**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

**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 MOBs 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 methane 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 CO2 before formate dehydrogenase (FDH) oxidized to CO2 (Hanson and Hanson, 1996). Formaldehyde has 3 fate: 1) further oxidation into CO2; 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 CO2 before formate dehydrogenase (FDH) oxidizes formate to CO2 (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.

A diagram of a molecule

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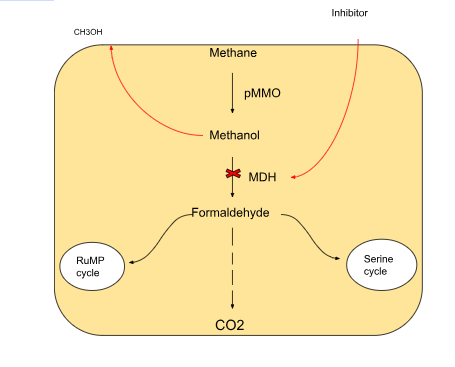
**Fig. 1. Methane Assimilation pathways in Methanotrophs. Abbreviations:** DHAP–dihydroxyacetone phosphate, EMC–methylmalonyl-coenzyme A, G3P–glyceraldehyde 3-phosphate, methylene-THF-methylene tetrahydrofolate, OAA–oxaloacetate, PEP–phosphoenolpyruvate, RuMP – ribulose monophosphate.

**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.

1. **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 CH4 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 (MgCl2), phosphate buffer and sodium chloride, and formate are necessary to reduce the subsequent methanol oxidation by MDH

**Table 1: Methanol production by methanotrophs**

|  |  |  |  |
| --- | --- | --- | --- |
| Strain | CH4 | Process | Reference |
| *Methylosinus trichosporium* OB3b | 30% | EDTA (0.5 mM), Na-formate (40 mM) | (Hwang et al., 2015) |
| *Methylosinus sporium* | 20% CH4 (raw biogas) | Copper (5 μM), Iron (10 μM) MgCl2 (20 mM) Copper (5 μM), Iron (10 μM) 62.3% CH4, 36.7% CO2, 0.13% H2S | (Patel et al., 2016b) |
| *Methylocystis bryophila* | 50% CH4 | Supplementation with H2 (10%) Na-formate (100 mM), MgCl2 (50 mM) | (Patel et al., 2016c) |
| *Methylocella tundrae* | 50% CH4 | Copper (5 μM), Iron (10 μM) Na-formate (50 mM), MgCl2 (50 mM) | (Mardina et al., 2016) |
| Co-culture of *Methylosinus sporium* *Methylocella tundrae* | 30% CH4 (simulated biogas CH4:CO2) | Copper (5 μM), Iron (10 μM) Encapsulated in silica gel Formate (100 mM), MgCl2 (50 mM) | (Patel et al., 2018b) |
| Immobilized co-culture of *Methylocystis bryophila* and *Methyloferula stellata* | 30% CH4 | Copper (5 μM), Iron (10 μM) Immobilised with polyvinyl alcohol (10%) in 3:2 ratio of *M. bryophila* and *M. stellate* Formate (100 mM), MgCl2 (20 mM) | (Patel et al., 2020b) |
| *M. tundrae* immobilized on banana leaves | 30% CH4 (raw biogas) | Formate (100 mM), MgCl2 (20 mM) | (Patel et al., 2021) |
| Encapsulated *M. album* Encapsulated *Methyloferula*  *stellate* | 30% CH4 (simulated biogas; CH4:CO2 at 2:1) | Copper (5 μM), Iron (10 μM) Encapsulated in polyvinyl alcohol (10%) Formate (100 mM), MgCl2 (50 mM) | (Patel et al., 2020c) |
| Strain AS1 isolated from active anaerobic sludge | 50% CH4 | 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). CO2 may also have inhibitory effects on MDH (Patel et al., 2018a; Xin et al., 2004).

1. **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](https://www.sciencedirect.com/topics/engineering/biopolymer) 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).

A diagram of a cycle

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**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).

1. **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).

A diagram of a formaldehyde

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**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 (QuornTM, 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 CH4).**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Strain | Methane | Process details | Assimilation | Reference |
| *Methylococcus capsulatus* | 60% CH4 | Nitrate as N source | 52% | (Steinberg et al., 2017) |
| *M. capsulatus (Bath)* | 60% CH4 | Batch, Ammonium as N source | 52.52% | (Rasouli et al., 2018) |
| Mixed-culture enriched in *Methylococcales* and *Methylophilales* | *O2:CH4 2:1 v/v* | Batch, filtrated-digestate as medium | 0.87 g DCW/g CH4 | (Khoshnevisan et al., 2019) |
| *Methylocapsa acidiphila*  Enriched methanotrophic and hydrogenotrophic culture | 60% CH4  26% CH4 | pH 5.7  Ammonium as N source | 58.6%  66.60% | (Xu et al., 2020) (Acosta et al., 2020) |

1. **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 theHalomonas 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, Marinococcushalotolerans, 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 (Fenizia 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 xylosidans* 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* |

1. **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 CH4 and O2 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 CH4 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 | *Methylomicrobium buryatense* 5GB1 | 20% CH4 | 10.7% | CSTR, pH 8.8 Oxygen limitation | (Gilman et al., 2015) |
| Lipids | *M. buryatense* 5GB1 | 20% CH4 | 9.5% | pH 9.0 Increased nitrate in the medium  – | (Dong et al., 2017) |
| Fatty Acids | *M. buryatense* 5G(B1) mutant train | 50%CH4 | 11% | - | (Demidenko et al., 2017) |
| FAME | *M. buryatense* 5GB1S engineered strain | 20% CH4 | 9% | Increased nitrate, phosphate, and trace elements in the medium | (Henard et al., 2017) |
| Lipids | *M. buryatense* 5GB1 mutant strain AP18 | 20%CH4 | 9.3% | pH 9.0 Increased nitrate, phosphate, and trace elements in the medium | (Fei et al., 2018) |

1. **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 | *Methylomicrobium buryatense* 5GB1S *pLhldh* mutant strain | 20% | Increased nitrate, phosphate, and trace elements in the medium | 0.808 | (Henard et al., 2016) |
| Lactic acid | *Methylomicrobium alcaliphilum* 20Z mutant strain | 20% | BCB | 0.027 | (Henard et al., 2018) |
| Lactic acid | *M. buryatense* 5GB1 mutant strain pAMR4 | 21% | Ammonium as N source | 0.50 | (Garg et al., 2018a) |
| Crotonic acid  Butyric acid | *M. buryatense* 5GB1C mutant strain  pCA09 | 25% | - | 0.08 | (Garg et al., 2018b) |
| D-lactic acid | *Methylomonas* sp*.* DH-1, LA- tolerant strain JHM80 | 20% | Increased nitrate | 0.09 | (Lee et al., 2019) |
| Muconic acid | *M. buryatense* 5GB1 mutant strain pMUC | 20% | CSTR Continuous gas supply | 0.012 | (Henard et al., 2019) |
| Succinic acid | *Methylomonas* sp. DH-1 mutant DS- GL strain | 30% | pH6.9 | 0.195 g/L | (Nguyen et al., 2019) |
| 3-HP acid (hydroxypropionic) | *Methylosinus trichosporium* OB3b mutant MCRMP strain | 30% | pH7.0 | 0.061 g/L | (Nguyen et al., 2020b) |
| 4-HB acid (hydroxybutyric) | *M. trichosporium* 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 | *Methylomicrobium alcaliphilum*  20ZM3/pNBM-Re mutant strain | 20% | pH 8.8 Oxygen-limitation | 0.086 g/L | (Nguyen et al., 2018) |
| Putrescine | *M. alcaliphilum* 20ZE4-pACO mutant strain | 30% | Supplementation with ammonium (2 mM) | 0.098 g/L | (Nguyen and Lee, 2019) |
| α-humulene (sesquiterpenoid) | *M. alcaliphilum* 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 | *Methylosinus trichosporium* OB3b/ cad4 mutant strain | 30% | pH 7.5 | 0.284 g/L | (Nguyen et al., 2020c) |
| α-humulene (sesquiterpenoid)  α-bisabolene (sesquiterpenoid) | *Methylotuvimicrobium alcaliphilum* 20Z pDXP-07 mutant strain  *Methylotuvimicrobium alcaliphilum* 20Z pBs-02 mutant strain | 50% | Two-phase cultivation 20% (v/v) dodecane as an organic phase | 0.56 mg/g DCW  12.24 ± 0.43 mg/g DCW (24.55 ± 0.86 mg/L) | (Nguyen et al., 2021) |

**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 (Chidambarampadmavathyet 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 Halomonasgrowth, mainly due to the use of low-cost CH4 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 (CH4)and carbon dioxide (CO2)) 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).

**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 fallingnatural 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.

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