Modeling Lipid and Carbohydrate accumulation in Green Algae, using Constraint Based Modeling

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Introduction

➢Green algae are presently being commercialized as a potential source of lipids and carbohydrates, to produce bio-fuels and bio-products.

➢Genomic and biochemical information have previously been used to re-construct the metabolic network of the green algae, *C. reinhardtii* (Boyle and Morgan, 2009).

➢The re-constructed network consists of 404 metabolites and 451 metabolic reactions (reversible and irreversible) organized into three metabolically active compartments representing the chloroplast, mitochondrion and cytosolic spaces.

➢Model parameters were determined through analyses of the lipid and starch content, with respect to other metabolic inputs and outputs.

➢Linear programming was used to study the behavior of the model organism under simulated growth conditions– including autotrophic, heterotrophic and mixotrophic growth.

➢This reconstruction led to the identification of metabolic and biochemical factors affecting the spectrum of carbohydrates and lipids in *C. reinhardtii* strain CC-400 cw15 mt+ and can be used to guide the engineering of new strains for lipid and carbohydrate production.

Metabolic Network Reconstruction

➢This model included pathways as shown in Figure 1.

➢Pyrenoid, responsible for starch storage, was considered to be a part of chloroplast. Starch was assumed to have 50 Glucose units.

➢Lipid synthesis was consistent with Figure 2 and assumed to have formed from 18-carbon fatty acids, and entirely, localized in chloroplast.

➢The biomass equation and photosynthetic reactions were extracted from the set of reactions presented by Boyle and Morgan (2009); and followed it up with ***KEGG Pathway*** queries to include Proton, Energy and Water balance.

Problem Formulation

➢The set of reactions (j) and metabolites (i) were transformed into a stoichiometric matrix (S) and a flux matrix (v). Eventually, we used these to solve a Linear Programming problem that would simulate a steady state mass balance.

➢The objective function was either maximization of biomass or minimization of energy.

➢A two step optimization strategy was followed for photoautotrophic growth. In the first step, biomass was maximized and light flux was left free. In the next step, the biomass formed was fixed and light flux was minimized.

$$\sum S_{ij} \cdot v_j = 0;\ for\ every\ i \in M_i$$
$$\sum S_{ij} \cdot v_j \le 0;\ for\ every\ i \in M_r$$
$$\sum S_{ij} \cdot v_j \ge 0;\ for\ every\ i \in M_p$$
$$a \le v_j \le b$$

M_r is a set of extracellular reactants,
M_p is a set of extracellular products, and
M_i is a set of intracellular metabolites

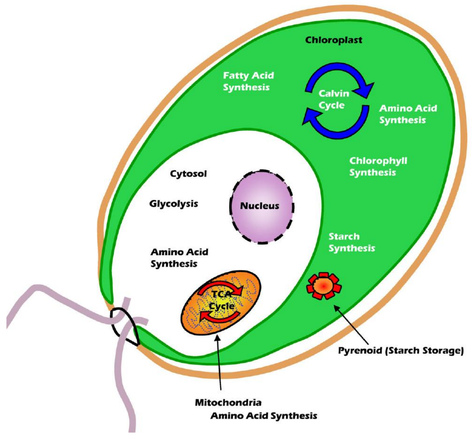

Figure 1. Localization of metabolic pathways, Adapted from Boyle and Morgan, BMC Sys. Biol., 2009

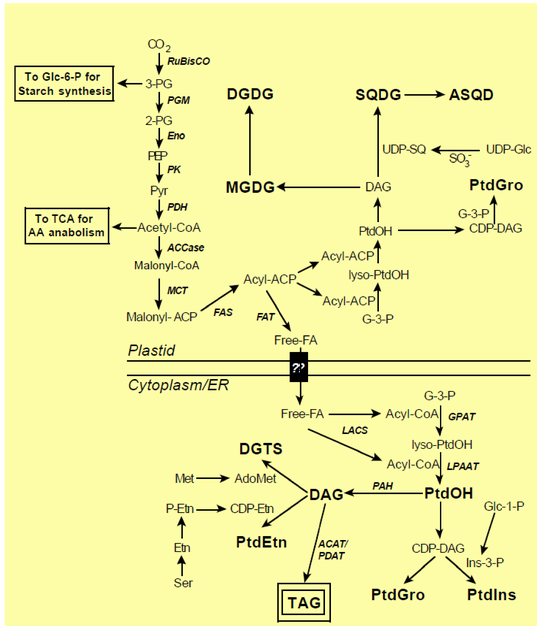

Figure 2. Lipid Metabolism in Chlamydomonas, Adapted from Reikhof, Sears, and Benning, Euk. Cell, 2005

Reaction	Photoautotrophic	Heterotrophic	Mixotrophic
Biomass yield (kg/hr)	2.900826	3.155824	3.394301
Oxygen	-126.904455	102.5253	-43.956488
Carbondioxide	100	-81.348863	-70.747457
Nitrate	9.7232	8.49763	12.110031
Sulphate	0.1082	0.0969	0.158038
Lipid	0.588287	0.968838	1.0115
Carbohydrate	0.116525	0.11058	0.10271
Acetate	0	100	100
Flux $v_i < 0$ indicates outside the system, while $v_i > 0$ indicates inside the system.			

Table 1. Fluxes through Input/output metabolites, Lipid and Carbohydrates (in moles/kg DW/hr)

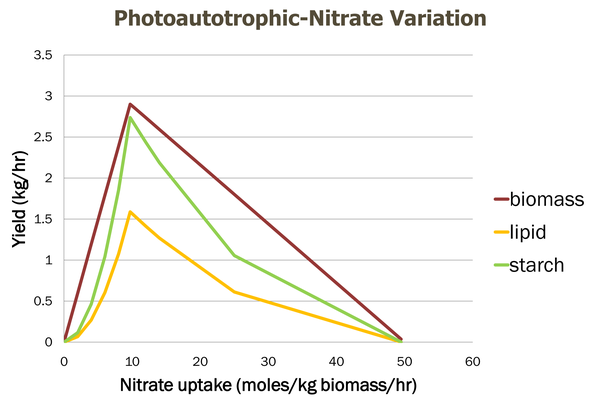

Figure 3. Changes in biomass composition (lipid and starch) with respect to Nitrate uptake

Results and Conclusion

➢Fluxes through various extracellular exchange reactions have been shown in Table 1. For mixotrophic growth, we observed different growth regimes with respect to nitrate uptake (data not shown). First growth regime involved uptake of O2 , while the second growth regime involved O2 and CO2 leaving the cell.

➢For mixotrophic growth, the cell allowed formation of Glycogen, which utilizes UTP to polymerize glucose. However, for heterotrophic and photoautotrophic growth, Amylopectin formation was favored. This is done to optimize the energy for total biomass formation than for starch production.

➢Further, we observed that nitrate starvation leads to accumulation of starch at a steep rate, as shown in Figure 3. It is known that some marine algae accumulate lipids, and are viewed as an potential source for lipid based biofuel production. However, there is lack of metabolic models for marine algal species.

References

1.Boyle NR, and Morgan JA,(2009), Flux Balance analysis of primary ,metabolism in *Chlamydomonas reinhardtii* . BMC Systems Biology. 3, p. 4

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