Energy Yields for Acetylenotrophy on Enceladus and Titan

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Highlights:

- Fermentation of acetylene (acetylenotrophy) is a ubiquitous and under-studied metabolism on Earth.
- Acetylene may be abundant on ocean worlds such as Titan and Enceladus.
- On ocean worlds, acetylenotrophy is more energy-yielding than hydrogenotrophic methanogenesis.
- Acetylenotrophy may be a critical catabolic strategy in establishing microbial communities in alien oceans.

Abstract

Saturn's moons Titan and Enceladus appear to satisfy three key criteria for life: presence of liquid water, nutrient availability, and metabolic energy sources. Consequently, these ocean worlds are the focus of astrobiology research investigations that aim to assess the potential for life beyond Earth. One plausible source of metabolic energy on these moons is acetylene (C_2H_2), a simple organic compound that is the second most abundant photochemical product in Titan's atmosphere and has been identified as a likely constituent of Enceladus' ocean. Acetylenotrophy, or the microbial fermentation of acetylene, is utilized by microbes on Earth in a number of environments. Here, we have calculated the energy yield of acetylenotrophy to be 69-78 kJ/mol C within the oceans of Titan and Enceladus, greater than the widely considered, hydrogenotrophic methanogenesis, at 25-65 kJ/mol C. Therefore, we propose acetylenotrophy as a potential metabolism on these moons that should be considered in future astrobiology studies on worlds with abundant acetylene.

Keywords: Titan, Enceladus, Astrobiology, Search for Extraterrestrial Life

1. Introduction & Background

Titan and Enceladus are Saturnian moons that likely contain global subsurface oceans (Mousis & Schmitt 2008; Sohl et al. 2014; Vance et al. 2018). Given the colocation of liquid water, organic molecules, and potential water-rock interactions, these oceans could source the crucial elements and energy required for life (Artemieva & Lunine 2003; Cable et al. 2021; Des Marais et al. 2008; Glein & Shock 2013; Hedgepeth et al. 2022; Hemingway & Mittal 2019; Hendrix et al. 2019; Iess et al. 2014; National Academies of Sciences 2022; Nixon et al. 2018; Postberg et al. 2023; Thomas et al. 2016; Waite et al. 2009, 2017, 2006). *Cassini-Huygens* and supplemental ground-based observations have identified hydrogen (H_2), acetylene (C_2H_2), and methane (CH_4) on these worlds (Singh et al. 2016; Vuitton et al. 2019; Waite et al. 2009, 2017). The detection of hydrogen and methane, in particular, has prompted many astrobiologists to propose hydrogenotrophic methanogenesis,

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad \textbf{(1)}$$

as a possible metabolic (energy-yielding) reaction on Titan, Enceladus, and other worlds (Affholder et al. 2021; Hoehler 2022; McKay 2016; McKay & Smith 2005; Porco et al. 2006; Ray et al. 2021; Sauterey et al. 2022; Steel et al. 2017; Taubner et al. 2018; Vance et al. 2016). In particular, evidence suggests a possible hydrothermal environment in Enceladus' ocean that could support methanogenesis ((Hsu et al. 2015; Waite et al. 2017)). However, no astrobiological study has considered the microbial fermentation of acetylene as a potential source of energy:

$$2C_2H_2 + 3H_2O \rightarrow CH_3CH_2OH + CH_3COO^- + H^+ \quad \textbf{(2)}$$

Sometimes followed by H_2 formation from H^+ and additional reactants depending on the species of acetylenotroph.

On Earth, acetylenotrophy and the corresponding microorganisms, acetylenotrophs, have been found in bioremediation sites, abandoned gold mines, and fresh water and marine sediments, despite low environmental concentrations of acetylene (Akob et al. 2018; Jameson et al. 2019; Lovley et al. 1995; Madrid et al. 2001; Mao et al. 2017; Oremland & Voytek 2008; Schink 1985; Xiao et al. 2007). Although the ocean compositions on Titan and Enceladus are not well-known, we can constrain the potential energy yields for Reactions (1) and (2) under plausible environmental conditions. This approach has proven to be a useful framework for exploring potential microbial activity in a range of environments on Earth (e.g. Amend & Shock 2001; Bradley et al. 2020; LaRowe & Amend 2019, 2015; Lu et al. 2021) and certain planetary bodies (e.g. Mars, Europa, and Enceladus, Jameson et al. 2019; McCollom 1999; Sauterey et al. 2022; Taubner et al. 2018). Here, we utilize best estimates of Titan and Enceladus oceans' physical and chemical properties to calculate values of Gibbs free energy for acetylenotrophy. To provide context for comparison, we also calculate the energy yields of hydrogenotrophic methanogenesis.

2. Materials & Methods

Gibbs energies of reaction, ΔG_r, were calculated using

$$\Delta G_r = \Delta G_r^0 - RT\ln(Q_r) \quad \textbf{(3)}$$

where ΔG_r^0 represents the standard state Gibbs energy of reaction, *R* denotes the gas constant, *T* stands for temperature in Kelvin and Q_r corresponds to the activity product, which is defined by

$$Q_r = \prod_i a_i^{\nu_i} \quad \textbf{(4)}$$

The symbols a_i and v_i refer to the activity and stoichiometric coefficient of the *i*th species in the chemical reactions of interest. Values of activity are calculated from

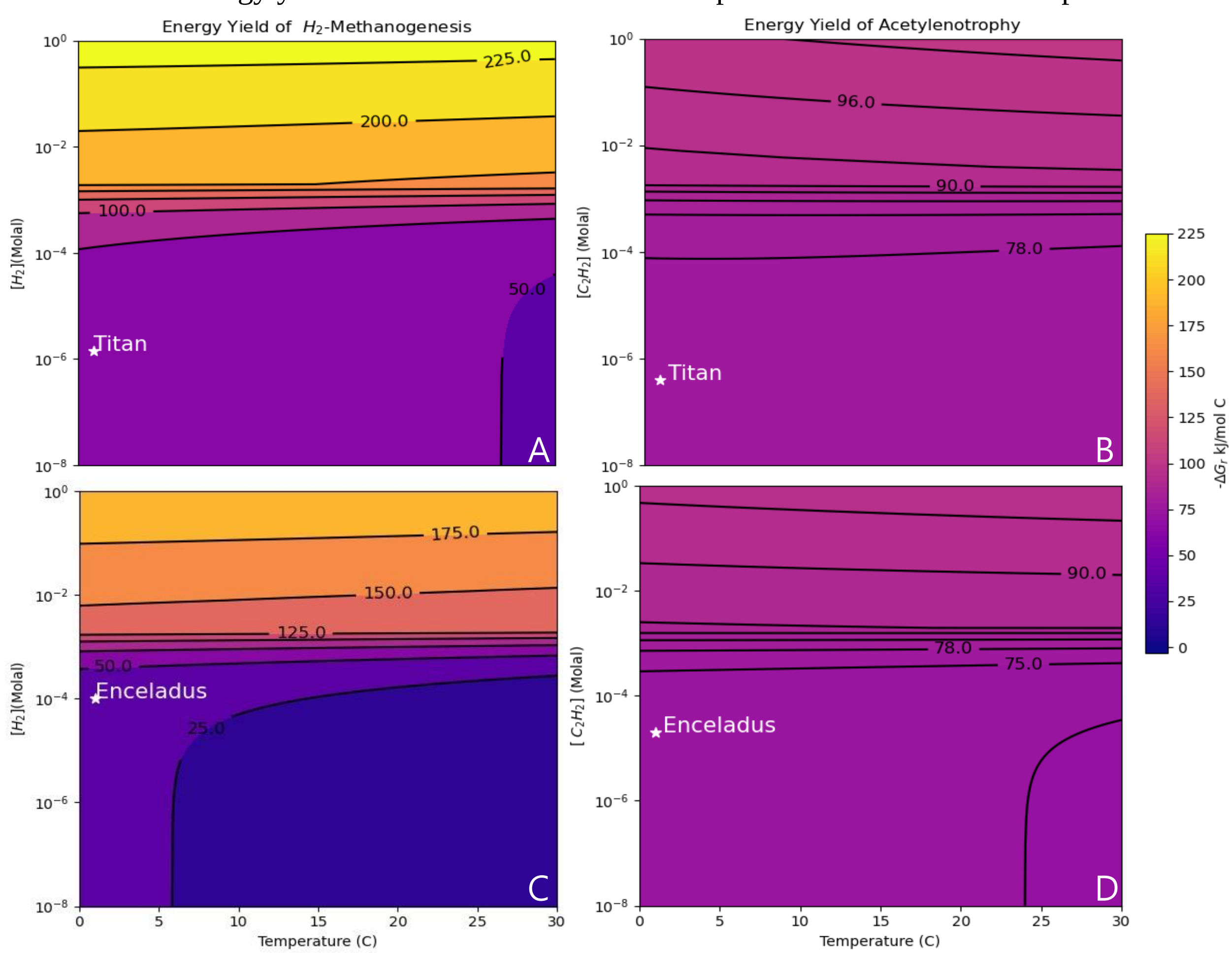
$$a_i = C_i / C_i^{\theta} * \gamma_i \quad \textbf{(5)}$$

where C_i and C_i^{θ} stand for the concentration and standard state concentration (one molal referenced to infinite dilution) of the *i*th species, respectively, in the reaction of interest, and γ_i designates the activity coefficient of the *i*th species. Values of ΔG_r^0 were calculated using the revised Helgeson-Kirkham-Flowers (HKF) equations of state (Helgeson 1981; Shock et al. 1992; Tanger & Helgeson 1988) with the *reaktoro* Python package, utilizing thermodynamic data from a number of sources (Gurvich et al. 1989; Johnson et al. 1992; Leal et al. 2016; Pennington & Kobe 1954; Wagner & Pruß 2002).

Energy yields were calculated at a pressure of 650 MPa for Titan (Figure 1a,b) and 10 MPa for Enceladus (Figure 1c,d) (Glein et al. 2015; Journaux et al. 2020; Postberg et al. 2023; Vance et al. 2018). In both cases, the ocean temperature was set at 1°C, similar to that predicted by Vance et al. (2018). The pH of Titan's ocean is hard to constrain; here we assumed neutrality (7.5 at 1°C). A slightly alkaline pH (8.5) was used for Enceladus (Glein et al. 2015; Glein & Waite 2020; Hoehler 2022; Postberg et al. 2023). Values of a_i were calculated as described in Amend & LaRowe (2019) for an ionic strength of 0.7 molal (*m*). Concentrations of H_2 and C_2H_2 were varied from 10^{-8} to 1*m*; the concentration of all other aqueous species were set at 10µ*m*. For Titan, our best estimates of aqueous H_2 (1.5 µ*m*) and C_2H_2 (0.4 µ*m*) concentrations were based on solubilities of atmospheric H_2 (0.1%) and C_2H_2 (5ppm) (Lorenz et al. 2019; Vuitton et al. 2019). For Enceladus, concentrations of hydrogen and acetylene were constrained by *Cassini* mission data. Based on material ejected into space through plumes, (Dougherty et al. 2006; Hansen et al. 2006; Nixon et al. 2018; Porco et al. 2006; Spahn et al. 2006; Tokar et al. 2006; Waite et al. 2006) an Enceladus ocean model and mixing ratios (Waite et al. 2009; 2017) yielded levels of 0.1 m*m* H_2 and 20 µ*m* C_2H_2.

3. Results & Discussion

Gibbs energy yields for Titan and Enceladus are plotted as a function of temperature and



activities of hydrogen (Reaction 1) or acetylene (Reaction 2) in Figure 1. At the conditions noted previously, energy yields for acetylenotrophy (Reaction 2) are 75-99 kJ/mol C in a Titan-like ocean, and 72-93 kJ/mol C in an Enceladus-like ocean. Note that energy yields are relatively insensitive to changes in temperature, but slightly higher in the elevated pressure environment posited for Titan (650MPa) compared to Enceladus (10MPa).

Figure 1: Filled contour plots of the Gibbs energies, $-\Delta G_r$, for hydrogenotrophic methanogenesis (a, c) and acetylenotrophy (b, d) as a function of temperature and substrate concentration (H_2 for panels a and c and C_2H_2 for panels b and d). Energy yields are shown in kJ/mol C. The top two panels represent Titan subsurface ocean pressure, 650 MPa, and neutral pH and the bottom two represent Enceladus' ocean pressure, 10 MPa, and pH = 8.5. The starred locations are representing the most likely substrate concentration for each ocean world considered, at a temperature of 1°C and assumed water activity = 1.0. Contour lines for panels a and c represent 25 kJ differences and those for panels b and d represent 3 kJ increments.

At the most likely conditions (white stars in Fig. 1), acetylenotrophy yields 75 and 72 kJ/mol C in the oceans of Titan and Enceladus, respectively. Energy yields for methanogenesis, by contrast, vary from very low (<25 kJ/mol C) to well above 150 kJ/mol C for the conditions considered in this study. At the most likely conditions (white stars), energy yields for methanogenesis in the oceans of Titan and Enceladus are 63 and 32 kJ/mol C, respectively. It should be noted that while the range of energy yields is much wider for methanogenesis, at the most likely ocean conditions for both Titan and Enceladus, acetylenotrophy is more exergonic. We conclude that acetylenotrophy is a viable catabolic strategy on those ocean worlds and may be a more tantalizing astrobiological target than methanogenesis.

These results illustrate the importance of determining species concentration to calculate reaction energetics, which in turn is critical for assessing the habitability of ocean worlds. Despite Titan having orders of magnitude lower concentrations of acetylene than Enceladus, its higher pressure environment yields more energy from acetylenotrophy. Within a global subsurface ocean, the concentration of the species of interest would be diluted, limiting the plausible locations for microbes to thrive. However, even very low acetylene activities lead to relatively large energy yields, so most any colocation of microbes and the species of interest ought to be sufficient for them to use that substrate. The same is not true for low activities of hydrogen, where the Gibbs energy of methanogenesis would represent an endergonic reaction.

Previous studies have calculated the energy yield of hydrogenotrophic methanogenesis for ocean worlds. Values determined for Enceladus include 40-125 kJ/mol CH_4 (Hoehler 2022; Porco et al. 2017; Ray et al. 2021; Steel et al. 2017; Taubner et al. 2018). One study explored a variety of Enceladus ocean conditions where methanogenesis would be exergonic (Affholder et al. 2021). The range of reported values represents different presumed ocean environments using carbonate disequilibrium (or other) models. The key distinguishing factor that would lead to a higher energy yield is the presumed concentration/activity of H_2 available within these modeled oceans, although the presumed pH and temperature of the ocean also varies across these models with less of an overall change. One ocean model was distinct: broad enough to predict endergonic as well exergonic methanogenesis, 0-150 kJ/mol (Higgins et al. 2021). Methanogenesis has been considered for other ocean worlds as well: a maximum energy yield of 125 kJ/mol C for Europa was computed, though the range varied depending on the model being used (McCollom 1999). Additional energetics studies on Europa considered many possible metabolisms, including methanogenesis, but not via Reaction (1) (Zolotov & Shock 2004). Acetylenotrophy has not been considered in these studies previously. We advocate that it ought to be included in these astrobiology analyses of other worlds, especially given it could be more exergonic than other metabolisms considered.

Here, we provide the first analyses of energy yields for acetylenotrophy under specific environmental conditions. We demonstrate that this metabolism is more exergonic than the much-considered hydrogenotrophic methanogenesis at the conditions applicable to Titan and Enceladus' oceans. We therefore suggest that future astrobiology missions, including *Dragonfly* to Titan (Barnes et al. 2021) and a future Enceladus flagship mission (MacKenzie et al. 2021), consider acetylenotrophy in their investigations in addition to other relevant metabolisms. Acetylenotrophy may have held a crucial role on early Earth, especially has acetylene may have been over a million times more abundant at that time (Oremland 1989; Oremland & Voytek 2008). The end products of acetylenotrophy (ethanol, acetate, and hydrogen gas) can be scavenged by methanogens, sulfate reducers and others for their own metabolisms (Miller et al. 2013). Acetylene also inhibits methanogens and sulfate reducers since it binds to their key catabolic enzymes (Mao et al. 2017). Early Earth's potential abundance of acetylene would have impeded methanogens and sulfate reducers that were present and attempting to colonize the soil environments; therefore acetylenotrophs would be needed to remove the acetylene and provide for a primordial microbial community (Oremland 1989; Oremland & Voytek 2008). If acetylene is abundant on an ocean world, acetylenotrophy may be a critical catabolic strategy in establishing microbial communities there. As we continue to understand the subsurface environments of ocean worlds and substrates available therein, we can better constrain energy yields for putative microbial lifestyles beyond Earth. We hope acetylenotrophy can be considered alongside other metabolisms of interest for future investigations of ocean world habitability.

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