



# Developing an individual-based model to study the bacterial denitrification process

Thesis submitted by

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# Context

Financial support



Research fellow

Ph.D.  
Thesis



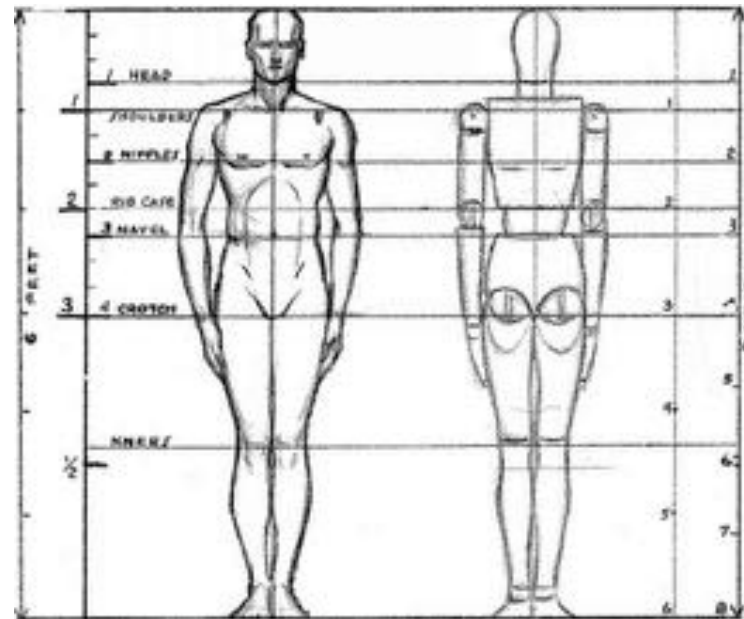
Research group  
BIOCOM-SC and the  
core model INDISIM  
Ginovart et al., 2002

Full experimental  
data set of two  
denitrifying bacteria  
Felgate et al., 2012

# What is a model?

- Abstraction of reality
- Simplification somehow
- Represent main features of reality

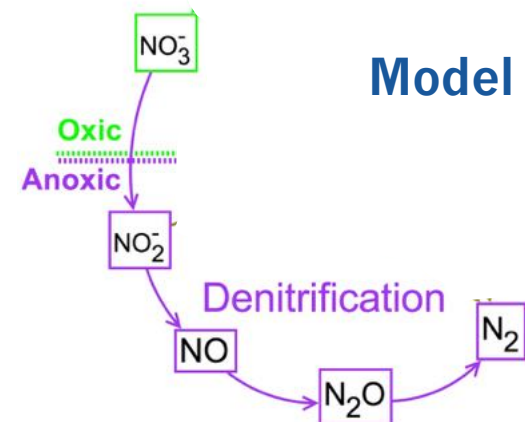
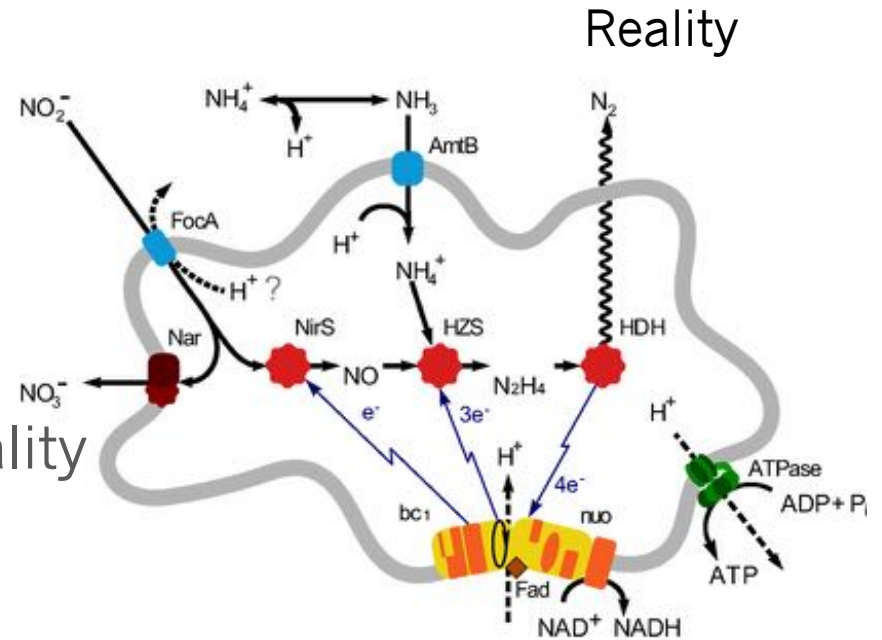
Reality



Model

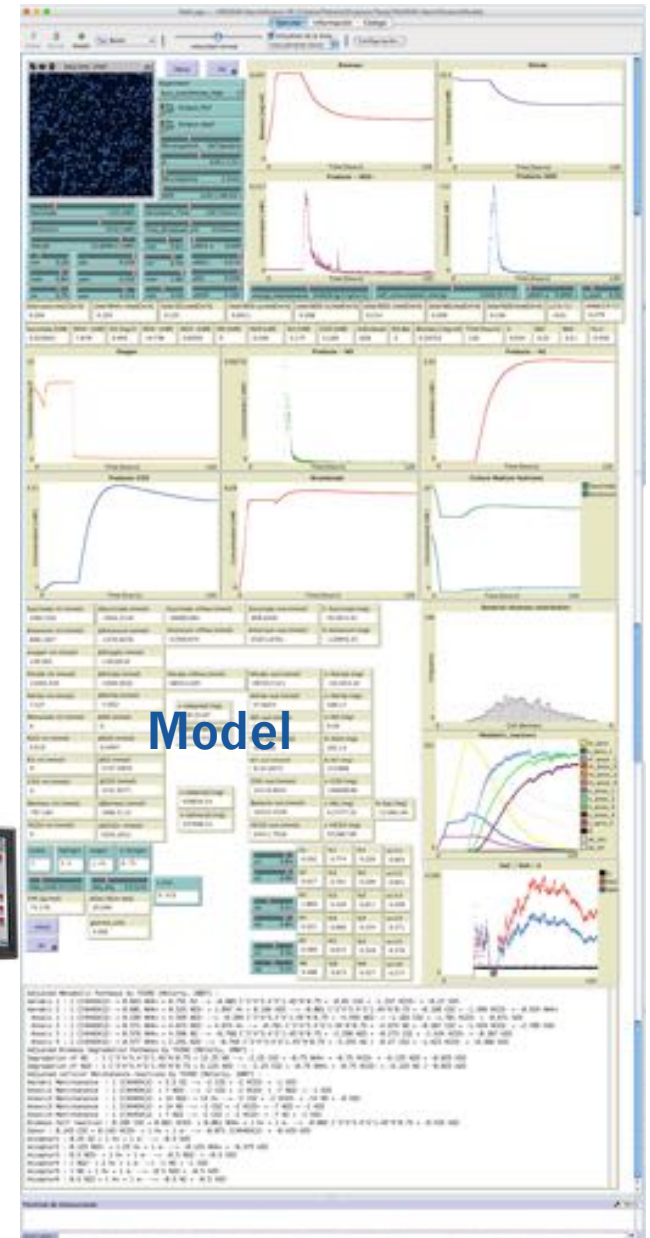
# What is a model?

- Abstraction of reality
- Simplification somehow
- Represent main features of reality



# What is a model?

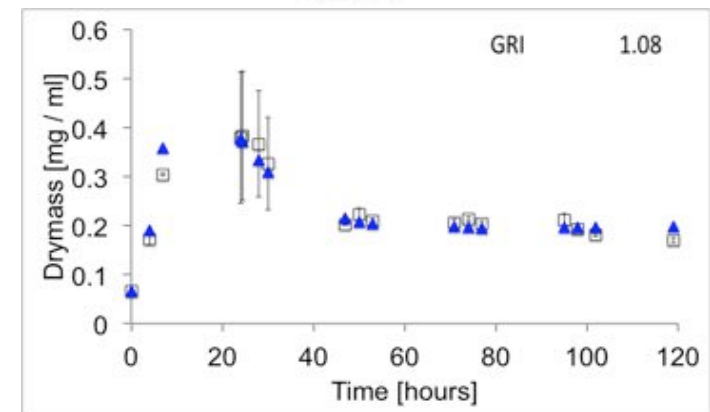
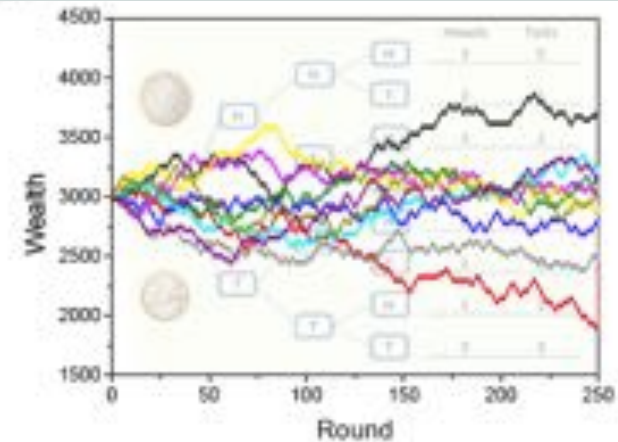
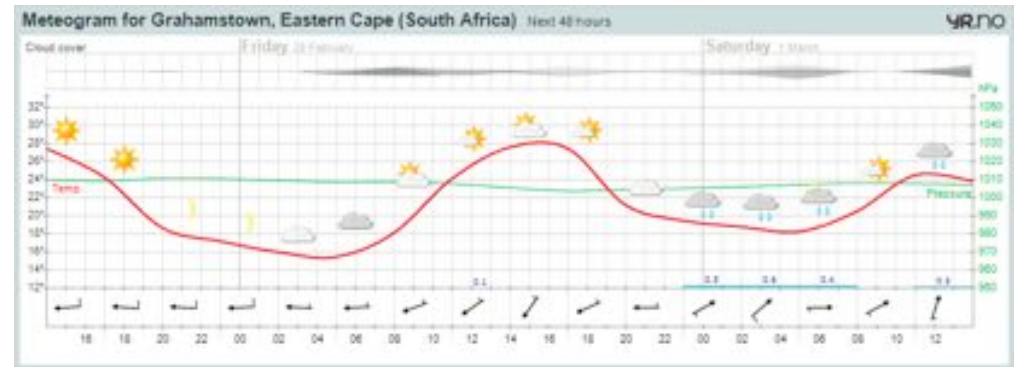
- Abstraction of reality
- Simplification somehow
- Represent main features of reality
- Representation of a phenomenon
- Description of complex processes



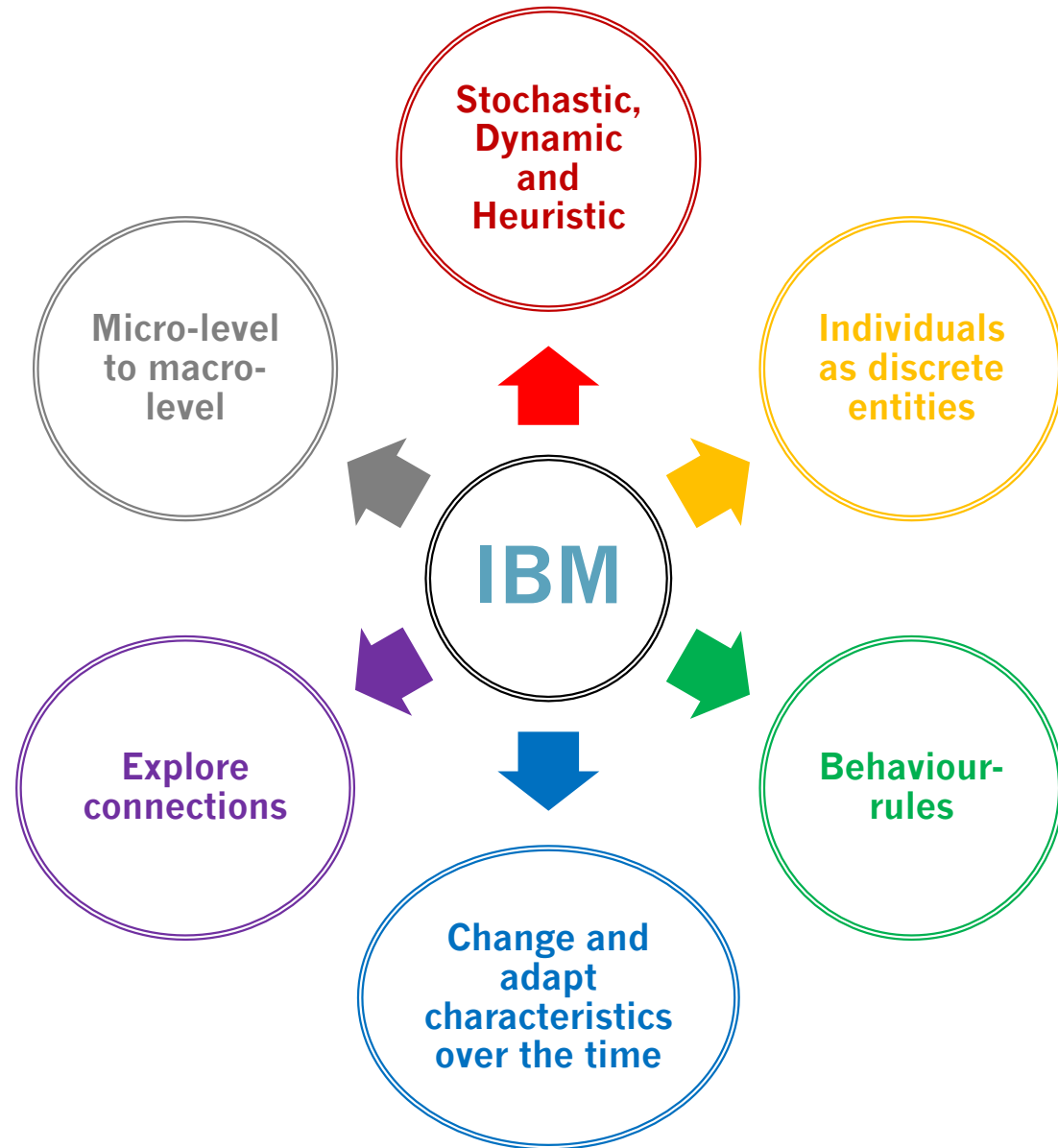
Reality

# What is a model?

- Abstraction of reality
- Simplification somehow
- Represent main features of reality
- Representation of a phenomenon
- Description of complex processes
- Controllable environment
- Science lab



# What is an Individual-Based Model?

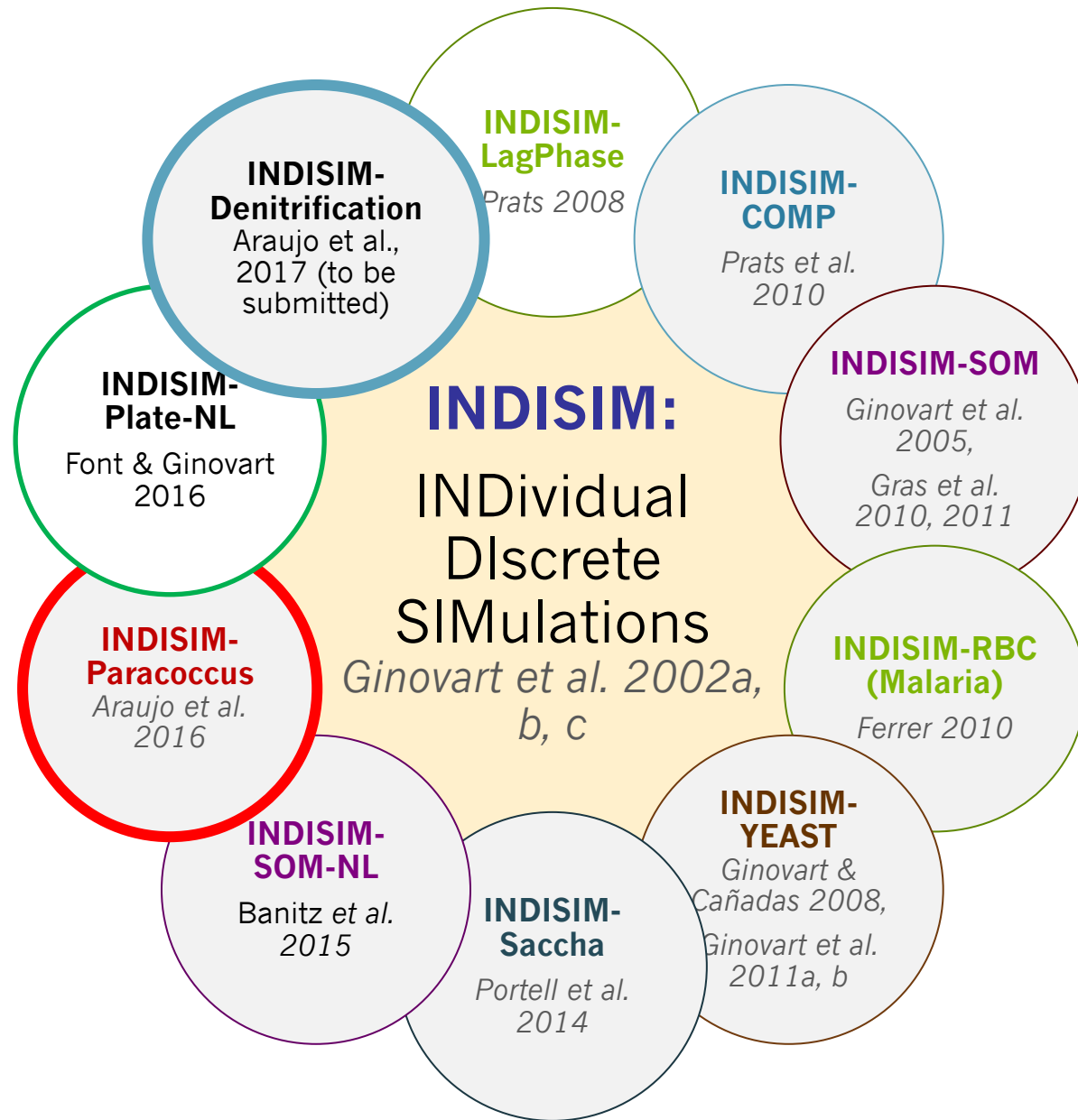




# Microbial IBMs

- ✓ BACSIM (Kreft et al., 1998)
- ✓ **INDISIM (Ginovart et al., 2002)**
- ✓ MIOR (Masse et al., 2007)
- ✓ MICRODIMS (Verhulst et al., 2011)
- ✓ ..... Among others

# INDISIM: our core model

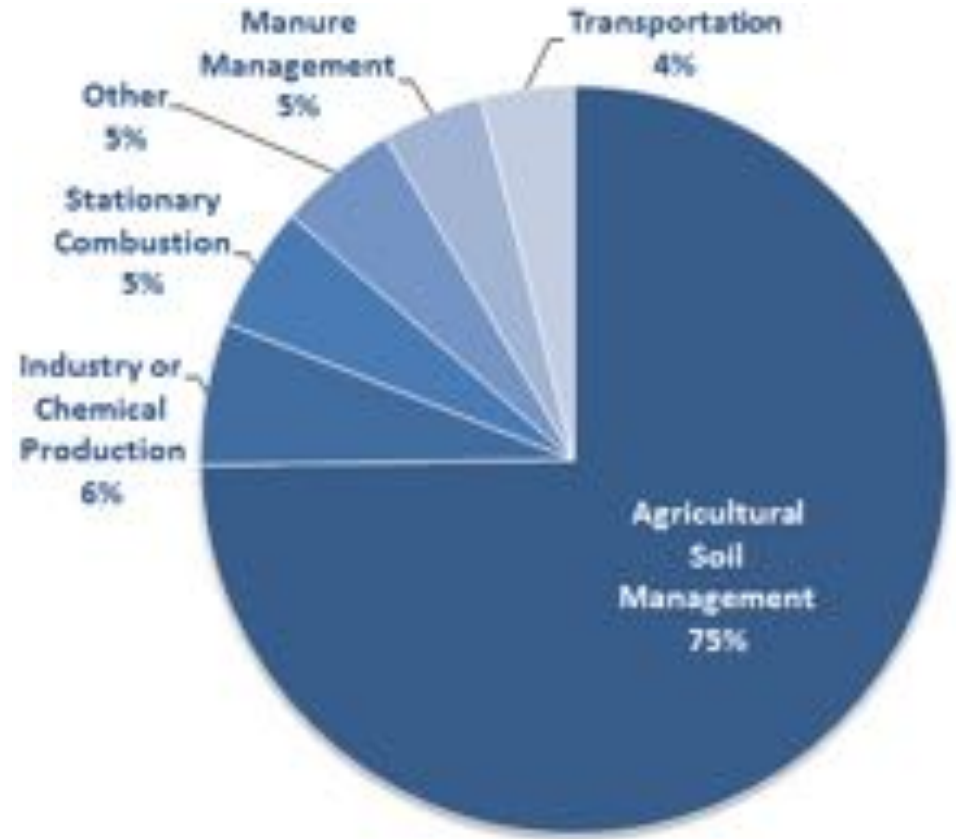
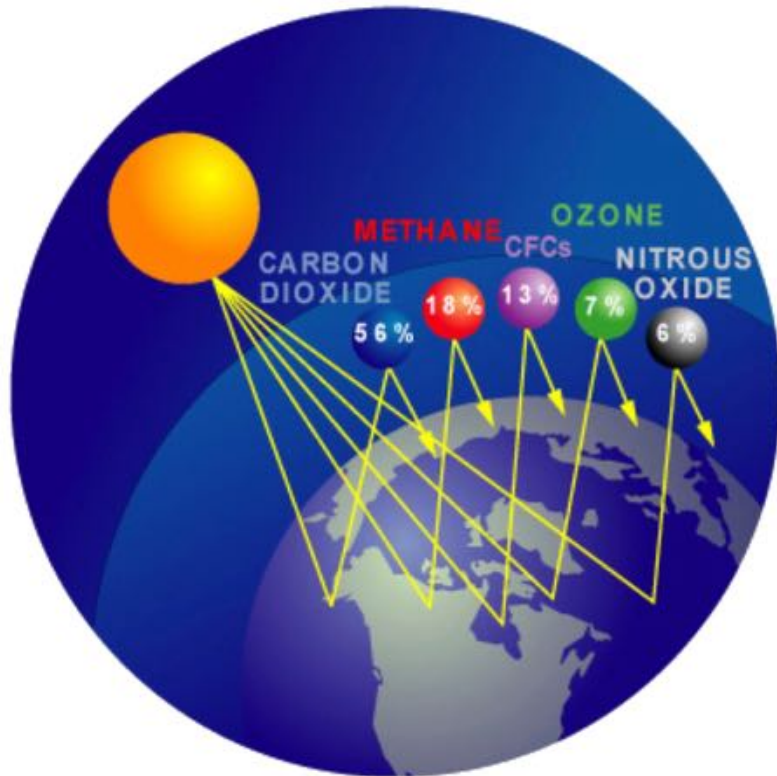


# Computational framework

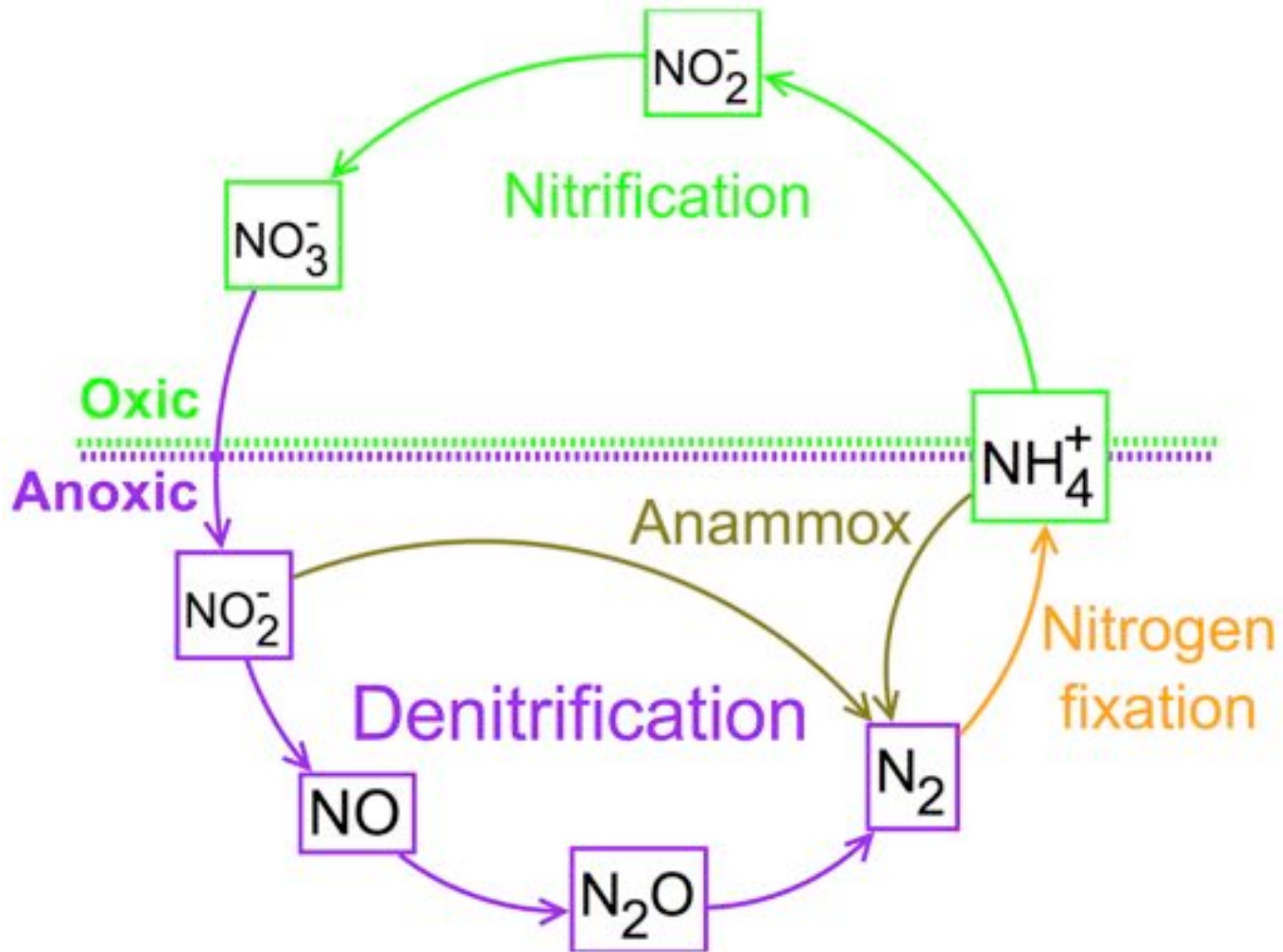
- NETLOGO
  - Open
  - Widespread
  - IBM
  - Friendly use



# Denitrification



# Denitrification – What is it?



# General Objective

To develop an IBM to study the denitrification process driven by denitrifying bacteria, using thermodynamic principles to write microbial metabolic reactions as the centre of the individual sub-model.

**MbT-Tool: An open-access tool based  
on Thermodynamic Electron  
Equivalents Model to obtain microbial-  
metabolic reactions to be used in  
biotechnological process**

# Objectives

## 1

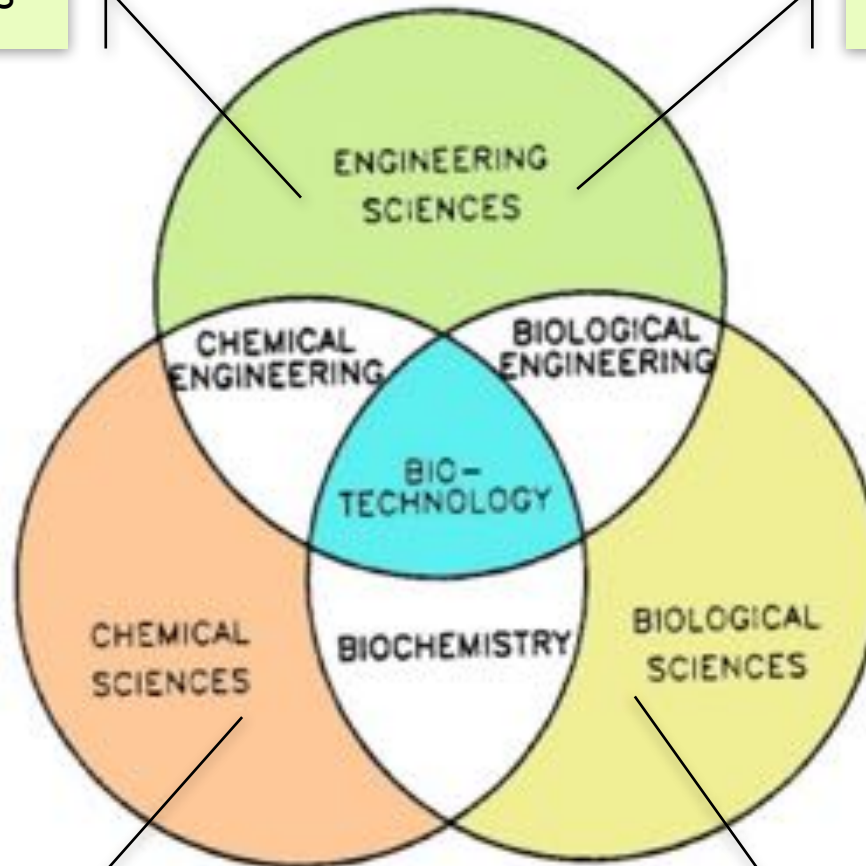
To develop an **open access and open source** computational tool to systematize the **writing** of **microbial metabolic reactions** based on the **thermodynamic** principles to be used as the starting point of modelling projects dealing with biotechnological process carried out by microbes.



# What do we need?

Thermodynamics

Reactor design



Stoichiometry

Pathways

# Thermodynamic approaches

Monod,  
1949

Mayberry et  
al., 1967

Battley,  
1987

Widdowson  
et al., 1988

Roels, 1981

Bedient and  
Rifai, 1992

McCarty,  
2007

Bauchp and  
Elsden,  
1960

McCarty,  
1975

MacQuarrie  
et al., 1990

Frind et al.,  
1990

VanBriesen &  
Rittmann,  
2000

Rittmann &  
McCarty,  
2001

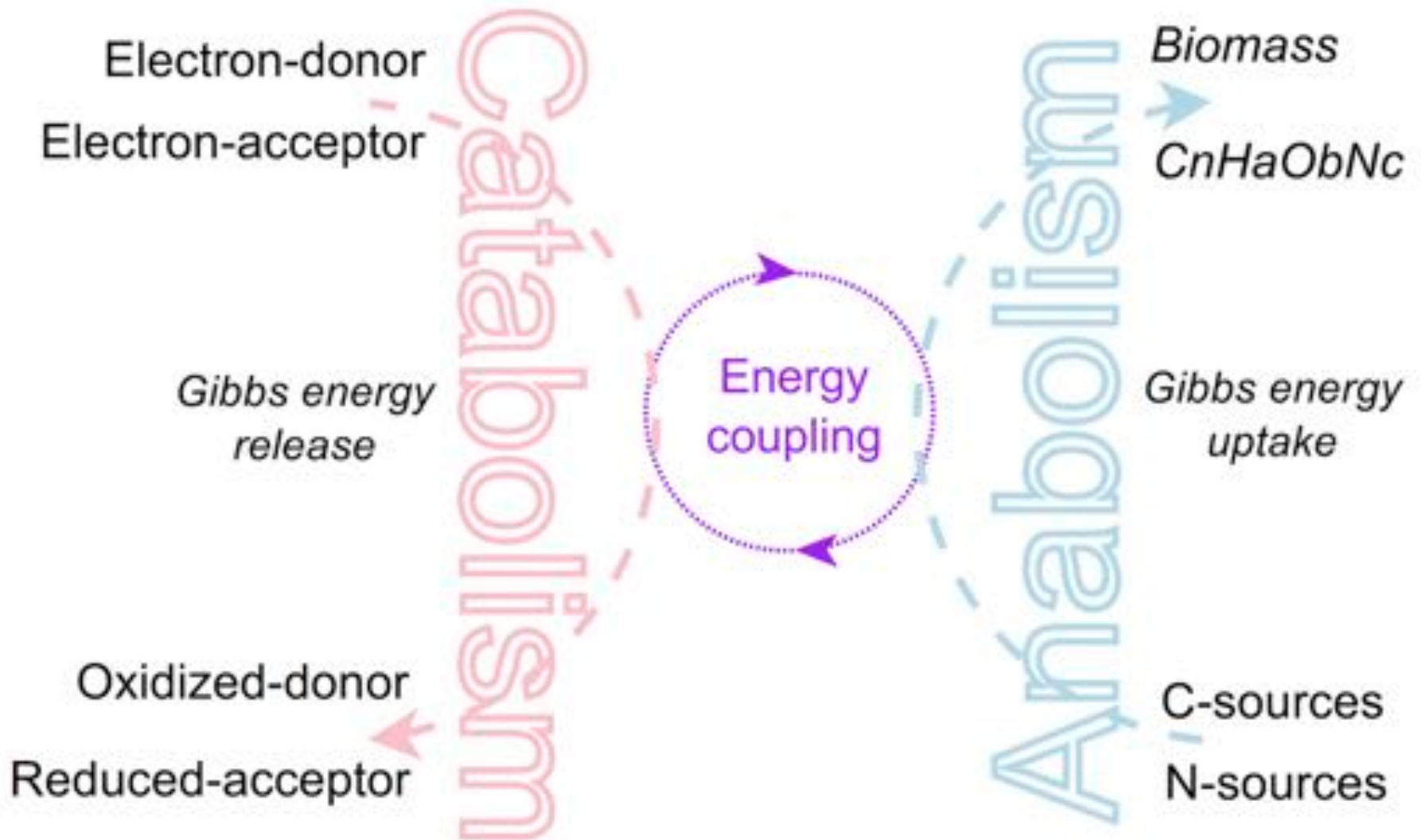
Minkevich  
and Eroshin,  
1973

Linton and  
Stephenson,  
1978

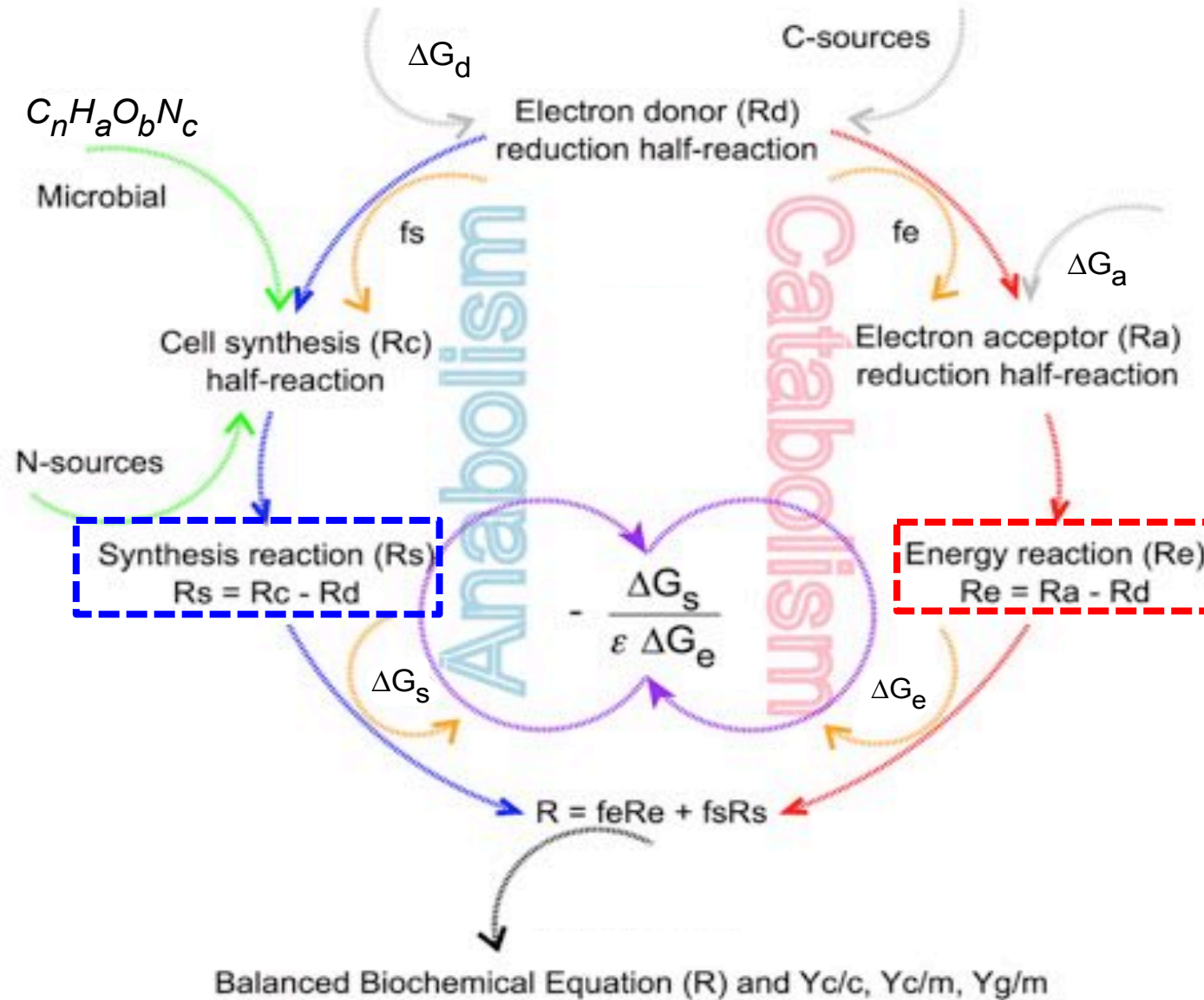
Borden  
et al., 1986

Heijnen &  
van Dijken,  
1992

# Thermodynamic approach



# Thermodynamic approach (McCarty, 2007)



# TEEM: Definitions (McCarty, 2007)

## TEEM 1

$$A = -\frac{\Delta G_s}{\varepsilon \Delta G_e} = \frac{\frac{(\Delta G_{in} - \Delta G_d)}{\varepsilon^n} + \frac{\Delta G_{pc}}{\varepsilon}}{\varepsilon(\Delta G_a - \Delta G_d)} = \frac{f_e^o}{f_s^o}$$

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

## TEEM 2

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

# Energy-Transfer-Efficiency ( $\varepsilon$ )

- Electrons distribution
  - Catabolism
  - Anabolism

If  $\Delta G_{ic} < 1$ , then  $n = -1$ , and

$$\varepsilon = \left( \frac{\Delta G_{pc}}{\Delta G_r [1 - (\gamma_d) / (\gamma_x Y_{C/C})] - \Delta G_{ic}} \right)^{0.5}$$

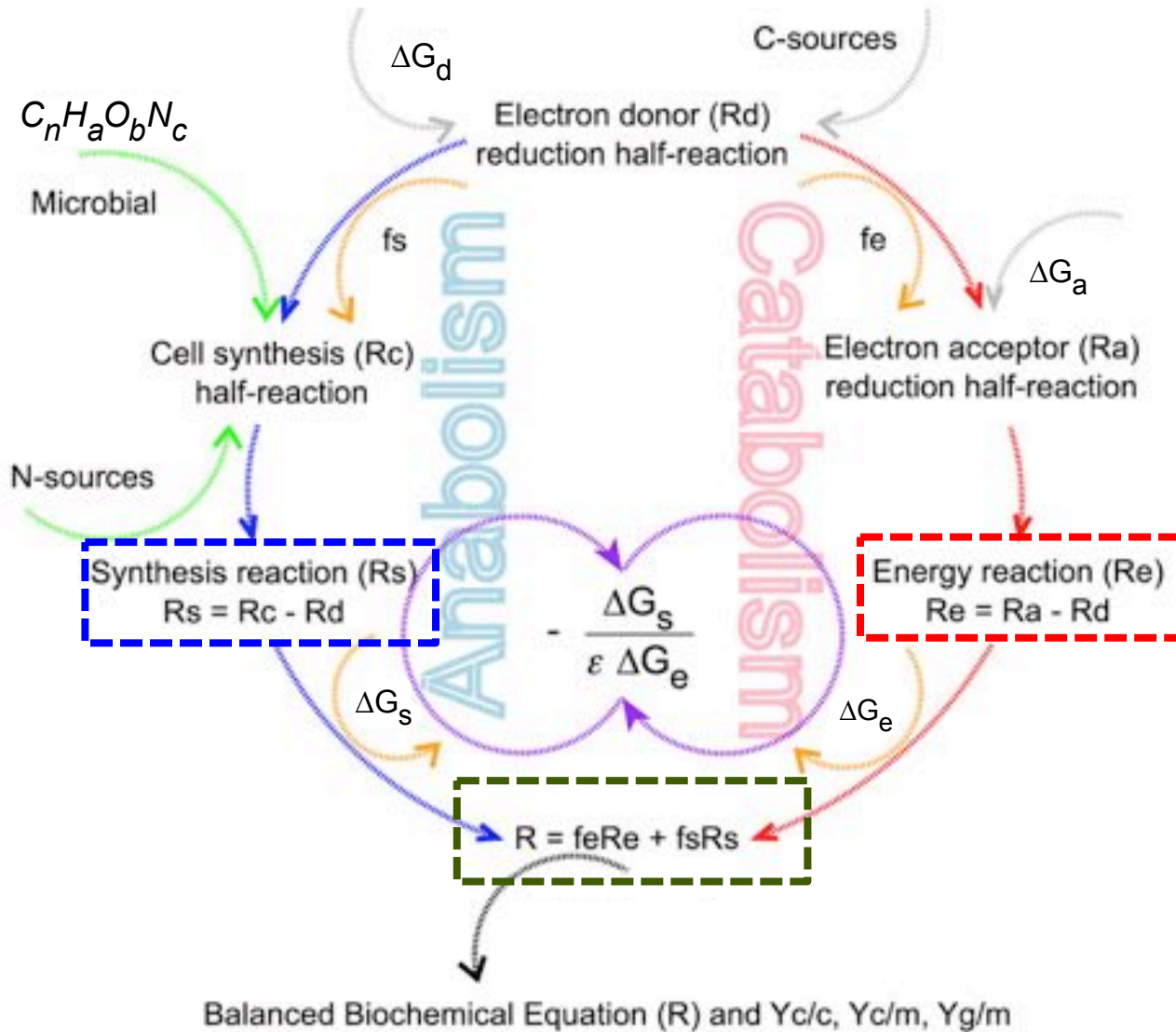
- $\varepsilon = 0.37$
- Standard deviation error of 15%

McCarty, 2007

If  $\Delta G_{ic} > 1$ , then  $n = +1$ , and

$$\varepsilon = \left( \frac{\Delta G_{pc} + \Delta G_{ic}}{\Delta G_r [1 - (\gamma_d) / (\gamma_x Y_{C/C})]} \right)^{0.5}$$

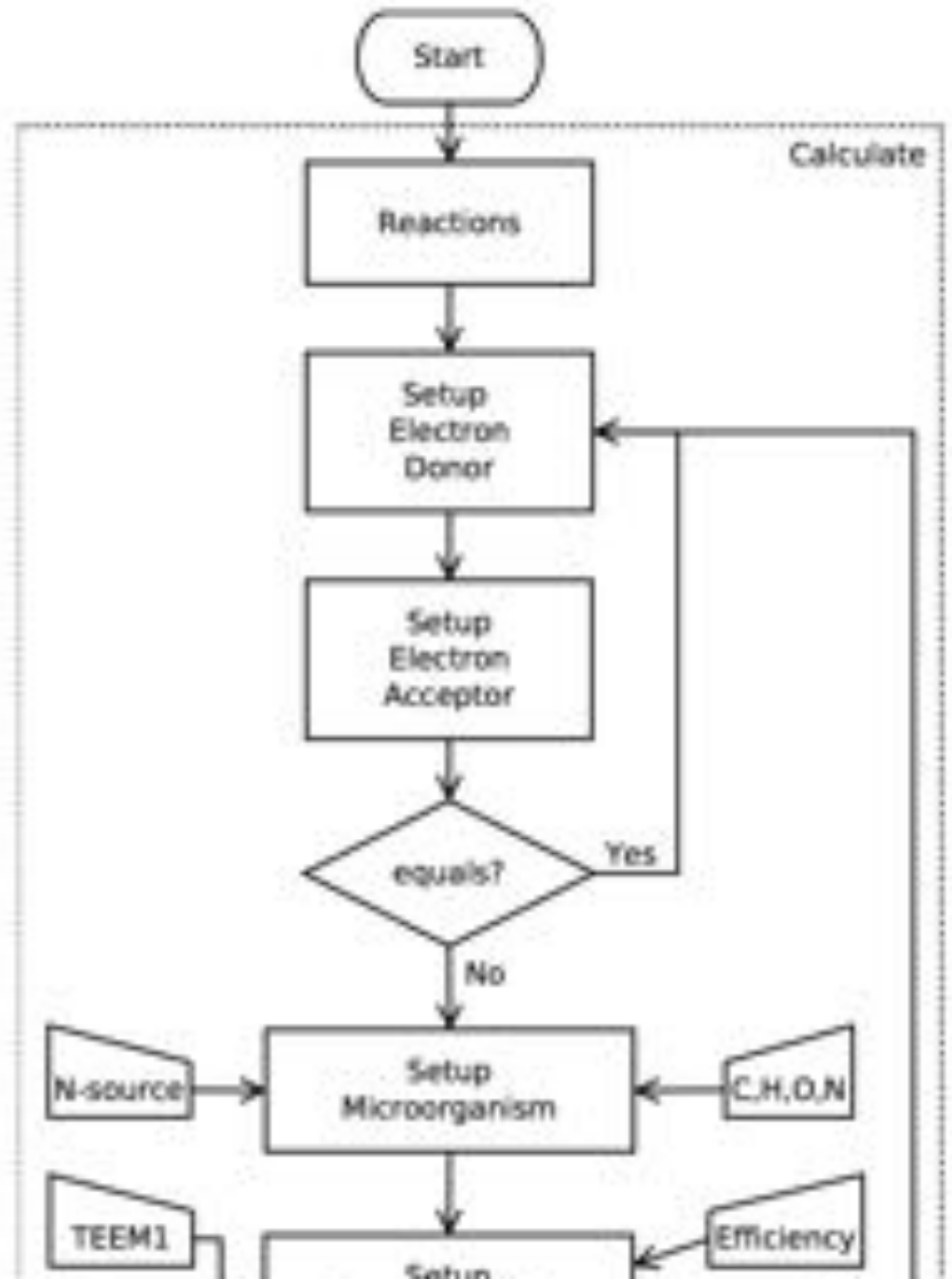
# Thermodynamic approach (McCarty, 2007)







# Flow Diagram



# Software development



# MbT-Tool: Metabolism-based on Thermodynamics



# MbT-Tool: Metabolism-based on Thermodynamics

Electron_donor	Microorganism
Glucose	C6.33H10.21O3.53N - Saccharomyce
--- Organic Reactions ---	
Acetate	N2O -> NO
Alanine	N2 -> N2O
Benzoate	H2S + HS- -> (SO4)2-
Citrate	H2S + HS- -> (SO3)2-
Ethanol	(SO3)2- -> (SO4)2-
Formate	S -> (SO4)2-
Glucose	(S2O3)2- -> (SO4)2-
Glutamate	H2O -> O2
Glycerol	
Glycine	
Lactate	
Methane	
Methanol	
Palmitate	
Propionate	
Pyruvate	
Succinate	
NTA - Acid nitrilotriacetic	
--- Inorganic Reactions ---	
NH4+ -> NO3-	
NH4+ -> NO2-	
NH4+ -> N2	
Fe2+ -> Fe3+	
H2 -> H+	
NO2- -> NO3-	
NO -> NO3-	
N2O -> NO3-	
NO + N2O -> NO3-	
N2 -> NO3-	
NO -> NO2-	
N2 -> NO2-	

**N-Source**

NH4+ ▼

NH4+

NO3-

NO2-

N2

Microorganism	N-Source
C6.33H10.21O3.53N - Saccharomyces cerevisiae, glucose	NH4+
C5H7O2N - Casein, aerobic	
C7H12O4N - Acetate, Ammonia N source aerobic	
C9H15O5N - Acetate, Nitrate N source aerobic	
C9H16O5N - Acetate, Nitrite N source aerobic	
C4.9H9.4O2.9N - Acetate, Methanogenic	
C4.7H7.7O2.1N - Octanoate, Methanogenic	
C4.9H9O3N - Glycine, Methanogenic	
C5H8.8O3.2N - Leucine, Methanogenic	
C4.1H6.8O2.2N - Nutrient	
C5.1H8.5O2.5N - Glucose, Nutrient	
C5.3H9.1O2.5N - Starch, Nutrient	
C5H8O2N - Bacteria, acetate	
C5H8.33O0.81N - Bacteria, acetate	
C4H8O2N - Bacteria, Undefined	
C4.17H7.42O1.38N - Aerobacterium aerogenes	
C4.54H7.91O1.95N - Klebsiella pneumoniae	
C4.17H7.21O1.79N - Klebsiella pneumoniae	
C4.16H8O1.25N - Escherichia coli, undefined	
C3.85H6.69O1.78N - Escherichia coli, glucose	
C6.33H10.21O3.53N - Saccharomyces cerevisiae, glucose	
C4H7.2O1.93N - Paracoccus denitrificans, succinate	
C5H9O2.5N - Agrobacterium tumefaciens, succinate	
C4.17H8O1.75N - Bacteria, Undefined	
C(n)H(a)O(b)N(c) - Bacteria, Generic	

**Thermodynamics**

TEEM\_2 ▼

TEEM\_1

TEEM\_2

# MbT-Tool

(rd) Electron donor --> NTA - Acid nitrilotriacetic :  
+ 0.0556 NH<sub>4</sub><sup>+</sup> + 0.3333 HCO<sub>3</sub><sup>-</sup> + 1.1111 H<sup>+</sup> + 1 e<sup>-</sup> --> + 0.0556 (C<sub>6</sub>H<sub>6</sub>O<sub>6</sub>N)<sub>3</sub><sup>-</sup> + 0.6667 H<sub>2</sub>O  
[ ΔG = 68.889 KJ/e-eq ]

(ra) Electron acceptor --> NO<sub>3</sub><sup>-</sup> -> N<sub>2</sub> :  
+ 0.2 NO<sub>3</sub><sup>-</sup> + 1.2 H<sup>+</sup> + 1 e<sup>-</sup> --> + 0.1 N<sub>2</sub> + 0.6 H<sub>2</sub>O [ ΔG = -72.2 KJ/e-eq ]

(rc) Biomass half reaction : C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N - Casein, aerobic , N-Source : NH<sub>4</sub><sup>+</sup>  
0.2 CO<sub>2</sub> + 0.05 HCO<sub>3</sub><sup>-</sup> + 0.05 NH<sub>4</sub><sup>+</sup> + 1 H<sup>+</sup> + 1 e<sup>-</sup> --> 0.05 C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N + 0.45 H<sub>2</sub>O  
[ ΔG = 18.799 KJ/e-eq ]

Energy reaction :  
+ 0.0556 (C<sub>6</sub>H<sub>6</sub>O<sub>6</sub>N)<sub>3</sub><sup>-</sup> + 0.0667 H<sub>2</sub>O + 0.2 NO<sub>3</sub><sup>-</sup> + 0.0889 H<sup>+</sup> --> + 0.0556 NH<sub>4</sub><sup>+</sup> + 0.3333 HCO<sub>3</sub><sup>-</sup> + 0.1 N<sub>2</sub>

Synthesis reaction :  
+ 0.0556 (C<sub>6</sub>H<sub>6</sub>O<sub>6</sub>N)<sub>3</sub><sup>-</sup> + 0.2 CO<sub>2</sub> + 0.2167 H<sub>2</sub>O --> 0.05 C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N + 0.1111 H<sup>+</sup> + 0.0056 NH<sub>4</sub><sup>+</sup> + 0.2833 HCO<sub>3</sub><sup>-</sup>

Balanced equation using TEEM\_1 :  
[ fe = 0.5 ] [ fs = 0.5 ] [ e = 0.33 ]  
+ 0.0556 (C<sub>6</sub>H<sub>6</sub>O<sub>6</sub>N)<sub>3</sub><sup>-</sup> + 0.1008 CO<sub>2</sub> + 0.1423 H<sub>2</sub>O + 0.0992 NO<sub>3</sub><sup>-</sup> --> 0.0252 C<sub>5</sub>H<sub>7</sub>O<sub>2</sub>N + 0.0119 H<sup>+</sup> + 0.0304 NH<sub>4</sub><sup>+</sup> + 0.3081 HCO<sub>3</sub><sup>-</sup> + 0.0496 N<sub>2</sub>

Yield prediction :  
Yg/m = 51.311 [ grams\_cells/mol\_donor ]  
Yc/m = 2.268 [ mol\_C\_cells/mol\_donor ]  
Yc/c = 0.378 [ mol\_C\_cell/mol\_C\_donor ]



COMPUTATIONAL  
AND STRUCTURAL  
BIOTECHNOLOGY  
JOURNAL

journal homepage: [www.elsevier.com/locate/csbj](http://www.elsevier.com/locate/csbj)



Communication

## MbT-Tool: An open-access tool based on Thermodynamic Electron Equivalents Model to obtain microbial-metabolic reactions to be used in biotechnological process

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<sup>b</sup> Department of Agri-Food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Edifici D4, Esteve Terradas 8, 08800 Castelldefels, Barcelona, Spain

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<http://dx.doi.org/10.1016/j.csbj.2016.08.001>

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**INDISIM-Paracoccus: An individual-based and thermodynamic model to deal with *Paracoccus denitrificans* in a bioreactor**

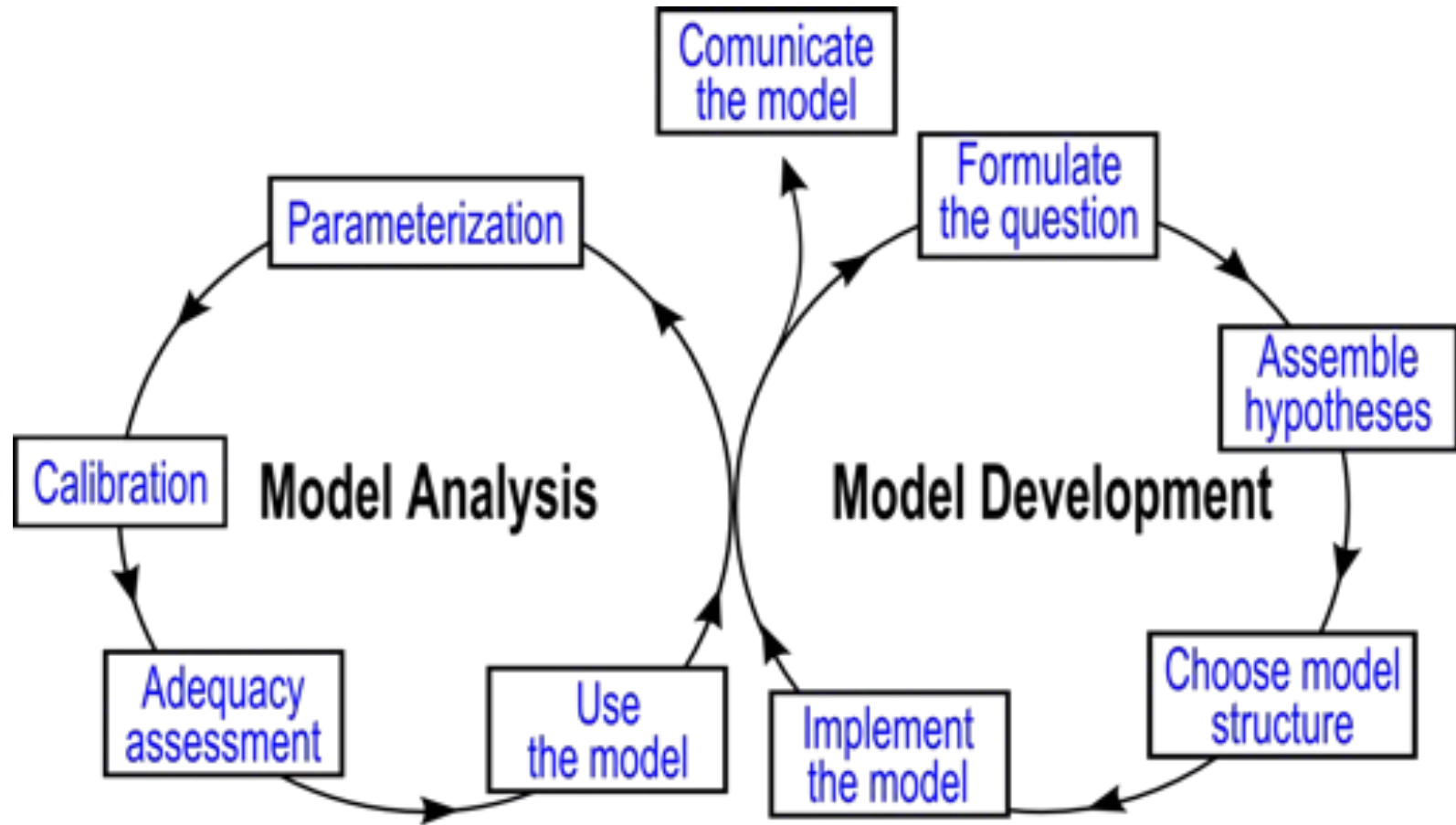
# Objective

2

To use **microbial metabolic reactions** in the design, implementation and parameterization of the **thermodynamic behaviour-rules** embedded in the metabolic sub-model of an IBM for denitrifying bacteria in the framework of **INDISIM**, implementing the model in an open-access programming platform to achieve a simulator that facilitates exploring the effects of **denitrifying bacterial** metabolic sub-model.



# Modelling cycle\*

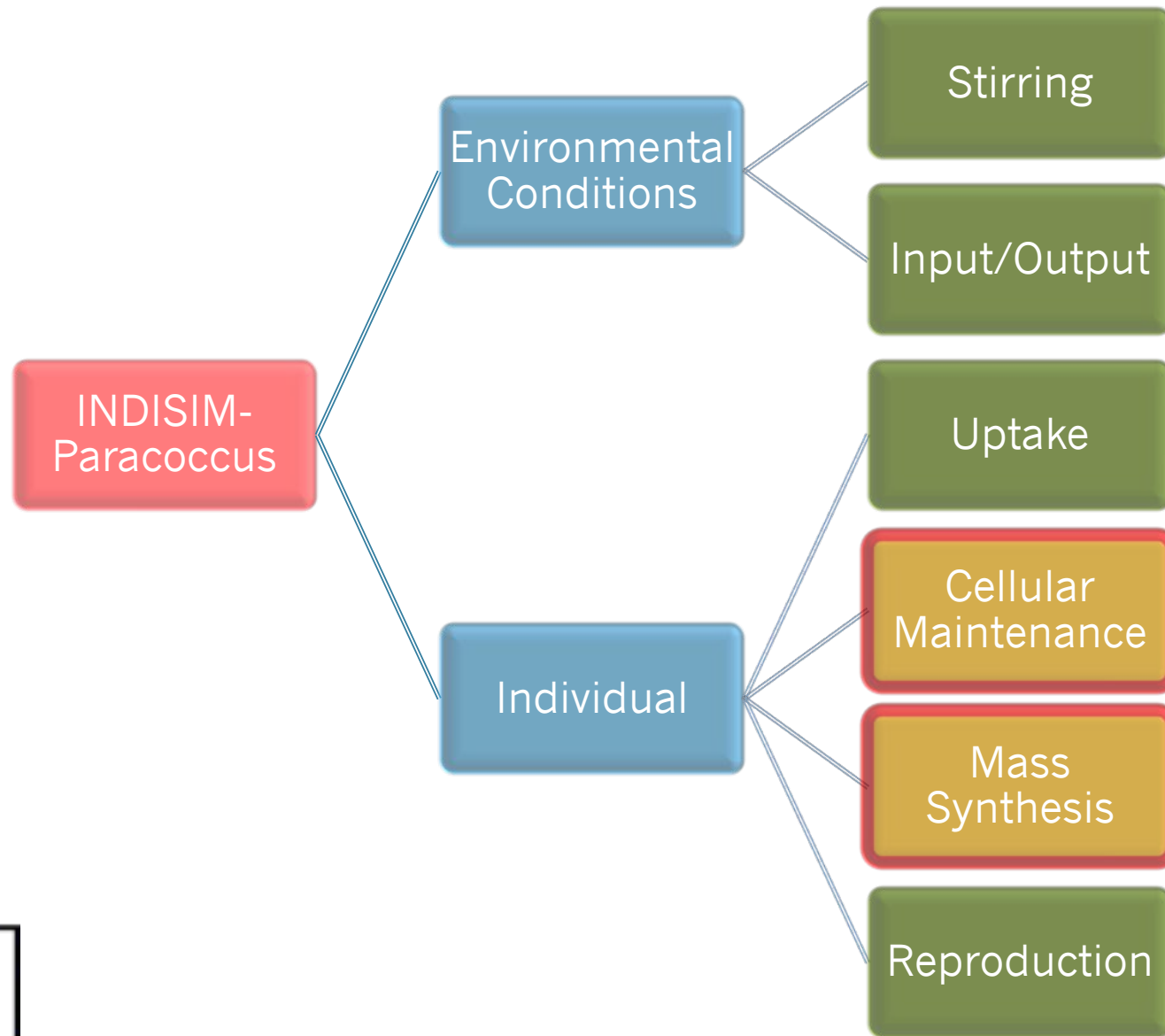


\*(Xavier Portell, 2014) PhD Thesis presentation. Individual-based observations and individual-based simulations to study *Saccharomyces cerevisiae* cultures.

Formulate the question

## INDISIM-Paracoccus:

# INDISIM-Paracoccus submodels



Assemble hypotheses

# Cellular maintenance *P. denitrificans*

Rd	Re	Ra
	$(C_4H_4O_4)^{2-} + 3.5 O_2 \rightarrow 2 CO_2 + 2 HCO_3^- + H_2O$	$O_2$
Succinate	$(C_4H_4O_4)^{2-} + 7 NO_3^- \rightarrow 2 CO_2 + 2 HCO_3^- + 7 NO_2^- + H_2O$	$NO_3^-$
	$(C_4H_4O_4)^{2-} + 14 NO_2^- + 14 H^+ \rightarrow 14 NO + 2 CO_2 + 2 HCO_3^- + 2 H_2O$	$NO_2^-$
	$(C_4H_4O_4)^{2-} + 14 NO \rightarrow 7 N_2O + 2 CO_2 + 2 HCO_3^- + H_2O$	$NO$
	$(C_4H_4O_4)^{2-} + 7 N_2O \rightarrow 7 N_2 + 2 CO_2 + 2 HCO_3^- + H_2O$	$N_2O$

Assemble hypotheses

(Araujo et al., 2015) Thermodynamic Behavior-Rules for a Bacterial Individual-Based Model to Study the Denitrification Process ([MathMod - February 2015 – IFAC,2015- V.8 - pp: 743-748](#))

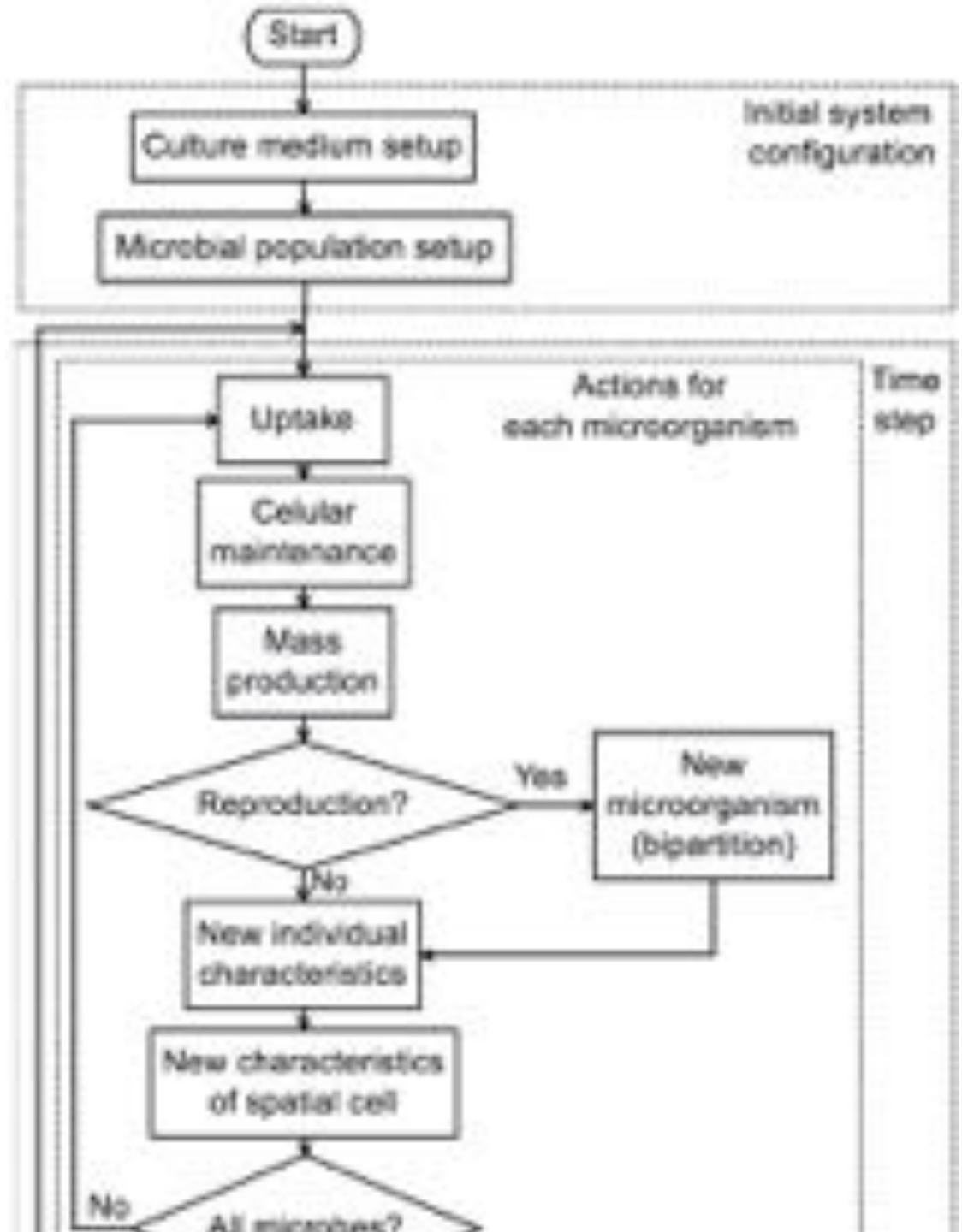
# Microbial Metabolic Reactions *P. denitrificans*

#	Microbial metabolic reactions (R)	$\epsilon$
R1	$(C_4H_4O_4)^{2-} + 0.60 NH_4^+ + 1.04 O_2$ $\rightarrow 0.81 C_3H_{5.4}O_{1.45}N_{0.75} + 0.19 CO_2 + 1.40 HCO_3^- + 0.34 H_2O$	0.84
R2	$(C_4H_4O_4)^{2-} + 0.08 NH_4^+ + 0.52 NO_3^- + 1.05 H^+ + 0.18 H_2O$ $\rightarrow 0.80 C_3H_{5.4}O_{1.45}N_{0.75} + 0.20 CO_2 + 1.40 HCO_3^-$	0.90
R3	$(C_4H_4O_4)^{2-} + 0.30 NH_4^+ + 4.55 NO_3^-$ $\rightarrow 0.40 C_3H_{5.4}O_{1.45}N_{0.75} + 4.55 NO_2^- + 1.10 CO_2 + 1.70 HCO_3^- + 0.67 H_2O$	0.41
R4	$(C_4H_4O_4)^{2-} + 0.58 NH_4^+ + 4.55 NO_2^- + 4.55 H^+$ $\rightarrow 0.77 C_3H_{5.4}O_{1.45}N_{0.75} + 4.55 NO + 0.26 CO_2 + 1.42 HCO_3^- + 2.64 H_2O$	0.84
R5	$(C_4H_4O_4)^{2-} + 0.58 NH_4^+ + 4.55 NO$ $\rightarrow 0.77 C_3H_{5.4}O_{1.45}N_{0.75} + 2.28 N_2O + 0.26 CO_2 + 1.42 HCO_3^- + 0.36 H_2O$	0.56
R6	$(C_4H_4O_4)^{2-} + 0.58 NH_4^+ + 2.28 N_2O$ $\rightarrow 0.77 C_3H_{5.4}O_{1.45}N_{0.75} + 2.28 N_2 + 0.26 CO_2 + 1.42 HCO_3^- + 0.36 H_2O$	0.53

Assemble hypotheses

(Araujo et al., 2015) Thermodynamic Behavior-Rules for a Bacterial Individual-Based Model to Study the Denitrification Process ([MathMod - February 2015 – IFAC,2015- V.8 - pp: 743-748](#))

# Flow Diagram



Choose model structure

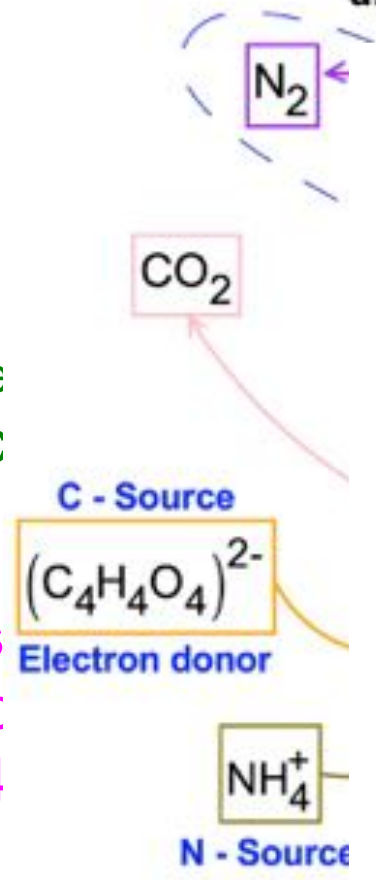
# Experimental conditions

Choose model structure

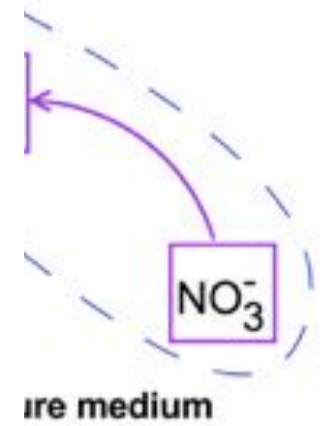
**INDISIM-Paracoccus: an Individual-Based and Thermodynamic model for a denitrifying bacterium**

- Batch culture
- ✓ Aerobic
- ✓ 24 hours

- Continuous
- ✓ Anoxic p
- ✓ From 24
- ✓ Dilution

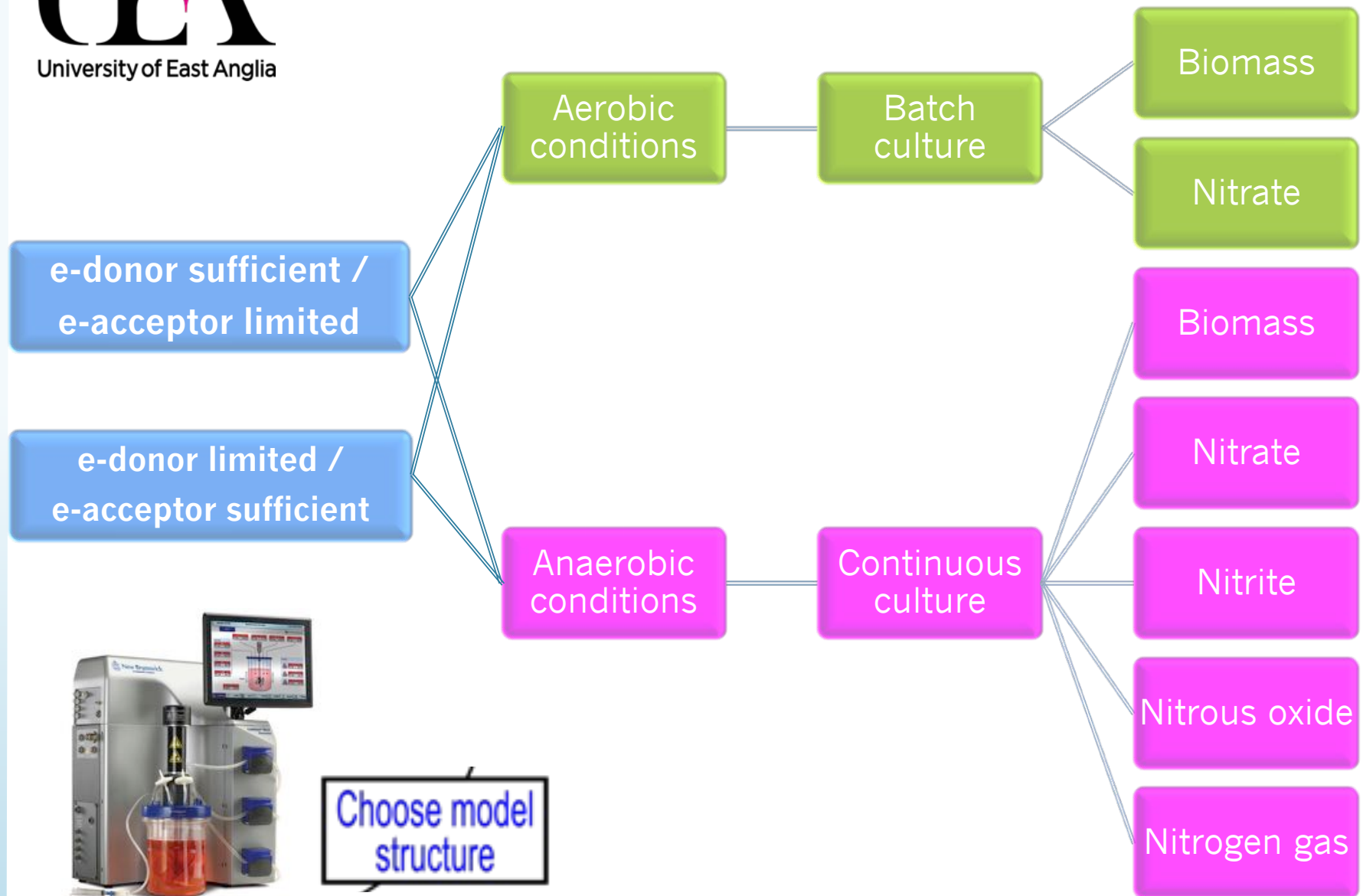


Electron acceptors



(Felgate et al., 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.

# Experimental data

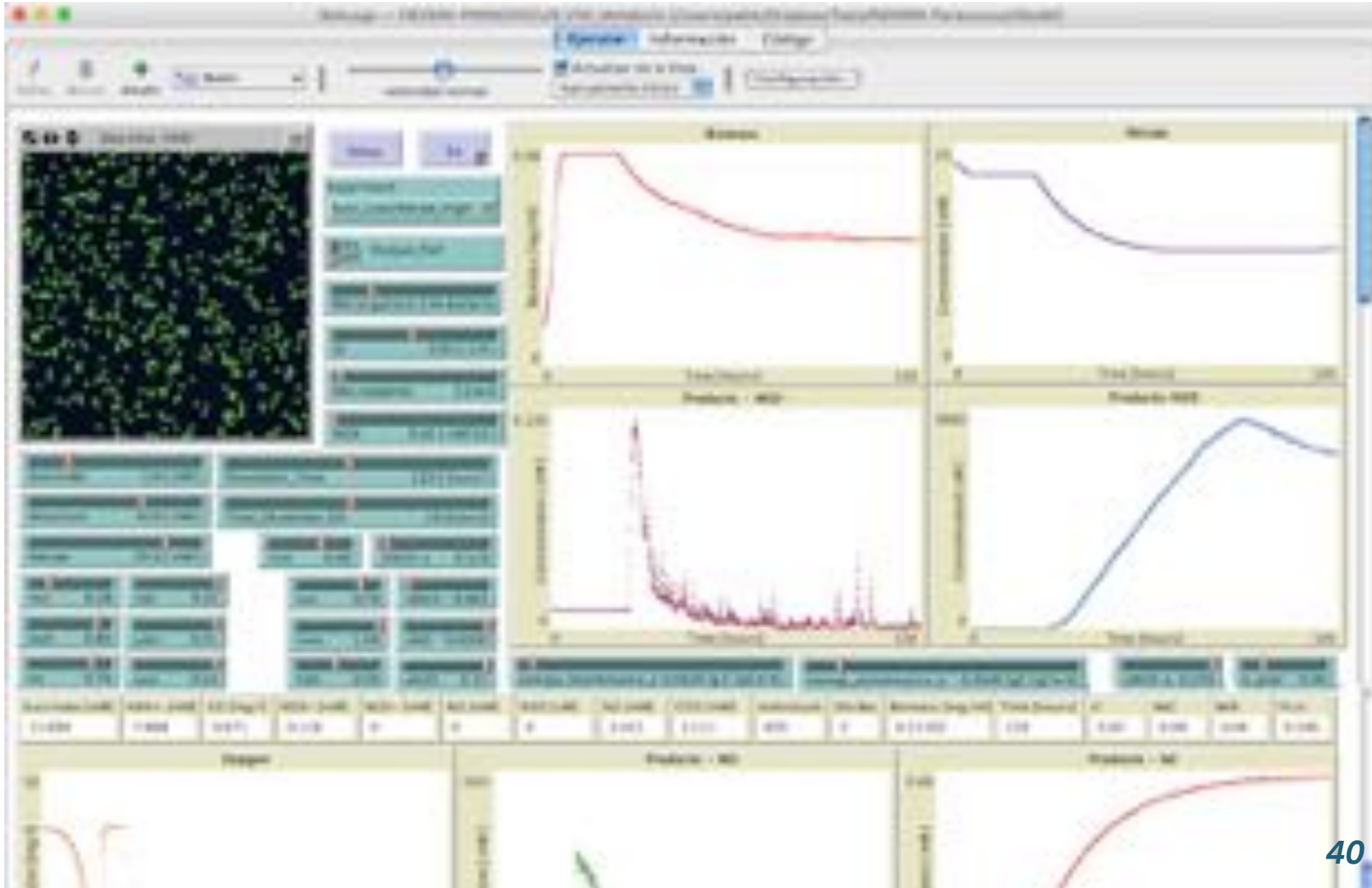


(Felgate et al., 2012) The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways.



Implement the model

# NetLogo implementation



Parameterization

# Model parameters

Nutrient	Culture medium initial concentration [mM] Felle et al. (2012)	Availability coefficient - $a_i$ ( $h^{-1}$ ) fixed according to Dab	Uptake-rate - $u_i$ ( $mol_{nutrient} \cdot mol_{mass}^{-1} \cdot h^{-1}$ )		
			Testing values		
			Low (L)	Medium (M)	High (H)
Succinate	5 - 10	0.28	0.065	0.13	0.52
Ammonium	5	0.63	-----	-----	0.31
Oxygen	236	0.5	-----	-----	0.54
Nitrate-a (aerobic)	9983 -	0.63	0.034	0.068	0.27
Nitrate-x (anaerobic)	1.6095	0.63	0.019	0.119	1.19
Nitrite	255 - 0.0112	0.79	0.0062	0.062	0.62
Nitric Oxide	-----	1.00	0.0000062	0.00062	0.62
Nitrous Oxide	0.003 - 0.000028	0.50	0.0031	0.031	0.31

Experimental Values

Model Values

Parameterization

# Model parameters

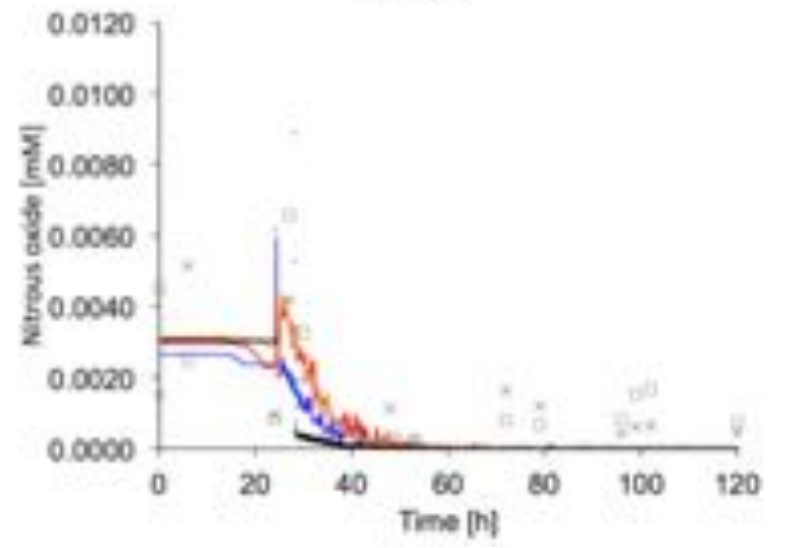
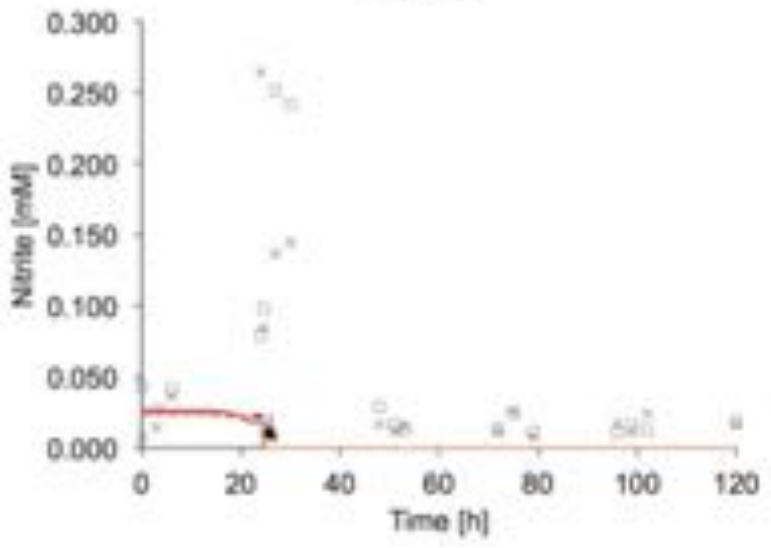
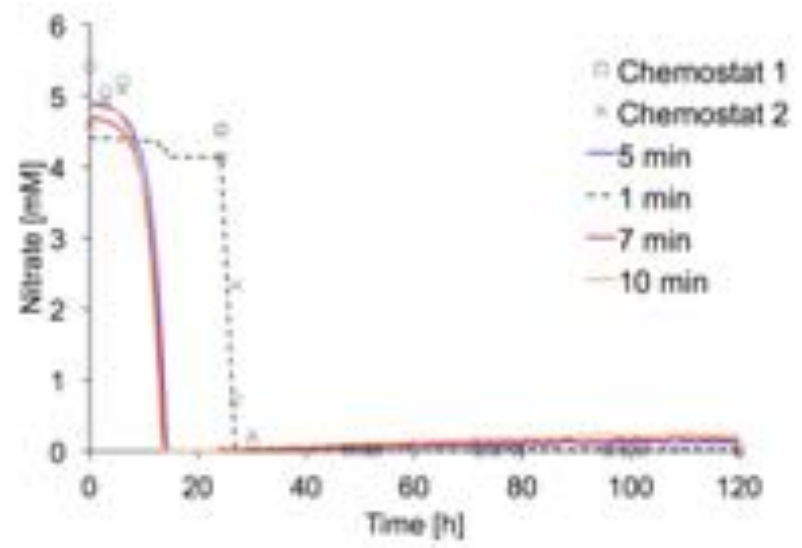
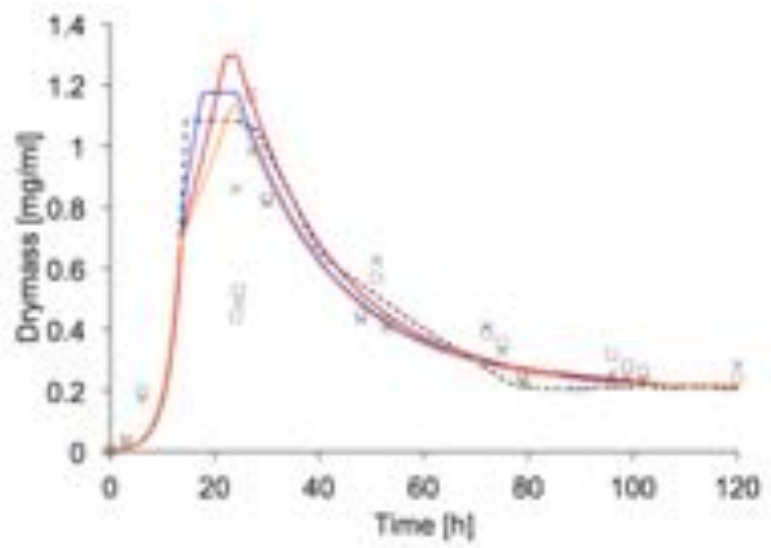
Other bacterial parameters			
Parameter	Testing range	Calibrated value	Reference
Cellular maintenance ( $gC_{donor} \cdot gC_{mic}^{-1} \cdot h^{-1}$ )	-----	0.0020 – 0.0040	Gras et al. (2011) and van Verseveld et al. (1983)
Mass split	-----	0.50 (15% coefficient of variation)	Derived from (Ginovart et al., 2002a)
Small bacterium size ( $\mu m$ )	0.4 – 0.6	0.5	Holt et al. (1994)
Big bacterium size ( $\mu m$ )	0.8 – 1.0	0.9	
Minimum bacterium size at reproduction	-----	75% of big bacterium size (15% coefficient of variation)	Derived from Gras et al., 2011 and (Ginovart et al., 2002a)

Biological published values

Calibration

# Model Pre-calibration

Step size



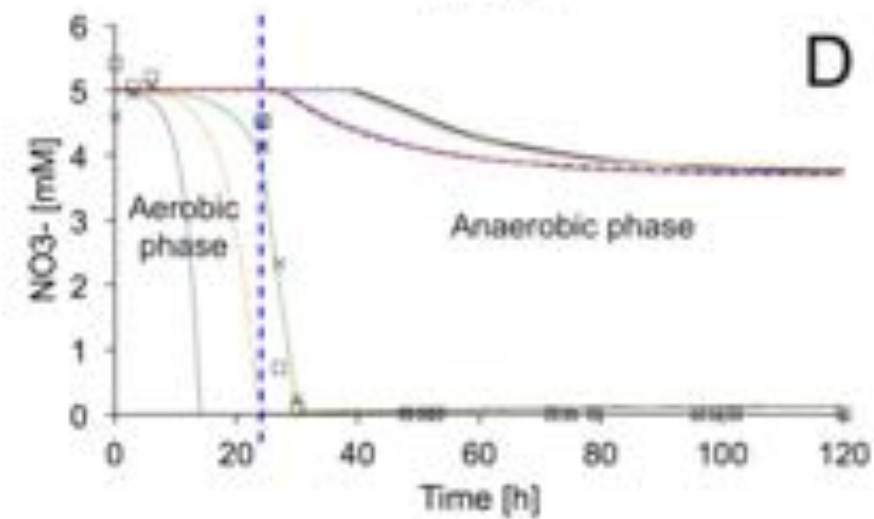
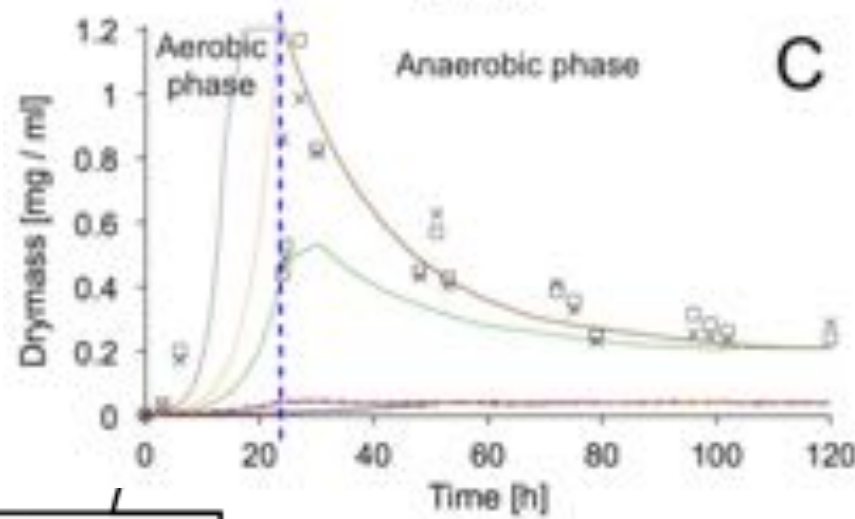
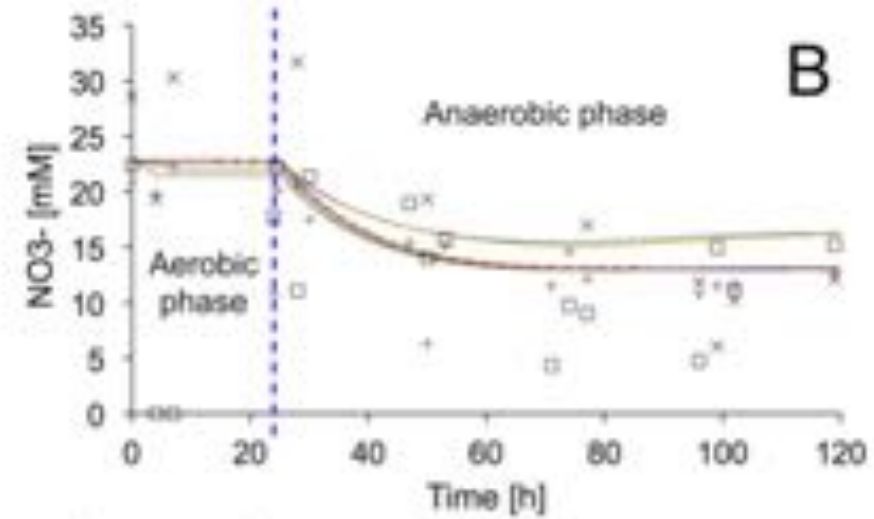
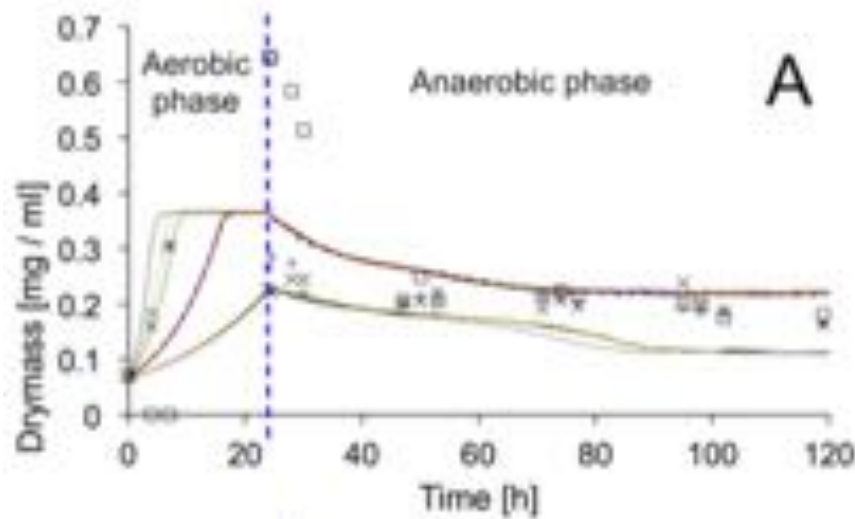
Parameterization

# Model parameters

Nutrient	Culture medium initial concentration [mM] Felgate et al. (2012)	Availability coefficient – $a_i$ ( $h^{-1}$ ) fixed according to <i>Dab</i>	Uptake-rate – $u_i$ ( $mol_{nutrient} \cdot mol_{mass}^{-1} \cdot h^{-1}$ )		
			Testing values		
			Low (L)	Medium (M)	High (H)
Succinate	5 – 20	0.28	0.065	0.13	0.52
Ammonium	10	0.84	-----	-----	0.31
Oxygen	0.236	0.79	-----	-----	0.54
Nitrate-a (aerobic)	4.9983 –	0.63	0.019	0.068	0.27
Nitrate-x (anaerobic)	21.6095		0.019	0.119	1.19
Nitrite	0.0255 – 0.0112	0.79	0.0062	0.062	0.62
Nitric Oxide	-----	1.00	0.0000062	0.00062	0.62
Nitrous Oxide	0.003 – 0.000028	0.50	0.0031	0.031	0.31

Testing Values

# Sensitivity Analysis – Aerobic phase

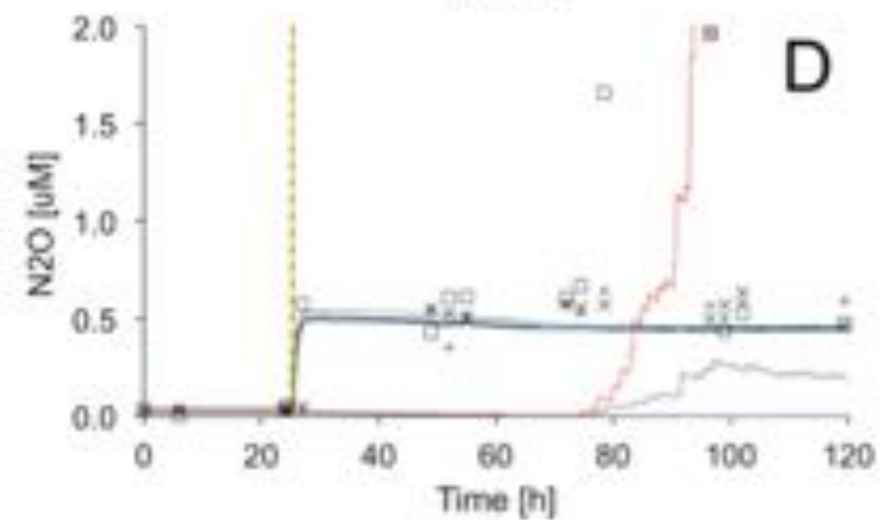
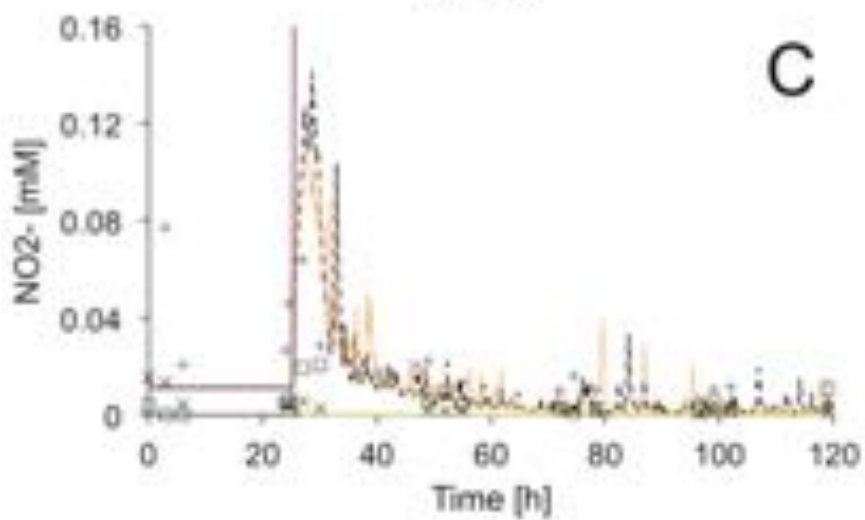
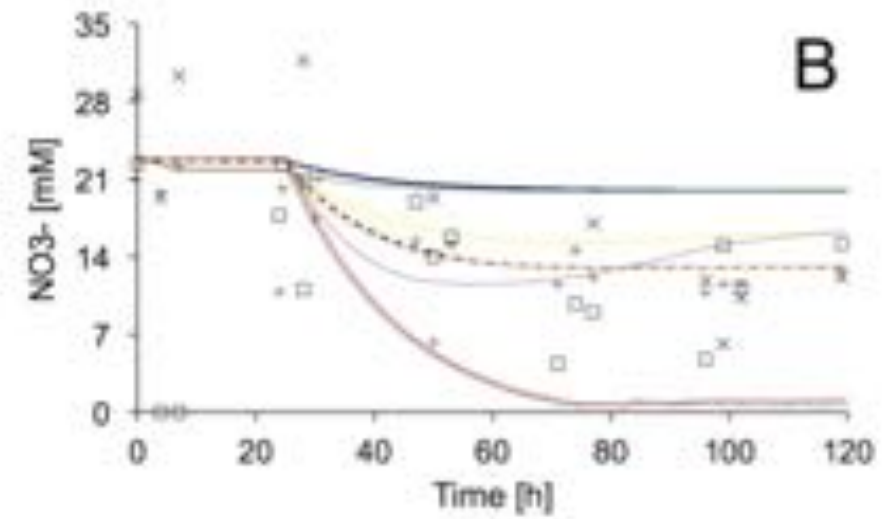
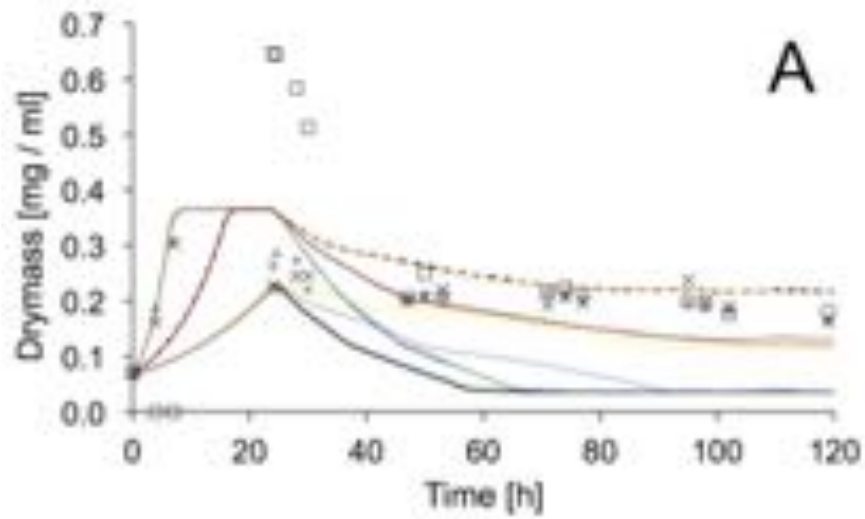


Calibration

Both experiments – Uptake-rates

Calibration

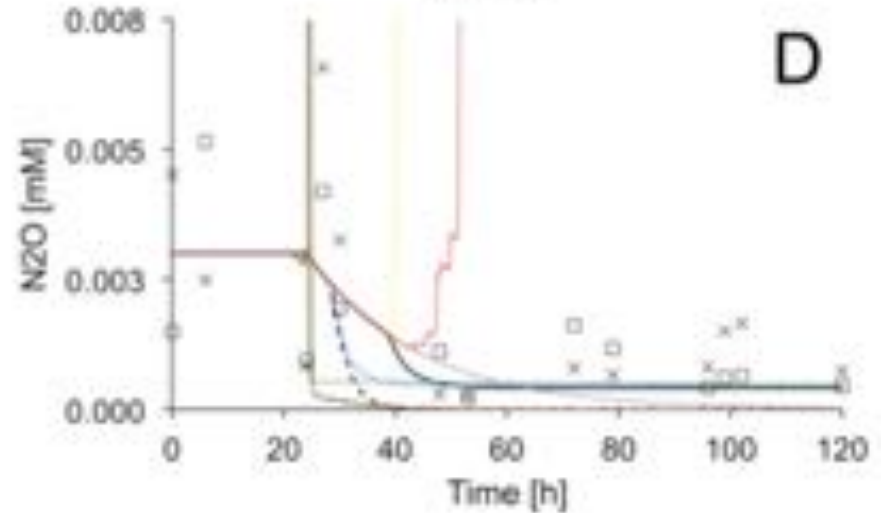
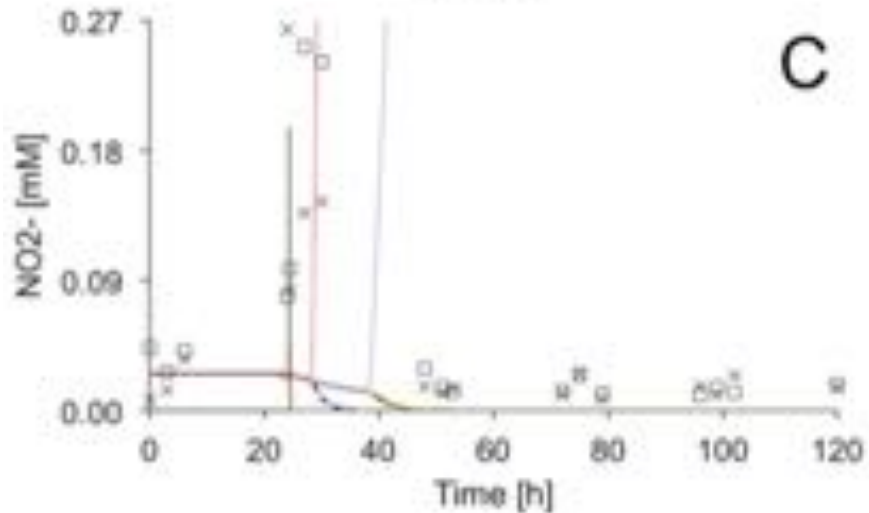
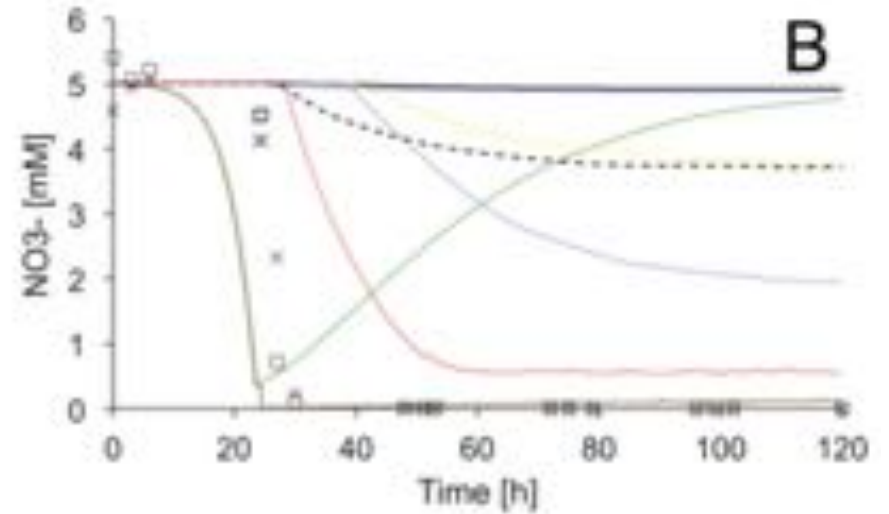
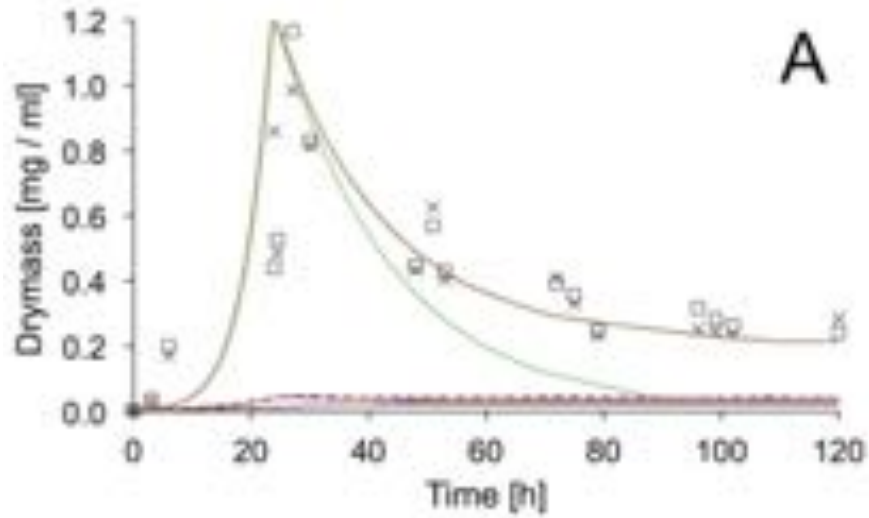
# Sensitivity Analysis – Anoxic phase



**$e^-$ -donor limited /  $e^-$ -acceptor sufficient**

**Calibration**

# Sensitivity Analysis – Anoxic phase



**e<sup>-</sup>-donor sufficient / e<sup>-</sup>-acceptor limited**



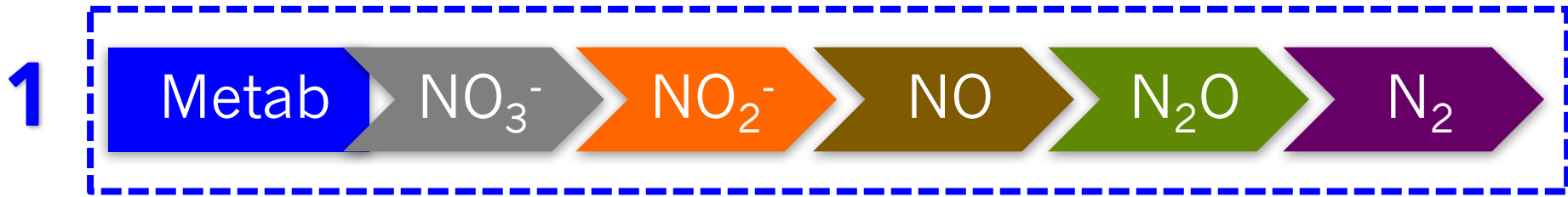
# Objective

## 3

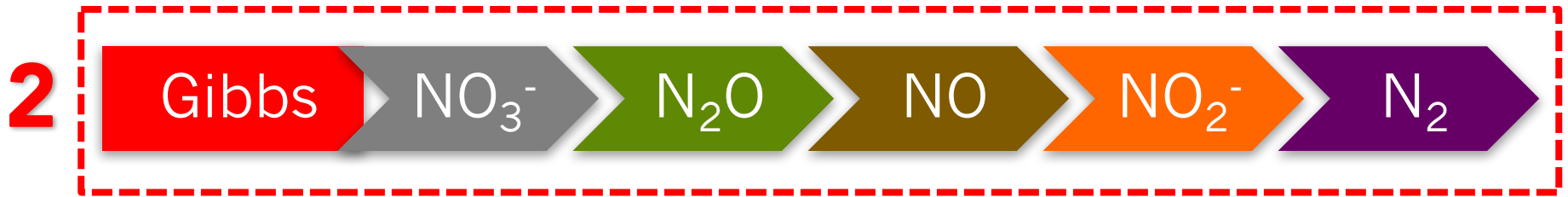
To use the simulator obtained to **test hypotheses and diverse metabolic strategies** for the **individual behaviour** of *Paracoccus denitrificans* in relation to the use of substrates growing in aerobic and anaerobic conditions in a bioreactor, testing the adequacy of the simulation outputs with **experimental published data**

# Two hypotheses to test

The **bacterium prioritizes the use** of those nitrogen oxides with a higher degree of oxidation over others



Denitrification process



The **bacterium goes first** for the more spontaneous reactions.

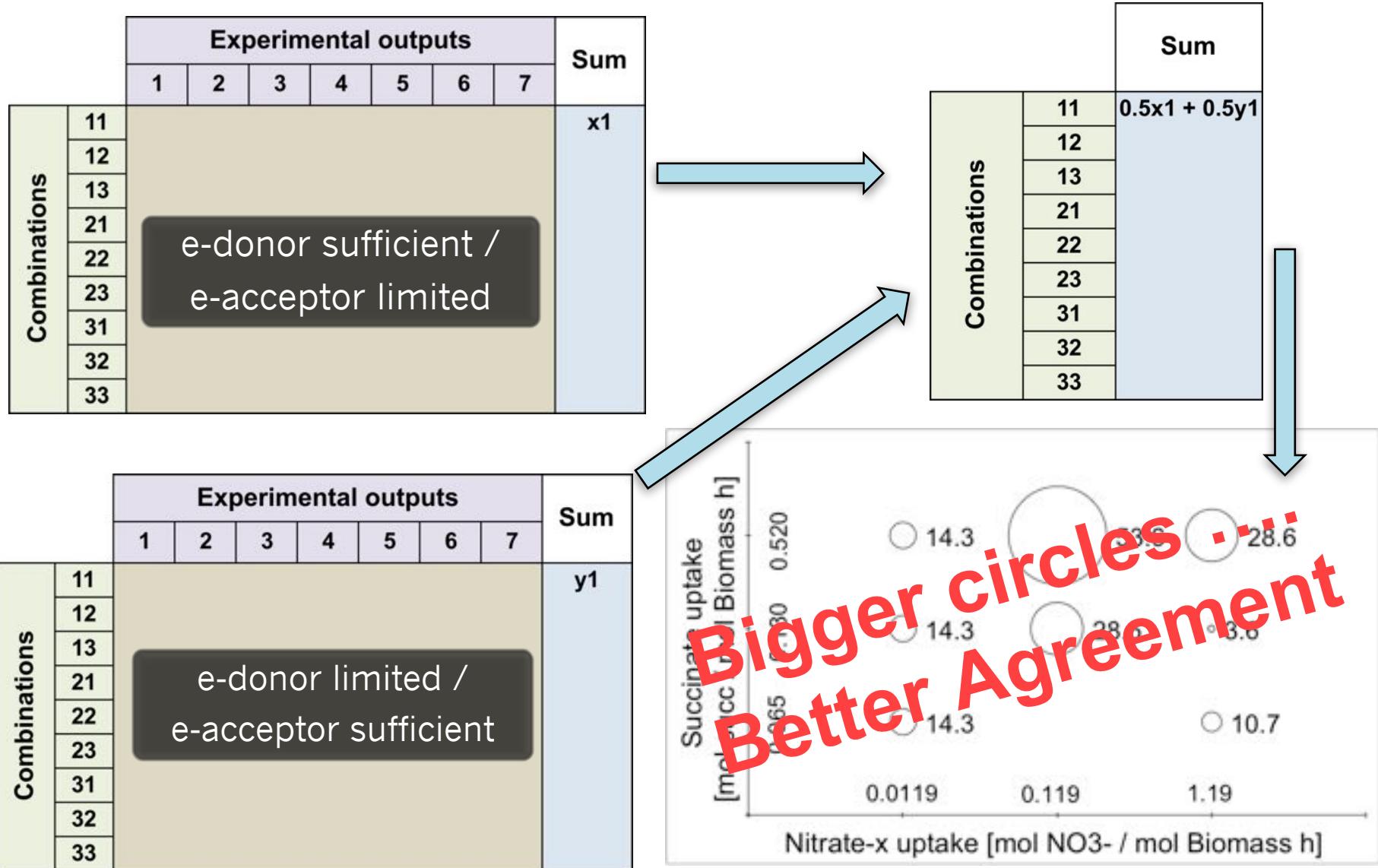
Adequacy  
assessment

# How did we evaluate our model?

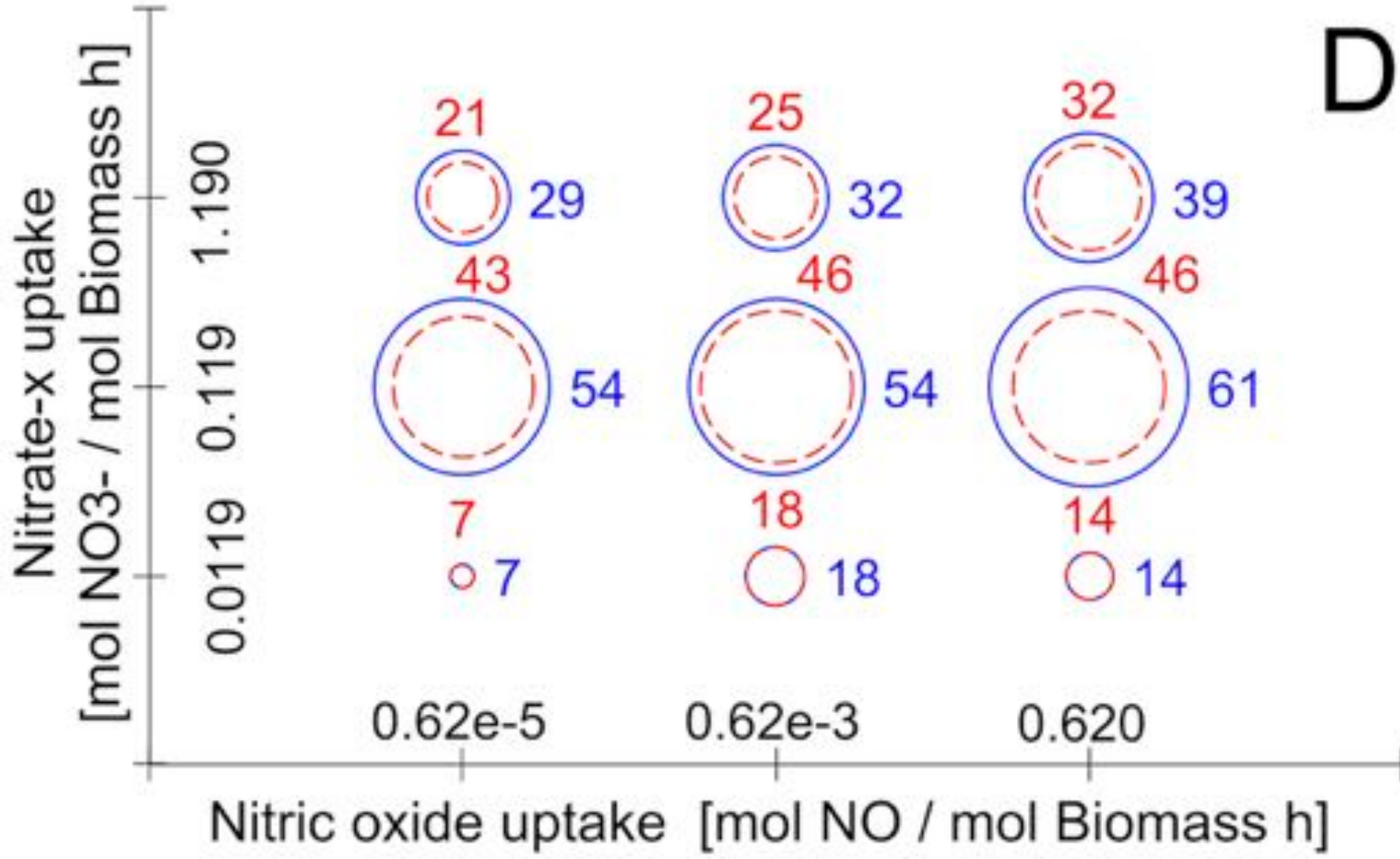
- **Factors:** Specific Uptake-rate (each nutrient - 6)
  - Aerobic phase: Succinate and  $\text{NO}_3^-_{(a)}$
  - Anaerobic phase: Succinate,  $\text{NO}_3^-_{(x)}$ ,  $\text{NO}_2^-$ , NO and  $\text{N}_2\text{O}$
- **Levels:** 3
- **Replicate:** 3
- **Response:** Score
  - 7 time evolutions (2 aerobic and 5 anaerobic phases)
  - 2 Hypotheses

Adequacy  
assessment

# Rating the parameters combination

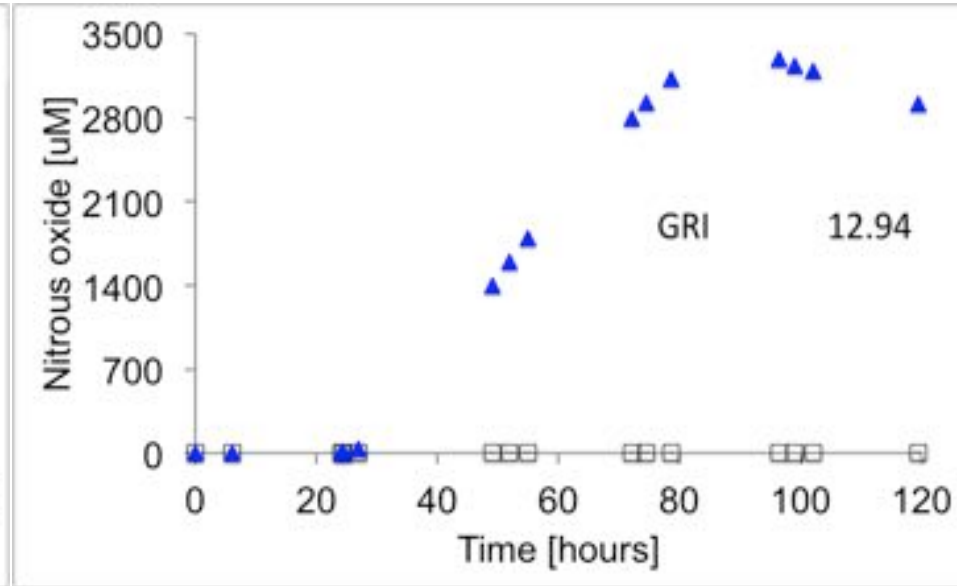
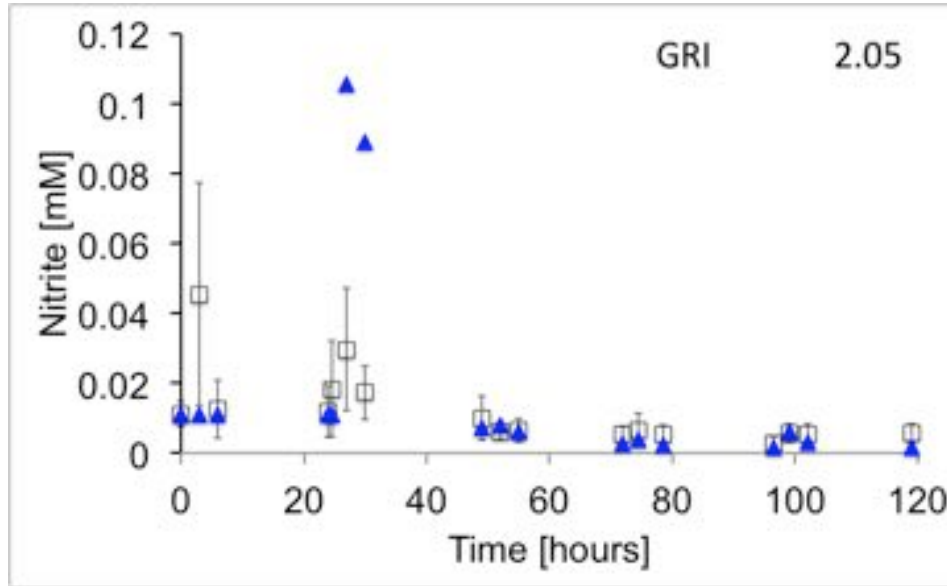
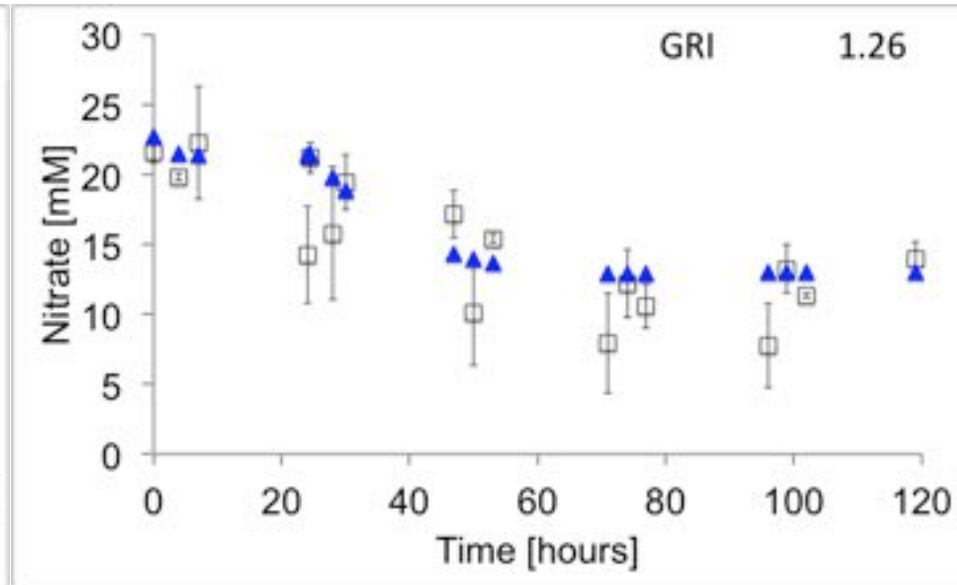
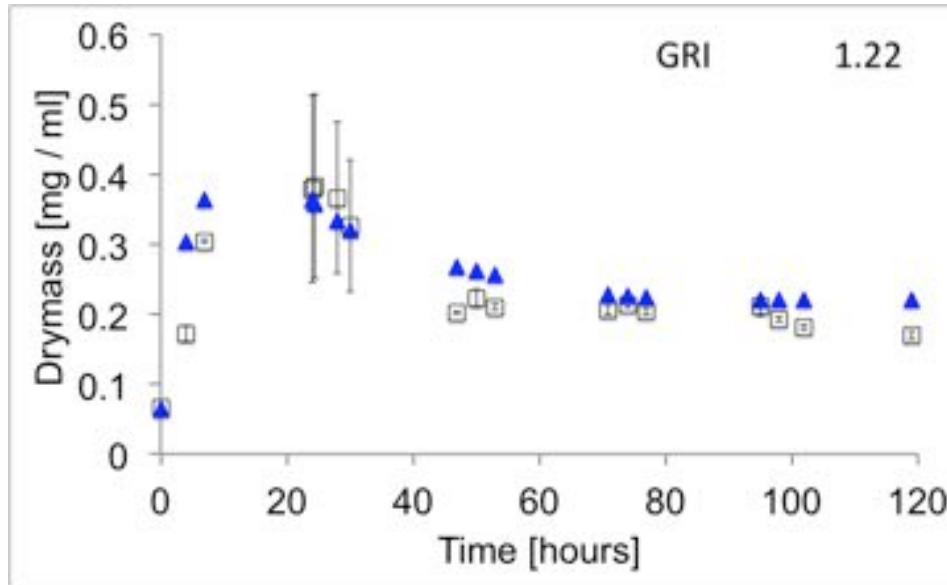


# Metabolic Hyp... Gibbs Hyp



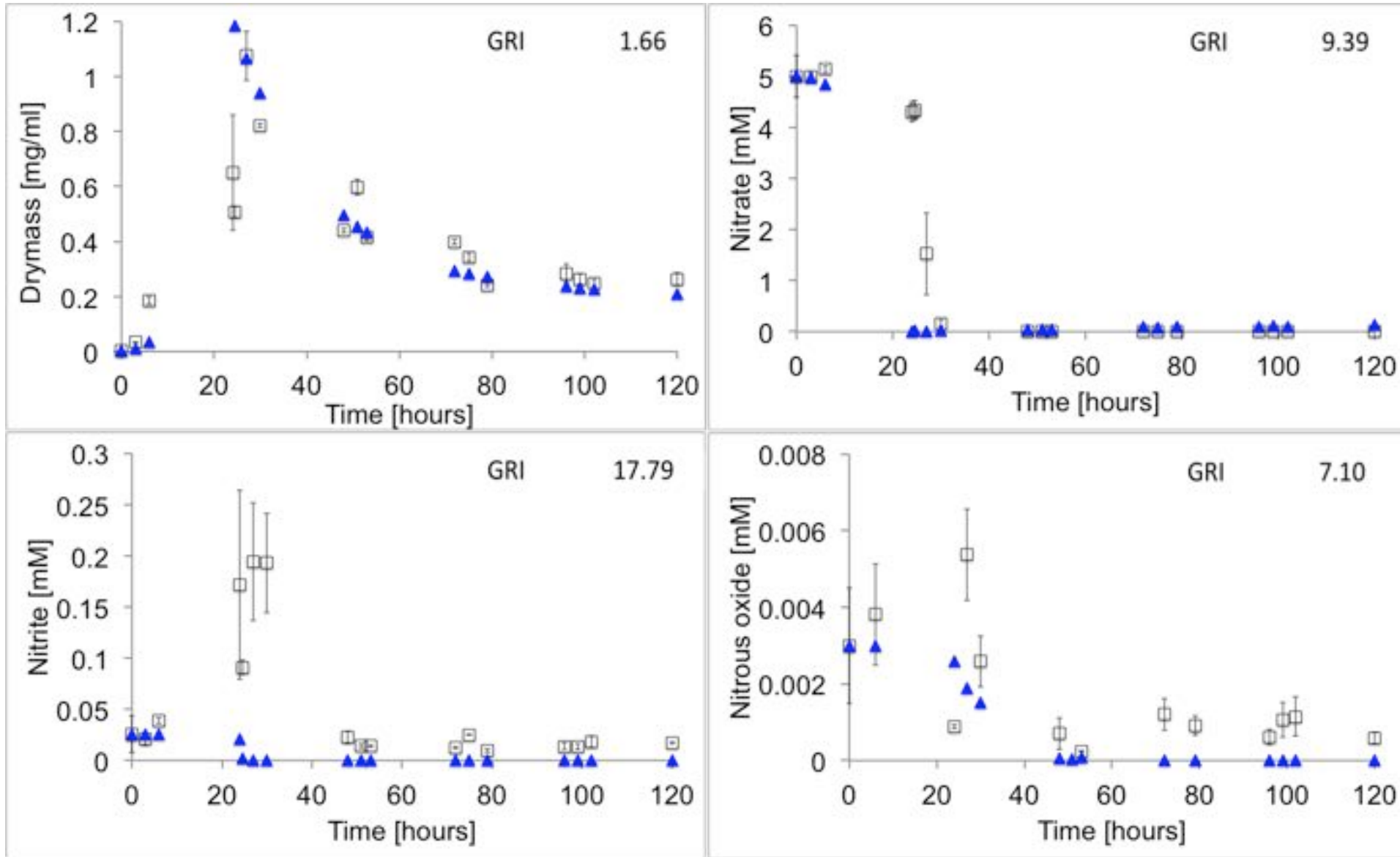
# e-donor limited / e-acceptor sufficient

INDISIM-Paracoccus: An IBM with thermodynamics inside



**GRI = Geometric Reliability Index** Simulations reasonably close to experimental observations shows **GRI factor 1 to 3**. (Jachner et al., 2007) and (Leggett and Williams, 1981).

# e<sup>-</sup>-donor sufficient / e<sup>-</sup>-acceptor limited



**GRI = Geometric Reliability Index** Simulations reasonably close to experimental observations shows **GRI factor 1 to 3**. (Jachner et al., 2007) and (Leggett and Williams, 1981).

Parameterization

# Model parameters

Nutrient	Culture medium initial concentration [mM] Felgate et al. (2012)	Availability coefficient – $a_i$ ( $h^{-1}$ ) fixed according to $D_{ab}$	Uptake-rate – $u_i$ ( $mol_{nutrient} \cdot mol_{mass}^{-1} \cdot h^{-1}$ )			
			Testing values			Calibrated Values
			Low (L)	Medium (M)	High (H)	
Succinate	5 – 20	0.28	0.065	0.13	0.52	0.52
Ammonium	10	0.84	-----	-----	0.31	0.31
Oxygen	0.236	0.79	-----	-----	0.54	0.54
Nitrate-a (aerobic)	4.9983 –	0.63	0.034	0.068	0.27	0.27
Nitrate-x (anaerobic)	21.6095		0.019	0.119	1.19	0.119
Nitrite	0.0255 – 0.0112	0.79	0.0062	0.062	0.62	0.062 – 0.62
Nitric Oxide	-----	1.00	0.0000062	0.00062	0.62	0.62
Nitrous Oxide	0.003 – 0.000028	0.50	0.0031	0.031	0.31	0.31





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## Journal of Theoretical Biology

journal homepage: [www.elsevier.com/locate/yjtbi](http://www.elsevier.com/locate/yjtbi)

## INDISIM-Paracoccus, an individual-based and thermodynamic model for a denitrifying bacterium



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<sup>b</sup> Department of Agri-food Engineering and Biotechnology, Universitat Politècnica de Catalunya, Edifici D4, Esteve Terradas 8, 08860 Castelldefels, Barcelona - Spain

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<sup>d</sup> School of Computing Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ - United Kingdom

### HIGHLIGHTS

- An IBM to study denitrification that uses thermodynamics for the cellular activity.
- The simulator facilitates interaction between modelers and experts in denitrification.
- The thermodynamic properties embedded into individual cells for modeling.

### GRAPHICAL ABSTRACT

The individual-based model approach with the thermodynamics embedded as an intracellular model defines the behavior-rule of the individual cell for maintenance and biomass generation to study the denitrification products dynamics, especially the greenhouse gas  $N_2O$ , carried out by denitrifying bacterium *Paracoccus denitrificans*.



Communicate  
the model

<http://dx.doi.org/10.1016/j.jtbi.2016.05.017>

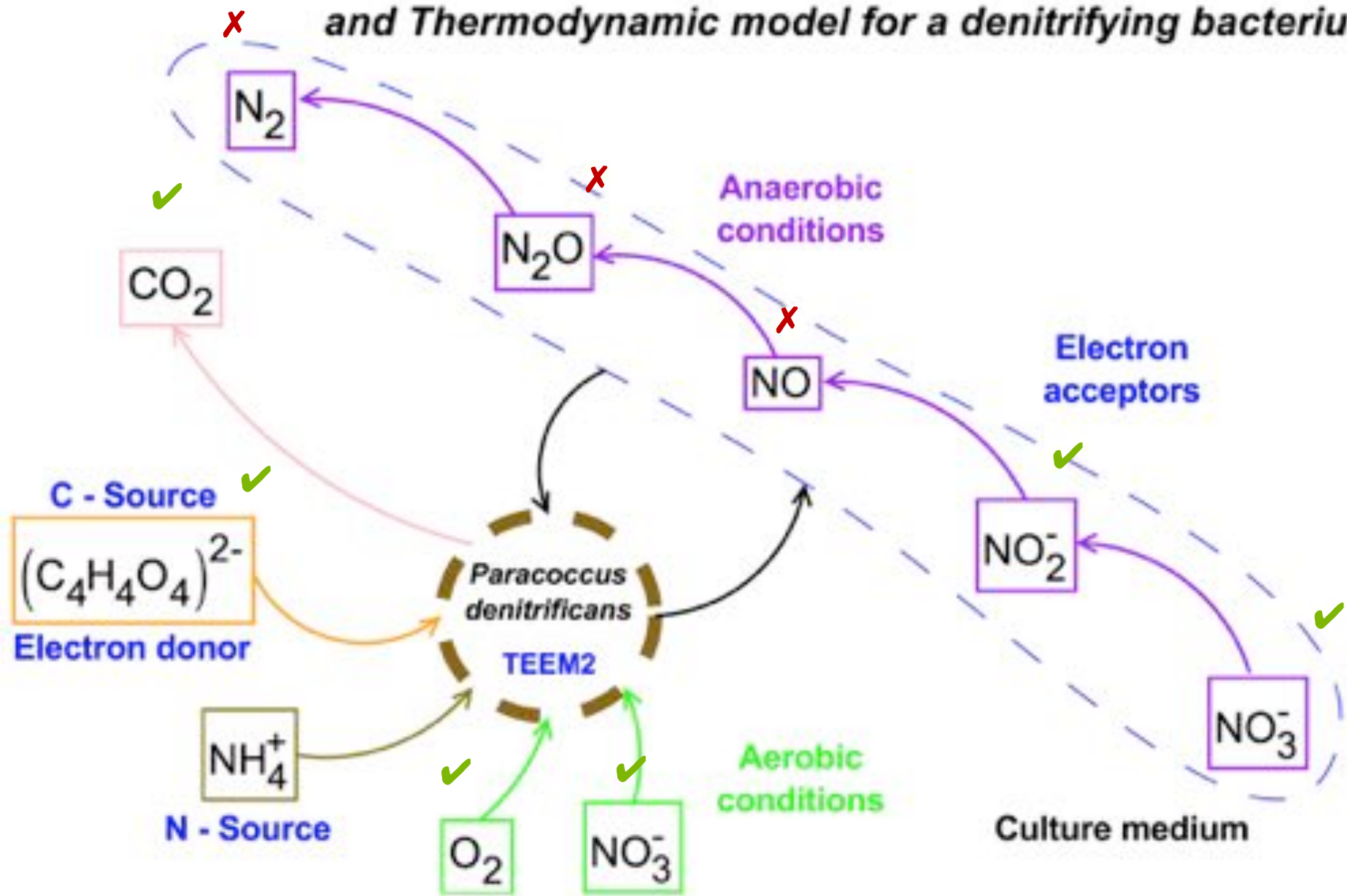
**INDISIM-Denitrification:  
an individual-based model  
for study the  
denitrification process**

# Objective

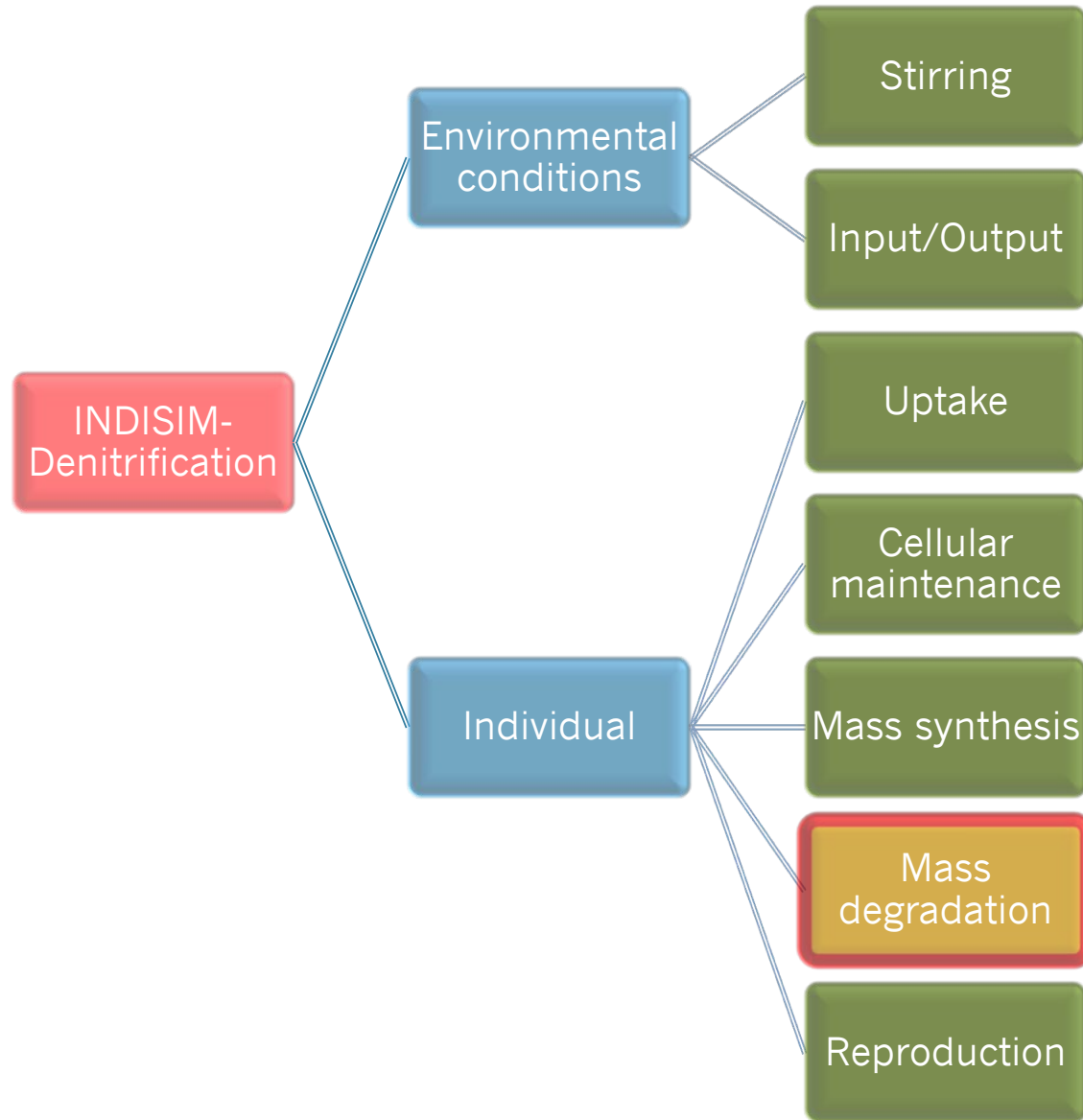
## 4

According to the results previously obtained, **to improve the model design**, modifying the individual rules required in the individual-based modelling context and **to generalize the model** to tackle other **denitrifying bacteria** using a wider set of published experimental data, performing the sensitivity analysis for some models' parameters in order to learn how the system works, and complete the modelling cycle.

# INDISIM-Paracoccus: an Individual-Based and Thermodynamic model for a denitrifying bacterium



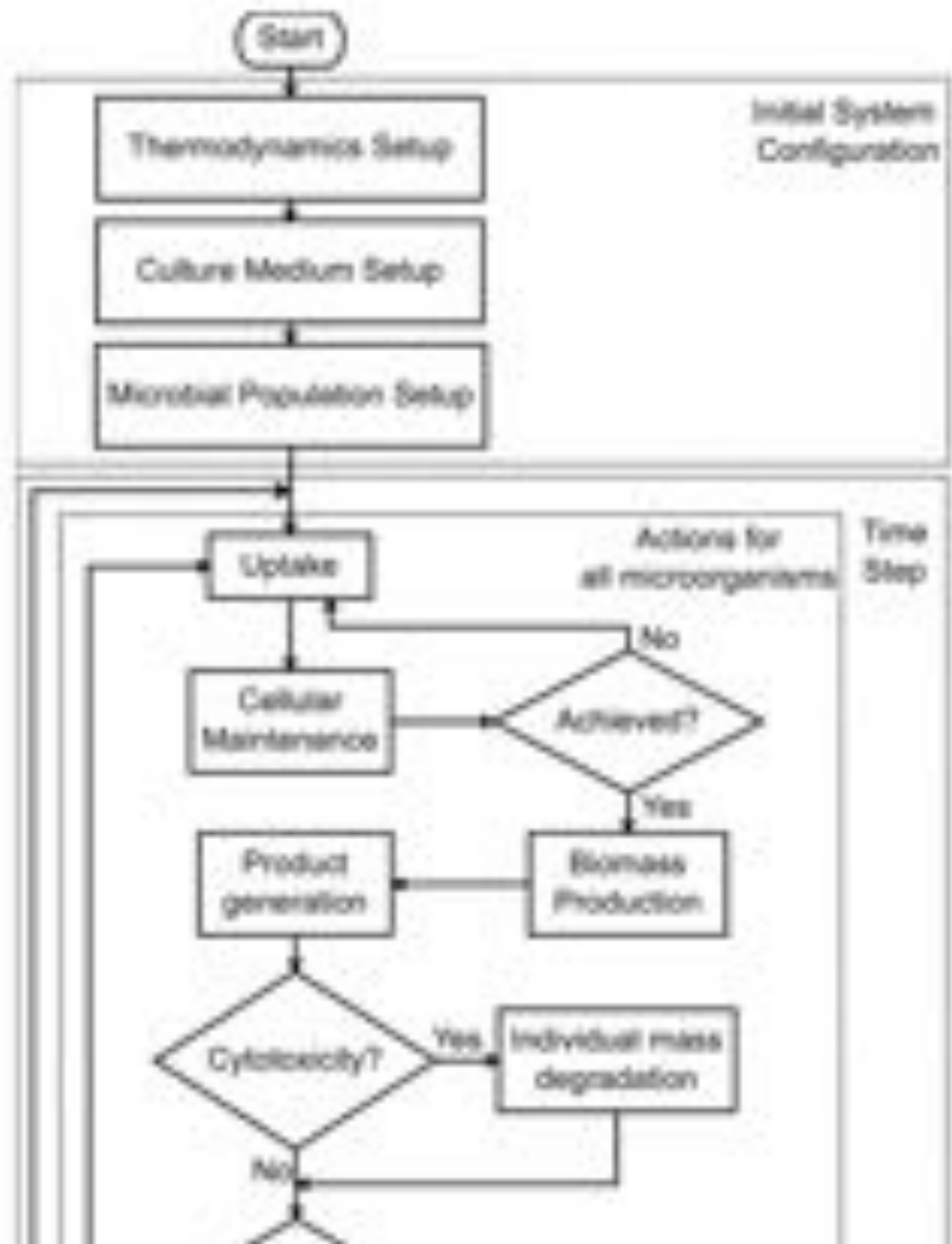
# INDISIM-Denitrification submodels



# More ideas to extend the model

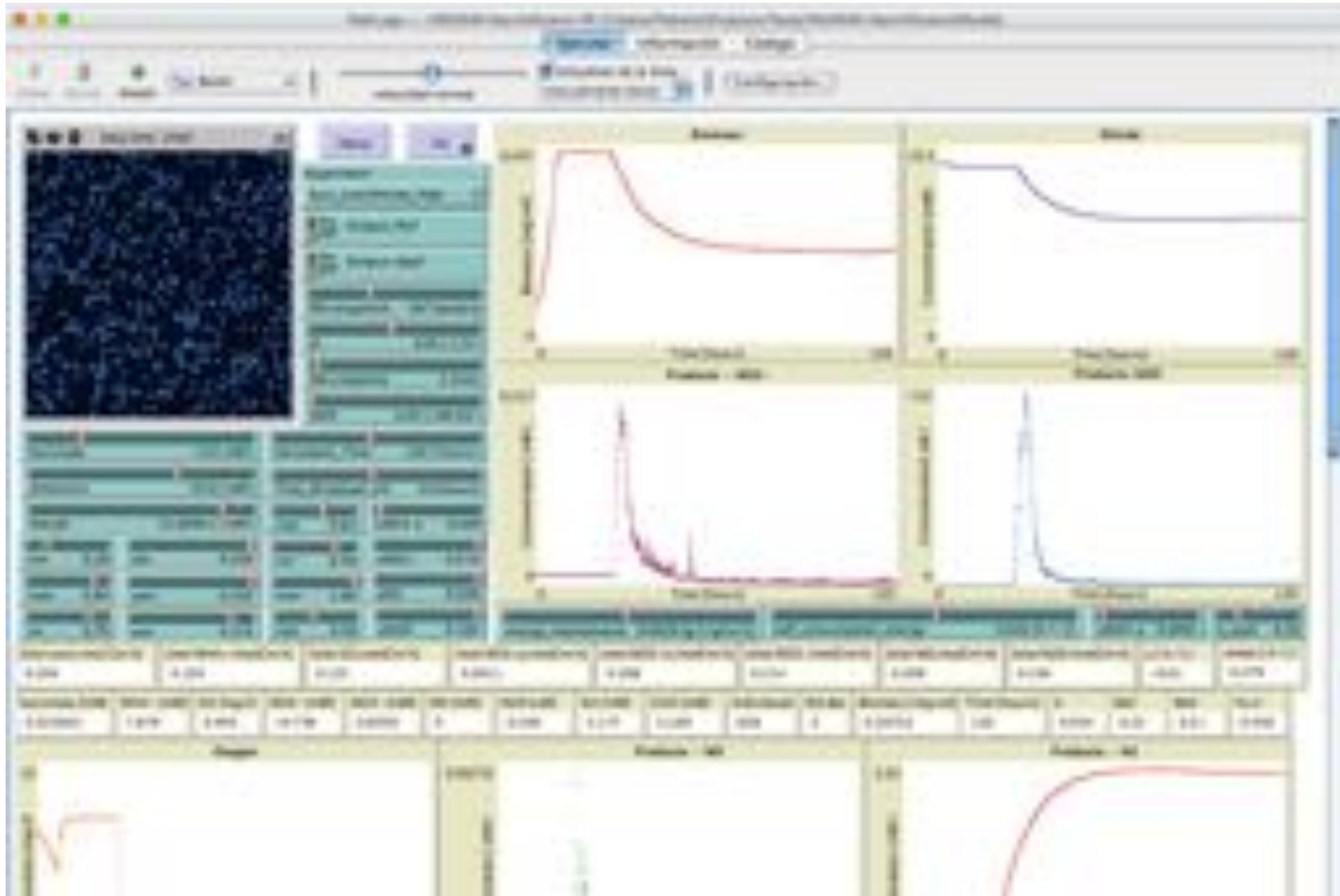
- **To include all thermodynamics calculations** into the implementation of the model
- **To change the microbial biomass**
  - Any denitrifying bacteria
- **To test the model with two denitrifying bacteria** with two experimental conditions and two bioreactor protocols
  - *Paracoccus denitrificans*
  - *Achromobacter xylosoxidans*

# Flow Diagram



# NetLogo implementation

INDISIM-Denitrification: the update of INDISIM-Paracoccus





# Metabolic pathways *A. xylosoxidans*

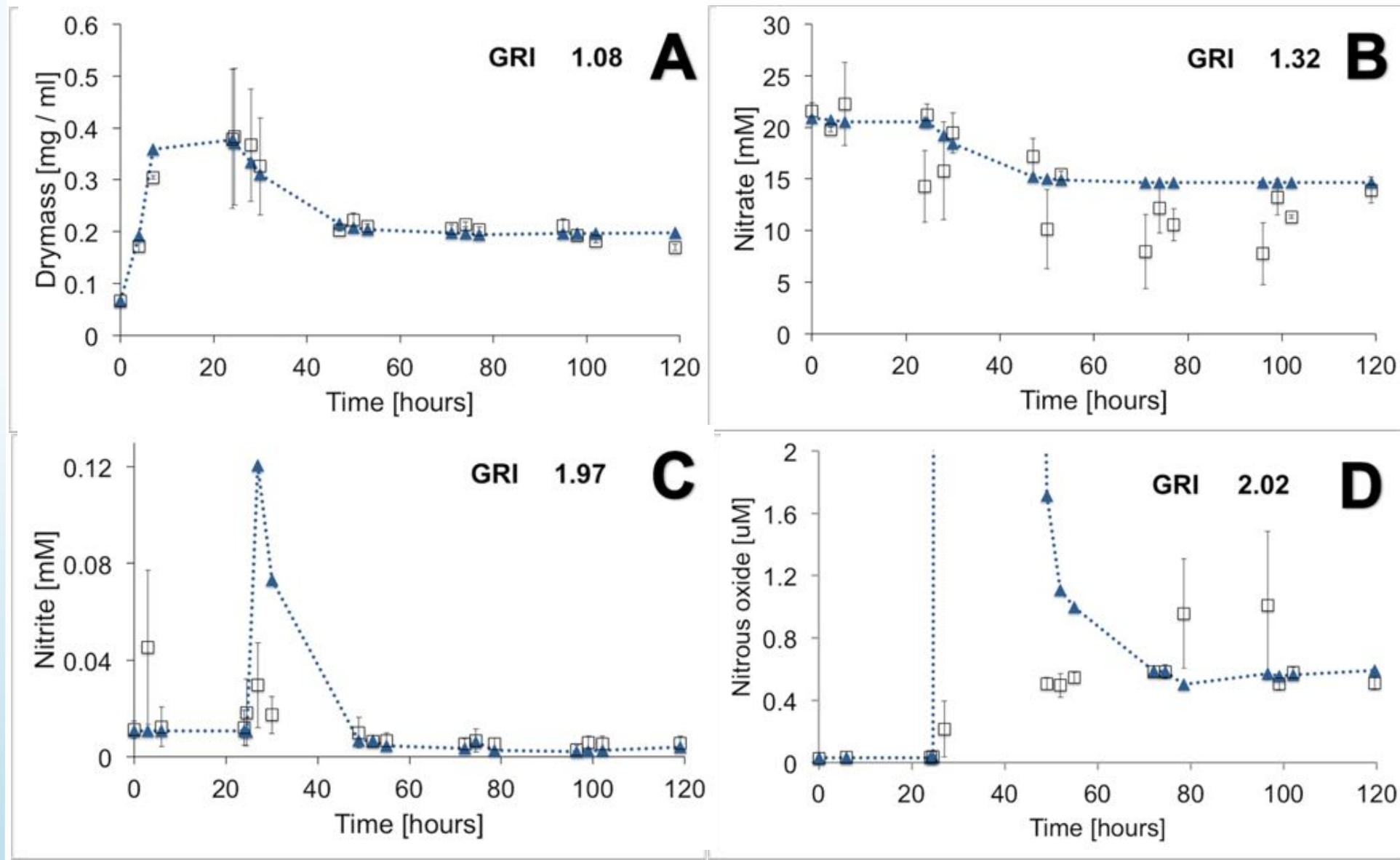
#	Microbial metabolic reactions (R)	ε
R1	$(C_4H_4O_4)^{2-} + 0.50 NH_4^+ + 0.89 O_2$ $\rightarrow 0.50 C_5H_9O_{2.5}N + 0.013 CO_2 + 1.50 HCO_3^- + 0.006 H_2O$	0.76
R2	$(C_4H_4O_4)^{2-} + 0.77 NO_3^- + 1.54 H^+ + 0.52 H_2O$ $\rightarrow 0.37 C_5H_9O_{2.5}N + 0.51 CO_2 + 1.63 HCO_3^- + 0.40 NH_4^+$	0.65
R3	$(C_4H_4O_4)^{2-} + 0.24 NH_4^+ + 4.49 NO_3^-$ $\rightarrow 0.24 C_5H_9O_{2.5}N + 4.49 NO_2^- + 1.05 CO_2 + 1.76 HCO_3^- + 0.52 H_2O$	0.41
R4	$(C_4H_4O_4)^{2-} + 0.45 NH_4^+ + 4.54 NO_2^- + 4.54 H^+$ $\rightarrow 0.45 C_5H_9O_{2.5}N + 4.54 NO + 0.20 CO_2 + 1.55 HCO_3^- + 2.37 H_2O$	0.84
R5	$(C_4H_4O_4)^{2-} + 0.50 NH_4^+ + 3.53 NO$ $\rightarrow 0.50 C_5H_9O_{2.5}N + 1.77 N_2O + 0.006 CO_2 + 1.50 HCO_3^- + 0.006 H_2O$	0.66
R6	$(C_4H_4O_4)^{2-} + 0.24 NH_4^+ + 4.50 N_2O$ $\rightarrow 0.24 C_5H_9O_{2.5}N + 4.50 N_2 + 1.05 CO_2 + 1.76 HCO_3^- + 0.52 H_2O$	0.27

# Microbial Metabolic Reactions for individual mass degradation

Bacteria	Cytotoxic gas	Microbial metabolic reaction (Rg)
<i>P. denitrificans</i>	NO	$C_3H_{5.4}O_{1.45}N_{0.75} + \frac{49}{4} NO \rightarrow \frac{9}{4} CO_2 + \frac{3}{4} HCO_3^- + \frac{49}{8} N_2O + \frac{3}{4} NH_4^+ + \frac{33}{40} H_2O$
	N <sub>2</sub> O	$C_3H_{5.4}O_{1.45}N_{0.75} + \frac{49}{8} N_2O \rightarrow \frac{9}{4} CO_2 + \frac{3}{4} HCO_3^- + \frac{49}{8} N_2 + \frac{3}{4} NH_4^+ + \frac{33}{40} H_2O$
<i>A. xylooxidans</i>	NO	$C_5H_9O_{2.5}N + 21 NO \rightarrow 4 CO_2 + HCO_3^- + \frac{21}{2} N_2O + NH_4^+ + 2 H_2O$
	N <sub>2</sub> O	$C_5H_9O_{2.5}N + \frac{21}{2} N_2O \rightarrow 4 CO_2 + HCO_3^- + \frac{21}{2} N_2 + NH_4^+ + 2 H_2O$

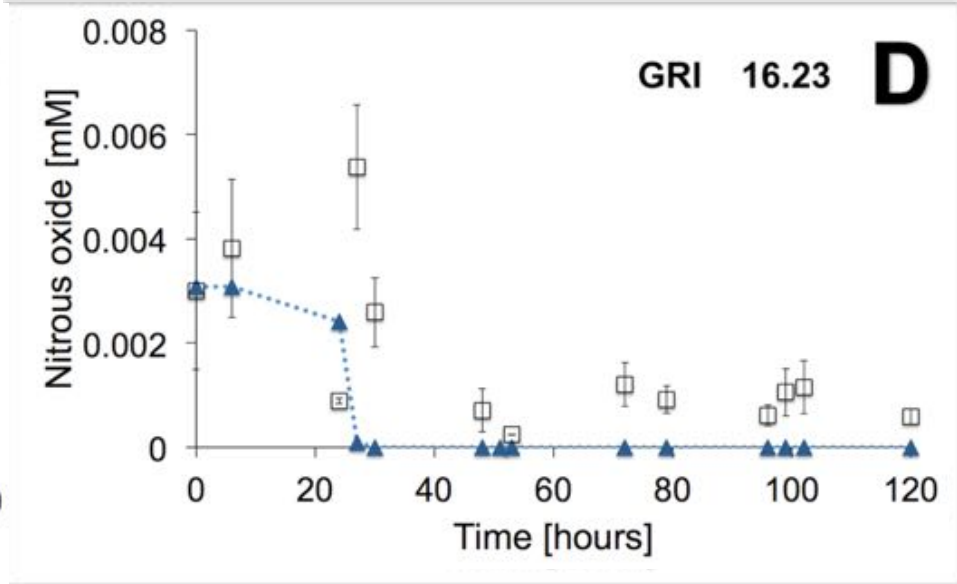
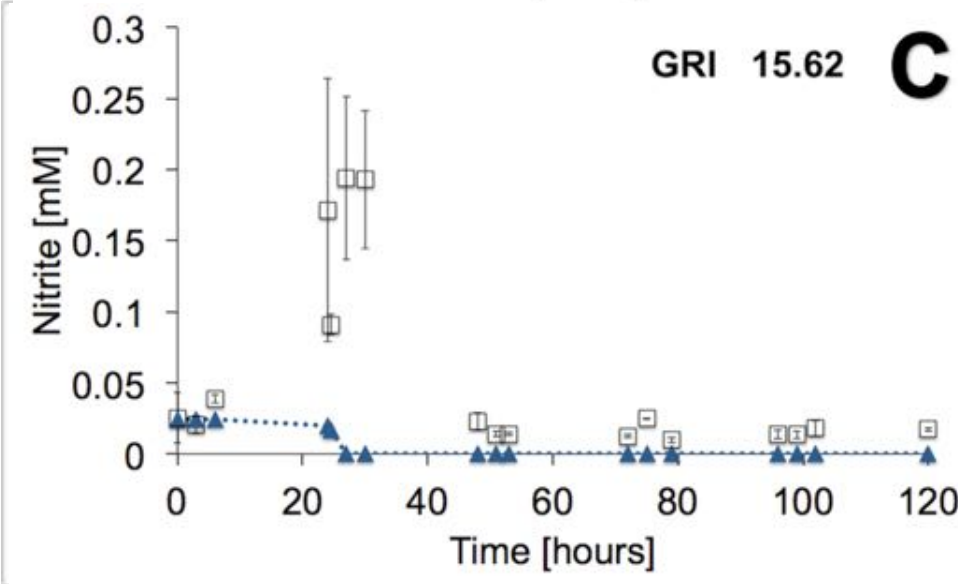
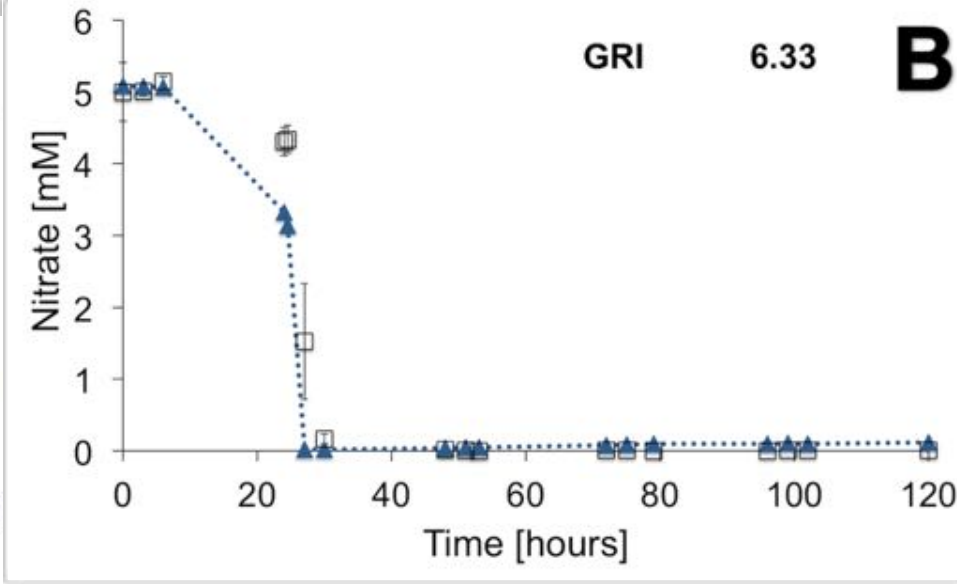
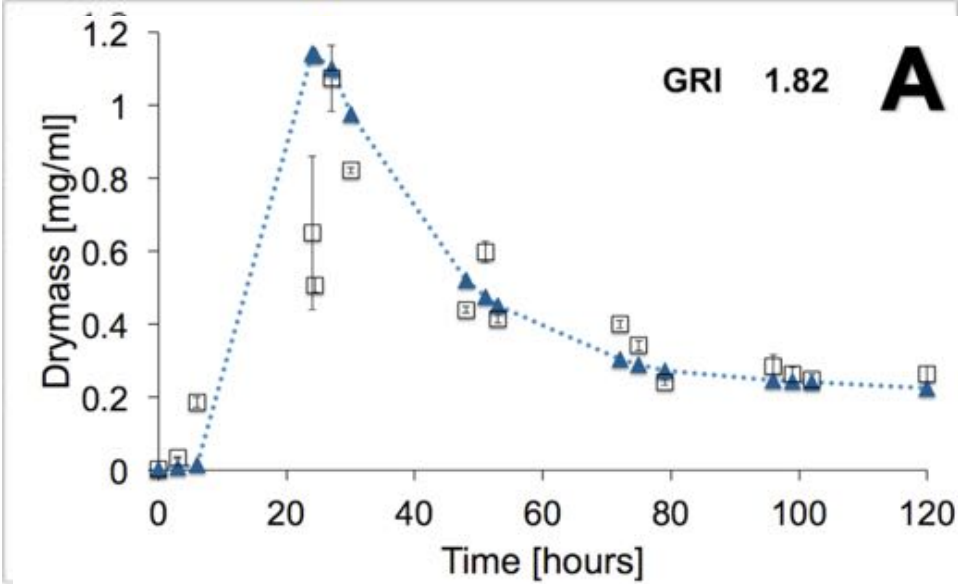
(Araujo et al., 2016) Mass degradation to reduce cytotoxic products as an individual behavior-rule embedded in a microbial model for the study of the denitrification process ([BioMicroWorld2015 – Proceedings book](#))

# *P. denitrificans*: e<sup>-</sup>-donor limited / e<sup>-</sup>-acceptor sufficient



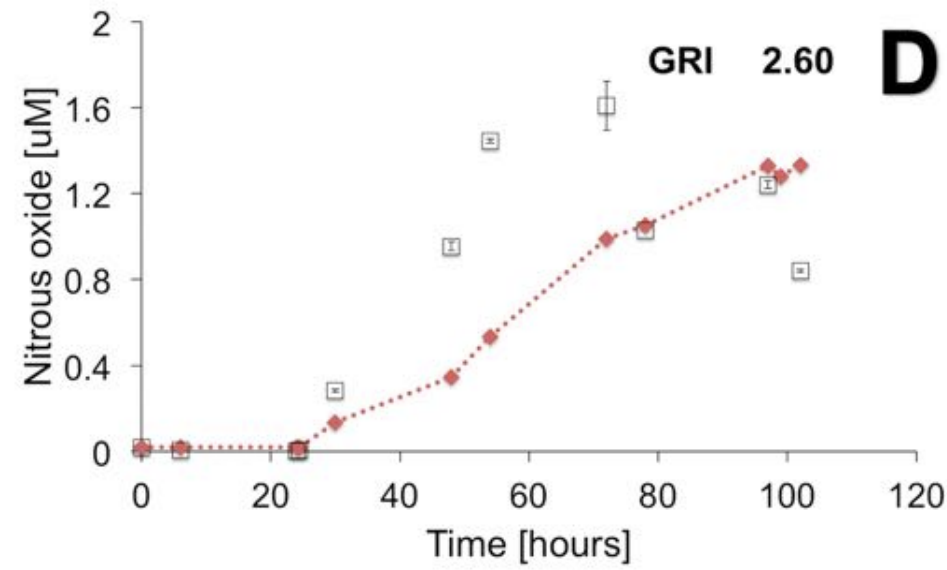
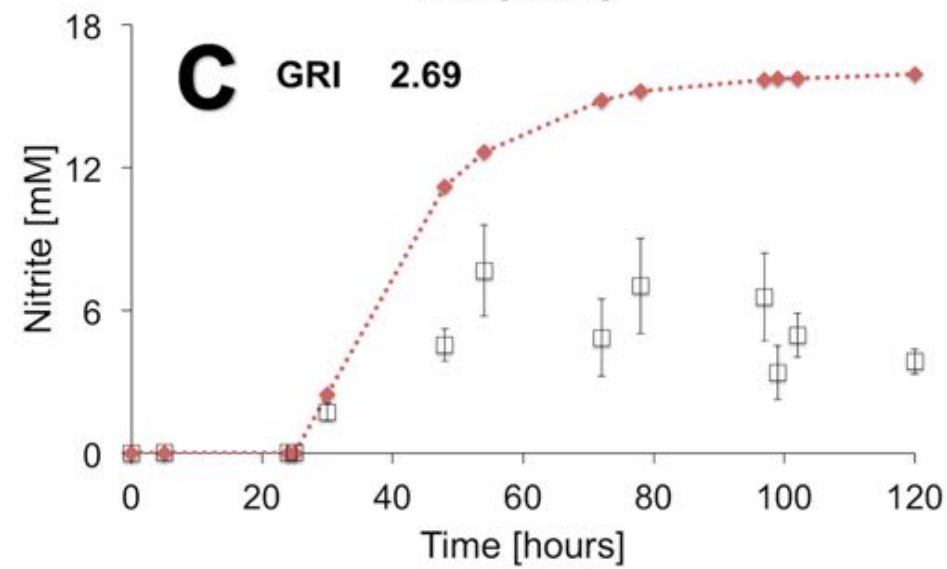
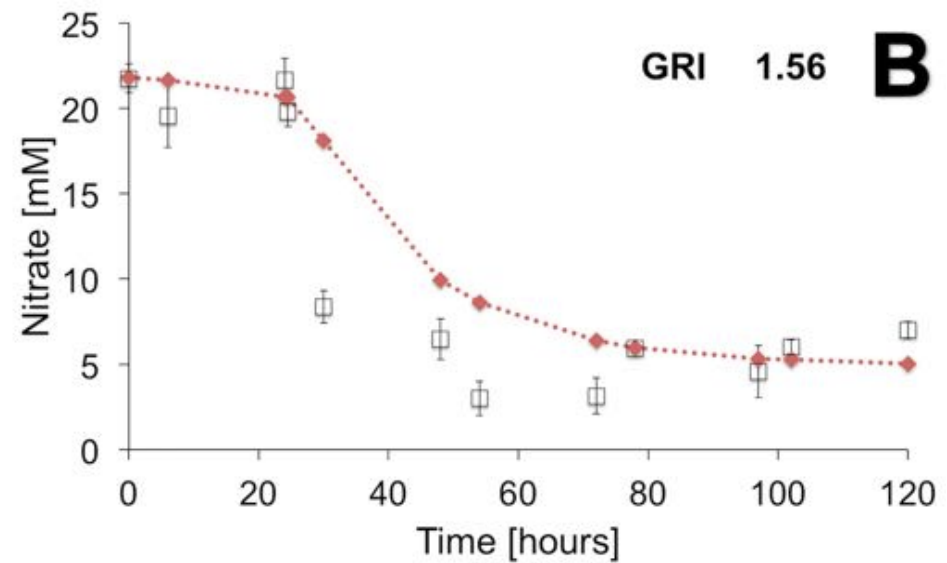
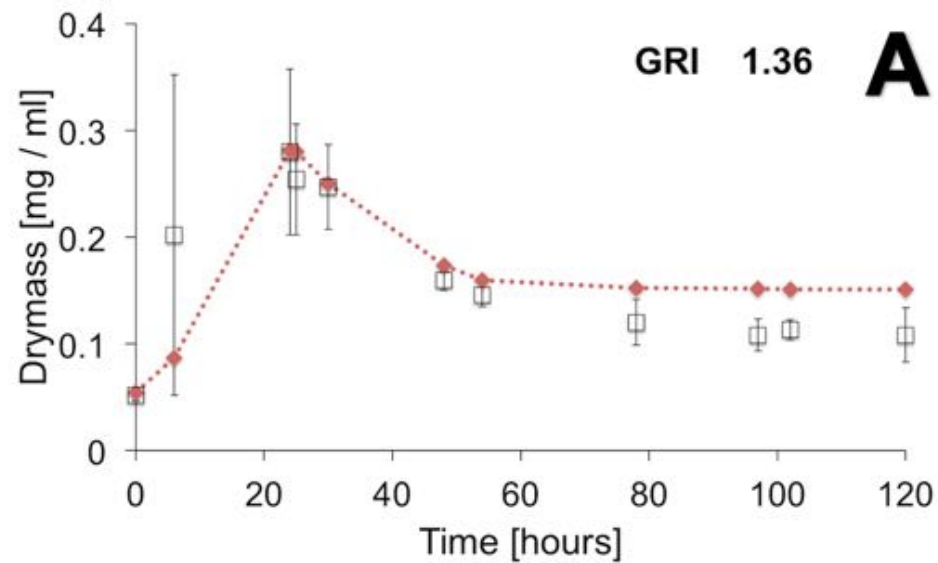
**GRI = Geometric Reliability Index** Simulations reasonably close to experimental observations shows **GRI factor 1 to 3**. (Jachner et al., 2007) and (Leggett and Williams, 1981).

# *P. denitrificans*: e<sup>-</sup>-donor sufficient / e<sup>-</sup>-acceptor limited



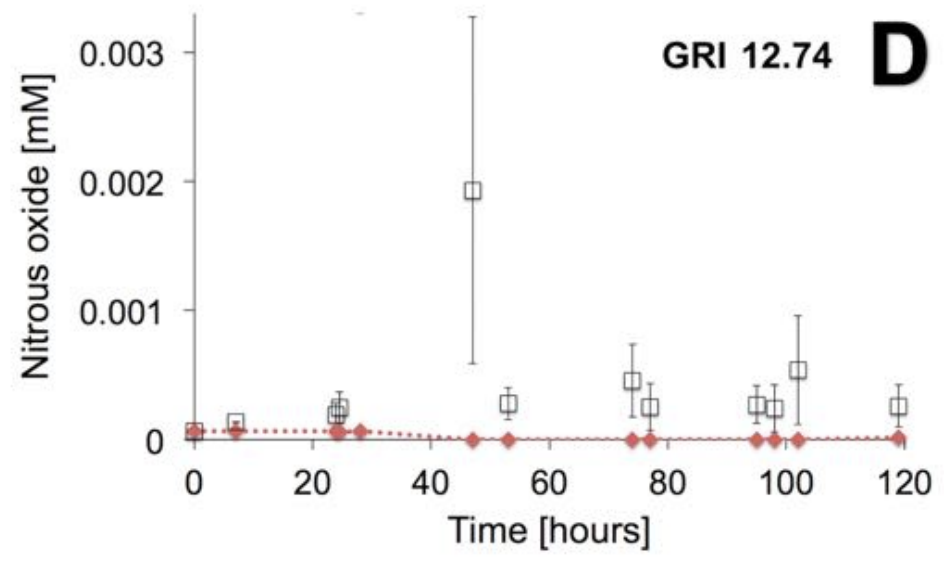
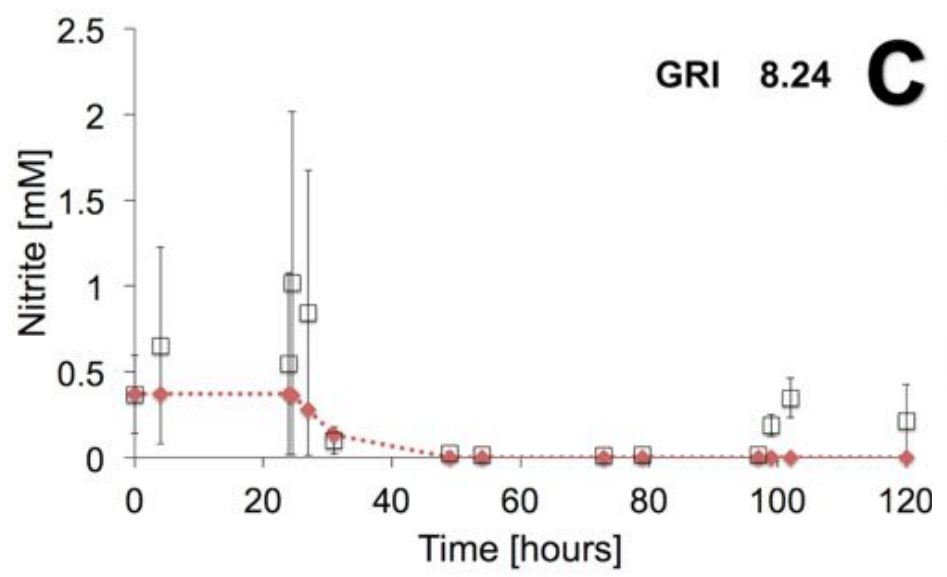
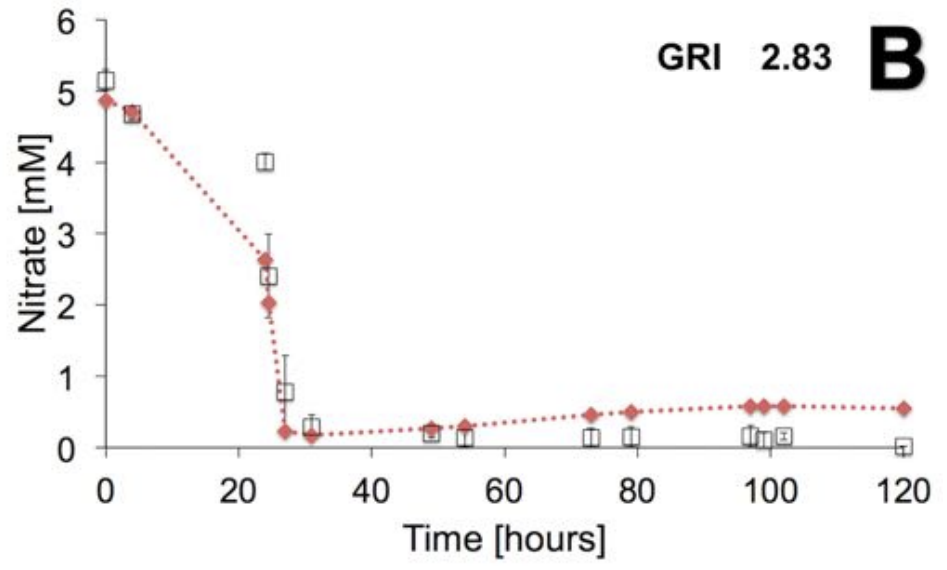
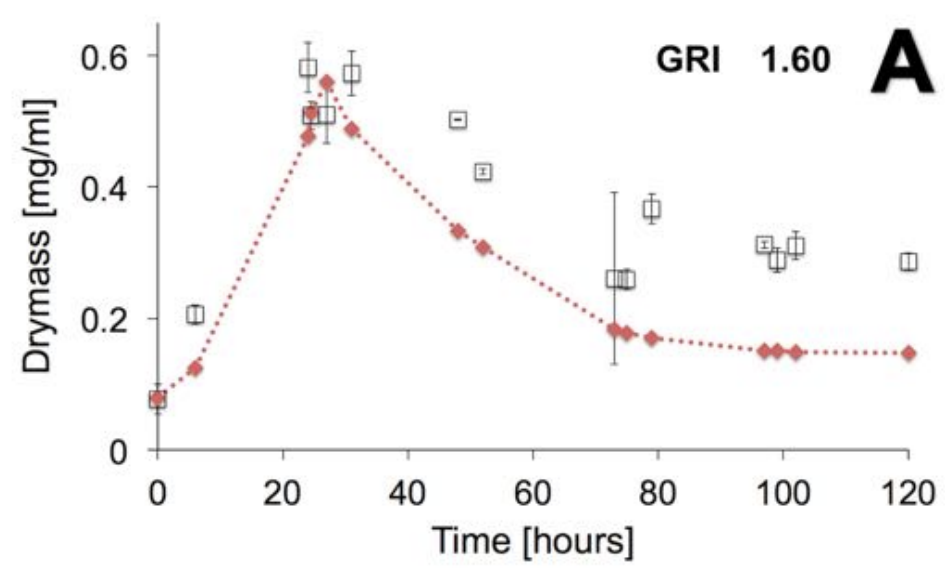
**GRI = Geometric Reliability Index** Simulations reasonably close to experimental observations shows **GRI factor 1 to 3**. (Jachner et al., 2007) and (Leggett and Williams, 1981).

# A. xyloxidans: e-donor limited / e-acceptor sufficient



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# A. xylooxidans: e<sup>-</sup>-donor sufficient / e<sup>-</sup>-acceptor limited

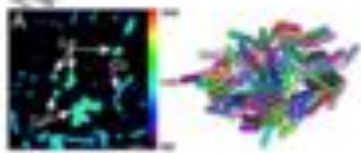


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**2016 @ASM Conference**  
**The Individual Microbe: Single-cell**  
**Analysis and Agent-based Modeling**

**March, 18<sup>th</sup> 2016**



## Research Topic

## The Individual Microbe: Single-Cell Analysis and Agent-Based

### Modelling

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## INDISIM-Denitrification: an individual-based model for study the denitrification process

**Pablo Araujo<sup>1,2,\*</sup>, Anna Gras<sup>2</sup>, Marta Ginovart<sup>3</sup>, Vincent Moulton<sup>4</sup>**

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4 School of Computing Sciences, University of East Anglia, Norwich Research Park, Norwich NR4 7TJ - United Kingdom; telephone: (44) 1603 592607; e-mail: [v.moulton@uea.ac.uk](mailto:v.moulton@uea.ac.uk)

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# Future perspectives



- The model can be **adapted to more complex systems**, e.g. wastewater treatments, soil management, and composting processes, among others.
- **INDISIM-Denitrification** could be incorporated into **INDISIM-SOM**, extending this soil model to complement the soil nitrogen cycle to deal with a **mixed microbial community**.
- There are some experimental works, which make reference to the role played by some elements in the **denitrification process such is copper and/or iron**, because they are a co-factor in activating some denitrifying enzymes. Study of this relation through the modeling process will be of great interest. Using a model such as **INDISIM-Denitrification** could be the next step to progress in knowledge of denitrification.

- We believe that the use of an approach in the field of **non-equilibrium thermodynamics to describe the microbial metabolism** has shown successful results and this methodology could be extended to other modelling frameworks.
- The use of **MbT-tool outputs** could be assumed as a starting point to design the metabolic sub models in other INDISIM branches **to improve** the design and parametrization of the model.
- Virtual experiments can be developed with some specific environmental characteristics where the bacteria execute a metabolic pathway **using some value of energy-transfer-efficiency** while in another environmental condition it executes the same pathway using a different  $\epsilon$  value.

# Conclusions

- **An open access and open source** tool has been developed to write microbial metabolic reactions based on Thermodynamic Electron Equivalents Model.
- The individual-based model named **INDISIM-Paracoccus** has been developed, including metabolic reactions as the basis of the individual behaviour-rules for the **cellular maintenance and biomass synthesis sub-model**, to deal with *Paracoccus denitrificans* growing in a bioreactor working as a batch and/or continuous culture. It has been verified that the corresponding simulator implemented in **NetLogo** platform works in accordance with its conceptual design.
- To improve the first simulator **INDISIM-Paracoccus**, a new individual-based model named **INDISIM-Denitrification** has been produced, which includes the new **individual rule to reduce cytotoxic products**, nitric oxide and/or nitrous oxide, through the degradation of individual mass.



**Thank you**