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Coding aliens: a study on potential microbes on Titan

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Abstract: Saturn's moon Titan hosts complex atmospheric processes and extraordinary surface features involving hydrocarbons instead of water. It thus provides room for research on the possibility of life in a non-Earthlike environment. A review of existing research on the topic is given; as well as calculations on the possible biomass density, based on the current unexplained methane abundance and on other models. Similarities between the results indicate that the assumptions of each model are mutually consistent. The values suggest that life on Titan according to the models should be detectable by *in situ* observations.

Contents

1 Introduction

Titan, the sixth and largest moon of Saturn, was discovered in 1655 by Christiaan Huygens. As the only other object in the Solar System with liquid on its surface apart from Earth, it also harbours a thick atmosphere where complex chemical cycles take place. Except on Titan, the chemical cycles involve hydrocarbons, instead of water as on Earth. They result in energy-rich molecules, made in the atmosphere with the help of sunlight, to rain down along with liquid methane, onto hydrocarbon lakes and dunes [1].

This complex hydrocarbon chemistry, along with unexpected observations of acetylene and hydrogen [2,3] plus a young surface [4] and atmosphere [1], all give room to observations and predictions on geochemical or even biological processes in a cryogenic, anhydrous, and anoxic environment. The possible presence of a subsurface (ammonia-water) ocean [5] and the hazy atmosphere may also yield clues about prebiotic Earth.

Research on this topic, especially conclusions that would also apply to "warm Titans", might expand the space of habitable planets beyond the Goldilocks zone, which would have implications on the Drake equation. The abundance of M-dwarf stars in the universe means that a majority of exoplanets with liquid on the surface should be more Titan-like than Earth-like [6], although the spectrum received on those planets would be different than what Titan receives from the Sun, a G-type star [7]. Thus, Titan presents a myriad of opportunities to further our understanding of how life could originate in the universe.

However, the origin of methane on Titan remains an open question. The simplest explanations of a source would be outgassing[1] from subsurface alkanofers[2] or from clathrates[3] in the crust; but the existence of these sources and the precise mechanism on Titan has yet to be confirmed [1]. In this paper, existing research is compiled to review the prospects for (microbial) life on Titan. A calculation of the biomass surface density based on the current methane abundance is also presented. It is hoped that the results would aid future missions to detect potential life on Titan.

2 Literature review

2.1 The atmosphere of Titan

Despite having a mass only 2.2% of Earth's, Titan has an atmosphere of 6.25×10^{18} kg, which is 1.19 times Earth's [8]. The haze in the atmosphere creates an anti-greenhouse effect, which lowers the temperatures and thus keeps the gases below their escape velocity (see Figure 1). Titan has no magnetosphere of its own to shield against solar winds, but they are less intense at Titan's distance from the Sun than at that of the inner planets. However, Titan does sometimes pass outside of Saturn's magnetosphere, and Saturn's (and thus its magnetosphere's) rotational period is faster than Titan's orbital period. These processes intensify the destructive effect of solar winds on Titan's atmosphere [9, 10]. Some gases, especially nitrogen, are thought to be replenished from Titan's interior [11, 12].

The atmosphere consists of 97% nitrogen (N_2), 1.4–1.5% methane (CH_4), 0.1–0.2% hydrogen (H_2) and small but still significant amounts of other molecules such as ethane (C_2H_6), hydrogen (H_2), acetylene (C_2H_2), and hydrogen cyanide (HCN). In particular, hydrogen and acetylene are produced with the help of solar radiation, consuming atmospheric methane in the process. As methane condenses into rain, these molecules are brought onto the surface [13], where the temperature (94 K) and pressure (1.5 atm) [8] are

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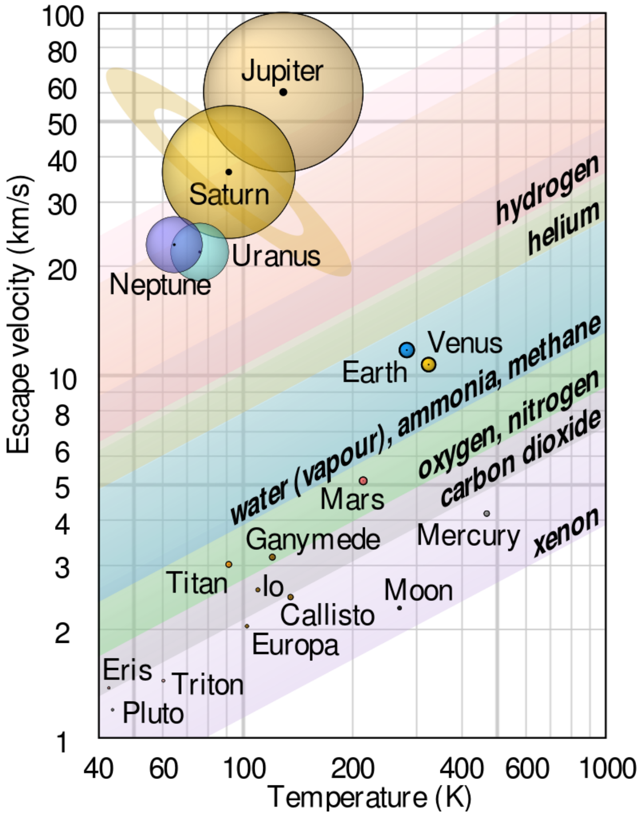
[1] Release of previously trapped gas.

[2] Underground reservoir of liquid hydrocarbons (instead of water as in aquifers).

[3] A crystal lattice with another molecule enclosed within the lattice space.

Figure 1: Escape velocity versus temperature. Regions indicate which gases are retained. Solar System bodies are plotted using surface temperature and drawn to scale. Source: Cmglee



near the triple point of methane, much like water on Earth [1], explaining the presence of methane lakes on the surface (see Section 2.2). Towards the surface, the mole fraction of methane increases to 4.9%, and that of nitrogen decreases to 95.1% [8].

Due to the photolytic processes mentioned above, the methane content in Titan's atmosphere should be short-lived [4], with the current abundance modelled to be destroyed within 2.13×10^7 years [14]. The photodissociation rate of methane was determined to be 5×10^{27} particle/sec based on INMS[4] measurements of H_2 outflow [15]. Various measurements and models indicate that the atmosphere as it is seen today should have an age between 3×10^8 to 6×10^8 years if methane is replenished, or to 10^9 years if it is not. However, the Copernican principle[5] means it is unlikely that we are in the time window to observe the last remnants of Titan's un-replenished methane [16]. As the lakes cannot contribute enough to the measured abundance [1], one other replenishing mechanism could be outgassing from Titan's interior. However the methane could also be from methanogenic life[6] on the surface, as outgassing has yet to be confirmed.

Furthermore, although ethane and acetylene should accumulate on the surface, no layer of these molecules have been found [2,17]. Based on observations, it has been predicted that a downflow of hydrogen should occur on Titan [3], contrary to intuition from simple physics. There are also measurements of nonuniform distributions of H_2 and other trace elements that are skewed towards the north [18,19]. These could be explained by a downward current in the north polar region, with the northern lakes acting as a sink, but could also be a pointer to biological activity in the south consuming these molecules [17] (see Section

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[4] The Ion and Neutral Mass Spectrometer instrument on the Cassini Orbiter

[5] That we are not privileged observers of the universe

[6] Organisms that produce methane as part of their metabolism.

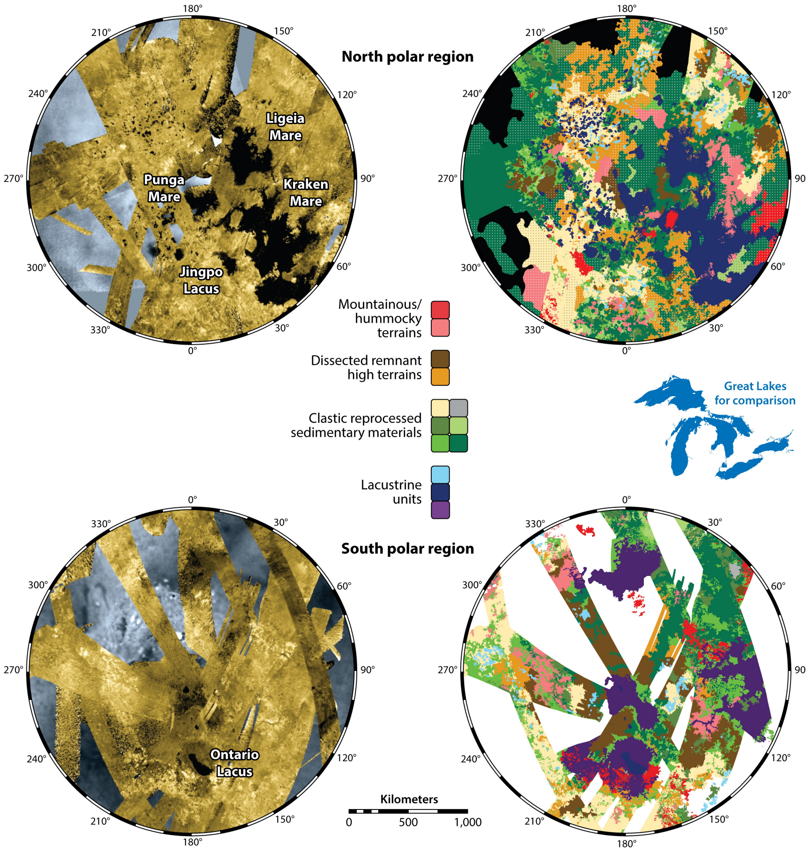
2.2).

Biological activity tend to prefer lighter isotopes of elements, since the chemical bonds would be easier to break and the energy thus easier to obtain [7,20]. This changes the isotopic ratios that are detectable in the atmosphere. On the surface of Titan, ${}^{12}_{6}\mathrm{C}/{}^{13}_{6}\mathrm{C} = 92.0 \pm 0.5$, which is close to the Solar System average [21] and within the constraints of Saturn (89^{+25}_{-18}) and Jupiter (94 ± 12) [15]. Thus, the observed isotopic ratios do not lend support to the presence of life on Titan. However, there is no strong isotopic effect resulting from the breakdown of N_2 on Earth either, and the recycling of CH_4 by organisms (see Table 1) should not necessarily result in an isotopic shift [17]. Further understanding of Titan's atmosphere is needed to explain the ratio [15], as well as the unexplained downflow of hydrogen in the atmosphere and deviation in abundance of acetylene at the surface.

2.2 The lakes of Titan

The hydrocarbon lakes of Titan are found at the polar regions only (Figure 2).

Figure 2: The polar regions on Titan. Source: [22]



Hayes AG. 2016.
Annu. Rev. Earth Planet. Sci. 44:57–83

While clouds [4] and rain [23,24] have been observed or modelled to occur at different latitudes, only the polar regions experience more precipitation than evaporation [24]. This difference is more exaggerated for the north pole than the south, due to hotter summers in the south during the present climate cycle [25], consistent with the observation that most filled lakes are in the north [22].

These lakes contain predominantly methane and ethane, which are liquid under the surface temperature of 94 K. The exact concentrations in each lake are still up to debate [7], but recent works suggest that those in the north generally have more methane than ethane [26], while the only large southern lake, Ontario Lacus, has ethane as its main component [27]. Unlike water, both methane and ethane are nonpolar solvents, which means any potential life in these liquids would need a completely different chemistry than water-based life (which depends on the ability of water to dissolve nutrients as a polar solvents). The nonpolarity combined with low temperatures would mean a low solubility for most molecules [7], which would be a challenge for life on Titan. However, carbon-based life in such an environment is not impossible, and might be more straightforward than that in Earth-like ones, considering that molecules would have no risk of hydrolysis nor thermal decomposition [17], that many known enzymes fucntion with a non-hydrophilic active site [28], and that nucleic acids may form faster in the absence of water [20].

Between ethane and methane, ethane is the better solvent [29]. A higher solubility means a bigger chance for life. Ethane is also less volatile, which would explain the sustained presence of Ontario Lacus despite the hotter summers. Speculatively, the resulting higher biological activity in Ontario Lacus would be an explanation for the lower concentration of H_2 and other trace elements in the south as mentioned in Section 2.1, if life on Titan consumes them for metabolism (see Section 2.4). [17]. However, the distribution of methane is modelled to alternate variably between the poles according to changes in the summer insolation[7] every 30 000 years, with a full period of 125 000 years, much like the Milankovitch cycles[8] on Earth [25]. Geologically this is a very short timescale, at least 1000 years shorter than the time it took for life to appear in the oceans of early Earth [30, 31]. Any potential life in Titan's lakes must therefore be able to survive changing concentrations of methane versus ethane, or even long dry periods if they reside in smaller lakes. On Earth, microbes respond to unfavourable conditions by forming cysts, which also helps in dispersal.

Along the edge of Ontario Lacus, there are “bathtub ring” deposits of acetylene, butane, and hydrogen cyanide among other hydrocarbons (see Figure 3), interpreted as evaporites that are left behind as methane evaporates from the lake [32,33] due to the present climate as discussed above (see also Figure 4). Models and observations agree on the existence of a acetylene-butane layer [34]. The recently discovered acetylene-butane co-crystal has been experimentally shown to be able to form in Titan conditions and be stable under ethane rain [35]. Such energy-dense molecules thus concentrated may be useful to potential life (see Section 2.4).

On the other hand, the stability and “water” levels of the northern lakes suggest that they are connected through underground reservoirs, similar to water tables on Earth [36]. This should contribute to the exchange of materials, which is beneficial to ecosystem processes. Indeed, the three largest lakes have been modelled to exhibit “salinity” gradients of ethane resulting from rainfall and tides due to Saturn [37].

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[7] Solar radiation received on the surface

[8] Periodic changes in climate, over thousands of years, due to orbital and axial movements

Figure 3: Composition and distribution of the deposits around Ontario Lacus, based on the Spectral Angle Mapper algorithm. The dark patch is the lake and the bright edge is identified as evaporites. The gridded images on the right side are satellite projections. Source: [33]

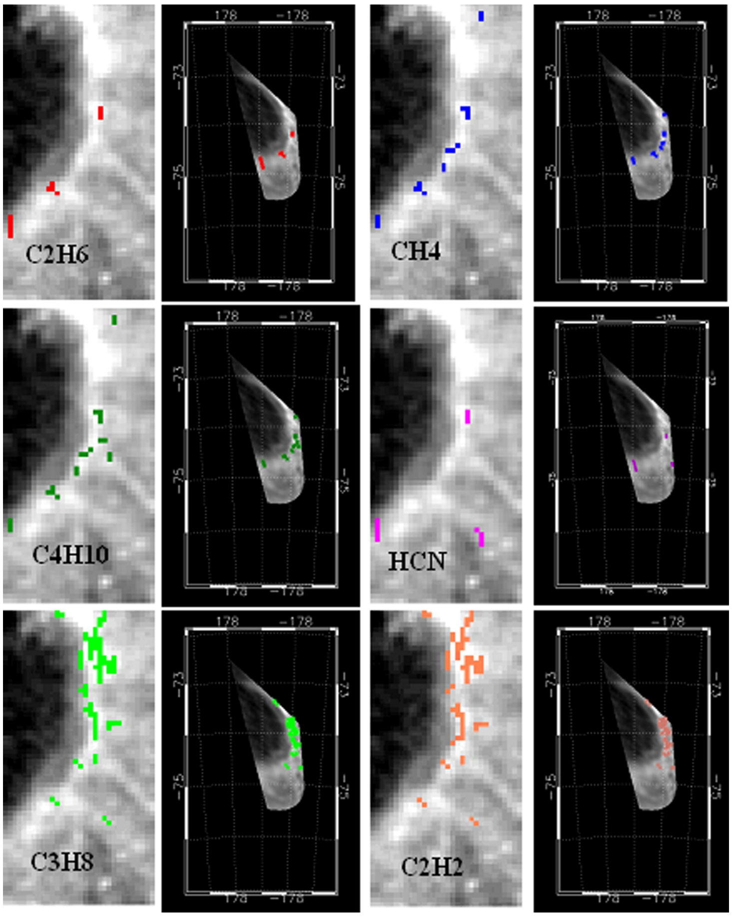
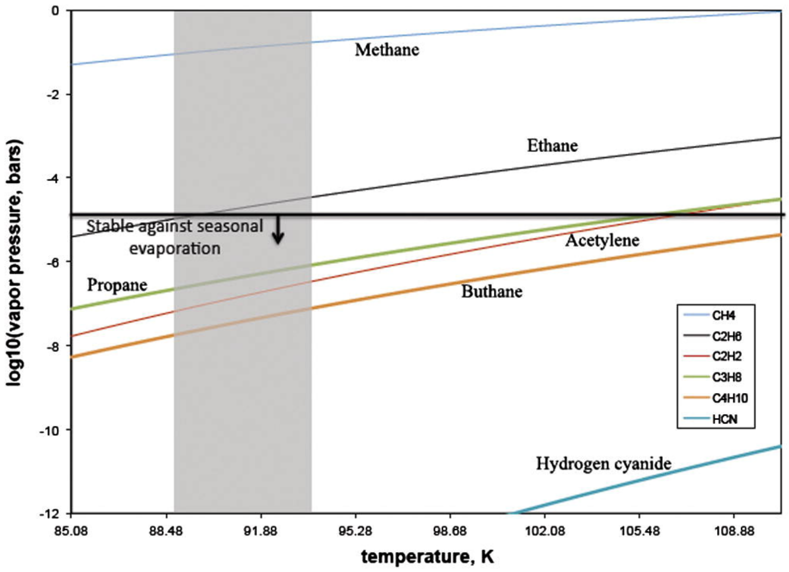


Figure 4: Vapor pressure versus temperature of materials observed on Titan. The grey bar is the range of temperatures of Titan lakes. The black line indicates which vapor pressures will lead to evaporation that is significant over seasonal timescales. As can be seen, methane would evaporate significantly from the lakes, while some ethane could also be affected by the seasons. Source: [33]



2.3 Definition of life

A discussion about potential alien life warrants an operational definition of "life". A general approach is that a lifeform shall be a distinctly bounded environment in disequilibrium with its surroundings, capable of transforming free energy from its surroundings to maintain itself and reproduce. [20, 38]. Thus, the chemical disequilibrium observed in Titan's atmosphere (Section 2.1) could be an indicator of a global biosphere.

2.4 Possible chemistry

For several reactions that generate methane via hydrogenation of molecules present on Titan, the Gibbs free energy has been calculated for Titan conditions, listed in Table 1 [39].

Table 1: Possible exothermic reactions on Titan

Reaction		$-\Delta G$ (kJ/mol)
$C_2H_2 + 3\,H_2 \longrightarrow 2\,CH_4$	{1}	334
$C_2H_6 + H_2 \longrightarrow 2\,CH_4$	{2}	57

These reactions are possible ways for potential lifeforms to gain energy while supplying methane to the atmosphere. Figure 6 shows how the reactions would play a role in the cycling of some gases in the atmosphere.

Figure 5: Titan's atmosphere. Source: [1]

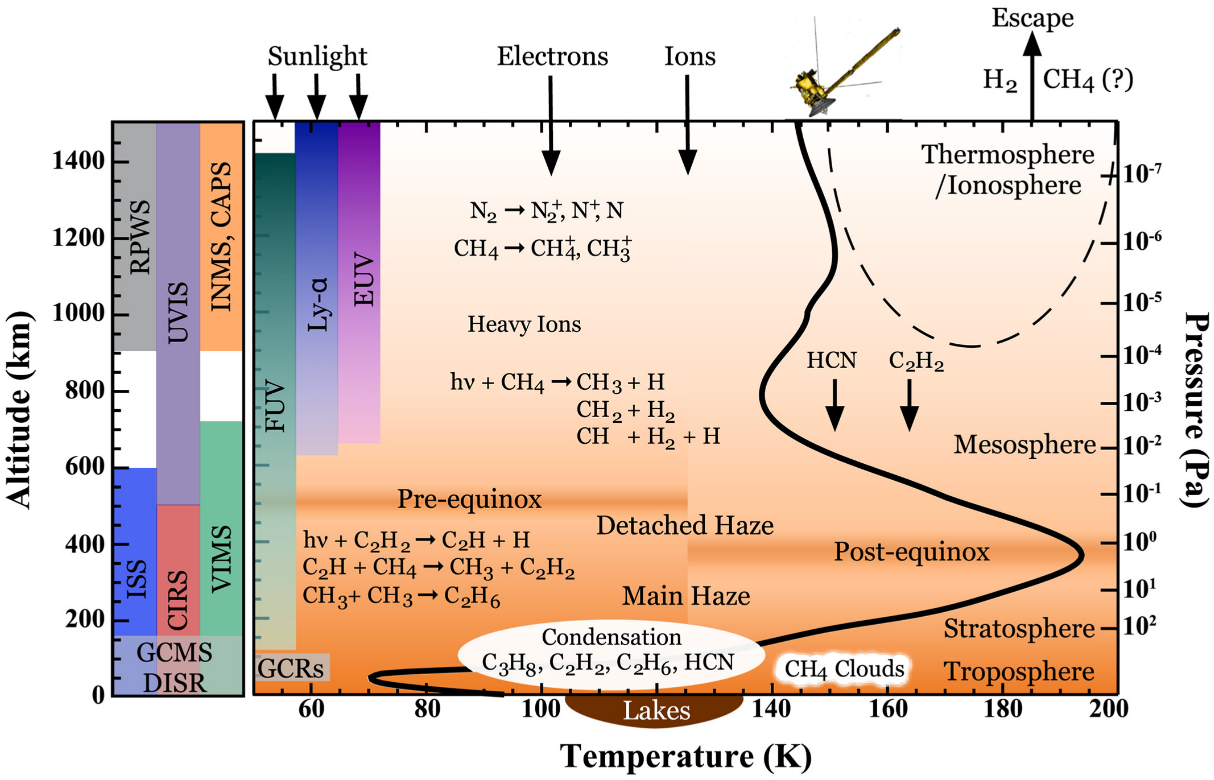
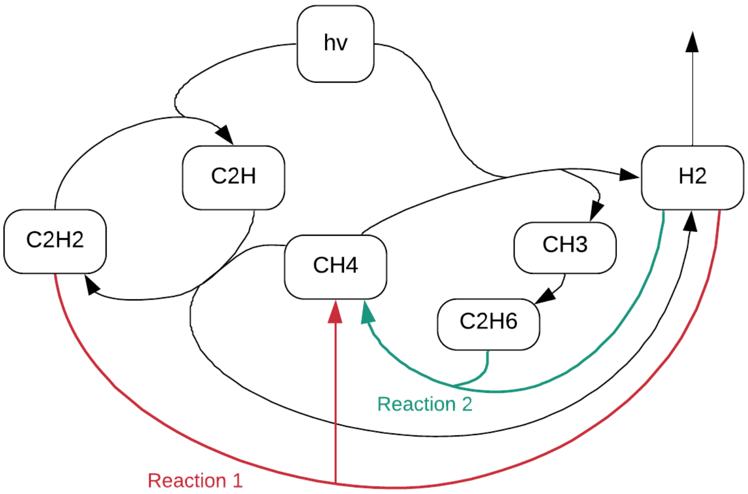


Figure 6: The cycling of some materials in the atmosphere together with Reactions 1 (red) and 2 (green). See also Figure 5 for the atmospheric processes.



If all the methane in the atmosphere is provided biologically by Reaction 1, then 2.2×10^{21} kJ of energy would have been produced during the course of 10^7 years, in order to keep the concentration constant [40]. This is consistent with the timescales mentioned in Section 2.1.

Aside from Reaction 1, acetylene (and its alkyl[9] substitute) can also be used in a reaction pathway to form alkyl nitrobenzene ($RC_6H_4NO_2$), a precursor to the biological building blocks on Earth (amino acids, fatty acids, sugars):

$$C_2HR + 2\,C_2H_2 + \text{Catalyst} \longrightarrow C_6H_5R \tag{3}$$

$$C_6H_5R + NO_2^+ \longrightarrow RC_6H_4NO_2 \tag{4}$$

The catalyst can be in the form of metals or aluminosilicate minerals[10], and R can be an alkyl group formed by radicals in the atmosphere. [41]

Titan's low temperatures mandate that covalent bonds would be much less relevant than they are on Earth, and hydrogen bonds should dominate chemistry instead. Furthermore, hydrogen bonds between imines and nitriles, and between amines and HCN, have binding energies of < 35 [kJ/mol], which is a requirement for interactions to occur over a reasonable timescale (within years) in Titan's cold environment. Since hydrogen bonds are highly directional, they would aid in the formation of structures. [7]

As for the information molecule, if the requirements are structural invariance with respect to the information encoded *and* solubility in the biosolvent, then a suitable candidate has yet to be found. Earthen DNA or RNA will not be viable on Titan. It is however reasonable to propose that the binding of the genetic code "bases" will use hydrogen bonds. [17]

HCN is regarded as an important ingredient to the formation of biomolecules. While Titan hosts an anhydrous and anoxic environment, many biomolecules known on Earth can theoretically function if replaced by their ammono-analogs, where the oxygen is replaced by NH [42]. Current detections suggest that HCN, abundant in the atmosphere of Titan, may have polymerised on the surface to form polyimine (pI), the most common polymer of HCN. This polymer can absorb photons, of wavelengths at which

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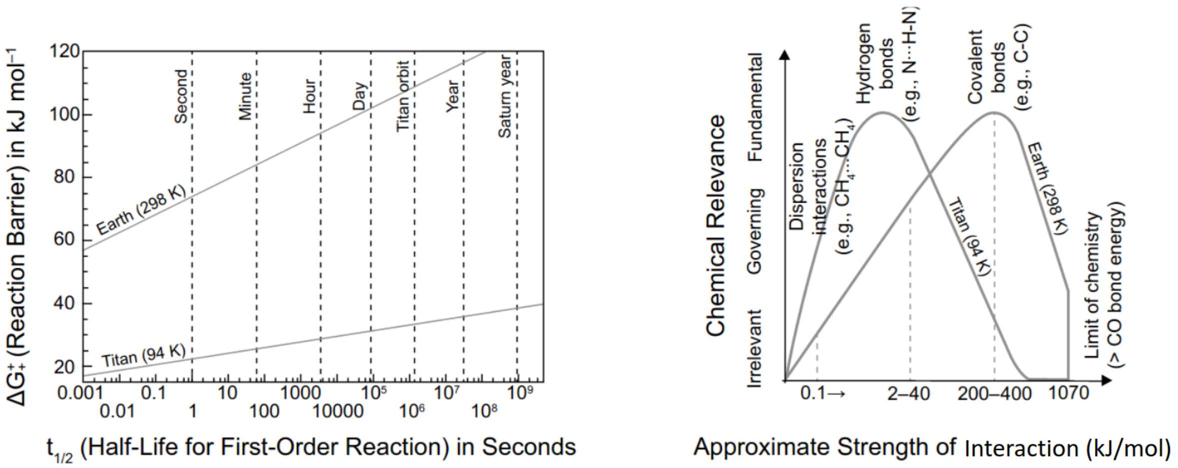
[9] An alkane (see footnote 12) without one hydrogen, i.e. molecules with the general formula $C_nH_{2n\pm1}$

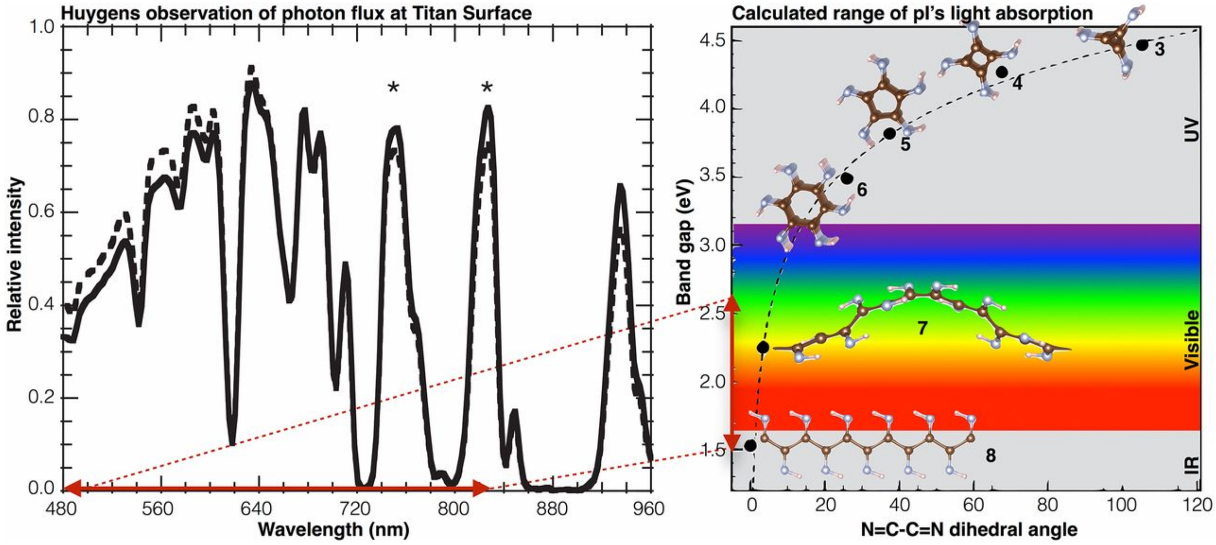
[10] A compound of aluminium, silicon, and oxygen.

Figure 7: Left: Reaction barriers versus reaction half-life for Earth and Titan temperatures. Right: Schematic showing the importance of types of reactions for Earth and Titan temperatures. Source: [7]



the Titan atmosphere is relatively transparent, to vary between polymorphs[11] (Figure 8) and hence drive chemistry or form structures. Even the addition and removal of an HCN molecule from pI have been shown to be thermodynamically possible with the help of photochemistry or catalysts. Since pI is able to make intermolecular hydrogen bonds with strengths of ∼4 [kJ/mol], they may be able to form alkanophobic monolayers in Titan's alkane[12]-rich environment, similar to hydrophobic molecules forming micelles on Earth. [43]

Figure 8: Left: Intensity of sunlight measured on Titan's surface, plotted against wavelength. Right: The polymorphs of pI plotted at their bandgap energies, which corresponds to a range of wavelengths. Source: [43]



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[11] Different crystalline forms of the same compound.

[12] Molecules with the formula C_nH_{2n+2}

2.5 Possible lifeforms

Simulations have also shown that bilayer membranes, made of acrylonitrile instead of lipids, are able to kinetically persist under Titan conditions [44]. Acrylonitrile has been detected in the atmosphere and the amount dissolved in the lakes are enough to form 10^7 vesicles per cm^3, based on an azotosome size of 10 µm [45]. Microbes on Titan could have large volumes compared to Earthen ones [40], especially since the size constraints imposed by the viscosity and surface tension of water would not be present in the cold hydrocarbon solvents of Titan.

Unfortunately, calculations have shown that acrylonitrile azotosomes are thermodynamically unfeasible on Titan, and thus cannot self-assemble like the lipid ones can on Earth [46]. It has yet to be found whether a membrane made of another molecule or configuration would be feasible on Titan. However, the cryogenic temperatures on Titan may favour microbes that are immobile, which means a membrane may hinder the diffusion of nutrients and waste; and would also make them less susceptible to damage by thermal reactivity, which means a membrane may not even be needed [46]. This does not necessarily violate the definition of life mentioned in Section 2.3, as the microbe can still be localised as an aggregate of molecules, but a lack of a membrane does make it difficult to imagine how such life could possess agency.

The immobility combined with the short (compared to Earth's Milankovitch cycles) climate cycles make it more favourable for microbes to reside on the edges or bottom of the lakes, where nutrients are deposited in large concentrations. The edges and also the surfaces of the lakes are favourable if the microbes rely on sunlight for part of their metabolism. This is feasible since the light level on Titan's surface is sufficient for photosynthesis for some Earthen organisms [7], and the sunlight could also be used for polyimine chemistry as mentioned above. It would also be favourable if the microbes rely on (the diffusion of) atmospheric gases, such as hydrogen in Reactions 1 and 2.

Methanogens exist also on Earth in hydrocarbon-rich liquid environments [47]. The fungus *Fusarium alkanophyllum* is able to extract water from its hydrocarbon surroundings in order to survive [48], and a strain of the fungus *Neosartorya fischeri* can even grow in purified asphaltenes[13], using that as its sole source of carbon and energy [49]. The marine and freshwater bacterium *Pelobacter acetylenicus* hydrolyses acetylene as an energy source and sole carbon source using an enzyme [35].

3 Model

The mass of the atmosphere is 6.25×10^{21} g [8]. With the mole fractions x_i of the atmosphere components near the surface and their molar masses M_i listed in Table 2, the current total amount of methane in the atmosphere N_0 [mol] can be calculated with Equations 1 and 2.

Table 2: Mole fractions and molar masses

Molecule	x_i (%)	M_i (g/mol)
N_2	95	28.0134
CH_4	4.9	16.04246
H_2	0.1	2.01588

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[13] Molecules found in crude oil that consist of carbon, hydrogen, nitrogen, oxygen, and sulfur.

The mass fraction (of methane) can be calculated as follows:

$$\frac{m_{\mathrm{CH4}}}{m_{\mathrm{tot}}} = x_{\mathrm{CH4}} \frac{M_{\mathrm{CH4}}}{\sum_i x_i M_i} \tag{1}$$

Then, the initial number of moles can be obtained via the molar mass

$$N_0 = \frac{m_{\mathrm{CH4}}}{M_{\mathrm{CH4}}} \tag{2}$$

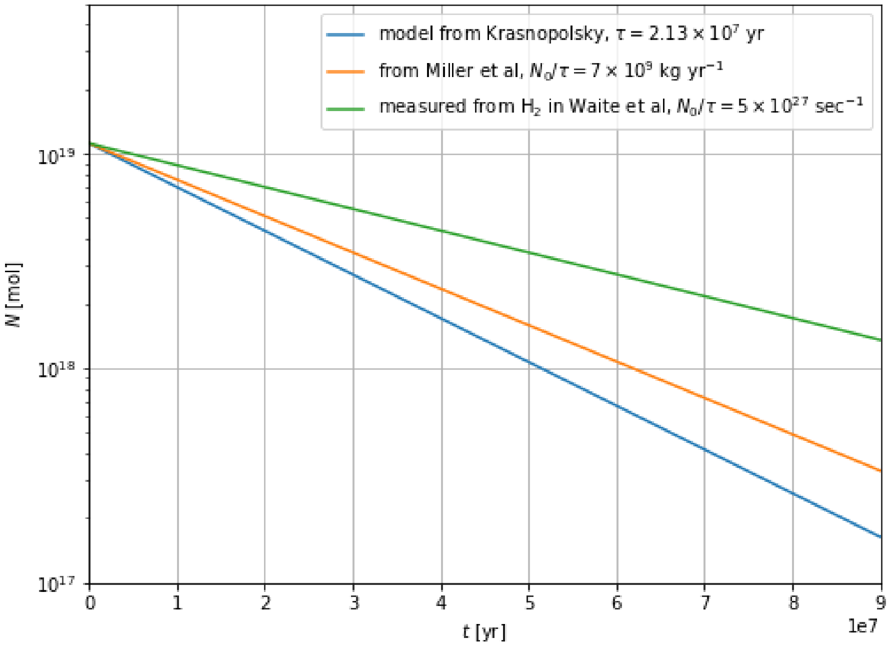
The methane loss in the atmosphere is assumed to follow an exponential decay model, since the absolute amount of methane destroyed is dependent on the amount remaining.

$$N(t) = N_0 e^{-t/\tau}$$
$$\frac{\mathrm{d}N}{\mathrm{d}t} = \frac{-N_0}{\tau} e^{-t/\tau} \tag{3}$$

In order to maintain the current concentration, the rate of methane *production* should be $-\frac{\mathrm{d}N}{\mathrm{d}t}$. Setting $t = 1\,\mathrm{yr}$ gives the amount of methane produced per year.

The lifetime τ can be taken as $2.13 \times 10^7\,\mathrm{yr}$ as modelled in [14]. The quantity $\frac{N_0}{\tau}$ can also be taken as $7 \times 10^9\,\mathrm{kg/yr}$ (from [11]) converted into mol/yr, or as the photodissociation rate of $5 \times 10^{27}\,\mathrm{s}^{-1}$ as inferred from H_2 outflow measurements [15]. The two values give rise to slightly different theoretical $N(t)$, shown in Figure 9.

Figure 9: Methane amount over time from now ($t = 0$), according to different parameter values, assuming exponential decay with no replenishment.



If the methanogens on Titan metabolise through either Reaction 1 or Reaction 2, both of which produces 2 molecules of methane per reaction, then the methane production rate from Equation 3 would correspond to an energy release rate depending on the ΔG of the reaction used. If Reaction 1 is used for all organisms, then the energy released is at a maximum, if Reaction 2 is used, then the energy released is at a minimum. If there are two species of microbes, each utilising one of the reactions, then the energy released would be in between the above limits.

As the lakes contain ethane as a main (non-volatile) component, and have "bathtub rings" where other components such as acetylene are concentrated, it is postulated in this model that the species using

Reaction 2 would live on the surface of the lakes, while the one using Reaction 1 would live on the edges of the lakes. Therefore, the lakes' area-to-perimeter ratio would give a reasonable estimate of how much one reaction may be preferred over the other, and thus how much energy is most likely to be released out of all possibilities within the above limits. To obtain this ratio, the perimeter lengths are multiplied with a width to make ribbons, since the "bathtub rings" indeed acquire a width as deposits build up over time as the "water" levels change. The lakes' perimeter and area data are obtained from [50], which is only a subset of 190 lakes out of all imaged lakes on Titan.

One reaction may occur faster than the other due to the physiological properties of the species that uses the reaction. Reaction 1 may be used more frequently than Reaction 2 because it is more energetic (larger $-\Delta G$), or less frequently because it uses more hydrogen (since the other component, either acetylene or ethane, are abundant in the respective locations) and already yields far more energy. The lakes and their shores may also be at different temperatures, a parameter on which the reaction rate could depend heavily (see also Figure 7), through the rate constant k.

$$k = Ae^{-\Delta G/RT} \tag{4}$$

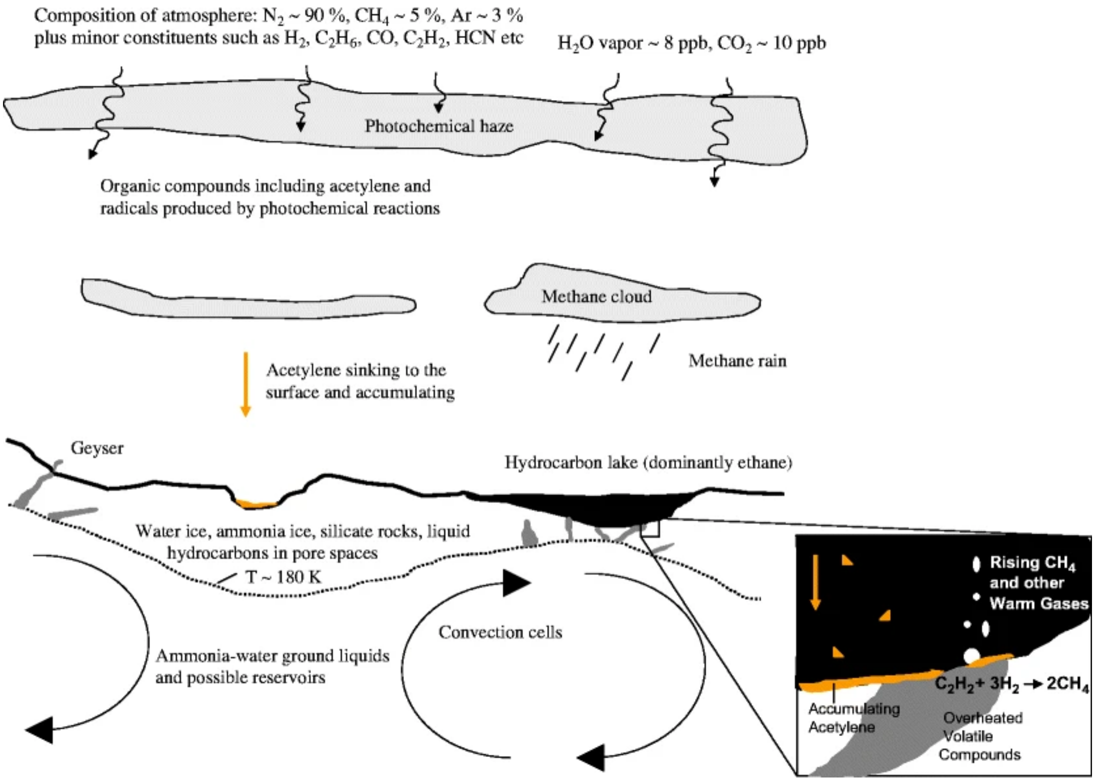
$$A = \rho Z \tag{5}$$

$$Z = 10^6 N_A^2\ [\mathrm{X}][\mathrm{Y}]\ \sigma_{\mathrm{XY}} \sqrt{\frac{8k_{\mathrm{B}}T}{\pi\,\mu_{\mathrm{XY}}}} \tag{6}$$

Here ρ is a scaling between the theoretical pre-exponential factor Z and the measured one A.

As there is no clear conclusion which reaction should occur faster than the other, it is assumed that they occur at the same rate, so that the lakes' perimeter-to-area ratio alone would give the ratio of methane produced via Reaction 1 versus via Reaction 2.

Figure 10: Schematic of the atmospheric and geological processes on Titan. Source: [51]. Note that the same methane concentration and reaction (inset) are used in this model, except it is postulated that the reaction (Reaction 1) should occur on the edge instead of bottom of the lakes, and Reaction 2 is also considered.



With an estimate of the energy released per year using the lake area-to-perimeter ratio, a biomass production rate in kg/yr can be obtained if the amount of energy used to produce a unit mass in J/kg is known. Multiply this by a generation time[14], then it is known how much biomass is on the lakes at one time.

Going further, the biomass can be divided by the 190 lakes' total surface area (including the perimeter ribbons) and a mean dry mass, to arrive at a microbe surface density. The dry mass can be the typical Earthen value of $2 \times 10^{-14}\,\mathrm{g}$ [40], or estimated based on acylonitrile vesicles discussed in Section 2.5. Titan's Ligeia Mare is approximately $14\,000\,\mathrm{km}^3$ and contains about 10^{13} to 10^{15} kg of acrylonitrile, enough for 3×10^7 azotosomes per cm^3 [45]. If the methanogens on Titan use a similar structure (that is thermodynamically and evolutionarily favourable), then they would have a mean minimum dry mass of

$$\frac{10^{14}\,\mathrm{kg}}{3 \times 10^7\,\mathrm{cm}^{-3} \cdot 14\,000\,\mathrm{km}^3} = 2.38 \times 10^{-10}\,\mathrm{g}.$$

This dry mass is a lower limit because it does not include any organelles and digestive material inside the microbe. However it is still only an estimate, since a cell membrane structure that can self-assemble on Titan has yet to be found.

Finally, the surface density thus obtained, could be seen as a measure of the detectability of such microbes on Titan. It could also be used to evaluate the significance of biological production of methane on Titan.

4 Results

The calculations described in Section 3 are implemented in a Python script, included in Appendix A. The input parameters and output results are summarised in Tables 3 and 4.

Table 3: Parameters used in this calculation

Parameter	Value	Explanation
Perimeter width	500 m	Smallest map scale used in [50], where the lakes surface area and perimeter data were found. This is within the widths of the deposit band observed around Ontario Lacus [32].
Energy demand	1.6 kJ/g	Average between 0 and 3.2 kJ/g, the latter of which is of the yeast *Saccharomyces cerevisiae* used in a similar calculation in [40]. The energy demand of Titan microbes should be less than Earthen ones. Microbes that require less energy should be favoured evolutionarily, because reactions on Titan yield less energy, and also if the microbes metabolise slowly as discussed above. This is also supported by the hypothesis that they might be immobile under the cold temperatures. (See Section 2.5.)
Generation time	0.5 yr	Average between 0 and 1 year, the latter of which is ≈ 1/30 of a seasonal cycle, as Titan's seasons are driven by Saturn's orbit, which takes 29.5 years. On Earth, microbes have much shorter generation times than 1/30 yr = 12 days. The long generation time taken here is plausible given that potential microbes on Titan are expected to metabolise slowly.

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[14] The amount of time it takes one generation to replace the previous one, taken as the typical reproducing age.

Table 4: Results of the calculation

Quantity	Value
Methane amount N_0	1.12×10^{19} mol
Total populated surface	290 487.726 km^2
Reaction 1 percentage	10.2%
Reaction 2 percentage	89.8%

Table 5: Results of the calculation that depend on τ and dry mass

Quantity \ τ (10^7 yr)	2.13	2.56	4.27	Units
Methane production rate $\frac{dN}{dt}$	5.25×10^{11}	4.36×10^{11}	2.62×10^{11}	mol/yr
Energy produced,				J/yr
min. (100% Reaction 2)	1.50×10^{16}	1.24×10^{16}	7.47×10^{15}	
max. (100% Reaction 1)	8.76×10^{16}	7.29×10^{16}	4.38×10^{16}	
according to percentages (Table 4)	2.24×10^{16}	1.86×10^{16}	1.12×10^{16}	
Biomass density	24.1	20.0	12.0	g/m^2
Number density, if dry mass =				microbes/m^2
2×10^{-14} g (Earth)	1.20×10^{15}	1.00×10^{15}	6.01×10^{14}	
2.38×10^{-10} g (azotosomes)	1.01×10^{11}	8.40×10^{10}	5.05×10^{10}	

5 Discussion

The methane amount of 1.12×10^{19} mol (according to this model, with data from [8]) translates to a mass of 1.80×10^{17} kg, which is smaller than the vertically integrated mass of 2×10^{17} kg from [11]. This is a surprising result. However, using the latter value to convert to N_0 would not change the numbers in Table 5 too much, since the values are still relatively close to each other.

The first two values of τ have similar results. The factor two difference for the third $\tau = 4.27 \times 10^7$ yr leads to corresponding differences between the results obtained in Table 5. This is because a change in τ implies a change of

$$\frac{\left.\frac{dN}{dt}\right|_{\tau=\tau_1, t=1\,\text{yr}}}{\left.\frac{dN}{dt}\right|_{\tau=\tau_2, t=1\,\text{yr}}} = \frac{\tau_2}{\tau_1} \exp\left(\frac{1\,\text{yr}}{\tau_2} - \frac{1\,\text{yr}}{\tau_1}\right) \tag{7}$$

in the methane production rate, the very first step of the calculation. For the large lifetimes considered here, the exponent tends to unity and the difference is approximately proportional to τ_2/τ_1. The long lifetime of $\tau = 4.27 \times 10^7$ yr does imply that the photodissociation rate derived from H_2 measurements cannot account for all the pathways through which CH_4 could be destroyed in the atmosphere according to models (see Figures 5 and 6), and in fact the photodissociation rate of 5×10^{27} particle/sec translates to 8303 mol/sec, while the general destuction rate of 7×10^9 kg/yr ($\tau = 2.56 \times 10^7$ yr) leads to 13 827 mol/sec. Thus, further measurements of atmospheric gases are required in order to confirm current models.

Unlike the factor of two difference resulting from changing τ, using an energy demand of 3.2 kJ/g and a generation time of 1 yr instead of the averages in Table 3 does not change the results significantly. Unless the latter parameters differ by orders of magnitude, similar results can be expected.

The maximum energy produced are all an order of magnitude less than the result of

$$\frac{2.2 \times 10^{21}\,\text{kJ}}{10^7\,\text{yr}} = 2.2 \times 10^{17}\,\text{J/yr} \tag{8}$$

from the calculation in [40], which also used Reaction 1 only (and the Earthen dry mass). Using the populated surface of 290 487.726 km^2 from this calculation, a surface density of

$$\frac{2.2 \times 10^{17}\,\mathrm{J/yr}}{3.2\,\mathrm{kJ/g/yr} \cdot 2 \times 10^{-14}\,\mathrm{g} \cdot 290\,487.726\,\mathrm{km}^2} = 1.18 \times 10^{16}\,\mathrm{microbes/m}^2 \tag{9}$$

is found. The values obtained with the Earthen dry mass are an order of magnitude (and a factor of two for the larger τ) smaller than (9), because the energy produced used in this calculation is also an order of magnitude smaller than (8).

A comparison can also be made with the *volume* density of 3×10^7 microbes/cm^3 in Ligeia Mare that is theoretically possible with the amount of acrylonitrile in the lake [45]. Assuming the microbes all live near the surface of the lake as discussed in Section 2.5, with Ligeia Mare's surface area of 126 000 km^2 [52] and aforementioned volume of 14 000 km^3 [45], a surface density of

$$\frac{3 \times 10^7\,\mathrm{microbes/cm}^3 \cdot 14\,000\,\mathrm{km}^3}{126\,000\,\mathrm{km}^2} = 3.33 \times 10^{15}\,\mathrm{microbes/m}^2 \tag{10}$$

would then be possible. The surface densities obtained with the Earthen dry mass (Table 5) and results 9 and 10 are all similar to the volume density of 4.1×10^{13} microbes/m^3 found in [40], which is a typical value in nutrient-poor environments on Earth, and hence should be detectable by *in situ* observations. The lakes of Titan might therefore be of interest for future expeditions.

The surface densities obtained with the dry mass based on acrylonitrile azotosomes have much lower values. This suggests the energy produced does not allow enough microbes to use up all of the acrylonitrile in the lakes of Titan, which is reasonable as the reactions feasible on Titan are less energetic due to the cold temperature. Moreover, the dry mass based on acrylonitrile azotosomes is much larger than the Earthen value, even though the values used to obtain that dry mass is based on an azotosome size of 10 µm. This is not inconsistent with the hypothesis that microbes on Titan might be large and immobile as discussed in Section 2.5, but the large disparity between the dry masses result in a similarly large range for the surface density. It is however noted that acrylonitrile membranes cannot self-assemble on Titan, so the derived results should be for reference only.

To improve the accuracy of the area-to-perimeter ratio, all lakes on Titan should be considered instead of only the 190 that were mapped. Before being included into percentage for each reaction, the smaller lakes should also be corrected for dry spells, which might slow down the rate of Reaction 2 since it depends on ethane. The rates for each reaction might also be estimated more precisely, perhaps with the help of the methanogens and acetylene-consuming organisms from Earth mentioned in Section 2.5.

6 Conclusion

Titan offers an exciting environment to make predictions about life "as we *don't* know it". There have been many predictions made related to the subject, based on conclusions drawn from recent data; here the predictions were compiled, and another one presented to be compared with them. The results presented were consistent with previous conclusions, however they could be improved with better atmospheric data and models, more imaging data of the lakes, and more research on hydrocarbon-dwelling methanogens. The similarities between the calculations suggest that the assumptions of each model are consistent with each other, and their values indicate that life thus modelled are detectable. The lakes of Titan are therefore an interesting location for *in situ* observations.

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8 References

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A Calculation code

```python
# Imports
from astropy import units as u
import numpy as np


# Constants
NA = 6.02214076e+23 / u.mol
TOTAL_MASS = 6.25e21 * u.gram  # Coustenis
DEMAND_YEAST = 3.2 * 1e3*u.joule / u.gram
GENTIME_MAX = 1 * u.year


# Classes
class Molecule:
    def __init__(self, name, mole_frac, molar_mass):
        '''
        mole_frac  : mole fraction of the molecule in the mixture (decimal, not %)
        molar_mass : molar mass of the molecule in g/mol
        '''
        self.name = name
        self.mole_frac = mole_frac
        self.molar_mass = molar_mass * u.gram/u.mol

class Reaction:
    def __init__(self, name, methane_produced, energy_per_mol):
        '''
        For reactions that produce methane only.
        methane_produced : number of methane produced as in the chemical formula
        energy_per_mol   : the energy released in kJ/mol
        '''
        self.name = name
        self.methane_produced = methane_produced
        self.energy_per_mol = energy_per_mol * 1e3 * u.joule / u.mol


# Globals used in functions
N2 = Molecule('N2', 0.95, 28.0134)
CH4 = Molecule('CH4', 0.049, 16.04246)
H2 = Molecule('H2', 0.001, 2.01588)
ATM_MOLECULES = [N2, CH4, H2]
R1 = Reaction('1', 2, 334)
R2 = Reaction('2', 2, 57)
ALL_REACTIONS = [R1, R2]


# Functions
def mass_in_mixture(molecule):
    '''
    Total mass of the molecule in the mixture
    molecule : Molecule object
    '''
    den = 0
    for m in ATM_MOLECULES:
        den += m.mole_frac * m.molar_mass
    num = molecule.mole_frac * molecule.molar_mass
    return num / den * TOTAL_MASS

def moles(molecule):
    '''
    Number of moles of the molecule (Molecule object) in the mixture
    '''
    return mass_in_mixture(molecule) / molecule.molar_mass

def production_rate_mol(molecule, tau):
    '''
```

```
    Production rate to balance out exponential decay, in mol/yr
    '''
    N0 = moles(molecule)
    t = 1 * u.year
    return N0 / tau * np.exp(- t / tau)

def get_reaction_ratios(areas, perimeters, peri_width):
    '''
    Given the inner areas and perimeter lengths of multiple lakes,
    gets the fractions of perimeter area and inner area over the total area,
    also returns the total area.
    areas      : array of the (inner) surface areas of lakes
    perimeters : array of the perimeter lengths of lakes
    peri_width : ribbon width of the perimeters
    '''
    perimeters = perimeters * peri_width
    surface_total = areas.sum() + perimeters.sum()
    acetylene = perimeters.sum() / surface_total
    ethane = areas.sum() / surface_total
    return acetylene, ethane, surface_total

def production_rate_kJ(reactions, reaction_ratios, tau):
    '''
    Converts production rate from mol/yr to kJ/yr
    reactions       : list of Reaction objects
    reaction_ratios : list of ratios for each reaction (percent taking place in
                      unit area w.r.t. all reactions)
    '''
    mol_per_yr = production_rate_mol(CH4, tau)
    mol_test = 0
    for i in range(len(reactions)):
        mol_test += reaction_ratios[i] * reactions[i].methane_produced
    energy = 0
    for i, reaction in enumerate(reactions):
        frac = reaction_ratios[i] * reaction.methane_produced / mol_test
        energy += mol_per_yr * frac / reaction.methane_produced * reaction.energy_per_mol
    return energy

def get_demand(energy_per_mass, generation_time):
    '''
    Gets the microbe's energy demand in (J/g/yr)
    energy_per_mass : energy it takes to build a unit mass of microbe
    generation_time : generation time of the microbe species
    '''
    return energy_per_mass / generation_time

def biomass_density(energy, demand, area):
    '''
    Gets the biomass surface density (g/m2)
    energy : energy produced per year (J/yr)
    demand : energy demand of the microbes (J/g/yr)
    area   : populated area (m2)
    '''
    return energy / demand / area

def get_dry_mass(choice):
    '''
    Returns dry mass of microbe based on acrylonitrile vesicles or Earth average
    '''
    if choice == 'acryl':
        # Palmer et al 2017
        acryl_ligeia = 1e14 * u.kilogram
        density_ligeia = (3e7 / u.cm**3).to(1/u.m**3)
        volume_ligeia = 14000 * u.kilometer**3

        dry_mass_acryl = acryl_ligeia / (density_ligeia * volume_ligeia)
        return dry_mass_acryl.to(u.gram)
```

```

    if choice == ’earth’:
        return 2e-14 * u.gram

def microbe_density(energy, demand, area, dry_mass):
    ’’’
    Calculates microbes number density
    energy   : energy produced per year (J/yr)
    demand   : energy demand of the microbes (J/g/yr)
    area     : populated area (m2)
    dry_mass : mean dry mass of microbes (g)
    ’’’
    return biomass_density(energy, demand, area) / dry_mass


# Main routine
def main(tau, peri_width, energy_per_mass, generation_time, choice):

    print(f"Moles of methane in atmosphere = {moles(CH4):.2e}")
    print(f"tau = {tau}")
    print(f"Moles of methane produced per year = {production_rate_mol(CH4, tau):.2e}")

    lake_data = np.loadtxt(’shoreline.txt’, unpack = True)
    areas = lake_data[3] * u.kilometer**2
    perimeters = lake_data[4] * u.kilometer
    R1_frac, R2_frac, POP_SURFACE = get_reaction_ratios(areas, perimeters, peri_width)
    print(f"Reaction 1 fraction = {R1_frac:.3f}")
    print(f"Reaction 2 fraction = {R2_frac:.3f}")
    print(f"Total populated surface = {POP_SURFACE}")

    energy = production_rate_kJ([R1, R2], [R1_frac, R2_frac], tau)
    energy_max = production_rate_kJ([R1], [1], tau)
    energy_min = production_rate_kJ([R2], [1], tau)
    print(f"Minimum energy (100% Reaction 2) = {energy_min:.2e}")
    print(f"Maximum energy (100% Reaction 1) = {energy_max:.2e}")
    print(f"According to fractions, energy = {energy:.2e}")

    demand = get_demand(energy_per_mass, generation_time)
    grams_per_area = biomass_density(energy, demand, POP_SURFACE).to(u.gram/u.meter**2)
    print(f"biomass density = {grams_per_area:.2e}")

    dry_mass = get_dry_mass(choice)
    print(f"with chosen dry mass as {choice} i.e. {dry_mass:.2e}")
    microbe_per_area = microbe_density(energy, demand, POP_SURFACE, dry_mass)
    print(f"number density = {microbe_per_area.to(1/u.meter**2):.2e}")
    print()


# Run main with different params
tau_kras = 2.13e7 * u.year
tau_waite = ((moles(CH4) * NA) / (5e27 / u.second)).to(u.year)
tau_miller = (moles(CH4) / (7e9 * u.kilogram / u.year / CH4.molar_mass)).to(u.year)

for i in [tau_kras, tau_waite, tau_miller]:
    for j in [’acryl’, ’earth’]:
        main(i, 0.5 * u.kilometer, DEMAND_YEAST / 2, GENTIME_MAX / 2, j)
```