INDISIM PARACOCCUS AN INDIVIDUAL BASED AND THERMODYNAMIC MODEL FOR A DENITRIFYING BACTERIUM

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Abstract

In this article, we combine a thermodynamic model for bacterial yield prediction with an individual-based model designed to describe the bacterial cell population dynamics. Our individual-based model use a culture medium containing succinate as a carbon source, ammonium as nitrogen source and various electron acceptors such as oxygen, nitrate, nitrite, nitric oxide and nitrous oxide to investigate in continuous or batch culture the different substrate-dependent cell growth kinetics and the environmental factors that control nitrous oxide production and consumption by the bacterium *Paracoccus denitrificans.* The individual behavior-rules that this microbe carries out for its nutrient uptake, cellular maintenance and reproduction cycle is according to the individual-based model INDISIM. Six metabolic pathways described for *Paracoccus denitrificans* were selected and translated into balanced chemical equations using the Thermodynamic Electron Equivalents Model (TEEM2). These equations are the basis of the individual behavior-rules that this microbe carries out for its biomass synthesis and denitrification products production. The simulation results achieved with the implementation of our model in NetLogo showed that it is possible investigate the microbial denitrification dynamics into a bioreactor because some of the outputs regarding the temporal evolutions of the nutrients and denitrification products are consistent with previous published experimental data.

Keywords: denitrification, bacterial yield prediction, individual-based model, Thermodynamic Electron Equivalents Model, NetLogo, INDISIM

Introduction

The global warming potential (GWP) of most significant greenhouse gases nitrous oxide (N_2O) and methane (CH_4) are 296 and 23 times greater, respectively, than a unit of carbon dioxide (CO_2). Among these three gases, N_2O may be the most important for fertilizer use because of its large CO_2 equivalent influence on GWP (Snyder et al., 2009; Woolfenden et al., 2013). Agriculture plays a substantial role for the majority of N_2O emissions especially when available soil N (in nitrate form) exceeds crop uptake, the risk of increased N_2O emissions rises. In conditions of low oxygen (O_2) availability, such as waterlogged soils, certain bacteria are able to use nitrate (NO_3^-) as a final electron acceptor (EA) and carry out respiratory metabolism in anaerobic conditions. These bacteria are known as denitrifying bacteria and are widespread in agricultural soils (Felgate et al., 2012; Richardson et al., 2009; Woolfenden et al., 2013).

Paracoccus denitrificans (*PD*) was first isolated in 1908 by Beijerink as a *Micrococcus denitrificans,* is a member of the α-proteobacteria and one of the best-characterized prokaryotes, is a gram-negative bacterium mainly found in soil and sewage sludge, is one of the preferred species for studies of the biochemistry and regulatory biology of denitrification because is capable of anaerobic growth in the presence of NO_3^-, nitrite (NO_2^-), nitric oxide (NO) or N_2O as EAs also can survive in ecosystems with fluctuating aerobic and anaerobic conditions, when molecular O_2 is present and preferred like terminal EA (Baumann et al., 1996; Beijerinck MW, 1910; Bergaust et al., 2010; Bergaust et al., 2012; van Verseveld et al., 1983). Four terminal enzymes are necessary to achieve this stepwise reduction and the genes encoding the denitrification enzymes have been identified in *PD*. Consequently,

understanding the environmental factors that control N_2O production and consumption by microbes is a challenge to the development practical mitigation strategies for N_2O emissions (Baker et al., 1998; Baumann et al., 1996; Felgate et al., 2012).

Some denitrification models were review by (Heinen, 2006). These models integrate a large number of parameters including NO_3^- and water (H_2O) content in soil and soil temperature and pH, in all cases the functions used are empirical and have been adjusted for own studies. In recent years (Kampschreur et al., 2012) and (Woolfenden et al., 2013) publish specific denitrification modeling to study the denitrification pathway carry out by microbes, a tool to study inhibiting and activating compounds and effect of copper availability, respectively, both of these models are differential equations based and the model parameters considered according Monod and Michaelis-Menten kinetics.

In other way is possible simulate the interactions of autonomous agents (individual and collective entities) with its environment, using a type of computational model called Agent-Based Models (ABMs) or more specifically using an Individual-based Models (IbMs) which defines special cases where the agent is assimilated to a living entity (Kreft et al., 2013). This modeling approach considering the population dynamics in which not all individuals are equivalent and their growth curve emerges from the interaction of the individuals with their environment (Fredrickson et al., 1967). In microorganism case with an extra source of complexity related to the fact that cellular properties are unevenly distributed among the cells of the population and the changes of extracellular environment constitute an important part in the cell population dynamics (Lee et al., 2009; Mantzaris, 2007). IbMs models consider individuals as discrete entities and offer basic rules that individual follows for interact with

his surrounding environment and other individuals, these interactions cause change in the individual and environmental characteristics, which allows study the intra-population variability with emergence of population behavior in systems to which the continuum hypothesis is not applicable (Ginovart and Prats, 2012). In past years (Giró et al., 1986) publish a simulation program to describe the spatial and time evolution of a population of living individuals under pre-assigned environmental conditions of energy. Further (Ferrer et al., 2008; Ginovart et al., 2002; Gras and Ginovart, 2006; Prats et al., 2010) create, develop, test and publish a discrete simulation model to study bacterial cultures called INDISIM and its different versions (INDISIM-SOM, INDISIM-YEAST and INDISIM-YEAST-SACCHA), have been used successfully to deal with diverse biological systems where microorganisms are the main agents driving the behavior of these systems.

Many different approaches to development a rigorous thermodynamic description for biomass yield prediction were reported by (Christensen and McCarty, 1975; Heijnen and Van Dijken, 1992; Liu et al., 2007; Maskow and von Stockar, 2005; McCarty, 1971; McCarty, 2007; Rittmann and McCarty, 2001; von Stockar and van der Wielen, 1997; Tijhuis et al., 1993; Xiao and VanBriesen, 2006). Considering the estimated Gibbs energy dissipation for cell synthesis from C-sources and N-source, the energy available from substrate transformation with the biomass specific Gibbs energy consumption for cellular maintenance and the efficiency of energy transfer for the overall growth process into a formal reaction for catabolism and anabolism, the thermodynamic analysis can be used to predict the bacterial yield and bio-product synthesis. Our model follow the Thermodynamic Electron Equivalents Model second version (TEEM2) proposed by McCarty (2007) to improve the individual

behavior rule for biomass synthesis, the TEEM2 implementation requires the specification of the C-source, N-source, electron donor (ED), EA and bio-products in the system, to accomplish four balances: carbon balance, nitrogen balance, electron balance and energy balance.

The main goals proposed in this work are: i) Design and describe a bacterial model for *PD* and a culture medium (CM) in which it develops and grows (batch or continuous culture, succinate-limited and NO_3^--limited) in the context of the IbMs methodology, ii) implement this computational model in NetLogo, a free access multi-agent programmable modeling environment and iii) compare the simulation outputs with some of the experimental results presented by Felgate et al. (2012).

Materials and Methods

The individual-based model INDISIM, a computational model to study bacterial cultures (Ginovart et al., 2005; Gras et al., 2011; Prats et al., 2008) was the core model, for that, our denitrifying bacteria model is called INDISIM-Paracoccus (IPa). INDISIM is discrete in space and time and controls a group of bacterial cells using a set of random-time-dependent variables for each bacterium at each time step program. For establishing IPa model, each individual, a single bacterial cell of simulated *PD*, follow individual rules of behavior described in the core model concerning with its motion, nutrient uptake, cellular maintenance and reproduction, the biomass synthesis follow six metabolic pathways described for *PD* by (Baker et al., 1998; Baumann et al., 1996; Beijerinck MW, 1910; Bergaust et al., 2010,2012; Richardson et al., 2009; Robertson, 1992; van Verseveld et al., 1983,1984) which are translated into balanced chemical equations according to TEEM2. The CM, the environment

to who interacts each *PD* simulated, follow the rules of nutrient availability accord to INDISIM and general chemostat procedures to batch or continuous culture with the possibility of different experiment for succinate-limited, NO_3^--limited in aerobic and anaerobic growth. IPa is developed using NetLogo a multi-agent programming language and modeling environment for simulating natural and social phenomena that allows ABMs implementation. It is particularly well suited for modeling complex systems evolving over time. This makes it possible to explore connections between micro-level behaviors of individuals (agents) to macro-level patterns that emerge from their interactions (Wilensky, 1999). The model description follows the ODD protocol, it stands for "Overview, Design concepts, and Details": the description starts with three elements that provide an overview of what the model is about and how it is designed, followed by an element of design concepts that depicts the ABMs essential characteristics, and it ends with three elements that provide the details necessary to make the description complete (Grimm, 1999; Grimm et al., 2010; Railsback and Grimm, 2011).

Model Description

Purpose: Modeling a bioreactor to grow *PD* in a CM with succinate, as ED and C-source, ammonium (NH_4^+) as N-source and various EAs as O_2, NO_3^-, NO_2^-, NO and N_2O, in order to simulate experiments with succinate-limited and NO_3^--limited in aerobic-anaerobic conditions in batch and continuous culture for compare simulated steady-states results of NO_3^-, N_2O rates and biomass yields with publish experimental data and identify factors, in the simulated metabolism or CM, which are significant to the dynamics of denitrification products, especially greenhouse gas N_2O.

Entities, State Variables, and Scales: IPa model has two kinds of entities: bacteria and square patches of CM. Each individual represents a unique bacterium of *PD* and has the variables: unique identification number, location (XY coordinates of the grid cell where it is), biomass, nutrient uptake capacities and counters for each reproduction cycle and metabolic pathway. To obtain the initial biomass, the model assumes that each bacterium has spherical shape with an internal diameter between 0,5 to 0,9 µm (Holt et al., 1994), then the individual biomass is deduced from cell volume by assuming a density of 1,1 $g \cdot cm^{-3}$ (Gras et al., 2011) and the elementary cell composition $C_3H_{5,4}N_{0,75}O_{1,45}$ (van Verseveld et al., 1979,1983). Therefore the smallest microorganism has a biomass of ~1 pmol and the highest microorganism has a biomass of ~6 pmol. Individuals has different uptake coefficient to capture nutrients through the cell membrane-associated proteins (Button, 1998). This uptake capacity is correlated with its individual biomass through the individual variable (*u*), which represents the amount of nutrient that could cross per unit of time, per unit of microbial biomass with the units ($mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$). The individual biomass updates every time step. The CM in IPa model is built by two-dimensional grid cells, that represent a bioreactor, in NetLogo is called "world" and is made by 31 x 31 cells called "patches" configured in "torus" mode (world without vertical and horizontal limits), each patch represents 1 nlitre, so the total volume is 961 nlitres. The CM variables are: unique position identifier in XY coordinates, nutrients and metabolic products quantity expressed in pmol. One time step represents 10 minutes and simulations were run for 120 hours (7200 minutes). With these units, pmol for biomass and nl for volume, IPa outputs (graphical and numerical) shows the concentration of nutrients and metabolic products in $mmol \cdot l^{-1}$ or $umol \cdot l^{-1}$ and dry biomass in $mg \cdot ml^{-1}$.

Process Overview and Scheduling: IPa is modeled as discrete time steps. In each time step the following processes are performed for each microorganism: uptake nutrient, cellular maintenance, new biomass synthesis and bipartition. The CM in each time step performed, for batch culture: initial concentration (normal distribution of nutrient concentration) and agitation (redistribute the nutrients homogeneously and randomly change microorganism position), in continuous culture: input nutrients flow (fresh CM is input to the bioreactor), agitation and output nutrients flow (randomly fraction of individuals and CM are removed from the bioreactor according to the dilution ratio). In each time step the time dependent variable of microorganism and CM are calculated, updating the graphics and digital outputs according to the time scale proposed. During the simulation process, entities are randomized generating an asynchronous update effect. Figure 1 shows the IPa schematic diagram.

Design Concepts - Basic Principles: The agent traits from which system dynamics emerge are: 1) individual biomass changes are possible if microorganism executes any of the six metabolic pathways adjusted by TEEM2, 2) the nutrient uptake capacity is accord to individual biomass and update each time step with the biomass changes, 3) the availability of nutrient CM is accord to Fick's law binary diffusion coefficient (Dab), 4) cellular maintenance value is appropriate requirement for heterotrophic microorganisms, 5) individual reproduction is accord to binary fission in bacteria and 6) dilution ratio and homogenous CM are the chemostat general procedures (Fig. 1).

Emergence: Outputs of the model are the result of the adaptation of individuals to the CM. The model was not forced to reproduce the results that appear at the system level. Model results are compared with some of the experimental results presented by Felgate et al. (2012)

in relation to biomass production, NO_3^- consumption rate and N_2O production rate in the succinate-limited and NO_3^--limited experiments in aerobic-anaerobic conditions with high copper concentration.

Adaptation: The individual identifies its biomass and uptake capacities. With this information the agent compare their uptake capacities with the nutrients availability and takes the lowest value. The first decision-making agent is about the response to the O_2 dissolved level in the CM, if the O_2 dissolved level is lower than NOX value the agent execute anaerobic metabolism and if is higher the agent execute aerobic metabolism. The second decision-making agent is performing metabolic pathways for growth and bio-product synthesis only if that reached their cellular maintenance in aerobic or anaerobic phase. The third decision-making agent is about reproduction that means not to make bipartitions if that has not reached the minimum reproduction mass.

Interaction and Collective: *PD* is the only bacterium in the virtual bioreactor, and only interacts with the CM. Simulated microorganisms do not develop aggregates; each agent acts uniquely.

Stochasticity: The initial individual position is randomly assigned, initial biomass (at the beginning of the simulation each biomass cell is determined from the normal distribution of which mean value is 3 pmol with standard deviation of 0.45 pmol), reproduction mass (the cell division event occurs when the individual biomass cell reaches a critical value determined from the normal distribution of which mean value is 3 pmol with standard deviation of 0.15 pmol then the biomass of the new-born cell is the half biomass of the original bacterial cell), the concentrations of nutrients in each grid cell at the beginning of the

simulation is determined from the normal distribution of which mean value is according to the experimental procedure (succinate-limited or NO_3^--limited with NH_4^+ and O_2) with standard deviation of 5% mean value, NOX value is fixed by the user in the range of 0.01 to 0.31 mM O_2 and is determined from the normal distribution of which mean is that fixed value with standard deviation of 5% mean value, individual position randomly change and randomly fraction of individuals are removed from the bioreactor according to the dilution ratio each time step.

Observation: The graphical and numerical outputs of the model are the concentration ($mmol \cdot l^{-1}$ or $umol \cdot l^{-1}$) of each CM component (succinate, NH_4^+, O_2, NO_3^- CO_2, NO_2^-, NO, N_2O and N_2) and microbial biomass ($mg \cdot ml^{-1}$) each time step. Additionally the user could obtain all simulated data in output file with extension ".txt".

Initialization: At the beginning of the simulation, the user can adjust: nutrient concentrations ($mmol \cdot l^{-1}$), dilution ratio (h^{-1}), initial amount of viable microorganisms (bacteria), simulation time (h), step time (min), all nutrients availability (h^{-1}), all nutrients uptake rates NOX value ($mmol \cdot l^{-1}$), time (h) for shutdown O_2 input flow and the maintenance energy requirement ($gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$).

Input data: Normal functioning external files are not necessary for the model to run simulated procedures.

Modeling or Theoretical Aspects

Sub models: The individual mathematical and theoretical sub-models considered are:

Maximum nutrient uptake: The maximum nutrient uptake $(Umax)_i$ is the amount of nutrient per unit of time, that a microorganism could use under non-limiting nutrient availability and

is correlated with the individual biomass (Ginovart et al., 2002; Gras et al., 2011), which is an appropriate IbM mechanism to explain observed heterogeneity (Kreft et al., 2013). It is defined as:

$$(\ _{"\#\$})_{\%} = \ \% \qquad (1)$$

In Eq.1, the sub-index *i* stand for the type of nutrient, the variable u_i represents the amount of nutrient that could cross per unit of time, per unit of microbial biomass and *m* is the individual biomass. To estimate this microscopic uptake parameter u_i we considering use macroscopic parameter μmax reported in specific literature for *PD* by van Verseveld et al. (1983) of μmax = 0,418 h^{-1}, value obtained in experiments anaerobic NO_3^--limited were shifted to aerobic succinate-limitation, with this value the maximum uptake for each nutrient is calculated according to the stoichiometric coefficients adjusted by TEEM2 for each metabolic pathway in each time step (Table I).

Maximum nutrient availability: In IPa model, $(Nmax)_i$ is the maximum quantity of nutrient *i* that is available for a microorganism per unit of time in the CM close of it. This parameter is directly related to the CM characteristics and not to the types of microorganisms involved, it is defined as:

$$(\ _{"\#\$})_{\%} = \ \% \ \% \quad (2)$$

In Eq. 2, S_i represents the maximum amount of nutrient in the spatial unit where the individual cell is located and n_i is a number between 0 and 1 that indicates the fraction of the total nutrient in CM is available for the individuals living in the spatial unit. In order to describe mass transfer effects between CM nutrients and the microorganism surface, to approximate n_i value, we considering Dab as a reference of the diffusive flux relative to the

average velocity of the fluid mixture in the absence or minor effects of any convective transport for each nutrient in H_2O, then assign the minimum value to 1 and comparing all values to it, thus the nutrient with minimum Dab will have the maximum availability per unit of time and the nutrient with maximum Dab will have less availability per unit of time, so these numerical coefficients n_i are obtained and their ascribed units (h^{-1}) and assigned to the corresponding nutrient (Table I).

If, $(Umax)_i \geq (Nmax)_i$, the microorganism uptake is $(Nmax)_i$ and if, $(Umax)_i < (Nmax)_i$, then the microorganism uptake is $(Umax)_i$.

Cellular maintenance (clm): Before biomass synthesis, it is necessary that a microorganism complete its basal metabolism in order to keep its structures. For *PD* in aerobic phase growth considering succinate as ED, van Verseveld et al. (1983) propose a maintenance coefficient of 0.017 $gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$ and Tijhuis et al. (1993) of 0.025 $gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$ and for *PD* in anaerobic phase growth considering succinate as ED and NO_3^- as EA, van Verseveld et al. (1977) publish a maintenance coefficient of 0.004 $gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$ and Gras et al. (2011) in INDISIM-SOM model considering an appropriate maintenance requirement for heterotrophic microorganisms of 0.002 $gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$. Therefore, to fit the IPa model, maintenance energy requirement are close of the published values, with these values and performing calculations with stoichiometric coefficients adjusted by TEEM2, it has been established the maintenance requirements for aerobic and anaerobic phases (Table I).

Biomass generation and denitrification products: According to (McCarty, 2007) TEEM2 can make an adjustment between cell synthesis energy and the energy reaction to predict bacterial

yield with the associated Gibbs free energies for these reactions. Microorganisms capture energy for growth and maintenance from redox reactions; electrons are obtained from an ED and transferred to intracellular electron carriers, (Rittmann and McCarty, 2001) proposed pyruvate for TEEM1 and (McCarty, 2007) proposed acetyl-CoA for TEEM2, carriers bring the electrons towards the EA; as a result the acceptor suffers a reduction reaction (energy reaction), which causes the regeneration of the initial carrier. Due to the previous reactions, thermodynamic free energy is lost at each transfer; this loss is considered by including a term for energy-transfer efficiency (*e*), (McCarty, 1971) consider *e* in the range of 0.2 to 0.8. (Christensen and McCarty, 1975; VanBriesen, 2002) suggested an *e* value of 0.2 to 0.3 for aerobic heterotrophs and (McCarty, 2007; Xiao and VanBriesen, 2006,2008) proposed *e* value of 0.4 to 0.7 for anaerobic heterotrophs. For *PD* in aerobic phase growth considering succinate as ED, (Heijnen and Van Dijken, 1992) propose a maximum population growth yield (Yc/c) of 0.48 C-$mol_{microbial}$·C-$mol_{succinate}^{-1}$ and (van Verseveld et al., 1983) of 0.51 C-$mol_{microbial}$·C-$mol_{succinate}^{-1}$ and for *PD* in anaerobic phase growth considering succinate as ED and NO_3^- as EA, (Heijnen and Van Dijken, 1992) publish a Yc/c of 0.387 C-$mol_{microbial}$·C-$mol_{succinate}^{-1}$ and (van Verseveld et al., 1977) of 0,352 C-$mol_{microbial}$·C-$mol_{succinate}^{-1}$. Therefore, to fit the IPa model, these macroscopic Yc/c parameters are used to estimate microscopic Yc/c values, whereas the population growth is a consequence of the stochasticity effects of the all individuals actions to interact with the CM, the Yc/c individual parameter is considering highest to Yc/c population parameter, so, it has been established succinate as ED and C-source with O_2 and NO_3^- as EAs to aerobic phase. NO_3^-, NO_2^-, NO and N_2O as EAs to anaerobic phase, also in both phases the cell synthesis reaction use NH_4^+ as N-source (RC),

which combined by TEEM2 with an specific *e* value for achieve an individual Yc/c that fit a maximum population growth yield according to the publish values, will originate the adjusted metabolic pathways for IPa model (Table II).

Results and Discussion

The simulations with IPa model reproduce faithfully the (Felgate et al., 2012) experimental protocols. The simulations are divided into two main sections; first the model is tested from the NO_3^--sufficient, carbon-limited for high copper levels *PD* results of Felgate et al. (2012), cultures were initially grown under batch conditions at 100% air saturation (236 µM) for 24h. The aeration was then switched off and the system switched to continuous culture with a dilution rate of 0,05 h^{-1}. The reservoir medium feed contained 20 mM NO_3^-, 5 mM succinate and 10 mM NH_4^+. During the aerated batch phase in the virtual bioreactor biomass grown until a steady state was achieved (Fig. 2A), NO_3^- levels remained constant at ~20 mM (Fig. 2B) and no significant net accumulation of N_2O was observed (Fig. 2C). Following the switch to continuous culture, the dissolved O_2 decreased to NOX level. The NO_3^- levels in the virtual bioreactor decreased in response to the culture switching to anaerobic metabolism and its residual level in steady state was ~10 mM (Fig. 2B), the biomass also decreased until a new steady state was achieved (Fig. 2A) and the N_2O production immediately began to increase reaching ~ 0.5 to 0.6 µM in the steady state (Fig. 2C). Second the model is tested from the NO_3^--limited, carbon-sufficient for high copper levels *PD* results (Felgate et al., 2012), as the previous experiment after 24h of aerobic batch growth the air supply was switched off and the system switched to continuous culture with a dilution rate of 0,05 h^{-1}. The reservoir medium feed contained 5 mM NO_3^-, 20 mM succinate and 10 mM NH_4^+.

Through the aerated batch phase in the virtual bioreactor biomass grown exponentially to a value of ~ 0,9 $mg \cdot ml^{-1}$ (Fig. 3A), NO_3^- levels decreased from ~ 5 mM (Fig. 3B) and no significant net accumulation of N_2O was observed (Fig. 3C). Following the switch to continuous culture, the dissolved O_2 decreased to NOX level. The NO_3^- residual level in steady state was ~ 0,4 to 0,6 mM (Fig. 3B), the biomass also decreased until a new steady state was achieved (Fig. 3A) and the N_2O production immediately began to decrease reaching to ~ 0.001 mM in the steady state (Fig. 3C). After calibration, it is possible to simulate the evolution of variables that were not experimentally measured, such as the simulated temporal evolution of succinate, NH_4^+, O_2 and CO_2 for both experiments (Fig. 2D and Fig. 3D).

The calibrated values of the microbiological parameters that produced the best fit of simulation results to experimental data for experimental protocols carbon-limited and NO_3^--limited for high copper levels *PD* results of Felgate et al. (2012) are listed in Table I. The maintenance rate that leads to the best fit for biomass evolution, NO_3^- rate and N_2O production is equal to 0.002 $gC_{donor} \cdot gC_{microbial}^{-1} \cdot h^{-1}$ for aerobic and anaerobic phases. The NO uptake rate that leads to the best fit of simulation results for N_2O production change from $3 \cdot 10^{-4}$ to $7 \cdot 10^{-4}$ $mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$ for each experiment respectively, and the NO_3^- uptake rate for the aerobic phase is equal to 0.07 $mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$ for both experiments. These values are lower than established for some metabolic pathways in this model.

The NetLogo simulator (Fig. 4) will require further adjustments in order to be closer of the denitrification enzymes concentration for microorganism reality and established connections between denitrification enzyme expressions with the environment of the microorganism.

With the fixed parameters in both experiments IPa shows a population yield Yc/c (after 120 simulated hours) of 0.46 C-$mol_{microbial}$·C-$mol_{succinate}^{-1}$ and for aerobic phase a μmax of 0.245 h^{-1} and for anaerobic phase a steady state according to the dilution ratio of 0.05 h^{-1}. These values are agreeing with technical literature of *PD* in experiments with succinate as ED and various EAs as referred in the sub models section.

TEEM2 seems to be a useful tool for modeling one of the behavior rules that bacterium follows in IPa model. TEEM2 is one of the thermodynamic model based on bioenergetics growth efficiency, its accept implicitly that part of the nutrient is catabolized and that biomass is then synthesized from the products of catabolism and the other part of the nutrient is used for energy generation, according to von Stockar et al. (2008) it is an approximation of the true biosynthetic reactions because the thermodynamic prediction structure is the dependence on values for energetic efficiency, further, Xiao and VanBriesen (2008) hypothesized that bacterial energy capture efficiency is not a constant, but rather is controlled by environmental conditions, readers are referred to (Heijnen and van Dijken, 1993; McCarty, 2007; von Stockar et al., 2008) for further details.

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Table I. Microbiological and culture medium parameters for INDISIM-Paracoccus model. Maximum culture medium availability (*n*) according to Fick's law binary diffusion coefficient. Maximum microorganism uptake capacities (*u*) according to μmax by van Verseveld et al. (1983), and maintenance energy requirement (*clm*) according to Gras et al. (2011) in INDISIM-SOM model.

Maximum culture medium availability			
Nutrient	*n* (h^{-1})	Dab ($cm^2 \cdot s^{-1}$)	Ref.
Succinate	0.275	$9.13 \cdot 10^{-6}$	Dab value as a reference for each nutrient in H_2O according to the Perry
Ammonium	0.841	$2.79 \cdot 10^{-5}$	
Oxygen	0.788	$2.61 \cdot 10^{-5}$	
Nitrate	0.633	$2.20 \cdot 10^{-5}$	
Nitrite	0.787	$2.61 \cdot 10^{-5}$	
Nitric Oxide	1.000	$3.32 \cdot 10^{-5}$	
Nitrous Oxide	0.496	$1.65 \cdot 10^{-5}$	

Maximum microorganism uptake capacities		
Nutrient	*u* ($mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$)	Ref.
Succinate	0.52	According to μmax = 0,418 h^{-1} reported by Van Verseveld et al. (1983)
Ammonium	0.31	
Oxygen	0.54	
Nitrate	$0.27^a - 4.75^b$	
Nitrite	2.46	
Nitric Oxide	2.46	
Nitrous Oxide	1.23	

Maintenance energy requirement				
Aerobic phase		Anaerobic phase		Ref
Nutrient	*clm* ($mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$)	Nutrient	*clm* ($mol_{nutrient} \cdot mol_{biomass}^{-1} \cdot h^{-1}$)	According to Gras et al. (2011)
Succinate	0.0015	Succinate	0.0015	
Oxygen	0.0016	Nitrate	0.0068	

(*a*) Maximum nitrate uptake in aerobic phase, (*b*) Maximum nitrate uptake in anaerobic phase

Table II. Inorganic and organic half-reactions and their Gibb's standard free energy at pH = 7.0 according to Rittmann and McCarty (2001) to obtain balanced chemical equations according to TEEM2 (McCarty, 2007) for *PD* metabolic pathways to improve the individual behavior rule for biomass synthesis in IPa model.

	Half-reaction	$\Delta G^{o,}$ (kJ/eeq)
ED	$^{1}/_{7}\ CO_2 + {}^{1}/_{7}\ HCO_3^- + H^+ + e^- \rightarrow {}^{1}/_{14}\ (C_4H_4O_4)^{2-} + {}^{3}/_{7}\ H_2O$	29.090
EA (1)	$¼\ O_2 + H^+ + e^- \rightarrow ½\ H_2O$	- 78.719
EA (2)	$^{1}/_{8}\ NO_3^- + {}^{5}/_{4}\ H^+ + e^- \rightarrow {}^{1}/_{8}\ NH_4^+ + {}^{3}/_{8}\ H_2O$	- 35.11
EA (3)	$½\ NO_3^- + H^+ + e^- \rightarrow ½\ NO_2^- + ½\ H_2O$	- 41.650
EA (4)	$2H^+ + NO_2^- + e^- \rightarrow NO + H_2O$	- 33.718
EA (5)	$H^+ + NO + e^- \rightarrow ½\ N_2O + ½\ H_2O$	- 115.829
EA (6)	$H^+ + ½\ N_2O + e^- \rightarrow ½\ N_2 + ½\ H_2O$	- 133.469
RC	$^{9}/_{49}\ CO_2 + {}^{3}/_{49}NH_4^+ + {}^{3}/_{49}\ HCO_3^- + H^+ + e^- \rightarrow {}^{4}/_{49}C_3H_{5,4}O_{1,45}N_{0,75} + {}^{106}/_{245}\ H_2O$	20.398[a]
Path.1	Aerobic conversion of succinate: $0.0714\ (C_4H_4O_4)^{2-} + 0.0431\ NH_4^+ + \mathbf{0.0740\ O_2} \rightarrow 0.0575\ C_3H_{5,4}O_{1,45}N_{0,75} + 0.0136\ CO_2 + 0.0998\ HCO_3^- + \mathbf{0.0240\ H_2O}$	
Path.2	NO_3^- conversion in aerobic phase: $0.0714\ (C_4H_4O_4)^{2-} + 0.0429\ NH_4^+ + \mathbf{0.0374\ NO_3^-} + 0.0748\ H^+ + 0.0132\ H_2O \rightarrow 0.0572\ C_3H_{5,4}O_{1,45}N_{0,75} + 0.0141\ CO_2 + 0.0999\ HCO_3^- + \mathbf{0.0374\ NH_4^+}$	
Path.3	NO_3^- reduction with succinate: $0.0714\ (C_4H_4O_4)^{2-} + 0.0214\ NH_4^+ + \mathbf{0.3250\ NO_3^-} \rightarrow 0.0286\ C_3H_{5,4}O_{1,45}N_{0,75} + \mathbf{0.3250\ NO_2^-} + 0.0786\ CO_2 + 0.1214\ HCO_3^- + 0.0479\ H_2O$	
Path.4	NO_2^- reduction with succinate: $0.0714\ (C_4H_4O_4)^{2-} + 0.0413\ NH_4^+ + \mathbf{0.3250\ NO_2^-} + 0.3250\ H^+$	

	® 0.0551 $C_3H_{5,4}O_{1,45}N_{0,75}$ + **0.3250 NO** + 0.0189 CO_2 + 0.1015 HCO_3^- + 0.1885 H_2O
Path.5	NO reduction with succinate: 0.0714 $(C_4H_4O_4)^{2-}$ + 0.0413 NH_4^+ + **0.3250 NO** ® 0.0551 $C_3H_{5,4}O_{1,45}N_{0,75}$ + **0.1625 N_2O** + 0.0189 CO_2 + 0.1015 HCO_3^- + 0.0260 H_2O
Path.6	N_2O reduction with succinate: 0.0714 $(C_4H_4O_4)^{2-}$ + 0.0413 NH_4^+ + **0.1625 N_2O** ® 0.0551 $C_3H_{5,4}O_{1,45}N_{0,75}$ + **0.1625 N_2** + 0.0189 CO_2 + 0.1015 HCO_3^- + 0.0260 H_2O

(a) This value was estimated from reported value of 18.8 kJ/eep for an assumed cell relative composition of $C_5H_7O_2N$ (McCarty, 2007) and considering RC when NH_4^+ serves as the source for cell synthesis of $C_3H_{5,4}O_{1,45}N_{0,75}$

Figure 1. Schematic diagram of the microbial activity as described in the Process Overview and Scheduling for INDISIM-Paracoccus model.

Figure 2. Comparison between INDISIM-Paracoccus model (lines) and the experiment NO_3^- - sufficient, carbon-limited for high copper levels *PD* results (points) of Felgate et al. (2012). (A) Biomass evolution in aerobic and anaerobic phase, (B) Nitrate consuming rate, (C) N_2O production rate and (D) the evolution of variables that were not experimentally measured, such as succinate, NH_4^+, CO_2 (left scale) and O_2 (right scale) for the experiment.

Figure 3. Comparison between INDISIM-Paracoccus model (lines) and the experiment the NO_3^--limited, carbon-sufficient for high copper levels *PD* results (points) of Felgate et al. (2012). (A) Biomass evolution in aerobic and anaerobic phase, (B) Nitrate consuming rate, (C) N_2O production rate and (D) the evolution of variables that were not experimentally measured, such as succinate, NH_4^+, CO_2 (left scale) and O_2 (right scale) for the experiment.

Figure 4. NetLogo simulator for INDISIM-Paracoccus model. Shows graphical and numerical outputs and sliders to setup and control the bioreactor operation with maximum microorganism uptake capacities and maximum culture medium availability.

Figure 1

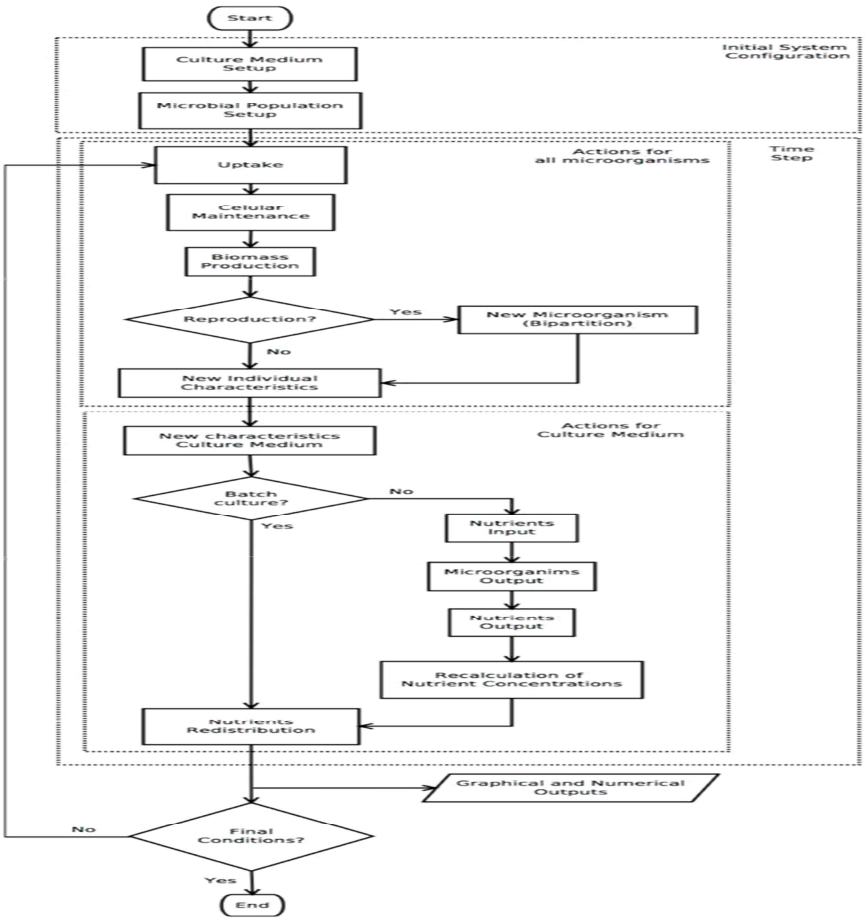


Figura 2

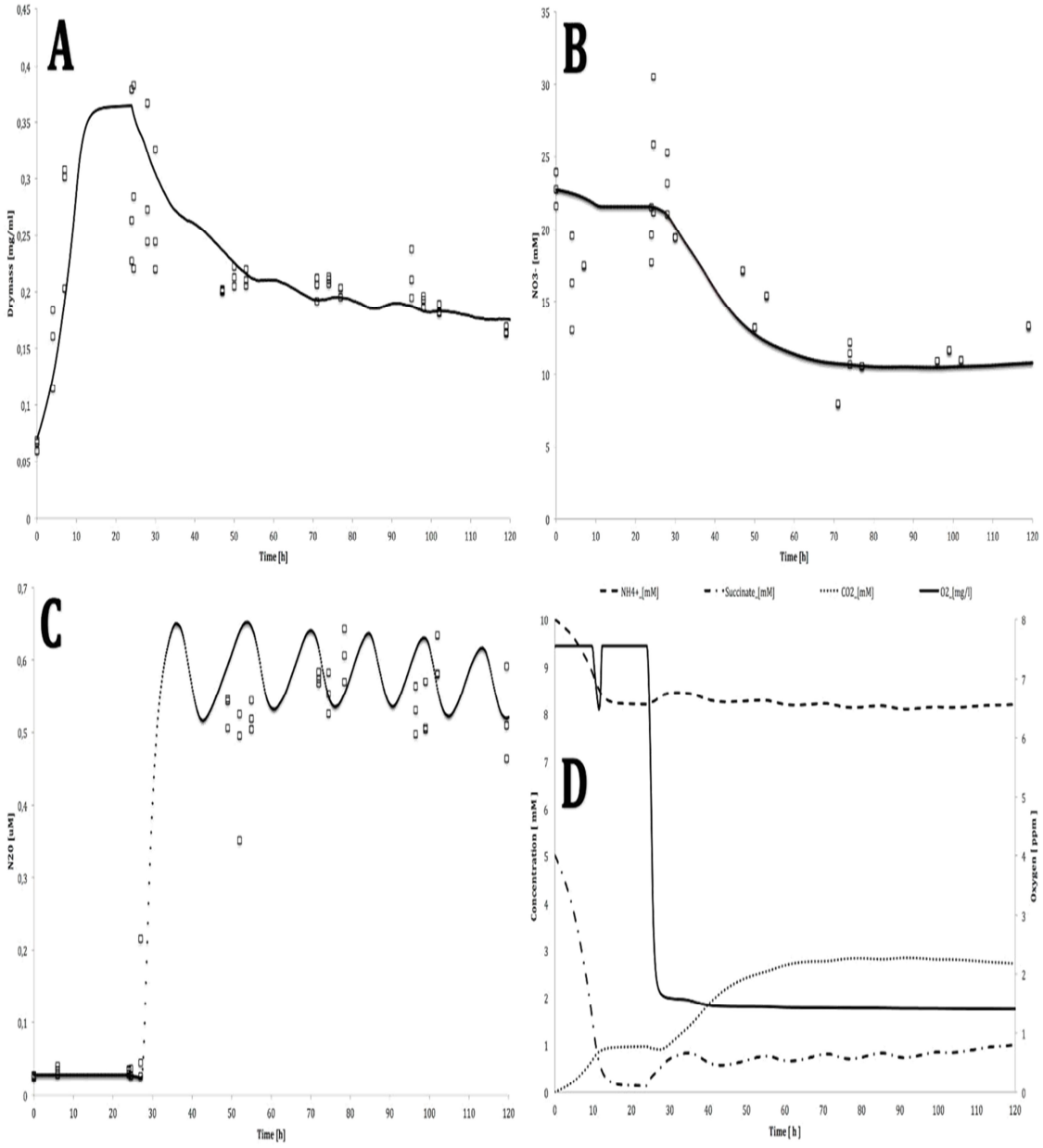

A
Drymass [mg/ml]
Time [h]
B
NO3- [mM]
Time [h]
C
N2O [uM]
Time [h]
D
NH4+_[mM]
Succinate_[mM]
CO2_[mM]
O2_[mg/l]
Concentration [mM]
Oxygen [ppm]
Time [h]

Figura 3

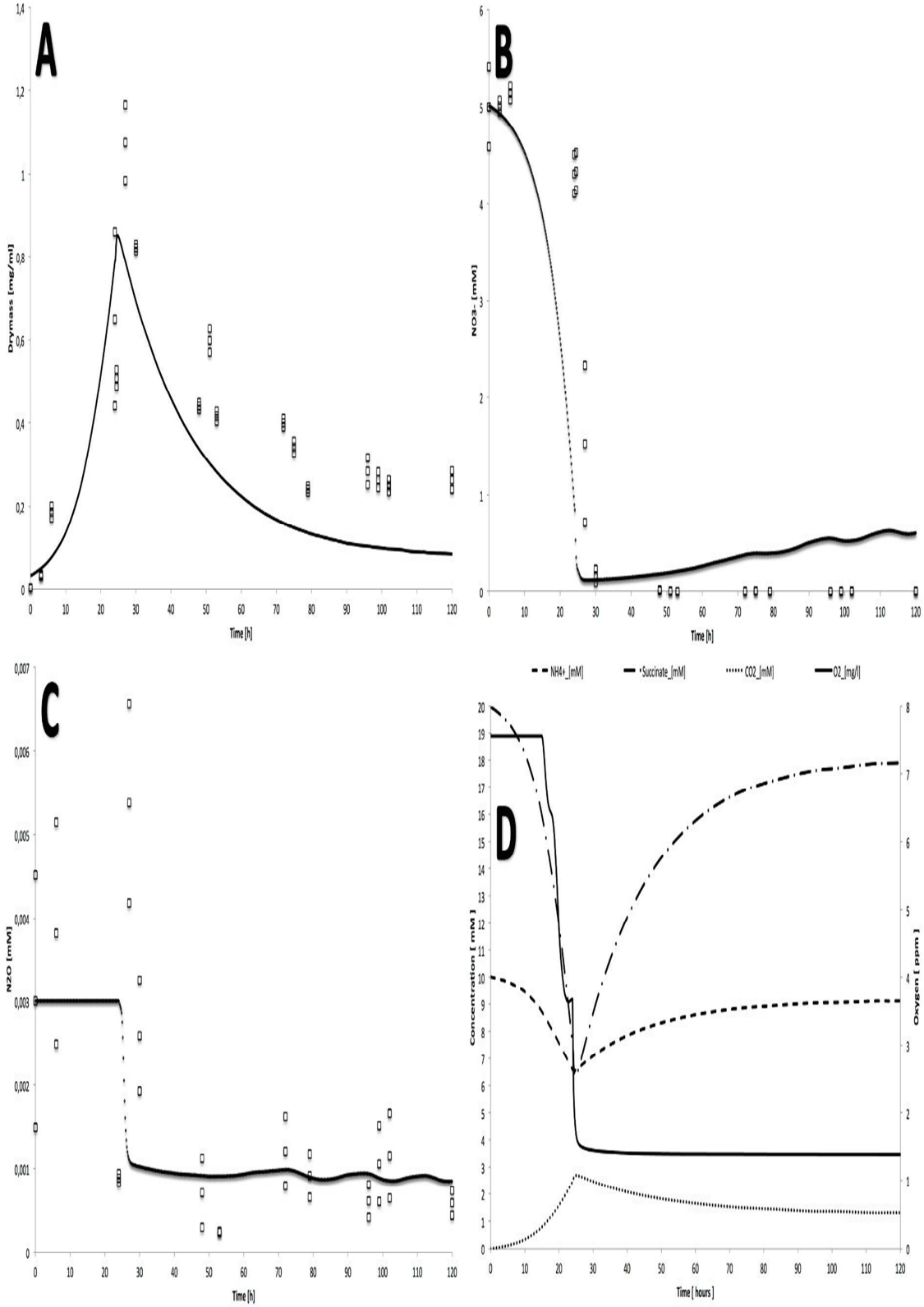
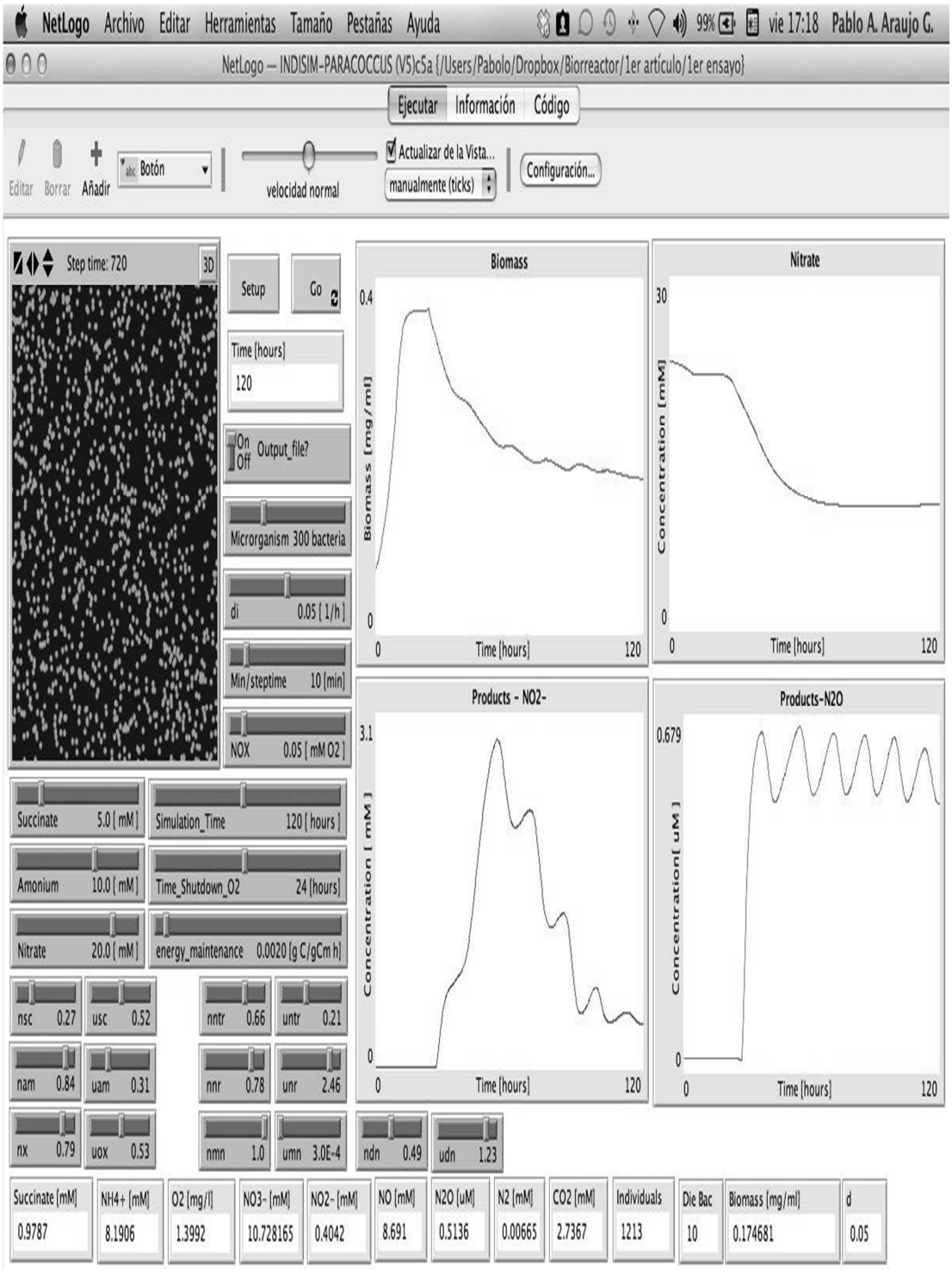

A
Dymass [mg/ml]
Time [h]
B
NO3- [mM]
Time [h]
C
N2O [mM]
Time [h]
D
NH4+_[mM]
Succinate_[mM]
CO2_[mM]
O2_[mg/l]
Concentration [mM]
Oxygen [ppm]
Time [hours]

Figura 4


NetLogo Archivo Editar Herramientas Tamaño Pestañas Ayuda
NetLogo – INDISIM-PARACOCCUS (V5)cSa {/Users/Pabolo/Dropbox/Biorreactor/1er artículo/1er ensayo}
Ejecutar Información Código
Editar Borrar Añadir Botón velocidad normal Actualizar de la Vista... manualmente (ticks) Configuración...
Step time: 720
3D
Setup
Go
Time [hours]
120
On Off Output_file?
Microrganism 300 bacteria
di 0.05 [1/h]
Min/steptime 10 [min]
NOX 0.05 [mM O2]
Succinate 5.0 [mM]
Simulation_Time 120 [hours]
Amonium 10.0 [mM]
Time_Shutdown_O2 24 [hours]
Nitrate 20.0 [mM]
energy_maintenance 0.0020 [g C/gCm h]
nsc 0.27
usc 0.52
nntr 0.66
untr 0.21
nam 0.84
uam 0.31
nnr 0.78
unr 2.46
nx 0.79
uox 0.53
nmn 1.0
umn 3.0E-4
ndn 0.49
udn 1.23
Biomass
Biomass [mg/ml]
0.4
0
Time [hours]
120
Nitrate
Concentration [mM]
30
0
Time [hours]
120
Products - NO2-
Concentration [mM]
3.1
0
Time [hours]
120
Products-N2O
Concentration[uM]
0.679
0
Time [hours]
120
Succinate [mM] 0.9787
NH4+ [mM] 8.1906
O2 [mg/l] 1.3992
NO3- [mM] 10.728165
NO2- [mM] 0.4042
NO [mM] 8.691
N2O [uM] 0.5136
N2 [mM] 0.00665
CO2 [mM] 2.7367
Individuals 1213
Die Bac 10
Biomass [mg/ml] 0.174681
d 0.05