Chapter 8
Bioconversion of Methane for Value-Added Products

Qiang Fei and Philip T. Pienkos

What Will You Learn from This Chapter?
This chapter will briefly summarize the background of methane production from natural gas and biogas together with an introduction to the biomolecular basis of methanotrophy and the actual and potential commercial applications. This chapter also discusses the safety considerations for using methane in laboratory and safe development of methane-based bioprocesses. In the end, this chapter will focus on the process development for biological conversion of methane into desired products with attention to the enhancement of mass transfer efficiency and the development of bioreactor designs.

8.1 Introduction

Methane (CH_4) is a colorless, odorless, nontoxic, and flammable gas and is the simplest and most energy dense alkane with a specific energy of 55 MJ/kg. It is one of the most common gases in the universe where it was produced as part of the same processes that formed the stars, planets, and other celestial bodies. On earth, CH_4 formed underground from organic material as a fossil fuel along with coal and petroleum. It is usually found in both wetlands and oceans, where it often finds its way to the surface and into the atmosphere. Approximately 36% of the CH_4 released into the atmosphere is due to natural geological activities (Bousquet et al. 2006).

Q. Fei (✉)
School of Chemical Engineering and Technology, Xi'an Jiaotong University, Xi'an, China
e-mail: feiqiang@mail.xjtu.edu.cn

P. T. Pienkos
National Renewable Energy Laboratory, National Bioenergy Center, Golden, CO, USA

R. K. Sani, N. K. Rathinam (eds.), *Extremophilic Microbial Processing of Lignocellulosic Feedstocks to Biofuels, Value-Added Products, and Usable Power*,
https://doi.org/10.1007/978-3-319-74459-9_8

However, most of the rest comes from human activities, such as burning fuel, leakage from natural gas systems, and raising livestock. In the USA, energy extraction (natural gas and petroleum), agriculture (enteric fermentation), and waste management (landfills) account for the highest CH_4 production (EPA 2015). CH_4 is a greenhouse gas, and its production has kept pace with the world's population growth. Due to its high global warming potential that is 80 times that of CO_2 over a 20-year time frame, more than 5.4 quads (1.47×10^5 million cubic meter) of natural gas has been flared annually at oil production sites around the globe (World Bank 2013). CH_4 can also be harnessed as an energy source, which has played a vital role of the world's supply of energy for years. Natural gas containing about 80–95% (v/v) CH_4 mixed with other heavier alkanes is one of the major sources for CH_4 production. With the heating value of 1020 BTU per standard cubic foot (BTU/scf), it is one of the major fuels used throughout the country. As a fossil fuel, natural gas is commonly used as an energy source for transportation, heating, cooking, and electricity generation. More than 24.6 quads (2.4×10^4 billion cubic feet) of natural gas has been extracted from the ground every year in the USA since 2010 (EIA 2016). The International Energy Agency estimates that the extraction of natural gas will keep increasing with projections that 25% of global energy will be derived from natural gas by 2035. This is due in large part to a tremendous increase in natural gas extraction in the USA since 2007 because of the shale gas development. Shale gas is one form of unconventional natural gas that is trapped within relatively nonporous shale formations, compared to the conventional sources found in multiple, relatively small, porous zones in various naturally occurring rock formations such as carbonates, sandstones, and siltstones. The unconventional gas reservoirs have large volumes that previously had been difficult to develop. However, advanced shale gas technologies, primarily hydraulic fracturing or "fracking," have not only improved the extraction capacity of natural gas but have also reduced natural gas costs from \$13/MM BTU to \$3/MM BTU with prices expected to remain stable for a long time (www.eia.gov), which allows CH_4 to be available as an economic substrate for bioprocesses. As the primary carbon source, glucose (corn syrup) was projected at a cost of approximately \$645/ton from a techno-economic analysis in 2011 cost (Davis et al. 2015), which is equal to \$0.194/carbon mole.[1] The price of wellhead natural gas was \$2.62/MM BTU (spot price of Henry Hub) in 2015 that gives \$146/ton or \$0.0023/carbon mole.[2] Therefore, the cost of CH_4 on per mole of carbon is eight times cheaper than the cost of glucose per mole of carbon. Taking account of CH_4 as the most reduced carbon source available, it is clearly a more suitable carbon source for the production of reduced products (e.g., fatty acids) compared with glucose.

[1] 1 ton glucose gives 33,333 (1,000,000 g/180 × 6) carbon mole. Glucose price = \$645/ton = \$645/33,333 carbon mole = \$0.0194/carbon mole.

[2] Natural gas (NG) price = \$2.62/MM BTU = \$2.62/1000 ft^3. Considering NG density of 0.7 kg/m^3 and 1 m^3 = 35 ft^3, NG price = \$2.62/20 kg = \$131/ton. Therefore CH_4 price is \$146/ton when NG contains 90% CH_4. 1 ton CH_4 gives 62,500 (1,000,000g/16) carbon mole. CH_4 price = \$146/ton = \$146/62,500carbon mole = \$0.0023/carbon mole.

Besides the fossil-based CH_4 derived from natural gas, biogas, a form of renewable energy produced from organic matter through the biological process of methanogenesis, is another major source of CH_4. Biogas has a lower energy content (400 BTU/scf) than natural gas and is mainly composed of 55–70% CH_4 and 30–45% carbon dioxide (CO_2). It may also contain small amounts of moisture, siloxane, hydrogen sulfide, ammonia, nitrogen, hydrogen, and aromatic hydrocarbons (Tsavkelova and Netrusov 2012). Biogas is commonly produced via a process called anaerobic digestion, which is a complex process that involves two stages. In the first stage, decomposition is performed by acidogenic bacteria that metabolize the waste components (primarily protein, carbohydrate, cellulose, and hemicellulose) into mainly volatile fatty acids (acetic, propionic, and butyric acid) and ammonia along with CO_2 and hydrogen (H_2) gases. In the second stage, most of the organic acids and all of the H_2 are metabolized by methanogens, with the end result being production of generating biogas.

An important source of biogas is agricultural activities typically generated from enteric fermentation taking place within ruminant animals (e.g., cows, sheep, goats, cattle, buffalo, and camels), which is responsible for 25% of anthropogenic CH_4 production (EPA 2015). Methanogenic microbial communities responsible for the enteric fermentation reside in the stomachs of these animals and convert organic matter into CH_4, which is exhaled, eructated, or released via flatus. Between 1990 and 2013, CH_4 production from agricultural activities increased by 11.3%, which is mainly due to the increased animal husbandry to provide increasing amounts of meat in the diet of the world's population. In 2014, more than 0.26 quads (257 billion cubic feet) of CH_4 was estimated to be produced from agriculture in the USA (USDA et al. 2014). Global livestock production has increased substantially since the 1960s and is expected to continue rising. Rice farming is another agricultural source of biogas. Rice paddies, which are essentially artificial wetlands, are characterized by high moisture content, oxygen depletion, and ample organic material. This creates a great environment for methanogenic communities that decompose the organic matter for CH_4 generation.

Landfill gas is another type of anthropogenic biogas typically derived from anaerobic methanogenic communities present in water and soil samples which can convert a diverse range of agricultural, industrial, domestic, and municipal wastes and biodegradable solid waste. Landfills are the third largest source of CH_4 production in the USA presenting 0.29 quads (284 billion cubic feet) of CH_4 produced in 2014 (USDA, EPA 2014). The peak production of biogas is from 5 to 7 years after the feedstock is dumped at landfills (EPA 2000). However, the bacteria will continue to decompose the buried waste and emit methane slowly for years after a landfill is closed.

8.2 Methanotrophic Bacteria

Methanotrophic bacteria are a group of bacteria that are capable of utilizing CH_4 as their sole carbon and energy source. Methanotrophs, first discovered in 1906, were isolated and characterized beginning in the 1970s by Whittenbury and his coworkers (Whittenbury et al. 1970), establishing the basis of the current classification of methanotrophic bacteria. Methanotrophic bacteria are gram-negative bacteria, and most were isolated from sewage, bogs, wetlands, lake basins, or ruminants (in other words, in environments where methane is plentiful), where they can grow well. Although methanotrophs are now known to be able to grow in both aerobic and anaerobic environments, most methanotrophs are usually specified as aerobic microorganisms that can oxidize methane to methanol and beyond for catabolism and anabolism. In this chapter, we will focus on aerobic methanotrophic bacteria and their cultivation. Readers who are interested in anaerobic methanotrophs can find more details from recent reviews (Caldwell et al. 2008; Knittel and Boetius 2009).

Methanotrophs are now classified into three groups replacing earlier categories such as Type I, Type II, and Type X (Fei et al. 2014b). Group I contains *Gammaproteobacteria* methanotrophs (formerly Type I and X) that are able to utilize the ribulose monophosphate (RuMP) cycle for single carbon assimilation, including the genera *Methylobacter Methylococcus*, *Methylohalobius*, *Methylomonas*, *Methanosphaera*, *Methylosoma*, *Methylomicrobium*, *Methanothermus*, *and Methanosarcina.* Group II contains *Alphaproteobacteria* methanotrophs (formerly Type II) capable of using the serine cycle to assimilate single carbon sources, including the genera *Methylosinus*, *Methylocapsa*, *Methylocella*, and *Methylocystis*. Recently, a new division of methanotrophs known as *Verrucomicrobia* methanotrophs have been assigned to Group III (Dunfield et al. 2007; Pol et al. 2007). This group can oxidize methane to generate metabolic energy to assimilate carbon at the level of CO_2 using the Calvin-Benson-Bassham cycle. All of the methanotrophs mentioned above are obligate methylotrophs that can only use the C1 molecules of methane, methanol, methylamine, or trimethylamine as the carbon and/or energy source. However, two recently discovered species, *Methylocella* and *Methylocystis*, are facultative methylotrophs, able to utilize not only methane/methanol as their sole energy source but also acetate and ethanol (Dedysh et al. 2005; Im et al. 2011).

Methanotrophs can metabolize CH_4, because of a unique enzyme, methane monooxygenase (MMO), which catalyzes the conversion of methane to methanol, the first intermediate in methane metabolism. Two forms of MMO are known as soluble methane monooxygenase (sMMO) present in the cytoplasm and particulate methane monooxygenase (pMMO) bound to intracellular membranes. Most methanotrophs employ pMMO when grown in the presence of copper and iron (needed for pMMO activity) (Dalton 1992), but the synthesis of sMMO is observed in copper-limited environments. In addition to copper and iron availability, a number of environmental factors can affect the type and activity level of MMO. These include nutrient availability including O_2, N, and P, gas transfer rate, pH, and temperature

(Fei et al. 2014b). There are two separate cycles (ribulose monophosphate (RuMP) cycle and serine cycle) by which formaldehyde, produced as an intermediate in methane oxidation, is assimilated into microbial biomass and metabolites (Fei et al. 2014b). The RuMP cycle is usually found in Group I methanotrophs for their C1 assimilation. These organisms have incomplete TCA cycles. Group II methanotrophs using the serine cycle to assimilate C1 substrates have functioning TCA cycles. However, some Group I methanotrophs not only use RuMP cycle but also possess genes for serine cycle enzymes (Lieberman and Rosenzweig 2004).

Metabolic engineering tools are beginning to play a critical role in the development of industrial methanotrophic biocatalysts. The development of mutagenesis techniques, gene transfer methods (knock-in and knockout of genes between chromosomal and plasmid DNA), and gene expression systems (development of promoters and other regulatory elements) offers a means to enhance nutrient uptake and alter metabolic flux toward desirable products, such as single-cell protein, chemicals, and fuels (biological conversion of gas to liquid fuels and chemicals or Bio-GTL&C), and other high-value co-products.

8.3 Applications

8.3.1 Single-Cell Protein (Amino Acids)

Because of the challenges to supply sufficient protein for the world's growing population in the 1960, the concept of using methane and methanol for the production of microbial biomass termed single-cell protein (SCP) was pursued by several companies including ICI, Hoechst, and Phillips Petroleum. SCP derived from biomass of methanotrophs is composed of nutritionally acceptable amino acids and has been mainly used as a promising replacement for animal nutrition supplement and human protein consumption. Enormous R&D efforts were devoted to SCP production by methanotrophs using CH_4 as the carbon source. *Methylococcus capsulatus* (Bath) has been researched considerably because of its high efficiency in production of SCP with CH_4 and some criteria such as amino acid composition, digestibility, and animal performance and health. Bewersdorff and Dostálek (Bewersdorff and Dostálek 1971) determined that 71% crude protein on dry cell weight base was produced in a mixed bacterial culture grown using CH_4 with a yield of 0.64 protein/g CH_4. In a continous cultivation with a dry cell weight of 2.2 g/L, the amino acid composition of the protein (g/100 g protein) was shown to be 10.2 of glutamic acid, 8.8 of aspartic acid, 7.7 of alanine, 7.0 of leucine, 5.9 of valine, 5.6 of glycine, 5.4 of lysine, 4.9 of isoleucine, and 4.3 of threonine along with smaller amounts of the other amino acids (Bewersdorff and Dostálek 1971). Protein from methanotrophic biomass has been used as animal supplement for pigs, chickens, mink, fox, dog, and fish (Øverland et al. 2010). Although several novel techniques for the production of SCP from methane were developed by Shell, viable commercial processes never materialized, largely due to economical considerations (Strong

et al. 2015). Although the increases in agricultural productivity brought on by advances made during the "Green Revolution" greatly mitigated the challenges of providing food for an increasing world population, this problem is beginning to surface again, leading to a reconsideration of the potential production of microbial protein. Due to the abundant supply and cheapness of natural gas, the production of methanotrophic protein from natural gas could be realistic on an industrial scale. UniBio A/S, a pioneer company of methanotrophic protein production, based in Odense, Denmark, opened its first commercial plant of SCP using natural gas as a nutritional food protein feed for animals in Nov 2016 (Vallenet et al. 2013). This plant improves UniBio's annual capacity of SCP production up to 80 ton. Calysta, Inc., a company located at Menlo Park, CA, USA, is also a major player focusing on the production of SCP using CH_4 as the sole carbon source. Calysta's FeedKind™ protein, a new fish and animal feed ingredient, has been targeting industrial markets of aquaculture and livestock feeds (Cantera et al. 2017).

8.3.2 Bio-Based Chemicals (Bio-GTC)

The same reason for renewed attention to the production of methanotrophic SCP makes it attractive as a potential source of biochemicals. Soluble metabolites produced by methanotrophs (e.g., biopolymer, organic acids, keto acids, carboxylic acids, ectoine, and vitamins) are all potential products from CH_4 with multiple industrial uses and high global demand. Recent interests in industrial applications have been focused on the bioconversion of natural gas/CH_4 into bulk chemicals (Bio-GTC). Methanol, formaldehyde, and formate are the initial products of CH_4 metabolism by methanotrophs. However, higher rates, yields, and titers would be necessary for economic processes. Poly-3-hydroxybutyrate (PHB) is a bio-derived and biodegradable plastic, a polyester made up of repeating units of 3-hydroxybutyric acid, with similar physical properties to other industrial plastics, such as polypropylene. The production of PHB has been observed in both Group I and Group II methanotrophs (Anthony 1982). However, the serine pathway used by Group II methanotrophs is the most efficient metabolic machinery for PHB biosynthesis (Wendlandt et al. 2001). When isocitrate dehydrogenase is used for NADPH regeneration, the theoretical yield of PHB is 0.56–0.67 g/g (Asenjo and Suk 1986; Yamane 1993), which is higher than that from glucose of 0.48 g/g and sucrose of 0.5 g/g (Yamane 1993), attesting to the higher energy content of methane compared to sugars. Wendlandt et al. reported that PHB content was as high as 51% in a continuous culture of *Methylocystis* sp. using methane as the sole carbon source (Wendlandt et al. 2001). Mango Materials, a new biology start-up founded in 2011, recently announced its new process of converting waste biogas into bioplastics instead of conventional petroleum-based plastics (Whitworth 2014). According to an economic model developed by researchers at Stanford University, the cost for PHB produced from Mango Materials' process could be as low as $1.2/kg (Roland-Holst et al. 2013).

Due to the development of genetic tools, manipulation of pathways to overproduce metabolic intermediates or compounds not naturally biosynthesized by methanotrophs is a topic of current interest. Recently, Calysta Energy announced that it has successfully demonstrated a lab-scale production of lactic acid from methane, under a research collaboration with NatureWorks (Calysta 2014). Intrexon's patent also indicates the feasibility of the production of 1-butanol, fatty alcohols, fatty acid esters, and 2,3-butanediol by metabolically engineered methanotrophs (Coleman et al. 2014). Besides the production of chemicals mentioned above, succinic acids, acetic acids, ectoine, vitamins, and astaxanthin are alternative value added products with large markets that could be produced by methanotrophs (Strong et al. 2015).

8.3.3 Biofuel (Bio-GTL)

Biofuel is another valuable product from methanotrophs with an enormous demand. A comprehensive review about the bioconversion of natural gas into liquid fuel (Bio-GTL) has been published discussing its opportunity and challenge (Fei et al. 2014b). However, until now there are still few studies regarding the feasibility of utilizing the Bio-GTL concept for the production of liquid fuels, due in large part to the lack of a robust, suitable production strain. A program developed by the Advanced Research Projects Agency-Energy (ARPA-E) of the US Department of Energy (DOE) known as Reducing Emissions using Methanotrophic Organisms for Transportation Energy (REMOTE) was initiated in 2013 to accelerate the development of economic Bio-GTL processes. A continuous gas delivery system with safety control and precautions has been developed at the National Renewable Energy Laboratory for converting CH_4 into lipids in high cell density culture of oleaginous methanotrophs—*Methylomicrobium buryatense* (Scanlon 2014). The microbial lipids can be catalytically upgraded to diesel blend stocks for biofuel. Downstream processing of microbial lipids for renewable diesel production follows a sequential catalytic upgrading process familiar to petroleum refining consisting of hydrotreating (hydroprocessing) followed by catalytic cracking and isomerization of the fatty acyl chains to fit the properties of renewable diesel. A techno-economic analysis (TEA) was performed for an integrated biorefinery process using biological conversion of methane considering such parameters as carbon yield, process efficiency, productivity (both lipid and acid), natural gas, and other raw material prices, etc. (Fei et al. 2014d). This preliminary cost analysis of Bio-GTL based solely on raw material costs and yields projected a CH_4-derived diesel cost range from \$0.7 to \$10.8/gal (Fei et al. 2014b).

Intrexon Corporation, a synthetic biology company, has formed a joint venture named Intrexon Energy Partners to scale up its Bio-GTL platform in 2014 targeting isobutanol for gasoline blending. Recently, Intrexon Corporation announced the achievement of farnesene production from CH_4, a potential feedstock for diesel fuel. Considering the recent volatility of crude oil prices and the potential for future

shortages, the utilization of CH_4 as a substrate for liquid fuel production has tremendous potential.

8.4 Safety Control

8.4.1 Explosive Limits

CH_4 is a combustible gas, which can cause fires or explosions in the presence of O_2 and an ignition source. As shown in the stoichiometric equation below, the combustion of CH_4 is highly exothermic.

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O \quad \Delta H = -890\,\mathrm{KJ/molA} \tag{8.1}$$

Therefore, the presence of CH_4 in the atmosphere is a potential hazard in industrial, commercial, and domestic environments. Due to the colorless and odorless nature of gaseous hydrocarbons, a trace amount of the organic sulfur compound methanethiol (CH_4S) is added to give commercial natural gas its distinctive smell. This safeguard makes gas leaks readily detectable and reduces the potential for serious explosion or asphyxiation. To support CH_4 combustion in air, however, the ratio of CH_4 and air/O_2 must be within well-defined explosive range, determined experimentally and referred to as explosive limits. The lower explosive limit (LEL) of CH_4 is the lowest CH_4 percentage by volume in air that can cause an explosion if ignited, and the upper explosive limit (UEL) of methane is the highest CH_4 percentage in air capable of producing an explosion (Gas 2013). These values are 5% for the LEL and 15% for the UEL. Below the LEL, CH_4 is too lean to burn, and above the UEL, CH_4 is too rich to burn. Therefore, CH_4 concentrations outside those limits are nonflammable. The potential for CH_4 explosions is also associated with the limiting oxygen concentration (LOC). The LOC is the minimum concentration of O_2 in a homogeneous mixture of CH_4, air, and an inert gas (e.g., N_2, CO_2) that will propagate a fire or explosion, independent of the concentration of CH_4 (Zlochower and Green 2009). The effect of adding an inert gas into a fuel gas mixture is to reach a level, below which this mixture can no longer be made flammable. Therefore, using air instead of pure O_2 can reduce the possibility of explosion and avoid the flammable mixture zone. The LOC for methane is 12.1 vol% and 14.6 vol% with N_2 and CO_2 as an inert gas, respectively (Hamer et al. 1967). Thus, by maintaining the O_2 concentration in a gas mixture below the LOC, certain safe operation can be predicted and guaranteed. A triangular diagram exhibits the regimes of flammability in a homogeneous mixture of CH_4, air, and N_2. This flammability diagram also depicts explosive limits (LEL and UEL), LOC, combustion stoichiometric line, and N_2 purge concentrations to avoid the flammable zone (orange-colored envelope determined experimentally). More explanations of this diagram are available (Hamer et al. 1967).

As discussed above, the flammability of CH_4 is calculated based upon its combustion stoichiometry (Eq. 8.1). However, during the culture of aerobic methanotrophic bacteria, the concentrations of CH_4 and O_2 vary with the gas specific utilization rate. For example, the utilization efficiency of CH_4 and O_2 will increase as the cell density increases and will either plateau or decrease in the stationary phase, which means the concentration of CH_4 and O_2 in the off-gas from a bioreactor will change with the growth phase of methanotrophs. Therefore the metabolic stoichiometry of methanotrophs should also be considered for the safe usage of gas mixtures during cultivation.

8.5 Control and Precaution

To grow aerobic methanotrophic bacteria, CH_4 is required as carbon source and energy source, and O_2 is required as source of the oxygen atom in methanol and as electron acceptor. Continuous supply of these two gases is mandatory during cultivation in order to maintain a healthy growth environment. Therefore, safety considerations for controls and precautions of continuous gas supply are needed before designing a bioprocess and carrying out experiments. Risk assessment is the first step to estimate the likelihood and magnitude of the occurrence of an unwanted event scenario and is used in such potentially dangerous fields, such as fire management, petroleum and natural gas extraction, processing and delivery, coal mine activity, and nuclear science and engineering. A risk assessment process for CH_4 usage in a laboratory should not only review restrictions, regulations, and standards for the institution but also consider the specifics of hazardous materials used for experiments, system pressure, size and ventilation of lab room, fire and explosion, volume/quantity, gas mixture composition, and flow rate of the gas mixture. A matrix shown in Table 8.1 is a simple tool to estimate the risk level of different scenarios.

Based on the assessment, risk management and control are also required for using CH_4 in laboratory (Schaufele 2013). In general, risk control can be categorized as administrative controls, engineering controls, and work practice controls (safe operating procedure). Administrative controls can be in the form of general policies or laboratory-specific standard operating procedures established at an administrative level and implemented by the principal investigator, laboratory supervisor, department chair, department safety committee, or office of environmental health and safety at an institute or a university. Engineering controls consist of various measures for eliminating hazards or reducing exposure to hazards at their sources before they are created. In the laboratory, examples of engineering controls for CH_4 usage include pressure relief valves, flow check valves, continuous leakage detection systems, use of nonflammable gas mixtures, limitation of maximum flow rates of CH_4 (e.g., installation of a restrictive flow orifice), and utilization of local exhaust systems such as a fume hood or ventilation system. A continuous gas delivery system for a Bio-GTL&C process can include a transient leakage detection system

Table 8.1 Risk assessment tool

	Risk Level				
	Insignificant (minor problem easily handed by normal day to day processes)	Minor (some disruption possible, e.g. damage equal to $500k)	Moderate (significant time/resources required, e.g. damage equal to $1 million)	Major (operations severely damaged, e.g. damage equal to $10 million)	Catastrophic (business survival is at risk damage, e.g. damage equal to $25 million)
Likelihood	Consequences				
Very likely (>90 chance)	High	High	Extreme	Extreme	Extreme
Likely (50-90% chance)	Moderate	High	High	Extreme	Extreme
Possible (10-50% chance)	Low	Moderate	High	Extreme	Extreme
Unlikely (3-10% chance)	Low	Low	Moderate	High	Extreme
Rare (<3% chance)	Low	Low	Moderate	High	High

that is able to turn off a solenoid valve at the CH_4 source if CH_4 is detected outside the cultivation system and an off-gas monitor system that is able to adjust the flow rate and ratio of CH_4 and air/O_2 through the mass flow controller to avoid flammable gas mixture (Hamer et al. 1967).

8.6 Cultivation of Methanotrophs

8.6.1 Operating Strategy of Methanotrophic Cultivation

Besides the safety management and control, one of the first decisions is the choice of the cultivation mode, which is essential to fermentation performances in terms of growth rate and productivity. The production of PHB and SCP by methanotrophs was a topic of interest for the last two decades. In those works, the researchers investigated batch, fed-batch, and continuous cultivation scheme for the methanotrophic culture. It is important to note that gaseous substrates (CH_4 and O_2) need to be supplied constantly during the cultivation of methanotroph to achieve high cell densities and productivities.

Batch cultivation in a closed culture system is the simplest mode for the production of cell mass and desired products (Fei et al. 2011b, 2013a, b, 2014a; Kennedy and Krouse 1999). Because nutrients (including CH_4 and O_2) are not added, nor waste products (other than CO_2) removed in the course of the entire fermentation, batch cultures can only allow a limited generation of microorganisms before growth stops. In the early stage of process development and research study, batch culture is a

good choice due to the low capital investment and simple operation. Serum vials and screw-cap bottles have been widely used in the methanotrophic cultivation for strain screening and genetic manipulation works (Whittenbury et al. 1970). In order to overcome the limitations of low cell density and productivity in batch cultivation, fed-batch cultures with controlled nutrient feeding are more suitable at large scale.

For the fed-batch culture of methanotrophs, one or more nutrients (especially CH_4, O_2, and nitrogen sources) are supplemented to the sealed container or bioreactor during cultivation, and the primary product remains in the bioreactor until the end of the fermentation (Fei et al. 2011a, 2015, 2016; Kennedy and Krouse 1999; Park et al. 2014). A higher cell density culture of methanotrophs can be achieved easily by only adding CH_4 and O_2 continuously (Fei et al. 2014c). In contrast to the closed/semi-closed system of batch/fed-batch culture, an open system with continuous cultivation provides steady-state cell growth rate by continuously supplying fresh medium as well as gaseous substrates such as CH_4 and O_2 for the methanotrophic cultivation.

This third option has several advantages over batch and fed-batch operations, including minimizing equipment downtime and production time lost due to the lag phase of the microbial culture. Shah et al. reported that both cell-specific growth rate and pMMO productivity were improved in a continuous culture of *M. trichosporium* OB3b (Park et al. 1992). Although the limitation of CH_4 and O_2 can be solved in fed-batch and continuous cultures, the mass transfer of these gases in the aqueous phase is the greatest technical challenge for the methanotrophic cultivation at large scale.

8.7 Mass Transfer Efficiency

Gas-liquid mass transfer efficiency is one of the primary technology obstacles in aerobic microorganism cultivation. The mass transfer efficiency, which can be expressed as the volumetric mass transfer coefficient $K_L a$, has been described by Klasson (Klasson et al. 1993), as shown in the following equation:

$$\boldsymbol{\gamma}_S^G = \frac{K_L a}{H}\left(P_S^G - P_S^L\right)$$

where $\boldsymbol{\gamma}_G$ (mol/L/h) is the volumetric mass transfer rate of the gaseous substrate; P_S^G and P_S^L (atm) are the partial pressures of the gaseous substrate in gas and liquid phase, respectively; and H (L×atm/mol) is Henry's law constant. According to the above equation, it is obvious that the partial pressure differential (between gas and liquid phase) is the main driving force for mass transfer and thus controls the availability of the substrate. Since H is a temperature-dependent constant, the mass transfer efficiency is affected by the culture temperature as well.

Because of the importance of gaseous substrates, CH_4 and O_2, gas mass transfer efficiency is one of the most important parameters in methanotrophic cultivation. The low solubility of CH_4 (22.7 mg/L) and O_2 (14.6 mg/L) in the water/fermentation

broth significantly limits their consumption and conversion efficiency for the cell growth of methanotrophs and calls for continuous transfer from the gas phase to the liquid phase. In order to improve the gas transfer rate, gas molecules need to travel into gas-liquid interface and subsequently diffuse efficiently through the culture media and then through the microbial cell surface, where they can participate in metabolic reactions. In an ideal situation, the bubble size of gaseous substrate should be small and uniform throughout the bioreactor. Therefore, significant effort has gone into the design of bioreactors that can provide a higher mass transfer rate by generating more gas-liquid-cell interfacial area from smaller and uniform gas bubbles.

8.8 Bioreactor Design

Because both CH_4 and O_2 acting as the electron donor and acceptor are gaseous and the solubility of these two gases is extremely low in the culture medium, the cultivation of methanotrophs will be severely limited by the mass transfer efficiency. Therefore, the choice of the bioreactor and the operating strategy for methanotrophic cultivation is one of the most important decisions in regard to the bioprocess development, which determines the final product titer, carbon conversion efficiency, and productivity and whether sustainable, reliable performance can be achieved. In addition to the bioreactor design and culture strategy, other aspects (e.g., the composition of gas mixture) should also be considered for high mass transfer. The gas mixture topics to be considered are air/pure O_2, pure methane, natural gas, biogas, and source of natural gas and biogas to provide workable mixtures of CH_4, higher alkanes, CO_2, and minor contaminants such as H_2S. Alvarez-Cohen reported that the highest cell growth of methanotrophs in a suspended cultivation is estimated at 130–200 mg/L/h with feeding CH_4/O_2 mixture and 40–70 mg/L/h with feeding CH_4/air mixture (Alvarez-Cohen 1993). The use of the water-immiscible organic solvent for improving methane transfer rate has been tested to achieve a high cell density culture (Han et al. 2009). Recently, a method of adding nanoparticles with functional groups into gas fermentation system has been developed to exploit the extensive adsorption capability of the functionalized nanoparticles to increase the mass transfer coefficiency (Zhu et al. 2010).

8.8.1 Continuous Stirred-Tank Bioreactors

Continuous stirred-tank bioreactors (CSTRs) are the most widely used bioreactors for growing methanotrophic bacteria. By increasing the agitation speed or modifying the bioreactor's impellor, smaller-size bubbles can be generated for increasing the gas-liquid interfacial area. However, high shear rates from excessive agitation could damage cells and inhibit growth rate, and power input for these strategies greatly

reduce the economic viability of industrial-scale fermentations. Consequently, microbubble sparging reactors, bubble column reactors, loop and airlift reactors, trickle-bed reactors, and membrane-based reactors are some of the other configurations developed for enhancing the mass transfer efficiency.

8.8.2 Microbubble Sparging CSTRs

In order to generate smaller gas bubbles, a microbubble distributor has been developed and equipped for CSTRs. A microbubble sparger breaks up the gaseous substrate into extremely fine and, in some cases, surfactant-stabilized bubbles in a high shear zone, which have lower rise velocities for longer liquid retention time (Bredwell et al. 1999). Compared with traditional CSTRs, the microbubble distributor provides a more energy efficient method to increase mass transfer efficiency.

8.8.3 Bubble Column Bioreactors

Bubble column bioreactors that are basically a cylindrical vessel with a gas sparging system at the bottom are widely used for industrial applications with large working volumes due to advantages in design and operation as compared to other reactors. Excellent heat and mass transfer, low maintenance requirements, and low operational costs are primary merits for methanotrophic cultivation. It was found that a bubble column reactor provided a higher conversion rate of methane than CSTR did, which resulted in a threefold greater propylene oxide titer (Hill et al. 1990).

8.8.4 Loop and Airlift Bioreactors

Similar to the mechanical agitation of bubble column bioreactors, loop and airlift bioreactors are characterized by recirculating the liquid in a defined cyclic pattern through channels that connect the gas-liquid separator on top of the main bubbling section or riser to its lower part. The patterns of fluid circulation are designed specifically for enhancing the mass transfer. It was found that a forced-liquid vertical tubular loop bioreactor can provide a twofold cell density improvement compared with an external airlift loop reactor and a horizontal tubular loop bioreactor (Yazdian et al. 2012). Rahnama et al. developed and compared a bubble column and a vertical loop reactor for the PHB production from natural gas, and a PHB content of 51% w/w was achieved in their vertical tubular loop bioreactor (Rahnama et al. 2012). UNIBIO, a company from Denmark, has also demonstrated commercial production of SCP as animal feed from methane using a patented U-loop bioreactor (UNIBIO 2011).

8.8.5 Trickle-Bed Bioreactor

Unlike bubble column bioreactors, gas and liquid in a trickle-bed bioreactor move cocurrently downward over a packed bed, or gas is fed countercurrently upward, while the liquid moves downward. Trickle-bed bioreactors have been mainly used for waste gas removal by methanotrophs (Reij et al. 1998). In the trickle-bed reactor, methanotrophic bacteria as catalysts are packed on the bed, which allows them to degrade the pollutants after the waste gas diffuses through the water phase. The mass transfer rate in the trickle-bed reactor is relatively dependent on the gas flow rate and operating temperature (Barton et al. 1999).

8.8.6 Monolithic Biofilm Bioreactors

Monolithic biofilm bioreactors resemble trickle-bed reactors, in which the gaseous substrate is allowed to pass through a bed of carrier material with low-pressure drop. Microorganisms are present as a biofilm attached on the bed, which have been used in CH_4 bioconversion platforms. In the culture of methanotrophs, attached methanotrophic bacteria in the biofilm utilize CH_4 efficiently for the production of extracellular products and cell mass (Arcangeli and Arvin 1999).

8.8.7 Membrane Biofilm Bioreactors

In a membrane biofilm bioreactor (MBfR), a biofilm is directly attached to a membrane instead of carrier media in monolithic biofilm bioreactors. Gases pass through the interior of a membrane to a biofilm growing on the membrane surface. Composite hollow fiber MBfRs have been proposed as technologically and economically feasible for nitrate and trichloroethylene (TCE) removal from contaminated waters with methane (Rishell et al. 2004). In the MBfRs, CH_4 and O_2 are diffused through the walls of membranes without forming bubbles. The methanotrophic biofilms on the outer wall of the membranes continuously consume gaseous substrates and pollutants for the development of a thick biofilm, which gave a TCE removal efficiency up to 90% (Clapp et al. 1999). This innovative MBfR offers a significant advantage in providing a high transfer rate of sparingly soluble gaseous substrates directly to the microorganisms, preventing losses to the atmosphere or effluent liquid and potentially reducing the gas volume (Henstra et al. 2007).

8.9 Conclusions and Future Perspectives

In this chapter, we have reviewed current knowledge of bioconversion of methane derived from either natural gas or biogas into high-value chemical products and biofuels instead of conventional methane application such as electricity generation or heating. Methane-derived products could replace a significant amount of petroleum-based commodities in the USA while at the same time capturing value from a wasted resource and mitigating climate change issues exacerbated by vented and flared natural gas. With the advent of economic and efficient tools of systems biology especially genomics, transcriptomics, and metabolomics, the potential to construct recombinant methanotrophic bacteria for methane-derived products is becoming much more accessible. Nevertheless, the challenges in moving from proof of concept to scale-up and commercialization still remain to be solved. The biggest technical challenges for methane bioconversion in commercial-scale application remain to be the mass transfer efficiency of poorly soluble gaseous substrates in the culture medium. Consequently, the development of bioreactors for methane fermentation has been the most popular research topic within recent years. CSTR, microbubble sparging CSTR, bubble columns reactors, loop reactors, airlift reactors, trickle-bed reactors, and membrane-based reactors are some of reactor types that have been investigated to improve mass transfer efficiency. The organic solvent and nanoparticle addition can also enhance the mass transfer but with a high cost. Meanwhile, efforts to optimize culture medium and fermentation conditions as well as exploring bioprocess technology are being pursued to enhance productivity and reduce production cost. Besides the consideration of the optimization of the bioprocess control, the safety assessment, risk management, and engineering and administrative control for CH_4 usage are also essential to the entire bioprocess.

Take-Home Message

- As a promising class of biocatalysts, methanotrophs are able to convert the CH_4 derived from natural gas or biogas into single-cell protein (SCP), biochemicals, and biofuels.
- Safety controls for CH_4 use are available and are a key factor for the development of bioconversion of CH_4 at both laboratory and industrial scales.
- To improve the mass transfer efficiency of CH_4 in batch, fed-batch, and continuous culture modes for the methanotrophic cultivation, bubble column bioreactor, loop and airlift bioreactor, trickle-bed bioreactor, monolithic biofilm bioreactor, and membrane biofilm bioreactor have been explored and have shown promise in enhancing both methanotroph growth and product kinetics.

Acknowledgments This work was supported by Key Research and Development Plan (2017GY-146) of Shaanxi Province of China and the Advanced Research Projects Agency-Energy (ARPA-E) of the US Department of Energy.

References

Alvarez-Cohen L (1993) Application of methanotrophic oxidations for the bioremediation of chlorinated organics. Microbial growth on C1 compounds, pp 337–350

Anthony C (1982) The biochemistry of methylotrophs. Academic, London

Arcangeli J-P, Arvin E (1999) Modelling the growth of a methanotrophic biofilm: estimation of parameters and variability. Biodegradation 10:177–191

Asenjo JA, Suk JS (1986) Microbial conversion of methane into poly-β-hydroxybutyrate (PHB): growth and intracellular product accumulation in a type II methanotroph. J Ferment Technol 64:271–278

Barton JW, Davison BH, Klasson KT, Gable CC (1999) Estimation of mass transfer and kinetics in operating trickle-bed bioreactors for removal of VOCS. Environ Prog 18:87–92

Bewersdorff M, Dostálek M (1971) The use of methane for production of bacterial protein. Biotechnol Bioeng 13:49–62

Bousquet P, Ciais P, Miller J, Dlugokencky E, Hauglustaine D, Prigent C et al (2006) Contribution of anthropogenic and natural sources to atmospheric methane variability. Nature 443:439–443

Bredwell MD, Srivastava P, Worden RM (1999) Reactor design issues for synthesis-gas fermentations. Biotechnol Prog 15:834–844

Caldwell SL, Laidler JR, Brewer EA, Eberly JO, Sandborgh SC, Colwell FS (2008) Anaerobic oxidation of methane: mechanisms, bioenergetics, and the ecology of associated microorganisms. Environ Sci Technol 42:6791–6799

Calysta I (2014) Calysta demonstrates lab-scale production of lactic acid from methane. http://calysta.com/2014/06/calysta-demonstrates-lab-scale-production-lactic-acid-methane-completes-successful-first-stage-multi-year-rd-development-collaboration-natureworks/2014

Cantera S, Lebrero R, Rodríguez E, García-Encina PA, Muñoz R (2017) Continuous abatement of methane coupled with ectoine production by Methylomicrobium alcaliphilum 20Z in stirred tank reactors: a step further towards greenhouse gas biorefineries. J Clean Prod 152:134–141

Clapp LW, Regan JM, Ali F, Newman JD, Park JK, Noguera DR (1999) Activity, structure, and stratification of membraneattached methanotrophic biofilms cometabolically degrading trichloroethylene. Water Sci Technol 39:153–161

Coleman WJ, Vidanes GM, Cottarel G, Muley S, Kamimura R, Javan AF, et al (2014) Biological conversion of multi-carbon compounds from methane. Google Patents

Dalton H (1992) Methane oxidation by methanotrophs. Physiological and mechanistic implications. Biotechnology handbook

Davis R, Tao L, Scarlata C, Tan E, Ross J, Lukas J, et al (2015) Process design and economics for the conversion of lignocellulosic biomass to hydrocarbons: dilute-acid and enzymatic. National Renewable Energy Laboratory

Dedysh SN, Knief C, Dunfield PF (2005) Methylocella species are facultatively methanotrophic. J Bacteriol 187:4665–4670

Dunfield PF, Yuryev A, Senin P, Smirnova AV, Stott MB, Hou S et al (2007) Methane oxidation by an extremely acidophilic bacterium of the phylum Verrucomicrobia. Nature 450:879–882

EIA (2016) Natural Gas Summary. US Department of Energy. http://www.eia.gov/dnav/ng/ng_sum_lsum_dcu_nus_a.htm

EPA (2000) Facts About Landfill Gas. United States Environmental Protection Agency. http://www.dem.ri.gov/programs/benviron/waste/central/lfgfact.pdf

EPA (2015) Inventory of U.S. greenhouse gas emissions and sinks: 1990-2013. US Environmental Protection Agency

Fei Q, Chang HN, Shang L, Choi J-D-R (2011a) Exploring low-cost carbon sources for microbial lipids production by fed-batch cultivation of Cryptococcus albidus. Biotechnol Bioprocess Eng 16:482–487

Fei Q, Chang HN, Shang L, Kim N, Kang J (2011b) The effect of volatile fatty acids as a sole carbon source on lipid accumulation by Cryptococcus albidus for biodiesel production. Bioresour Technol 102:2695–2701

Fei Q, Brigham CJ, Lu J, Sinskey AJ (2013a) Production of branched-chain alcohols by recombinant Ralstonia eutropha in fed-batch cultivation. Biomass Bioenergy 56:334–341

Fei Q, Smith H, Dowe N, Pienkos PT (2013b) Effects of culture medium ingredients on cell growth in batch cultures of Methylomicrobium buryatense with methane as a sole carbon source. Recent Advances in Fermentation Technology (RAFT). http://sim.confex.com/sim/raft10/webprogrampreliminary/Paper25952.html

Fei Q, Fu R, Shang L, Brigham CJ, Chang HN (2014a) Lipid production by microalgae Chlorella protothecoides with volatile fatty acids (VFAs) as carbon sources in heterotrophic cultivation and its economic assessment. Bioprocess Biosyst Eng:1–10

Fei Q, Guarnieri MT, Tao L, Laurens LML, Dowe N, Pienkos PT (2014b) Bioconversion of natural gas to liquid fuel: opportunities and challenges. Biotechnol Adv 32:596–614

Fei Q, Smith H, Dowe N, Pienkos PT (2014c) Effects of culture conditions on cell growth and lipid production in the cultivation of Methylomicrobium buryatense with CH4 as the sole carbon source. SIMB annual meeting and exhibition, July 20–24, 2014, St. Louis

Fei Q, Tao L, Pienkos PT, Guarnieri M, Palou-Rivera I (2014d) Techno-economic analysis of bioconversion of methane into biofuel and biochemical. The 11th bioprocess international conference and exhibition, Oct 20–24 2014. BioProcess International, Boston

Fei Q, Wewetzer SJ, Kurosawa K, Rha C, Sinskey AJ (2015) High-cell-density cultivation of an engineered Rhodococcus opacus strain for lipid production via co-fermentation of glucose and xylose. Process Biochem 50:500–506

Fei Q, O'Brien M, Nelson R, Chen X, Lowell A, Dowe N (2016) Enhanced lipid production by Rhodosporidium toruloides using different fed-batch feeding strategies with lignocellulosic hydrolysate as the sole carbon source. Biotechnol Biofuels 9:130

Gas L (2013) Lower and upper explosive limits for flammable gases and vapors (LEL/UEL). Matheson Gas Products, p 22

Hamer G, Hedén CG, Carenberg CO (1967) Methane as a carbon substrate for the production of microbial cells. Biotechnol Bioeng 9:499–514

Han B, Su T, Wu H, Gou Z, Xing X-H, Jiang H et al (2009) Paraffin oil as a "methane vector" for rapid and high cell density cultivation of Methylosinus trichosporium OB3b. Appl Microbiol Biotechnol 83:669–677

Henstra AM, Sipma J, Rinzema A, Stams AJ (2007) Microbiology of synthesis gas fermentation for biofuel production. Curr Opin Biotechnol 18:200–206

Hill A, Kelley R, Srivastava V, Akin C, Hayes T, Frank J (1990) Bench-scale co-oxidative production of propylene oxide by methanotrophs. Institute of Gas Technology, Chicago, IL

Im J, Lee SW, Yoon S, DiSpirito AA, Semrau JD (2011) Characterization of a novel facultative Methylocystis species capable of growth on methane, acetate and ethanol. Environ Microbiol Rep 3:174–181

Kennedy M, Krouse D (1999) Strategies for improving fermentation medium performance: a review. J Ind Microbiol Biotechnol 23:456–475

Klasson K, Gupta A, Clausen E, Gaddy J (1993) Evaluation of mass-transfer and kinetic parameters for Rhodospirillum rubrum in a continuous stirred tank reactor. Appl Biochem Biotechnol 39:549–557

Knittel K, Boetius A (2009) Anaerobic oxidation of methane: progress with an unknown process. Annu Rev Microbiol 63:311–334

Lieberman RL, Rosenzweig AC (2004) Biological methane oxidation: regulation, biochemistry, and active site structure of particulate methane monooxygenase. Crit Rev Biochem Mol Biol 39:147–164

Øverland M, Tauson A-H, Shearer K, Skrede A (2010) Evaluation of methane-utilising bacteria products as feed ingredients for monogastric animals. Arch Anim Nutr 64:171–189

Park S, Hanna ML, Taylor RT, Droege MW (1992) Batch cultivation of Methylosinus trichosporium OB3b .3. Production of particulate methane monooxygenase in continuous culture. Biotechnol Bioeng 40:705–712

Park GW, Fei Q, Jung K, Chang HN, Kim Y-C, Kim N-j et al (2014) Volatile fatty acids derived from waste organics provide an economical carbon source for microbial lipids/biodiesel production. Biotechnol J 9:1536–1546

Pol A, Heijmans K, Harhangi HR, Tedesco D, Jetten MS, den Camp HJO (2007) Methanotrophy below pH 1 by a new Verrucomicrobia species. Nature 450:874–878

Rahnama F, Vasheghani-Farahani E, Yazdian F, Shojaosadati SA (2012) PHB production by Methylocystis hirsuta from natural gas in a bubble column and a vertical loop bioreactor. Biochem Eng J 65:51–56

Reij MW, Keurentjes JT, Hartmans S (1998) Membrane bioreactors for waste gas treatment. J Biotechnol 59:155–167

Rishell S, Casey E, Glennon B, Hamer G (2004) Characteristics of a methanotrophic culture in a membrane-aerated biofilm reactor. Biotechnol Prog 20:1082–1090

Roland-Holst D, Triolo R, Heft-Neal S, Bayrami B (2013) Bioplastics in California

Scanlon B (2014) NREL working to clean air in fracking process. http://www.nrel.gov/news/features/feature_detail.cfm/feature_id=73002014

Schaufele ME (2013) Toxic and flammable gases in research laboratories: considerations for controls and continuous leak detection. J Chem Health Saf 20:8–14

Strong P, Xie S, Clarke WP (2015) Methane as a resource: can the methanotrophs add value? Environ Sci Technol 49:4001–4018

Tsavkelova E, Netrusov A (2012) Biogas production from cellulose-containing substrates: a review. Appl Biochem Microbiol 48:421–433

UNIBIO (2011) UniProtein. UniBio A/S. http://www.unibio.dk/?page_id=673

USDA, EPA, DOE (2014) Biogas opportunities roadmap. In: U.S. Department of Agriculture, U.S. Environmental Protection Agency, Energy USDo, editors. www.usda.gov

Vallenet D, Belda E, Calteau A, Cruveiller S, Engelen S, Lajus A et al (2013) MicroScope – an integrated microbial resource for the curation and comparative analysis of genomic and metabolic data. Nucleic Acids Res 41:D636–DD47

Wendlandt KD, Jechorek M, Helm J, Stottmeister U (2001) Producing poly-3-hydroxybutyrate with a high molecular mass from methane. J Biotechnol 86:127–133

Whittenbury R, Phillips K, Wilkinson J (1970) Enrichment, isolation and some properties of methane-utilizing bacteria. J Gen Microbiol 61:205–218

Whitworth J (2014) Mango materials moves on methane gas for bioplastics. http://www.foodproductiondaily.com/Innovations/Mango-Materials-moves-on-methane-gas-for-bioplastics: http://www.foodproductiondaily.com

World Bank (2013) Global gas flaring reduction partnership. http://go.worldbank.org/016TLXI7N0

Yamane T (1993) Yield of poly-D(-)-3-hydroxybutyrate from various carbon-sources – a theoretical-study. Biotechnol Bioeng 41:165–170

Yazdian F, Shojaosadati SA, Nosrati M (2012) Mixing studies in loop bioreactors for production of biomass from natural gas. Iran J Chem Chem Eng 31:91

Zhu H, Shanks BH, Choi DW, Heindel TJ (2010) Effect of functionalized MCM41 nanoparticles on syngas fermentation. Biomass Bioenergy 34:1624–1627

Zlochower IA, Green GM (2009) The limiting oxygen concentration and flammability limits of gases and gas mixtures. J Loss Prev Process Ind 22:499–505