Communication

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

Pablo Araujo Granda a,b,⁎, Anna Gras b, Marta Ginovart c

⁎ Corresponding author.
E-mail addresses: pablo@araujo.ec, paraujo@iquce.edu.ec (P. Araujo Granda), anna.gras@upc.edu (A. Gras), marta.ginovart@upc.edu (M. Ginovart).

1. Introduction

For the construction and development of models of living organisms, it is necessary to describe their physical constants, physiological reactions, interactions and responses to the environment. A robust model of any living system must include chemicals and conservation principles, and relate stoichiometric mass balance with the laws of conservation of energy. Most biochemical processes that involve energy transduction close to the balance can be studied using a non-equilibrium thermodynamic approach. Intuitively, a complete model has a number of sub-models that take into account the compartmentalized structure of living organisms; and specifically the metabolic sub-model should include descriptions of different parts and functions of their developmental stages [1–3].

The use of microbial metabolic reactions (MMRs) that include the microbial bio-mass as a product or as a reactant is a relevant task for modelling microbial activity. An MMR is the written version of a biochemical process. The stoichiometry is a part of the chemistry that permits the writing of balanced equations because it studies the molar relationship between the reactants and the products in a chemical reaction. There are several reasons to suggest that the presence of microorganisms in a biochemical process complicates the stoichiometry. First, biochemical reactions often involve oxidation and reduction of more than one chemical species. Second, the micro-organisms have two different roles: they perform the reaction and also they are the final products of it. Third, the micro-organisms produce several chemical species.

© 2016 The Authors. Published by Elsevier B.V. on behalf of Research Network of Computational and Structural Biotechnology. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
for biomass yield prediction based on the energy-transfer-efficiency (ε) between catabolism and anabolism [4,5,7–14], while other thermodynamic approaches correlate the biomass yield calculations with the Gibbs energy dissipations [6,15–21]. In brief, the thermodynamic approaches explain how catabolic and anabolic reactions are coupled to predict the biomass yield in terms of standard Gibbs energy. Considering these diverse approaches in the bio-thermodynamics field, this study takes into consideration the one that is based on ε, because it includes: i) the standard Gibbs energy for cell synthesis from different carbon-sources and nitrogen-sources, ii) the energy available from substrate transformation, and iii) the ε to the overall metabolic process. In addition, choosing the thermodynamic approach allows the prediction of full stoichiometric relationships for biological reactions in the absence of experimental yield values [5,12].

One powerful tool that is currently revolutionizing the study of microbial biotechnology is the mathematical and computing modelling that explicitly takes into account the microbial diversity and the exchanges of nutrients and materials within the micro-organism community and its surrounding environment [22,23]. Models such as individual-based models (IBMs) [24–27], metabolic energy-based modelling (EBMs) [28], reaction-centric models (RCMs) [29], and even, deterministic-population models (DPMs) [30] can obtain useful information from a MMR. The reason is that these reactions, or the stoichiometry between chemical species described in the metabolic sub-model, can help in understanding how the microbial interactions happen and why microbial systems behave as they do.

Environmental microbiologists could take advantage of the knowledge of metabolic reactions because usually the chemical resources for the micro-organisms are the pollutants that technicians must control [4]. In other words, the information provided by MMRs could be of importance in other engineering fields because they provide information about the quantities of chemicals required to satisfy microbial requirements as well as the quantities of the generated end products [5].

In this context, the principal aim of this contribution is to develop a computational tool to write MMRs for processes driven by a range of microbes based on the Thermodynamic Electron Equivalents Model (TEEM), a methodology proposed by Perry L. McCarty [4,7–9,11], which is the baseline of many current bio-technological studies [4,27,31].

The specific objectives are: (i) to implement the tool on the open-access platform NetLogo to distribute the source-code among the scientific community, (ii) to promote its use and to encourage future extensions and adaptations; and (iii) to test the computational tool by writing of some MMRs.

This manuscript presents the following structure: Section 2 describes the TEEM thermodynamic approach, the basic concepts about the empirical chemical composition used to represent the microorganisms and the open-access software used to implement the computational tool; Section 3 describes the design, performance and application of the computational tool and presents some MMRs for diverse metabolic functional groups of micro-organisms; and finally Section 4 presents some final remarks and possible future applications for this work.

2. Materials and Methods

2.1. TEEM: the thermodynamic approach

The Thermodynamic Electron Equivalents Model (TEEM) is designed to study the stoichiometry and kinetics of a wide variety of biological treatments of wastewaters. TEEM writes a stoichiometric reaction to describe the overall cellular metabolism from reduction-half-reactions for: the electron-donor (RD), electron-acceptor (Ra) and cell synthesis (Rc). TEEM is based on terms of the standard Gibbs free energy involved in these reactions and in how the energy between catabolism and anabolism is coupled [4,7–11].

For the use of TEEM, no specific and detailed knowledge of metabolism is required. First, we have to identify the electron donor(s) (eD) and the electron acceptor(s) (eA) and write reduction-half-reactions for each one of them. Second, it is necessary to establish the N-source for biomass synthesis, and third, it is essential to determine the empirical chemical formula that will represent the microbial cells.

TEEM has two versions, the first one, TEEM1 [4], considers a realistic formulation of the anabolic reaction taking into account different N-sources such as ammonium (NH₄⁺), nitrate (NO₃⁻), nitrite (NO₂⁻) and di-nitrogen (N₂), and a complete explanation of ε between catabolism and anabolism. The second version, TEEM2 [11], complements TEEM1 because it considers oxygenase reactions involved and the aerobic heterotrophic oxidation of C₁ organic compounds.

According to TEEM, the metabolic energy is obtained from the redox reaction between an eD with an eA. The electrons are obtained from eD and transferred to intra-cellular intermediates. In TEEM1 the intermediate is the pyruvate with its half-reaction standard Gibbs free energy equal to 35.09 kJ/eeq, and in TEEM2 the intermediate is acetyl-CoA with its half-reaction standard Gibbs free energy equal to 30.9 kJ/eeq. The intermediate compounds bring the electrons towards the eA, which is being reduced causing the initial carrier regeneration [11].

TEEM calculates microbial yield (Yc/c) using a relation between the standard Gibbs free energy of the catabolic and anabolic reactions and an appropriate ε value. The microbial catabolism is represented by the energy reaction (Re). To write it we have to combine Rd with Ra. Once Re is known, it is necessary to represent the microbial anabolism by writing the reaction for microbial biomass synthesis (Rs), and to do this we have to combine Rd with Rc.

Rc is a hypothetical half-reaction, which considers as reactants the N-source (NH₄⁺, NO₃⁻, NO₂⁻, or N₂), carbon dioxide (CO₂) and bicarbonate (HCO₃⁻), and as products water and the microbial biomass represented by an empirical chemical formula of cells (CₙHₒₓOₙNₓ). This empirical chemical formula considers the molar relationships only for four basic elements: Carbon (c), Hydrogen (a), Oxygen (b) and Nitrogen (c). To establish the adequacy of this formula, researchers compared theoretical thermodynamic calculations using the cell’s empirical chemical formula with the thermodynamics of growth of the same micro-organism on several substrates using batch cultures growing in the exponential phase at pHₙₐₜₐ [32–35]. If the formula only considers four elements, the fitness is close to 95%, but if we include two more elements, e.g. phosphorous and sulphur, the fitness increases its value to around 98–99% [36,37].

To estimate the standard Gibbs free energy of Rc (ΔGₚₑ), TEEM proposes a value of 3.33 KJ per gram cells [4,9,11], which is related to one generic microbial cell composition C₉H₁₀O₇N when NH₄ serves as the N-source for cell synthesis. The ΔGₚₑ value is valid in the context in which TEEM was developed. This does not make a great deal of difference in calculated microbial yields, but is felt to be an acceptable theoretical choice [11]. In other research fields, e.g. bio-geochemical processes in marine environments, and taking into account different pressure, temperature, pH and N-sources for cell synthesis the reported ΔGₚₑ value is 302 J per gram cells [3,37,38]. Moreover, TEEM considers in its internal structure the possibility of using four different N-sources to write Rc [4]. The theoretical explanation is that micro-organisms prefer to use ammonium as an inorganic nitrogen source for cell synthesis, because it is already in the (-III) oxidation state, the status of organic nitrogen within the cell. However, when ammonium is not available for cell synthesis, many prokaryotic cells could use oxidized forms of nitrogen as alternatives. Therefore, nitrate (NO₃⁻), nitrite (NO₂⁻) and di-nitrogen (N₂) are included as nitrogen sources. When an oxidized form of nitrogen is used, micro-organisms must reduce it to the (-I) oxidation state of ammonium, a process that requires electrons and energy, thus reducing their availability for synthesis. Therefore, different N-sources will obtain different results in the microbial yield [4,11].

To couple the energy from catabolism to anabolism, TEEM considers a relation between the electrons involved. The electrons that come from
the eD will be divided into two portions. The first portion (feo) is transferred to the eA to generate energy (catabolism) and the other portion of electrons (fso) is transferred to the N-source for cell synthesis (anabolism). TEEM calculates the relationship between feo and fso using: (i) standard Gibbs free energy of Rd, Ra and Rc, (ii) standard Gibbs free energy of the intracellular intermediates compounds, and (iii) an appropriate ε value. This term is included because TEEM considers that a fraction of the thermodynamic free energy involved is lost at each energy transfer between catabolism and anabolism [13,14].

With this information, an MMR (R = feoRa + fsoRc − Rd) is written. This reaction represents the full stoichiometric relationships for a biological process without the experimental yield values commonly required to obtain it [12]. For a detailed description of this thermodynamic approach the reader can refer to McCarty (2007) and Rittmann and McCarty (2001).

2. The NetLogo platform

The computational tool is implemented in the widely used, free and open source program NetLogo, a multi-agent programmable modelling environment [39]. NetLogo is licensed under GPL (GNU General Public Licence) and it was chosen mainly for the way this platform is organized: the source-code is very well documented, open and easy to read, giving the option to share this developed tool with other researchers without difficulty. This straightforward interaction, in the near future, will facilitate the upgrading of the computational tool by the scientific community interested in writing and using metabolic reactions for microbial processes.

3. The developed computational tool: MbT-Tool

We have named the computational tool as MbT-Tool, standing for Metabolism based on Thermodynamics. MbT-Tool is the interactive tool developed to study and model microbial metabolism based on two versions of TEEM: TEEM1 [4] and TEEM2 [11]. With the MbT-Tool it is possible to write MMRs conducted by microbes. Moreover, using the MbT-Tool it is possible to predict the microbial yield in standard thermodynamic conditions, e.g. P = 1 bar, temperature = 25 °C and pH = 7.0.

The MbT-Tool user interface displays all variables and parameters regarding the composition of the microbial biomass and the substrates that can be selected to describe the metabolic processes, avoiding the complex and tedious calculations of the implemented thermodynamic model by the user. The composition of the microbial biomass, which is represented by CnH2OcNc, seems to be related to the substrates where the micro-organism grows and it can be slightly different depending on the C-sources and N-sources [14]. However, Battley (2013) asserts that the empirical composition of the same cells metabolizing any given substrate is expected to be the same. Therefore, the MbT-Tool considers Battley’s hypothesis as valid and allows use of the same microbial biomass formula and writing MMR using different growing substrates.

Fig. 1 shows the schematic description of how the MbT-Tool is based on TEEM with a brief explanation of TEEM concepts which are helpful in understanding the main terms involved in it, especially how energy from catabolism is coupled to anabolism and as a result of these combinations a MMR is obtained. Fig. 2 shows the schematic programming structure of MbT-Tool using a flow diagram. Fig. 3 shows a screenshot of the MbT-Tool, which includes: interface, info and code tabs. The interface tab is where the reader can obtain the outputs. It also has inputs-options that can be used to set up the MbT-Tool. The info tab provides an introduction to the MbT-Tool. It explains how it was created and how to use it. The code tab is where the code is stored. The reader can find the lists of organic and inorganic reactions, TEEM equations with theoretical half-reactions of biomass formation taking into account different N-sources, and other programmed details of the MbT-Tool in the supplementary material.

Fig. 1. MbT-Tool is a computational tool to represent the microbial metabolism through a microbial metabolic reaction (R) using TEEM as thermodynamic approach which is based on the energy-transfer-efficiency between catabolic and anabolic processes.
3.1. How to use MbT-Tool

To use the developed computational tool, it is necessary to install NetLogo (at least NetLogo 5.0) [https://ccl.northwestern.edu/netlogo/download.shtml], and download the MbT-Tool [http://mosimbio.upc.edu/en/publications/publications-by-year/years-2010-now]. It runs on any computer that supports JAVA with an operating system (OS): Windows, Mac OS X and Linux [22,39,40].

On opening MbT-Tool, the interface window appears (Fig. 3). On the first screen the user has to select: one or two electron donor(s) and one electron acceptor from the organic (in supplementary material, Table S1) or inorganic (in supplementary material, Table S2) reduction-half-reactions, and the empirical chemical formula of the cells (in supplementary material, Table S3) or can introduce the molar relationship between the four main elements (C, H, O and N), the N-source to the cell synthesis half-reaction. It is possible to choose between four sources, NH4+, NO3−, NO2− or N2 (please refer to the supplementary material, Table S4).

With this setup information, the user has to select the thermodynamic approach TEEM1 or TEEM2 (in supplementary material, Table S5) to write the MMRs. If the user chooses TEEM2 it is necessary to define the number of oxygenase reactions per mole of substrate, introducing an integer number in the “q” parameter. Finally, the user has to fix the ε value for the process. With all this data, the MbT-Tool displays the following outputs: the Rd, Ra and Rc half-reactions, the energy reaction (Re), the synthesis reaction (Rs), feo, fso, ε, the MMR (R) and the calculated microbial yield (Fig. 3). This MMR is the reaction that McCarty defines as global reaction, which arises from the combination between: the catabolic reaction with the anabolic reaction and the value of ε [11]. The user could also download these outputs in an archive with a “.txt” extension. The file name is written with the information of the Rd, Ra, Rc, N-source and the thermodynamic approach used (TEEM1 or TEEM2).

To avoid potential errors when using the MbT-Tool, we recommend not selecting the same chemical species to the eD and to the eA; they must be different. If eD and eA are the same, the result of the calculations using their standard Gibbs free energy could provoke an inconsistent value on the fraction of electrons destined for cellular synthesis or electrons destined for energy. An inconsistent value will be obtained when these fractions of electrons are greater than one or below zero. If this numerical inconsistency occurs, the MbT-Tool stops and shows an alert message to the user.

Regarding the decision of using TEEM1 or TEEM2, it all depends on: (i) the growth substrate, (ii) the microorganism(s) involved, and (iii) the metabolic pathway that will be represented using the MbT-Tool. For instance, if the growth substrate is a C1 compound, it is better to use TEEM2 over TEEM1. If one of the intermediates metabolic compounds in the pathway is pyruvate, it is better to use TEEM1 over TEEM2. If Acetyl-CoA is an intermediate compound in the pathway, it is better to use TEEM2 over TEEM1. Finally, if oxygenase reactions are involved, it is better to use TEEM2 over TEEM1. Basically, microorganisms utilize the oxygenase reactions to create more biodegradable forms of substrates. Some examples are: when alkanes are hydroxylated [41], alkenes are converted into the corresponding epoxides [42], carbon monoxide is oxidized to carbon dioxide [43], ammonia is oxidized to hydroxylamine [44], and some aromatic compounds and cyclic alkanes are hydroxylated [45]. To determine the “q” value, the user must establish if the reaction is mono-oxygenase-catalyzed or dioxygenase-catalyzed. But in the most common cases, the “q” value ranges from 0 to 4. For a detailed description of this procedure the reader can refer to Xiao and VanBriesen (2006, 2008) [13,14].

3.2. Case-studies

Some different microbial processes have been selected to demonstrate the potential and versatility of the MbT-Tool showing the output.
of the simulator, which is a set of MMRs with the corresponding yield prediction value.

In the experiments carried out by Battley (2013, 2007, 1995) [32,35,36] related to the Saccharomyces cerevisiae, he has established C6.33H10.21O3.53N as the empirical chemical formula for this yeast. Considering this information, using the MbT-Tool it is possible to represent two metabolic pathways: the biomass synthesis from glucose, and the biomass synthesis from pyruvate to ethanol. Before executing the MbT-Tool, it is necessary to determine some parameters. We used TEEM1 because we consider that there is no oxygenase involved and the c-source is not a C1 compound. We used NH4+ because this chemical species is the universal N-source to biomass synthesis, and for ε we used a value that allows us to obtain the reported cell yield growing on glucose of the 0.098 mol Ccells/mol Cdonor [36].

Using the MbT-Tool with the established inputs, theoretical considerations and TEEM1 with ε value of 0.57 for the first pathway and ε value of 0.84 for the second one, the MMRs and the yields are:

**Biomass synthesis from glucose:**

\[
\begin{align*}
\text{CH}_3\text{COCOO}^- + 0.047 \text{NH}_4^+ + 2.25 \text{HCO}_3^- & \rightarrow 0.094 \text{C}_6\text{H}_5\text{O}_6 + 2.16 \text{CH}_3\text{COO}^- + 1.17 \text{CO}_2 + 3.59 \text{H}_2\text{O} \quad \text{with } \text{Yc/c} = 0.099 \text{ mol Ccells/mol Cdonor}. \\
\end{align*}
\]

**Biomass synthesis from pyruvate to ethanol:**

\[
\begin{align*}
\text{CH}_3\text{COCOO}^- + 0.047 \text{NH}_4^+ + 1.325 \text{H}_2\text{O} & \rightarrow 0.047 \text{C}_6\text{H}_{10.21}\text{O}_{3.53}\text{N} + 0.734 \text{CH}_3\text{CH}_2\text{OH} + 0.283 \text{CO}_2 + 0.953 \text{HCO}_3^- \quad \text{with } \text{Yc/c} = 0.098 \text{ mol Ccells/mol Cdonor}. \\
\end{align*}
\]

In the published research carried out by H.W. van Verseveld [46–48] related to the growth of Paracoccus denitrificans, and considering succinate as electron donor and various final electron acceptors, the reported formula for this denitrifying bacterium was established as C4H4O4N0.75. To write MMRs, we considered succinate as C-source, NH4+ as N-source for biomass synthesis and the main electron acceptors involved in the de-nitrification pathway. Taking into account this information we used the MbT-Tool with TEEM2 and different ε values to represent a sequence of four reduction reactions (NO3− → NO2− → NO → N2O → N2) using MMRs for this denitrifying bacterium.

First reaction (NO3− → NO2−): (C4H4O4)2− + 0.30 NH4+ + 4.55 NO3− → 0.40 C3H5.4O1.45N0.75 + 4.55 NO2− + 1.10 CO2 + 1.70 HCO3− + 0.67 H2O. (ε = 0.41).

Second reaction (NO2− → NO): (C4H4O4)2− + 0.58 NH4+ + 4.55 NO2− + 4.55 H+ → 0.77 C3H5.4O1.45N0.75 + 4.55 NO + 0.26 CO2 + 1.42 HCO3− + 2.64 H2O. (ε = 0.84).

Third reaction (NO → N2O): (C4H4O4)2− + 0.58 NH4+ + 4.55 NO → 0.77 C3H5.4O1.45N0.75 + 2.28 N2O + 0.26 CO2 + 1.42 HCO3− + 0.36 H2O. (ε = 0.56).

Finally, the reaction (N2O → N2): (C4H4O4)2− + 0.58 NH4+ + 2.28 N2O → 0.77 C3H5.4O1.45N0.75 + 2.28 N2 + 0.26 CO2 + 1.42 HCO3− + 0.36 H2O. (ε = 0.53).

Using these MMRs we created an IBM called INDISIM-Paracoccus [27,31,48,49], which is designed to study the de-nitrification process carried out by the bacteria P. denitrificans in order to explore the consequence of different priorities in the individual use of electron-acceptors on the denitriﬁcation pathway [27].

Battley (1987) [33] established C3.85H6.69O1.78N as the empirical chemical formula for Escherichia coli. Considering this information, using the MbT-Tool it is possible to represent its diauxic growth on glucose and lactose. With the obtained MMR, it is possible to begin a modelling project related to the quantitative determination of
metabolic fluxes during co-utilization of two C-sources [49] or a modelling project related to the organization of metabolic reaction networks [50]. We used TEEM2 because we consider that at least one mono-oxygenase-catalyzed reaction is involved (q = 1) [13,14]. We used NH4 as N-source to biomass synthesis. We used two eDs (glucose and lactate) and one eA (oxygen), and for e we used a 0.37 value [11]. Using the MbT-Tool with the established inputs, the MMRs and the yields are:

C6H12O6 + CH3CHOHCOO− + 5.05 O2 + 1.018 NH4 + 0.018 HCO3− → 1.018 C6H8O6NH4+ + 5.097 CO2 + 7.133 H2O.

Bacterial yield: Yg/m = 97.139 (gramcells/mol donor); Yc/m = 3.917 (molcell/mol donor) and Yc/e = 0.435 (molcell/molc donor).

In the MbT-Tool, we selected a gram-negative bacterium with the empirical formula of C6H2O2N [5]. This bacterium degrades nitritotriacetic acid (NTA) in the absence of molecular oxygen. To write the metabolic equation using nitrate as eA, NTA is used as the eD, and TEEM1 is used as the thermodynamic approach with an ε value of 0.33 [4,9,11,13,14]. Wanner et al. [51] reported a cell yield achieves an MMR in which one of the products is the chemical composition of the micro-organism involved in the process and some values of its bacterial yield. With these reactions, the user can start to construct a model, ii) incorporate the information into an existing model or iii) start the calculations of the mass balance for a bioreactor.

However, this tool has a limitation: it is not possible to compare the MbT-Tool’s outputs directly with experimental results. We consider that results obtained through the MbT-Tool could be the starting point to deal with a modelling project in the framework of microbial ecology or biotechnological processes. The non-expert user achieves an MMR in which one of the products is the chemical composition of the micro-organism involved in the process and some values of its bacterial yield. With these reactions, the user can start to construct a model, ii) incorporate the information into an existing model or iii) start the calculations of the mass balance for a bioreactor.

4. Final remarks

The main purpose of this contribution is to present the development of the MbT-Tool and make it available to a wide spectrum of readers, showing how this tool could be used in different research frameworks. This tool is a tangible way of achieving the compression of a thermodynamic approach for the construction of living models could be a time-consuming task. In contrast, a person with experience in these MMRs could have enough criteria to use and interpret the output reactions from MbT-Tool in his academic or research context.

In the research field on IBMs, using the INDISIM methodology as a core model, for instance [24–26,52–54], we realized that is essential to know the metabolic reactions carried out by the micro-organisms to increase or decrease their own biomass. These metabolic reactions could be used to design the individual metabolism model as well as to

(rd) Electron donor --> NTA - Acid nitritotriacetic :
+ 0.0556 NH4+ + 0.3333 HC03− + 1.1111 H+ + 1 e− --> + 0.0556 (C6H6O6N)3− + 0.6667 H2O
[ ΔG = 68.889 KJ/eq ]

(ra) Electron acceptor --> NO3− --> N2 :
+ 0.2 NO3− + 1.2 H+ + 1 e− --> + 1 N2 + 0.6 H2O [ ΔG = -72.2 KJ/eq ]

(rc) Biomass half reaction : C5H7O2N - Casein, aerobic , N-Source : NH4+
0.2 CO2 + 0.05 HC03− + 0.05 NH4+ + 1 H+ + 1 e− --> + 0.05 C5’H’7’O’2’N’1 + 0.45 H2O
[ ΔG = 18.799 KJ/eq ]

Energy reaction :
+ 0.0556 (C6H6O6N)3− + 0.0667 H2O + 0.2 NO3− + 0.0889 H+ --> + 0.0556 NH4+ + 0.3333 HC03− + 0.1 N2

Synthesis reaction :
+ 0.0556 (C6H6O6N)3− + 0.2 CO2 + 0.2167 H2O --> + 0.05 C5’H’7’O’2’N’1 + 0.1111 H+ + 0.8856 NH4+ + 0.2833 HC03−

Balanced equation using TEEM_1 :
[ fe = 0.5 ] [ fs = 0.5 ] [ e = 0.33 ]
+ 0.0556 (C6H6O6N)3− + 0.1008 CO2 + 0.1423 H2O + 0.0992 NO3− --> + 0.0252 C5’H’7’O’2’N’1 + 0.0119 H+ + 0.0304 NH4+ + 0.3801 HC03− + 0.0496 N2

Yield prediction :
Yg/m = 51.311 [ gms cells/mol donor ]
Yc/m = 2.268 [ mol_C_cells/mol donor ]
Yc/e = 0.378 [ mol_C_cells/mol_c_donors ]

Fig. 4. General outputs of MbT-Tool for the calculations related to the degradation of nitritotriacetic acid in the absence of molecular oxygen (using nitrate as electron acceptor) by a gram-negative bacterium.
parameterize the IBM models. In this sense, we think that the MBT-Tool’s outputs are a convenient means to advance with this type of model [27,31,55].

Finally, we consider that the user could take advantage of the MMRs provided by the MBT-Tool in a wide range of academic or research fields, such as, for example: i) as a source to design processes that take advantage of the microbial system [4], and ii) as the bedrock to make a connection between the microbial biomass and the substrates used by the micro-organism for pollution control [10,56]. Additionally, the NetLogo’s rather flat learning curve and comprehensive documentation [57] make this a user-friendly tool, easily accessible to chemists, biologists, ecologists, engineers and modellers, among others. Users without extensive programming experience can modify the code, introducing new chemical species to write new reduction-half-reactions, as well as other options for investigating alternative metabolic pathways or adapting certain processes according to specific studies.

In conclusion, the description of the cellular metabolism by means of thermodynamic concepts is strategic for investigating microbial activity and modelling bio-technological processes. We are convinced that the MBT-Tool will facilitate users to think about the biochemistry of metabolism due to its simplicity of use, and its results could be an interesting starting point for a microbial modelling approach.

Acknowledgments

The financial support of SENESCYT - Ecuador National Secretory of Higher Education, Science, Technology and Innovation (Grant no. Convocatoria Abierta 2011 – No. 94-2012) is gratefully acknowledged.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.csbj.2016.08.001.

References

[1] Doelle HW. Bacterial metabolism. Elsevier Science; 1969.

[2] von Stockar U, Vojinović V, Maskow T, Liu J-S. Can microbial growth yield be estimated using simple thermodynamic analogies to technical processes? Chem Eng Process Process Intensif 2008;47:980–90. http://dx.doi.org/10.1016/j.cep.2007.02.016.

[3] Amend JP, LaRoe DE, McCollum TM, Stock EL. The energetics of organic synthesis inside and outside the cell. Philos Trans R Soc Lond B Biol Sci 2013;368:2012055. http://dx.doi.org/10.1098/rstb.2012.0255.

[4] Rittmann BE, McCarty PL. Environmental biotechnology: principles and applications; 2001.

[5] VanBriesen JM, Rittmann BE. Mathematical description of microbial reactions involving intermediates. Biotechnol Bioeng 2000;67:35–52.

[6] von Stockar U. Biothermodynamics of live cells: A tool for biotechnology and biochemical engineering. J Non-Equilib Thermodyn 2010.

[7] McCarty PL. Thermodynamics of biological synthesis and growth. Adv Water Pollut Res 1965;2:169–87.

[8] Stratton FE, McCarty PL. Graphical evaluation of the kinetic parameters for bacterial growth. Can J Microbiol 1969;15:1201–5. http://dx.doi.org/10.1139/m69-217.

[9] McCarty PL. Energetics and bacterial growth. Org Compd Aquat Environ 1971;1:157–72.

[10] Christiansen D, McCarty PL. Multi-process biological treatment model. J Water Pollut Control Fed 1975;47:2562–64.

[11] McCarty PL. Thermodynamic electron equivalents model for bacterial yield prediction: Modifications and comparative evaluations. Biotechnol Bioeng 2007;97:377–88. http://dx.doi.org/10.1002/bit.

[12] VanBriesen JM. Evaluation of methods to predict bacterial yield using thermodynamics. Biodegradation 2002;13:171–90. http://dx.doi.org/10.1023/A:1020887214787.

[13] Xiao J, VanBriesen JM. Expanded thermodynamic models for microbial true yield prediction: Biotechnol Bioeng 2006;93:110–21. http://dx.doi.org/10.1002/bit.20700.

[14] Xiao J, VanBriesen JM. Expanded thermodynamic true yield prediction model: Adjustments and limitations. Biodegradation 2008;19:99–127. http://dx.doi.org/10.1007/s10532-007-9119-5.

[15] Heijnen JJ, Van Dijken JP. In search of a thermodynamic description of biomass yields. Biotechnol Bioeng 1992;39:833–58. http://dx.doi.org/10.1002/bit.26093006.

[16] Heijnen JJ. Thermodynamics of microbial growth. Técyl bioprocess technofer biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal bioprocess ferment biocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresssbiocatal biopresss
[49] Wendisch VF, De Graaf AA, Sahm H, Eikmanns BJ. Quantitative determination of metabolic fluxes during cointilization of two carbon sources: comparative analyses with Corynebacterium glutamicum during growth on acetate and/or glucose. J Bacteriol 2000;182:3088–96. http://dx.doi.org/10.1128/JB.182.11.3088-3096.2000.

[50] Kremling A, Bettenbrock K, Laube B, Jahreis K, Lengeler JW, Gilles ED. The organization of metabolic reaction networks. Metab Eng 2001;3:362–79. http://dx.doi.org/10.1006/mben.2001.0199.

[51] Wanner U, Kemmler J, Weilenmann HU, Egli T, El-Banna T, Auling G. Isolation and growth of a bacterium able to degrade nitrilotriacetic acid under denitrifying conditions. Biodegradation 1990;1:31–41.

[52] Ginovart M, Cañadas JC. INDISIM-YEAST: An individual-based simulator on a website for experimenting and investigating diverse dynamics of yeast populations in liquid media. J Ind Microbiol Biotechnol 2008;35:1359–66. http://dx.doi.org/10.1007/s10295-008-0436-4.

[53] Ginovart M, López D, Gras A. Individual-based modelling of microbial activity to study mineralization of C and N and nitrification process in soil. Nonlinear Anal Real World Appl 2005;6:773–85. http://dx.doi.org/10.1016/j.nonrwa.2004.12.005.

[54] Araujo P, Gras A, Ginovart M. Discrete simulation model to study heterotrophic microbial activity in soil. Appl Simul Model. IASTED Acta ...; 2004.

[55] Araujo P, Gras A, Ginovart M. Mass degradation to reduce cytotoxic products as an individual behavior-rule embedded in a microbial model for the study of the denitrification process. In: Méndez-Vilas A, editor. Microbes spotlight recent Prog. Underst. Benf. Harmf. Microorg. BrownWalker Press; 2016. p. 444–8 [both included].

[56] Henze M, Gujer W, Mino T, van Loosdrecht M. Activated sludge models ASM1, ASM2, ASM2d and ASM3; 2006.

[57] Railsback SF, Grimm V. Agent-based and individual-based modeling: a practical introduction. Princeton University Press; 2012.