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# Mathematical Modeling of Photosynthetic Eukaryotic Microorganisms Using Metabolic Networks

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**Abstract:** Metabolic modeling is a powerful tool for understanding microbial metabolism and is particularly appealing to a wide range of applications, from biotechnology and medicine to environmental science and sustainability. In that context, the elaboration of metabolic networks is essential despite the challenges underlying their reconstruction. While the development of genome-scale networks is computationally costly, small networks are often oversimplified, limiting their use in industrial applications. For this purpose, this paper suggests a method to identify metabolic networks of intermediate size by combining biological knowledge and a series of constraint-based methods in an iterative strategy allowing the refinement of the network definition. The present study focuses on the mathematical modeling of photosynthetic eukaryotic organisms and leads to a detailed network including energy aspects such as the proton motive force. The procedure is effective, yielding promising results while metabolic analyses provide consistent predictive capabilities of the network, in concordance with existing studies.



*Keywords:* Metabolic modeling, metabolic flux analysis, metabolic network, photosynthetic organisms, static regime

## 1. INTRODUCTION

Photosynthetic eukaryotic cells are of huge importance both ecologically and economically and play key roles in energy production, oxygen generation, carbon cycling, and supporting biodiversity. For instance, *Tisochrysis lutea* is a species of microalgae which is a primary producer in marine ecosystems ; *Chlorella species* are green algae widely recognized for their potential biotechnological applications and nutritional value (rich in proteins, vitamins, minerals and essential fatty acids) ; and *Botryoccoccus braunii* is a species of green microalgae able to produce renewable biofuels and other valuable hydrocarbons, making it a key organism for sustainable energy solutions. With such impressive advantages, photosynthetic eukaryotic organisms have become one of the preferred choices for a sustainable photobiological cell factory.

Mathematical modeling of microbial metabolism is a crucial aspect of biotechnology, system biology and medicine and is extensively used in industrial, environmental and medical applications. In this context, metabolic modeling is becoming a powerful tool to understand, predict and optimize the complex metabolic processes of microorganisms (Stephanopoulos et al., 1998) and the elaboration of metabolic networks is required. However, the identification of such networks is not an easy task since they are highly interconnected making their reconstruction time-consuming and their analyses computationally intensive. Besides, it requires integrating high-quality, reliable and available data from genomics, transcriptomics, proteomics and metabolomics and an iterative process of model refinement is needed to ensure their accuracy and predictive capability, comparing model predictions with experimental observations.

In metabolic modeling, the size of the network is of crucial importance, since it impacts both the model's overall complexity and the dimensionality of the solution space. For this reason, the elaboration of metabolic networks of appropriate size, that is large enough to cover the whole metabolism of the cell but small enough to avoid high computational resources, is still a major research focus. On the one hand, the advances in sequencing techniques and genome annotation methods have enabled the development of genome-scale metabolic models (Covert et al., 2001; Price et al., 2003; Reed and Palsson, 2003). Nevertheless, to ensure network consistency and comprehend metabolic interactions within cellular networks, examination of the structural and topological properties of metabolic networks is needed (Klamt et al., 2003). Frameworks based on convex analyses have been developed and methods based on a series of constraints that govern the operation of the network at steady-state have emerged. Among the realm of methods, flux balance analysis (FBA) and flux variability analysis (FVA) are popular approaches as well as techniques relying on extreme pathways and elementary modes to assess the network robustness (Schuster and Hilgetag, 1994; Schilling et al., 2000; Orth et al., 2010). The latter methods are effective but can become computationally costly because of the size of the network. On the other hand, small-size metabolic networks have been deduced to facilitate the analyses and the application of control strategies. These smaller networks are based on biological knowledge and focus only on the main metabolic functions of microorganisms (Provost and Bastin, 2004;


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Zamorano et al., 2013; Fernandes de Sousa et al., 2016). However, such models are often oversimplified and important metabolic interactions are omitted.
Regarding the modeling of photosynthetic eukaryotic cells, genome-scale metabolic models have been reconstructed in the recent years (Zuniga et al., 2016; Loira et al., 2017) and smaller models have emerged (Baroukh et al., 2014, 2017; Assis Pessi et al., 2023). However, while the genome-scale networks are difficult to manage (several thousand reactions), the smaller networks (around a hundred reactions) lack of biological realism and have a limited predictive power. In such cases, the development of a mid-size network is attractive, so that the metabolic model can be applied to a wider range of biological questions (broader scope of applications).
For this purpose, this study proposes a method for the elaboration of a mid-size metabolic network structure, typically comprised of a couple hundred reactions, which is detailed enough to capture the complexity of the cellular metabolism, yet remains straightforward to analyze at the process level. The methodology is iterative and consists of combining biological knowledge and mathematical methods, using in-silico data to quantify intracellular mechanisms and refine the network definition. The original aspect of this study lies in the combination and sequential, iterative exploitation of mathematical methods, as illustrated in Fig. 1, which will be detailed in the sequel of the article. The procedure leads to a metabolic network composed of 166 metabolites and 192 reactions that includes energy aspects, barely outlined in existing studies.
The paper is organized as follows. Sec. 2 presents the modeling procedure and details the algorithmic scheme of the mathematical methods. Sec. 3 focuses on the modeling of photosynthetic eukaryotic organisms. Sec. 4 discusses the results using in-silico data under different light regimes. Finally, conclusions are drawn in Sec. 5.

## 2. METHOD

The modeling procedure is depicted in Fig. 1. First, an initial set of metabolic reactions must be selected. The main metabolic pathways are common for most microorganisms and can be found in literature and biochemistry textbooks (Nelson and Cox, 2008). At this point, genomic studies may be very useful to detect specific metabolic functions and identify the corresponding intracellular reactions, if available. However, to reduce the network's dimensionality and complexity, lumping techniques must be applied. In this case, lumping involves nothing but grouping similar chemical species or reactions into a single entry to simplify the mathematical modeling (Martinez, 1990).
Furthermore, the reversibility of chemical reactions needs to be analyzed to ensure the consistency of the network. This issue is often bypassed in most studies dealing with mathematical modeling. However, as it will be discussed in the following sections, reactions reversibility can have a massive influence on metabolic analyses' outcomes. Determining whether a reaction is reversible involves several considerations, such that thermodynamics, kinetics and physiological context. In this study, the standard free energy change $\Delta$G is used and depends on the actual concentrations of reactants and products as well as the temperature prevailing during the reaction. In that respect, the

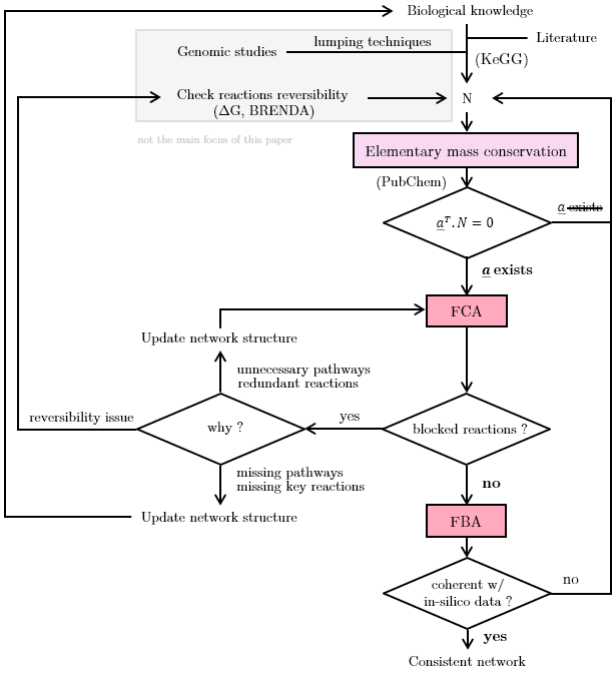


Fig. 1. Methodology and algorithmic scheme of the mathematical methods to get mid-size metabolic networks

reaction tends to proceed in the forward direction when $\Delta$G is large and negative, in the reverse direction when $\Delta$G is large and positive and the reaction is considered as reversible if this quantity is small and close to zero. In this work, reactions reversibility is stated on the basis of $\Delta$G but also on the enzyme regulation, i.e. the enzymes capability to catalyze a reaction in both directions depending on cellular conditions. The latter information can be found using the enzyme database BRENDA.
At this stage, a first set of metabolic reactions is gathered into a matrix representation $N$ defining the stoichiometric matrix of the metabolic network. It is a $m \times n$ matrix where $m$ represents the number of metabolites and $n$ corresponds to the number of metabolic reactions ; $N_{ij}$ is related to the stoichiometry of the metabolite $i$ taking part in the $j^{th}$ reaction.

As mentioned previously, the original aspect of the procedure lies in the use of mathematical methods in a sequential and iterative way while ensuring the consistency of the network. The latter are explained below and then, an application of the workflow is proposed in Sec. 3. Note that the use of the lumping techniques and the study of reactions reversibility are not the main focus of this article and will be described in future research papers.

### *2.1 Elementary Mass Conservation*

Network consistency involves elementary mass conservation. For this purpose, a variable $z$ is defined and represents the total number of relevant atoms involved in the metabolic reactions and taking part in the cellular metabolism. Based on the mass balance principle and considering the influence of dilution due to cell growth is negligible, the general equation of internal metabolites dynamics is expressed :

$$\frac{d\underline{C}}{dt} = N\underline{v}(\underline{C}) \tag{1}$$

Where $\underline{C} \in \mathcal{R}^m$ denotes the vector of metabolites concentrations and $\underline{v} \in \mathcal{R}^n$ is the vector of network fluxes.
A non-negative vector $\underline{a}$ is introduced and contains information about the number of atoms (of carbon, nitrogen, or phosphorus) in each metabolite : $\underline{z} = \underline{a}^T\underline{C}$. Therefore, if mass conservation applies, $\underline{z}$ must be constant, so its time derivative is null. Hence, $\underline{a}^T N = 0$. If such vector $\underline{a}$ exists, then there is mass conservation when constructing the network. For mass balance of biomass and macromolecules, an average atomic composition is considered.

### *2.2 Constraint-Based Methods*

To limit the possible solutions and explore the solution space, constraint-based methods are established. It consists in adding a series of constraints that govern the operation of the network at steady-state. By applying the pseudo-steady state assumption, the following system of linear equations is obtained :

$$N\underline{v} = 0 \tag{2}$$

In addition, network fluxes are often subject to positivity constraints assuming that direct reactions prevail over their reverse counterparts :

$$\underline{v} \geq 0 \tag{3}$$

*Flux Balance Analysis* It is undoubtedly the most popular constraint-based method. It supposes an optimal behaviour of the cell and consists in computing an optimal flux distribution $\underline{v}$ which maximizes or minimizes an objective function $Z = \underline{c}^T\underline{v}$ such that :

$$\underline{v}^{opt} = \max_{\underline{v}} \Big(Z\Big) \; s.t. \; \{N\underline{v} = 0 \; ; \; \underline{v} \geq 0 \; ; \; \underline{v}_l \leq \underline{v} \leq \underline{v}_u\} \tag{4}$$

where $\underline{c}$ is a vector of weights translating how much each reaction contributes to the objective function, and $\underline{v}_l$, and $\underline{v}_u$ are vectors of lower and upper bounds, respectively. Note that the optimal flux distribution may not correspond to the actual flux distribution and might not be unique.

*Flux Coupling Analysis* This framework appears particularly useful to study the topological and flux connectivity features of large metabolic networks. This approach requires the solution of a sequence of linear programs and allows reducing the dimensionality of the network by finding pairs of metabolic fluxes. Three types of couplings are commonly characterized : directional, partial and full coupling. More information can be found in (Burgard et al., 2004). Pairs not belonging to one of these categories are classified as uncoupled, defining blocked reactions.
In order to reduce the number of linear programs to solve, this work suggests to enhance the existing method and identify only the blocked reactions, defined as reactions incapable of carrying a flux under steady-state conditions for a particular uptake scenario. In this way, blocked reactions are exploited to identify incomplete pathways, to pinpoint errors or omissions in the metabolic reconstruction and possibly to detect issues regarding reactions reversibility. Furthermore, inconsistencies in the network and redundant reactions might be identified. Mathematically, it consists in solving the following linear programming problem once for every flux :

$$maximize \quad v_j \tag{5}$$

$$subject\ to \quad \sum_{j=1}^{N} N_{ij}v_j = 0, \; \forall i \in M \tag{6}$$

$$v_j^{uptake} \leq v_j^{uptake^{max}}, \; \forall j \in N_{transport} \tag{7}$$

$$v_j \geq 0, \; \forall j \in N \tag{8}$$

In this formulation, reversible reactions are expressed as two irreversible reactions in opposite direction, constraining all the fluxes to positive values. Therefore, if the maximum value of the flux is zero, then the reaction is said unusable or blocked. Also, the solution of the above linear program depends on the steady-state assumption and imposed uptake-secretion scenarios. Therefore, a blocked reaction does not necessarily imply that the reaction is incorrect, inconsistent, or irrelevant in describing the cellular metabolism ; it could only indicate that the reaction is not activated under the current uptake-secretion conditions.

## 3. APPLICATION

This section is devoted to the application of the procedure for the purpose of obtaining a mid-size metabolic network structure representative of the metabolism of photosynthetic eukaryotic cells. First, the main metabolic pathways describing the metabolism of such organisms are mentioned, with a focus on the reactions characterizing ATP synthesis. Then, the iterative process depicted in Fig. 1 is applied and a couple of iterations are presented.

### *3.1 Analysis of Metabolic Pathways*

As discussed in Sec. 2, most organisms have metabolic pathways in common. As a matter of fact, glycolysis occupies a central position in their metabolism. Basically, one molecule of glucose produces two molecules of pyruvate. Then, pyruvate is oxidized into acetyl groups to enter the so-called tricarboxylic acid cycle. Also, glucose (specifically glucose-6-phosphate) can be oxidized via the pentose phosphate pathway to form ribose-5-phosphate, essential to synthesize RNA, DNA and coenzymes. In addition, the GS-GOGAT pathway is a crucial biochemical pathway for nitrogen assimilation in many microorganisms and allows the incorporation of ammonium which is toxic to cells at high concentrations, into organic molecules, specifically amino acids. Another way to dispose of toxic ammonium is via the urea cycle, also called ornithine cycle. Indeed, although photosynthetic organisms mostly manage nitrogen through the GS-GOGAT pathway, certain species can produce urea under specific conditions.
The previous pathways are catabolic, meaning the major metabolic fuels are degraded to release chemical energy and yield the synthesis of ATP. Nevertheless, anabolic pathways are also necessary to describe the whole metabolism of an organism. In this case, these pathways use chemical energy in the form of ATP, NADH or NADPH to synthesize cellular components from simple precursor molecules. In that respect, the network must also contain routes characterizing the biosynthesis of carbohydrates, lipids, amino acids and nucleotides, as well as information pathways (proteins, DNA and RNA metabolism) in order to form biomass.

In contrast with animal cells, plants and photosynthetic organisms can synthesize organic compounds like glucose, directly from $CO_2$ and water, during a process called $CO_2$ assimilation or the Calvin-Benson cycle. This process takes place in the stroma of chloroplasts, which are distinct organelles within the cells, proper to eukaryotic plants and algae.

### *3.2 ATP Synthesis*

Energetic reactions are fundamental for the proper operation of living organisms. As cited in the previous section, ATP is required for anabolic pathways and is also needed for the reduction of $CO_2$ during the Calvin-Benson cycle in photosynthetic organisms. ATP can be synthesized through diverse mechanisms depending on the presence of light and oxygen availability.
First, ATP can be generated in all living organisms through enzymatic reactions during glycolysis and tricarboxylic acid cycle. This process, called substrate-level phosphorylation, enables a fast production of ATP in the cytoplasm, particularly under anaerobic conditions or when light is not available for photosynthesis.
However, the most significant step in ATP synthesis is the process of oxidative phosphorylation, alongside the process of photophosphorylation for photosynthetic organisms in the presence of light. During these metabolic processes, the synthesis of ATP (from ADP and $P_i$) is driven by the proton motive force (PMF), which is an electrochemical gradient. This proton gradient is created across a membrane and is caused by the movement of electrons through the electron-transport-chain (based on the chemiosmotic theory (Nelson and Cox, 2008)). Regarding photophosphorylation, light energy is captured by chlorophyll and other pigments during the so-called oxygenic photosynthesis. During this process, electrons flow through a series of membrane-bound carriers in the thylakoid membrane of chloroplasts. As the electrons move through the electron-transport-chain, ions $H^+$ are pumped across the membrane, creating a higher concentration of $H^+$ inside the membrane, producing a proton gradient that will serve as the driving force for ATP synthesis. Regarding the oxidative phosphorylation, the same mechanism occurs but takes place across the inner mitochondrial membrane. In this case, the proton gradient is created by the movement of the electrons from NADH and $FADH_2$ generated in glycolysis and the citric acid cycle.
Since the electrochemical gradient is poorly modeled in existing metabolic networks, this study suggests to differentiate the cytoplasmic protons, i.e. outside the membrane, $H^+_{\mathbf{n}}$ (namely $H^+$) and the periplasmic protons $H^+_{\mathbf{p}}$, i.e. inside the membrane (thylakoid membrane of chloroplasts or mitochondrial membrane). It leads to the following metabolic reactions :

$$ADP + P_i + \eta\ H^+_{\mathbf{p}} \rightarrow ATP + H_2O + \eta\ H^+_{\mathbf{n}}$$
$$2H_2O + 2NADP^+ + 10\ \gamma^* \rightarrow O_2 + 2NADPH + 12H^+_{\mathbf{p}}$$
$$NADH + 11H^+_{\mathbf{n}} + 0.5\ O_2 \rightarrow NAD^+ + 10H^+_{\mathbf{p}} + H_2O$$
$$FADH_2 + 6H^+_{\mathbf{n}} + 0.5\ O_2 \rightarrow FAD^+ + 6H^+_{\mathbf{p}} + H_2O$$

The first reaction represents the synthesis of ATP from ADP and $P_i$, the second is relative to photophosphorylation, the third and fourth reactions are relative to oxidative phosphorylation. $\gamma^*$ represents a mole of photon and $\eta$ is the number of protons required to produce one molecule of ATP. For photosynthetic eukaryotic organisms, $\eta = 4$. By combining the previous metabolic reactions, it follows that 2.5 molecules of ATP are produced per NADH, 1.5 molecules of ATP are produced per $FADH_2$ and 3 molecules of ATP with photophosphorylation per $O_2$ produced. These results are in concordance with the approximate stoichiometry established in (Nelson and Cox, 2008).

### *3.3 Iterative Strategy*

For the first iteration of the strategy, the main metabolic pathways identified in Sec. 3.1 are considered and the stoichiometric matrix of the metabolic network $N$ is built. Note that metabolic reactions have been established by using biochemistry textbooks and genomic studies and reactions reversibility is accounted for by considering the $\Delta$G value and enzyme regulation. Future research papers will detail these parts. The present work focuses mainly on the algorithmic scheme of the mathematical methods. Doing so, a network composed of 169 metabolites and 178 reactions is obtained. Then, following the scheme shown in Fig. 1, elementary-mass conservation is checked and constraint-based methods can be applied. For this purpose, a realistic uptake scenario is considered for which light, a carbon source ($HCO_3^-$) and a nitrogen source ($HNO_3^-$) are available. Flux balance analysis is performed with biomass optimization and flux coupling analysis is exploited to prevent large computation times.
After analysis, the network provides inconsistent results. A few dozen reactions are blocked leading to a biomass production rate equals to zero. Indeed, during daytime, photosynthetic organisms reduce $CO_2$ to triose sugars before their conversion into glucose via the Calvin-Benson cycle and gluconeogenesis. The latter pathway is missing and needs to be added. Also, by analyzing the outcomes of the flux coupling analysis, several missing reactions enabling the formation of key intermediate metabolites have been emphasized and an issue with the reversibility of a Lewis acid-base reaction has been identified.

After adding the missing reactions and pathways, the structure of the network is updated and elementary-mass conservation is verified yet again. The second iteration leads to a network containing 182 metabolites and 202 reactions. Flux coupling analysis is performed and unusable reactions are still detected. However, in this case, these unusable reactions are due to a too detailed description of some pathways conducting to the formation of unnecessary metabolites. Therefore, it is not caused by inconsistencies in the network structure and in that context, flux coupling analysis turns out to be particularly interesting to simplify over-detailed networks, as noticed in (Burgard et al., 2004). Because it possibly impacts the network calculability, all unnecessary reactions are removed from the network. However, as mentioned in Sec. 2.2, discarding such reactions does not mean they are incorrect, inconsistent or irrelevant.

More iterations are performed and are detailed in Table 1. A third iteration is carried out to remove unnecessary reactions. The fourth iteration consists in removing redundant

reactions due to lumping techniques or conflicting nomenclature of chemical compounds from literature. The fifth iteration discards futile intermediate metabolites while the sixth is for the addition of cofactors metabolism reactions. And so on. In this way, this application shows the benefits of the iterative procedure and the merits of using several mathematical methods together to build a metabolic network structure. Finally, the ultimate network is comprised of 166 metabolites (internal metabolites and intracellular energetic cofactors) and 192 reactions (intracellular and transport reactions). The latter can be provided by the authors upon request.

Table 1. Information related to the metabolic networks using the iterative procedure

| iter. | # met. | # rnx | # blocked rnx |
|---|---|---|---|
| 1 | 169 | 178 | 39 |
| 2 | 182 | 202 | 16 |
| 3 | 173 | 193 | 7 |
| 4 | 173 | 190 | 7 |
| 5 | 166 | 183 | 0 |
| 6 | 166 | 192 | 0 |

## 4. RESULTS AND DISCUSSION

This section presents the results of metabolic flux analyses using in-silico data to validate the metabolic network reconstruction. This study focuses only on the quasi-steady state under constant light regimes enabling the use of classical metabolic analysis tools. *CellDesigner* (Funahashi et al., 2003) is used to have a graphical representation of the network and derive the flux map. Different scenarios are addressed to assess the consistency of the network structure.

### *4.1 Daylight Period*

The case study covered in this section is related to an autotrophic regime where the organisms use sunlight, $CO_2$ and $H_2O$ to produce organic compounds essentially. In the network, light energy is modeled through an amount of moles of photons ; however, light is commonly expressed in watts per square meter. For simulations, a light with a wavelength of 600 $n$m is assumed with a power density of 150 W.m$^{-2}$, which provides a photon flux density equal to 7,5185.10$^{-4}$ moles of photons per second per square meter, using the Planck equation. Thereafter, the transport reaction related to light is set to 10 photons, equivalent to a light exposure of 3,7 hours.
In addition, a carbon source and a nitrogen source are considered as available and simulations are performed for a net assimilation of 100 mol of bicarbonate ion $HCO_3^-$ and 10 mol of nitrate $NO_3^-$. As illustrated on the flux map in Fig. 2, autotrophy is characterized by high fluxes in the photosynthetic pathways and the activation of the Calvin-Benson cycle taking place in chloroplasts. Upper glycolysis operates in the glyconeogenic direction to produce carbohydrates and sugar precursors metabolites, essential for growth. The pentose phosphate pathway is in the reductive mode and ATP synthesis is driven by the PMF and is synthesized via photophosphorylation and oxidative phosphorylation (in addition to substrate-level phosphorylation). Furthermore, the analysis of the complete map shows the activation of the citric acid cycle to synthesize metabolite precursors for biomass growth. Lastly, the nitrogen source enables the biosynthesis of amino-acids and the activation of the nucleotide salvage pathway. The GS-GOGAT pathway and the urea cycle are involved for nitrogen assimilation and biomass is produced.

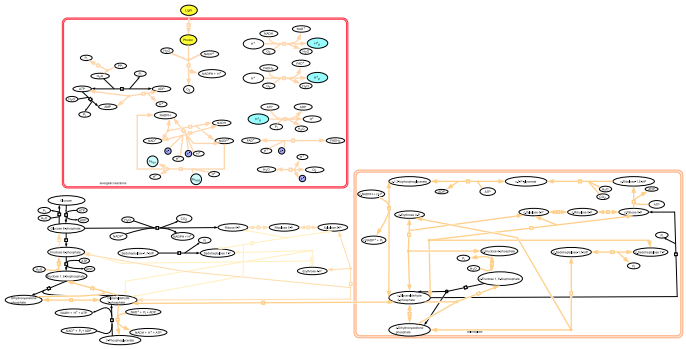

Fig. 2. Flux map during the day phase representative of autotrophic regime (online reading is advised)

### *4.2 Nighttime Period*

The scenario explored in this section aims at representing a heterotrophic regime, meaning that carbon storage molecules are degraded into precursors metabolites and energy for growth and maintenance essentially. To allow a comparison with existing studies, the heterotrophic regime (equivalent to a chemotrophic regime in this case) is simulated by considering a net assimilation of 100 mol of glucose and no light. A source of nitrogen is also considered. As illustrated in Fig. 3, the absence of light inhibits the activation of the Calvin-Benson cycle. When glucose is used as carbon source, upper glycolysis is in the downward direction and the pentose phosphate pathway is in the oxidative mode. A further analysis of the whole flux map shows that the primary carbon flux is through the acid citric cycle which generates precursor metabolites for growth and energy via oxidative phosphorylation and substrate-level phosphorylation (no photophosphorylation during the dark phase).

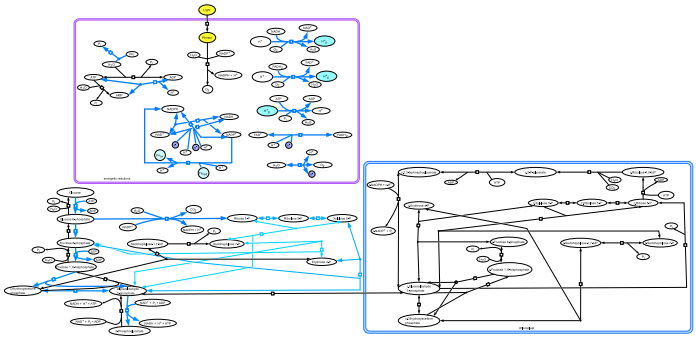

Fig. 3. Flux map during the night phase representative of heterotrophic regime (online reading is advised)

### *4.3 Discussion*

The results discussed in the previous sections are in concordance with existing studies (Baroukh et al., 2014, 2017;

Assis Pessi et al., 2023) in which smaller networks have been identified. This paper allows the elaboration of a more detailed network structure which improves the understanding of the metabolism of photosynthetic eukaryotic organisms, notably by modeling the PMF. In this way, these results demonstrate the merits of the proposed procedure to identify a metabolic network and confirm the network coherence. Nevertheless, it is important to acknowledge the limitation of in-silico data and the importance of experimental data to gain reliable insights into the operation of the organisms. Moreover, the more data, the better the flux predictions provided by metabolic analyses. Indeed, the identified network is underdetermined and a calculability analysis might be conducted to determine the number of fluxes that can be uniquely calculated.
Besides, autotrophic and heterotrophic fluxes differ only in the arrangement of the core carbon network (Calvin-Benson cycle, glycolysis, pentose phosphate pathway, citric acid cycle). Indeed, the remaining pathways show relatively consistent flux patterns regardless of growth conditions. Accordingly, it confirms that anabolic processes operate independently of growth conditions, which is explained by the bow tie structure of microorganisms.

## 5. CONCLUSION

This work proposes the elaboration of a mid-size metabolic network structure representative of the metabolism of photosynthetic eukaryotic microorganisms. The strategy combines biological knowledge and a series of constraint-based methods while respecting elementary mass conservation and exploring the issue of reactions reversibility. Furthermore, the identified network includes key energy aspects and metabolic flux analyses provide convincing results in concordance with existing studies. This enables highlighting the merits of the procedure to elaborate consistent metabolic networks. Even though the present study is static, it offers first insights into the metabolism of organisms submitted to light changes. Further research should entail dynamic modeling approaches and the consideration of experimental data in order to consolidate the observations and refine the network definition.

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