2) to increase the genetic diversity as an evolutionary engineering selection strategy. Cells were inoculated in 2% Yeast Minimal Media (YMM), and the extracellular concentrations of glucose, ethanol, glycerol and acetate were measured at different time points. OD_{600} values were determined by a spectrophotometer. Additionally, cell dry weight analysis was conducted to determine biomass production. Acquired extracellular metabolite concentrations, OD_{600} values and dry weights of the reference strain (without mutagenesis) were used in this study are collected in Table 2.1 and Table 2.2.

2.1.2. Determination of Rates

As the slope of the curve of $\ln OD_{600}$ as a function of time gives the growth rates, natural logarithm of OD_{600} values were calculated to obtain specific growth rates by using the equation 2.1.

$$\mu = \frac{\Delta \ln OD_{600}}{\Delta t} \tag{2.1}$$

In order to determine uptake and secretion rates of the metabolites, the steady-state assumption is applied in 3 hours intervals. Metabolite rates in the units of mmol/Lh are obtained and further converted to mmol/gDWh to be used as flux values.

Table 2.1. Measurements of extracellular concentrations.

Time (h)	**Glucose (g/L)**	**Ethanol (g/L)**	**Glycerol (g/L)**	**Acetate (g/L)**
0	19.99	0	0	1.08
3	17.98	0.58	0.02	1.24
6	15.85	1.2	0.06	1.16
9	12.21	3.39	0.18	1.37
12	9.18	7.97	0.61	2.45
15	0.4	8.17	0.69	2.46
27	0	8.28	0.76	2.6
46	0	8	0.77	2.45
50	0	6.62	0.64	2.02
54	0	5.74	0.55	1.73
58	0	5.46	0.54	1.74
72	0	3.72	0.49	1.33

Table 2.2. Measured OD_{600} and cell dry weight values of reference strain.

Time (h)	OD600	ln(OD600)	Cell DW (g/L)
0	0.21	-1.560647748	-
3	0.53	-0.634878272	-
6	1.76	0.565313809	0.9
7.5	2.66	0.978326123	-
9	4.46	1.495148766	1.9
12	5.31	1.669591835	-
15	5.88	1.771556762	-
18	5.83	1.763017	2.32
21	6.07	1.803358605	-
24	5.87	1.769854634	-
30	6.14	1.814824742	2.26
40	6.44	1.86252854	-
46	6.36	1.850028377	-
50	6.3	1.840549633	-
54	6.55	1.87946505	-
63	6.54	1.877937165	-
67	6.88	1.928618652	-
72	6.97	1.941615225	2.66

Table 2.3. Calculated flux values.

Time	Metabolite fluxes in mmol/gDWh				Growth h-1
	Glucose	Ethanol	Glycerol	Acetate	Biomass
0-3	-12.3963884	13.98837518	0.24131	2.960496	0.30859
3-6	-4.378823762	4.984363569	0.160873	-0.49342	0.400064

2.2. Model Selection

iAN50, a stoichiometric model of intermediary metabolism including glycolysis, the pentose phosphate pathway, anaerobic excretion, citric acid cycle (TCA cycle), oxidative phosphorylation, and uptake pathways for galactose, ethanol and acetate is used to simulate [4].

2.3. Flux Balance Analysis

2.4. Simulation of Batch Conditions

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