Calculation of biomass yields for various substrates under aerobic and anaerobic conditions using stoichiometric models

Gisela Martí Guerrero

14th April, 2023

1 Introduction

In this report, we present the results of biomass yield calculations for three substrates: fructose, glutamine, and succinate, under both aerobic and anaerobic conditions. These calculations were performed using the Optflux software, which is a computational tool for metabolic flux analysis that uses stoichiometric models. This report is organized as follows. First, a brief overview of the methodology used for biomass yield calculations and the Optflux software is provided. Next, we present our results for the biomass yields of fructose, glutamine, and succinate under both aerobic and anaerobic conditions. Finally, we compare our computed yields with the previously known values for glucose and discuss the implications of our findings.

The biomass yield is the amount of organic matter or biological material that can be obtained from a given area or volume. The quantification of biomass yield is crucial in understanding the metabolic behavior of microorganisms. In general, higher biomass yields are desirable as they indicate greater productivity of a given biological process. The biomass yield can vary depending on the substrate and the growth conditions, such as aerobic or anaerobic. In this report, in addition to the three substrates mentioned above, we also compared our computed biomass yields with the previously known values for glucose. The comparison of the biomass yields of these substrates with that of glucose will provide insights into their metabolic behavior.

Stoichiometric models are mathematical models that describe the mass balance and stoichiometry of biochemical reaction networks. These models are based on the principle that the total mass of reactants and products in a chemical reaction must be conserved. The behavior of metabolic networks is analyzed using these models, which also offer a quantitative knowledge of the fluxes and concentrations of metabolites within a cell or organism.In the context of this report, stoichiometric models are particularly relevant because they are used to calculate the biomass yield of different substrates under different growth conditions. The models used in this report are based on the principle of mass balance and take into account the stoichiometry of the metabolic reactions involved in the conversion of substrates into biomass. Stoichiometric models can be created and examined using the software Optflux, which calculates the fluxes of metabolites via the metabolic network and optimizes the network using programming techniques. The biomass yield is then calculated from the optimized flux distribution.

The difference between stoichiometric models and other types of models used in systems biology such as kinetik models, is that stoichiometric models assume that the metabolic reactions are at steady-state and do not consider the dynamics of the metabolic network over time. This is why they are useful for analyzing steady-state metabolic behavior and predicting the effects of genetic or environmental perturbations on the metabolic network. In contrast, kinetics models describe the dynamics of biochemical reactions over time, using detailed mathematical equations that take into account the enzyme kinetics and reaction rates. These models can provide more detailed insights into the temporal behavior of the system, such as the time course of substrate and product concentrations, and can capture the effects of regulatory mechanisms and feedback loops on the metabolic network. Kinetics models are useful for analyzing the transient behavior of metabolic pathways, such as the response of the network to changes in substrate concentrations or enzyme activity.

2 Results

In this section we present the results obtained from the simulations made with the OptFlux program of the sbml model *Ecoli_core_model.xml*, using a flux balance analysis (FBA). FBA is a mathematical modeling approach used to predict the metabolic fluxes and steady-state behavior of biological systems. It is first explained how

the produced biomass value is computed under aerobic conditions (oxygen present in the reaction), and then under anaerobic conditions (absence of oxygen). The necessary computations are then described in order to determine the biomass yield for each substrate under both conditions based on the values obtained.

2.1 Produced biomass calculation

For the each substrate in aerobic conditions, we set the environmental conditions to:

- Glucose drain reaction lower bound to **0**. This prevents the model from consuming this metabolite (i.e. Glucose is not present in the medium).
- A lower bound of **-10** to the alternative substrate (Fructose/Glutamine/Succinate). This allows the model to consume these metabolites (i.e. the metabolite is present in the medium).

For the anaerobic simulations, we set the environmental conditions to:

- Glucose and Oxygen drain reaction lower bound to **0**, so that there is neither glucose nor oxygen in the medium.
- A lower bound of **-10** of the alternative substrate (Fructose/Glutamine/Succinate), so that these metabolites are present.

The resulting biomass production values obtained from these simulations are can be found in *Table* 1. All numerical values have units of $g \cdot gDW^{-1} \cdot h^{-1}$. [1]

Substrate	**Biomass Value**	
	Aerobic	**Anaerobic**
Glucose	0.8739	0.2117
Fructose	0.8739	0.2117
Glutamine	0.5593	NaN
Succinate	0.3976	NaN

Table 1: Produced biomass values.

2.2 Biomass yield calculation

Once we have the values for the produced biomass, we can calculate the total biomass yield for each metabolite. The consumed biomass corresponds to the value of the flux of the limiting substrate, which is the substrate that restricts the maximum growth rate of the simulation. In our case, the limiting substrate of all the simulations is the same substrate that the lower bound is set to $-10\frac{mmol}{gDW \cdot h}$ (i.e. in the fructose simulation, the limiting substrate is fructose, etc). To calculate the consumed biomass, it is necessary to make a change of units, using the molar mass of each substrate. Glucose and Fructose have molar masses of $180\, g/mol$, Glutamine $146\, g/mol$ and Succinate $118\, g/mol$.

The steps required to compute the biomass yield of fructose are presented below. For the other substrates, the same calculations are made using their initial produced biomass values and their respective molar masses. The final values of biomass yield for each susbtrate are shown in *Table* 2.

$$10\frac{mmol}{gDW \cdot h} \cdot \frac{1\, mol}{1000\, mmol} \cdot \frac{180\, g}{1\, mol} = 1.8\frac{g}{gDW \cdot h}$$

$$Biomass\, Yield\, (aerobic) = \frac{0.8739\, g \cdot gDW^{-1} \cdot h^{-1}}{1.8\, g \cdot gDW^{-1} \cdot h^{-1}} = 0.4860$$

$$Biomass\, Yield\, (anaerobic) = \frac{0.2117\, g \cdot gDW^{-1} \cdot h^{-1}}{1.8\, g \cdot gDW^{-1} \cdot h^{-1}} = 0.1176$$

[1] $g \cdot gDW^{-1} \cdot h^{-1}$ means grams per grams of dry weight per hour.

Substrate	Biomass Yield	
	Aerobic	Anaerobic
Glucose	0.4860	0.1176
Fructose	0.4860	0.1176
Glutamine	0.3831	NaN
Succinate	0.3369	NaN

Table 2: Biomass yield for the substrates Fructose, Glutamine and Succinate in aerobic and anaerobic conditions.

3 Discussion

The biomass yield obtained in a flux balance analysis represents the amount of biomass produced per unit of substrate consumed, under the given constraints and conditions. The stoichiometry of the metabolic network, the accessibility of substrates and nutrients, and the effectiveness of energy and biomass conversion pathways are a few of the variables that affect biomass yield.

First of all we can see how the simulations for glucose and fructose give exactly the same results; their biomass yield is 0.4860 in aerobic conditions, and 0.1176 in anaerobic conditions. This indicates that the metabolic pathways for these substrates are similarly efficient at producing biomass. This could be due to the fact that glucose and fructose are isomers with similar chemical structures, and can be metabolized by similar enzymes and pathways in many organisms. Since they have the same molar mass, and the lower bound of the drain reaction was set to $-10\frac{mmol}{gDW \cdot h}$ for both of them, their final biomass yield values are the same.

The changes in the organism's energy metabolism under these two situations can be used to explain the difference in biomass yield between aerobic and anaerobic settings that was seen in the simulation. Aerobic circumstances enable the organism to employ oxygen as an electron acceptor in the electron transport chain, enabling effective oxidative phosphorylation and ATP synthesis. While nitrate and sulfate are less effective at generating ATP, they may be used by the cell in anaerobic conditions. As the synthesis of biomass molecules requires energy in the form of ATP, the reduced ATP generation in anaerobic conditions may be a constraint on the biomass production. As a result, under anaerobic conditions, the organism might have to dedicate more resources to producing ATP, which can decrease the overall biomass yield. An example of the respiration and fermentation processes for glucose is shown below. *Figure* 1 shows a schematic view of the respiration process, while *Figure* 2 shows the fermentation process.

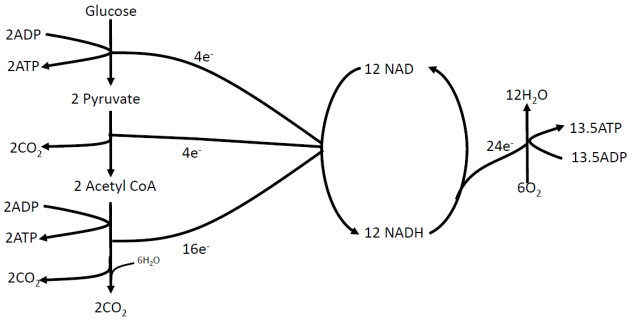

Figure 1: Glucose respiration

The glucose respiration process has the the following chemical reaction:

$$C_6H_{12}O_6 + 6\,O_2 + 17.5\,ADP \longrightarrow 6\,CO_2 + 6\,H_2O + 17.5\,ATP$$

In this process glucose reacts with oxygen, which is used as an electron acceptor, forming a total of 17.5 ATP's that can be later used by the cell. Carbon dioxide and water are created as byproducts of the reaction.

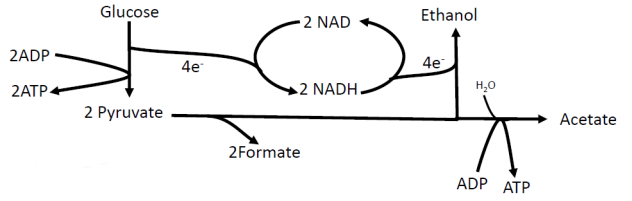

Figure 2: Glucose fermentation

The glucose Fermentation process has the following chemical reaction:

$$C_6H_{12}O_6 + H_2O + 3\,ADP \longrightarrow 2\,CH_2O_2 + C_2H_4O_2 + C_2H_6O + 3\,ATP$$

Fermentation is a partial breakdown of glucose producing only 3 net ATP's per glucose by phosphorylation. Since there is no oxygen available in anaerobic conditions, the ATP generation is less efficient than in the previous case.

Going back to the simulation results, we can see that glutamine has a biomass yield of 0.3831 in aerobic conditions and a null value NaN in anaerobic conditions. The reason why the biomass yield value in aerobic conditions is lower than the one of glucose and fructose in the same conditions may be because glutamine is not as efficient in producing biomass, meaning that it requires additional metabolic steps to be converted into usable biomolecules, while the other substrates can be rapidly incorporated into many biosynthetic pathways. The same thing happens with succinate, which has an even lower biomass yield of 0.3369 in aerobic conditions.

However, in both molecules of glutamine and succinate, the simulation gives a null value for the total flux of the reaction, which means that these substrates are not fermentable. A substrate that is not fermentable cannot be metabolized by an organism through fermentation, and it can only be utilized as a source of energy using different metabolic pathways or environmental conditions, such as aerobic conditions. This way, a substrate is fermentable only if it allows to have a net production of ATP (ATP production coupled to enzymatic reactions) while maintaining the redox balance in anaerobic conditions. It is possible, however, that the ATP production of glutamine and succinate fermentation is not 0, but since there is an initial amount of only $10\frac{mmol}{gDW \cdot h}$, the cell cannot be sustained with such a low ATP yield.

We may also compare this analyses' use of stoichiometric models against the option of utilizing kinetic models to carry out the same analysis. Each of these models has advantages and disadvantages. The steady-state behavior of the metabolic network may be studied using stoichiometric models, which can also be used for predicting how changes in the environment or genetics would affect the system. Stoichiometric models, however, do not account for the complex kinetics of individual network reactions and do not offer data on the temporal dynamics of the system. The exact kinetics of each process in the metabolic network are described in kinetics models, which can also provide information on the system's temporal dynamics, such as the time course of substrate and product concentrations. However, kinetic models can be computationally expensive and require a great deal of specific information. Stoichiometric models, such as flux balance analysis, are adequate because the purpose of this study is solely to predict the steady-state behavior of the various metabolites and the effect of environmental perturbations. The kinetics models may be more suitable if a more in-depth examination of these substrates' behavior and the development of their concentrations over time was required. To completely comprehend the behavior of the metabolic network, a combination of stoichiometric and kinetics models may be required.