**Steps for how to reparameterize ctRBA**

Notes on how to reparametrize C. thermocellum Resource Allocation Model. Note that the workflow is not optimized as the focus has been getting the work done, not making it streamlined.

1. Update relevant files in build\_model/input.
2. Ensure that the protein concentration data is located in build\_model/input/get\_kapps/[insert].
3. Run Jupyter notebooks beginning with A01 to A05 in the build\_model folder. A01 to A03 creates the file essential for the RBA model (apart from the value), whereas A04 and A05 creates the essential files for beginning the procedure to calculate values.
4. Move all files in model to the cluster (in order to run GAMS code). Generally, I move just the GAMS subfolder, as the whole file system is a bit large.
5. Run the file “get\_essen\_v\_inactive.gms”. This code identifies the inactive reactions that are essential. Note that reactions that have “UNKNOWN” GPR are considered inactive, yet those with “SPONT” GRP are considered active.
6. Previous step will result in a filed called “minimal\_essential\_inactives.txt”, which is a list of the inactive reactions essential for the model to function. Running the code “make\_remaining\_inactives.pl” will create a new file “model/remaining\_inactive\_rxns.txt” which will remove all essential inactive reactions from the “inactive\_rxns.txt” list.
7. Run the code “GAMS/get\_v\_min.gms”. This code will return the list of minimal reaction rates.
8. Move the resulting “get\_v\_min\_flux\_all.txt” file back to your local system. Copy this into the excel worksheet “ctherm\_RBA/build\_model/input/get\_kapp/v\_min\_rxn.xlsx”.
9. Run the jupyter notebook “get\_kapps\_02\_15\_2024”. This calculate s on a per-enzyme basis, and assigns them to the correct reactions in an output file that GAMS can read. As part of running of the notebook, near the end, should be a cell which reports the number of reactions with calculate values and the median value. These are sanity checks, please note these values.
10. The previous step should create a GAMS-compatible parameter file “kapp\_vals.txt”. Move this file to “GAMS/model” on the cluster.
11. From here, you should be able to run “runRBA.gms”.

**Run RBA naming conventions**

each run RBA is given a few indicators to describe its settings concisely. The format is something like:

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**runRBA\_1\_2\_3\_4\_5\_6\_7.gms**

**position 1: states how growth rate is determined**

b - bisection method

f - fixed

**position 2: whether or not carbon yields is enforced**

c - cellulosome synthesis enforced

n - cellulosome synthesis not enforced

**position 3: carbon source**

cb - cellobiose

av - microcrystalline cellulose or avicel

**position 4: objective**

et - maximize ethanol objective

etmin - maximize ethanol objective

ac - maximize acetate objective

ae - maximize acetate + ethanol objective

mih2 - minimize hydrogen gas (H2) objective

mah2 - maximize hydrogen gas (H2) objective

mig - minimize synthesis of glycolytic proteins

mag - minimize synthesis of glycolytic proteins

mxg - mixed glycolysis objective (min upper, max lower)

**position 5: whether or not malate shunt/ppdk ratio was enforced in parameterization run of the kapps used**

r - ratio was enforced

nr - ratio was not enforced

**position 6: cellulosome synthesis enforcement**

e - product yield ranges from MFA enforced

ne - product yield ranges from MFA not enforced

**position 7: model used**

b - base model

w - base\_wo\_glc model

g - GAPN model

a - atp\_pfk model

r - adhE\_rep model

d - adhE-D494G model