Introduction

This supplementary material was developed by the CNPEM.Brazil Bioinformatics group in order to insert all equations that had not been included on gapseq software. The pathways chosen were simulated using the cobrapy library by calling functions and classes through the python programming language. The modeling assumptions were not sufficient to succeed, and the objective of inducing the CO2 uptake couldn't be achieved. It's important to highlight that the vast majority of the students had never had any experience in this kind of subject and after a period of DIY effort it's consensual that all group members have learned a lot, either by means of research immersion, bioinformatics modeling, conventional modeling and so forth.

```
! pip install cobra
import cobra
import pandas as pd

from cobra import Model, Reaction, Metabolite

!ls

#model=cobra.io.read_sbml_model('ecoli-DH5alpha-adapt-WL.xml',
warning=False)
model=cobra.io.read_sbml_model('/content/ecoli-DH5alpha-adapt-
WL.xml')

"""# **Wood-ljundhal** Pathway"""

reaction = Reaction('rxn16153')
reaction.name='carbon monoxide dehydrogenase/acetyl-CoA synthase
(CODH/ACS)'
reaction.subsystem="Cell growth"
reaction.lower_bound=-1000
reaction.upper_bound=1000

co2_c = Metabolite(
    'cpd00011_c',
    formula='CO2',
    name='CO2',
    compartment='c')

h_c = Metabolite(
    'cpd00067_c',
    formula='H',
    name='H',
    compartment='c')

e_c = Metabolite(
    'cpd12713_c',
    formula='E',
    name='E',
```

```
        compartment='c')

    h2o_c = Metabolite(
        'cpd00001',
        formula='H2O',
        name='Water',
        compartment='c')

    co_c = Metabolite(
        'cpd00204',
        formula='CO',
        name='Carbon monoxide',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        co2_c:-1.0,
        h_c:-1.0,
        e_c: -1.0,
        h2o_c: 1.0,
        co_c:1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    '''
    Equation with compound IDs
    cpd00010 + cpd00011 + cpd00067 + (2) cpd12713 + cpd12809 <= cpd00001
+ cpd00022 + cpd12810
    '''
    reaction = Reaction('rxn16164')
    reaction.name='R09317'
    reaction.subsystem="Cell growth"
    reaction.lower_bound=0
    reaction.upper_bound=1000

    coa_c= Metabolite(
        'cpd00010_c',
        formula='C21H32N7O16P3S',
        name='Coenzyme A',
        compartment='c')

    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')

    h_c = Metabolite(
        'cpd00067',
        formula='H',
        name='H',
        compartment='c')

    e_c = Metabolite(
```

```
        'cpd12713_c',
        formula='E',
        name='E',
        compartment='c')

    methyl_c = Metabolite(
        'cpd12809_c',
        name='Methylcorrinoid',
        formula='C20H3CoN4R21',
        compartment='c')


    agua_c = Metabolite(
        'cpd00001_c',
        name='Water',
        formula='H20',
        compartment='c')

    aCoa_c = Metabolite(
        'cpd00022_c',
        name='Acetyl-CoA',
        formula='',
        compartment='c')

    corrinoid = Metabolite(
        'cpd12810_c',
        name='Corrinoid',
        formula='C19CoN4R21',
        compartment='c')



    # Adding metabolites into a reaction
    reaction.add_metabolites({
        coa_c:1.0,
        co2_c:1.0,
        h_c:1.0,
        e_c:2.0,
        methyl_c:1.0,
        agua_c:-1.0,
        aCoa_c:-1.0,
        corrinoid:-1.0})

    model.add_reactions([reaction])

    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    reaction = Reaction('rxn17445')
    reaction.name='formylmethanofuran-tetrahydromethanopterin
formyltransferase'
    reaction.subsystem="Cell growth"
    reaction.lower_bound=0
    reaction.upper_bound=1000

    hydrogen=Metabolite(
        'cpd00067_c',
```

```
    formula='H',
    name='H',
    compartment='c')

tetraHydro=Metabolite(
    'cpd00895_c',
    formula='C30H42N6O16P',
    name='5,6,7,8-tetrahydromethanopterin',
    compartment='c') ##c?

formyMeta=Metabolite(
    'cpd27090_c',
    formula='C25H29N4O9',
    name='Formyl-methanofurans4',
    compartment='c')

formylHPT5=Metabolite(
    'cpd00936_c',
    formula='C31H45N6O17P',
    name='5-Formyl-H4MPT',
    compartment='c')

metanoFurans = Metabolite(
    'cpd27506_c',
    formula='C24H30N4O8',
    name='Methanofuran',
    compartment='c')

reaction.add_metabolites({
    hydrogen:-1,
    tetraHydro:-1,
    formyMeta:-1,
    formylHPT5:1,
    metanoFurans:1
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn19033')
reaction.name='acetyl-CoA:corrinoid protein O-acetyltransferase'
reaction.subsystem="Cell growth"
reaction.lower_bound=-1000
reaction.upper_bound=1000



accoa_c = Metabolite(
    'cpd00022_c',
    formula='C23H34N7O17P3S',
    name='Acetyl-CoA',
    compartment='c')



CoFeSP = Metabolite(
```

```
    'cpd26754_c',
    formula='C54H79CoN14O9',
    name='Co(I) corrinoid Fe-S protein',
    compartment='c')



coa = Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
    name='CoenzymeA',
    compartment='c')



co = Metabolite(
    'cpd00204_c',
    formula='CO',
    compartment='c')



FeSP = Metabolite(
    'cpd27517_c',
    formula='C55H82CoN14O9',
    compartment='c')



# Adding metabolites into a reaction
reaction.add_metabolites({
    accoa_c:-1.0,
    CoFeSP:-1.0,
    coa: 1.0,
    co: 1.0,
    FeSP: 1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn17208')
reaction.name='carbon-monoxide dehydrogenase (ferredoxin)'
reaction.subsystem="Cell growth"
reaction.lower_bound=-1000
reaction.upper_bound=1000


h2o_c = Metabolite(
    'cpd00001_c',
    formula='H2O',
    name='Water',
    compartment='c')


co_c = Metabolite(
```

```
        'cpd00204_c',
        formula='CO',
        name='Carbon monoxide',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',           #           available           at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')


    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')


    h_c = Metabolite(
        'cpd00067_c',
        formula='H',
        name='H',
        compartment='c')


    red_ferr_c = Metabolite(
        'cpd28082_c',
        formula='Fe2S2',
        name='Reduced-ferredoxins',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        h2o_c:-1.0,
        co_c:-1.0,
        ox_ferr_c: -1.0,
        co2_c: 1.0,
        h_c: 3.0,
        red_ferr_c: 1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    reaction = Reaction('rxn16328')
    reaction.name='oxalate:ferredoxin oxidoreductase'
    reaction.subsystem="Cell growth"
    reaction.lower_bound=0
    reaction.upper_bound=1000


    oxa_c = Metabolite(
        'cpd00180_c',
```

```
        formula='C2O4',
        name='Oxalate',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd11621_c',
        formula='Fe2S2',           #           available           at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')


    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')


    red_ferr_c = Metabolite(
        'cpd28082_c',
        formula='Fe2S2',
        name='Reduced-ferredoxins',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        oxa_c:-1.0,
        ox_ferr_c: -2.0,
        co2_c: 2.0,
        red_ferr_c: 2.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    import tempfile
    from pprint import pprint
    from cobra.io import write_sbml_model, validate_sbml_model
    with      tempfile.NamedTemporaryFile(suffix='ecoli-DH5alpha-adapt-
WL.xml') as f_sbml:
        write_sbml_model(model, filename=f_sbml.name)
        report = validate_sbml_model(filename=f_sbml.name)

    pprint(report)

    model.reactions.get_by_id('EX_cpd11416_c0')

    print(model.objective.expression)

    model.optimize()

    model.summary()

    # co2_flux = model.problem.Constraint(
```

```
      #     model.reactions.EX_cpd00011_e0.flux_expression,
      #     lb=-20,
      #     ub=-19)
      # model.add_cons_vars(co2_flux)

      # gluc_flux = model.problem.Constraint(
      #     model.reactions.EX_cpd00027_e0.flux_expression,
      #     lb=0,
      #     ub=10000)
      # model.add_cons_vars(gluc_flux)

      with model:
          model.objective = {model.reactions.bio1: 1}
          model.optimize()
          print(model.reactions.EX_cpd00011_e0.flux)

      import cobra.test
      co2_flux = model.problem.Constraint(
          model.reactions.EX_cpd00011_e0.flux_expression,
          lb=-20,
          ub=-19.99)
      model.add_cons_vars(co2_flux)

      gluc_flux = model.problem.Constraint(
          model.reactions.EX_cpd00027_e0.flux_expression,
          lb=0,
          ub=10000)
      model.add_cons_vars(gluc_flux)

      # model.objective_direction = 'min'
      # model.objective='bio1'
      print(model.objective.expression)
      print(model.objective.direction)


      solution=model.optimize().objective_value
      print(solution)

      # from cobra.flux_analysis import gapfill

      # def filling_gaps(x):
      #   universal = cobra.Model(model)
      #   co2_reaction = model.reactions.get_by_id('rxn16164')
      #   model.objective= co2_reaction
      #   solution= model.optimize().objective_value

      #   solution = gapfill(model, universal, demand_reactions=False)
      #   for reaction in solution[0]:
      #       print(reaction.id)

      #     result  =  gapfill(model,  universal,  demand_reactions=False,
iterations=4)

      #   with model:
      #     model.objective = model.add_boundary(model.metabolites.cpd_c,
type='demand')
      #     solution = gapfill(model, universal)
      #     for reaction in solution[0]:
```

```
#         print(reaction.id)

#   return solution

# filling_gaps(model)


# solution = gapfill(model, universal, demand_reactions=True)
# for reactions in solution[0]:
#   print(reaction.id)

# from cobra.flux_analysis import gapfill
# universal = cobra.Model('universal_reactions')
# for i in [i.id for i in model.metabolites.cpd00011_c.reactions]:
#   reaction = model.reactions.get_by_id(i)

"""# **Calvin Cycle** Pathway"""

reaction = Reaction('rxn16153')
reaction.name='carbon  monoxide  dehydrogenase/acetyl-CoA  synthase
(CODH/ACS)'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000

co2_c = Metabolite(
    'cpd00011_c',
    formula='CO2',
    name='CO2',
    compartment='c')

h_c = Metabolite(
    'cpd00067_c',
    formula='H',
    name='H',
    compartment='c')

e_c = Metabolite(
    'cpd12713_c',
    formula='E',
    name='E',
    compartment='c')

h2o_c = Metabolite(
    'cpd00001',
    formula='H2O',
    name='Water',
    compartment='c')

co_c = Metabolite(
    'cpd00204',
    formula='CO',
    name='Carbon monoxide',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    co2_c:-1.0,
```

```
        h_c:-1.0,
        e_c: -1.0,
        h2o_c: 1.0,
        co_c:1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    '''
    Equation with compound IDs
    cpd00010 + cpd00011 + cpd00067 + (2) cpd12713 + cpd12809 <= cpd00001
+ cpd00022 + cpd12810
    '''
    reaction = Reaction('rxn16164')
    reaction.name='R09317'
    reaction.subsystem=''
    reaction.lower_bound=0
    reaction.upper_bound=1000

    coa_c= Metabolite(
        'cpd00010_c',
        formula='C21H32N7O16P3S',
        name='Coenzyme A',
        compartment='c')

    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')

    h_c = Metabolite(
        'cpd00067',
        formula='H',
        name='H',
        compartment='c')

    e_c = Metabolite(
        'cpd12713_c',
        name='E',
        formula='E',
        compartment='c')

    methyl_c = Metabolite(
        'cpd12809_c',
        name='Methylcorrinoid',
        formula='C20H3CoN4R21',
        compartment='c')


    agua_c = Metabolite(
        'cpd00001_c',
        name='Water',
        formula='H20',
        compartment='c')
```

```
aCoa_c = Metabolite(
    'cpd00022_c',
    name='Acetyl-CoA',
    formula='',
    compartment='c')

corrinoid = Metabolite(
    'cpd12810_c',
    name='Corrinoid',
    formula='C19CoN4R21',
    compartment='c')



# Adding metabolites into a reaction
reaction.add_metabolites({
    coa_c:1.0,
    co2_c:1.0,
    h_c:1.0,
    e_c:2.0,
    methyl_c:1.0,
    agua_c:-1.0,
    aCoa_c:-1.0,
    corrinoid:-1.0})

model.add_reactions([reaction])

'''def reverse_reaction():
  rxn = coa_c + co2_c + h_c + 2 * e_c + methyl_c <= agua_c + aCoa_c
+ corrinoid
  return rxn

from cobra import flux_analysis
cobra.flux_analysis.fastcc._flip_coefficients(reverse_reaction,
rxn)'''

print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn17208')
reaction.name='carbon-monoxide dehydrogenase (ferredoxin)'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


h2o_c = Metabolite(
    'cpd00001_c',
    formula='H2O',
    name='Water',
    compartment='c')


co_c = Metabolite(
    'cpd00204_c',
    formula='CO',
```

```
        name='Carbon monoxide',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',            #            available            at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')


    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')


    h_c = Metabolite(
        'cpd00067_c',
        formula='H',
        name='H',
        compartment='c')


    red_ferr_c = Metabolite(
        'cpd28082_c',
        formula='Fe2S2',
        name='Reduced-ferredoxins',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        h2o_c:-1.0,
        co_c:-1.0,
        ox_ferr_c: -1.0,
        co2_c: 1.0,
        h_c: 3.0,
        red_ferr_c: 1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    reaction = Reaction('rxn19033')
    reaction.name='acetyl-CoA:corrinoid protein O-acetyltransferase'
    reaction.subsystem=''
    reaction.lower_bound=-1000
    reaction.upper_bound=1000



    accoa_c = Metabolite(
        'cpd00022_c',
        formula='C23H34N7O17P3S',
```

```
    name='Acetyl-CoA',
    compartment='c')



CoFeSP = Metabolite(
    'cpd26754_c',
    formula='C54H79CoN14O9',
    name='Co(I) corrinoid Fe-S protein',
    compartment='c')



coa = Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
    name='CoenzymeA',
    compartment='c')



co = Metabolite(
    'cpd00204_c',
    formula='CO',
    compartment='c')



FeSP = Metabolite(
    'cpd27517_c',
    formula='C55H82CoN14O9',
    compartment='c')



# Adding metabolites into a reaction
reaction.add_metabolites({
    accoa_c:-1.0,
    CoFeSP:-1.0,
    coa: 1.0,
    co: 1.0,
    FeSP: 1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn00909')
reaction.name='methylenetetrahydrofolate reductase 1 (MTHFR1)'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


mlthf_c = Metabolite(
    'cpd00125_c',
```

```
    formula='C20H21N7O6',
    name='5,10-Methylenetetrahydrofolate',
    compartment='c')


fadh2_c = Metabolite(
    'cpd00982_c',
    formula='C27H33N9O15P2',
    name='Flavin adenine dinucleotide reduced',
    compartment='c')


fad_c = Metabolite(
    'cpd00015_c',
    formula='C27H31N9O15P2',
    name='Flavin adenine dinucleotide oxidized',
    compartment='c')


mthf5_c = Metabolite(
    'cpd00345_c',
    formula='C20H24N7O6',
    compartment='c')


# Adding metabolites into a reaction
reaction.add_metabolites({
    mlthf_c:-1.0,
    fadh2_c:-1.0,
    fad_c: 1.0,
    mthf5_c: 1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn14396')
reaction.name='5-methyltetrahydrofolate:ferredoxin oxidoreductase'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


h_c = Metabolite(
    'cpd00067_c',
    formula='H',
    name='H',
    compartment='c')


mlthf_c = Metabolite(
    'cpd00125_c',
    formula='C20H21N7O6',
    name='5,10-Methylenetetrahydrofolate',
    compartment='c')
```

```
    red_ferr_c = Metabolite(
        'cpd11620_c',
        formula='Fe2S2*',
        name='Reduced-ferredoxins',
        compartment='c')


    mthf5_c = Metabolite(
        'cpd00345_c',
        formula='C20H24N7O6',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',            #            available            at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        h_c:-2.0,
        mlthf_c:-1.0,
        red_ferr_c:-2.0,
        mthf5_c:1.0,
        ox_ferr_c:2.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    reaction = Reaction('rxn24034')
    reaction.name='5-methyltetrahydrofolate:ferredoxin oxidoreductase'
    reaction.subsystem=''
    reaction.lower_bound=-1000
    reaction.upper_bound=1000


    mthf5_c = Metabolite(
        'cpd00345_c',
        formula='C20H24N7O6',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',            #            available            at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')


    h_c = Metabolite(
        'cpd00067_c',
```

```
    formula='H',
    name='H',
    compartment='c')


mlthf_c = Metabolite(
    'cpd00125_c',
    formula='C20H21N7O6',
    name='5,10-Methylenetetrahydrofolate',
    compartment='c')



# Adding metabolites into a reaction
reaction.add_metabolites({
    h_c:-2.0,
    mlthf_c:-1.0,
    red_ferr_c:-2.0,
    mthf5_c:1.0,
    ox_ferr_c:2.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn24147')
reaction.name='RXN-6282.c'
reaction.subsystem=''
reaction.lower_bound=0
reaction.upper_bound=1000


h2o_c = Metabolite(
    'cpd00001_c',
    formula='H2O',
    name='Water',
    compartment='c')


mlthf_c = Metabolite(
    'cpd24592_c',
    formula='C20H213N7O6',
    name='5,10-Methenyltetrahydrofolates',
    compartment='c')


formyl_c = Metabolite(
    'cpd27039_c',
    formula='C20H23N7O7',
    name='10-Formyltetrahydrofolate',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    h2o_c:-1.0,
```

```
    mlthf_c:-1.0,
    formyl_c:-1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn24148')
reaction.name='RXN-6283.c'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


adp_c = Metabolite(
    'cpd00008_c',
    formula='C10H12N5O10P2',
    name='ADP',
    compartment='c')


phos_c = Metabolite(
    'cpd00009_c',
    formula='HO4P',
    name='Phosphate',
    compartment='c')


formyl_c = Metabolite(
    'cpd27039_c',
    formula='C20H23N7O7',
    name='10-Formyltetrahydrofolate',
    compartment='c')


atp_c = Metabolite(
    'cpd00002_c',
    formula='C10H12N5O13P3',
    name='ATP',
    compartment='c')


for_c = Metabolite(
    'cpd00047_c',
    formula='CH1O2',
    name='Formate',
    compartment='c')


tetrf_c = Metabolite(
    'cpd28218_c',
    formula='C19H23N7O6',
    name='acid tetrahydrofolate',
    compartment='c')

# Adding metabolites into a reaction
```

```
reaction.add_metabolites({
    adp_c:-1.0,
    phos_c:-1.0,
    formyl_c:-1.0,
    atp_c:1.0,
    for_c:1.0,
    tetrf_c:1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn24148')
reaction.name='RXN-6283.c'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


adp_c = Metabolite(
    'cpd00008_c',
    formula='C10H12N5O10P2',
    name='ADP',
    compartment='c')


phos_c = Metabolite(
    'cpd00009_c',
    formula='HO4P',
    name='Phosphate',
    compartment='c')


formyl_c = Metabolite(
    'cpd27039_c',
    formula='C20H23N7O7',
    name='10-Formyltetrahydrofolate',
    compartment='c')


atp_c = Metabolite(
    'cpd00002_c',
    formula='C10H12N5O13P3',
    name='ATP',
    compartment='c')


for_c = Metabolite(
    'cpd00047_c',
    formula='CH1O2',
    name='Formate',
    compartment='c')


tetrf_c = Metabolite(
    'cpd28218_c',
```

```
        formula='C19H23N7O6',
        name='acid tetrahydrofolate',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        adp_c:-1.0,
        phos_c:-1.0,
        formyl_c:-1.0,
        atp_c:1.0,
        for_c:1.0,
        tetrf_c:1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    reaction = Reaction('rxn28000')
    reaction.name='RXN-5061.c.riceexp.Oxidized-ferredoxins_Reduced-
ferredoxins'
    reaction.subsystem=''
    reaction.lower_bound=0
    reaction.upper_bound=1000


    h_c = Metabolite(
        'cpd00067_c',
        formula='H',
        name='H',
        compartment='c')


    mlthf_c = Metabolite(
        'cpd00125_c',
        formula='C20H21N7O6',
        name='5,10-Methylenetetrahydrofolate',
        compartment='c')


    red_ferr_c = Metabolite(
        'cpd28082_c',
        formula='Fe2S2',
        name='Reduced-ferredoxins',
        compartment='c')


    mthf5_c = Metabolite(
        'cpd00345_c',
        formula='C20H24N7O6',
        compartment='c')


    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',            #           available           at:
https://www.metanetx.org/chem_info/MNXM588581
```

```
    name='Oxidized-ferredoxins',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    mthf5_c:-1.0,
    ox_ferr_c:-1.0,
    h_c:2.0,
    mlthf_c:1.0,
    red_ferr_c:2.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn34053')
reaction.name='Methenyltetrahydrofolate cyclohydrolase'
reaction.subsystem=''
reaction.lower_bound=0
reaction.upper_bound=1000

h2o_c = Metabolite(
    'cpd00001',
    formula='H2O',
    name='Water',
    compartment='c')


mthf5_c = Metabolite(
    'cpd00345_c',
    formula='C20H24N7O6',
    compartment='c')


h_c = Metabolite(
    'cpd00067_c',
    formula='H',
    name='H',
    compartment='c')


fthf10_c = Metabolite(
    'cpd00201_c',
    formula='C20H21N7O7',
    name='10-Formyltetrahydrofolate',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    h2o_c:1.0,
    mthf5_c:-1.0,
    h_c:4.0,
    fthf10_c:1.0
})

model.add_reactions([reaction])
```

```
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

"""# **TCA Reverse** Pathway"""

## https://modelseed.org/biochem/reactions/rxn20754
reaction = Reaction('rxn20745')
reaction.name='FRD; fumarate reductase (menaquinone); menaquinol-fumarate oxidoreductase; succinate dehydrogenase (menaquinone); succinate:menaquinone oxidoreductase'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000

fum_c = Metabolite(
    'cpd00106',
    formula='C4H2O4',
    name='Fumarate',
    compartment='c')


mqh2_c = Metabolite(
    'cpd27500_c',
    formula='C16H18O2',
    name='Menaquinols',
    compartment='c')  #confirmar se Ã© 'c' o compartment (nÃ£o consegui achar...)


succ_c = Metabolite(
    'cpd00036_c',
    formula='C4H4O4',
    name='Succinate',
    compartment='c')


mq_c = Metabolite(
    'cpd27501_c',
    formula='C16H12O2*',
    name='Menaquinones',
    compartment='c')  #confirmar se Ã© 'c' o compartment (nÃ£o consegui achar...)

# Adding metabolites into a reaction
reaction.add_metabolites({
    fum_c:-1.0,
    mqh2_c:-1.0,
    succ_c:1.0,
    mq_c:1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

## https://modelseed.org/biochem/reactions/rxn33854
```

```
reaction = Reaction('rxn33854')
reaction.name='Succinate --CoA Ligase (ADP forming)'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=0

atp_c = Metabolite(
    'cpd00002',
    formula='C10H12N5O13P3',
    name='ATP',
    compartment='c')


coa_c= Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
    name='Coenzyme A',
    compartment='c')


succ_c = Metabolite(
    'cpd00036_c',
    formula='C4H4O4',
    name='Succinate',
    compartment='c')


adp_c = Metabolite(
    'cpd00008_c',
    formula='C10H12N5O10P2',
    name='ADP',
    compartment='c')

succoa_c = Metabolite(
    'cpd00078_c',
    formula='C25H35N7O19P3S',
    name='Succinyl-CoA',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    atp_c:1.0,
    coa_c:1.0,
    succ_c:1.0,
    adp_c:-1.0,
    succoa_c:-1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

## https://modelseed.org/biochem/reactions/rxn33856
reaction = Reaction('rxn33856')
reaction.name='Fumarate Hydratase'
reaction.subsystem=''
reaction.lower_bound=-1000
```

```
reaction.upper_bound=0

mal__L_c = Metabolite(
    'cpd00130',
    formula='C4H4O5',
    name='L-Malate',
    compartment='c')


h2o_c = Metabolite(
    'cpd00001',
    formula='H2O',
    name='Water',
    compartment='c')


fum_c = Metabolite(
    'cpd00106',
    formula='C4H2O4',
    name='Fumarate',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    mal__L_c:1.0,
    h2o_c:-1.0,
    fum_c:-1.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

reaction = Reaction('rxn17399')
reaction.name='2-oxoglutarate ferredoxin oxidoreductase'
reaction.subsystem=''
reaction.lower_bound=0
reaction.upper_bound=1000

#CoA + 2-Oxoglutarate + 2 Oxidized-ferredoxins ->  CO2 + H+ +
Succinyl-CoA + 2 Reduced-ferredoxins

coa_c= Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
    name='Coenzyme A',
    compartment='c')

#2-Oxoglutarate
oxoglutarate_c = Metabolite(
    'cpd00024_c',
    formula='C5H4O5',
    name='2-Oxoglutarate',
    compartment='c')

ox_ferr_c = Metabolite(
    'cpd27757_c',
```

```
          formula='Fe2S2',           #           available           at:
https://www.metanetx.org/chem_info/MNXM588581
          name='Oxidized-ferredoxins',
          compartment='c')

      co2_c = Metabolite(
          'cpd00011_c',
          formula='CO2',
          name='CO2',
          compartment='c')

      h_c = Metabolite(
          'cpd00067_c',
          formula='H',
          name='H',
          compartment='c')

      #Succinyl-CoA
      succinyl_coa_c = Metabolite(
          'cpd00078_c',
          formula='C25H35N7O19P3S',
          name='Succinyl-CoA',
          compartment='c')

      red_ferr_c = Metabolite(
          'cpd28082_c',
          formula='Fe2S2',
          name='Reduced-ferredoxins',
          compartment='c')

      #CoA  +  2-Oxoglutarate  +  2  Oxidized-ferredoxins  ->   CO2  +  H+  +
Succinyl-CoA + 2 Reduced-ferredoxins
      # Adding metabolites into a reaction
      reaction.add_metabolites({
         coa_c:-1.0,
         oxoglutarate_c:-1.0,
         ox_ferr_c:-2.0,
         co2_c:1.0,
         h_c:1.0,
         succinyl_coa_c:1.0,
         red_ferr_c:2.0
      })
      model.add_reactions([reaction])
      print(f'{len(model.reactions)} reactions')
      print(f'{len(model.metabolites)} metabolites')
      print(f'{len(model.genes)} genes')


      #
      #rxn20508 - Gabi
      #Phosphate + Oxaloacetate  Phosphoenolpyruvate + H2CO3
      #cpd00009 + cpd00032 <=> cpd00061 + cpd00242

      reaction = Reaction('rxn20508')
      reaction.name='phosphate:oxaloacetate                  carboxy-lyase
(phosphorylating)'
      reaction.subsystem=''
      reaction.lower_bound=-1000
```

```
reaction.upper_bound=1000

phos_c = Metabolite(
    'cpd00009_c',
    formula='HO4P',
    name='Phosphate',
    compartment='c')

oaa_c = Metabolite(
    'cpd00032_c',
    formula='C4H2O5',
    name='Oxaloacetate',
    compartment='c')

pep_c = Metabolite(
    'cpd00061_c',
    formula='C3H2O6P',
    name='Phosphoenolpyruvate',
    compartment='c')


h2co3_c = Metabolite(
    'cpd00242_c',
    formula='H2CO3',
    name='Carbonic acid',
    compartment='c')

#Phosphate + Oxaloacetate  Phosphoenolpyruvate + H2CO3
#cpd00009 + cpd00032 <=> cpd00061 + cpd00242
reaction.add_metabolites({
   phos_c:-1.0,
   oaa_c:-1.0,
   pep_c:1.0,
   h2co3_c:1.0
})
model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')



#rxn20651 - Gabi
#pyruvate oxidoreductase
#CoA + Pyruvate + Oxidized-ferredoxins => CO2 + Acetyl-CoA + H+ +
Reduced-ferredoxins
#cpd00010 + cpd00020 + cpd27757 => cpd00011 + cpd00022 + cpd00067 +
cpd28082

reaction = Reaction('rxn20651')
reaction.name='pyruvate oxidoreductase'
reaction.subsystem=''
reaction.lower_bound=0
reaction.upper_bound=1000

coa_c = Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
```

```
        name='Coenzyme A',
        compartment='c')

    #Pyruvate
    pyr_c = Metabolite(
        'cpd00010_c', #ID incorreto?
        formula='C3H3O3',
        name='Pyruvate',
        compartment='c')

    ox_ferr_c = Metabolite(
        'cpd27757_c',
        formula='Fe2S2',            #            available            at:
https://www.metanetx.org/chem_info/MNXM588581
        name='Oxidized-ferredoxins',
        compartment='c')

    co2_c = Metabolite(
        'cpd00011_c',
        formula='CO2',
        name='CO2',
        compartment='c')

    accoa_c = Metabolite(
        'cpd00022_c',
        formula='C23H34N7O17P3S',
        name='Acetyl-CoA',
        compartment='c')

    h_c = Metabolite(
        'cpd00067_c',
        formula='H',
        name='H',
        compartment='c')

    red_ferr_c = Metabolite(
        'cpd28082_c',
        formula='Fe2S2',
        name='Reduced-ferredoxins',
        compartment='c')

    #CoA + Pyruvate + Oxidized-ferredoxins => CO2 + Acetyl-CoA + H+ +
Reduced-ferredoxins
    #cpd00010 + cpd00020 + cpd27757 => cpd00011 + cpd00022 + cpd00067 +
cpd28082
    reaction.add_metabolites({
       coa_c:-1.0,
       pyr_c:-1.0,
       ox_ferr_c:-1.0,
       co2_c:1.0,
       accoa_c:1.0,
       h_c:1.0,
       red_ferr_c:1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
```

```
print(f'{len(model.genes)} genes')

#rxn05794
reaction = Reaction('rxn05794')
reaction.name='oxalosuccinate     carboxy-lyase     (2-oxoglutarate-
forming)'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


nadp_c = Metabolite(
    'cpd00006_c',
    formula='C21H25N7O17P3',
    name='Nicotinamide adenine dinucleotide phosphate',
    compartment='c')


h_c = Metabolite(
    'cpd00067_c',
    formula='H',
    name='H',
    compartment='c')


oxalosuccinate_c = Metabolite(
    'cpd03187_c',
    formula='C6H3O7',
    name='Oxidized-oxalosuccinate',
    compartment='c')


nadph_c = Metabolite(
    'cpd00005_c',
    formula='C21H26N7O17P3',
    name='Nicotinamide adenine dinucleotide phosphate - reduced',
    compartment='c')


co2_c = Metabolite(
    'cpd00011_c',
    formula='CO2',
    name='CO2',
    compartment='c')


akg_c = Metabolite(
    'cpd00024_c',
    formula='C5H4O5',
    name='2-Oxoglutarate',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    nadp_c:-1.0,
    h_c:-2.0,
    oxalosuccinate_c: -1.0,
    nadph_c: 1.0,
```

```
        co2_c: 1.0,
        akg_c: 1.0
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    ##################

    #rxn07824
    reaction = Reaction('rxn07824')
    reaction.name='Nitrotoluene degradation'
    reaction.subsystem=''
    reaction.lower_bound=-1000
    reaction.upper_bound=1000


    C16396_c = Metabolite(
        'cpd15102_c',
        formula='C7H9N3O2',
        name='2,4-Diamino-6-nitrotoluene',
        compartment='c')


    C16399_c = Metabolite(
        'cpd15105_c',
        formula='C7H11N3O',
        name='2,4-Diamino-6-hydroxylaminotoluene',
        compartment='c')


    # Adding metabolites into a reaction
    reaction.add_metabolites({
        C16396_c:-1.0,
        C16399_c :1.0,
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')


    ########

    #rxn13974
    reaction = Reaction('rxn13974')
    reaction.name='pyruvate:ferredoxin      2-oxidoreductase      (CoA-
acetylating)'
    reaction.subsystem=''
    reaction.lower_bound=-1000
    reaction.upper_bound=0


    co2_c = Metabolite(
        'cpd00011_c',
```

```
    formula='CO2',
    name='CO2',
    compartment='c')


aCoa_c = Metabolite(
    'cpd00022_c',
    name='Acetyl-CoA',
    formula='',
    compartment='c')


red_ferr_c = Metabolite(
    'cpd11620_c',
    formula='Fe2S2*',
    name='Reduced-ferredoxins',
    compartment='c')


coa_c= Metabolite(
    'cpd00010_c',
    formula='C21H32N7O16P3S',
    name='Coenzyme A',
    compartment='c')


pyr_c = Metabolite(
    'cpd00020_c',
    formula='C3H3O3',
    name='Pyruvate',
    compartment='c')


C00139_c = Metabolite(
    'cpd11621_c',
    formula='C3H3O3',
    name='Oxidizedferredoxin',
    compartment='c')


# Adding metabolites into a reaction
reaction.add_metabolites({
    co2_c:-1.0,
    h_c:-1.0,
    aCoa_c: -1.0,
    red_ferr_c: -2.0,
    coa_c: 1.0,
    pyr_c: 1.0,
    C00139_c: 2.0
})

model.add_reactions([reaction])
print(f'{len(model.reactions)} reactions')
print(f'{len(model.metabolites)} metabolites')
print(f'{len(model.genes)} genes')

## rxn14048
```

```
      # CO2 + H+ + Succinyl-CoA + (2) Reducedferredoxin <= CoA + 2-
Oxoglutarate + (2) Oxidizedferredoxin
      reaction = Reaction('rxn14048')
      reaction.name='2-oxoglutarate:ferredoxin              oxidoreductase
(decarboxylating)'
      reaction.subsystem=''
      reaction.lower_bound=-1000
      reaction.upper_bound=0


      coa_c= Metabolite(
          'cpd00010_c',
          formula='C21H32N7O16P3S',
          name='Coenzyme A',
          compartment='c')


      h_c = Metabolite(
          'cpd00067_c',
          name='H',
          formula='H',
          compartment='c')


      succinyl_coa_c = Metabolite(
          'cpd00078_c',
          formula='C25H35N7O19P3S',
          name='Succinyl-CoA',
          compartment='c')


      red_ferr_c = Metabolite(
          'cpd28082_c',
          formula='Fe2S2',
          name='Reduced-ferredoxins',
          compartment='c')


      coa_c= Metabolite(
          'cpd00010_c',
          formula='C21H32N7O16P3S',
          name='Coenzyme A',
          compartment='c')


      akg_c = Metabolite(
          'cpd00024_c',
          formula='C5H4O5',
          name='2-Oxoglutarate',
          compartment='c')


      C00139_c = Metabolite(
          'cpd11621_c',
          formula='C3H3O3',
          name='Oxidizedferredoxin',
          compartment='c')
```

```
      # Adding metabolites into a reaction
      reaction.add_metabolites({
          co2_c:1.0,
          h_c:1.0,
          succinyl_coa_c: 1.0,
          red_ferr_c: 2.0,
          coa_c: 1.0,
          akg_c: 1.0,
          ox_ferr_c: 2.0
      })


      ################################################################


      ## rxn14178
      #  CoA  +  2-Oxobutyrate  +  (2)  Oxidizedferredoxin  =>  CO2  +  H+  +
Propionyl-CoA + (2) Reducedferredoxin
      reaction = Reaction('rxn14178')
      reaction.name='2-oxobutanoate:ferredoxin   2-oxidoreductase   (CoA-
propanoylating)'
      reaction.subsystem=''
      reaction.lower_bound=0
      reaction.upper_bound=1000


      co2_c = Metabolite(
          'cpd00011_c',
          formula='CO2',
          name='CO2',
          compartment='c')


      obut2_c = Metabolite(
          'cpd00094_c',
          name='2-Oxobutanoate',
          formula='C4H5O3',
          compartment='c')


      C00139_c = Metabolite(
          'cpd11621_c',
          formula='C3H3O3',
          name='Oxidizedferredoxin',
          compartment='c')


      co2_c = Metabolite(
          'cpd00011_c',
          formula='CO2',
          name='CO2',
          compartment='c')


      h_c = Metabolite(
          'cpd00067_c',
          name='H',
          formula='H',
```

```
    compartment='c')


ppcoa_c = Metabolite(
    'cpd00086_c',
    formula='C24H36N7O17P3S',
    name='Propanoyl-CoA',
    compartment='c')


red_ferr_c = Metabolite(
    'cpd28082_c',
    formula='Fe2S2',
    name='Reduced-ferredoxins',
    compartment='c')

# Adding metabolites into a reaction
reaction.add_metabolites({
    co2_c:-1.0,
    obut2_c:-1.0,
    C00139_c:-2.0,
    co2_c:1.0,
    h_c:1.0,
    ppcoa_c: 1.0,
    red_ferr_c: 2.0
})


################################################################


## rxn16332
# Succinate + mql7 <=> Fumarate + Menaquinol
reaction = Reaction('rxn16332')
reaction.name='succinate:menaquinone oxidoreductase'
reaction.subsystem=''
reaction.lower_bound=-1000
reaction.upper_bound=1000


succ_c = Metabolite(
    'cpd00036_c',
    formula='C4H4O4',
    name='Succinate',
    compartment='c')


mql7_c = Metabolite(
    'cpd11451_c',
    name='Menaquinone',
    formula='C16H16O2(C5H8)',
    compartment='c')


fum_c = Metabolite(
    'cpd00106',
    formula='C4H2O4',
    name='Fumarate',
```

```
        compartment='c')


    mqol_c = Metabolite(
        'cpd00011_c',
        formula='C16H18O2(C5H8)',
        name='Menaquinol',
        compartment='c')

    # Adding metabolites into a reaction
    reaction.add_metabolites({
        succ_c:-1.0,
        mql7_c:-1.0,
        fum_c:1.0,
        mqol_c:1.0,
    })

    model.add_reactions([reaction])
    print(f'{len(model.reactions)} reactions')
    print(f'{len(model.metabolites)} metabolites')
    print(f'{len(model.genes)} genes')

    """# Exporting Models"""

    model.reactions.rxn16332

    cobra.io.save_matlab_model(model,'ecoli-DH5alpha-adapt-CC.mat')

    cobra.io.write_sbml_model(model,          'ecoli-DH5alpha-adapt-WL-
GPP.out.xml')
```

Original file is located at
https://colab.research.google.com/drive/1dFzKUpITTLGlU_6panRIjTni_I3G-7PK