STOICHIOMETRY II: NITROGEN IN ENERGY METABOLISM AND CELL SYNTHESIS

AMMONIA OXIDATION IN WASTEWATER TREATMENT

All terms in stoichiometric relations expressed in units of nitrogen (N, MW = 14 g/mole)

Oxidation of ammonium-nitrogen (NH_4-N)

Bacteria carrying out ammonia oxidation to nitrate are autotrophs (chemolithotrophs) which use reduced nitrogen species for energy (electron donor): ammonia oxidizing bacteria (AOB) use ammonia, NH_3/NH_4^+ (oxidation state -3) and nitrite oxidizing bacteria (NOB) use nitrite, NO_2^- (oxidation state +3) as electron donors. Product of AOB metabolism is nitrite; product of NOB metabolism is nitrate (NO_3^-). Generally, but not always, oxygen is the electron acceptor. Chemolithotrophs fix CO_2 for cell compound synthesis.

Recall half-reaction method (units are moles):

For oxidation of ammonia to nitrite, oxidation of electron donor half-reaction:

1. Show reduced reactant and oxidized product and balance for reactant (e-donor)

$$NH_4^+ \rightarrow NO_2^-$$

2. Balance oxygen with water

$$NH_4^+ + 2\ H_2O \rightarrow NO_2^-$$

3. Balance hydrogen with protons

$$NH_4^+ + 2\ H_2O \rightarrow NO_2^- + 8H^+$$

4. Balance charge with electrons

$$NH_4^+ + 2\ H_2O \rightarrow NO_2^- + 8H^+ + 6e^-$$

For complete oxidation-reduction reaction, couple with reduction of electron acceptor (oxygen)

1. Same as oxidation step – first show oxidized reactant and reduced product reaction and balance for reactant (e-acceptor)

$$O_2 \rightarrow 2\ H_2O$$

2. Step 2 not necessary since reactant is oxygen

3. Balance hydrogen with protons

$$O_2 + 4\ H^+ \rightarrow H_2O$$

4. Balance charge with electrons

$$O_2 + 4\ H^+ + 4e^- \rightarrow H_2O$$

Combine half-reactions to eliminate electrons, since there are not free electrons in aqueous system (see next stoichiometry)

<u>AOB</u> (common genera: *Nitrosomonas, Nitrosococcus, Nitrosolobus*)

Molar stoichiometry for oxidation of ammonia to nitrite (no synthesis uptake):

$$\mathbf{NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O} \qquad \textbf{(1)}$$

(Microbiologists think that unionized ammonia, NH_3, is the actual substrate)

<u>NOB</u> (common genera: *Nitrobacter, Nitrospira, Nitrospina*)

Molar stoichiometry for oxidation (no synthesis uptake)

$$\mathbf{NO_2^- + 0.5O_2 \rightarrow NO_3^-} \qquad \textbf{(2)}$$

Usually AOB and NOB grow in proximity since nitrite intermediate is consumed by NOB. Nitrite is fairly unstable and accumulation in treatment processes (or natural environments) is evidence of inhibition or process upset caused by oxygen deficiency, toxicity or other conditions. High concentrations of both ammonia and nitrite have been reported to inhibit AOB and NOB.

Overall molar stoichiometry for complete nitrification:

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad (3 = (1) + (2))$$

Mass stoichiometry (NH_4^+ is reference compound)

$$1.00NH_4^+ + 3.555O_2 \rightarrow 3.444NO_3^- + 0.111H^+ + 1.00H_2O \qquad (4)$$

$\psi_1 = 1.00$ g
$\psi_2 = a_2MW_2/MW_1 = 2(32)/18 = 3.555$ g
$\psi_3 = a_3MW_3/MW_1 = 1(62)18 =3.444$ g
$\psi_4 = a_4MW_4/MW_1 = 2(1)/18 = 0.111$ g
$\psi_2 = a_5MW_5/MW_1 = 1(18)/18 = 1.00$ g

Check: 1.00+3.555 = 3.444 + 0.111 + 1.00
4.555 = 4.555 ✓

COD equivalent stoichiometry, NH_4 as reference:

COD equivalents of NH_4 = 3.555 g-COD/g-NH_4
COD equivalents of O_2 = -1g-COD/g-O_2
COD equivalents of NO_3^-, H^+, and H_20 = 0

$$1\ NH_4\text{-COD} – 1\ O_2\text{-COD} \rightarrow 0NO_3^- + 0H^+ + 0H_2O \qquad (5)$$

$Y_1 = \psi_1 (COD_1/COD_1) = 1.00 (3.55/3.55) = 1$
$Y_2 = \psi_2(COD_2/COD_1) = 3.555(-1.00)/3.555 = -1$
$Y_3 = \psi_3 (COD_3/COD_1) = 3.444(0)/3.555 = 0$
$Y_4 = \psi_4 (COD_4/COD_1) = 0.111(0)/3.55 = 0$
$Y_5 = \psi_5 (COD_5/COD_1) = 1.00(0)/3.55 = 0$

Check: 1 – 1 = 0 ✓

COD of ammonium expressed as nitrogen from equation 4.

$COD_{NH4\text{-}\underline{N}}$ = 3.555 (g-COD/g-NH_4)(18g-NH_4/14g NH_4-N) = **4.57 g-COD/g-NH_4-N**

CELL SYNTHESIS WITH SIMPLE CARBOHYDRATE AS SOURCE

Molar stoichiometry, reference compound is product cells ($C_5H_7NO_2$)

$$5CH_2O + HCO_3^- + NH_4^+ \rightarrow C_5H_7NO_2 + 4H_2O + CO_2 \qquad (6)$$

Mass stoichiometry with cells as reference (MW = 113)

$$1.327CH_2O + 0.540HCO_3^- + 0.159NH_4^+ \rightarrow 1C_5H_7NO_2 + 0.637H_2O + 0.389CO_2 \qquad (7)$$

$\psi_1 = 1$ (cells) then numbering from left to right:
$\psi_2 = a_2MW_2/MW_1 = 5(30)/113 = 1.327$ g
$\psi_3 = a_3MW_3/MW_1 = 1(61)113 = 0.540$ g
$\psi_4 = a_4MW_4/MW_1 = 1(18)/113 = 0.159$ g
$\psi_5 = a_5MW_5/MW_1 = 4(18)/113 = 0.637$ g
$\psi_6 = a_6MW_6/MW_1 = 1(44)/113 = 0.389$ g

Check: 1.327 + 0.540 + 0.159 = 2.026 = 1 + 0.637 + 0.389 ✓

COD stoichiometry with cells as reference

$$1CH_2O\text{-}COD + 0HCO_3^- + 0NH_4^+ \rightarrow 1C_5H_7NO_2\text{-}COD + 0H_2O + 0CO_2 \qquad (8)$$

COD equivalents of CH_2O= 1.07 g-COD/g-CH_2O
COD equivalents of $C_5H_7NO_2$ = 1.42 g-COD/g-$C_5H_7NO_2$
COD equivalents of HCO_3^-, CO_2, and H_2O = 0
COD equivalents of NH_4 = 0 g-COD/g-NH_4 <u>in this reaction</u> (no e^- transfer since oxidation level of cell nitrogen = -3, same as ammonia)

$Y_1 = \psi_1 (COD_1/COD_1) = 1.00 (1.42/1.42) = 1$
$Y_2 = \psi_2(COD_2/COD_1) = 1.327(1.07)/1.42 = 1$
$Y_3 = \psi_3 (COD_3/COD_1) = 0.540(0)/1.42 = 0$
$Y_4 = \psi_4 (COD_4/COD_1) = 0.159(0)/1.42 = 0$
$Y_5 = \psi_5 (COD_5/COD_1) = 0.637(0)/1.42 = 0$
$Y_6 = \psi_6 (COD_6/COD_1) = 0.389(0)/1.42 = 0$

NITROGEN UPTAKE IN CELL SYNTHESIS

KEY POINT: As with COD, a portion of wastewater ammonia consumed in a biological treatment process is used for energy generation (nitrification) and a portion is used for cell synthesis. The mass stoichiometry for cell synthesis above gives the nitrogen requirement for cell growth, expressed as the ratio of g-N/g-cells = i_{NHX}, where i is a like a yield factor, like Y for COD. From (7)

i_{NHX} = 0.159 (g NH_4^+/g-cells)(14 g-NH_4-N/18g- NH_4^+) = 0.124 g-NH_4-N/g-cells

the ratio i for nitrogen used in cell synthesis can be expressed in units of cell COD (COD_X) also:

i_{NHX} = 0.124 g-NH_4-N/g-cells)/1.42 g-COD/g-cells = 0.087 g NH_4-N/g-COD_X

Nitrogen requirements for cell synthesis are assumed to be similar for heterotrophic and autotrophic bacteria. However, the cell yield for autotrophic bacteria (g-X_{BA}/g-NH_4-N) is much lower than the substrate yield for heterotrophic bacteria (g-X_{BH}/g-COD_S), so the nitrogen requirement for autotrophic growth is often neglected in process modeling.