A bioenergetic model to predict habitability, biomass and biosignatures in astrobiology and extreme conditions

P. M. Higgins and C. S. Cockell

2020

This is the author's accepted manuscript. Please cite this work as:

Higgins P. M. and Cockell C. S. 2020 "A bioenergetic model to predict habitability, biomass and biosignatures in astrobiology and extreme conditions" *J. R. Soc. Interface.* **17** 20200588 DOI: 10.1098/rsif.2020.0588

Abstract

In order to grow, reproduce and evolve life requires a supply of energy and nutrients. Astrobiology has the challenge of studying life on Earth in environments which are poorly characterised or extreme, usually both, and predicting the habitability of extraterrestrial environments. We have developed a general astrobiological model for assessing the energetic and nutrient availability of poorly characterised environments to predict their potential biological productivity. NutMEG [Nutrients, Maintenance, Energy and Growth] can be used to estimate how much biomass an environment could host, and how that life might affect the local chemistry. It requires only an overall catabolic reaction and some knowledge of the local environment to begin making estimations, with many more customisable parameters, such as microbial adaptation. In this study, the model was configured to replicate laboratory data on the growth of methanogens. It was used to predict the effect of temperature and energy/nutrient limitation on their microbial growth rates, total biomass levels, and total biosignature production in laboratory-like conditions to explore how it could be applied to astrobiological problems. As temperature rises from 280 to 330 K, NutMEG predicts exponential drops in final biomass (10^9—10^6 cells L^{-1}) and total methane production (62—3 μM) despite an increase in peak growth rates (0.007—0.14 hr^{-1}) for a typical methanogen in ideal conditions. This is caused by the increasing cost of microbial maintenance diverting energy away from growth processes. Restricting energy and nutrients exacerbates this trend. With minimal assumptions NutMEG can reliably replicate microbial growth behaviour, but better understanding of the synthesis and maintenance costs life must overcome in different extremes is required to improve its results further. NutMEG can help us assess the theoretical habitability of extraterrestrial environments and predict potential biomass and biosignature production, for example on exoplanets using minimum input parameters to guide observations.

1 Introduction

Astrobiology has the challenge of studying life on Earth in environments which are poorly characterised or extreme, usually both, and predicting the habitability of extraterrestrial environments. A habitable environment can allow an organism to undergo metabolic activity and hence maintain itself and grow or reproduce [1]. Locations of interest across the field vary: from extreme conditions on Earth [2], to the Martian surface [3] and subsurface [4–7], icy moons (e.g. Enceladus [8]) and even exoplanets [9]. The extreme conditions expected in these targets are typically under-studied and often difficult to replicate in the laboratory. We set out to create a computational model which can help take steps towards answering three key questions in astrobiology using a minimal number of input assumptions:

- *How much biomass can we expect in a given environment?* If the availability of energy and nutrients is known, what biomass could theoretically be sustained and would it reach detectable levels? Where, in the myriad of astrobiological targets is the best place to look for it?
- *What are life's minimum requirements?* Beyond water, life also needs a supply of energy and nutrients in order to grow and reproduce [10, 11], but what is the minimum supply of these required to remain viable?
- *What markers could extraterrestrial life be leaving for us to search for, and will they be in detectable quantities?* If the minimum requirements are met and we can expect some biomass, what signatures could life be leaving? Biosignatures can take many forms, and regardless of whether we look to the solar system or beyond to exoplanets [12], we must focus on those which are most likely to be above detection limits.

Biogeochemical modelling has been used extensively in an attempt to understand regions of the Earth that are not easily accessible, such as hydrothermal vents [e.g. 13–16], the marine subsurface [e.g. 17–19], drylands [e.g. 20], and the atmosphere [e.g. 21]. These modern ecological models can provide very effective results, but have high specificity and still require a large number of potentially complex input parameters and knowledge of the systems they aim to replicate.

Alternatively, there are many routes to estimating biomass yields from a more theoretical basis [e.g. 4, 22, 23], but often little attention is paid to differing environments and the effect of extremes such as temperature and salinity. In geomicrobiology and environmental microbiology, many of these extremes are more commonplace. A comprehensive method of modelling growth energetically in these natural settings is described by LaRowe and Amend [17, 24], in which the rate of change in total biomass is calculated by considering the balance between energetic supply and demand. Such a general approach is required for astrobiology, albeit more agnostic and requiring fewer known variables because often little is known about astrobiological targets. Furthermore, extraterrestrial life, if it exists at all, may not necessarily behave in the same way as Earth life. Nonetheless, it must be bound by the laws of thermodynamics and we could reasonably take this as a starting point to assess the biological potential of any extraterrestrial environment.

This study focusses on assessing habitability using an energetic and kinetic approach. The energetic approach to habitability [10, 25] parameterises the way in which biology interacts with its environment in terms of energy, based on the notion that an appropriate supply of energy and nutrients is necessary for life to synthesise the complex structures required for metabolism, growth and reproduction. A multitude of energy sources are possible, but when one thinks of energy as a fuel for life the most common method of uptake is through chemical pathways featuring redox couples, occasionally involving the absorption of radiation [10]. This energetic uptake can be quantified by calculating the free energy ΔG [J mol^{-1}] of the relevant process(es). ΔG is a measure of the spontaneity of a chemical reaction, demonstrating whether it is a net source or sink of energy. A negative ΔG describes a spontaneous process — one in which energy is released — such as in the break down of polymers or simple metabolisms, and a positive ΔG requires net energetic input, such as building new biomacromolecules. Free energies vary with the local composition, temperature and pressure [26].

Organisms grow and reproduce by using overall energy-yielding reactions with negative ΔG to drive energy-consuming reactions with positive ΔG [10]. More important, however, is the rate at which the organisms can access this energy — the available power P [W] [11]. To calculate this one must also

be aware of the kinetics of these biologically-mediated interactions, which are strongly dependent on the energetic availability and physiochemical environment [27]. Principally, the metabolic reaction must provide enough power to overcome the energetic costs of survival [11] (for example, overcoming rates of amino acid racemization with temperature [28]).

A simple organism can direct its energy supply into either growth or maintenance processes [24]. For this work, growth processes are those concerning the energetic cost of biosynthesis (i.e. building new biomass), and all other mechanisms of energetic loss are considered maintenance processes. In reality, the computation of both these contributions is complex, with the compounding effects of temperature [e.g. 29, 30], pressure [e.g. 31], salinity [e.g. 32], pH [e.g. 33], nutrient availability [e.g. 34] *etc.* each contributing to the organism's ultimate fate [10, 35, 36]. The actual power organisms use for maintenance also varies with growth phase and substrate availability [37].

We present a novel computational model which can estimate maintenance costs in extreme and/or nutrient limited environments from theory or empirical data. It can predict theoretical biomass levels and rates of biosignature production from as little as an overall catabolic reaction in poorly characterised, extreme (extra)terrestrial environments and other locations of astrobiological interest. A collection of organisms and their local environment are considered as self-contained objects communicating only via energy and nutrient exchange. The notion of modelling certain bioenergetic processes such as metabolism in a modular fashion has been suggested before [38] although it has also been recognised that modelling life in extreme environments, for which there is little information, is difficult [10]. The model's simplicity does come with some caveats and these will be discussed.

2 Methods

2.1 An efficiency based model for microbial growth (The core concept)

For any given organism, the power going into growth processes must be a fraction of the power supply from metabolism, as other energetic processes necessary for maintenance must take precedence [10, 27]. Beyond this primary limitation due to maintenance, the ability of an organism to grow new biomass can also be restricted by the availability of key nutrients. The majority of biological matter is made up of six elements: carbon, hydrogen, nitrogen, oxygen, phosphorus and sulphur, hitherto referred to as 'CHNOPS' elements [35].

We have developed Nutrients, Maintenance, Energy, and Growth [NutMEG][1], an open source Python module for predicting growth behaviour of life in poorly characterised environments. To do so, it estimates the free energy ΔG of internal cellular processes corresponding to: metabolism (Sec 5.2); growth (Sec 5.4) and optionally maintenance, and the rate of these processes (Sec 5.3). This novel schema offers several benefits. The use of kinetics allows for more realistic consideration of the thermodynamics at play governing metabolic rates [27] rather than simply using static total energetic availability to predict biomass. By monitoring metabolic rates and nutrient uptake, ΔG is dynamically corrected as the organism(s) interact with the environment (via Equation 6, Sec. 5.2). This allows us to also consider external effects on the metabolic energy yield with time, such as an abiotic source or sink of substrate. The minimum required input parameters for a growth prediction are shown in Table 1.

Organism Parameter	**Symbol**	**Unit**
Rate constant of catabolism	k_{cat}	$\mathrm{M}^{(1-z)}\ \mathrm{s}^{-1}$
Rate constants of nutrient uptake	k_x	s^{-1}
Catabolic reaction	–	–
Environment Parameter	**Symbol**	**Unit**
Composition activities or molarities	[x]	– or M
Temperature	T	K
Pressure	p	Pa
Volume	V	m^3
pH	pH	–
NutMEG Output	**Symbol**	**Unit**
Population	N	cells
Population volume	V_{BM}	m^3
Free energy of catabolism	$-\Delta G_A$	J $(\mathrm{mol\ CO_2})^{-1}$
Free energy of ATP production	ΔG_C	J $(\mathrm{mol\ CO_2})^{-1}$
Rate of catabolism	r	$(\mathrm{M\ CO_2})\ \mathrm{s}^{-1}$
Available power supply	P_S	W cell^{-1}
Maintenance as a fraction of supply	$1-\epsilon_M$	–
Power available for growth	P_G	W cell^{-1}
Growth rate	r_G	s^{-1}
Composition activities or molarities	[x]	– or M
Rates of nutrient uptake	r_x	$\mathrm{M\ s}^{-1}$

Table 1: Minimal organism and environment parameters required to initialise a growth prediction and default parameters for NutMEG to output in time series. There are many more parameters included in the model for organisms which allow for fine tuning of a well-characterised species or an exploration of possible parameter spaces for others. Further information on what can be changed can be found in NutMEG's documentation. By default, *E. coli*-like parameters are chosen unless otherwise specified. Where these are unavailable, other model organisms are used and these are noted in the documentation. Using inheritance, one can increase specificity for organisms or environments allowing new parameters or adaptations to be introduced. The order of the catabolic reaction is given by z.

[1] `https://github.com/pmhiggins/NutMEG`

NutMEG considers cells as small engines whose growth is throttled by a maintenance efficiency $\epsilon_M \in [0, 1]$ (equivalent to 1 − the fractional energetic cost of maintenance vs. the energetic supply). Hence, with an instantaneous power supply $P_S(t)$ and maintenance cost $P_M(t)$, both in W cell^{-1}, the net power available as fuel for growth, $P_F(t)$ [W cell^{-1}] is:

$$P_F \quad = \quad P_S - P_M \quad = \quad \epsilon_M P_S \tag{1}$$

Growth also necessitates the availability of nutrients to build the new biomass. If nutrient uptake can also be characterised as an efficiency $\epsilon_{UT} \in [0, 1]$ then the final power which can be used for biomass production, $P_G(t)$ [W cell^{-1}], is:

$$P_G(t) = \epsilon_{UT} \left[\epsilon_M \left[P_S(t)\right]\right] \tag{2}$$

Further details on how these efficiencies are parameterised and calculated can be found in Section 5. This schema is shown in Figure 1.

To convert this growth power into new biomass, one must know the energy required to synthesise each cell from the nutrients available E_{syn} [J cell^{-1}] (Sec 5.4). Then, if either the energy or nutrient supply is the limiting factor in growth and not the rate of biomass production, for a suitably small time step dt [s], the total biomass $N(t)$ [cells] is:

$$N(t + dt) = N(t) \left(1 + \frac{E_G(t, dt)}{E_{\text{syn}}}\right) \tag{3}$$

where $E_G(t, dt) = P_G(t)dt$ [J (cell s)$^{-1}$] is the total energy each cell can contribute to growth per time step.

At first glance the simplicity of this model may give the impression of limited applicability. We argue that sorting processes into groups concerning supply, maintenance, nutrient uptake and growth is useful for determining how these factors could affect habitability. One can introduce unique traits of organisms without compromising computational efficiency (allowing for trivial inclusion of: life spans; unique rates for nutrient uptake, growth, metabolism; competition between organisms *etc.* (Sec 4, 5)). At the same time, NutMEG allows flexibility in input for conditions which have limited data or large uncertainties (e.g. extraterrestrial environments), and can highlight areas in which a given environment or organism requires better understanding. For example, in-keeping with the concepts outlined above, one could define upper and lower efficiencies required for life in various environments, i.e. best-case and worst-case habitability assessments.

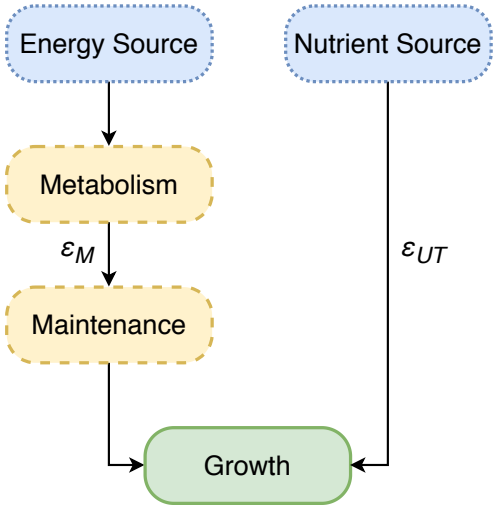

Figure 1: **Flowchart to demonstrate the concept of the model.** ϵ_{M} and ϵ_{UT} are the energetic efficiencies of maintenance and nutrient uptake respectively.

2.2 Configuration against empirical methanogenic growth data

By either calculating or estimating the total efficiency ($\epsilon_{UT}\epsilon_M$), NutMEG can be used to simulate both extreme environments in which specific maintenance calculations need to be performed, and those which have limited data sets. This flexibility makes NutMEG an appropriate model for examining the habitability of extraterrestrial and extreme environments.

To demonstrate the model concept and explore how restricting the availability of energy or nutrients can affect microbial growth behaviour, a simple model of methanogens growing in both optimal and energy/nutrient limited environments was produced. Hydrogenotrophic methanogens were selected because their metabolism is thought to be ancient, and their use of hydrogen and carbon dioxide has made them of interest in astrobiology. Methanogens could use these primordial gases on other planetary bodies as a redox couple:

$$CO_2 + 4\,H_2 \rightleftharpoons 2\,H_2O + CH_4$$

Methanogens can grow in numerous extreme environments, including at a wide range of temperatures, pH extremes, pressures and salinities [39]. There are multiple locations in the solar system whose physicochemical environments are known or suspected to overlap with those habitable to methanogens on Earth, including Mars [7] and Enceladus [8].

First, an organism is selected and parameterised in its optimum environment for use with NutMEG, then energy and nutrient limitation is simulated to demonstrate how that affects growth rates, biomass levels, and biosignature (methane) production.

2.2.1 Modelling Empirical Methanogens

To ensure simulation results were realistic reflections of biological behaviour, we matched NutMEG's results to empirical data for methanogens. NutMEG was used to reparameterise data from hydrotrophic methanogen growth experiments into the required format and find P_M in optimal conditions for a variety of temperatures. To achieve this, growth prediction simulations (Sec 5.1) were performed on methanogens for which data on: cell volume, optimal growth rate, optimal temperature, optimal pH, optimal pressure, substrate headspace composition, and growth rate calculation methods were available from the phymet2 database [40]. The growth rate calculation methods were found in the publications referring to specific species. Substrate concentrations were inferred from the headspace composition [41], which for all methanogens was H_2:CO_2 = 80:20.

The reaction quotient and rate constant of methanogenesis are also necessary for the growth prediction (Sec 5.2, 5.3). Computing the quotient requires the concentration of CH_4, and the rate constant can be estimated from its rate of change. Of the methanogens in the database, 40% of growth rates were calculated using Powell (1983)'s method [42], which uses CH_4 rates of change, with the remainder being from OD measurements or cell counts. Powell's technique was used in reverse to estimate the rate of change of [CH_4] for each empirical methanogen. The methane concentration was set at 3×10^{-8} M, as this corresponds to a maintenance power equivalent to Tijhuis et al. (1993)'s prediction [43] at 300 K. We chose to match to this value as Tijhuis et al. (1993)'s maintenance costs are based on empirical data, the majority of which are around this temperature. It was used as a conservative benchmark; recent analyses suggest it may be an overestimate of maintenance [37].

As this data pertains to methanogens growing optimally in growth media, it is assumed that they are not nutrient limited e.g. $\epsilon_{UT} \sim 1$. It follows that in optimum conditions the difference between P_G and P_S for these organisms is the maintenance power P_M [W cell^{-1}].

2.2.2 A Typical Optimal Methanogen [TOM]

When outside their optimal growth temperature range, each of the methanogens would be affected in different ways depending on their unique physiology. This is due to differences between the methanogens themselves (e.g. their size, adaptations for maintenance, synthesis energy (Sec 5.4) *etc.*), and uncertainty

in the empirical data. The data used originates from a variety of experimental procedures performed at various times over the past 50 years, many of which without reported confidence values [40]. Some of the methanogens in the database were also noted as requiring extra nutrients than standard media (15%), formate or acetate as additional substrates (8%), or were also observed reducing sulphur (6%).

To isolate the effect of temperature on energy and nutrient limitation, a *typical optimal methanogen* [TOM] was created. The TOM is an artificial methanogen, designed to behave as if any given temperature were its optimum by mimicking the growth behaviour of a real methanogen which is well-adapted to said temperature. It had the mean of each of: cell volume (3.44 μm^3), optimal pH (6.9), and optimal local pressure (182 kPa) of the database methanogens as described above. Maintenance powers were estimated using an identical procedure to the empirical methanogens; the calculated P_M for the TOM is the reduction in P_S required to match its growth rate. This growth rate is estimated from a linear regression of the database methanogens' optimal growth rates with temperature (Figure S2).

3 Results

3.1 Maintenance costs of methanogens

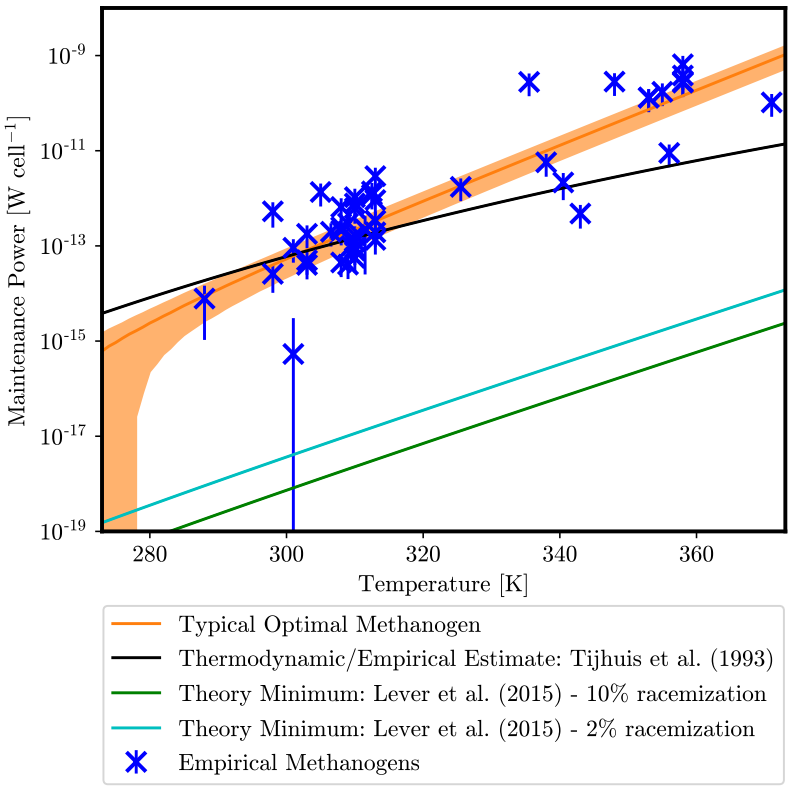

Figure 2: **Predicted maintenance power required for the empirical methanogens and typical optimal methanogen [TOM]**. This shows the required maintenance cost [W cell^{-1}] for the TOM (orange line) and empirical methanogens (blue crosses) to grow optimally at various temperatures. For both, error bounds indicate possible variations in power supply due to variation in ATP yield per CO_2 metabolised; between 0.5 and 1.5 [44, 45]. Initial [CH_4] was unknown in the data, so a value which yielded a maintenance cost equivalent to Tijhuis et al. (1993) [43] at 300 K for the TOM was used.

Values of P_M were selected which yielded the empirical growth rates for each empirical methanogen (Figure 2, blue crosses). While the different methanogen species clearly have different maintenance powers, an exponential trend upwards with increasing temperature can be seen, consistent with previous studies [43]. The maintenance power of the TOM follows a similar trend (Figure 2, orange bar).

Below temperatures of 280 K, one bound of the TOM's maintenance power range (corresponding to an ATP yield of 0.5 mol per mol CO_2) drops significantly (i.e. $\epsilon_M \to 1$, $P_M \to 0$). A P_M of 0 is nonphysical, and this reflects a scenario in which NutMEG predicts that the P_S is less than the P_G required to match the target growth rate. This suggests that methanogens at low temperatures must be metabolically efficient and able to metabolise more moles of ATP per mole of CO_2 or have lower energies of cell synthesis. It should be noted however that this occurs outwith the temperature range for which methanogen data was available (288–371 K) [40] and our logarithmic extrapolation of target growth rates (Figure S2) could be unreliable.

These maintenance power predictions can be compared with previous theoretical and empirical estimations. Tijhuis et al. (1993) [43] used empirical data to estimate the maintenance power requirement with temperature for *E. coli*. Shown in black in Figure 2 is the Tijhuis prediction for a cell with the same size as the TOM. Also shown in Figure 2 are theoretical estimates generated using Lever et al. (2015)'s method whereby minimum thermodynamic maintenance predictions are based on protein replacement after racemization [28]. The lines on Figure 2 represent the cost of protein replacement to the TOM after 2% or 10% racemization of the amino acids in an average protein.

3.2 Energy and Nutrient Limitation of the TOM

NutMEG was used to artificially limit the availability of energy and nutrients to explore the effect they would have on the predicted growth rates, biomass levels, and total CH_4 produced by the TOM. Figure 3 shows growth curves for the TOM with its calculated maintenance cost at 280 K, 300 K and 330 K. Figure 4 shows the peak growth rate, final biomass, and total CH_4 production for temperatures between 280 K and 330 K. For both figures, each column represents the effect of a specific limiting factor on the organism. These include reducing the initial concentration of dissolved metabolic substrates CO_2 and H_2, or nutrient sources P (the sole phosphorus source), or limiting the rate constant with which the organism can access phosphorus (Sec 5.5). All simulations were performed in 1 L 'vessels' saturated in nutrients apart from the phosphorus limiting simulations.

Growth Curves
When the concentration of either CO_2 or H_2 is restricted with no other changes to the organism or environment, growth curves become more characteristic of inhibited microbial growth as shown in Figure 3. For higher temperatures, the effect of limiting CO_2 and H_2 becomes more pronounced, with little to no growth at 90% optimal [CO_2] at 330 K compared to almost optimum growth at 280 K. This is due to the higher maintenance power at higher temperatures (Figure 2).

Limiting the concentration of available nutrients places a cap on the growth curves when all of the available phosphorus is locked in biomass for the cooler environments with slower growth (Fig 3 centre-right, top & middle). This demonstrates that the rate of uptake of phosphorus is not the key limiting factor until there is too little remaining to sustain exponential growth of a large culture. At 330 K, the growth rate is also limited by a lower [P], due to the overall uptake rate (below, Sec 5.5) running slower than the metabolism.

If instead of changing the concentration of available phosphorus the uptake rate constant k_P was changed (parameterising phosphorus' bioavailability and microbial adaptation to its uptake), the growth rate slows but there is little impact on biomass levels. This is because the growth rate becomes nutrient limited i.e. phosphorus uptake rather than the metabolism is determining the rate of biomass production, but there remains enough phosphorus to continue building large cultures. The final biomass is still lower than the optimal alternative because the methanogens are surviving for a longer period of time and hence more of the available energy in the system has been diverted to maintenance costs before reaching the limit of microbial growth.

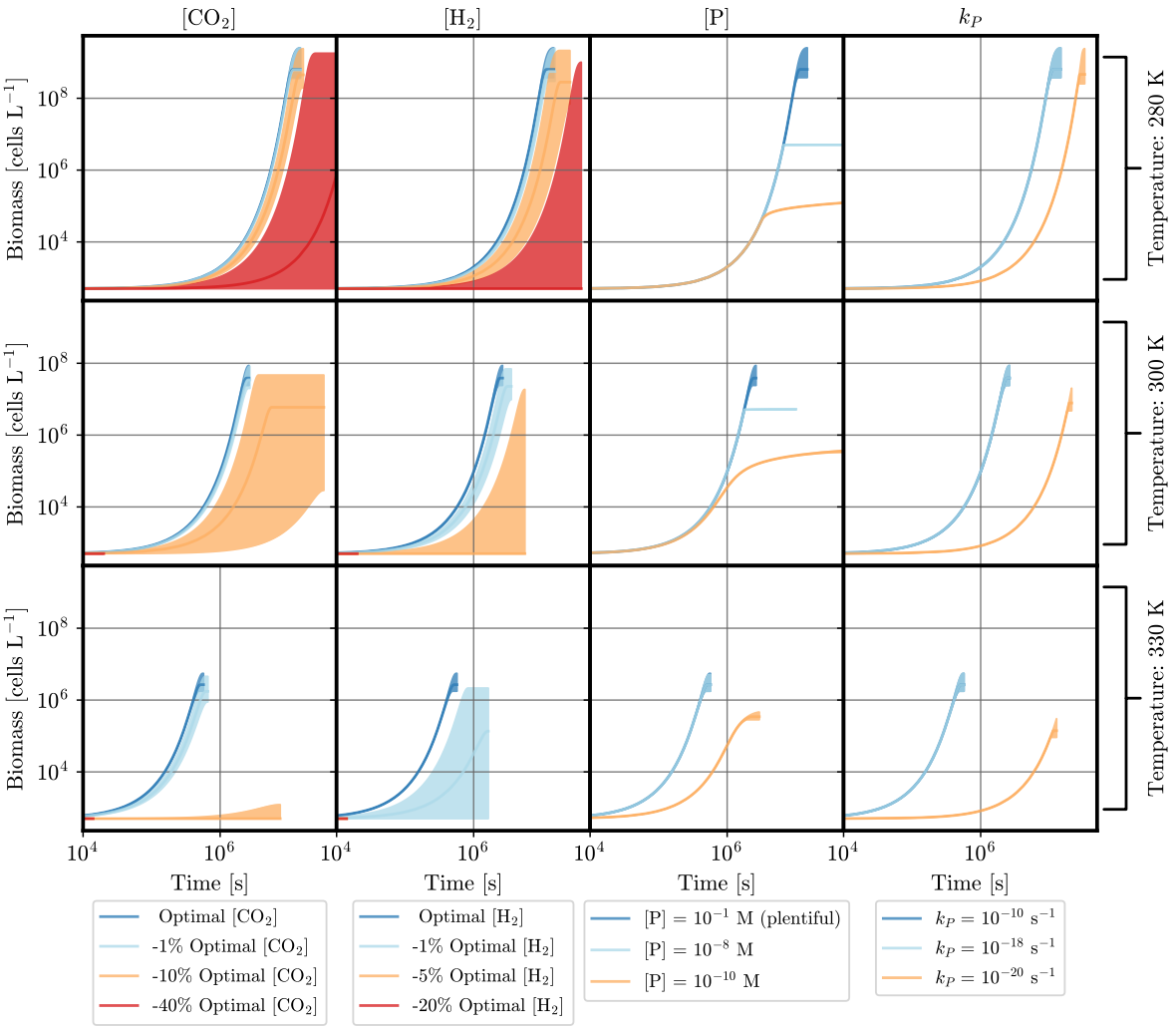

Figure 3: **Growth curves of simulated typical optimal methanogens [TOM] under energy or nutrient limitation.** The TOM, methanogens which exhibit maximum growth rates across given temperatures, are shown growing in their optimal conditions in each subplot and growing in energy or nutrient limited conditions at various temperatures. Columns from left to right show the effect of $[CO_2]$, $[H_2]$, [P], and k_P, the latter the rate constant of phosphorus uptake. Each row shows the same changes at different temperatures. The optimal dissolved $[CO_2]$ and $[H_2]$ vary with temperature (Figure S3) so changes are shown with a % change on the optimal concentration at that temperature. The filled-in segments show variation in growth curves at various yields of ATP per mol CO_2 — between 0.5 and 1.5. Where the dark blue curve appears absent it is obstructed by the light blue curve; for these cases $\epsilon_{UT} = 1$ and energy is the main limiting factor, as increasing nutrient availability does not increase growth rates.

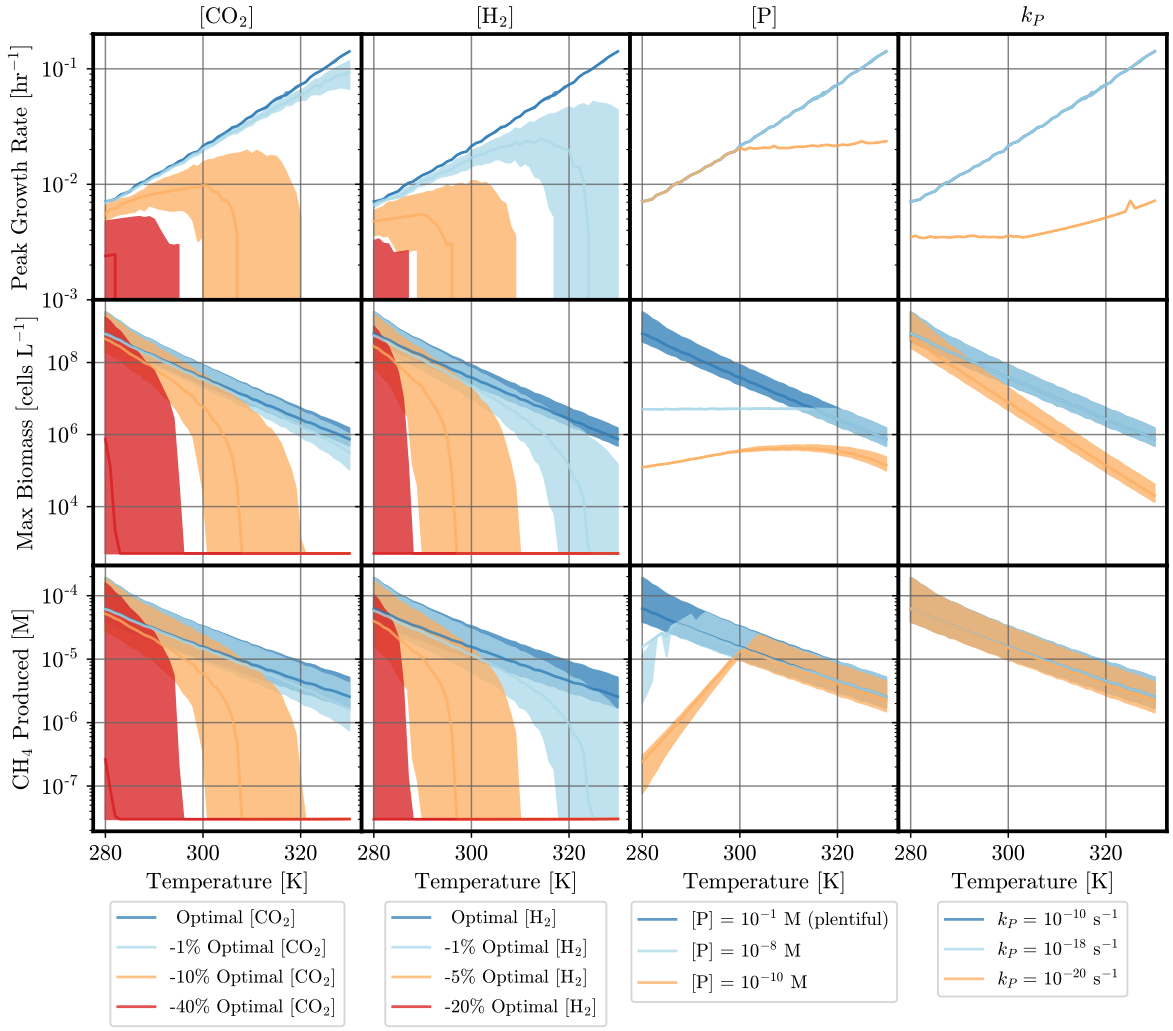

Figure 4: **Peak growth rates, final biomass values and total CH_4 production of the simulated typical optimal methanogens [TOM], growing under energy or nutrient limitation.** The TOM, methanogens which exhibit maximum growth rates across given temperatures, growing in their optimal conditions are shown by the dark blue line in each subplot and growing in energy or nutrient limited conditions at various temperatures. For the TOM in energy and nutrient saturated conditions, as temperature rises from 280 to 330 K NutMEG predicts exponential drops in final biomass (10^9—10^6 cells L^{-1}) and total methane production (62—3 μM) despite an increase in peak growth rates (0.007—0.14 hr^{-1}). Equivalent doubling times are ∼100—5 hr. Columns from left to right show the effect of $[CO_2]$, $[H_2]$, [P], and k_P, the latter the rate constant of phosphorus uptake. The optimal dissolved CO_2 and H_2 vary with temperature (Figure S3) so changes are shown with a % change on the optimal concentration at that temperature. The filled-in segments show variation in growth curves at various yields of ATP per mol CO_2 — between 0.5 and 1.5. Where the dark blue curve appears absent it is obstructed by the light blue curve; for these cases $\epsilon_{UT} = 1$ and energy is the main limiting factor, as increasing nutrient availability does not increase growth rates.

Specific characteristics of the growth curves and how they change with temperatures can be examined in further detail:

Growth Rates
There is an exponential increase in peak growth rate with temperature for the TOM in its optimal conditions (Figure 4, top row). When restricting substrate concentration to a fraction of the optimal dissolved amount, the effect on growth rate becomes more pronounced at higher temperatures. This is due to the higher maintenance power predicted for the TOM at higher temperatures (Figure 2). The initial ϵ_M value decreases with increasing temperature because maintenance costs become a larger fraction of power supply. As $\epsilon_M \to 0$ the TOM can tolerate smaller deviations in substrate concentration before maintenance power exceeds power supply and the growth rate $\to 0$.

With limiting nutrients, different effects are seen depending on the extent to which we restrict [P]. For this example, when restricted to 10^{-8} M there is no slow down in growth rate meaning the TOM remains energy limited at all temperatures while in its exponential phase. When restricted instead to 10^{-10} M the growth is nutrient limited for temperatures > 300 K meaning the rate of uptake of phosphorus cannot meet the demands for biosynthesis made by P_F (Sec 5.1). For all temperatures above 300 K the growth rate is then restricted by this uptake rate. Changing the phosphorus uptake rate constant more explicitly limits the nutrient availability for reasons discussed above.

Biomass Levels
With increasing temperature the final expected biomass in the vessel decreases exponentially. This is due to the effects on ϵ_M described above. Reducing substrate availability exacerbates this by decreasing net power supply while maintenance power remains constant, decreasing ϵ_M further until the energy limitation renders the environment uninhabitable to the TOM at these maintenance powers. It has been postulated, however, that in extreme energy-limited systems some organisms focus on other processes at the expense of repairing non-fatal protein degradation, reducing the maintenance by up to 95% [28, 37].

The availability of phosphorus also affects the final biomass levels. When [P] = 10^{-8} M, while growth rates in the exponential phase are the same as the optimum, growth arrest begins sooner, but not until phosphorus is completely exhausted at lower temperatures (Figure 3 centre-right top). At temperatures >315 K, growth stops before all of the phosphorus has been consumed, hence there is only energy limitation in this scenario. When [P] = 10^{-10} M, there is a gentle increase in final biomass with temperature because at lower temperatures there is slower growth and more energy overall is used for maintenance before nutrient limitation becomes important. However, there is a peak in biomass at ∼310 K after which the energy limitation also becomes important, as above for the 10^{-8} simulation. When decreasing k_P the final biomass falls with temperature at an increased rate due to a larger amount of energy being used for maintenance in total throughout the lifetime of the simulation.

CH_4 Production
The total CH_4 production has a qualitatively similar trend to total biomass production with temperature: at increased temperature the final methane concentration decreases exponentially. This suggests that net biosignature production of thermophilic methanogens in closed systems could be lower than their psychrophilic counterparts. It is important to note though that the faster metabolism and growth rate with temperature means that the rate of CH_4 production is higher at higher temperatures but growth arrest occurs sooner leading to this lower overall CH_4 production. In natural systems with substrate replenishment this may not be the case (e.g. hydrothermal vents).

Restricting [P] reduces total CH_4 production at lower temperatures (Figure 4, centre-right bottom). At the lower P_M at these temperatures, the organism is nutrient limited because less energy is required for maintenance. The CH_4 production increases for these nutrient limited organisms with temperature until energy becomes limiting (at 285–295 K for [P] = 10^{-8} M and 300–305 K for [P] = 10^{-10} M) and total CH_4 production begins to decrease again, for reasons discussed above. These results show that as extreme environments require more energy to survive, biosignatures produced by the overall metabolism (methane in this case), will be produced at a higher rate per unit biomass.

3.3 Alternative explanation: synthesis energy

One input variable to NutMEG which is difficult to estimate is the energy required to synthesise a cell E_{syn}. For this study it was estimated by combining the energetic cost of amino acid synthesis [46] in anoxic environments and the cost of protein synthesis from these amino acids [47, 48, Sec 5.4], and increases with the local temperature (Figure S1). Were E_{syn} larger, a lower maintenance power would be required for NutMEG's output to match the empirical growth data (Sec 3.1). Figure 5 shows the proportion with which E_{syn} needs to be increased such that the maintenance power required to match empirical growth rates would be zero — a scenario classically considered by Monod kinetics [49], though thermodynamically unrealistic [10]. At temperatures exceeding 320 K E_{syn} would need to be corrected by an order of magnitude or more to minimise P_M, but in these conditions the maintenance cost would be non-zero [10, 28, 43, 50]. At less extreme temperatures, such as 300 K, a correction of less than 10x would minimize the maintenance contribution required. Even if this were the case, one would expect that the growth curves and biosignatures in Figures 3 and 4 would be similar — perhaps with more CH_4 production if $\epsilon_M \to 0$ after more substrate usage due to the lower maintenance power.

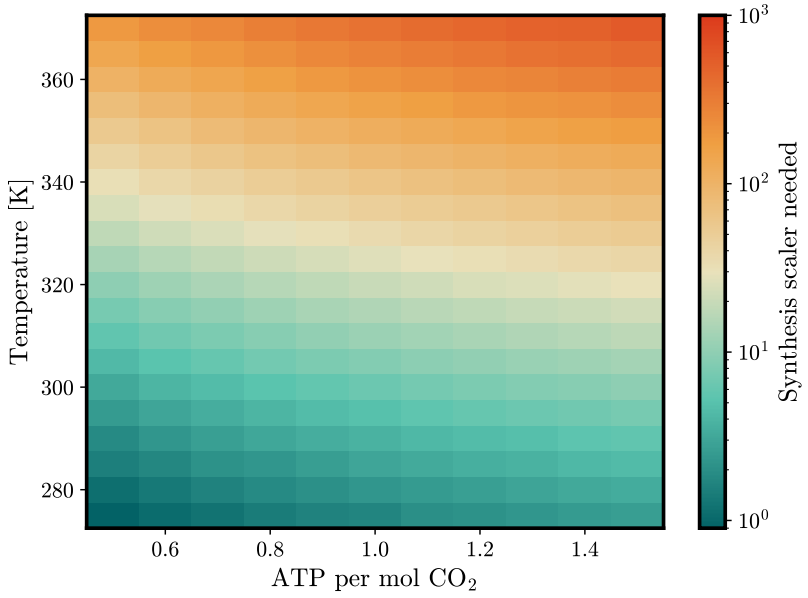

Figure 5: **Factor by which the synthesis energy of the typical optimal methanogen needs to be increased for the optimal maintenance power to be zero.** The correction to the E_{syn} needed for the maintenance to be zero while still returning empirical growth rates. More realistic E_{syn} values could be between the values used for this study and the values on this contour.

4 Discussion and Conclusions

A common problem for astrobiologists is investigating the habitability of extreme environments on Earth or extraterrestrial environments for which we have very little information. We have developed a simple bioenergetic model for the dynamic monitoring of both organism and environment as they interact with one another, allowing for the study of growth rates, biomass production, biosignature (e.g. gas) production and other parameters. By matching the model predictions to laboratory observations we have investigated how placing a theoretical methanogen under either energy or nutrient limitation can affect the rates of microbial growth, total biomass production, and total CH_4 production in closed, well-mixed systems. Increased energy and nutrient limitation restrict the total biomass expected — an important measure of habitability — increasingly at higher temperatures owing to the low energy yield of the methanogenesis metabolism and higher maintenance power. They also limit the total CH_4 production, which would be an important biosignature for detecting methanogens on other planets, such as on exoplanets. The model's simplicity does mean that these results come with some caveats.

First, our results rely on the assumption that in optimal conditions the uptake of CO_2 and H_2 is the rate-determining step in the metabolism; a reasonable assumption for such a low-energy reaction. When calculating the effects of energy limitation there are two major effects on the simulation parameters. First is the free energy yield of the metabolism (Equation 6), second is the rate of the metabolism $r = r_+ F_T$ where r_+ is the forwards rate of the metabolic pathway's rate-determining step, and F_T is a thermodynamic scaling factor [27, Sec. 5.3]. Here we assume that the rate-determining step is the uptake of H_2 or CO_2, e.g. $r_+ = k_M[CO_2][H_2]^4$ where k_M is the rate constant of methanogenesis, but it could be from another step in the metabolism such as the phosphorylation of ATP or an enzyme-controlled interaction in the pathway [27]. If this were the case, and in our optimal conditions $[CO_2]$ and/or $[H_2]$ were well above the value at which their uptake would be the rate-limiting step, they could be lowered without effect until this were reached and energy limitation becomes important. From that point on, one would achieve results qualitatively similar to those reported in this paper. Further laboratory experiments are required to help better understand at which $[CO_2]$ and $[H_2]$ accessing these substrates will become the rate-determining step, but in the meantime our results provide a qualitative view of what will happen at that stage.

The maintenance power was used as a single temperature dependent value, whereas in reality it would reflect the energy change from a series of chemical transactions the organism has made. For extreme conditions, the maintenance power should be computed explicitly for each adaptation to every given extreme [10] and NutMEG does allow for this. For example, the energetic cost of maintaining cellular pH against a gradient ΔpH could be estimated as $\Delta G = -2.3RT\Delta\text{pH}$ where R is the universal gas constant [10, 33]. The power cost can then be computed, if the rate of the mechanism is known. This knowledge is not available for many extremes, and different organisms will use different pathways with varying efficiencies. As a result, further understanding of microbial adaptations to extremes is required — either by predicting such pathways or finding their net minimum thermodynamically viable energetic cost [10, 36].

The maintenance power can also vary with the organism's growth phase and the local energetic availability [e.g. 37]. Most empirical data, including that used in this study, is based on measurements made in the exponential phase which are not representative of life in natural systems. In energy-limited systems, maintenance costs could be substantially lower than those calculated here [37], for example a recent study suggests that in Earth's deep subsurface most methanogens could be surviving on energy fluxes between $\sim 10^{-19}$–10^{-21} W cell^{-1} [51]. For NutMEG to successfully simulate natural systems, better understanding of how organisms can survive on such low energy fluxes is required.

The synthesis energy was calculated by combining the cost of amino acid and protein synthesis, but our analyses would benefit from a more comprehensive complement of biomacromolecules. Proteins account for approximately 55% the dry mass content of *E. coli* in the exponential growth phase [48, 52], and the inclusion of other biomolecules such as RNA and lipids will improve the quality of this estimation. Similarly, protein repair is a key factor in maintenance costs but the cost of RNA repair becomes more important with increasing cell size [50]. Biomolecular content also varies significantly between species and growth phase and this should also be accounted for [52, 53].

NutMEG primarily predicts growth arrest when the maintenance power exceeds the supply power among other criteria, but there are other explanations of what could cause growth to stop. An example is the potential role of quorum sensing in some bacteria [e.g 54], which means that some microbes will not grow until a critical cell density is achieved, even though theoretically the energy and substrates for growth are available. The problem with the specifics of growth arrest is that it is often tied to the specific physiology of particular species. NutMEG's philosophy in part relies on having minimal ties to the peculiarities of particular terrestrial organisms and instead opts for a general approach. Nevertheless, these specificities could be included if one was modelling organisms in which one wished to investigate the influence of such characteristics on biomass or biosignature production,

While the simulations we performed did predict growth, none reached a steady state with the environment. This is because there was no cell death or net inflow of substrates to sustain the methanogen population after $\epsilon_M \to 0$. This would, inevitably, lead to the death of all of the organisms. This issue is mitigated in this study, as we explicitly considered laboratory-like 'vessels' as well-mixed closed systems for analysis. Mixing and diffusion effects will also need to be considered in wider astrobiological applications which would aim to achieve a steady state, such as the simulation of hot springs or oceans. Some small steps can be made with NutMEG to mitigate these issues, such as including net inflows or outflows of substrates. Omission of a cell death rate is common in simulations of cell dynamics, but it has been shown to be an important factor in maintenance considerations. Characterising its effects along with other kinetic factors such as growth rate are some of the major problems in the current understanding of maintenance requirements [36].

Other parameters within NutMEG can be altered and have a significant effect on growth rates, biomass, and biosignature production. To explore them we also trialled some simulations including: varying the maintenance power to see how less well-adapted organisms could be quantified or to what extent a new extreme could be tolerated; including a life span for the methanogens after which they become inactive; and including net sources/sinks of CO_2 and H_2 to dynamically change the energetic availability with time (Supplemental Animation). Increasing the maintenance power by up to 20% has a similar effect to the energy limitation across different temperatures. Inclusion of a lifespan reduces peak biomass values (and final active biomass falls to zero) but it did not significantly affect CH_4 production.

Adding a net inflow of CO_2 or H_2 increased all three of the peak growth rates, biomass levels, and biosignature production as this is fundamentally the opposite of energy limitation. However, reservations must be made when considering these predictions, as the growth of real methanogens is likely limited by the rate-determining step, such that decreasing the availability of nutrients may slow growth down, but increasing nutrients will not necessarily enhance growth any further. Adding a sink of CO_2 or H_2, for example by abiotic processes or a competing organism, has a similar effect to energy limitation. There are examples of natural systems exhibiting higher cell concentrations than those achievable in the laboratory [e.g 55]. Properly including sources and sinks of potential substrates and nutrients could help explain these observations; our results suggest that sometimes slower peak growth rates could produce higher concentrations of biomass given enough time.

As some of the results presented in Section 3.2 predict growth on the scale of years, resupply of substrates will be of particular importance when estimating how much biomass such an environment could sustain for these long periods of time. There could also be a significant influence from other CHNOPS elements, nutrients and micro-nutrients, which should be included if they act as a significant limiting factor. For example, observations from the Cassini spacecraft suggest Enceladus' subsurface ocean may contain the major ingredients of methanogenesis in an energy-yielding configuration [e.g. 8]. Phosphorus is the only CHNOPS element yet to be directly detected [8, 56], but a recent study suggests it could be as low as $\sim 10^{-10}$ M [57], a level at which our results suggest nutrient limitation could be important. This tenuous energetic and nutrient landscape provides a salient example of how NutMEG could be applied to astrobiological problems.

When attempting to model extraterrestrial environments, existing studies generally infer possible energetic habitability from the free energy values alone [e.g. 8], or predict maximum biomass values from the total free energy available [e.g. 7]. NutMEG can offer valuable additions to such calcuations by dynamically calculating the kinetics of metabolism, maintenance costs, and variation in free energy yield as the local composition changes with time. Monitoring of the organisms and environment also allows us to see the trends in biomass and biosignatures with time.

In conclusion, NutMEG offers a new computational technique to investigate habitability and potential growth, biomass and biosignature production in little understood terrestrial and extraterrestrial environments. It was used to predict that in energy and nutrient saturated conditions, there is an exponential drop in final biomass (10^9—10^6 cells L^{-1}) and total methane production (62—3 μM) despite an increase in peak growth rates (0.007—0.14 hr^{-1}) as temperature rises from 280 to 330 K for methanogens in ideal conditions. When placed in energy or nutrient limiting environments, all three parameters are further reduced.

In the future, NutMEG's credibility can be bolstered by comparing its first order predictions with empirical evidence from real extreme environments on the Earth. In extraterrestrial environments it can provide a basis for prioritising locations to be studied. Example applications could be to explore energy and nutrient availability on Saturn's moon Enceladus or in the subsurface of Mars. Given the minimal observations and inferences available from exoplanets, NutMEG can also be used to explore the possible parameter space for expected biomass and gas production which can impact, and therefore be used to explain, exoplanetary spectra.

5 Extended Methods

NutMEG has been developed as a wide ranging model to assess the key requirements of energetic habitability and a full complement of its applicability with further examples is available in its documentation. Here, we will summarise the key calculations performed for the results in this work.

5.1 Implementation of Growth Prediction

To contextualise the calculations presented here, the reader may wish to first review NutMEG's most basic overall function — to predict growth curves. If a time step dt [s] is not manually input, it is determined by the model as the amount of time it would take for the slowest growing microbial community to increase by 0.1%. Then, each time step consists of the following:

- Increase age of all cells by dt
- Update local composition data if there is an inflow/outflow included.
- Update the reaction quotient for the catabolic reaction using local composition data, and calculate its free energy (Sec 5.2).
- Use thermodynamically limited biochemical kinetics to calculate the conservable power supply $P_S(t)$ (Sec 5.3).
- Maintenance contributions
  - Calculate (where necessary) and sum the contributions to maintenance power $P_{M,i}(t)$. In this work, the maintenance power was entered explicitly as the temperature-dependent values inferred from the growth rates of methanogens in optimal conditions (Sec 2.2.2, Fig 2). The actual power organisms use for maintenance varies with growth phase, substrate availability and other environmental parameters [37], though this was not included in this analysis.
  - The maintenance efficiency is then $\epsilon_M = 1 - \sum P_{M,i}(t)/P_S(t)$
- Nutrient contributions
  - From maintenance calculations, we have the total instantaneous *power* available for growth, $\epsilon_M P_S(t)$
  - Using their activity and uptake rate constants, work out which of the CHNOPS elements would be limiting (i.e. the limiting contribution to making cells per unit time — we could have enough C for 6 cells hr^{-1} but only enough phosphorus for 2 cells hr^{-1} for example.)
  - Set the uptake rate for all of the elements such that they would match that production rate.
  - Given the total quantity of nutrients that could be collected in dt, compute the biomass this could correspond to: $g_{UT}(t)$.
  - Using this and the biomass which the energy can create $g_E(t)$, compute ϵ_{UT}:

$$\epsilon_{UT}(t) = \begin{cases} 1\ , & g_E(t) < g_{UT}(t) \\ \frac{g_{UT}(t)}{g_E(t)}\ , & g_E(t) > g_{UT}(t) \end{cases} \tag{4}$$

- Perform the metabolic reaction with the local environment.
  - If $\epsilon_{UT} = 1$, all of the available power supply is being utilised for maintenance and growth, and the molar concentration of reagents for each organism to react in the catabolism [M cell^1] is simply $r(t)dt$, where $r(t)$ [M substrate s^{-1}] is the thermodynamically limited rate of the catabolic reaction (Sec 5.3).
  - However, if $\epsilon_{UT} < 1$, growth is also restricted by the availability of nutrients (i.e. nutrient uptake is the growth rate-determining step). This means the organism need not collect all of the power available, as it cannot be used for growth and would otherwise be wasted. The power supply needed in this scenario is then $(1 - (1 - \epsilon_{UT})\epsilon_M)P_S$.

– In either of the cases above, the total molar concentration of reagents to react in the catabolism [M] can be expressed as:

$$(1 - (1 - \epsilon_{UT})\epsilon_M) \times N(t) \times r(t) \times dt \tag{5}$$

- Increase the biomass volume, mass and cell count by the number of new cells created: $(P_G(t) \times dt)/E_{\text{syn}}$
- If the organism has a set life span, deactivate all cells that have exceeded it.

The simulation can be ended when either user-defined criteria are met, or alternatively upon the death of all organisms or sufficiently low growth rate variability. For this work, the simulation ends when $\epsilon_M = 0$ for more than 10 steps, the metabolic rate drops below 10^{-40} M CO_2 (cell s)$^{-1}$ for 50 steps, or the cell population reaches 0.

5.2 Free energy availability in natural systems

The free energy of a chemical interaction can be expressed as:

$$\Delta G = \Delta G^{\circ} + RT \ln Q \tag{6}$$

where ΔG° [J mol^{-1}] is the standard free energy of the interaction, R [J (mol K)$^{-1}$] is the universal gas constant, and Q the reaction quotient all at temperature T [K]. Standard free energies of metabolisms are calculated using an SUPCRT92 database [58] and the reaktoro package for chemical systems[2], which implements the revised HKF equations [59]. The free energy changes dynamically with the composition, so as the composition is updated in the implementation (Sec 5.1) the available free energy per mole of substrate changes over time.

5.3 Thermodynamically limited biochemical kinetics

The free energy of a process is not enough to be used by an organism as is, it must first be converted and stored as a 'fuel' to use for future processes. All currently understood life forms use the molecule ATP for this purpose, utilising the high-energy phosphorylation reaction of ADP. This phosphorylation must be considered to calculate the rate of a multi-step (bio)chemical process, and activated complex theory can be used to both calculate this and correct for non-standard thermodynamic conditions using a thermodynamic limiter F_T [27, 60]. The thermodynamic limiter is applied as a correction to the forward reaction rate of the pathway r_+:

$$r = r_+ F_T \tag{7}$$

$$F_T = 1 - e^{-\frac{f}{\chi RT}} \tag{8}$$

$$f = \Delta G_A - \Delta G_C \tag{9}$$

where $f = -\Delta G_{net}$ is the 'thermodynamic driving force' or the difference between the amount of free energy available from methanogenesis ΔG_A (the negative of the free energy of the methanogenesis reaction) and the amount of free energy conserved by the organism during the pathway ΔG_C. χ is the 'average stoichiometric number', i.e. the average number of times each step has taken place in the pathway (usually 1) [27]. This could be thought of as akin to a heat engine. It is impossible for the cell to be 100% efficient because otherwise there would be little thermodynamic reason for the reaction to take place resulting in very slow kinetics. Hence, an organism must find a 'sweet-spot' of energetic efficiency against rate of uptake.

[2] https://reaktoro.org/index.html

5.4 The Energetic cost of cell synthesis

Upon initialisation of an organism object, the model estimates one or both of the following: the energy required to build all of the amino acids in one cell (based on calculations in McCollom and Amend (2005) [46]), and the energy required to form all of the biomacromolecules in a cell from their constituent amino acids [47, 48]. For this study, the cost of both was considered and is shown in Figure S1.

For the latter calculation, it is assumed that the total cost of polymerisation of one dry gram of cells is broadly comparable to the cost of polymerisation of one dry gram of that organism's constituent proteins [28, 61]. If the synthesis of protein P_n is a string of condensation reactions of amino acids AA, the overall reaction will take the form:

$$\sum_{i=1}^{20} n_i \mathrm{AA}_i \rightarrow \mathrm{P}_n + (n-1)\mathrm{H_2O} \tag{10}$$

where $n = \sum n_i$ is the number of amino acids in the chain. The free energy of this reaction can be estimated using a group contribution algorithm [47]. The standard free energy of formation of protein P_n $\Delta G_f^\circ[P]$ can be estimated as the sum of the standard free energies of formation of its constituent parts:

$$\Delta G_f^\circ[P] = \Delta G_f^\circ[AABB] + (n_{AA} - n_{GLY} - 1)\Delta G_f^\circ[PBB] + \sum_{i=1}^{19} m_i \Delta G_f^\circ[R_i] + n_{GLY}\Delta G_f^\circ[GLY] \tag{11}$$

where AABB, PBB, GLY, and R represent the amino acid 'backbone' (H_2N—CH—COOH), protein 'backbone' (HN—CH—C=0), glycine, and the R group of non-glycine amino acids respectively. n_{GLY} is the number of glycines in the chain, n_{AA} is the number of non-GLY amino acids in the chain, and m_i acts as a counter for the number of occurrences of each non-GLY amino acid i in the chain. Overall, the standard free energy of reaction for this protein synthesis is:

$$\Delta G_r^\circ = (n-1)\Delta G_f^\circ[\mathrm{H_2O}] + \Delta G_f^\circ[P] - \sum_{i=1}^{20} n_i \Delta G_f^\circ[\mathrm{AA}_i] \tag{12}$$

In principle equation 12 should be used for every single protein in the cell, but the reality is that any cell can contain millions or hundreds of millions of proteins, the exact structures of which is largely unknown if they are even detected [62, 63].

The model builds the mean constituent protein of the organism, for this study *E. coli* was used [62]. The free energy of synthesis of a protein with the mean length of amino acids (144), built proportional to the typical abundances of amino acids was calculated per equation 12, corrected to nonstandard conditions using the local temperature, amino acid abundance and a protein concentration of 10^{-12} M (both from [46]) and then converted to be the cost to produce one dry gram. This is then taken to approximate the cost of polymerisation of one dry gram of cells.

5.5 Nutrient Limitation

In order to successfully build or repair a cell, an influx of CHNOPS elements is required. To calculate the possible rate of nutrient uptake, NutMEG requires the concentration of each nutrient x [mol L^{-1}] in the local environment, and the associated rate constant for its uptake, k_x [s^{-1}]. This rate constant could be extracted from empirical data using a similar technique to that used to extract the rate constant of methanogenesis (Sec 2.2.2) if such data is available, though for this analysis an artificially high rate constant was used for each CHNOPS element as to separate it from other limiting factors (apart from when it was deliberately lowered e.g. Figures 3 and 4).

The uptake rate required to reach maximum efficiency for each CHNOPS element varies depending on how much is required in the cell itself. Table 2 summarises the standard dry weight percentage population by element for bacteria, slightly adapted to remove the 4% population of other elements. This composition was assumed for the simulated methanogens.

Element	Actual average % dry wt	Used Average % dry wt	corresponding mol per dry gram
C	50	50	0.4167
H	8	9	0.09
N	14	15	0.0107
O	20	20	0.0125
P	3	4	0.0013
S	1	2	0.0006

Table 2: Average makeup of a microbe compiled in [64], adapted slightly to 100% as there are other contributions from non-CHNOPS elements.

Acknowledgements

This work was supported through the Science and Technology Facilities Council (STFC) grant ST/R000875/1 and an STFC studentship to PMH. We thank three reviewers for their detailed and constructive comments.

Author Contributions

PMH and CSC conceived the core concepts of the model. PMH developed the methodology and code, performed the simulations and analysis, and drafted the manuscript. CSC offered astrobiology and microbiology insight and guidance throughout the study and helped with the structure and editing of the manuscript.

Data Accessibility

All of the code required to replicate these results, as well as data files containing the data plotted in all figures are available in the NutMEG-Implementations GitHub repository, in the TOM directory: `https://github.com/pmhiggins/NutMEG-Implementations`

NutMEG itself is available in the NutMEG public GitHub repository: `https://github.com/pmhiggins/NutMEG`

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