In simulating microbe-mediated reactions, you need additional two more database files other than the original database file. One is the aqueous.dbs, which specifies the reaction stoichiometry and kinetics. The other is the CatabolicPathways.dbs, which

Step 1: entering blocks in the database

Aerobic oxidation and nitrate reducing reaction are aqueous kinetic reactions, named as *Aerobic_oxidation* and *Nitrate_reduction_inhib_hw5* in the model through the input and database files, respectively. To fully represent the anabolic (biomass growth) and catabolic (energy-yielding) pathways in microbe-mediated reactions, *Aerobic_oxidation_half* (catabolic pathway) and *C5H7O2N_Ace_NH4_O2* (anabolic pathway) are named for *Aerobic_oxidation*. Similarly, for *Nitrate_reduction_inhib_hw5*, *Nitrate_reduction_inhib_half* and *C5H7O2N_Ace_NH4_NO3* represent the anabolic and catabolic pathways, respectively. As such, you need to make sure the specialized database for kinetic aqueous reactions "aqueous.dbs" has the details of the stoichiometry, thermodynamics, and kinetic parameters of the microbe-mediated reactions, including both catabolic and anabolic pathways:

********* ***Define stoichiometry and thermodynamic parameters of aqueous kinetic reactions*** *********

```
&Aqueous
 name        =  Aerobic_oxidation_half  (Define the catabolic pathway for aerobic reaction, i.e., Ra-Rd)
 type        =   catabolic
 stoichiometry = -0.250 'O2(aq)'  -0.125 Acetate  0.250 HCO3-    0.125 H+
 keq         = 500.00
/

&Aqueous
 name         = C5H7O2N_Ace_NH4_O2 (Define the anabolic pathway for aerobic reaction, i.e., Rc-Rd)
 type        = anabolic
 stoichiometry = -0.050 NH4+  -0.125 Acetate   -0.075 H+   0.050 'C5H7O2NO2(aq)'   0.150 H2O
 keq         = 0.00
/


&Aqueous
 name        =  Nitrate_reduction_inhib_hw5 (Define the catabolic pathway for nitrate reducing reaction,
                                              i.e., Ra-Rd)
 type        =  catabolic
 stoichiometry = -0.125 Acetate  -0.200 NO3-  -0.075 H+   0.100 H2O  0.250 HCO3-  0.100 'N2(aq)'
 keq         = 500.0
/


&Aqueous
 name         = C5H7O2N_Ace_NH4_NO3 (Define the anabolic pathway for nitrate reducing reaction,
                                              i.e., Rc-Rd)
 type        = anabolic
 stoichiometry = -0.050 NH4+  -0.125 Acetate   -0.075 H+   0.050 'C5H7O2NNO3(aq)'   0.150 H2O
 keq         = 0.00
/
```

********** ***Define kinetic parameters of aqueous kinetic reactions*** **********

```
&AqueousKinetics
  name        = Aerobic_oxidation    (Define the overall aerobic reaction)
  label       = default
  type        = MonodBiomass
  rate25C     = 50000.0        (The maximum specific growth rate μmax for aerobic reaction,
                                mol/mol-biomass/yr)
  monod_terms  = 'tot_Acetate' 1.00E-3  'O2(aq)' 1.04E-4    (The half-saturation coefficient of the
                                                    electron donor and acceptors, Km,acceptor and Km,donor)
  biomass     = 'C5H7O2NO2(s)'              (The immobile biomass, aerobic bacteria)
  bq          = -0.0
  chi         = 1
  direction   =-1
/

&AqueousKinetics
  name       = Nitrate_reduction_inhib_hw5  (Define the overall nitrate reducing reaction)
  label      = default
  type       = MonodBiomass
  rate25C    = 20000                (The maximum specific growth rate μmax for nitrate reducing reaction,
                                     mol/mol-biomass/yr)
  monod_terms  = 'tot_Acetate' 1.0E-03 'tot_NO3-' 1.0E-03 (The half-saturation coefficient of the
                                                    electron donor and acceptors, Km,acceptor and Km,donor)
  inhibition = 'O2(aq)' 1.00E-06    (The inhibition coefficient for the inhibiting chemical, KI,O2(aq))
  biomass  = 'C5H7O2NNO3(s)'    (The immobile biomass, nitrate reducing bacteria)
  chi      = 1
  bq       = -0.0
  direction = -1
/
```

Step 2: setting up in the input file.
Then, you would work on the input file to make sure CrunchTope would pick up the reactions (catabolic and anabolic pathways) you need from the database:

```
AQUEOUS_KINETICS     (Tell the code Aerobic_oxidation and Nitrate_reduction_inhib_hw5 are
                      kinetic aqueous reactions so that it will use the specialized database
                      "aqueous,dbs". Otherwise it would only use the general database
                      "OldRifleDatabaseHang.dbs";
                      Besides, you also need to tell the code how to calculate the overall
                      stoichiometry of microbe-mediated reactions through weighting the portion
                      of electron equivalent for corresponding catabolic and anabolic pathways)
Aerobic_oxidation    -pathway  Aerobic_oxidation_half   0.40  -pathway C5H7O2N_Ace_NH4_O2  0.60
-rate 50000.00
                    (–pathway is followed by the name of the &Aqueous namelist containing
                    the stoichiometry, and the portion of electron equivalent assign to that pathway.
                    Here, for aerobic oxidation Aerobic_oxidation,
                    0.40 electron equivalent is assigned to the catabolic pathway Aerobic_oxidation_half
                    while the left 0.60 is assigned to the anabolic pathway C5H7O2N_Ace_NH4_O2.
                    In other words, 40% of energy (from the redox reactions) is used for
                    energy production (fe) to maintain microbial life and the left 60% is used
```

for cell synthesis (f_s). 50000 is the maximum specific growth rate μ_{max} for the overall aerobic oxidation, with a unit of mol/mol-biomass/year. This value will overwrite the rate value provided in the database "aqueous.dbs".)

Nitrate_reduction_inhib_hw5 –pathway Nitrate_reduction_inhib_half 0.45 -pathway C5H7O2N_Ace_NH4_NO3 0.55 -rate 20000.00

(Here, for nitrate reducing reaction *Nitrate_reduction_inhib_hw5*, 0.45 electron equivalent is assigned to *Nitrate_reduction_inhib_half* while the left 0.55 is assigned to *C5H7O2N_Ace_NH4_NO3*; In other words, 45% of energy (from the redox reactions) is used for energy production (f_e) to maintain microbial life and the left 55% is used for cell synthesis (f_s). 20000 is the maximum specific growth rate μ_{max} for the overall nitrate reducing reaction, with a unit of mol/mol-biomass/year. This value will overwrite the rate value provided in the database "aqueous.dbs".)

END

MINERALS (Related database is the general database "OldRifleDatabaseHang.dbs")

C5H7O2NO2(s) -label default -rate -2.0 (Define the reaction rate between the immobile and mobile biomass *$C5H7O2N_{AOB(s)}$ and $C5H7O2N_{AOB(aq)}$*. In the current setup, the produced *$C5H7O2N_{AOB(aq)}$* transforms into *$C5H7O2N_{AOB(s)}$* very quickly.)

C5H7O2NNO3(s) -label default -rate -2.0 (Define the reaction rate between the immobile and mobile biomass *$C5H7O2N_{NRB(s)}$ and $C5H7O2N_{NRB(aq)}$*. In the current setup, the produced *$C5H7O2N_{NRB(aq)}$* transforms into *$C5H7O2N_{NRB(s)}$* very quickly.)

END

Note that the species involved in the kinetic aqueous reaction *Aerobic_oxidation* and *Nitrate_reduction_inhib_hw5* needs to be specified in the keyword block "*PRIMARY_SPECIES*", and "Condition" in the input file, and also needed to be listed at primary species in the general database "OldRifleDatabaseHang.dbs":

For example, in the database "OldRifleDatabaseHang.dbs":

*****Listed as primary species***********

'C5H7O2NO2(aq)' 1.0 0.0 113.12 (Format: <'SpeciesName'> <Debye-Huckel size parameter> <Charge> <Molecular weight>)

'C5H7O2NNO3(aq)' 1.0 0.0 113.12 (Format: <'SpeciesName'> <Debye-Huckel size parameter> <Charge> <Molecular weight>)

'N2(aq)' 3.0 0.0 28.0134 (Format: <'SpeciesName'> <Debye-Huckel size parameter> <Charge> <Molecular weight>)

'NO3-' 3.0 -1.0 62.0049 (Format: <'SpeciesName'> <Debye-Huckel size parameter> <Charge> <Molecular weight>)

********** Define thermodynamic parameters of reactions************

'C5H7O2NO2(s)' 565.0 1 1.000 'C5H7O2NO2(aq)' 500.0 -25.0 500.00 500.00 500.00 500.00 500.00 500.00 113.0 (Define the transformation between the immobile and mobile aerobatic biomass.
Format: <'Immobile Biomass Name' > <Molar Volume>
<Number of species in reaction> <Stoichiometric coefficient>
<'SpeciesName'>.<Log K array> <Molecular Weight>.
Here the <Log K array> is referred to equilibrium constant at
eight temperature points, 0, 25, 60, 100, 150, 200, 250 and 300 ^{0}C.
In the current example, the temperature is 25 ^{0}C. Therefore LogK = -25.0)

'C5H7O2NNO3(s)' 565.0 1 1.000 'C5H7O2NNO3(aq)' 500.0 -25.0 500.00 500.00 500.00 500.00 500.00 500.00 113.0 (Define the transformation between the immobile and mobile nitrate
reducing biomass.
Format: <'Immobile Biomass Name' > <Molar Volume>
<Number of species in reaction> <Stoichiometric coefficient>
<'SpeciesName'> <Log K array> <Molecular Weight>.
Here the <Log K array> is referred to equilibrium constant at
eight temperature points, 0, 25, 60, 100, 150, 200, 250, and 300 ^{0}C.
In the current example, the temperature is 25 ^{0}C. Therefore LogK = -25.0)

********** Define kinetic parameters of solid-water reactions**********

C5H7O2NO2(s) (Transformation reaction for aerobic biomass)
label = default
type = tst (Reaction type)
rate(25C) = -2.00 !cis (Kinetic rate constant, which will be overwritten by the value in the input file)
activation = 15.0 (kcal/mole) (Activation energy)
+----------
C5H7O2NNO3(s) (Transformation reaction for nitrate reducing biomass)
label = default
type = tst
rate(25C) = -2.00
activation = 15.0 (kcal/mole)
+----------