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Microalgae Metabolic Modelling: Formulation, Analyses, Applications, and Challenges

Maryam Afarin[1], Hasan Mahfouzi[2], Zahra Rahmani[2], Taraneh Ghoreyshi[1], Fereshteh Naimpoor[3*]

1PhD Student;2MSc student; 3Academic staff

School of Chemical, Petroleum and Gas Engineering, Iran University of Science and Technology (IUST)

**fnaeim@iust.ac.ir*

Objective and background

Microalgae have recently become the focus of many studies due to their broad biotechnological applications. They have been considered as the sustainable energy resources for the third-generation biofuels as well as cell-factories of valuable bio-based products. Photoautotrophic microalgae use CO2 as C-source and light as energy (E)-source for their growth. This can mitigate atmospheric CO2, the cause of global climate change, and yield neutral lipids or carbohydrates (Park, Nguyen, and Jin 2019). Additionally, microalgae have complex metabolism and can produce many valuable compounds such as proteins, pigments, medicines and cosmetics with commercial demand. On the other hand, organic compounds can be consumed by heterotrophic/mixotrophic microalgae to produce cell mass rich in carbohydrate/lipid. This is advantageous since wastewaters rich in organic compounds can support microalgal growth and their product formation (Patel et al. 2019).

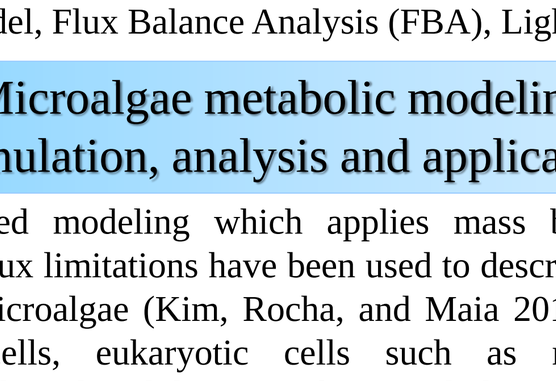
To make the microalgal based-processes commercial, many studies have been carried out to make the processes cost effective (Benavente-Valdés et al. 2016). Efforts have been devoted to optimizing the culture medium and operating conditions as well as discovering the superior strains. On the other hand, metabolic models have been generated by reconstruction of biochemical reaction networks alongside metabolic balance analysis (FBA) using linear programming to predict the cellular behavior. This allows strain engineering by thorough investigation of cell activities and finding the optimal gene manipulations as well as flux redirection towards the desired cell characteristics. Using these models along with critical analyses may enhance microalgal productivities and this in turn can reduce the costs and support commercialization of their products (Tibocha-Bonilla et al. 2018b).

Keywords: Microalgae, Photoautotroph, Heterotroph, Mixotroph, Metabolic Model, Flux Balance Analysis (FBA), Light Cycle

Microalgae metabolic modeling: formulation, analysis and applications

Constraint-based modeling which applies mass balance, energy balance, and flux limitations have been used to describe the potential behavior of microalgae (Kim, Rocha, and Maia 2018). Contrary to prokaryotic cells, eukaryotic cells such as microalgae are compartmented and exhibit specific metabolic activities within compartments. Despite this division, the performance of the whole cell is highly dependent on the interactions among compartments (Wahrheit, Nicolae, and Heinzle 2011). Table 1 describes the various features of the eukaryotic models. Microalgae have light-dependent metabolic pathways and hence more organelles due to their photosynthetic nature compared to other unicellular eukaryotes. Therefore, more challenges exist on reconstruction of their metabolic network and modelling. More than 40 metabolic models for photosynthetic microorganisms (cyanobacteria and microalgae) have been reported (Tibocha-Bonilla et al. 2018a). Table 2 lists the features of these models.

Table 1. Eukaryotic metabolic model features



Components		Description	Method of identification	Scheme
Compartments		No.: 2-15, based on complexity	Gene localization techniques	○
Metabolites	Dedicated	In just one organelle		△
	Shared	In more than one organelle		⬟
Reactions	Transport	Between organelles	Biochemical information	←
	Exchange	With extracellular space		⮌

Table 2. Features of microalgal metabolic models

Features	Descriptions				References
Model Scale[+]	Core	no G	24-280 R	19-278 M	(Chang et al. 2011)
	Genomic	151-2249 G	24-280 R	19-278 M	
Compartments	**No.**: 1-14 cytoplasm, mitochondrion, chloroplast, thylakoid, glyoxysome , endoplasmic reticulum, amyloplast, chromoplast ,nucleus, peroxisome, plasma membrane, lysosome, Golgi apparatus, and extracellular space				(Manichaikul et al. 2009) (Kliphuis et al. 2012) (Loira et al. 2017) (Mekanik et al. 2019)
Metabolisms	Photoautotrophic, heterotrophic, mixotrophic				
Lighting	Light (L), Dark (D) and cyclic L/D				
Computational Analysis	FBA: Flux balance analysis, a constraint-based modelling approach				(Zuñiga et al. 2016)
	MFA: Metabolic flux analysis, using isotopic flux measurements with FBA				(Boyle, Sengupta, and Morgan 2017)
	pFBA: Parsimonious flux balance analysis, minimizing the fluxes through the network				(Sarkar et al. 2019)
	FVA: Flux variability analysis, finding min and max of each flux				(Shene, Asenjo, and Chisti 2018)
	dFBA: Dynamic flux balance analysis, assuming faster intracellular compared to extracellular dynamics				(Scott et al. 2018) (Baroukh et al. 2016)
	MPA: metabolic pathway analysis. Characterization of all feasible flux distributions: Elementary modes(EM) and extreme pathways(EP)				(Rügen et al. 2012)
Aims	High-value products productivity				(Wu et al. 2015)
	Effect of light intensity, spectrum and light cycle				(Sarkar et al. 2019)
	Validation				(Krumholz et al. 2012)
	Flux distribution within network				(Boyle and Morgan 2009)
	Growth under various metabolism				(Shene, Asenjo, and Chisti 2018)
	Cell growth over time				(Baroukh et al. 2016)

[+]: G: Genes R: Reactions M: Metabolites

Microalgae metabolic modeling: challenges

Microalgae in nature follow the cyclic lifestyle. On day light, they use photosynthesis to accumulate storage compounds while in the dark they grow heterotrophically on their storage material. Therefore, the light/dark cycle should be considered while modelling microalgal. This issue has been tackled by a few studies. dFBA was used to predict the accumulation of storage molecules in microalgae (Bordbar et al. 2017). Accumulation of the energy-rich compounds and growth on these storage compounds for cell maintenance in the dark were studied by ((Knoop et al. 2013a), (Shene, Asenjo, and Chisti 2018)). Dynamic biomass objective functions was used by (Zuñiga et al. 2018). Although some of the models were able to simulate the growth in the dark, the rate of their consumption greatly exceeded the rate of their synthesis under the light (Shene, Asenjo, and Chisti 2018). Another challenge on modelling of microalgae is the high level of compartmentalization and the fact that the localization of a number of reactions/pathways are not known. Additionally, limited information regarding the exchange of metabolites between the compartments are available. Furthermore, as the model considers more details, the metabolic network becomes larger making the simulation computationally burdensome. Therefore, a new challenge is to minimize the size of the microalgae metabolic network while the essential information would not be missed (Parichehreh et al. 2019). Another point to consider is the fact that microalgae always coexist with other microorganisms such as bacteria in natural environments instead of pure cultures in the laboratories. Bacteria are benefited from the microalgae exudates such as oxygen and starch. On the other hand, microalgae growth is promoted by bacterial products such as CO2, inorganic substances and some growth factors. Metabolic modeling of these communities and the interactions among the members is another challenge to predict microalgae behavior in ecosystems more realistically (Guerra-Renteria et al. 2019).

Conclusion

Metabolic modeling has been proven critical for our understanding of complex metabolism in microalgae. Metabolic models have progressed from core models to genome-scale metabolic models, now including detailed compartmentalization. A variety of model analyses have exploited to identify boundaries for light and nutrient conditions. The 40 metabolic models of microalgae presented are mostly based on similar principles and also has the similar limitations. Future studies should overcome existing models limitations.

References

Benavente-Valdés, Juan Roberto, Cristóbal Aguilar, Juan Carlos Contreras-Esquivel, Alejandro Méndez-Zavala, and Julio Montañez. 2016. 'Strategies to enhance the production of photosynthetic pigments and lipids in chlorophycae species', Biotechnology Reports, 10: 117-25.

Kim, Osvaldo D., Miguel Rocha, and Paulo Maia. 2018. 'A Review of Dynamic Modeling Approaches and Their Application in Computational Strain Optimization for Metabolic Engineering', Frontiers in Microbiology, 9.

Park, Seunghye, Thu Ha Thi Nguyen, and EonSeon Jin. 2019. 'Improving lipid production by strain development in microalgae: Strategies, challenges and perspectives', Bioresource Technology, 292: 121953.

Patel, Anil Kumar, Jae Min Joun, Min Eui Hong, and Sang Jun Sim. 2019. 'Effect of light conditions on mixotrophic cultivation of green microalgae', Bioresource technology, 282: 245-53.