8th Vienna International Conference on Mathematical Modelling
February 18 - 20, 2015. Vienna University of Technology, Vienna, Austria

IFAC
INTERNATIONAL FEDERATION OF AUTOMATIC CONTROL

Thermodynamic behavior-rules for a bacterial individual-based model to study the denitrification process

Pablo Araujo [*,], Anna Gras [**] and Marta Ginovart [***]**

** Chemical Engineering Faculty, Central University of Ecuador, Ciudad Universitaria Francisco Viteri y Gatto Sobral, P.O.Box. 17-01-3972, Quito - Ecuador; (Tel: (34) 636261560; e-mail: paraujo@iquce.edu.ec / pablo@araujo.ec).*
*** Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Edifici D4, Esteve Terradas 8, 08860 Castelldefels, Barcelona - Spain; (Tel: (34) 935521224; e-mail: anna.gras@upc.edu).*
**** Department of Applied Mathematics III, Universitat Politècnica de Catalunya, Edifici D4, Esteve Terradas 8, 08860 Castelldefels, Barcelona - Spain; (Tel: (34) 935521133; e-mail: marta.ginovart@upc.edu).*

Abstract: The individual's adaptive behavior to environmental conditions through different behavior-rules is one of the strongest aspects of an individual-based model (IBM). Microbial IBMs consider individuals as discrete entities that follow behavior-rules that dictate how microorganisms interact with their surrounding environment and other microbes, so that the microorganisms and the environment can change their characteristics. This makes it possible to explore connections between micro-level microorganism behaviors and macro-level patterns that emerge from their interactions. INDISIM-Paracoccus is a bacterial IBM used to model the growth and development of the bacteria *Paracoccus denitrificans* in batch and continuous cultures under aerobic and anaerobic conditions. It embeds thermodynamic properties in individual cells, which can simulate the behavior of the cell population more realistically and mechanistically than other approaches. The IBM's development and application with some intracellular detail and complexity constitute a key advantage in the investigation and understanding of the different steps of denitrification carried out by a denitrifying bacterium.

Keywords: denitrification, *Paracoccus denitrificans*, bacterial yield prediction, individual-based model, Thermodynamic Electron Equivalents Model, INDISIM.

1. INTRODUCTION

Denitrification is the process in which bacteria, for instance *Paracoccus denitrificans,* one of the frequently chosen species for biochemistry studies, use nitrate as a final electron acceptor and carry out respiratory metabolism in anaerobic conditions. Denitrification reduces the nitrate content of soil, so that fewer nitrates can leach downwards and root uptake may be hindered (Heinen, 2006). Denitrification is also a source of environmental burden, in agricultural soils; nitrous oxide (N_2O) emissions are very important due to the large amount of N-fertilizer in crops and soil organic matter mineralization (Snyder et al., 2009). N_2O is a powerful greenhouse gas that can persist for up to 150 years while it is slowly broken down in the stratosphere (Richardson et al., 2009). To study the effects of denitrification on the nitrogen balance in agricultural systems controlled experiments in bioreactors and simulation models can be helpful tools (Felgate et al., 2012).

About 25 years ago an interesting modeling approach paradigm was implemented, which is an alternative to the population-level approach (Grimm, 1999). This modeling approach is called "individual-based modeling" (Individual-based Models, IBMs), with which it is possible to simulate the interactions of the agents (individuals and/or collective entities) with their environment. Microbial IBMs offer some advantages over the traditional population-level models (Ferrer et al., 2008; Hellweger & Bucci, 2009; Kreft et al., 2013).

We are developing an IBM for denitrifying bacteria called INDISIM-Paracoccus (Araujo et al., 2014). The model assumes a culture medium containing succinate as a carbon source, ammonium as a nitrogen source and various electron acceptors such as oxygen, nitrate, nitrite, nitric oxide and nitrous oxide to simulate continuous or batch cultures under diverse substrate-dependent cell growth of the bacterium *P. denitrificans*. The model embeds a Thermodynamic Electron Equivalents Model (TEEM2) (McCarty, 2007) for bacterial growth prediction within the IBM INDISIM (Ginovart et al., 2002). The obtained stoichiometric reactions are an intracellular model for generating the microorganism behavior-rules.

In the INDISM-Paracoccus framework, the objectives of this study are to: i) show how balanced energy reactions are incorporated into the behavior-rules for cellular maintenance and for biomass synthesis following a thermodynamic approach, and ii) implement the model on NetLogo and test two hypotheses about the order in which the reactions are followed by the bacteria while the denitrification process occurs. Temporal evolutions of some system variables will be analyzed and compared.

2. MATERIALS AND METHODS

2.1 Metabolic pathways

P. denitrificans can survive in ecosystems with fluctuating aerobic and anaerobic conditions, because it can use molecular oxygen dissolved in the medium; thus in the aerobic phase it can execute "*Aerobic respiration*" with oxygen (O_2) as the electron acceptor (Reaction 1) and "*Nitrate reduction - Dissimilatory*" as the nitrate (NO_3^-) electron acceptor (Reaction 2) (Baker et al., 1998; Beijerinck, 1910; Caspi et al., 2012).

$\mathbf{O_2} + 4\ H^+ + 4\ e^- \rightarrow 2\ \mathbf{H_2O}$ (Reaction 1)
$\mathbf{NO_3^-} + 10\ H^+ + 8\ e^- \rightarrow \mathbf{NH_4^+} + 3\ H_2O$ (Reaction 2)

Further *P. denitrificans* in anoxic conditions executes "*Nitrate reduction - Denitrification process*" because it is capable of anaerobic growth in the presence of NO_3^-, nitrite (NO_2^-), nitric oxide (NO) or N_2O as electron acceptors (Reactions 3 to 6) (Baumann et al., 1996; Bergaust et al., 2010; Bergaust et al., 2012; Caspi et al., 2012; van Verseveld et al., 1983).

$2\ \mathbf{NO_3^-} + 4\ H^+ + 4\ e^- \rightarrow 2\ \mathbf{NO_2^-} + 2\ H_2O$ (Reaction 3)
$4\ H^+ + 2\ \mathbf{NO_2^-} + 2\ e^- \rightarrow 2\ \mathbf{NO} + 2\ H_2O$ (Reaction 4)
$2\ H^+ + 2\ \mathbf{NO} + 2\ e^- \rightarrow \mathbf{N_2O} + H_2O$ (Reaction 5)
$2\ H^+ + \mathbf{N_2O} + 2\ e^- \rightarrow \mathbf{N_2} + H_2O$ (Reaction 6)

2.2 Thermodynamic electron equivalents model second version – TEEM2

Microorganisms capture energy released by redox reactions for maintenance and growth. Redox reactions always involve an electron donor and an electron acceptor. The electrons are obtained from an electron donor and transferred to intracellular electron carriers. Carriers bring the electrons towards the electron acceptor; as a result the acceptor suffers a reduction reaction that causes the regeneration of the initial carrier. When microorganisms use an electron-donor substrate for synthesis, a portion of their electrons (fe^o) is transferred to the electron acceptor to generate energy and metabolic products and the other portion of electrons (fs^o) is transferred to the N-source for cell synthesis (Rittmann & McCarty, 2001) (Fig 1).

TEEM2 is a thermodynamic model based on bioenergetics growth efficiency that can make an adjustment between cell synthesis reaction (Rs) and energy reaction (Re) to predict bacterial yield with the associated Gibbs free energies for these reactions (McCarty, 2007).

Re is the combination of the half-reaction for the electron donor (Rd) and the half-reaction for the electron acceptor (Ra). **Rs** is the combination of Rd with the half-reaction for the biomass synthesis (Rc) that considers ammonium or other nitrogen sources for new biomass generation (Rittmann & McCarty, 2001).

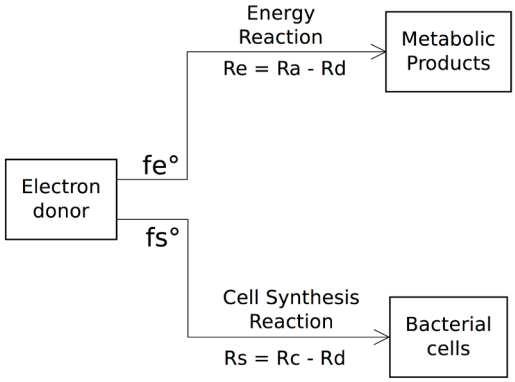

Fig 1. Electron donor utilization for energy production and cell synthesis. Adapted from Rittmann & McCarty (2001).

Equations (1) and (2) show how TEEM2 calculates the relationship between (fe^o) and (fs^o) with Rd, Ra and Rc half-reactions and their Gibbs standard free energy along with other Gibbs energy potential terms, considering that thermodynamic free energy is lost at each transfer by including a term for energy-transfer efficiency (ε). Equation (3) shows how TEEM2 calculates the maximum bacterial yield Yc/c (McCarty, 2007).

$$A = -\frac{\Delta G_s}{\varepsilon \Delta G_e} = \frac{\frac{(\Delta G_{fa} - \Delta G_d)}{\varepsilon^m} + \frac{(\Delta G_{in} - \Delta G_{fa})}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon\left(\Delta G_a - \Delta G_d - \frac{q}{p}\Delta G_{xy}\right)} = \frac{f_e^o}{f_s^o} \quad (1)$$

$$f_s^o = \frac{1}{1+A} \ ; \ f_e^o = \frac{A}{1+A} \ ; \ f_s^o + f_e^o = 1 \quad (2)$$

$$Y_{c/c} = \frac{\gamma_d}{\gamma_x} f_s^o \quad (3)$$

Where,

- fs^o = Fraction of electron-donor electrons converted for synthesis (eeq cells/eeq donor).
- fe^o = Fraction of electron-donor electrons used for energy and converted to reaction products (eeq products/eeq donor).
- ΔG_e = Gibbs free energy for energy reaction (kJ/eeq).
- ΔG_s = Gibbs free energy for cell synthesis reaction (kJ/eeq).
- ΔG_a = Reduction potential for electron acceptor half-reaction (kJ/eeq).
- ΔG_d = Reduction potential for electron donor half-reaction (kJ/eeq).
- ΔG_{xy} = Reduction potential for NADH oxidation (219.2 kJ/mol).
- ΔG_{in} = Reduction potential for Acetyl-CoA half-reaction (30.9 kJ/eeq).
- ΔG_{fa} = Reduction potential for formaldehyde half-reaction (46.53 kJ/eeq for C1 compounds, 0 for others).
- ΔG_{pc} = Gibbs free energy for intermediate conversion to cells (kJ/eeq) = 3.33 kJ/gcells (Molecular weight Cells/pcells) = 3.33(113/20) = 18.8 kJ/eeq with ammonia as nitrogen source and cell formulation of $C_5H_7O_2N$. With nitrate, nitrite, or N_2 as nitrogen source, pcells equals 28, 26 and 23 kJ/eeq, respectively (Rittmann & McCarty, 2001).
- ε = Energy transfer efficiency.

- m = +1 if $\Delta G_{fa} > 0$, otherwise = n.
- n = +1 if $m = n$ and $(\Delta G_{in} - \Delta G_d) > 0$, otherwise n = -1.
- p = Number of electron equivalents per mole of substrate from half-reaction reduction equation.
- q = Number of oxygenase reactions per mole substrate.
- γ_d = Degree of reduction of electron donor.
- γ_x = Degree of reduction of cells.
- Yc/c = Maximum bacterial yield ($molC_{mic}/molC_{substrate}$).

3. RESULTS

3.1 Basic model description

INDISIM-Paracoccus is an IBM for the denitrification carried out by the bacteria *P. denitrificans* growing in batch and continuous culture in aerobic and anaerobic growing conditions. The model has two entities: individuals and square patches of culture medium. An individual represents a unique bacterium of *P. denitrificans* and has the following variables: an identification number, location, mass, reproduction mass, internal product amounts and counters for each metabolic pathway and reproduction cycle. The smallest microorganism has a mass of ~ 1 pmol and the largest microorganism has a mass of ~ 6 pmol. A two-dimensional lattice of 31x31 grid cells represents the bioreactor that contains the culture medium; one spatial cell represents 1 nl, so the total bioreactor volume is 961 nl. Their variables are: position identifier in XY coordinates, total amount of each nutrient, succinate, NH_4^+, O_2, NO_3^-, and metabolic products, NO_2^-, NO, N_2O, N_2 and CO_2. All microbial and culture medium processes are discretized in time steps. One time step represents 10 min. At each time step all the individuals are controlled by a set of time-dependent variables, and they perform the following processes: nutrient uptake, cellular maintenance, biomass synthesis, metabolic products generation and bipartition. Culture medium processes are different depending on the bioreactor management protocol. At the beginning of the simulation the bioreactor works as a batch culture with oxygen saturated conditions, and the user manages at what time this phase ends and switches to continuous culture in anoxic conditions.

A microbe in INDISIM-Paracoccus checks the local oxygen-dissolved level and if it is lower than a threshold value (O_2-MIN) the microbe uses the anaerobic metabolism; otherwise it uses aerobic metabolism. This change is discrete for each bacterium in the time step; therefore there is a gradual translation for the population. Having selected the metabolism, the microbe carries out its maintenance according to the energy reactions and its specific maintenance requirements. After maintenance, if the succinate intake and the quantity of some electron acceptors are greater than zero, the bacterium can perform biomass synthesis. With the nutrient intakes updated the microbe divides the amount of each nutrient by its respective stoichiometric coefficient and selects the smallest value. This information provides the demands of the other nutrients for new biomass and metabolic products generation. After this, if there are remaining electron donors and some electron acceptor intakes, the microbe can perform the next metabolic reaction. Otherwise the remaining unused intakes are expelled to the medium. The bipartition process is an INDISIM sub-model (Ginovart et al., 2002). The sub-models related to the bioreactor's procedure are: i) Agitation: Nutrients and metabolic products are redistributed in the culture medium and microorganism positions change randomly, ii) Input flow: The bioreactor is refilled with fresh culture medium and iii) Output flow: A fraction of individuals and culture medium are randomly removed.

The model design has been implemented in the NetLogo multi-agent programmable modeling environment (Wilensky, 1999), and the simulator may be obtained from the authors on request.

3.2 Stoichiometric coefficients for cellular maintenance energy reaction and biomass synthesis.

The energy reactions consider that succinate and some electron acceptors were obtained according to TEEM2 for aerobic and anaerobic maintenance (Table I). See appendix A for detail calculations. The stoichiometric coefficients, for a metabolic pathway, were obtained from Gibbs free energy for a half-reaction (reactions 1 to 6) with an assigned ε value in the range proposed by McCarty (1971, 2007) (Table II). See appendix B for detail calculations.

Table I. Balanced energy reactions (Re) for cellular maintenance in aerobic and anaerobic phase. (Re = Ra – Rd) according to (Rittmann & McCarty, 2001).

Chemical species ($x10^{-2}$)	Succinate with				
	Oxygen	Nitrate	Nitrite	Nitric oxide	Nitrous Oxide
$C_4H_4O_4^{2-}$	7.14	7.14	7.14	7.14	7.14
O_2	0.25	---------	---------	---------	---------
NO_3^-	---------	50	---------	---------	---------
NO_2^-	---------	(50)	100	--------	---------
NO	---------	---------	(100)	100	---------
N_2O	---------	---------	---------	(50)	50
N_2	---------	---------	---------	---------	(50)
CO_2	(14.3)	(14.3)	(14.3)	(14.3)	(14.3)
HCO_3^-	(14.3)	(14.3)	(14.3)	(14.3)	(14.3)
H_2O	(7.1)	(7.1)	(14.3)	(7.1)	(7.1)
H^+	---------	---------	100	---------	---------
Numbers between parenthesis are reaction products					

Table II. Balanced chemical equations for biomass ($C_3H_{5,4}O_{1,45}N_{0,75}$) synthesis in aerobic and anaerobic phase. (R = $fe^oRa + fs^oRc - Rd$) according to TEEM2 (McCarty, 2007).

Chemical species ($x10^{-2}$)	Reaction					
	1	2	3	4	5	6
$C_4H_4O_4^{2-}$	7.14	7.14	7.14	7.14	7.14	7.14
NH_4^+	4.31	0.55	2.14	4.13	4.13	4.13
O_2	7.40	------	------	------	------	-------
Biomass	(5.75)	(5.72)	(2.86)	(5.51)	(5.51)	(5.51)
NO_3^-	------	3.74	32.5	------	-------	------
NO_2^-	---------	---------	(32.5)	32.5	------	------
NO	---------	---------	---------	(32.5)	32.5	------
N_2O	------	------	------	------	(16.25)	------
N_2	------	------	------	---------	---------	16.25
CO_2	(1.36)	(1.41)	(7.86)	(1.89)	(1.89)	(1.989)
HCO_3^-	(9.98)	(9.99)	(12.14)	(10.15)	(10.15)	(10.15)
H_2O	(2.40)	1.32	(4.79)	(18.85)	(2.60)	(2.60)
H^+	------	7.48	------	32.5	------	------
Numbers between parenthesis are reaction products						

3.3 Preliminary simulation results with INDISIM-Paracoccus

For biomass synthesis in anaerobic phase the bacterium has the possibility to execute the denitrification process that is carried out with four reactions. To investigate the effect of the priority in the use of different electron acceptors at the microbial level two hypotheses were formulated. The first hypothesis is that the four reactions succeed according to their standard Gibbs energy because this indicates the spontaneity of reaction occurrence in comparison to the others. Reactions with lower Gibbs energy are expected to occur first. In this case the order is: Reaction 3, Reaction 6, Reaction 5 and Reaction 4. The second hypothesis is that the four reactions succeed according to the nitrogen oxides reduction level. In this case the order is: Reaction 3, Reaction 4, Reaction 5 and Reaction 6. INDISIM-Paracoccus IBM allows us to investigate and compare the two hypotheses thorough outputs of some system variables such as biomass, nitrate, nitrite, nitric oxide, nitrous oxide, nitrogen, oxygen, carbon dioxide, succinate and ammonium (Fig. 2).

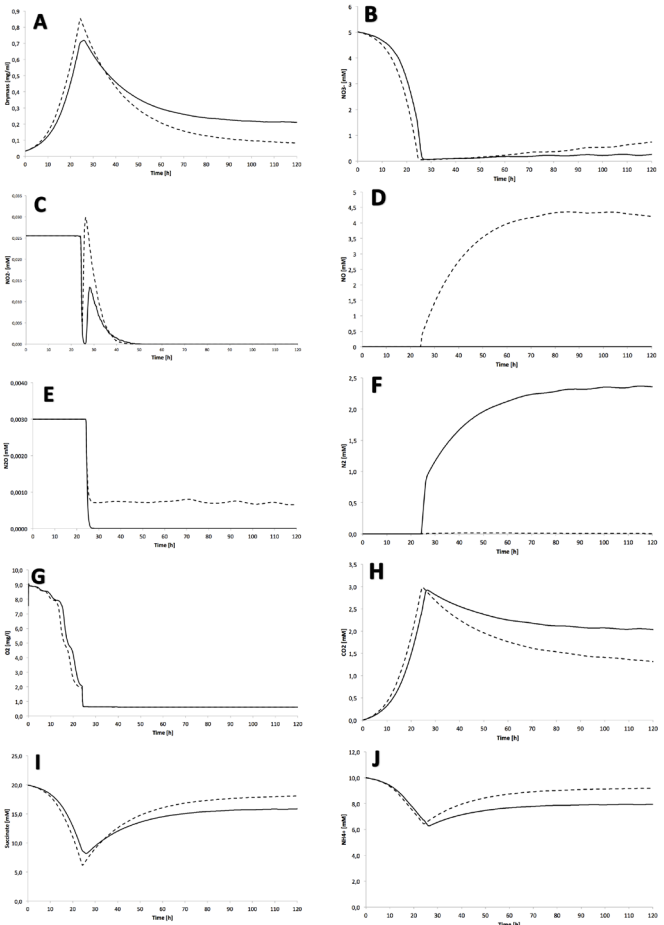

Fig. 2. Temporal evolutions of: A) Biomass, B) NO_3^-, C) NO_2, D) NO, E) N_2O, F) N_2, G) O_2, H) CO_2, I) Succinate, J) NH_4^+. Reactions occur according to: Dashed line: their standard Gibbs energy. Continuous line: the nitrogen oxides reduction.

For all temporal evolutions in the aerobic phase (first 24 hours) the simulated results are similar for the two hypotheses. After switching to the anaerobic phase, the Gibbs order hypothesis shows the presence of intermediate nitrogen oxides and low N_2 production, and the hypothesis that the reactions occur according to the nitrogen oxides reduction shows the absence of intermediate nitrogen oxides and high N_2 production. In the long term both scenarios achieve a steady state according to the dilution rate of 0.05 h^1.

4. DISCUSSION

INDISIM-Paracoccus offers the possibility of interpreting, understanding and investigating the dynamics of *P. denitrificans* growing in a controlled condition. The simulator allows us to treat the intrinsic variability of the microbes, each of which has particular characteristics and acts according to specific behavior-rules related to its biological guidelines. INDISIM-Paracoccus model is implemented in the widely used, free and open source IBM software platform NetLogo that facilitates interaction among researchers, modelers and academics. When converting the reactions that represent metabolic pathways into a balanced chemical equation by applying the TEEM2, the individual growth yield obtained is higher than published population yields, but the population growth yield is in accordance with reported *P. denitrificans* values. TEEM2 appears to be a useful tool for modeling the individual behavior-rules in the INDISIM-Paracoccus model. The hypothesis that the reactions in the bacterium occur according to their standard Gibbs energy does not seem plausible, because NO production reaches higher values than those reported by experimentalists (Felgate et al., 2012). But it was useful in the first steps of our investigation to develop and parameterize the model. Further work will be needed in making adjustments in order to deal with the denitrification process according to the nitrogen oxide reduction and to include denitrification enzyme expression as a response to the environmental conditions.

REFERENCES

Araujo, P., Gras, A., & Ginovart, M. (2014). An Individual-based model for the study of Paracoccus denitrificans, a denitrifying bacterium. In A. Méndez-Vilas (Ed.), *Industrial, medical and environmental applications of microorganisms. Current status and trends* (pp. 28–33). Wageningen Academic Publishers.

Baker, S. C., Ferguson, S. J., Ludwig, B., Page, M. D., Richter, O.-M. H., & van Spanning, R. J. M. (1998). Molecular genetics of the genus paracoccus: metabolically versatile bacteria with bioenergetic flexibility. *Microbiol. Mol. Biol. Rev.*, *62*(4), 1046–1078.

Baumann, B., Snozzi, M., Zehnder, A., & Van Der Meer, J. (1996). Dynamics of denitrification activity of Paracoccus denitrificans in continuous culture during aerobic-anaerobic changes. *J. Bacteriol.*, *178*(15), 4367–4374.

Beijerinck MW, M. D. (1910). Bildung und Verbrauch von Stickoxydul durch Bakterien. *International Journal of Medical Microbiology*, *2*(II), 30–63.

Bergaust, L., Mao, Y., Bakken, L. R., & Frostegård, A. (2010). Denitrification response patterns during the

transition to anoxic respiration and posttranscriptional effects of suboptimal pH on nitrous [corrected] oxide reductase in Paracoccus denitrificans. *Applied and Environmental Microbiology*, *76*(19), 6387–96. doi:10.1128/AEM.00608-10

Bergaust, L., van Spanning, R. J. M., Frostegård, A., & Bakken, L. R. (2012). Expression of nitrous oxide reductase in Paracoccus denitrificans is regulated by oxygen and nitric oxide through FnrP and NNR. *Microbiology (Reading, England)*, *158*(Pt 3), 826–34. doi:10.1099/mic.0.054148-0

Caspi, R., Altman, T., Dreher, K., Fulcher, C. A., Subhraveti, P., Keseler, I. M., … Karp, P. D. (2012). The MetaCyc database of metabolic pathways and enzymes and the BioCyc collection of pathway/genome databases. *Nucleic Acids Research*, *40*(Database issue), D742–53. doi:10.1093/nar/gkr1014

Felgate, H., Giannopoulos, G., Sullivan, M. J., Gates, A. J., Clarke, T. A., Baggs, E., … Richardson, D. J. (2012). The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. *Environmental Microbiology*, *14*(7), 1788–800. doi:10.1111/j.1462-2920.2012.02789.x

Ferrer, J., Prats, C., & López, D. (2008). Individual-based modelling: an essential tool for microbiology. *Journal of Biological Physics*, *34*(1-2), 19–37. doi:10.1007/s10867-008-9082-3

Ginovart, M., López, D., & Valls, J. (2002). INDISIM, an individual-based discrete simulation model to study bacterial cultures. *Journal of Theoretical Biology*, *214*(2), 305–19. doi:10.1006/jtbi.2001.2466

Gras, A., Ginovart, M., Valls, J., & Baveye, P. C. (2011). Individual-based modelling of carbon and nitrogen dynamics in soils: Parameterization and sensitivity analysis of microbial components. *Ecological Modelling*, *222*(12), 1998–2010. doi:10.1016/j.ecolmodel.2011.03.009

Grimm, V. (1999). Ten years of individual-based modelling in ecology: what have we learned and what could we learn in the future? *Ecological Modelling*, *115*(2-3), 129–148. doi:10.1016/S0304-3800(98)00188-4

Heinen, M. (2006). Simplified denitrification models: Overview and properties. *Geoderma*, *133*(3-4), 444–463. doi:10.1016/j.geoderma.2005.06.010

Hellweger, F. L., & Bucci, V. (2009). A bunch of tiny individuals—Individual-based modeling for microbes. *Ecological Modelling*, *220*(1), 8–22. doi:10.1016/j.ecolmodel.2008.09.004

Kreft, J.-U., Plugge, C. M., Grimm, V., Prats, C., Leveau, J. H. J., Banitz, T., … Hellweger, F. L. (2013). Mighty small: Observing and modeling individual microbes becomes big science. *Proceedings of the National Academy of Sciences of the United States of America*, *110*(45), 18027–8. doi:10.1073/pnas.1317472110

McCarty, P. (1971). Energetics and bacterial growth. *Organic Compounds in Aquatic Environments*, *1*, 157–172.

McCarty, P. L. (2007). Thermodynamic electron equivalents model for bacterial yield prediction: modifications and comparative evaluations. *Biotechnology and Bioengineering*, *97*(2), 377–388. doi:10.1002/bit

Richardson, D., Felgate, H., Watmough, N., Thomson, A., & Baggs, E. (2009). Mitigating release of the potent greenhouse gas N(2)O from the nitrogen cycle—could enzymic regulation hold the key? *Trends in Biotechnology*, *27*(7), 388–97. doi:10.1016/j.tibtech.2009.03.009

Rittmann, B. E., & McCarty, P. L. (2001). *Environmental Biotechnology: Principles and Applications*. *Biotechnology* (p. 463).

Snyder, C. S., Bruulsema, T. W., Jensen, T. L., & Fixen, P. E. (2009). Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agriculture, Ecosystems & Environment*, *133*(3-4), 247–266. doi:10.1016/j.agee.2009.04.021

Van Verseveld, H. W., Boon, J. P., & Stouthamer, A. H. (1979). Growth yields and the efficiency of oxidative phosphorylation of Paracoccus denitrificans during two- (carbon) substrate-limited growth. *Archives of Microbiology*, *121*(3), 213–223. doi:10.1007/BF00425058

Van Verseveld, H. W., Braster, M., Boogerd, F. C., Chance, B., & Stouthamer, A. H. (1983). Energetic aspects of growth of Paracoccus denitrificans: oxygen-limitation and shift from anaerobic nitrate-limination to aerobic succinate-limitation. *Archives of Microbiology*, *135*(3), 229–236. doi:10.1007/BF00414485

Wilensky, U. (1999). NetLogo. *Center for Connected Learning and Computer-Based Modeling, Northwestern University, Evanston, IL.* Retrieved March 05, 2014, from http://ccl.northwestern.edu/netlogo/

Appendix A. EXAMPLE OF CALCULATIONS OF ENERGY REACTIONS FOR CELLULAR MAINTENANCE IN INDISIM-PARACOCCUS MODEL

The metabolic reactions were written considering the elementary cell composition for *P. denitrificans* ($C_3H_{5.4}N_{0.75}O_{1.45}$) proposed by van Verseveld et al. (1979, 1983).

Before biomass synthesis, each bacterium executes a behavior-rule for cellular maintenance. The maintenance requirements are different for the aerobic and anaerobic phases. So to establish the individual behavior-rule for the aerobic phase we assume a maintenance requirement of 0.002 $gC_{donor} \cdot gC_{mic}^{-1} \cdot h^{-1}$ proposed by Gras et al. (2011) and write the energy reaction (Re) with succinate and oxygen as follows:

(i) Write inorganic and organic half-reactions for electron donor and electron acceptor.

Electron donor (succinate) half-reaction (Rd):

$^1/_7\ CO_2 + {}^1/_7\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{14}\ (C_4H_4O_4)^{2-} + {}^3/_7\ H_2O$

Electron acceptor (oxygen) half-reaction (Ra):

$¼\ O_2 + H^+ + e^- \rightarrow ½\ H_2O$

(ii) According to Rittmann & McCarty (2001) following equation (4) a balanced stoichiometric equation can be written for the energy reaction.

$$Re = Ra - Rd \qquad (4)$$

Ra $\quad 0.25\ O_2 + H^+ + e^- \rightarrow 0.50\ H_2O$

– Rd $\quad 0.0714\ (C_4H_4O_4)^{2-} + 0.4285\ H_2O \rightarrow 0.1428\ CO_2 + 0.1428\ HCO_3^- + H^+ + e^-$

Re $\quad$ **$0.0714\ (C_4H_4O_4)^{2-} + 0.25\ O_2 \rightarrow 0.1428\ CO_2 + 0.1428\ HCO_3^- + 0.0714\ H_2O$**

Re is the balanced chemical equation for the energy reaction to determine the individual behavior-rule for aerobic maintenance in the INDISIM-Paracoccus model.

(iii) Computation of specific maintenance requirements for the aerobic phase gives:

$$0.002\frac{gC_{succinate}}{gC_{mic}\cdot h}\times\frac{1\ mol\ Succinate}{48gC_{Succinate}}\times\frac{36gC_{mic}}{1\ mol\ C_3H_{5.4}O_{1.45}N_{0.75}}=0.0015\frac{mol\ Succinate}{mol\ Biomass\cdot h}$$

$$0.0015\frac{mol\ Succinate}{mol\ Biomass\cdot h}\times\frac{0.25\ mol\ Oxygen}{0.0714 mol\ Succinate}=0.0052\frac{mol\ Oxygen}{mol\ Biomass\cdot h}$$

Appendix B. EXAMPLE OF CALCULATIONS OF BALANCED CHEMICAL EQUATIONS FOR BIOMASS SYNTHESIS

If cellular maintenance is accomplished a microbe runs a metabolic reaction to synthesize biomass and produce denitrification products. Therefore it is necessary to transform the reaction that represents the metabolic pathway into a balanced chemical reaction using TEEM2. In all reactions succinate is the universal electron donor (Rd) and C-source, and ammonia is the universal N-source (Rc) for cell synthesis. The electron acceptors (Ra) used are different; in aerobic conditions they are O_2 and NO_3^- while in anaerobic conditions they are NO_3^-, NO_2^-, NO and N_2O. With this and the Gibbs free energy for each half-reaction with an appropriate ε value, in the range proposed for McCarty (2007), the stoichiometric coefficients for a metabolic reaction and its individual growth yield, Yc/c, are obtained. For example, to establish the first step of the denitrification represented by Reaction 3, according to TEEM2 methodology, we proceed as follows:

(i) Write inorganic and organic half-reactions and their Gibbs standard free energy for electron donor and electron acceptor.

Electron donor (succinate) half-reaction (Rd):

$^1/_7\ CO_2 + {}^1/_7\ HCO_3^- + H^+ + e^- \rightarrow {}^1/_{14}\ (C_4H_4O_4)^{2-} + {}^3/_7\ H_2O$

$\Delta G_d = 29.090$ kJ/eeq

Electron acceptor (nitrate) half-reaction (Ra):

$½\ NO_3^- + H^+ + e^- \rightarrow ½\ NO_2^- + ½\ H_2O$

$\Delta G_a = -\ 41.650$ kJ/eeq

(ii) With equation (5) write the cell synthesis half-reaction (Rc) where $n = 3$, $a = 5.4$, $b = 1.45$, $c = 0.75$ and $d = 4n+a-2b-3c = 12.25$, and adjust ΔG_{pc} following the methodology:

$$\frac{(n-c)}{d}CO_2+\frac{c}{d}NH_4^+ +\frac{c}{d}HCO_3^- + H^+ + e^- \rightarrow \frac{1}{d}C_nH_aO_bN_c+\frac{2n-b+c}{d}H_2O \qquad (5)$$

$^9/_{49}\ CO_2 + {}^3/_{49}\ NH_4^+ + {}^3/_{49}\ HCO_3^- + H^+ + e^- \rightarrow {}^4/_{49}\ C_3H_{5.4}O_{1.45}N_{0.75} + {}^{106}/_{245}\ H_2O$

$\Delta G_{pc} = 20.398$ kJ/eeq

(iii) Degree of reduction computation for electron donor and cells:

$$\gamma_d = \frac{\text{electrons donor}}{\text{carbon donor}} = \frac{14}{4} = 3.5 \text{ and } \gamma_x = \frac{\text{electrons cells}}{\text{carbon cells}} = \frac{49/4}{3} = 4.083$$

(iv) Following equations (1), (2) and (3) computation of f_s^o, f_e^o and Yc/c according to McCarty (2007):

$$A = \frac{\frac{(0-29.09)}{0.41^1}+\frac{(30.90-0)}{0.41^1}+\frac{20.398}{0.41}}{0.41(-41.65-29.09-0)} = 1.857$$

$$f_s^o = \frac{1}{1+1.857} = 0.35$$

$$f_e^o = 1.857 \times 0.35 = 0.65$$

$$Y_{c/c} = \frac{3.5}{4.083}\times 0.35 = 0.30\ \left[\frac{molC_{mic}}{molC_{succinate}}\right]$$

(v) According to Rittmann & McCarty (2001), following equation (6) a balanced stoichiometric equation can be written.

$$R = f_e^o\,Ra + f_s^o\,Rc - Rd \qquad (6)$$

f_e^oRa $\quad 0.3250\ NO_3^- + 0.65\ H^+ + 0.65\ e^- \rightarrow 0.3250\ NO_2^- + 0.3250\ H_2O$

f_s^oRc $\quad 0.064\ CO_2 + 0.0214\ NH_4^+ + 0.021\ HCO_3^- + 0.35\ H^+ + 0.35\ e^- \rightarrow 0.0286\ C_3H_{5.4}O_{1.45}N_{0.75} + 0.1514\ H_2O$

– Rd $\quad 0.0714\ (C_4H_4O_4)^{2-} + 0.4285\ H_2O \rightarrow 0.1428\ CO_2 + 0.1428\ HCO_3^- + 1.00\ H^+ + 1.00\ e^-$

R $\quad$ **$0.0714\ (C_4H_4O_4)^{2-} + 0.0214\ NH_4^+ + 0.3250\ NO_3^- \rightarrow 0.0286\ C_3H_{5.4}O_{1.45}N_{0.75} + 0.3250\ NO_2^- + 0.0786\ CO_2 + 0.1214\ HCO_3^- + 0.0479\ H_2O$**

R is the balanced chemical equation using the TEEM2 to determine the individual behavior-rule for biomass generation in the first step of the denitrification process represented by Reaction 3.