Elemental balance based methodology to establish reaction stoichiometry in environmental modeling

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Abstract Kinetic models in activated sludge, anaerobic digestion and other environmental modeling fields rely on the proper formulation of stoichiometric coefficients. Elemental balancing provides a simple and rigorous way to establish the stoichiometric coefficients of reactions represented in the Gujer matrix. The deduction of these coefficients is frequently trivial, from basic mass balancing considerations. In more complex cases, such as the Anammox growth reaction, rigorous elemental balancing is required to establish the proper formulation. This paper demonstrates the methodology based on a simple aerobic heterotrophic growth reaction where stoichiometry coefficients (such as the $(1-Y_H)/Y_H$ term for oxygen) are well known. In the second step the methodology is applied for the Anammox growth reaction. The fraction of N_2 gas in current models originates from the NH_4^+ and the NO_2^- electron donor/acceptor pair in equal proportion. This paper demonstrates that this stoichiometry is a simplification leading to elemental balance errors. The proper stoichiometric coefficients are derived.
Keywords Anammox; elemental balancing; Gujer matrix; stoichiometry

Introduction

Strous *et al*. (1998, 1999) described a newly discovered lithotroph, anaerobically converting ammonia and nitrite to nitrogen gas according to the following stoichiometry:

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+$$

$$= 0.26NO_3^- + 1.02N_2 + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O$$
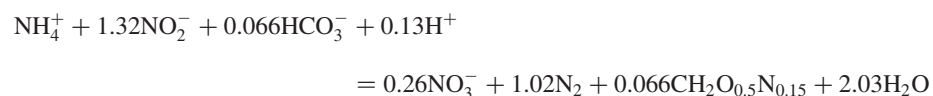
This new microorganism, a planctomycete, is an anaerobic ammonia oxidizer and was named Anammox.

A kinetic model for the growth of the Anammox biomass was proposed by Koch *et al* (2002). Only the stoichiometry is investigated here. The basis of growth rate is 1 g Anammox biomass COD. The stoichiometric coefficients in the Koch model are shown in row 1 of Table 1.

A full elemental balance cannot be prepared on this model since carbon (bicarbonate) is not included. Also, N for synthesis is not included. The authors recognize this but claim that this causes only a small error due to low biomass production. The remaining N balance is trivial and agrees only approximately with Strous *et al* (1999). Concerning N_2 production, half of the N comes from NH_3 and half from NO_2^-. The nitrate production stoichiometry is significantly different from Strous *et al* (1999): 0.88 gNO$_3$-N is produced

Table 1 Proposed stoichiometric coefficients for the Anammox growth reaction

Components →	Ammonia S_{NH}	Nitrite S_{NO2}	Nitrate S_{NO3}	Nitrogen gas S_{N2}
Koch *et al.* (2002)	$-1/Y_{NH3}$	$-1/Y_{NH3}$ - 1/1.14	1/1.14	$2/Y_{NH3}$
Hao *et al.* (2002)	$-1/Y_{NH3}$ -i_{NBN}	$-1/Y_{NH3}$ - 1/1.14	1/1.14	$2/Y_{NH3}$
Dapena-Mora *et al.* (2004)	$-1/Y_{NH3}$ -i_{NBN}	$-1/Y_{NH3}$ - 1/1.52	1.52	$2/Y_{NH3}$

Y_{NH3} = 0.114, i_{NBN} = 0.087

per g biomass COD instead of 1.52 gNO$_3$-N. Since one mole of this biomass representation contains 36.4 g COD, 0.26 mole of nitrate production per 0.066 mole of biomass converts to 1.52 g NO3-N produced per g biomass COD (0.26 x 14.0/(0.066 x 36.4)) according to Equation 1.

Hao *et al*. (2002) included N for synthesis (adding a -i_{NBN} term to the S_{NH} column) and kept the same stoichiometry (Table 1).

Dapena-Mora *et al*. (2004) retained the same ammonia and nitrite stoichiometry (but introduced a typo missing the stoichiometric coefficient in the Anammox state variable column). The authors based on the original Strous *et al* (1998) publication revised the 1/1.14 nitrate production stoichiometry to the correct 1.52 (Table 1).

An assumption in these modeling approaches is that half the nitrogen in the nitrogen gas produced is originating from ammonia, and the other half from nitrate. Elemental balancing can provide further insight into this assumption and help identify the proper stoichiometric coefficients.

Method description (example: ASM1 aerobic heterotrophic growth)

The well known heterotrophic growth reaction under aerobic conditions in ASM1 (Henze *et al.*, 2000) is used to demonstrate the principle of the proposed elemental balancing method. The proposed methodology relies on the following steps:

(1) Establish the elemental composition of each state variable. In this section, the composition as developed in Takács (2006) for the extended ASM1 is used. Biomass composition is $C_5H_7O_2N$ and substrate composition is $C_{2.43}H_{3.96}O$.

(2) Establish the COD content of the state variables (using the chemical reaction for complete oxidation). In this case, 1 mole of biomass is equivalent to 5 moles of O_2, and 1 mole of substrate is 2.92 moles of O_2.

$$C_5H_7O_2N + 5O_2 = 5CO_2 + NH_3 + 2H_2O$$

$$C_{2.43}H_{3.96}O + 2.92O_2 = 2.43CO_2 + 1.98H_2O$$
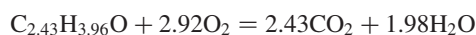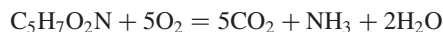
(3) Set up an elementally balanced reaction with unknown stoichiometry to generate (an arbitrarily selected) 1 mole of biomass. This example is for aerobic heterotrophic growth, in the neutral form as described in Takács (2006), specifically for elemental balancing:

$$\gamma_{Ss}C_{2.43}H_{3.96}O + \gamma_{O2}O_2 + NH_3 = \gamma_{CO2}CO_2 + C_5H_7O_2N + \gamma_{H2O}H_2O$$
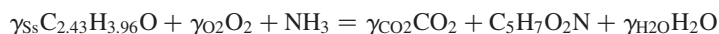
Note that synthesis ammonia is explicitly identified, resulting in a trivial N balance. The reaction can be expressed in the usual charged form or using uncharged species. The uncharged form yields simpler symbolic manipulation and the resulting stoichiometric coefficients will be the same. Charge balance is maintained using exclusively neutral, undissociated or unionized components.
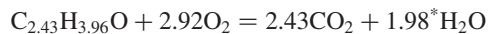
(4) There are four unknowns (γ_{Ss}, γ_{O2}, γ_{CO2}, γ_{H2O}) in the equation and three elemental balances can be set up. The degree of freedom is one – the measured yield. Set up three elemental balances and the expression for yield.

- Carbon balance: $\gamma_{Ss} * 2.43 = \gamma_{CO2} + 5$;
- Oxygen balance $\gamma_{Ss} + \gamma_{O2} * 2 = \gamma_{CO2} * 2 + 2 + \gamma_{H2O}$;
- Hydrogen balance $\gamma_{Ss} * 3.96 + 3 = 7 + \gamma_{H2O} * 2$;
- Yield expression $Y = (5 * O_2)/(\gamma_{Ss} * 2.92 * O_2)$; (necessarily incorporating biomass and substrate COD equivalents, $O_2 = 32$ g/mole).

(5) Solve the linear equation system (manually or preferably using a symbolic solver e.g. Maple). In ASM1 we are only interested in substrate, oxygen and biomass coefficients (though the method provides coefficients for CO_2 and H_2O as well), thus: For substrate, $\gamma_{Ss} = 1.712328767/Y_H$, for oxygen, $\gamma_{O2} = 5 * (1-Y_H)/Y_H$, for biomass, the coefficient is 1.

(6) Do a mass balance check on the reaction to verify results.

(7) The coefficients in 5) are per mole of biomass. A conversion to grams of biomass COD (which is the basis of the ASM1 process rate) is necessary, using values from 2). This results in:
- Substrate coefficient: $1/Y_H$
- O_2 coefficient: $(1-Y_H)/Y_H$
- Biomass coefficient: 1
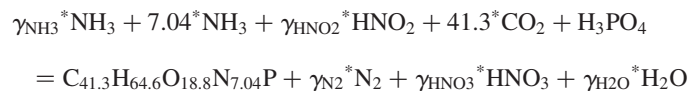- Synthesis N coefficient: $1 * N/(5 * O_2) = 0.087$ ($N = 14$ g/mole, $O_2 = 32$ g/mole)

Method application (Anammox example)

The elemental balance methodology described in the first section was applied to the Anammox growth reaction.

(1) The biomass composition was selected as: $C_{41.3}H_{64.6}O_{18.8}N_{7.04}P$. This elemental composition includes P and results in the following, widely accepted stoichiometric values for biomass: VSS/COD = 1.42, N/COD = 0.07, P/COD = 0.022, C/VSS = 0.50 (Van Denmark and Batzing, 1987). The biomass used in Koch *et al.* (2002) had a different composition, $CH_2O_{0.5}N_{0.15}$. This biomass results in the following, slightly different ratios: VSS/COD = 1.51, N/COD = 0.09, C/VSS = 0.50, and does not contain phosphorus.

(2) 1 mole of biomass is equivalent to 44.02 moles of COD, and 1 mole of substrate is 2.92 moles of COD.

$$C_{41.3}H_{64.6}O_{18.8}N_{7.04}P + 44.02O_2 = 41.3CO_2 + 18.98H_2O + 7.04NH_3 + H_3PO_4$$

$$C_{2.43}H_{3.96}O + 2.92O_2 = 2.43CO_2 + 1.98^*H_2O$$

(3) The Anammox reaction is set up as:

$$\gamma_{NH3}{}^*NH_3 + 7.04^*NH_3 + \gamma_{HNO2}{}^*HNO_2 + 41.3^*CO_2 + H_3PO_4$$

$$= C_{41.3}H_{64.6}O_{18.8}N_{7.04}P + \gamma_{N2}{}^*N_2 + \gamma_{HNO3}{}^*HNO_3 + \gamma_{H2O}{}^*H_2O$$

(4) There are five unknowns (since C and P balances are trivial) and three elemental balances for H, O and N. The degree of freedom is 2, selected as:
- the amount of biomass COD grown on one mg electron-donor ammonia N (Y_{NH3}), according to Koch (2002), and
- the amount of nitrate N produced on one mg electron-donor ammonia N used for growth (Y_{NO3}), which describes the 1/1.14 or 1.52 constants in previous works.

(5) The resulting molar coefficients ($44.02 * O_2$ is the COD of one mole of biomass)

$$\gamma_{NH3} = (44.02^*O_2)/(Y_{NH3}{}^*N)$$

$$\gamma_{HNO2} = (44.02^*O_2)/(Y_{NH3}{}^*N) + (73.367^*Y_{NO3}{}^*O_2)/(Y_{NH3}{}^*N) - 58.693$$

$$\gamma_{N2} = (44.02^*O_2)/(Y_{NH3}{}^*N) + (14.673^*Y_{NO3}{}^*O_2)/(Y_{NH3}{}^*N) - 29.3467$$

$$\gamma_{HNO3} = Y_{NO3}{}^*(44.02^*O_2)/(Y_{NH3}{}^*N)$$

$$\gamma_{H2O} = 2^*(44.02^*O_2)/(Y_{NH3}{}^*N) + (14.673^*Y_{NO3}{}^*O_2)/(Y_{NH3}{}^*N) - 49.5867$$

Considering $N = 14.0067$ and $O_2 = 35.9988$, the reaction becomes:

$$889.2NH_3 + 1205.7HNO_2 + 41.3CO_2 + H_3PO_4$$

$$= C_{41.3}H_{64.6}O_{18.8}N_{7.04}P + 929.3N_2 + 229.4HNO_3 + 1791.2H_2O$$
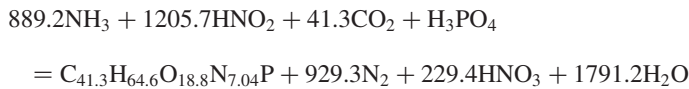
Converting this back to one mole of ammonia used:

$$NH_3 + 1.37HNO_2 + 0.047CO_2 = 0.0011C_{41.3}H_{64.6}O_{18.8}N_{7.04}P + 1.05N_2$$

$$+ 0.26HNO_3 + 2.03H_2O$$
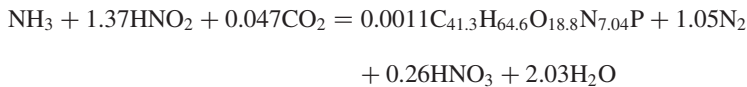
This reaction is rather similar to the original Strous equation. The main differences are due to the "higher molecular weight" of the biomass and the inclusion of P (as well as the slightly different elemental composition of the biomass).

(6) These coefficients must be expressed for 1 g COD biomass to be included in the Anammox growth reaction. The resulting row in the Gujer matrix is presented in Table 2.

Some of the stoichiometric coefficients are different from the literature:

S_{NH}: same as in Hao et al. (2002)
S_{NO2}: new expression (the value of the $4/3 * N/O2$ term is 0.584)
S_{NO3}: same as Dapena-Mora et al. (2004) if $Y_{NO3} = 1.52$
S_{N2}: new expression

These stoichiometric coefficients can be developed, using the same methodology, for a biomass of arbitrary composition, i.e. $C_{\alpha,C}H_{\alpha,H}O_{\alpha,O}N_{\alpha,N}P$. (Using elemental balancing, the COD of one mole of this biomass is $\alpha,C + 1/4 * \alpha,H -1/2 * \alpha,O -3/4 * \alpha,N + 5/4$ moles of O_2). All elemental molar factions (α,C, etc.) cancel out during the development, resulting in the same formulation as in Table 2., proving that all of these stoichiometric coefficients are independent of biomass composition.

Based on calculations using this stoichiometry and $Y_{NH3} = 0.114$ and $Y_{NO3} = 1.52$, 47.5% of the nitrogen in the N_2 gas originates from ammonia, the rest from nitrite.

Table 2 Proposed stoichiometric coefficients for the Anammox growth reaction

	S_{NH}	S_{NO2}	S_{NO3}	S_{N2}
Anammox growth	$-1/Y_{NH3}-i_{NBN}$	$-1/Y_{NH3} - 5/3^*Y_{NO3} +4/3^*N/O_2$	Y_{NO3}	$2/Y_{NH3} + 2/3^*Y_{NO3}-4/3^*N/O_2$

Conclusions

A method based on elemental balances simplifies deriving stoichiometric coefficients and serves as a rigorous check on reaction continuity. The method's steps are easy to follow, and the result it produces is demonstrated to agree with stoichiometry of well known reactions such as the aerobic heterotrophic growth on readily degradable substrate. The method is applied to develop stoichiometric coefficients for the Anammox growth reaction. It is shown that the currently published models use simplifying assumptions for these stoichiometric coefficients.

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