An individual-based model for the study of *Paracoccus denitrificans*, a denitrifying bacterium

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In order to understand some environmental factors that control N_2O production by microbes in agricultural soils, a virtual bioreactor for *Paracoccus denitrificans* was designed using a culture medium containing succinate as a carbon source, ammonium as nitrogen source and various electron acceptors such as oxygen, nitrate, nitrite, nitrogen monoxide and dinitrogen oxide. INDISIM was the core individual-based model for the bacterial behavior and five metabolic pathways were selected and translated into balanced chemical equations using the Thermodynamic Electron Equivalents Model. This thermodynamic approach is the basis of the individual metabolism that this microbe carries out for its cellular maintenance and production of new biomass. The preliminary simulation results achieved with the implementation of this model in NetLogo showed that it is possible to investigate the behavior of this denitrifying bacterium and some of the outputs regarding the temporal evolutions of the diverse substrates are consistent with previous experimental data carried out with it.

Keywords Denitrification, *Paracoccus denitrificans*, Individual-based model, Thermodymanic Electron Equivalents Model, NetLogo

1. Introduction

Agriculture plays a substantial role in the balance of the three most significant greenhouse gases whose emissions are influenced by humankind. The three gases are CO_2, N_2O and CH_4. The global warming potential (GWP) of each of these gases can be expressed in CO_2 equivalents. The GWPs of N_2O and CH4 are 296 and 23 times greater, respectively, than a unit of 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 [1]. A majority of studies have shown that soil conditions such as water filled pore space, temperature, and soluble C availability have a dominant influence on N_2O emissions. Fertilizer source and crop management factors may affect N_2O emissions, but due to interactions with soil conditions it is difficult to make general conclusions. When N-fertilizer is applied above the economic optimum N rate, or when available soil N (especially in NO_3^- form) exceeds crop uptake, the risk of increased N_2O emissions rises. In conditions of low oxygen availability, such as waterlogged soils, certain bacteria are able to use the NO_3^- as a final electron acceptor and carry out respiratory metabolism in anaerobic conditions. These bacteria are known as denitrifying bacteria and are widespread in agricultural soils.

Denitrifying bacteria through an anaerobic process in which the nitrous oxide is an intermediate (N_2O) use nitrate (NO_3^-) and reduce it to nitrogen gas (N_2) via nitrite (NO_2^-). Consequently, understanding the environmental factors that control N_2O production and consumption by microbes is a challenge to the development of practical mitigation strategies for N_2O emissions [2].

ABMs (Agent Based Models), a type of computational model, simulate the interactions of autonomous agents (individual and collective entities) and the environment, in order to evaluate their effects on the system as a whole. This process serves to create and predict the complex phenomena. IBMs (Individual Based Models) are special cases where the agent is assimilated to a living entity. Therefore, the rules of simple behavior for different discrete microbes can generate a complex system behavior. The discrete modeling of microorganisms that are part of a system permits us to widen our knowledge of them and expand our understanding of the interactions that arise among them. As a result, we are able to study phenomena of competition, synergies and antagonisms.

The main goals proposed in this work are: i) Design and describe a bacterial model for *Paracoccus denitrificans,* one of the most important denitrifying bacteria in soils, and a culture medium in which it develops and grows (batch or continuous culture) in the context of the individual-based model methodology, identifying the main factors involved in the denitrification process driven by this microbe 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 and co-authors [2].

2. Materials and Methods

The individual-based model INDISIM, a computational model to study bacterial cultures [3-4] was the core model, for that, this denitrifying bacteria model is called INDISIM-Paracoccus. For establishing this model, the biomass synthesis and maintenance are identified as driving factors for denitrification process. Consequently, five metabolic pathways were selected and translated into balanced chemical equations following the methodology Thermodynamic Electron Equivalents Model for Bacterial Yield Prediction (TEEM2), a thermodynamic model for prediction of maximum microbial yields. The model is based on energy release and consumption as determined from the reduction potential or Gibbs free energy of half-reaction reduction equations together with losses of energy during energy transfer [5].

NetLogo is 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) and macro-level patterns that emerge from their interactions [6].

3. Results

3.1 INDISIM-Paracoccus model

The model description follows the ODD protocol, it stands for "Overview, Design concepts, and Details": the protocol 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 [7].

3.1.1 Purpose

Modeling a bioreactor to grow *P. denitrificans* in a culture medium with succinate, as electron donor and C-source, ammonium as N-source and various electron acceptors as oxygen, nitrate, nitrite, nitrogen monoxide and nitrous oxide, in order to identify those factors which are significant to the dynamics of denitrification products, especially greenhouse gas N_2O (Fig. 1).

3.1.2 Entities, State Variables, and Scales

The model has two kinds of entities: bacteria and square patches of culture medium. The bacteria are active individuals of *P. denitrificans*, which has the ability to perform five catabolic pathways in aerobic and anaerobic phases and fulfills its activities: maintenance, biomass generation and reproduction. The bioreactor is modeled as a two-dimensional grid cell, containing the culture medium and the metabolic products. Individual variables are included for each bacterium: unique identification number, location (XY coordinates of the grid cell where it is), biomass, rates of nutrient intake and counters for each reproduction cycle and metabolic pathway. Local variables for grid cells are: unique position identifier in XY coordinates, concentration of nutrients and metabolic products. The simulation time can be setup between 1 to 200 hours, with recommended the 120 hours as contrasted well with the experimental results.

3.1.3 Process Overview and Scheduling

In each simulation step the following processes are performed for each microorganism: nutrient intake, basal metabolism, when it is achieved then it synthetize new biomass following the thermodynamically feasible pathway. If the individual biomass has reached the reproduction mass value the individual is divided into two identical microorganisms. To model the bioreactor agitation, it proceeds to redistribute the nutrients homogeneously in the culture medium after each time step. To model the experimental protocol as a continuous culture it is assumed that a fraction of individuals and a fraction of all substrates are removed from the bioreactor according to the dilution ratio. In each time step the time dependent variable of microorganism and medium are calculated, updating the graphics and digital outputs according to the time scale proposed. A program step is equivalent to 30 minutes. During the simulation process, entities are randomized generating an asynchronous update effect.

3.1.4 Design Concepts

Basic Principles: The model incorporates five metabolic pathways adjusted by TEEM2 with the nutrient intake postulate and cell maintenance needs.

Emergence: Outputs of the model are the result of the adaptation of individuals to the culture medium. 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 and co-authors [2] in relation to the denitrification products concentration, biomass production, nitrate consumption rate, and nitrite production rate.

Adaptation: The microorganism can execute different metabolic pathways. Pathway selection depends on the prosperity of the thermodynamics and whether or not the surrounding nutrient concentration is enough for cell maintenance. The follow decision-making agent is about reproduction that means not to make bipartitions if that has not reached the minimum reproduction mass.

Sensing: The agent identifies only its biomass. With this information the agent takes nutrients from the medium for cellular maintenance. If it is completed, what keeps on to perform metabolic pathways of growth and generate denitrification products. The agent executes bipartition if reaches the minimum reproduction mass required.

Interaction: *P. denitrificans* is the only bacteria in the virtual bioreactor, and only interacts with the culture medium. In each simulation step, the agents arrive randomly to a grid cell of culture medium. If the concentrations of nutrients in the medium allow their cellular maintenance, will run their metabolic activity following the pathways modeled.

Stochasticity: Randomly assigned to individuals: initial individual position, initial biomass, changes in position and a fraction of them are removed from the bioreactor according to the dilution ratio. The concentrations of nutrients in each grid cell at the beginning of the model are located on a normal distribution with a standard deviation that is known and fixed by the user.

Collective: Simulated microorganisms do not develop aggregates; each agent acts uniquely.

Observation: The outputs of the model are the concentration of each component of the culture medium (succinate, ammonium, oxygen, nitrate) and all microbial denitrification products (biomass, carbon dioxide, nitrite, nitric oxide, oxide dinitrogen and nitrogen).

3.1.5 Initialization

At the beginning of the simulation, the user can adjust: nutrient concentrations in the culture medium, nutrients input flow that fix the bioreactor dilution ratio, initial amount of viable microorganisms, oxygen dissolved level in the culture medium for change the bacterial metabolic pathway between aerobic and anaerobic phase.

3.1.6 Input data

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

3.1.7 Sub models

Some of the individual sub-models considered are:

Nutrient intakes: The cell membrane-associated proteins transport specific nutrients. Active sites cover a part of the membrane surface, so the microorganism has the ability to capture nutrients dissolved in the spatial cell where it develops. The intake parameter is negative correlated with the biomass in order to assume that the smaller individuals have greater nutritional requirement. The intake of each nutrient is calculated according to the individual biomass and assigned according to the metabolic pathway that uses and only part of nutrient concentration in the culture medium cross the cell membrane. For INDISIM-Paracoccus three different intake rates were established, one for the aerobic phase and two for anaerobic phases. Intakes during the anaerobic phase were higher compared to the aerobic phase, because the *P. denitrificans* is a denitrifying bacterium.

Cellular maintenance: Before biomass synthesis, it is necessary that a microorganism complete its basal metabolism in order to keep its structures. It is assumed that a maintenance requirement of 0.002 g C donor/(g C microbial h) is appropriate for heterotrophic microorganisms [3]. With this value and performing calculations with stoichiometric coefficients adjusted with TEEM2, it has been established the INDISIM-Paracoccus maintenance requirements.

Biomass generation and denitrification products: Microorganisms capture energy released by redox reactions. Electrons are obtained from a primary donor and transferred to intracellular electron carriers. Carriers bring the electrons towards the terminal acceptor. As a result the acceptor suffers a reduction reaction, which causes the regeneration of the initial carrier. Due to the previous reactions, thermodynamic free energy is lost at each transfer. [5]. TEEM2 can make an adjustment between cell synthesis energy and the energy lost in the

carriers. Gibbs free energy determines this approach in the half-reactions reduction considering energy losses during the process of energy transfer [8]. Bacterial growth involves two basic redox reactions, one for the production of energy and the other for cell synthesis. Succinate is the electron donor, it supplies electrons to the acceptor, depending on the chosen metabolic pathway, oxygen in aerobic phase, and nitrogen species for the anoxic phase. For energy production, a half-reaction cell synthesis that use ammonium as N-source is necessary [5]. Therefore, it has been established the half-reaction reduction for electron donor, the electron acceptor and cell synthesis, which combined by TEEM2, will originate the adjusted metabolic pathways for INDISIM-Paracoccus model (Fig.1).

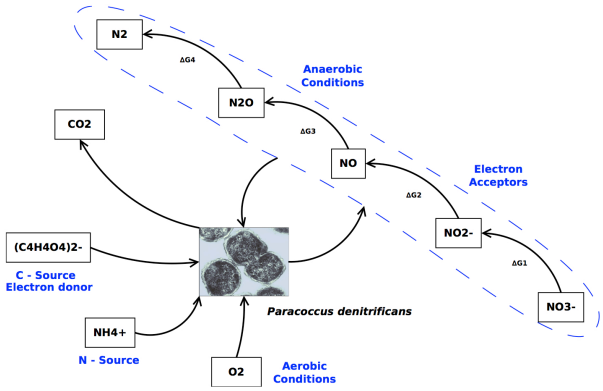

Fig. 1 Schematic representation of INDISIM-Paracoccus model shows the electron donor (C-source), ammonium as N-source and, the different electron acceptors, interacting with the microorganism, which execute their metabolic pathways to obtain denitrification products.

3.2 INDISIM-Paracoccus simulator

Using the INDISIM-Paracoccus model implemented in Netlogo plaform, a virtual bioreactor was generated (Fig. 2). The outputs generated are compared with some of the experimental data presented by Felgate and co-authors [2].

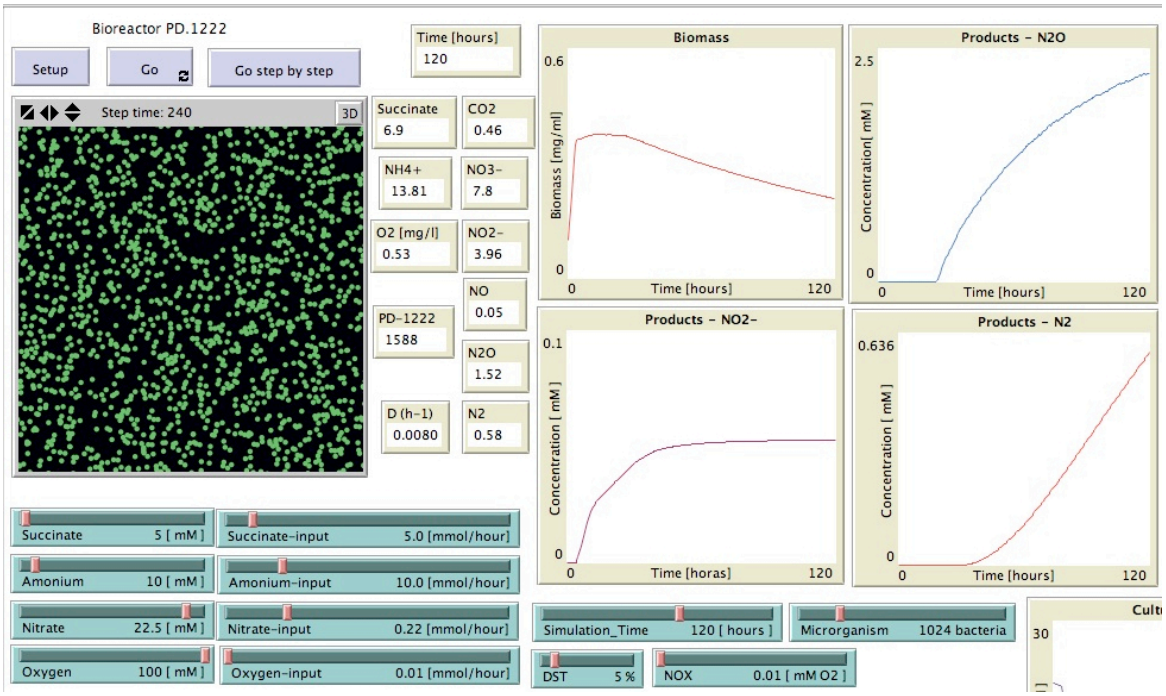

Fig. 2. A screenshot of INDISIM-Paracoccus simulator**.** Sliders to control values for the model parameters with graphical and numerical outputs.

4. Discussion

The simulator developed allows to work with batch or continuous cultures, being the user able to: define the simulation time, formulate different culture medium, adjust the dilution ratio, setup the initial number of viable microorganisms, setup the oxygen dissolved level in the culture medium which is the key factor to carry out the aerobic and anaerobic metabolic pathways.

The INDISIM-Paracoccus simulator graphical outputs are consistent with previous experimental data carried out with *P. denitrificans* by Felgate and co-authors [2] regarding the microbial biomass, nitrous oxide production and nitrite production rate, in continuous culture at 120 simulated hours.

The nutrient intake rates established for some metabolic pathways in this model and simulator will require further adjustments and modifications of some model parameters in order to be closer of the microorganism reality.

TEEM2 seems to be an useful tool for modeling bacterial metabolism, but it is necessary further work to better adjust the energy transfer efficiency parameter for each metabolic pathway.

The IBM developed allows modeling complex systems of interaction between microorganisms and their culture medium under different management protocols with this bioreactor. NetLogo platform offers the possibility to develop a friendly simulator like INDISIM-Paracoccus to be used with different goals.

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