**Simple Instructions in how to perform the DFBA simulations**

In order to run a DFBA simulation, first, open the files simulation.m, FBA.m and DFBA.m. On the simulation.m file, enter the initial concentrations of biomass (g/L), carbon source (mmol/L), ammonia (mmol/L), PHB (mmol/L), acetate (mmol/L), lactate (mmol/L), ethanol (mmol/L), formate (mmol/L), succinate (mmol/L), and the initial volume (L), respectively. It is also possible to remove or add new metabolites to be tracked by the simulations, but that will require changes in other sections of the scripts as well. The sections that would require changes in this instance are marked in the m.files. Next, enter the final time of the simulation (in hours) and the number of elements, which in turn will define the number of steps used. The final time can be any amount of time that is enough for all carbon source to be consumed. Uncomment the kinetic parameters for the model of the microorganism that will be used in the simulation, and define the feed carbon source concentration. The feeding profile used is the exponential feeding profile (default), but other feeding profiles can be used such as constant feed or no feed, so long as the appropriate changes to the code are carried out.

Now, go to the FBA.m file. Uncomment the indexes and load the model of the microorganism that will be used in the simulation. For growth associated PHB production simulations that explore the trade-off between biomass and PHB formation, keep the biomass objective function (default), and on the “constrains for the linprog function” section, fix the flux to PHB synthesis to any desired number, within the limits of the maximum PHB yield of the chosen microorganism. For non growth-associated PHB production simulations, change the objective function to PHB synthesis. For that, just uncomment the block of code that changes the objective function, as marked in the FBA.m file itself.

Go to the DFBA.m file. Uncomment the maximum oxygen uptake rate for the model of the microorganism that will be used in the simulation. Finally, go back to the simulation.m file and run. Running the simulation.m file will create a concentrations matrix (y) and a time vector (t) variable, and a plot showing the biomass and metabolites as a function of time is created. With that, the final biomass, final yield, final titer, and the time to reach the final titer (and hence, the productivity) can be identified.