

## UTILISATION OF METHANOTROPHS FOR CONVERSION OF METHANE TO BIOPRODUCTS

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### ABSTRACT

By using methane as the sole carbon source, methanotrophic bacteria can produce bioplastics, biofuels, feed additives, ectoines, and a variety of other high-value compounds. A lot of strains, including the co-cultured ones along with engineered strains have shown potential benefits to the production of bioproducts. It will be a breakthrough in research if these processes can be commercialized. Despite certain limitations to known production strategies that make commercialization of methane-based products difficult, a lot of attention is currently focused on promising advances.

**Keywords:** Methanotrophs, Bioproducts, Methane, Microbial protein

### 1. INTRODUCTION

Harmful gases in the atmosphere have always been an area of concern. In addition to reducing harmful gas emissions that contribute to climate change, using greenhouse gasses (GHGs) as a carbon source for the manufacturing of chemicals, fuels, and other components increases process economy by displacing expensive sugar-based carbon sources and addresses the problem of food security. One appealing alternative as a feedstock for biotechnological processes is methane, one of the most prevalent GHGs with a global warming potential substantially larger than carbon dioxide, Abbasi et al., (2012). By utilizing methanotrophic bacteria as cell factories, methane can be directly utilized. A class of common bacteria called methanotrophs have the unusual ability to oxidize methane and use it as their only source of carbon and energy, Hanson and Hanson, (1996). For instance, Strong et al.(2015) discussed the possibility of using methanotrophic bacteria to produce methanol as well as other soluble products like formaldehyde or organic acids, while Kalyuzhnaya et al. (2015) propose genetic modification of methanotroph strains to remove the methanol dehydrogenase protein and increase methanol yields. Other workers have shown the feasibility of using methane consuming methanotrophs to produce lipids that could be harvested for biofuel production.

At the initial stage of their metabolic process, methanotrophs—obligate methane-using bacteria—produce methanol. The majority of methanotrophs are mesophilic, growing best in aerobic environments at atmospheric pressure and temperatures between 25 and 30 °C (Gilman A et al.) Metabolism of methane begins when it is oxidized to methanol, a process catalyzed by methane monooxygenase (MMO). The reducing equivalents of this energy-demanding step are generated through the metabolic pathway after the oxidation of methanol to formaldehyde, and some of the reducing equivalents are transported back to the MMO, Hanson et al. (1996). In addition to the basic cultivation parameters affecting the microbial processes, such as temperature and pH, different environmental requirements for methane bioproduct conversion determine some other important factors to be optimized in the process. In this review, recent advances and trends in methanotrophic bioproducts were compiled and overviewed.

#### Methane Assimilation

Methanotrophic bacteria are unique in that they can use methane as their sole source of carbon and energy. The first reports on methanotrophs began to appear at the beginning of the 20th century, but the real breakthrough in the study of this field occurred in 1970, when Whittenbury et al. (1970) isolated more than 100 Gram-negative methane-oxidizing bacteria (MOB). Methanotrophy was originally announced in 1906 as an oxygen-dependent process, and for almost a century, aerobic methanotrophs was considered the only biological way to oxidize methane, and that all methanotrophs belonged to the Proteobacteria family. About a third of this is used for the production of formaldehyde, followed by the synthesis of olefins for the plastics industry (Da Silva, 2016). Biogenic methane produced by anaerobic digestion can be a source of methanol production by methanotrophs. These MOB can be isolated from many habitats, typically from ecosystems where methane is released, such as oil fields or coalfields, landfills, sewage sludge, freshwater and marine sediments, wetlands, aquifers, wastewater treatment and biogas plants, as well as extremes. sources temperature, pH or salinity (Knief, 2015; Ross and Rosenzweig, 2017; Strong et al., 2015).

Methanotrophs are generally classified into two different types, such as type I and type II, based on their different metabolic mechanisms for the assimilation of C1 compounds. Type I methanotrophs use the ribulose monophosphate (RuMP) pathway and type II methanotrophs use the serine pathway. The preferred concentration of methane for growth differs between type I and type II methanotrophs. Different enzymes used for methane oxidation lead to different

assimilation pathways of one-carbon substrates into important metabolic pathways. In methanotrophs, methane substrate and glycolysis of cell material are linked through methane assimilation processes.

Methane metabolism in methanotrophs cells begins from methane oxidation into methanol in the reaction catalyzed by methane monooxygenase (MMO) whose presence and activity is a defining characteristic of methanotrophic bacteria, Hanson and Hanson, (1996). The use of MMO enzyme complex enables activation of a strong C–H bond in methane and its oxidation into methanol using oxygen at ambient temperature and pressure, Ross and Rosenzweig, (2017). Methanol is converted to methylene in the first step, and after that it is oxidized to formaldehyde, which can then be used as a substrate for cellular carbon fixation pathways or further oxidized. Formaldehyde is first converted into formate by formaldehyde dehydrogenase (FADH) in the case of the oxidation into CO<sub>2</sub> before formate dehydrogenase (FDH) oxidized to CO<sub>2</sub> (Hanson and Hanson, 1996). Formaldehyde has 3 fate: 1) further oxidation into CO<sub>2</sub>; 2) ribulose monophosphate (RuMP) cycle assimilation; or 3) assimilation via serine cycle. Formaldehyde is first converted into formate by formaldehyde dehydrogenase (FADH) in the case of the oxidation into CO<sub>2</sub> before formate dehydrogenase (FDH) oxidizes formate to CO<sub>2</sub> (Hanson and Hanson, 1996). Formaldehyde is used in the RuMP pathway to create fructose-6-phosphate, which is then converted into ribulose-5-phosphate by means of specific enzymes.

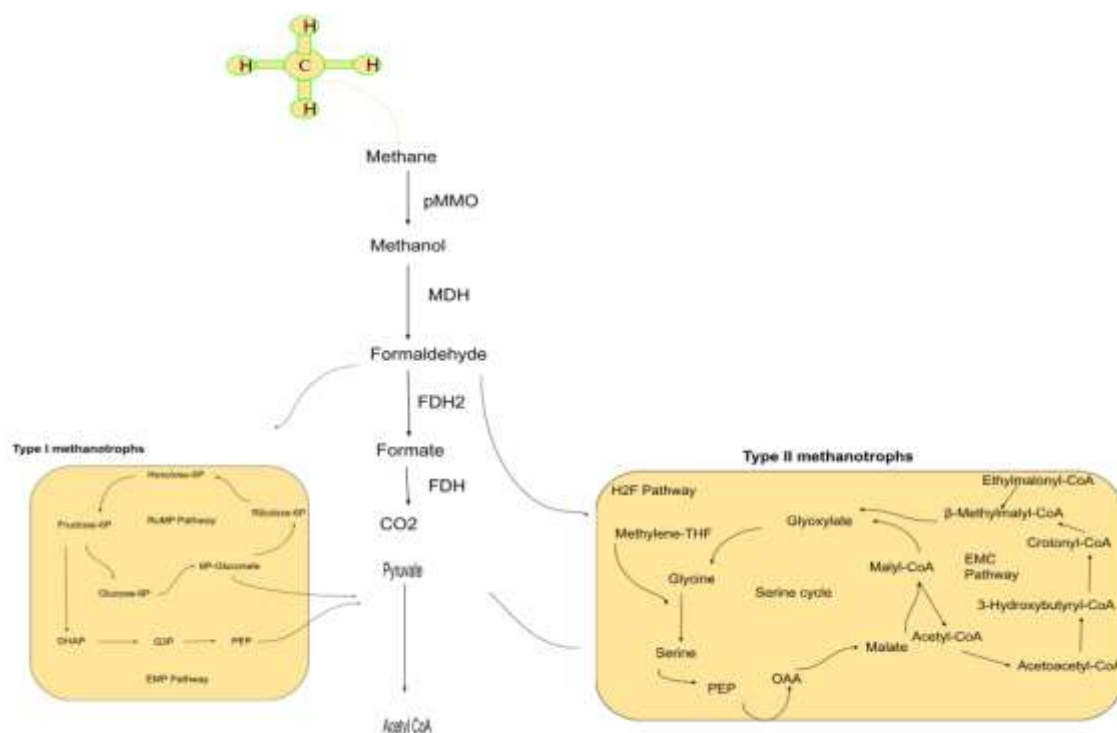


Fig. 1. Methane Assimilation pathways in Methanotrophs.

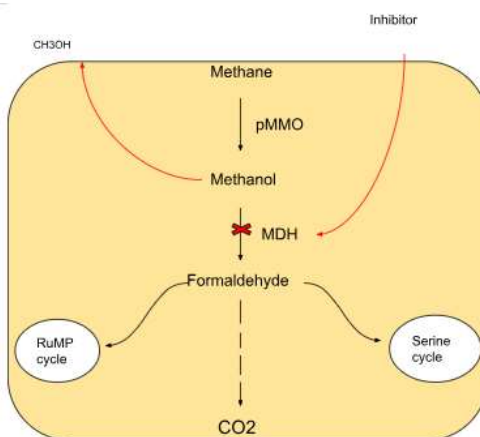
**Abbreviations-** DHAP–dihydroxyacetone phosphate, EMC–methylmalonyl-coenzyme A, G3P–glyceraldehyde 3-phosphate, methylene-THF–methylene tetrahydrofolate, OAA–oxaloacetate, PEP–phosphoenolpyruvate, RuMP – ribulose monophosphate.

### Bioproducts

Methanotrophs are obligate methane-utilizing bacteria that have been commercially exploited to produce a range of products: bioplastics, biofuels, feed additives, ectoine and a variety of other high-value chemical compounds. Methanotrophs are versatile bacteria that use methane as a source of energy and can be genetically modified to produce a wide range of products. For instance, Strong et al. discussed the possibility of using methanotrophic bacteria to produce methanol as well as other soluble products like formaldehyde or organic acids, while Kalyuzhnaya et al. propose genetically modifying methanotroph strains to remove the methanol dehydrogenase protein and increase methanol yields. Some co-workers also suggested that methanotrophs could be used to produce lipids to make biofuels, and Haynes et.al., (2014) suggests using genetically modified organisms to bioactivate methane to make butanol. Using the halophilic methanotroph, methane can be used as a cheap and plentiful resource for the production of ectoine. Bacterial biopolymers derived from methane can be used as environmentally friendly substitutes for chemical polymers in the plastics industry or hydrocarbons in the fuel industry. They can even help meet the world's protein demand.

### A. METHANOL

Over the years, two major ways of methanol production have been implemented: a) the immobilization of cells for process improvement b) the use of biogas as a carbon source. Methanol has been a highly exploited product due to its usage as a precursor for biofuels and other chemicals, organic acids, formaldehyde, olefins, Bjorck et al., (2018). At the initial stage of methane oxidation, methanotrophs produce methanol. Also the extra accumulation of alcohol can be achieved by inhibition of the enzyme MDH that converts methanol to formaldehyde



**Fig. 2.** Methanol Production in Methanotrophic bacteria

Methanotrophs are a special class of microbes that use CH<sub>4</sub> as a source of carbon and energy and may even convert it to methanol (Fei et al., 2014; Haque et al., 2019; Patel et al., 2020; Su et al., 2019). Addition of MDH inhibitors, such as ammonium chloride, ethylenediaminetetraacetate (EDTA), magnesium chloride (MgCl<sub>2</sub>), phosphate buffer and sodium chloride, and formate are necessary to reduce the subsequent methanol oxidation by MDH

**Table 1:** Methanol production by methanotrophs

Strain	CH <sub>4</sub>	Process	Reference
<i>Methylosinus trichosporium</i> OB3b	30%	EDTA (0.5 mM), Na-formate (40 mM)	(Hwang et al., 2015)
<i>Methylosinus sporium</i>	20% CH <sub>4</sub> (raw biogas)	Copper (5 μM), Iron (10 μM) MgCl <sub>2</sub> (20 mM) Copper (5 μM), Iron (10 μM) 62.3% CH <sub>4</sub> , 36.7% CO <sub>2</sub> , 0.13% H <sub>2</sub> S	(Patel et al., 2016b)
<i>Methylocystis bryophila</i>	50% CH <sub>4</sub>	Supplementation with H <sub>2</sub> (10%) Na-formate (100 mM), MgCl <sub>2</sub> (50 mM)	(Patel et al., 2016c)
<i>Methylocella tundrae</i>	50% CH <sub>4</sub>	Copper (5 μM), Iron (10 μM) Na-formate (50 mM), MgCl <sub>2</sub> (50 mM)	(Mardina et al., 2016)
Co-culture of <i>Methylosinus sporium</i> <i>Methylocella tundrae</i>	30% CH <sub>4</sub> (simulated biogas CH <sub>4</sub> :CO <sub>2</sub> )	Copper (5 μM), Iron (10 μM) Encapsulated in silica gel Formate (100 mM), MgCl <sub>2</sub> (50 mM)	(Patel et al., 2018b)
Immobilized co-culture of <i>Methylocystis bryophila</i> and <i>Methyloferula stellata</i>	30% CH <sub>4</sub>	Copper (5 μM), Iron (10 μM) Immobilised with polyvinyl alcohol (10%) in 3:2 ratio of <i>M. bryophila</i> and <i>M. stellata</i> Formate (100 mM), MgCl <sub>2</sub> (20 mM)	(Patel et al., 2020b)

<i>M. tundrae</i> immobilized on banana leaves	30% CH <sub>4</sub> (raw biogas)	Formate (100 mM), MgCl <sub>2</sub> (20 mM)	(Patel et al., 2021)
Encapsulated <i>M. album</i> Encapsulated <i>Methyloferula stellate</i>	30% CH <sub>4</sub> (simulated biogas; CH <sub>4</sub> :CO <sub>2</sub> at 2:1)	Copper (5 μM), Iron (10 μM) Encapsulated in polyvinyl alcohol (10%) Formate (100 mM), MgCl <sub>2</sub> (50 mM)	(Patel et al., 2020c)
Strain AS1 isolated from active anaerobic sludge	50% CH <sub>4</sub>	Copper (5 μM), Iron (10 μM) Sequencing mode in external- loop airlift bioreactor.	(Ghaz-Jahanian et al., 2018)

Table 1: Data was mostly conducted by researchers from Konkuk University, Re- public of Korea that showed advantages of using biogas over pure methane (Patel et al., 2020a, 2020c; Patel et al., 2018b; Patel et al., 2016b). CO<sub>2</sub> may also have inhibitory effects on MDH (Patel et al., 2018a; Xin et al., 2004).

## B. BIOPOLYMERS

Biopolymers are very promising materials for highly sensitive and selective gas and vapour sensors because of their abundance, biocompatibility, and special properties. Due to their biodegradability, biocompatibility, and environmentally friendly manufacturing methods, biopolymers are significant replacements for petroleum-based plastics. PHAs are biodegradable polyesters made from hydroxyalkanoates (HA), which are compounds that are stored as intracellular carbon and energy in microorganisms. Methane-derived bacterial biopolymers can be used as sustainable alternatives to chemical polymers in the plastic industry. PHAs may be used for a variety of purposes, ranging from industrial uses as an alternative to petroleum-based plastic for packaging to biomedicine and pharmacy as well as agriculture (Liu et al., 2020; Wendlandt et al., 2005). These applications are dependent on the specific properties of the polyester produced. PHAs are recognized as a unique type of bioplastic that can be chemically altered or bioengineered to function as biomaterials, such as scaffolds for tissue engineering, sutures, particulate vaccines, and drug carriers, or low-value bioplastics, Moradali M.F. et al., (2020).

Poly-3-hydroxybutyrate (PHB), the primary type of PHA produced naturally, has a limited application due to its brittleness, low thermal stability, and propensity to embrittle over time. The simplest and most common form of PHA, poly-3-hydroxybutyrate (PHB), is produced industrially by accumulating polymer in pure strains using plant-derived carbon sources as the feedstock, typically sugars. Under nutrient-limited conditions, some methanotrophs can synthesize the PHB homopolymer from methane, Karthikeyan O et al.,(2015). Since its description for several strains in the seminal work by Whittenbury, et al. in 1970, methanotrophic and methylotrophic bacteria have been of constant interest for decades, Vecherskaya M et al., (2001), Karthikeyan O et al.,(2015), Zhang Y.X. et al.,(2008). Three essential enzymes catalyze reactions for the PHB synthesis in methanotrophic bacterial cells: β-ketothiolase, acetoacetyl-CoA reductase and PHA synthase (Cantera et al., 2019a).

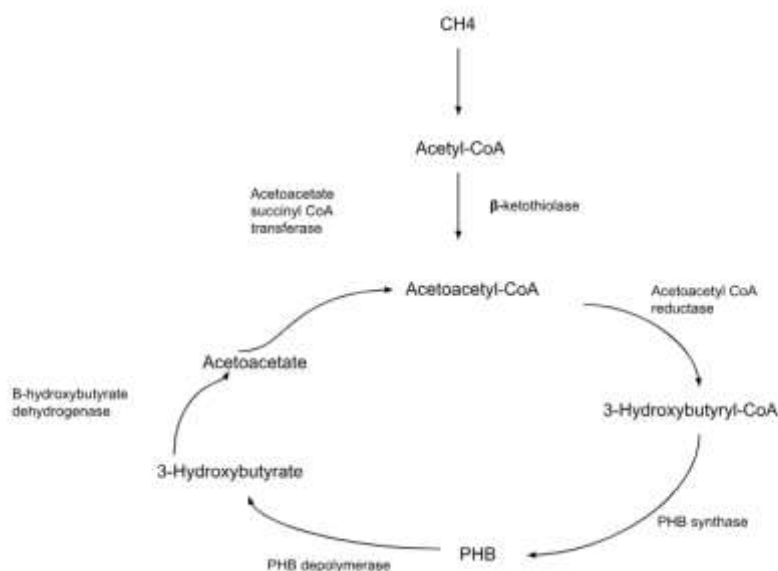


FIG. 3. PHB Synthesis in Methanotrophic Bacteria

Methanotrophic bacteria are thought to be a potent pathway for the microbial production of PHB from methane because they offer a combined solution for three significant environmental issues:

- (1) A potential strategy for reducing GHG emissions and carbon emissions
- (2) the production of biodegradable polymers to replace conventional plastics derived from fossil fuels
- (3) a decrease in the use of organic carbon sources like sugars for the production of PHB. Karthikeyan O et al.,(2015)

In an elegant series of experiments with a methanotroph (*Methylocystis parvus* OBBP), PHB was used as a source of reducing power to aid methane consumption, as opposed to the supply of C2 units for synthesis. PHB can serve as the sole growth substrate in aerobic cultures enriched on acetate during periods of carbon deficiency, Pieja A.J. et al., (2011). Numerous studies have compared the advantages of mixed cultures and pure strains for PHA accumulation in general, some of which are specifically focused on the use of methane as a carbon source Wendlandt K.D. et al., (2010), Helm J et al., (2006), Chidambarampadmavathy K et al., (2015), Pieja A.J et al., (2012). One method to produce biopolymers with more desirable properties during biological synthesis involves co-polymerizing 3-hydroxybutyrate (3HB) with different hydroxyalkanoate (HA) monomers. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV), a polymer that may be tougher and significantly more elastic than PHB, is an illustration of such a copolymer. Ziga et al. also successfully produced PHBV copolymers from methane in *Methylobacterium organophilum* CZ-2 using citrate or propionate as a co-substrate. A *Methylocystis* species-dominated enriched culture was used by Myung et al. to demonstrate the capability of tailoring poly(3-hydroxybutyrate-co-3-hydroxyvalerate) from methane under non-aseptic conditions, Zúñiga C et al.,(2013). Due to the organism-specific nature of biopolymer production, it is still necessary to conduct optimization research for each individual inoculum used in the process under study (Rostkowski et al., 2013).

### c. MICROBIAL PROTEIN

Methanotrophs are desirable among the various microbes used for microbial protein production because they allow for the recovery and upcycling of nutrients and carbon from low grade residual streams (Rasouli et al., 2018, Tsapekos et al., 2019) and have been proven to be feed components for various animal formulae (Verland et al., 2006; Skrede et al., 1998).

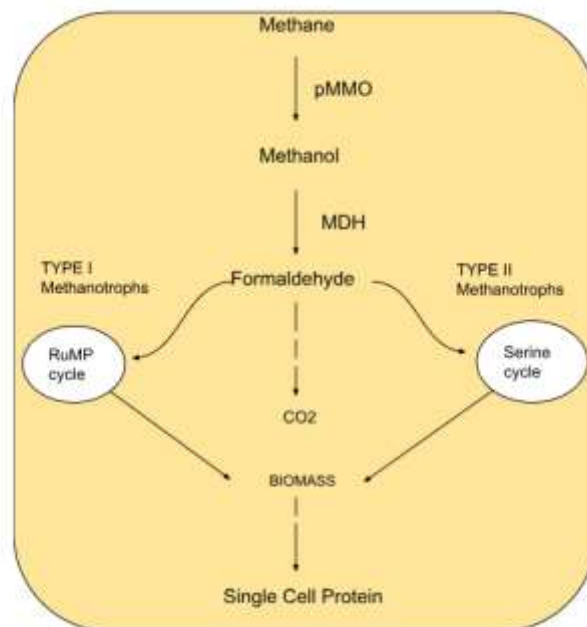


Fig. 4. Single Cell Protein production in Methanotrophic Bacteria.

*Methylococcus capsulatus*, which for years was the most frequently regarded methanotroph for SCP production, has been shown to produce microbial protein up to 70% of dry weight when grown in growth-optimal conditions (Pieja et al., 2017). Different sources have been used as feedstock when producing SCPs. For instance, natural gas, Ritala A et al., (2017); Yazdian F et al.,(2005); Schøyen HF et al.,(2007), agro-industrial wastes, Khadijah Hanim AR et al.,(2016); Bacha U et al.,(2011); Pandey A et al.,(2000), and edible sugars (Quorn™, Marlow Foods Ltd, UK). The use of these feedstocks for SCP production does come with some difficulties, though: The direct use of agro-industrial wastes may cause heavy metals to accumulate in the SCP, which may make it less palatable and make the production of SCP based on edible sugars less economically viable. Natural gas-based SCP production processes are also becoming less desirable due to their reliance on unsustainable fossil fuels.

**Table 2:** Microbial protein (SCP) production by methanotrophic bacteria since 2015, expressed as accumulation of microbial protein in cells (%DCW) or as SCP yield on methane (g DCW/g CH<sub>4</sub>).

Strain	Methane	Process details	Assimilation	Reference
<i>Methylococcus capsulatus</i>	60% CH <sub>4</sub>	Nitrate as N source	52%	(Steinberg et al., 2017)
<i>M. capsulatus (Bath)</i>	60% CH <sub>4</sub>	Batch, Ammonium as N source	52.52%	(Rasouli et al., 2018)
Mixed-culture enriched in <i>Methylococcales</i> and <i>Methylophilales</i>	O <sub>2</sub> :CH <sub>4</sub> 2:1 v/v	Batch, filtrated-digestate as medium	0.87 g DCW/g CH <sub>4</sub>	(Khoshnevisan et al., 2019)
<i>Methylocapsa acidiphila</i> Enriched methanotrophic and hydrogenotrophic culture	60% CH <sub>4</sub> 26% CH <sub>4</sub>	pH 5.7 Ammonium as N source	58.6% 66.60%	(Xu et al., 2020) (Acosta et al., 2020)

#### D. ECTOINE

Ectoines (ectoine and hydroxyectoine) are one of the most profitable bioproducts synthesized by microorganisms. These metabolites are compatible solutes produced by bacteria to resist salt stress (Lang et al., 2011; Pastor et al., 2010; Strong et al., 2016). They are used as stabilizers in medicines, nutrition, cosmetology due to their high Dna-protein complexes.(Poli et al.,2017).There are many microorganisms that synthesize ectoines under various stress conditions, such as halobacteria, actinomycetes, and Firmicutes (Pastor, J.et al., 2010). Ectoines are also found in some methylotrophic bacteria with a more salt-tolerant four-gene cluster that synthesizes ectoine independently of other amino acid biosynthetic pathways (Khmelenina, V.N et al 2010, Carmona-Martínez et al., 2021, Cantera et al., 2020). Examples of methylotrophic bacteria that synthesize ectoines include members of the genera *Methylomicrobium*, *Methylobacter*, and *Methylophaga*. In addition, some archaeal species also produce ectoine and hydroxyectoine (Widderich et al.,2016). The most common ectoine-synthesizing halophiles used as industrial producers are members of the *Halomonas* group, such as *Halomonas salina* (Chen, Q et al.,2014), *Halomonas boliviensis* (Van-Thuoc et al.,2010), and *Halomonas elongata* (Schwibbert et al 2011).In addition, so are some ectoine-synthesizing bacteria such as *Brevibacterium album*, *Marinococcus halotolerans*, *Virgibacillus salexigens*, and *Halomonas* sp. It also co-synthesizes hydroxyectoine under certain stresses in the presence of the enzyme ectD (Widderich, et al., 2014). Studies have also shown that some microalgae (Fenzia et al., 2020) and biogas production (Pérez et al.2022, Rodero et al., 2022) produce less ectoine. For example, ectoine can increase the catalytic efficiency of the enzyme lipase in biodiesel production,resulting in improved overall biodiesel yields (Wang, et al.,2010). The yield of methyl esters using a solvent-free methanolysis system consisting of cottonseed oil was significantly increased by 20.9% by adding ectoine to the immobilized lipase. Ectoine can act as an enzyme stabilizer that can preserve the structural conformation of lipases, thereby improving the overall production efficiency of methyl esters (Wang, et al.,2010).

**Table3:** Summary of some potential microbial ectoine

Phylum	Microorganism
Actinobacteria	Brachybacterium faecium DSM 4810 Brevibacterium album DSM 18261 Gordonia terrae NBRC 100016 Kytococcus sedentarius DSM 20547 Streptomyces coelicolor A3 (2)
Firmicutes	Bacillus halodurans DSM 497T Bacillus pseudofirmus OF4

	Halobacillus halophilus DSMZ 2266T Marinococcus halotolerans DSM 16375 Marinococcus sp. M52 Virgibacillus salexigens DSM 11438
Proteobacteria	Achromobacter xyloosidans A8 Acidiphilum crytum JF-5 Cellvibrio japonicus Ueda107 Chromohalobacter salexigens DSM 3043 Halomonas boliviensis Halomonas elongata DSM 2581 Nitrosococcus oceani ATCC 19707 Pseudomonas stutzeri A1501 Roseobacter sp. MED193 Vibrio cholerae O395
Archaea	Nitrosopumilus sp. AR2 Candidatus

#### f. BIODIESEL

Research into biodiesel production using methanotrophs is still in its early stages and is limited to a few laboratory strains such as *Methylomicrobium buryatense* and *Methylocystis* sp (Gilman et al.,2015, Dong et al., 2017) exclusive. Modifications made to the wild-type strain were aimed at overcoming bottlenecks in the production of higher fatty acids and lipids. B. Intracellular fatty acid degradation and acetyl- and malonyl-CoA levels as precursors of adipogenesis (Demidenko et al., 2017; Henard et al., 2017). Gilman et al., investigated the effects of CH<sub>4</sub> and O<sub>2</sub> limitation on the growth and lipid production of *M.buryatense* in continuous culture and found that oxygen-limited conditions resulted in a maximum dry cell weight (DCW) of 0.79 g/L and a lipid content of 10.7%. found ( w/w delivered) In 2013, a program called Reducing Emissions Using Methanotrophs for Transportation Energy (REMOTE),announced by the U.S. The Department of Energy’s Advanced Research Projects Agency (ARPA-E) was designed to convert CH<sub>4</sub> into liquids. was launched to accelerate the development of the economic process of fuel (Fei Q et al.,2014). Demidenko et al. only. (2017) were able to produce slightly more than 11% extractable FAMES on a dry weight basis due to altered regulation of fatty acid biosynthesis.

**Table 4:** Fatty acids and lipids production by methanotrophs since 2015, expressed as accumulation in cells (% DCW).

	Strain	Methane	Assimilation	Process details	Reference
FAME	<i>Methylomicrobium buryatense</i> 5GB1	20% CH <sub>4</sub>	10.7%	CSTR, pH 8.8 Oxygen limitation	(Gilman et al., 2015)
Lipids	<i>M. buryatense</i> 5GB1	20% CH <sub>4</sub>	9.5%	pH 9.0 Increased nitrate in the medium	(Dong et al., 2017)
Fatty Acids	<i>M. buryatense</i> 5G(B1) mutant train	50%CH <sub>4</sub>	11%	-	(Demidenko et al., 2017)
FAME	<i>M. buryatense</i> 5GB1S engineered strain	20% CH <sub>4</sub>	9%	Increased nitrate, phosphate, and trace elements in the medium	(Henard et al., 2017)
Lipids	<i>M. buryatense</i> 5GB1 mutant strain AP18	20%CH <sub>4</sub>	9.3%	pH 9.0 Increased nitrate, phosphate, and trace elements in the medium	(Fei et al., 2018)

### e. ORGANIC ACIDS

With advances in detailed metabolic pathway discovery and genome sequencing, genetic modification of wild methanotrophic strains to improve yields or synthesize unnatural organic acids is becoming increasingly common. Of particular interest in recent years has been the production of lactate, which is naturally produced from pyruvate by lactate dehydrogenase (LDH) but occurs at low yields due to the toxic effects of lactate concentrations on cells (Henard et al., 2016). Lactate tolerant strains were constructed, L-lactate in this case is ensured by genetic engineering aimed at deletion of competing signaling pathways (e.g., downregulation of phosphoketolase signaling pathway genes to reduce flux to acetyl-CoA formation). can be achieved (Henard CA et al., 2017). Other organic acids that can be achieved via the acetyl-CoA node include acetic acid, C-4 carboxylic acids such as crotonic acid and butyric acid, succinic acid, and 3-hydroxypropionic acid (Cai et al., 2019; Garg et al., 2018b; Nguyen et al., 2020b; Nguyen et al., 2019).

**Table 5:** Organic acids production by methanotrophic bacteria since 2015, expressed in titers (g/L).

Product	Strain	Methane	Process Details	Titer(g/L)	References
Lactic acid	<i>Methylobacterium buryatense</i> 5GB1S <i>pLhdh</i> mutant strain	20%	Increased nitrate, phosphate, and trace elements in the medium	0.808	(Henard et al., 2016)
Lactic acid	<i>Methylobacterium alcaliphilum</i> 20Z mutant strain	20%	BCB	0.027	(Henard et al., 2018)
Lactic acid	<i>M. buryatense</i> 5GB1 mutant strain pAMR4	21%	Ammonium as N source	0.50	(Garg et al., 2018a)
Crotonic acid Butyric acid	<i>M. buryatense</i> 5GB1C mutant strain pCA09	25%	-	0.08	(Garg et al., 2018b)
D-lactic acid	<i>Methylomonas</i> sp. DH-1, LA- tolerant strain JHM80	20%	Increased nitrate	0.09	(Lee et al., 2019)
Muconic acid	<i>M. buryatense</i> 5GB1 mutant strain pMUC	20%	CSTR Continuous gas supply	0.012	(Henard et al., 2019)
Succinic acid	<i>Methylomonas</i> sp. DH-1 mutant DS- GL strain	30%	pH6.9	0.195 g/L	(Nguyen et al., 2019)
3-HP acid (hydroxypropionic)	<i>Methylosinus trichosporium</i> OB3b mutant MCRMP strain	30%	pH7.0	0.061 g/L	(Nguyen et al., 2020b)
4-HB acid (hydroxybutyric)	<i>M. trichosporium</i> OB3b 4HB-SY4 mutant strain	40%	-	0.011 g/L (10.5 mg/L)	(Nguyen et al., 2020c)

### OTHER PRODUCTS:

In recent years, a research team at Kyung Hee University, South Korea has been actively working on the genetic engineering of methanotrophs for the heterologous production and improvement of existing metabolic pathways of various compounds, from the above organic acids to other new products. starting. Examples of alpha-humulene and alpha-bisabolene include 3-butanediol, putrescine, cadaverine or sesquiterpenoids. These compounds have the potential



to be used in a wide range of applications. As biofuel (2,3-butanediol), monomer for bioplastics (putrescine, cadaverine), pesticide (putrescine), medicine (putrescine, cadaverine, sesquiterpenoids) or industry (cadaverine, sesquiterpenoids) (Nguyen et al., 2020a; Nguyen et al., 2021; Nguyen et al., 2018; Nguyen and Lee, 2019; Nguyen et al., 2020c).

**Table 6:** Other new products of methanotrophs achieved by engineered strains since 2015, expressed as accumulation in cells (mg/g DCW) for internally produced compounds or as titer (g/L) for externally produced compounds.

Product	Strain	Methane	Process Details	Titer(g/L)	References
2,3-butanediol	<i>Methylobacterium alcaliphilum</i> 20ZM3/pNBM-Re mutant strain	20%	pH 8.8 Oxygen-limitation	0.086 g/L	(Nguyen et al., 2018)
Putrescine	<i>M. alcaliphilum</i> 20ZE4-pACO mutant strain	30%	Supplementation with ammonium (2 mM)	0.098 g/L	(Nguyen and Lee, 2019)
$\alpha$ -humulene (sesquiterpenoid)	<i>M. alcaliphilum</i> 20Z SQ08 mutant strain	50%	Two-phase cultivation 20% (v/v) dodecane as an organic phase	0.75 mg/g DCW	(Nguyen et al., 2020a)
Cadavarine	<i>Methylosinus trichosporium</i> OB3b/cad4 mutant strain	30%	pH 7.5	0.284 g/L	(Nguyen et al., 2020c)
$\alpha$ -humulene (sesquiterpenoid) $\alpha$ -bisabolene (sesquiterpenoid)	<i>Methylotuvimicrobium alcaliphilum</i> 20Z pDXP-07 mutant strain <i>Methylotuvimicrobium alcaliphilum</i> 20Z pBs-02 mutant strain	50%	Two-phase cultivation 20% (v/v) dodecane as an organic phase	0.56 mg/g DCW 12.24 $\pm$ 0.43 mg/g DCW (24.55 $\pm$ 0.86 mg/L)	(Nguyen et al., 2021)

## 2. FUTURE STUDIES

These new techniques were developed by Kwon et al. Compiled and checked. (2019) include high-throughput extinction cultivation, a soil substrate membrane system, and his CSTR screening method for isolating rapidly growing methanotrophs. For increased methane oxidation rates, concerted methods of both methanotroph and non-methanotrophic microorganisms can be stimulated (Ho et al., 2014). In addition to the basic culture parameters that affect microbial processes, such as temperature and pH, the various environmental requirements for converting methane into bioproducts introduce other important factors that need to be optimized in the process. Defined. Biopolymer production yields depend on nutrient-limiting conditions that induce PHA accumulation. Nitrogen and carbon sources and copper as essential ions for PHB synthesis are key parameters that need to be optimized (Chidambaram padmavathy et al., 2015b; Karthikeyan et al., 2015a).

The break-even price of Ectoine is 3-6 times lower than the cost of producing Ectoine in the best and worst possible scenarios compared to current production from long-term fermentation with *Halomonas* growth, mainly due to the use of low-cost CH<sub>4</sub> biogas. resulting in a doubling of the reduction. Carbonsubstrate for the growth of halo alkaline bacteria. Production of biomethane (renewable natural gas) and platform chemicals such as methanol, polyhydroxyalkanoates (PHAs) and single-cell proteins from biogas components (mainly methane (CH<sub>4</sub>) and carbon dioxide (CO<sub>2</sub>)) Alternative routes for biogas utilization are being developed (Pieja et al., 2017). In processes aimed at SCP production, it is important to adapt optimal conditions for cell growth in a short period of time. Studies should be conducted to ensure that all nutrients required for cell growth are adequate (Zha et al., 2021).

### 3. CONCLUSION

It has been suggested that methane is the feedstock of the future. Chemical production has historically taken place in large, centralized facilities, and this could be the case if natural gas methane is used. Methanotrophs have been studied for more than 30 years (Q. Fei *et al.*, 2014) for use in bioremediation, and natural gas was briefly used to produce single-cell proteins for animal feed in the 1970s, before oil prices soared. Recent Moves to circumvent the "food-to-fuel" concept, coupled with rising biomethane production and falling natural gas prices, have led research institutions and companies to turn methane into products such as biopolymers and single-cell proteins. Focused on biological conversion, biofuels and value-added chemicals.

### 4. REFERENCES

- [1] Abbasi, T., Tauseef, S.M., Abbasi, S.A., 2012. Anaerobic digestion for global warming control and energy generation - an overview. *Renew. Sust. Energ. Rev.* 16, 3228–3242. <https://doi.org/10.1016/j.rser.2012.02.046>.
- [2] Bacha, U., Nasir, M., Khalique, A., Anjum, A. A., & Jabbar, M. A. (2011). Comparative assessment of various agro-industrial wastes for *Saccharomyces cerevisiae* biomass production and its quality evaluation as single cell protein. *Journal of Animal and Plant Sciences*, 21(4), 844-849.
- [3] Cal, A. J., Sikkema, W. D., Ponce, M. I., Franqui-Villanueva, D., Riiff, T. J., Orts, W. J., Pieja, A. J., & Lee, C. C. (2016). Methanotrophic production of polyhydroxybutyrate-co-hydroxyvalerate with high hydroxyvalerate content. *International Journal of Biological Macromolecules*, 87, 302–307. <https://doi.org/10.1016/j.ijbiomac.2016.02.056>
- [4] Cantera, S., Bordel, S., Lebrero, R., Gancedo, J., García-Encina, P.A., Muñoz, R., 2019a. Bio-conversion of methane into high profit margin compounds: an innovative, environmentally friendly and cost-effective platform for methane abatement. *World J. Microbiol. Biotechnol.* 35, 1–10. <https://doi.org/10.1007/s11274-018-2587-4>.
- [5] Cantera, S., Phandanouvong-Lozano, V., Pascual, C., García-Encina, P. A., Lebrero, R., Hay, A., & Muñoz, R. (2020). A systematic comparison of ectoine production from upgraded biogas using *Methylobacterium alcaliphilum* and a mixed haloalkaliphilic consortium. *Waste Management*, 102, 773–781. <https://doi.org/10.1016/j.wasman.2020.01.033>
- [6] Carmona-Martínez, A. A., Marcos-Rodrigo, E., Bordel, S., Marín, D., Herrero-Lobo, R., García-Encina, P. A., & Muñoz, R. (2021). Elucidating the key environmental parameters during the production of ectoines from biogas by mixed methanotrophic consortia. *Journal of Environmental Management*, 298, 113462. <https://doi.org/10.1016/j.jenvman.2021.113462>
- [7] Chen, Q.; Zhang, L.; Li, X.; Liu, S.; Li, D. Poly- $\beta$ -hydroxybutyrate/ectoine co-production by ectoine-excreting strain *Halomonas salina*. *Process Biochem.* 2014, 49, 33–37.
- [8] Chidambarampadmavathy, K., Karthikeyan, O. P., & Heimann, K. (2015). Biopolymers made from methane in bioreactors. *Engineering in Life Sciences*, 15(6), 689-699. <https://doi.org/10.1002/elsc.201400203>
- [9] Chidambarampadmavathy, K., Karthikeyan, O. P., Huerlimann, R., Maes, G. E., & Heimann, K. (2017). Response of mixed methanotrophic consortia to different methane to oxygen ratios. *Waste Management*, 61, 220–228. <https://doi.org/10.1016/j.wasman.2016.11.007>
- [10] Chidambarampadmavathy, K., Obulisamy, P. K., & Heimann, K. (2015). Role of copper and iron in methane oxidation and bacterial biopolymer accumulation. *Engineering Life Sciences*, 15, 387–399. <https://doi.org/10.1002/elsc.201400127>
- [11] Da Silva, M. J. (2016). Synthesis of methanol from methane: Challenges and advances on the multi-step (syngas) and one-step routes (DMTM). *Fuel Processing Technology*, 145, 42. <https://doi.org/10.1016/j.fuproc.2016.01.023>
- [12] Demidenko, A., Akberdin, I. R., Allemann, M., Allen, E. E., & Kalyuzhnaya, M. G. (2017). Fatty acid biosynthesis pathways in *Methylobacterium buryatense* 5GB1. *Frontiers in Microbiology*, 7, 2167. <https://doi.org/10.3389/fmicb.2016.02167>
- [13] Demidenko, A., Akberdin, I. R., Allemann, M., Allen, E. E., & Kalyuzhnaya, M. G. (2017). Fatty acid biosynthesis pathways in *Methylobacterium buryatense* 5GB1. *Frontiers in Microbiology*, 7, 2167. <https://doi.org/10.3389/fmicb.2016.02167>
- [14] Dong, T., Fei, Q., Genlot, M., Smith, H., Laurens, L. M. L., Watson, M. J., & Pienkos, P. T. (2017). A novel integrated biorefinery process for diesel fuel blendstock production using lipids from the methanotroph, *Methylobacterium buryatense*. *Energy Conversion and Management*, 140, 62-70. <https://doi.org/10.1016/j.enconman.2017.02.006>

- [15] Fei, Q., Guarnieri, M. T., Tao, L., Laurens, L. M. L., Dowe, N., & Pienkos, P. T. (2014). Bioconversion of natural gas to liquid fuel: Opportunities and challenges. *Biotechnology Advances*, 32(3), 596–614. <https://doi.org/10.1016/j.biotechadv.2013.12.005>
- [16] Fei, Q., Guarnieri, M. T., Tao, L., Laurens, L. M. L., Dowe, N., & Pienkos, P. T. (2014). Bioconversion of natural gas to liquid fuel: Opportunities and challenges. *Biotechnology Advances*, 32(3), 596–614. <https://doi.org/10.1016/j.biotechadv.2013.12.005>
- [17] Fei, Q., Guarnieri, M. T., Tao, L., Laurens, L. M., Dowe, N., & Pienkos, P. T. (2014). Bioconversion of natural gas to liquid fuel: opportunities and challenges. *Biotechnology advances*, 32(3), 596–614. <https://doi.org/10.1016/j.biotechadv.2014.03.011>
- [18] Fenizia, S., Thume, K., Wirgenings, M., & Pohnert, G. (2020). Ectoine from bacterial and algal origin is a compatible solute in microalgae. *Marine Drugs*, 18(1), 42. <https://doi.org/10.3390/md18010042>
- [19] Garg, S., Wu, H., Clomburg, J. M., & Bennett, G. N. (2018). Bioconversion of methane to C-4 carboxylic acids using carbon flux through acetyl-CoA in engineered *Methylobacterium buryatense* 5GB1C. *Metabolic Engineering*, 48, 175–183. <https://doi.org/10.1016/j.ymben.2018.06.001>
- [20] Ghaz-Jahani, M. A., Khoshfetrat, A. B., Hosseini Rostami, M., & Haghighi, M. (2018). An innovative bioprocess for methane conversion to methanol using an efficient methane transfer chamber coupled with an airlift bioreactor. *Chemical Engineering Research and Design*, 134, 80–89. <https://doi.org/10.1016/j.cherd.2018.03.039>
- [21] Gilman, A., Laurens, L. M., Puri, A. W., Chu, F., Pienkos, P. T., & Lidstrom, M. E. (2015). Bioreactor performance parameters for an industrially-promising methanotroph *Methylobacterium buryatense* 5GB1. *Microbial cell factories*, 14, 182. <https://doi.org/10.1186/s12934-015-0372-8>
- [22] Gilman, A., Laurens, L. M., Puri, A. W., Chu, F., Pienkos, P. T., & Lidstrom, M. E. (2015). Bioreactor performance parameters for an industrially-promising methanotroph *Methylobacterium buryatense* 5GB1. *Microbial Cell Factories*, 14, 182. <https://doi.org/10.1186/s12934-015-0390-4>
- [23] Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological Reviews*, 60(2), 439–471. <https://doi.org/10.1128/mr.60.2.439-471.1996>
- [24] Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological reviews*, 60(2), 439–471. <https://doi.org/10.1128/mr.60.2.439-471.1996>
- [25] Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological reviews*, 60(2), 439–471. <https://doi.org/10.1128/mr.60.2.439-471.1996>
- [26] Haynes, C. A., & Gonzalez, R. (2014). Rethinking biological activation of methane and conversion to liquid fuels. *Nature chemical biology*, 10(5), 331–339. <https://doi.org/10.1038/nchembio.1509>
- [27] Helm, J., Wendlandt, K. D., Rogge, G., & Kappelmeyer, U. (2006). Characterizing a stable methane-utilizing mixed culture used in the synthesis of a high-quality biopolymer in an open system. *Journal of Applied Microbiology*, 101(3), 387–395. <https://doi.org/10.1111/j.1365-2672.2006.02960.x>
- [28] Henard, C. A., Smith, H. K., & Guarnieri, M. T. (2017). Phosphoketolase overexpression increases biomass and lipid yield from methane in an obligate methanotrophic biocatalyst. *Metabolic Engineering*, 41, 152–158. <https://doi.org/10.1016/j.ymben.2017.03.007>
- [29] Henard, C. A., Smith, H., Dowe, N., Kalyuzhnaya, M. G., Pienkos, P. T., & Guarnieri, M. T. (2016). Bioconversion of methane to lactate by an obligate methanotrophic bacterium. *Scientific Reports*, 6, 21585. <https://doi.org/10.1038/srep21585>
- [30] Hwang, I. Y., Hur, D. H., Lee, J. H., Park, C. H., Chang, I. S., Lee, J. W., & Lee, E. Y. (2015). Batch conversion of methane to methanol using *Methylobacterium trichosporium* OB3b as biocatalyst. *Journal of microbiology and biotechnology*, 25(3), 375–380. <https://doi.org/10.4014/jmb.1412.12007>
- [31] Kalyuzhnaya, M. G., Puri, A. W., & Lidstrom, M. E. (2015). Metabolic engineering in methanotrophic bacteria. *Metabolic engineering*, 29, 142–152. <https://doi.org/10.1016/j.ymben.2015.03.010>
- [32] Kalyuzhnaya, M. G., Puri, A. W., & Lidstrom, M. E. (2015). Metabolic engineering in methanotrophic bacteria. *Metabolic engineering*, 29, 142–152. <https://doi.org/10.1016/j.ymben.2015.03.010>
- [33] Karthikeyan, O. P., Chidambarampadmavathy, K., Cirés, S., & Heimann, K. R. (2015). Review of sustainable methane mitigation and biopolymer production. *CRITICAL REVIEWS IN ENVIRONMENTAL SCIENCE AND TECHNOLOGY*, 45(15), 1579–1610. <https://doi.org/10.1080/10643389.2014.966422>

- [34] Khadijah Hanim, A. R., Jamilah, H., & Zakaria, Z. (2016). Bioproteins production from palm oil agro-industrial wastes by *Aspergillus terreus* UniMAP AA-1. *Pertanika Journal of Tropical Agricultural Science*, 39(1), 30-40.
- [35] Khmelenina, V. N., Mustakhimov, I. I., Reshetnikov, A. S., Kalyuzhnaya, M. G., & Trotsenko, Y. A. (2010). Genetic and biochemical aspects of ectoine biosynthesis in moderately halophilic and halotolerant methylotrophic bacteria. *Journal of Agricultural and Biological Sciences*, 5(3), 446–458.
- [36] Kwon, M., Ho, A., & Yoon, S. (2019). Novel approaches and reasons to isolate methanotrophic bacteria with biotechnological potentials: Recent achievements and perspectives. *Applied Microbiology and Biotechnology*, 103, 1–8. <https://doi.org/10.1007/s00253-018-9435-1>
- [37] Lang, Y., Bai, L., Ren, Y., Zhang, L., & Nagata, S. (2011). Production of ectoine through a combined process that uses both growing and resting cells of *Halomonas salina* DSM 5928T. *Extremophiles*, 15(3), 303-310.
- [38] Moradali, M. F., & Rehm, B. H. A. (2020). Bacterial biopolymers: from pathogenesis to advanced materials. *Nature reviews. Microbiology*, 18(4), 195–210. <https://doi.org/10.1038/s41579-019-0313-3>
- [39] Myung, J., Galega, W. M., van Nostrand, J. D., Yuan, T., Zhou, J. Z., & Criddle, C. S. (2015). Long-term cultivation of a stable *Methylocystis*-dominated methanotrophic enrichment enabling tailored production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate). *Bioresource Technology*, 198, 811-818. <https://doi.org/10.1016/j.biortech.2015.09.094>
- [40] Nguyen, A. D., & Lee, E. Y. (2021). Engineered methanotrophy: A sustainable solution for methane-based industrial biomanufacturing. *Trends in Biotechnology*, 39, 381–396. <https://doi.org/10.1016/j.tibtech.2020.07.007>
- [41] Nguyen, L. T., & Lee, E. Y. (2019). Biological conversion of methane to putrescine using genome-scale model-guided metabolic engineering of a methanotrophic bacterium *Methylomicrobium alcaliphilum* 20Z. *Biotechnology for Biofuels*, 12, 1-12. <https://doi.org/10.1186/s13068-019-1490-z>
- [42] Øverland, M., et al. (2006). Apparent total tract digestibility of unprocessed and extruded diets containing basic and autolyzed bacterial protein meal grown on natural gas in mink and rainbow trout. *Animal Feed Science and Technology*.
- [43] Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P. S., & Mohan, R. (2000). Biotechnological potential of agro-industrial residues II: Cassava bagasse. *Bioresource Technology*, 74(1), 81-87.
- [44] Pastor, J. M., Salvador, M., Argandoña, M., Bernal, V., Reina-Bueno, M., Csonka, L. N., Iborra, J. L., Vargas, C., Nieto, J. J., & Cánovas, M. J. (2010). Ectoines in cell stress protection: Uses and biotechnological production. *Biotechnology Advances*, 28(6), 782–801.
- [45] Patel, S. K. S., Gupta, R. K., Kalia, V. C., & Lee, J. K. (2021). Integrating anaerobic digestion of potato peels to methanol production by methanotrophs immobilized on banana leaves. *Bioresource technology*, 323, 124550. <https://doi.org/10.1016/j.biortech.2020.124550>
- [46] Patel, S. K. S., Gupta, R. K., Kumar, V., Kondaveeti, S., Kumar, A., Das, D., Kalia, V. C., & Lee, J. K. (2020). Biomethanol Production from Methane by Immobilized Co-cultures of Methanotrophs. *Indian journal of microbiology*, 60(3), 318–324. <https://doi.org/10.1007/s12088-020-00883-6>
- [47] Patel, S. K. S., Kalia, V. C., Joo, J. B., Kang, Y. C., & Lee, J. K. (2020). Biotransformation of methane into methanol by methanotrophs immobilized on coconut coir. *Bioresource technology*, 297, 122433. <https://doi.org/10.1016/j.biortech.2019.122433>
- [48] Patel, S. K. S., Kumar, V., Mardina, P., Li, J., Lestari, R., Kalia, V. C., & Lee, J. K. (2018). Methanol production from simulated biogas mixtures by co-immobilized *Methylomonas methanica* and *Methylocella tundrae*. *Bioresource technology*, 263, 25–32. <https://doi.org/10.1016/j.biortech.2018.04.096>
- [49] Patel, S. K., Jeong, J. H., Mehariya, S., Otari, S. V., Madan, B., Haw, J. R., Lee, J. K., Zhang, L., & Kim, I. W. (2016). Production of Methanol from Methane by Encapsulated *Methylosinus sporium*. *Journal of microbiology and biotechnology*, 26(12), 2098–2105. <https://doi.org/10.4014/jmb.1608.08053>
- [50] Patel, S. K., Mardina, P., Kim, S. Y., Lee, J. K., & Kim, I. W. (2016). Biological Methanol Production by a Type II Methanotroph *Methylocystis bryophila*. *Journal of microbiology and biotechnology*, 26(4), 717–724. <https://doi.org/10.4014/jmb.1601.01013>
- [51] Pérez, V., Moltó, J. L., Lebrero, R., & Muñoz, R. (2022). Ectoine production from biogas: A sensitivity analysis. Effect of local commodity prices, economy of scale, market trends, and biotechnological limitations. *Journal of Cleaner Production*, 369, 133440. <https://doi.org/10.1016/j.jclepro.2022.133440>

- [52] Pieja A.J., Sundstrom E.R., Criddle C.S. Poly-3-hydroxybutyrate metabolism in the type II methanotroph *Methylocystis parvus* OBBP. *Appl. Environ. Microbiol.* 2011;77:6012–6019. doi: 10.1128/AEM.00509-11. [PMC free article][PubMed] [CrossRef] [Google Scholar] [Ref list]
- [53] Pieja, A. J., Morse, M. C., & Cal, A. J. (2017). Methane to bioproducts: The future of the bioeconomy? *Current Opinion in Chemical Biology*, 41, 123-131. <https://doi.org/10.1016/j.cbpa.2017.10.024>
- [54] Pieja, A. J., Morse, M. C., & Cal, A. J. (2017). Methane to bioproducts: The future of the bioeconomy? *Current Opinion in Chemical Biology*, 41, 123–131. <https://doi.org/10.1016/j.cbpa.2017.10.024>
- [55] Pieja, A. J., Sundstrom, E. R., & Criddle, C. S. (2012). Cyclic, alternating methane and nitrogen limitation increases PHB production in a methanotrophic community. *Bioresource Technology*, 107, 385-392. <https://doi.org/10.1016/j.biortech.2011.12.044>
- [56] Poli, A., Finore, I., Romano, I., Gioiello, A., Lama, L., & Nicolaus, B. (2017). Microbial diversity in extreme marine habitats and their biomolecules. *Microorganisms*, 5(2), 25. <https://doi.org/10.3390/microorganisms5020025>
- [57] Rasouli, Z., et al. (2018). Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs. *Biochemical Engineering Journal*.
- [58] Ritala, A., Häkkinen, S. T., Toivari, M., & Wiebe, M. G. (2017). Single cell protein: State-of-the-art, industrial landscape and patents 2001–2016. *Frontiers in Microbiology*, 8, 2009.
- [59] Rodero, M. D. R., Herrero-Lobo, R., Pérez, V., & Muñoz, R. (2022). Influence of operational conditions on the performance of biogas bioconversion into ectoines in pilot bubble column bioreactors. *Bioresource Technology*, 358, 127398. <https://doi.org/10.1016/j.biortech.2022.127398>
- [60] Rodríguez, Y., Firmino, P. I. M., Arnáiz, E., Lebrero, R., & Muñoz, R. (2020). Elucidating the influence of environmental factors on biogas-based polyhydroxybutyrate production by *Methylocystis hirsuta* CSC1. *The Science of the total environment*, 706, 135136. <https://doi.org/10.1016/j.scitotenv.2019.135136>
- [61] Rostkowski, K. H., Pfluger, A. R., & Criddle, C. S. (2013). Stoichiometry and kinetics of the PHB-producing type II methanotrophs *Methylosinus trichosporium* OB3b and *Methylocystis parvus* OBBP. *Bioresource Technology*, 132, 71-77. <https://doi.org/10.1016/j.biortech.2012.12.129>
- [62] Schøyen, H. F., Svihus, B., Storebakken, T., & Skrede, A. (2007). Bacterial protein meal produced on natural gas replacing soybean meal or fish meal in broiler chicken diets. *Archives of Animal Nutrition*, 61(4), 276–291.
- [63] Schwibbert, K., Marin-Sanguino, A., Bagyan, I., Heidrich, G., Lentzen, G., Seitz, H., Rampp, M., Schuster, S. C., Klenk, H. P., & Pfeiffer, F. (2011). A blueprint of ectoine metabolism from the genome of the industrial producer *Halomonas elongata* DSM 2581T. *Environmental Microbiology*, 13(12), 1973-1994. <https://doi.org/10.1111/j.1462-2920.2011.02507.x>
- [64] Strong, P. J., Xie, S., & Clarke, W. P. (2015). Methane as a resource: can the methanotrophs add value?. *Environmental science & technology*, 49(7), 4001–4018. <https://doi.org/10.1021/es504242n>
- [65] Strong, P. J., Xie, S., & Clarke, W. P. (2015). Methane as a resource: can the methanotrophs add value?. *Environmental science & technology*, 49(7), 4001–4018. <https://doi.org/10.1021/es504242n>
- [66] Tsapekos, P., et al. (2019). Methane oxidizing bacteria to upcycle effluent streams from anaerobic digestion of municipal biowaste. *Journal of Environmental Management*.
- [67] Van-Thuoc, D., Guzmán, H., Quillaguamán, J., & Hatti-Kaul, R. (2010). High productivity of ectoines by *Halomonas boliviensis* using a combined two-step fed-batch culture and milking process. *Journal of Biotechnology*, 147(1), 46-51. <https://doi.org/10.1016/j.jbiotec.2010.03.016>
- [68] Vecharskaya, M., Dijkema, C., & Stams, A. J. (2001). Intracellular PHB conversion in a Type II methanotroph studied by <sup>13</sup>C NMR. *Journal of industrial microbiology & biotechnology*, 26(1-2), 15–21.
- [69] Wang, Y., & Zhang, L. (2010). Ectoine improves yield of biodiesel catalyzed by immobilized lipase. *Journal of Molecular Catalysis B: Enzymatic*, 62(1-2), 90-95. <https://doi.org/10.1016/j.molcatb.2010.02.008>
- [70] Wendlandt, K. D., Stottmeister, U., Helm, J., Soltmann, B., Jechorek, M., & Beck, M. (2010). The potential of methane-oxidizing bacteria for applications in environmental biotechnology. *Engineering in Life Sciences*, 10(2), 87-102. <https://doi.org/10.1002/elsc.200900093>
- [71] Whittenbury, R., Phillips, K. C., & Wilkinson, J. F. (1970). Enrichment, isolation and some properties of methane-utilizing bacteria. *Journal of general microbiology*, 61(2), 205–218. <https://doi.org/10.1099/00221287-61-2-205>

- [72] Widderich, N., Czech, L., Elling, F. J., Konneke, M., Stoveken, N., Pittelkow, M., Riclea, R., Dickschat, J. S., Heider, J., & Bremer, E. (2016). Strangers in the archaeal world: Osmostress-responsive biosynthesis of ectoine and hydroxyectoine by the marine thaumarchaeon *Nitrosopumilus maritimus*. *Environmental Microbiology*, 18(4), 1227–1248. <https://doi.org/10.1111/1462-2920.13137>
- [73] Widderich, N., Hoppner, A., Pittelkow, M., Heider, J., Smits, S. H., & Bremer, E. (2014). Biochemical properties of ectoine hydroxylases from extremophiles and their wider taxonomic distribution among microorganisms. *PLoS ONE*, 9(12), e93809. <https://doi.org/10.1371/journal.pone.0093809>
- [74] Xin, J., Cui, J., Niu, J., Hua, S., Xia, C., Li, S., & Zhu, L. (2004). Production of methanol from methane by methanotrophic bacteria. *Biocatalysis and Biotransformation*, 22, 225 - 229.
- [75] Yazdian, F., Hajizadeh, S., Shojaosadati, S. A., Khalilzadeh, R., Jahanshahi, M., & Nosrati, M. (2005). Production of single cell protein from natural gas: Parameter optimization and RNA evaluation. *Iranian Journal of Biotechnology*, 3(4), 235-242.
- [76] Zha, X., Tsapekos, P., Zhu, X., Khoshnevisan, B., Lu, X., & Angelidaki, I. (2021). Bioconversion of wastewater to single cell protein by methanotrophic bacteria. *Bioresource Technology*, 320, 124351. <https://doi.org/10.1016/j.biortech.2020.124351>
- [77] Zhang, Y. X., Xin, J. Y., Chen, L. L., Song, H., & Xia, C. U. (2008). Biosynthesis of poly-3-hydroxybutyrate with a high molecular weight by methanotroph from methane and methanol. *Journal of Natural Gas Chemistry*, 17(1), 103-109. [https://doi.org/10.1016/S1003-9953\(08\)60034-1](https://doi.org/10.1016/S1003-9953(08)60034-1)
- [78] Zúñiga, C., Morales, M., & Revah, S. (2013). Polyhydroxyalkanoates accumulation by *Methylobacterium organophilum* CZ-2 during methane degradation using citrate or propionate as cosubstrates. *Bioresource Technology*, 129, 686-689. <https://doi.org/10.1016/j.biortech.2012.11.120>