Opportunities
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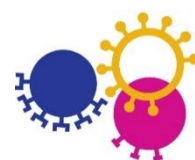# Methane Fermentation
for the
UK Bioeconomy

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Competing Interests

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Executive Summary

Methane is a strong driver of climate change. As a greenhouse gas, it is 25 times more potent than CO_2. Mitigation of methane emissions should therefore be considered important to reach climate targets. Methanotrophs are bacteria that are capable of converting methane to CO_2. Their ability to produce valuable chemical commodities from gas is proposed herein. In order to be economical as "bio-factories", the feedstock methane must come at a low cost.

Natural gas from gas and oil extraction is the main source of methane. Natural gas is 90% methane. As a feedstock, the cost effectiveness of this source is linked with traditional gas demand and energy prices. Alternative sources, such as gas flares and vents from extraction rigs, could be more cost effective as demonstrated in US. Unfortunately in the UK, flaring and venting is highly regulated and reduced to a minimum during exploration, and is only allowed in emergencies during production. Therefore gas flares and vents from extraction rigs shall not be considered as a feedstock for biological conversion of methane in UK.

Other potential sources of methane are anaerobic digestion and landfill gas. Landfill gas is currently captured and used for heat or electricity. Thus landfill gas for bioconversion would have to be bought at current energy prices, and the establishment of new landfill sites with the associated infrastructure requires capital investment. Anaerobic digesters, on the other hand, can be set up without large demand on land and technology investment, provided feedstocks, such as waste water treatment sludge, farm waste or food waste, are available. This could provide a viable option for bioconversion of gas by building on the established anaerobic digestion economy and increasing value of off-gas by methanotrophic conversion to higher value chemicals.

Current chemical conversion of methane to liquid fuels relies on highly energy intensive chemical conversions via syngas. This technology is reliant on large volumes of methane and is not economical on a small scale. Thus on a small scale, biological conversion of methane to fuels, such as *n*-butanol, ethanol or biodiesel, from lipids is favourable. However, the current low market value of petrochemically derived fuels, which leads to small gross margins, has discouraged investment in these products. In addition, syngas technology is not yet market ready and requires further R&D. Specialist or platform chemicals that do not compete directly with petrochemical sources can increase gross margin. Selecting molecules that are naturally produced by methanotrophs circumvents the need for expensive genetic engineering of the bacteria. This was recognised by US company Calysta, which changed its mission from the methanotrophic production of fuels to the production of single cell proteins for use as fish food. Similarly, the production of lipids for biodiesel or dietary supplements is technically feasible, however both are more demanding at downstream processing stages.

An interesting potential product from methanotrophs is methanol. Methanol is produced directly in one step via the methane monooxygenase (MMO), the initial enzyme of methanotroph metabolism. Even though methanol is produced naturally by the organism, it is directly converted further to formaldehyde where it enters the metabolism. As a result, yields

achieved in fermentation are too small to be industrially viable. However, it is conceivable that bioengineering of the host could increase production (Conrado et al. 2014). This could lead to methanotrophic fermentation becoming an important pillar of the methanol economy, as was proposed by Nobel Prize winner George Olah and co-workers (Olah et al. 2009). In this scenario, methanol acts as a central molecule for energy storage, which can be used as a fuel (either as a drop-in fuel in petrol engines or in specific methanol engines), or can be further converted to higher hydrocarbons.

In many sectors, biotechnology for methane conversion is not developed to be economically viable, largely due to the market turbulence of natural gas and small scale of current biogas production in UK. However, political incentives could help to develop this promising field. One such incentive would be to include liquid fuels, derived from otherwise lost methane, under the Renewable Transport Fuels Obligation (RTFO). This would make the development and use of such derived fuels more attractive.

At present, the methane bioeconomy is in the very early stages of development. There are companies that claim to be within reach of market entry. Calysta has facilities running within the UK to produce sample amounts of their proprietary fish food. Other companies are producing platform chemicals, and a US research program (ARPA-E REMOTE) has invested $39 million into R&D to develop biological methane as a transportation fuel conversion technology, with results due imminently. For a methane bioeconomy to be viable, the sources to be used have to be either emission based or renewable. It was shown that gas from flaring and venting at extraction sites is difficult to access, while other emissions cannot be captured at present. It can be concluded that anaerobic digestion is thus the only renewable source of feedstock for methane production, and that dual purpose facilities running on organic waste are conceivable, producing fertiliser and higher value chemicals from the off-gas.

Contents

1. Methane

Methane is the most abundant organic gas in the Earth's atmosphere (Cicerone 1988). It is formed from one carbon and four hydrogen atoms (CH_4). The heat of combustion (HOC) for methane is 890.71 ± 0.38 kJ/mol. Nowadays, methane as a part of natural gas is used directly in domestic and industrial appliances or is used to generate heat and electricity.

Methane is 25 times more potent as a greenhouse gas (GHG) than CO_2 when measured over a 100 year period. While CO_2 constitutes 61% of man-made GHG, methane makes up 16%. While methane concentrations in the atmosphere have doubled in the last two centuries, this increase has slowed in the past ten years. It is thought that reduction of methane in the atmosphere will have a high short-term impact on climate change mitigation, due to its short atmospheric lifespan (Kerr & Hershman 2009).

1.1. Sources

Natural gas with a typical methane content of over 90% (Demirbas 2010), is mainly produced from fossil sources. It is the fastest growing primary energy source and has significantly lower CO_2 emissions per unit of energy delivered than coal or oil. The UK produced 460TWh and imported another 490TWh worth of natural gas in 2015 with production increasing by 7.6% compared to the previous year. This gas is predominantly used for generation of electricity and in domestic consumption, but production of higher value chemical commodities can be considered. However, since this feedstock is not a waste product, it comes at a considerable price that the end product needs to absorb. In the following text, the possibility of utilising waste gas, emitted to the environment from different sources, is investigated.

According to the statistics of the Department for Business, Energy & Industrial Strategy (DBEIS 2017) methane is the second most important GHG emitted in the UK after CO_2. The agricultural sector contributes the biggest share of methane emissions with 27.7 $MtCO_2e$ (mega tonnes CO_2 equivalents), compared to 16.5 $MtCO_2e$ in the waste management sector and 6.7 $MtCO_2e$ in the energy supply sector. These three sectors contribute over 97.5% (50.9 $MtCO_2e$) of the total methane emissions of the UK (52.2 $MtCO_2e$). Since this methane counts as an emission, it can be regarded a waste product and if technology allows its capture, it could be obtained at a lower cost than established sources.

Even though agricultural emissions are mainly methane, this source is not easily accessible as it originates from enteric fermentation in livestock, in particular cattle. This source would have to be made accessible through novel technologies, such as that proposed in the eccentric 'cow bag packs' project, which collects methane directly from the cow's rumen (INTA 2013). On the other hand, research is ongoing to reduce methane production from cattle with food additives, such as algae (Kinley et al. 2016), lipids, grains, organic acids, tannins and oils (Beauchemin et al. 2009). This could reduce the methane available from this source in the near future. Another agricultural, non-emission source of methane is anaerobic digesters, in which micro-organisms break down organic matter. Anaerobic digestion (AD) uses agricultural waste

material such as food and drink waste, processing residues (*e.g.* bakery or brewery waste), agricultural residues (*e.g.* manure), crops, and sewage sludge. However, when considering AD as a methane source, the feedstock has to be considered carefully, as different biogas yields can be expected depending on the starting material (CROPGEN 2005). Using AD as a source of methane to drive methane fermentation would shift the question of feedstock availability towards organic waste. AD is a mature technology[1] and major investments are underway in the UK[2]. Implementation of a dual-purpose facility of AD and methane fermentation could be explored.

Methane emissions from waste management mainly consist of landfill sites (DBEIS 2017). Municipal solid waste (MSW) at landfill sites generated more than 15.5 $MtCO_2e$ of methane in 2010, with values decreasing since 1990 due to received waste becoming less biodegradable, and better systems for the capture of methane at landfill sites. Currently, landfill sites are able to capture around 75% of methane emissions, which corresponds to approximately 46.5 $MtCO_2e$[3]. This gas is used to produce electricity, heat or combined energy (electricity and heat) via combustion in a gas turbine, creating mechanical power that is used by a generator to produce electricity. The major drawback of this process is contaminated methane with organic matters that damage the mechanical components. In many cases, it's not cost-effective to use it as a fuel for electricity production and is just flared off at the site, causing emission of GHG and environmental damage. The quality of gas also reduces to point where useful energy generation or even simple flaring become impractical. A bioconversion process for methane, therefore, has to be economically favourable compared to this process in order to compete as mentioned above, also should make use of the low quality of gases.

Although the energy supply sector is responsible for over 29% of UK GHG emissions, methane contributes less than 5% to the supply sector emissions. The source of this methane is losses in exploration, production and transport of gas (58%) and losses in coal mining and handling (21%). Apart from production flaring during exploration, these gas sources are virtually inaccessible. Methane emissions from offshore gas flaring and venting (0.3 MtCO2e and 0.7 MtCO2e, respectively) combined only constitute 15% of energy supply methane emissions (Penistone 2017). Considering a 98% destruction removal efficiency (DRM) (Epa 1996; Caulton et al. 2014) of natural gas flares, this would mean that 15 MtCO2e of methane are flared by the UK offshore oil industry. Furthermore, it is estimated that flaring wastes 3.5%[4] of the worldwide gas supply (Tollefson 2016), which amounts to about 143 billion m^3. If such flares are accessible, this would constitute a virtually free source of gas to use as a feedstock for bioconversion to higher value chemicals. However, accessing this source would demand significant investment to build biological conversion facilities on existing oil rigs, coupled with

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[1] http://www.biogas-info.co.uk/

[2] http://www.anaerobicdigestionnet.com/

[3] Calculated from values in (CCC 2012)

[4] Estimates from satellite data as per 2012 (Elvidge et al. 2015)

substantial transport costs to the end user. In addition, accessing this source would pose a significant technological challenge. Further challenges to accessing methane from offshore flaring include transport of the natural gas, which is often inconvenient for oil producers due to long transport ways, and costly liquefaction that can deem it uneconomical. Oil producers are often contractually obliged to use the gas instead of flaring it. This could attach a price to gas giving it a negative value for the producer. It would thus be passed on for free, or paid for to be removed (Lunsford 2000). Such possibilities could exist in UK off-shore gas extraction but have to be clarified on a case by case basis.

Unconventional gas extraction from shale gas, coal bed methane and gasified coal is at an early stage in the UK, and potential levels of production are unknown (POoST 2013). Furthermore, based on current policy only very small amounts of gas are expected to be flared onshore, and only during exploration or emergencies during production.

The ease of availability of the feedstock and the volumes that can be accessed will influence the choice of product. It is obvious that with increasing cost of the feedstock the value of the product increases. As shown above the access to methane in the UK is currently restricted to acquisitions from the general gas grid or coupled to high investments to access alternative sources. The associated costs need to be covered by the envisaged product. The gas volume which is available at any location can further be used to determine the feasibility of production of a commodity. Dictated by simple economies of scale a facility with access to larger gas volumes will be able to economically produce a lower value product, whereas lower methane volumes demand higher value products. Table 1 shows the gas price industrial clients of different purchase volume and for wholesale consumers in 2016 (1 kWh = 0.09 m^3, at 0°C and 1 bar). This shows that the price for the feedstock can vary over 2.2 times depending on the purchasing volume.

The price of the feedstock will directly influence the economics of the product as can be seen in Table 2. By calculating a price for a given product based on the cost of the feedstock only, not considering other manufacturing costs such as depreciation of the

Table 1: Gas price average of 2016 for different sizes of industrial users. Data from (DfBEIS, 2017) and wholesale price calculated as yearly average from day-ahead contract[5].

Use of [1000 kWh/y]	Gas price [pence/kWh]
<1500	2.618
1500-8800	2.245
>8800	1.437
Wholesale	1.183

Table 2: Base price of currently feasible products from methanotroph conversion (compare with Table 4). The asking price of the product is based only on the price of the feedstock with current conversion yields (according to (Strong et al. 2015). Other manufacturing costs were not taken into account but will add substantially to the final cost of the product. 1 Conversion of natural gas to methane by physical means as comparison. 2 Conversion efficiency of thermal conversion of gas to electricity. [3](Alibaba whole sale 2017), [4](García Prieto et al. 2017), [5](Christodoulou 2017), [6](Klein et al. 2017), [7](Bonk et al. 2017).

User acquisition volume of natural gas [1000 kWh/y]	Price of feedstock [£/kg CH4]	Single Cell Protein [£/kg]	PHB [£/kg]	Formate [£/kg]	Acetate [£/kg]	Succinate [£/kg]	Lactate [£/kg]	Electricity[1] [£/kWh]

Current yield [kg/kg CH4]		0.02	0.54	0.09	0.03	0.0008	0.0009	30%[2]
<1500	0.48	24.17	0.90	5.37	16.11	604.19	537.06	785.40
1500-8800	0.41	20.72	0.77	4.61	13.82	518.11	460.54	673.50
>8800	0.27	13.27	0.49	2.95	8.84	331.63	294.79	431.10
Wholesale	0.22	10.92	0.40	2.43	7.28	273.02	242.68	354.90
Current market price [£/kg]		0.30[3]	4.63[4]	0.38[5]	0.48[5]	6.66[6]	1.33[7]	53.43[4]

facility and salaries, it becomes clear that with current yields only polyhydroxybutyrate (PHB) is a viable product with a mark-up of 500- 1100%. Other naturally obtained products from methanotrophs still need notable increase in production yields to become economical or the feedstock price needs to be decreased as mentioned above.

This analysis of gas sources in the UK market shows that natural gas cannot be accessed directly as a virtually "free" source from flares without large investment in off-shore technology. However, in other countries with less stringent policies on onshore flaring this may not be true. It can thus be concluded that in the UK methane comes with an attached price, which will be the natural gas market price that will, in turn, directly influence the price of products from biological conversion.

Table 3: Non-conventional gas resources in the UK with estimated availability in Mt. [1]calculated from emissions with 21 Mt CO2e/Mt CH4 according to (Baggott et al. 2006) [2]calculated form biogas production (NNFCC 2016) with an estimated 50% methane portion.

Methane source	Availability per year in the UK [1] [Mt]	Accessibility
Off-shore flaring	0.7	accessible with high investment costs
Off-shore venting	0.03	accessible with high investment costs
Landfill currently captured	2.2	accessible through the grid
Landfill currently lost	0.6	accessible on site
Enteric fermentation	1.3	not currently accessible
Anaerobic digestion[2]	52	Currently used as heat, heat and electricity, biogas or biomethane

1.2. Current Chemical Conversion of Methane

Currently there are two industrially viable ways to convert methane to higher hydrocarbons, olefins and petrol: the methanol-to-gasoline (MTG) route and Fischer-Tropsch (FT) synthesis. For both routes methane is first converted into synthesis gas (syngas), a mixture of carbon monoxide (CO) and hydrogen (H_2), by steam reforming. This process proceeds at high temperatures, which is coupled to high energy costs. The following conversion by FT synthesis is again dependent on high temperatures, high pressure and catalysts and the product is highly variable in hydrocarbon chain length. Methane is used in a multistep process via the MTG route, converting it via steam reforming to syngas and further via a high temperature, high pressure process to methanol. The methanol can then be used with a zeolite catalyst to produce

petrol in a process developed by the Mobil Oil Corporation (now ExxonMobil) (Marsh et al. 1988). To improve the gas to liquid (GTL) route via MTG, there is ongoing research to develop one step methane to methanol processes and to improve the selectivity to make such an operation economical on an industrial scale (Silva 2016). Furthermore, small scale GTL technology has been developed by Compact GTL Limited, which enables smaller facilities, and therefore non-capital intensive FT GTL. Compact GTL technology is largely similar to conventional FT GTL technology, but does not produce the high value distillate products due to the lack of refinement capabilities to distil crude oil (Wood et al. 2012).

Improving the methane to methanol process could further increase the chance of a proposed methanol economy (Olah et al. 2009). Methanol can be used as a drop in fuel at 15% in common petrol without any changes to current engines necessary, or in so called flex-fuel vehicles optimised for the more corrosive fuels methanol and ethanol.
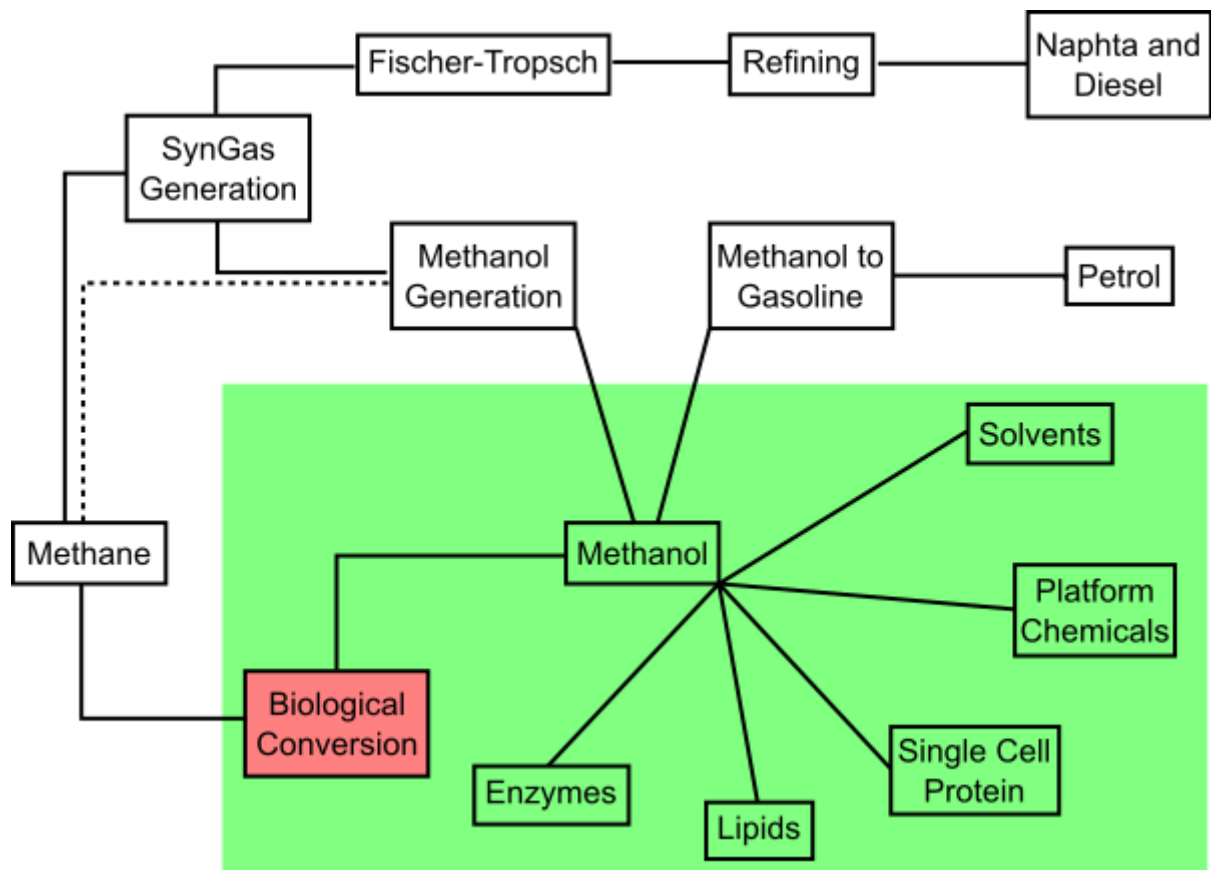

Figure 1: Currently available chemical technologies for methane conversion (top half) and possibilities for methane conversion via biological routes (shaded in green).

2. Policy

To secure investment in a new technology, investors' confidence in the process and the legislation has to be ensured. Policymakers must ascertain stable legislation for a sector while remaining flexible enough to integrate innovation. This can be reached by offering incentives to promote preferred technologies alongside penalties to discourage unfavourable ones.

While for production of value added chemicals there is currently no policy to support innovation from sustainable or waste resources like methane, the GTL route could potentially be covered by the Renewable Transport Fuels Obligation[6] (RTFO). The RTFO is the UK government's main policy to reduce GHG emissions from road and non-road mobile machinery transportation by encouraging the supply of renewable fuels. Under the RTFO, fuel suppliers are obliged to provide a certain percentage of renewable fuel, or to 'buy-out' of this obligation by paying a fee on every litre of non-renewable fuel supplied. Suppliers can meet their obligation towards the RTFO by redeeming Renewable Transport Fuel Certificates (RTFCs), which are valued against the type of substrate used in fuel production. Policies laid out by the EU Renewable Energy Directive[7] (RED) concerning transportation fuels are covered within the RTFO. Currently, only hydrocarbons derived from biomass are eligible for RTFCs (DfT 2017b). Liquid fuels include methanol, ethanol and butanol, but also fatty-acid-methyl-esters (FAME) and other biodiesels. Gaseous commodities are methane, propane and butane derived from biomass only. Feedstocks qualified for renewable fuel production are categorised in two groups depending on their value as a product (single count RTFC) or as wastes (double count RTFC) (DfT 2017a). No gaseous feedstocks are listed or recognised so far, a fact which was observed by a previous report received by C1Net (E4Tech 2016) regarding the use of waste gas for production of biocommodities.

The UK government foresees increased usage of natural gas due to the anticipated phase-out of coal for the production of electricity by 2025. This is likely to reduce availability for secondary gas usage such as biochemical conversion. However, a recent report (McGlade et al. 2016) suggests that gas use following the current policy, especially without support for new carbon capture and storage (CCS) technology, is at odds with the long-term carbon targets being pursued. The report suggests that to reach the environmental goals, gas can only be used as a 'bridge' from 2015- 2020 until low- or zero-carbon energy sources are developed. It remains to be seen if this report has an influence on current policy and if changes in prospective gas usage can be anticipated.

3. Available Biotechnological Conversion

3.1. Methanotrophs

Methanotrophic bacteria catalyse the conversion of methane to methanol at ambient pressures and temperatures using methane monooxygenases (MMO). There are two iso-enzymes that catalyse this reaction; the soluble MMO (sMMO) and the particulate MMO (pMMO). The sMMO is a cytoplasmic enzyme complex consisting of a reductase, hydroxylase and a regulatory protein (Iguchi et al. 2010). The pMMO is found in nearly all methanotrophs and is an integral membrane protein bound to the intracytoplasmic membrane (ICM)

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[6] https://www.gov.uk/government/collections/renewable-transport-fuels-obligation-rtfo-orders

[7] https://ec.europa.eu/energy/en/topics/renewable-energy/renewable-energy-directive

(Lieberman & Rosenzweig 2005). This ICM structure is found in gammaproteobacteria or type I methanotrophs as bundles of vesicular stacks within the cells and in alphaproteobacteria or Type II methanotrophs as parallel membranes running the periphery of the cell membrane (Semrau et al. 2010). Type I and type II organisms also differ in carbon assimilatory pathways, of which type I use the ribulose monophosphate (RuMP) cycle and type II use the serine pathway. Further to this, carbon storage under nutrient limiting conditions varies in type I and type II. Type I organisms typically accumulate glycogen (Linton & Cripps 1978) whereas type II accumulate poly-3-hydroxybutyrate (Pieja et al. 2011) as intracellular carbon storage.

Genetics and molecular biology in methanotrophs has come a long way. DNA transfer is successfully implemented (Baani & Liesack 2008; Crombie & Murrell 2011) and insertions and deletions are easily achieved by marker exchange through standard homologous recombination. Random transposon mutant libraries are produced using transposon systems. Furthermore, replicating vectors which have broad host range, such as IncP, IncQ and pBBR, have been reported. A wiki created by academic researchers as well as Calysta contains a comprehensive list of tools used in the study and manipulation of methanotrophs[8].

These tools can be used to alter the native metabolism of methanotrophs for production of chemicals of interest. Pyruvate and acetyl-CoA are produced in high flux in Type 1 and 2 methanotrophs, respectively, which can be used as starting points for the biosynthesis of other products. By deletion or overexpression of native genes, the flux towards these central products can be increased. Furthermore, heterologous genes not naturally present in the organisms can be expressed to produce novel products. *N*-butanol is an important biofuel that attracted much attention recently due to its superior qualities when compared to ethanol (Swana et al. 2011). Methanotrophs are not naturally able to produce this solvent. However, genes coding for enzymes required for *n*-butanol synthesis have been expressed in *Methylobacterium extorquens* AM1, which has similar enzymes to methanotrophs but does not possess a MMO (Hu & Lidstrom 2014). Iso-butanol production was recently demonstrated in metabolically engineered *M. capsulatus* using a 5-step reaction (Coleman et al. 2016).

3.2. Possible Products from Methanotrophic Fermentation

Natural products from microbial conversion of methane include single-cell protein, biopolymers, vitamins, methanol, ectoine, formaldehyde, organic acids, enzymes and lipids for biodiesel or dietary supplements. Furthermore, modern synthetic biology methods could be used to engineer methanotrophs to produce novel metabolites. Products attracting most interest are fuels and *n*-butanol in particular. Although conversion is theoretically possible (Haynes & Gonzalez 2014), it is technically challenging and market realisation is not expected soon. More effective methane activation pathways have to be developed and the pathways to the commodity of interest introduced to the organism. Not only are these tasks challenging in their own right, but they are amplified by the demands of working with slow growing organisms that

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[8] http://methanotroph.org/wiki/genetics/

are difficult to access genetically. These difficulties are exemplified in the reorientation from GTL technology to SCP by US company Calysta (compare Section 4.2). Furthermore, many projects in ARPA-E's REMOTE (Reducing Emissions using Methanotrophic Organisms for Transportation Energy) program, which started in 2013 and which aimed to "seek to enable highly efficient biological conversion of methane to liquid fuels for small-scale deployment", have to date failed to deliver on their high expectations. However, some of these projects are still ongoing, and most finished only recently so outcome reports are not yet available.

Another compound of interest from methanotrophic fermentation is methanol due to its central role in the methanotrophic metabolism. Methanol is formed from methane in one step by MMO. This step is low in energy and carbon efficiency which leads to the need of engineering a more efficient methane activation (Conrado et al. 2014). The highest published methanol titre to date is 1.34 g/l from a natural isolate of *Methylomonas* (Hur et al. 2017). Several strategies to improve the natural production have been proposed. These must be proven before a methanol economy based on methanotrophs, as proposed by George Olah (Olah et al. 2009), can be envisaged.

Single-cell protein (SCP) is the furthest developed product from methanotrophic conversion to date. The protein content of bacterial species including methanotrophs ranges from 50- 65 % which is high compared to other organisms with 30- 60 % protein content. Appropriate process development can optimise for high protein yields. SCP has been used to feed agri- and aquacultural species for human consumption or production of value added products. Species include pigs, chicken, mink, fox, Atlantic salmon, rainbow trout and Atlantic halibut (Øverland et al. 2010). The biggest challenge in bacterial SCP production is the high nucleic acid content of micro-organisms (8- 12 %) which can cause kidney and bladder stones when consumed in large volumes. SCP consumption without purification of the protein from nucleic acid is therefore only recommended for short lived species. As a feedstock for longer lived species - including the use for human consumption - downstream purification by heat-treatment of hydrolysis is essential. (Strong et al. 2015; P. J. Strong et al. 2016)

Methanotrophs accumulate internal storage granules when other nutrients then carbon is limiting in the culture. These granules consist of polyhydroxy-alkanoates (PHA) such as poly(3-hydroxybutyrate) (PHB). PHB is a potential substitute for plastic with beneficial properties such as biodegradability, biocompatibility and thermoplasticity (P. J. Strong et al. 2016). The structure of these polymers can be enhanced by co-substrates such as hydroxyl-acid precursors or citrate or proprionate to produce poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) which is tougher and more elastic than PHB (P. Strong et al. 2016).

Even though a high PHB content of up to 70 % was reached in cultures of *Methyolcystis pavus* to date the high production cost of methanotrophic PHA compared to petrochemical plastic production limits a commercial application. Additional yield or increase of molecular weight could be achieved by metabolic engineering. However, in Alphaproteobacteria the biosynthesis is linked to core metabolic pathways which exacerbates genetic engineering (P. J. Strong et al. 2016). Initial commercial success could lead from higher value polymers for use

in biomedical applications can be envisaged due to the biodegradability and biocompatibility of PHAs. (Strong et al. 2015)

Lactic acid, as a precursor for poly-lactic acid (PLA) for use on bioplastics is a can potentially be produced from genetically engineered methanotrophic species. Henard et al. produced lactate up to the inhibitory concentration (1.3g/l) by providing a heterologous lactate dehydrogenase (*ldh*). They also showed that lactic acid can be concurrently produced with fatty acids without negative effect on fatty acid yield for biodiesel conversion (fatty acid methyl ester, FAME) (Henard et al. 2016). The relatively low lactate yield compared to yields reached in other species which can be up to 100 times higher might be increased by additional strain engineering and bioprocess optimisation.

Methanotrophs produce internal lipid membranes. The intracellular lipid content is found to be up to 20 % with a content of 35 % being deemed economical for methane to biodiesel conversion (Conrado et al. 2014). A higher intracellular lipid content might be reached with bioprocess optimisation. The lipids can be extracted and used to produce biodiesel such as FAME or fatty acid ethyl esters (FAEE). Alternatively higher value products could be generated fit for human consumption as health supplements (Müller et al. 2004; MÜLLER et al. 2005; P. J. Strong et al. 2016). However, downstream processing and purification will increase considerably due to higher standards for human nutritional supplements.

Haynes and Gonzalez analysed the possibility for liquid fuel production from methane. The calculation was based on the conversion of methane to *n*-butanol as a higher value alcohol which can be used as a drop-in fuel in the current petrol infrastructure. However, calculations would be similar for other solvents such as ethanol, acetone or isopropanol. The paper concluded that conversion can be economically feasible if several technological hurdles can be overcome. Most importantly the activation of methane to methanol and formaldehyde which needs to be energetically balanced. This can be achieved with alternative methane monooxygenases, via methane addition to fumarate, anaerobic hydroxylation of methane with water or anaerobic activation via methylation of benzene. The application of a comparably efficient activation of methane would lead to an 'excess' of reducing equivalents compared to traditional methane activation. The reducing equivalents can then be incorporated in downstream products such as butanol. (Haynes & Gonzalez 2014)

There are many hurdles to methanotrophic GTL fuel production which need to be overcome by genetic engineering before an economic production can be envisaged. Incorporation of heterologous enzymes for methane activation in either methanotrophs or the establishment of methane uptake pathways in non-methanotrophic organisms is far from trivial. Furthermore, incorporation of pathways with oxygen-sensitive enzymes into aerobic methanotrophs is technically very challenging. However, as shown (Haynes & Gonzalez 2014) these challenges are theoretically manageable.

Table 4: Overview of possible products from biological methane fermentation. *PHA: Polyhydroxyalkanoate; PHB: polyhydroxybutyrate. Sources: (Strong et al. 2015; EC 2015)

Product	Relative Value	Global Annual Demand	Notes
Naturally produced by known methanotrophs			
Single Cell Protein	low	> 25 kt	Close to market. Sample production in Teeside UK
Internal storage proteins (PHAs*)	low	17 kt	Promising bioplastic precursor. PHB* concentrations of up to 70% w/w reached [Asenjo 1986]. Price decreasing due to increased production from sugars.
Lipids; biodiesel	low	1.4×10^9 L	Proof of concept stage [Hill 2017]
Lipids; dietary supplement	high	kg- t	
Ectoine	very high	1- 10 t	
Methanol	low	90×10^6 L	Highly cytotoxic. Need of genetic engineering to improve yields
Formaldehyde	low	35×10^9 L	Need of genetic engineering to improve yields
Acetic acid	low	13,000 kt	Need of genetic engineering to improve yields
Surface layers	medium- high	unknown	
Extracellular polysaccharides (EPS)	low- medium	> 10 t	
Growth media and Vitamin B12	medium- high	< 1 t	
Enzymes	low- high	kg- t	
Genetic modification needed			
Ethanol	low	77,000 kt	
n-butanol	low	3,000 kt	Production energetically possible [Haynes & Gonzalez 2014] but technologically demanding
Carotinoids	low- medium	>1 tonne	
Farnesene	low	12 kt	Lab-scale production (Intrexon) possible. Promising chemical building block.
Biotransformation of propene to prolpylene	medium- high	9- 10 t	
Lactic acid	low	472 kt	Toxic at low levels. Demand increasing for the chemical building block lactic acid but especially polylactic acid (PLA) as bioplastic precursor. Commercialisation from methane foreseen in the next decade (NatureWorks)
Succinic acid	low	76 kt	Promising platform chemical. Bio-succinate currently competitively produced from sugars.

4. Company Case Studies

4.1. UniBio

UniBio was founded in 2001 and has headquarters in London and Denmark. The company produces a highly concentrated protein product, UniProtein, which can be used as a direct supplement in feed for animals. UniProtein is a single-cell protein from *Methylococcus capsulatus.* The organism is grown in a patented U-loop fermenter (Larsen 2000) which mixes the gaseous feedstock methane into the fermentation broth. This technology allows the harvest of 4kg of product per m^3 of reactor per hour of fermentation as compared to 1.8kg/m^3/h in conventional *M. capsulatus* fermentations. In the fermenter the organism grows continuously on methane from natural gas, technically pure oxygen, the nitrogen source ammonia and several essential minerals in water. In the downstream process the bacterial protein is separated from the broth by centrifugation and finally spray dried. The final product is a protein-rich biomass consisting of 72.9% protein and can be used directly as animal feed supplement. UniProtein has been tested as feed stock for salmon, chicken, calves and pigs as a close substitute to fish-meal.

The company's strategy is to partly produce protein itself and partly licensing the technology to international investors for production from surplus gas from oil production. Six years after establishment of the company and after development of the U-loop reactor the first pilot plant was built in Trinidad and Tobago together with the University of Trinidad and Tobago. The first fermentation at the pilot plant was completed in 2013, and was reported a success. In October 2016, UniBio opened their first commercial scale plant for which production is expected to commence in the second half of 2017, with the full production being sold to Vestjyllands Andel, a Danish animal feed manufacturer.

4.2. Calysta

The Californian company Calysta focuses on production of fish-feed (trade name Feedkind) from methane metabolising *Methylococcus capsulatus* (Bath). Originally the company aimed to develop biological gas-to-liquid and gas-to-chemical routes. Both of these goals became redundant with the acquisition of the Norwegian company BioProtein A/S and a concomitant reorientation towards gas-to-feed in 2014. Feedkind was shown to have superior nutritional value to conventional fish-feed (fish-meal) and does not provoke gut inflammation like soy-meal. Furthermore, the amino acid composition can be adapted to the needs of the customer by, for example, increasing histidine concentration.

In September 2016 Calysta opened a market introduction facility for production of sample quantities of Feedkind in Teesside, North East England. Future plans include the opening of a world-scale plant in Tennesee (US) in conjunction with the animal feed producer Cargill.

Research efforts of Calysta with world-wide partners include: sustainable production of Omega 3 fatty acids, using their gas-to-chemicals knowledge; partnering with the University

of Nottingham and Nottingham-based company, CHAIN Biotech; and the production of lactic acid as a chemical building block in collaboration with Natureworks (see below).

4.3. Intrexon

Founded in 1998, Intrexon is a company specialised in synthetic biology applications, with a wide portfolio in Health, Food, Energy, Environment and Consumer sectors. Intrexon Energy's aim is to develop a gas-to-liquid conversion using methanotrophs. The initial focus is on the production of iso-butanol as a "drop-in" fuel, which can be blended with standard petrol and used with the existing petroleum infrastructure. Intrexon has reported success in producing iso-butanol and farnesene in methanotrophs (Intrexon 2016) but titres have not been disclosed. Commercialisation of this venture is planned for 2018. Further plans include the production of the chemical building block 1,4-butanediol with the developed methane bioconversion platform.

Doubts were raised about the economic feasibility of the gas-to-liquid route taken by Intrexon. The company's plan to take the platform from bench to plant in two years was questioned (Chatsko 2016). However other sources believe that the expertise in the company will be sufficient to deliver on their promises (Lane 2016).

4.4. Mango Materials

Mango Materials, which was founded in 2010 as a spin-out licensee from Stanford University (Slavin 2015), concentrates on the production of poly-hydroxybutryate (PHB) from methane (Mango Materials n.d.). PHB is a polyhydroxyalkanoate (PHA), which can be used like polypropylene and polyethylene as a common plastic. The advantage of PHAs is their biodegradability in natural environments. In countries where synthetic plastic microbeads have been banned (due to their negative impact on the environment, especially the sea), microbeads from PHB are still permitted (Department for Environment, Food & Rural Affairs 2016). Biodegradable microbeads are the first product aimed at by Mango Materials.

Mango Materials strategy is a decentralised set-up - their reactors are located next to sources of methane. Initially, production was planned next to waste-water treatment plants that are already fitted with systems to re-route the methane produced by the treatment process. Further developments will include landfills, agricultural facilities and other industries where methane is produced as a waste product of the operation.

The company is currently refining their downstream product recovery technology with a grant from the Office of Energy Efficiency and Renewable Energy (US) (Office of Energy Efficiency & Renewable Energy 2017).

4.5. NatureWorks

NatureWorks' established product, Ingeo, consists of poly-lactic acid (PLA), which has been shown to be biodegradable in composting facilities and does not degrade in landfill. The

company currently uses first generation plant (sugar) feedstocks for microbial conversion to lactic acid and further chemically into PLA. In 2013 NatureWorks entered a joint venture with Calysta (see above) to develop a methane to lactic acid research project. Having proven that the fermentation process was feasible, this project was awarded $2.5 million from the United States Department of Energy. This allowed NatureWorks to open a gas-fermentation laboratory at their headquarters. Further plans include the opening of a 2323 m^2 pilot plant. Commercialisation is foreseen in the next decade (NatureWorks 2016).

4.1. StringBio

StringBio is a company based in Bangalore, India which was founded in 2013. StringBio concentrates on the development of solutions to produce chemical building blocks from methane with a focus on waste management. Their current targets are lactic acid for PLA production (see Section 4.5), succinic acid for bioplastics and solvents, as well as the use of biomass for animal feed. With several patents protecting their expertise, the company has won several start-up grants.

4.2. ARPA-E REMOTE Program

The US Government's Advanced Research Projects Agency-Energy (ARPA-E) started the REMOTE program (an acronym for Reducing Emissions using Methanotrophic Organisms for Transportation Energy) in 2013. REMOTE's aim is to "seek to enable highly efficient biological conversion of methane to liquid fuels for small-scale deployment". The program's focus is on the improvement of biological routes from methane to transportation fuel, but also examining high-productivity methane conversion processes and bioreactor technologies[9]. The wider aim of the project is to develop technologies that can efficiently ($> 60\%$) convert methane into energy dense (higher or equal to n-butanol) liquid fuels in a process that can be deployed at different scales (CapEx $< \$50,000$/bpd). This can be achieved by more efficient methods of methane activation. Further, by using the activated methane in engineered metabolic pathways to higher value fuels. And finally by optimising bioprocess technology by, for example, increasing methane solubility. (ARPA-E 2013)

The REMOTE program has granted $39 million to 19 projects that address these issues, and has funded academic as well as industrial partners. Projects funded under REMOTE have finished and final reports can be expected shortly.

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[9] http://arpa-e.energy.gov/?q=arpa-e-programs/remote

5. Recommendations

Methane conversion to higher value products by biotechnological means is an interesting, and in parts, promising technology. In theory it offers a means of converting a volatile compound into liquid or solid commodities, which are easier to store and often have higher value than the feedstock. However, to make the technology economically viable, feedstock costs and products value must be considered. Proposed sources of lower cost methane include AD biogas and landfill gases, of which dual-purpose facilities could be the key to generate the bio methane for methanotrophic fermentation to higher value chemicals.

Whichever feedstock will be favoured by industrial users, policy makers will have to ensure incentives are in place. At the time of writing, liquid fuel from gas is not eligible for credits under the RTFO, despite it being a potential waste product. If biomethane from AD is used for methanotrophic conversion, which in itself is eligible for RTFCs, it has to be ascertained that this will hold true for products thereof.

The development of biological methane fermentation for most products is still at an early stage. In view of potential future applications in onshore unconventional gas extraction, there is a mutual interest for policy makers and funding bodies to support further research into these promising organisms.

Abbreviations

MtCO$_2$e	Mega tonnes CO$_2$ equivalents
GHG	Green house gas
MSW	Municipal solid waste
DRM	destruction removal efficiency
HOC	heat of combustion
MTG	methanol-to-gasoline
FT	Fischer-Tropsch
RTFO	Renewable Transport Fuels Obligation
RED	Renewable Energy Directive
RTFC	Renewable Transport Fuel Certificate
MMO	methane monooxygenase
sMMO	soluble methane monooxygenase
pMMO	particulate methane monooxygenase
ICM	intracytoplasmic membrane
PHA	polyhydroxyalkanoate
PHB	polyhydroxybutyrate
SCP	single-cell protein

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