BioGTL Platform for the Conversion of Natural Gas to Fuels and Chemicals

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Abstract

Methane, from a variety of sources including natural gas, represents an abundant domestic resource. Chemical approaches developing gas-to-liquids (GTL) technology to improve the use of methane as a fuel have met with only limited success to date despite significant investment. In contrast, little effort has been expended to deploy modern bioengineering approaches towards GTL process development. Several limitations, most notably the cost of sugar feedstocks, have prevented the economical production of biofuels using microbial systems. Exploiting inexpensive, domestically abundant carbon feedstocks such as methane represents an attractive strategy towards economically sustainable biofuel production

Important progress has recently been made toward engineering a number of phototrophic and fermentative microorganisms for the production of fuels and chemicals. Several limitations, most notably the ever-increasing cost of sugar feedstocks, currently prevent the economical production of fuels from microbial systems. Exploiting methane, an inexpensive, domestically abundant carbon feedstock, represents an attractive strategy towards economically sustainable production of next generation transportation fuels. Calysta Energy has developed a genetic engineering platform for host organisms (methanotrophs) capable of metabolizing methane to a variety of biofuels and biochemicals. The genetic tools, together with innovative fermentation and bioprocess approaches, enable the rapid implementation of well-characterized pathways to utilize natural gas as a biological feedstock instead of sugar.

Introduction

Important progress has recently been made toward engineering a number of phototrophic, heterotrophic, and fermentative microorganisms for fuels production. While phototrophic algae systems appear to have many advantages for fuel production, scale-up to commercial production is currently challenging. Fermentation approaches are better characterized, but several limitations, most notably the increasing cost of sugar feedstocks (Fig. 1), currently prevent the economical production of fuels from such systems. Interestingly, sugar and other food crop prices have become closely linked to oil prices since at least 2006, implying that these feedstocks are not well-suited to the production of petroleum replacements. Using biology to exploit alternative carbon feedstocks which are relatively inexpensive and domestically abundant represents an attractive alternative to both traditional petrochemical processes and sugar based bioprocesses.

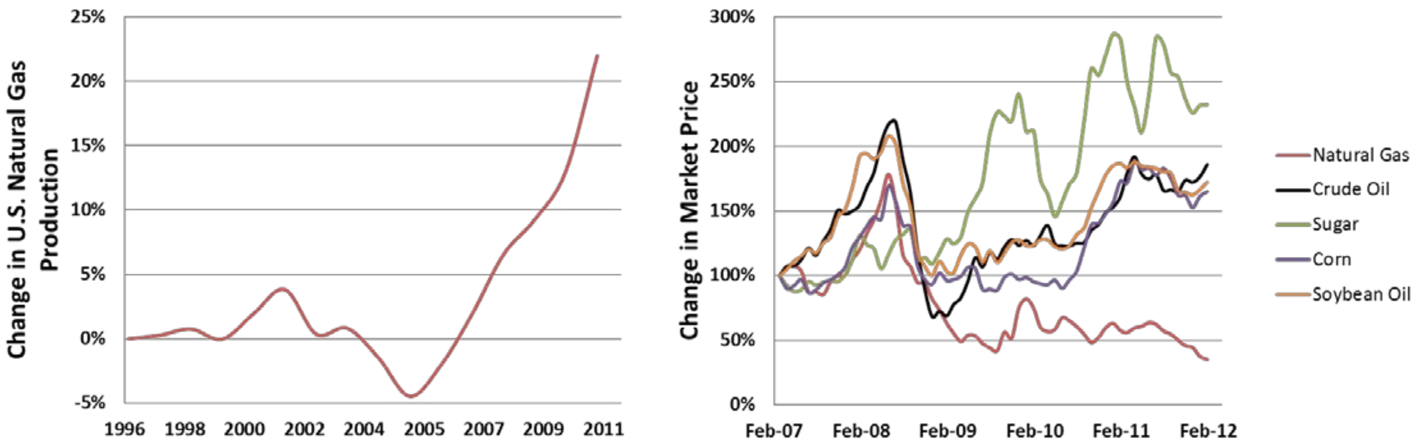

Figure 1. Historical domestic natural gas production and pricing. [1]

Methane State-of-the-Art Applications and Economics

One of the most abundant domestic carbon feedstocks is methane, sourced primarily from natural gas. The recent rise in domestic production of methane (from 48bft^3/day in 2006 to 65bft^3/day in 2012) has driven the cost of natural gas to record lows (from ~$14.00/MMBTU in 2006 to ~$2.50/MMBTU in 2012). Importantly, in sharp contrast to sugar, corn, and other biomass feedstocks, natural gas prices are now decoupled from crude oil prices in the U.S. (Fig. 1). Domestic natural gas is primarily produced by hydraulic fracturing ("fracking"), but methane can also be obtained from other sources such as landfills and sewage. Methane's 34x higher greenhouse gas contribution relative to CO_2 implies that capturing these sources will have a significant environmental benefit. Longer term, biomass-to-methane strategies may eventually enable a fully renewable carbon cycle if 'green' methane-based technologies are developed.

Methane's gaseous nature and volatility make transportation and direct usage as a vehicle fuel problematic. For this reason, there is a strong incentive to convert the gas to a liquid form to allow for easy transport to the point of use. Two main approaches are currently being pursued: liquefaction leading to liquefied natural gas (LNG) and chemical conversion to convert gas-to-liquid (GTL) [2]. The Fischer Tropsch (F-T) process is currently the most prevalent approach for converting methane from natural gas to higher-order hydrocarbons[2]. Note that the F-T process takes syngas as an input which is produced from natural gas by steam reforming (syngas can also be sourced from coal gasification, by high-temperature reaction with water and oxygen). The F-T process yields petroleum products consistent with today's fuel supply, but suffers from a number of drawbacks, including low yields, poor selectivity (making downstream utilization complex), and requires significant capital expenditure and scale to achieve economical production[3]. The massive scale required for an F-T plant (>$2B capital cost for a typical plant[2]) also represents a significant limitation due to the large amount of methane feedstock required to supply continuous operation of the plant. As methane transportation is prohibitively expensive in most cases, such a plant must be co-located with either a large gas source or a pipeline. An additional cost and scaling factor is the economics of gas-scrubbing technologies[3], as F-T catalysts are

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[1] Meyer, G. & Chazen, G. "US natural gas prices fall to decade low." Financial Times, January 20, 2012.

[2] Patel, B., 2005. "Gas Monetisation: A Techno-Economic Comparison of Gas to Liquid and LNG." 7th World Congress of Chemical Engineering, Glasgow, Scotland, UK.

[3] Spath, P.L. & Dayton, D.C. Preliminary Screening — Technical and Economic Assessment of Synthesis Gas to Fuels and Chemicals with Emphasis on the Potential for Biomass-Derived Syngas December 2003 NREL/TP-510-34929.

highly sensitive to common contaminants in natural gas which survive the syngas conversion process (see Table 1).

Impurity	Tolerance Level	Reference
Sulfur	0.2 ppm 1ppmv 60 ppb	4 5 6
Halides	10 ppb	5
Nitrogen	10 ppmv NH_3 0.2 ppmv NO_x 10 ppb HCN	6

Table 1. Syngas Impurities and Tolerances for Fischer-Tropsch

(Adapted from Ref 7). Note that these limits refer to syngas sourced from natural gas, as coal-derived syngas tends to have fewer/different contaminants.

The requirements for ready access to large volumes of clean gas, combined with massive capital investment, currently limit natural gas based F-T plants to successful operation in only a few locations world-wide[3]. Additionally, high transportation costs and the low density of methane frequently prevent the use of smaller sources of natural gas. These sources are generally referred to as "stranded gas," which is usually vented and burned at its source. Examples include off shore oil wells, traditional oil wells with small quantities of associated gas, or methane releases from landfills.

F-T plants have been in operation semi-continuously since 1938. Despite significant research and development over the last 70+ years, the limitations of F-T technology prevent broad adoption of commercial GTL processes. In contrast, essentially no effort has been expended to apply modern bioengineering approaches (including emerging synthetic biology tools and biofuel pathway information) towards GTL technology development. In particular, the low capital cost and scalability of a bioprocess would be particularly amenable to addressing stranded domestic gas sources.

Methanotrophic Bacteria

To create a biological GTL platform, it is logical to begin with an organism that can already metabolize methane, as introduction of an entirely new carbon assimilation pathway in a model organism is unlikely to succeed on a reasonable timescale. Methanotrophs are prokaryotes that utilize methane as their sole source of carbon and energy[7]. Methanotrophs have been observed in a wide range of environments, including both aerobic and anaerobic, typically in association with natural methane sources such as degrading biomass or petroleum offgas. Methanotrophs are a

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[4] Dry, M.E. (1981). "The Fischer-Tropsch Synthesis," in Catalysis, Science and Technology, (J.R. Anderson, M. Boudart, eds.), Springer-Verlag, Vol. 1, pp. 159-256.

[5] Boerrigter, H., den Uil, H., and Calis, H.-P. (2002). "Green Diesel from Biomass via Fischer-Tropsch Synthesis: New Insights in Gas Cleaning and Process Design." Paper presented a Pyrolysis and Gasification of Biomass and waste, Expert Meeting, 30 September, 2002, Strasbourg, FR.

[6] Turk, B.S., Merkel, T., Lopez-Ortiz, A., Gupta, R.P. Portzer, J.W., Kishnam, G., Freeman, B.D., and Fleming, G.K. (2001). "Novel TEchnologies for Gaseous Contaminants Control." Final Reprot for DOE Contract No. DE-AC26-99FT40675, September 2001.

[7] Hanson, R. S.; Hanson, T. E. (1996). "Methanotrophic bacteria." Microbiological reviews 60 (2): 439–471.

significant contributor to the global methane balance[8], recently illustrated by their rapid response in consuming the significant methane releases from the Deepwater Horizon oil spill[9]. Importantly, this demonstrates their tolerance of contaminants in natural gas relative to F-T processes (Table 1), which may represent a significant cost advantage for a commercial fuel process.

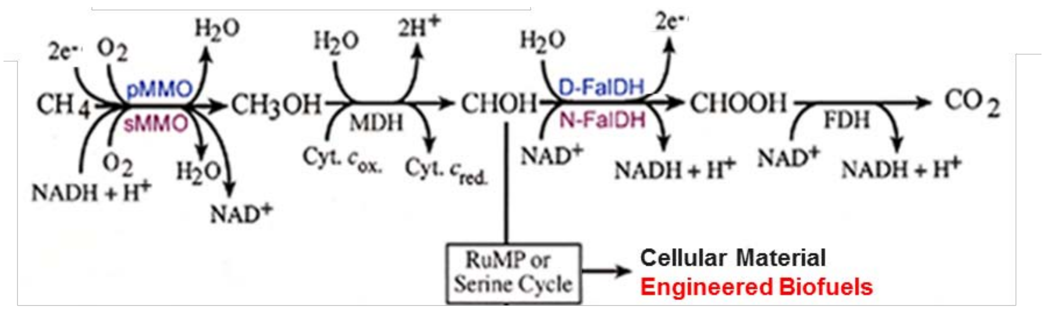

Figure 2. Methane metabolism for carbon assimilation in methanotrophic bacteria.

Aerobic methanotrophs metabolize methane through a specific enzyme, methane monoxygenase (MMO). Methanotrophs convert methane to methanol and subsequently formaldehyde (Fig. 2). Formaldehyde can be further oxidized to CO_2 to provide energy to the cell in the form of reducing equivalents (NADH), or incorporated into biomass through either the RuMP or Serine cycles[7] which are directly analogous to carbon assimilation pathways in photosynthetic organisms.

Due to the low solubility of methane in water, methanotrophs have evolved extensive membrane structures to enable efficient capture and utilization of gaseous feedstocks[7]. Two representative methanotroph species are depicted in Figure 3, highlighting the significant membrane enrichment common in this class of organism, which compares favorably with many species of algae[10]. Analogous to current fuel production in algae systems, these membrane lipids represent an attractive source of hydrocarbons for the production of biofuels. It is important to note that since the methanotroph cells are grown with methane as the sole source of energy and carbon, these lipids are derived almost entirely from methane. Thus, lipid accumulation in methanotrophs represents a natural GTL process which can be harnessed for commercial purposes.

[8] Oremland, R. S.; Culbertson, C. W. (1992). "Importance of methane-oxidizing bacteria in the methane budget as revealed by the use of a specific inhibitor". Nature 356 (6368): 421.
[9] Kessler, et al. (2011) “A Persistent Oxygen Anomaly Reveals the Fate of Spilled Methane in the Deep Gulf of Mexico.” Science 21: 312-315.
[10] Davis, R. et al. (2011) “Techno-economic analysis of autotrophic microalgae for fuel production.” Applied Energy 88 3524–3531.

Methylosinus trichosporium OB3b *Methylococcus capsulatus* Bath

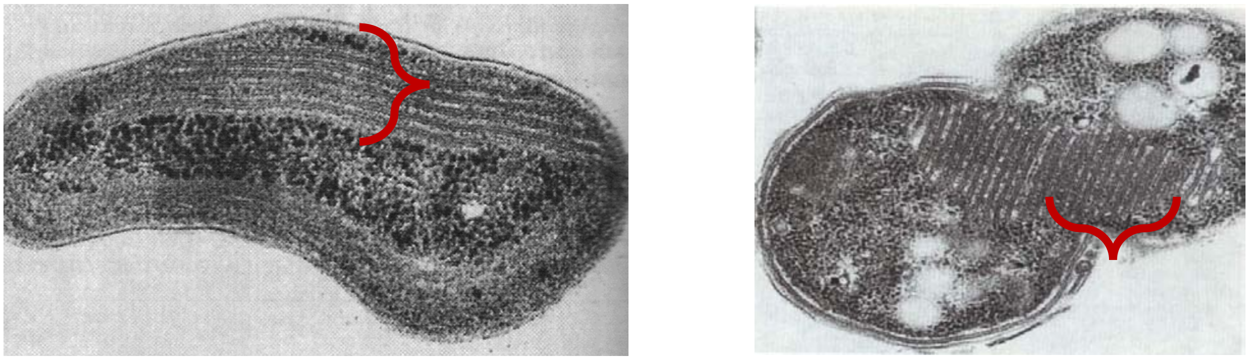

Figure 3. Micrographs of methanotrophic bacteria.
Examples of stacked membrane structures are highlighted by a red bracket in each panel.

Biotechnology Platform Benchmarks

Although a variety of approaches to biofuel and biochemical production have been explored over the past decade, for the purposes of comparison we will focus on three systems currently deemed to be close to commercial viability, namely open algal ponds, fermentation of cellulosic sugars, and biomass pyrolysis and reforming. Table 2 shows a comparison of the theoretical yield of these processes from their respective feedstocks to diesel (as a representative, highly reduced petrochemical replacement target). It is important to note that both CO_2 and biomass-derived sugars contain a significant mass fraction of oxygen (illustrated by the low %C for these feedstocks in Table 2), which is lost during the processing to a reduced hydrocarbon. This leads to low theoretical conversion yields and represents a major challenge to develop an economical petrochemical replacement. In comparison, methane contains no wasted mass as oxygen, which results in extremely high theoretical conversion yields. Coupled with the low cost of methane from natural gas and other sources, this observation suggests that a methane-based bioprocess would be highly competitive with current biotechnology platforms.

Carbon Feedstock	MW	%C	Conversion Method	Theoretical Diesel Yield (g/g)
CO_2	44	27%	Algae (Open Ponds)	21%
Sugar/ Biomass	~180	40%	Fermentation	31%
			Pyrolysis	47%
CH_4	**16**	**75%**	**Fermentation**	**59%**

Table 2 Comparison of biofuel platform efficiency

Several current research efforts are focused on developing specialty chemicals using engineered pathways in microbial systems (reviewed in 11, 12). Importantly, the yield advantages shown in Table 2 suggest that these approaches would benefit significantly when

[11] Zhang F, Rodriguez S, Keasling JD. (2011) Metabolic engineering of microbial pathways for advanced biofuels production. Curr Opin Biotechnol. 2011 Dec;22(6):775-83.
[12] Lamsen EN, Atsumi S. (2012) "Recent progress in synthetic biology for microbial production of C3-C10 alcohols." Front Microbiol. 2012;3:196.

implemented in a methanotroph system. Fermentation-based approaches to biofuel production have enjoyed a high level of technical success. Most recently, Solazyme Inc. has produced drop-in biofuels from algal lipids derived from sugar which successfully powered a battleship[13]. However, despite impressive yields and productivity from their fermentation system, Solazyme's biodiesel is priced at approximately $26/gallon[14] and is not competitive with petroleum fuels. Similarly, Amyris Inc. recently announced that its yeast fermentation biofuel platform could produce fuels at no less than $29/gal.[15] High production costs are an inherent problem with traditional fermentation systems, as the low theoretical efficiency from sugar (Table 2), coupled with competing food uses and the relatively high price of sugar, represent an inherent cost floor for production that cannot be overcome by scale or engineering.

While significant current attention has been focused on open-pond algae and biomass-based approaches (i.e. pyrolysis) to overcome the limitations of traditional fermentation, a methane-based fermentation process offers a number of advantages relative to both. A high-level comparison is summarized in Table 3. In general, methane fermentation would provide similar advantages to algal systems over biomass fermentation in terms of a low-cost, well-characterized feedstock that does not compete with human food sources. However, methane fermentation would allow for more flexible production plant locations, reduced land use, and higher density cultures due to the lack of a dependence on continuous availability of sunlight. Further, methane fermentation would be conducted in a closed fermenter system, allowing for more flexibility in the use of genetically modified organisms, as well as drastically reducing the water consumption of the production process.

Metric	Algae (Open Ponds)	Methane Fermentation	Biomass Fermentation
Capital Investment	Low	**High**	High
Ease of scale-up	Requires land use	**Good**	Good
Feedstock availability	Mod. (year-round sun)	**Good (nat. gas / biogas)**	Low (food competition)
Feedstock sensitivity	Low	**Low**	High (inhibitors)
Feedstock processing cost	Low	**Low**	High (release sugars)
Downstream processing cost	High (dilute culture)	**Low (dense culture)**	Low (dense culture)
Flexibility to strain selection	Low (open system)	**High (closed system)**	High (closed system)
Water use	High (evaporation)	**Low**	Low

Table 3 Comparison of biotechnology platforms (adapted from 10)

Feasibility

A critical requirement for development of a biotechnology platform is the availability of tools for the directed manipulation and modification of methanotroph metabolism. Although such tools are commonplace for model organisms (e.g. *Escherichia coli* or *Saccharomyces cerevisiae*), relatively little effort has been expended to develop similar capabilities in methanotrophs. However, Calysta Energy has successfully developed a suite of tools for the expression of heterologous proteins in methanotrophs (Fig. 4), as well as tools for the efficient targeted manipulation of the methanotroph genome. As Calysta continues to improve the technical capability to modify methanotrophic organisms, Calysta is also making the tools available at no cost to the academic research community to help build interest and critical mass in the field.

[13] Watson, J. (2011) "Navy launches its largest biofuel test for ship." Associated Press, Nov 16, 2011.
[14] Herndon, A. (2011) "Navy to Buy $12 Million of Advanced Biofuels in Record Purchase." Bloomberg News, Dec 5, 2011.
[15] Bullis, K. (2012) "Why Amyris is Focusing on Moisturizers, Not Fuel, for Now." MIT Tech Review, May 9, 2012.

It is important to note that the central metabolism of methanotrophs is comparable to that of most model organisms, in that it proceeds through typical metabolites such as pyruvate and acetyl-coA. This means that metabolic pathways which have been developed, characterized, and validated in other host organisms can be adapted for use in methanotrophs. Using this approach, Calysta has successfully demonstrated production of a variety of chemicals from methane via engineered metabolic pathways (not shown).

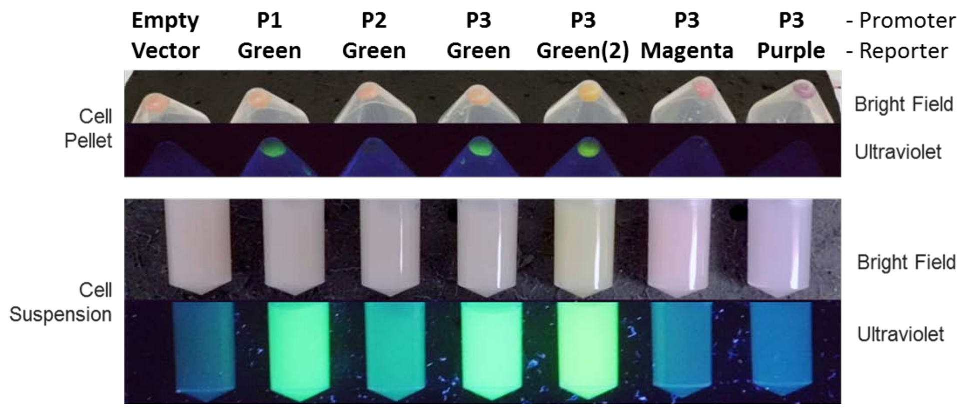

Figure 4. Demonstration of genetic manipulation in methanotrophs.
Methylococcus capsulatus was transformed using proprietary methods with plasmids containing a combination of promoters (P1, P2, P3) and reporter proteins (two versions of Green, Magenta, Purple). The two Green reporters are fluorescent in ultraviolet light, while Magenta and Purple absorb in the visible spectrum only.

Economic Impact

Detailed examination of the costs associated with other bioprocesses reveals that the methanotroph process improves upon systemic limitations in those processes. Traditional fermentation approaches that convert sugars to products suffer from the high cost of the sugar feedstock (or high processing costs if attempting to use a cellulosic sugar source), low theoretical yield due to the highly oxidized state of the carbon feedstock, and macroeconomic factors, such as transportation costs, competition with human food sources, and potential environmental impacts due to land use. An example alternative process that does not use sugar is open-pond algae. However, major cost drivers in an algae bioprocess include operating costs in the form of water usage and CO_2 delivery, as well as capital costs due to the dependence of yield on surface area rather than volume in an open pond system. Several groups are spearheading efforts to develop bioprocesses based on syngas to overcome some of the above issues, but, as noted above, syngas production is capital-intensive as well as requiring additional costs for removing contaminants from the feedstock stream.

By comparison, a methanotroph bioprocess overcomes the feedstock cost issues of traditional fermentation processes, while reducing capital and operating costs compared to algae or syngas-based processes. Further, total capital costs and minimum operating scale of a methanotroph biofuel plant are significantly lower than a F-T plant. This should allow implementation of economical, scaled-down GTL plants in a wide range of currently infeasible locations, especially in the United States using domestic methane sources. For these reasons, a methanotroph platform represents a truly disruptive improvement in cost and performance relative to current state-of-the-art methods. The demonstrated flexibility of biological systems to produce, specifically and at high yield, a wide range of chemicals allows the production of drop-in chemicals to replace those currently produced from petroleum. The ability to use methane as a biological feedstock represents an opportunity for these drop-in replacements to compete directly with petroleum chemicals on price, while simultaneously providing an outlet for stranded or waste methane which would otherwise have a major detrimental impact in terms of greenhouse gas emissions.