A Mathematical Model of Denitrification in *Pseudomonas aeruginosa*

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MOTIVATION

Lake Erie, one of the Great Lakes in North America, has witnessed recurrent summertime low oxygen dead zones for decades. This is a yearly phenomenon that causes microbial production of the greenhouse gas nitrous oxide from denitrification. *Pseudomonas aeruginosa* is abundant in the microbial community in Lake Erie and can perform denitrification under anaerobic conditions. **This study aims to capture the effect of phosphate on a denitrication metabolic network of *P. aeruginosa* in order to shed light on the reason of greenhouse gas nitrous oxide accumulation during oxygen depletion in Lake Erie.**

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INTRODUCTION

Pseudomonas aeruginosa is a metabolically flexible member of the Gammaproteobacteria. Under anaerobic conditions and the presence of nitrate, *P. aeruginosa* can perform (complete) denitrification, a respiratory process of dissimilatory nitrate reduction to nitrogen gas via nitrite (NO_2), nitric oxide (NO) and nitrous oxide (N_2O). This study focuses on a mathematical model of a metabolic network in *P. aeruginosa* under denitrification and testable hypotheses generation. To our knowledge, this is the ***first*** mathematical model of denitrification for this bacterium. Analysis of the long-term behavior of the system changing the concentration level of oxygen (O_2), nitrate (NO_3), and phosphate (PO_4) suggests that PO_4 highly affects the denitrification performance of the network.

MATHEMATICAL MODEL

Let $X = \{low, medium, high\}$, n be the number of variables in the system, and f_i be an update function of the i^{th} variable. Then,

$$f = (f_1, f_2, \ldots, f_n): X^n \rightarrow X^n$$

is a transition function whose iteration represents evolution of model over time.

Model simulation is ***stochastic*** at the update function level under the assumption that even if the expression levels of the variables of an update rule guarantee activation or degradation, there is a probability that the process will not occur due to stochastic effects.

An update function of each variable can be specified by transition tables.

O_2 (t)	NO_3 (t)	PO_4 (t)	DNR (t+1)
low	low	low	medium
low	low	high	low
low	high	low	high
low	high	high	medium
high	low	low	low
high	low	high	low
high	high	low	low
high	high	high	low

Table 1. Transition table of DNR

DNR (t)	NO (t)	NOS(t+1)
low	low	low
low	high	low
medium	low	low
medium	high	high
high	low	low
high	high	high

Table 2. Transition table of NOS

RESULTS

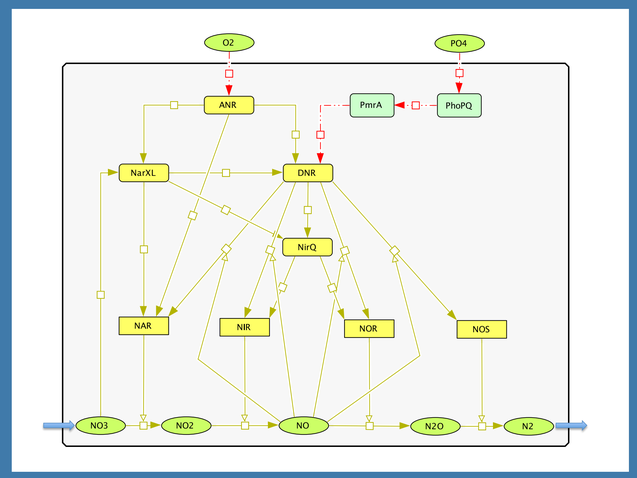

Figure 1: Denitrification metabolic network of *P. aeruginosa*. Green solid arrows indicate activation and red dashed arrows indicate inhibition. This is an open system, and the entire process occurs intracellularly.

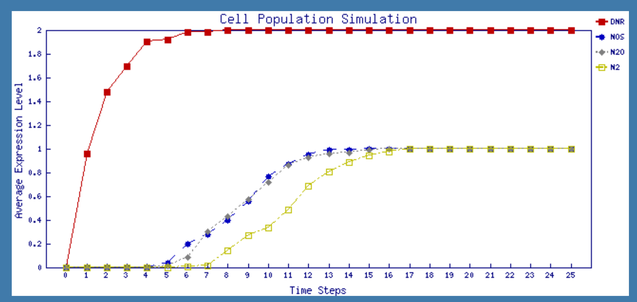

Figure 2. Cell population dynamics of *P. aeruginosa* during denitrification. With 100 simulations and randomly update schedule, the long-term behavior of DNR, NOS, N_2O, and N_2 is shown under the perfect condition for denitrification: low O_2 and PO_4, and high NO_3.

O_2	NO_3	PO_4	Steady State
low	low	low	No denitrification – high NO_3 tension is not sensed
low	low	high	No denitrification
low	high	low	High denitrification performance
low	high	high	Low denitrification performance
high	low	low	No denitrification – low O_2 tension is not sensed
high	low	high	No denitrification – low O_2 tension is not sensed
high	high	low	No denitrification – low O_2 tension is not sensed
high	high	high	No denitrification – low O_2 tension is not sensed

Table 3. Steady state analysis of the system under different environmental conditions. All results except the blue ones confirm pertinent biological literature.

MODEL PREDICTIONS

✧ High PO_4 prevents the activation of DNR via ANR.

✧ High PO_4 prevents the induction of NOS via DNR.

These hypotheses (the effect of PO_4 into the network) are currently being tested using gas chromatography (GC) and reverse transcriptase quantitative PCR (qRT-PCR).

- GC is used for measuring the output of N_2O from cultures grown under increasing PO_4 conditions.
- qRT-PCR is utilized to detect levels of mRNA from target genes such as denitrification genes and *dnr*.

INITIAL MODEL VALIDATION

Culture (mM P)	$[N_2O]$ ppm, 24 h	$[N_2O]$ ppm, 72 h
1.0 mM	760.3 +/- 109.3	813.8 +/- 52.1
3.0 mM	856.0 +/- 121.5	872.3 +/- 63.3
7.5 mM	1484.0 +/- 146.2	1786 +/- 98.0

Table 4. NO_2 concentrations in *P. aeruginosa cultures grown in glucose minimal medium at varying PO_4 concentrations*, normalized to 10^8 cells

✓ **More PO_4 causes less reduction of NO_2**

REFERENCES

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