# Annex – Dry lab iGEM Toulouse 2021

## ➢ Model assumptions

- The different phases (liquid and gas) contained in the reactor are homogenous.

- $O_2$ produced by the cyanobacteria through photosynthesis coupled to the air bubbling in the reactor should result in saturated concentration of $O_2$ in the liquid phase, hence $O_2$ is considered as non-limiting, and its transfer is not included in the model. This hypothesis was verified during our bioreactor experiment.

- At each time interval, each microorganism must be in a state of metabolic equilibrium, hence the stoichiometric modelling. This assumption is justified by the fact that the time intervals are very small and that the overall dynamics of the system can therefore be considered as a succession of infinitesimal stationary states.

## ➢ Details of parameter calculation

### ▪ CO2 concentration of the gas input $CO_2{}^{gas}{}_{in}$

We use the ideal gas law to calculate this concentration:

$$PV=nRT$$

$$\rightarrow CO_2{}^{gas}{}_{in} = n/V = P/RT = 41\ mM$$

At atmospheric pressure and 35°C.

### ▪ Production yield of sucrose $R_{CO2\ ,Sucrose}$

In the work carried out by (Lin et al. 2020), the authors were able to measure that for the sucrose-secreting strain *S. elongatus UTEX 2973* CscB, approximately 80% on average of the fixed CO2 goes to sucrose upon induction.

Hence: $R_{CO2\ ,\ Sucrose}$ = 0,8 Cmole sucrose / Cmole CO2 = 0,067 mmol sucrose / mmol CO2

### ▪ Production yield of the violet leaf aldehydes (nonadienal and nonadienol)

The precursors of these molecules is alpha-linolenic acid (ALA). Under optimized conditions, it was shown that *S. elongatus* can reach a lipid content of 29.0 ± 2.1% w/w (Silva et al. 2014). This means that the one gram of biomass contains 290 mg of lipids.

Moreover, approximately 3.5% of these lipids are ALA (Santos-Merino et al. 2018). There is therefore 10,15 mg ALA/gDCW. By assuming that all this precursor will be consumed in the LOX pathway and using the growth rate of *S. elongatus UTEX 2973,* we find:

$$q_{nonadienol/al} = 0,012\ mmol/gDCW/h$$

We finally divide this result by the maximum uptake rate of $CO_2$ which gives:

$$R_{CO2\ ,Nonadienol/al} = 0{,}00032 \text{ mmol nonadienal / mmol CO2}$$

- ## Production yield of the violet terpenes

Due to the strong link of our dry lab work and our Supporting Entrepreneurship section, we chose to be as close as possible of an industrial production framework. The highest lycopene producing yeast strain described to date is able of accumulating 73.3 mg/g DCW (Ma et al. 2019).

By multiplying this by the growth rate of our strain (0,5 $h^{-1}$), we obtain:

$$q_{lycopene}=0{,}00745 \text{ mmol/gDCW/h}$$

We split this between the four terpenes (and normalize with the carbon number of each molecule).

$$q_{alpha\_ionone} = 0{,}0171 \text{ mmol/gDCW/h}$$

$$q_{beta\_ionone} = 0{,}0171 \text{ mmol/gDCW/h}$$

$$q_{dihydroionone} = 0{,}0171 \text{ mmol/gDCW/h}$$

$$q_{linalool} = 0{,}0683 \text{ mmol/gDCW/h}$$

We finally divide by $q_{sucrose}max$ and obtain:

$$R_{Sucrose,\alpha ionone} = 0{,}002$$

$$R_{Sucrose,\beta ionone} = 0{,}002$$

$$R_{Sucrose,Dihydroionone} = 0{,}002$$

$$R_{Sucrose,Linalool} = 0{,}008$$

## References:


Lin P-C, Zhang F, Pakrasi HB. 2020. Enhanced production of sucrose in the fast-growing cyanobacterium Synechococcus elongatus UTEX 2973. Scientific Reports. 10(1):390. doi:10.1038/s41598-019-57319-5.

Ma T, Shi B, Ye Z, Li X, Liu M, Chen Y, Xia J, Nielsen J, Deng Z, Liu T. 2019. Lipid engineering combined with systematic metabolic engineering of Saccharomyces cerevisiae for high-yield production of lycopene. Metabolic Engineering. 52:134–142. doi:10.1016/j.ymben.2018.11.009.

Santos-Merino M, Garcillán-Barcia MP, de la Cruz F. 2018. Engineering the fatty acid synthesis pathway in Synechococcus elongatus PCC 7942 improves omega-3 fatty acid production. Biotechnol Biofuels. 11:239. doi:10.1186/s13068-018-1243-4.

Silva CSP, Silva-Stenico ME, Fiore MF, de Castro HF, Da Rós PCM. 2014. Optimization of the cultivation conditions for Synechococcus sp. PCC7942 (cyanobacterium) to be used as feedstock for biodiesel production. Algal Research. 3:1–7. doi:10.1016/j.algal.2013.11.012.