# A Thermodynamic Analysis Of Intermediary Metabolic Steps And Nitrous Oxide Production In Ammonium-Oxidizing Bacteria


**Michelle N. Young**[*,1], Joshua P. Boltz[*], Andrew Marcus[*], Jose A. Jimenez[**], Ahmed Al-Omari[***], Imre Takács[****], and Bruce E. Rittmann[*]

[*]Biodesign Swette Center for Environmental Biotechnology, Arizona State University, Tempe, AZ 85287-5701
[**]Brown and Caldwell, 351 Lucien Way, Ste. 250, Maitland, FL, 32751
[***]Brown and Caldwell, 1725 Duke St. Suite 250 Alexandria, VA 22314
[****]Dynamita, 2015 route d'Aiglun, 06910 Sigale France
[1]Email: Michelle.N.Young@asu.edu


## *WE USE THE LATEST KNOWLEDGE OF AMMONIUM OXIDIZING BACTERIA (AOB) METABOLISM TO ESTIMATE AOB ENERGETICS AND KINETICS*

The latest understanding of the metabolic pathways for AOB and thermodynamics electron equivalents modelling (TEEM) were integrated to estimate kinetic and stoichiometric parameters for the AOB's multi-step nitrification and autotrophic denitrification reactions. An energetics analysis for each reaction in the oxidation and reduction pathways resulted in an understanding of which intermediate pathways are important for respiration and biomass synthesis, how $N_2O$ is linked to biomass growth and detoxification, and individual pathway stoichiometry and kinetics determined by TEEM can be normalized to the number of electrons used in the respiration chain to determine whole cell kinetics. This normalized TEEM approach can be applied to a variety of bacteria besides AOB to provide kinetic insights into performance.

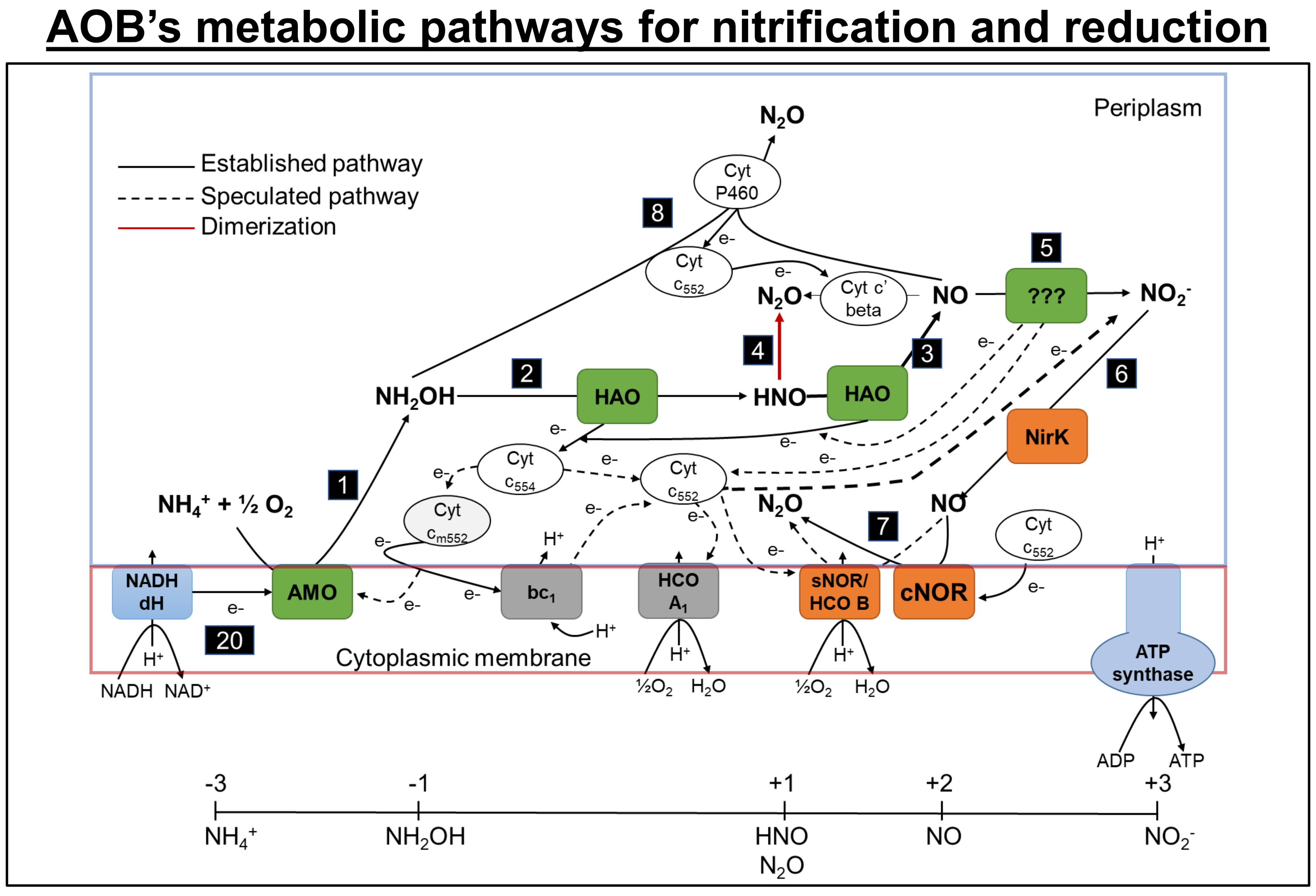
## Background

Nitrous oxide ($N_2O$) is 300 times more potent than $CO_2$ as a greenhouse gas[1] and contributes 6% of annual global greenhouse gas emissions[2]. > 85% of $N_2O$ emissions are from biotic processes, like agricultural soil management, wastewater treatment, and manure management[3]. Ammonium-oxidizing bacteria (AOB) are the primary bacteria producing $N_2O$ and important for biological nutrient removal during wastewater treatment. AOB are generally perceived as obligate aerobes that oxidize ammonium ($NH_4^+$) to nitrate ($NO_2^-$). Recent advances in the understanding AOB metabolism have elucidated key mechanisms AOB nitrification, $N_2O$ production, and $NO_2^-$ reduction[4-6]. **No model has described AOB metabolism based on all the intermediate steps and their thermodynamic viability.**

## AOB Metabolism is Complex

**$N_2O$ formation:**
- Dimerization during nitrification
- Detoxification when $NH_2OH$ reacts with NO through cyt P460
- NO reduction using NOR enzyme for respiration/biomass synthesis

### AOB's metabolic pathways for nitrification and reduction



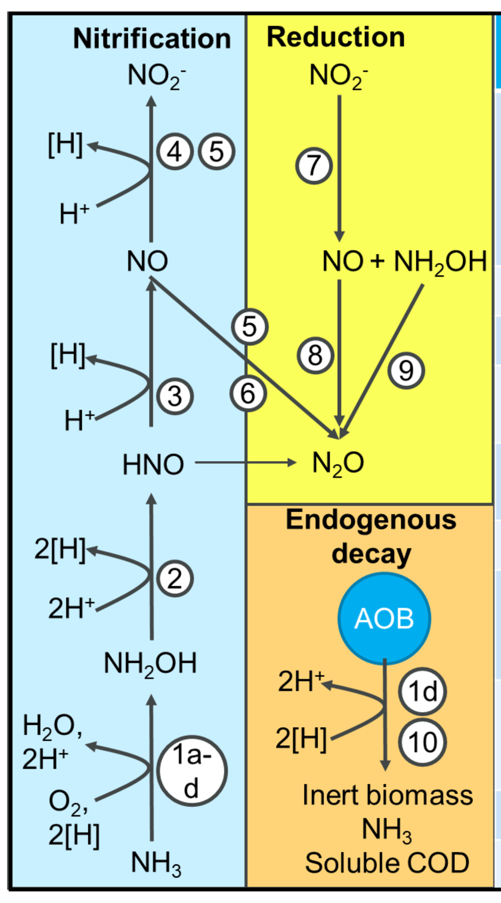

### $NH_4^+$ Monooxygenation (AMO)

$$NH_4^+ + O_2 + 2[H] \rightarrow NH_2OH + H_2O + H^+$$

**where [H] = electron equivalent**

- 4 out of 6 electrons produced during nitrification are used in this step
- 2 electrons must be provided from downstream nitrification products ($NH_2OH$, HNO, or NO)
- No energy for cell maintenance or biomass synthesis from AMO

### A model of AOB metabolism




| Pathway description | Biomass formation? |
|---|---|
| 1. Oxidation of $NH_3$ using<br>a) $NH_2OH$ as the electron source<br>b) HNO as the electron source<br>c) NO as the electron source<br>d) Electrons from biomass decay | No |
| 2. $NH_2OH$ oxidation to HNO | Yes |
| 3. HNO oxidation to NO | Yes |
| 4. NO oxidation to $NO_2^-$ under high DO conditions | Yes |
| 5. NO oxidation to $NO_2^-$ under low DO conditions | No |
| 6. Dimerization of HNO to $N_2O$ | No |
| 7. Reduction of $NO_2^-$ to NO using<br>a) $NH_2OH$ as the electron source<br>b) HNO as the electron source | No |
| 8. Reduction of NO to $N_2O$<br>a) $NH_2OH$ as the electron source<br>b) HNO as the electron source | Yes |
| 9. $N_2O$ formation from $NH_2OH$ and NO | No |
| 10. Endogenous decay | No |

Respiration/ biomass synthesis does not occur during $NH_4^+$ monooxygenation (Rxn 1) or $NO_2^-$ reduction to NO (Rxn 7)

## Hybrid Thermodynamic Electron Equivalents Modeling (TEEM)

TEEM utilizes thermodynamics of the electron donor and acceptor half reactions and carbon assimilation processes to estimate the fraction of electrons used for biomass synthesis based on Gibbs free energy

Fraction of electrons to biomass synthesis, $f_s^0$

$$f_s^0 = \frac{1}{1+A} \text{ and } A = -\frac{\Delta G_s}{\varepsilon \Delta G_r}$$

where $\Delta G_s$ = energy required to incorporate carbon into biomass
$\Delta G_r$ = overall energy reaction between the acceptor and donor

Yield $$Y = \frac{f_s^0 M_c}{n_e \gamma_{N,i}}$$

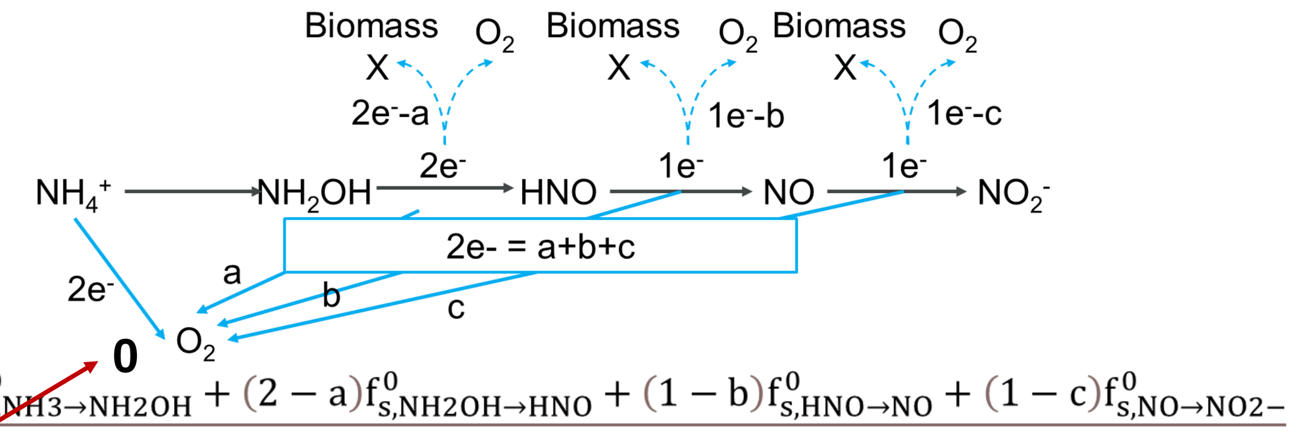
where $M_c$ = cells molecular weight = 113 g/mol,
$n_{e^-}$ = number of electron equivalents
$\gamma_{N,i}$ = mass of nitrogen in a donor reaction per electron equivalent

Maximum substrate utilization rate $$\hat{q}_i = \frac{1\ e^-\ eq\ A}{g X_a\ d}\left(\frac{e^-\ eq\ D}{(1-f_s^0)e^-\ eq\ A}\right)(\gamma_{N,i})$$

Maximum specific growth rate $$\hat{\mu}_i = \hat{q}_i Y_i$$

**Hybrid TEEM:**
1. Calculate the stoichiometry/kinetics for each individual pathway that produces biomass
2. Normalize the individual pathway value based on the number of electrons contributed to overall biomass growth

### Normalization of nitrification pathway




$$f^0_{s,tot} = \frac{\cancel{f^0_{s,NH3\to NH2OH}}\ 0 + (2-a)f^0_{s,NH2OH\to HNO} + (1-b)f^0_{s,HNO\to NO} + (1-c)f^0_{s,NO\to NO2-}}{6}$$

Normalization can also be applied to the denitrification pathway.

## Normalized Whole-Cell Kinetics

### Nitrification pathway

| Electron source for the AMO reaction: $NH_2OH$ | HNO | NO | $f^0_{s,tot}$ eeq cells/eeq donor | $Y_{tot}$ mg $X_a$ /mg N | $\hat{q}_{tot}$ mg N/ mg $X_a$/d | $\hat{\mu}_{tot}$ 1/d |
|---|---|---|---|---|---|---|
| **0** | 1 | 1 | 0.057 | 0.14 | 3.7 | 0.70 |
| **1** | 1 | 0 | 0.067 | 0.16 | 5.0 | 0.94 |
| **1** | 0 | 1 | 0.067 | 0.16 | 5.0 | 0.94 |
| **2** | 0 | 0 | 0.078 | 0.19 | 7.5 | 1.42 |
| **For comparison, literature values are:** | | | | **0.15-0.44** | **3.7-7.5** | **0.7-1.4** |

### Reduction pathway

| Electron source for the NOR reaction | $f^0_{s,tot}$ eeq cells/ eeq donor | $Y_{tot}$ mg $X_a$ /mg N | $\hat{q}_{tot}$ mg N/ mg $X_a$/d | $\hat{\mu}_{tot}$ 1/d |
|---|---|---|---|---|
| **$NH_2OH$** | 0.12 | 0.10 | 8.0 | 0.77 |
| **HNO** | 0.15 | 0.12 | 8.2 | 0.96 |
| **Mathematical modeling literature values are:** | | **0.02** | **1.3** | **0.15-0.28** |

- Using electron donors $NH_2OH$ for AMO oxidation and HNO for NO reduction is most energetically favorable
- NO reduction to $N_2O$ appears to be thermodynamically favorable, but the need for $O_2$ in $NH_4^+$ monooxygenation likely precludes NO reduction to $N_2O$ from becoming a major pathway
- $N_2O$ is energetically favorable in the reduction pathway and a detoxification step in aerobic processes


**References:** [1]IPCC (2013). *Climate Change: The Physical Science Basis*. [2]IPCC, 2014. Climate Change 2014: Synthesis Report. [3]US EPA, 2021. Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2019. [4]Sedlacek, C.J., et al., 2020. *mSystems* 5:e00562-19. [5]Mellbye, B.L., et al. 2018. *mSystems* 3:e00170-17. [6]Kozlowski, J.A., et al. 2014. *Appl. Environ. Microbiology* 80(16), 4930-4935.



**Acknowledgements:** Funding provided by Brown and Caldwell. Modeling support provided by Dynamita. All work was performed at the Biodesign Swette Center for Environmental Biotechnology at Arizona State University.