SynBio

Review

Can *Methylococcus capsulatus* Revolutionize Methane Capture and Utilization for Sustainable Energy Production?

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Abstract: Methane is the second largest contributor to global warming after carbon dioxide. Once it is released into the atmosphere, methane lingers for over 10 years, during which it traps heat, contributes to the formation of ground-level ozone, and affects air quality adversely. Conversely, methane has some benefits that could be harnessed to address its impact on the environment while utilizing it for good. Methane's significant role in global warming and potential for energy production and other beneficial applications necessitate the adoption of innovative solutions to remediate the gas from the atmosphere and harness some of its benefits. This article explores *Methylococcus capsulatus*, a methanotrophic bacterium, and its potential for revolutionizing sustainable methane capture and utilization. With its unique metabolic abilities, *M. capsulatus* efficiently oxidizes methane, making it a promising candidate for biotechnological applications. We review current research in its current and potential applications in methane capture and utilization, emphasizing key characteristics, implementation challenges, benefits, and limitations in methane capture and conversion. We also highlight the importance of interdisciplinary collaborations and technological advancements in synthetic biology to maximize its energy production potential. Our article analyzes *M. capsulatus*' role in addressing methane-related environmental concerns and advancing sustainable energy solutions.

Keywords: methane capture; biotechnology; sustainable energy; methane utilization

Citation: Akinsemolu, A.A.; Onyeaka, H.N. Can *Methylococcus capsulatus* Revolutionize Methane Capture and Utilization for Sustainable Energy Production? *SynBio* **2024**, *2*, 311–328. https://doi.org/10.3390/synbio2030019

Received: 24 June 2024
Revised: 27 August 2024
Accepted: 30 August 2024
Published: 4 September 2024

1. Introduction

The escalating concentrations of atmospheric methane resulting from various anthropogenic activities have spurred an urgent need for effective climate change mitigation strategies. Methane, recognized as an exceptionally potent greenhouse gas with a 25-fold greater global warming potential than carbon dioxide, demands focused attention in climate mitigation endeavors [1–4]. Contributing factors such as the burning of biomass, industrial treatment processes, coal mining, and unsustainable agricultural practices have substantially augmented atmospheric methane levels [5–9]. Efforts to capture and utilize methane are pivotal for environmental preservation. While effective, albeit to a limited extent, traditional methods of methane capture and utilization, including containment and transportation for use or combustion, face limitations in terms of efficiency, cost-effectiveness, and environmental impact [10]. Thus, alternative approaches are imperative, especially those that maximize methane capture while mitigating environmental concerns [11,12]. In this context, microbial-based biotechnologies have emerged as promising solutions for methane capture and utilization [13]. Among these biotechnologies, *Methylococcus capsulatus* has attracted widespread interest due to its ability to use methane as both a carbon and energy source. Additionally, the bacteria contain methane monooxygenase (MMO), an enzyme that facilitates the conversion of methane into methanol precursor for biofuel production [14,15]. Its distinct attributes include a high-affinity methane uptake system and the presence of particulate methane monooxygenase (pMMO), which positions the bacteria as a promising candidate for methane capture and utilization [16,17].

However, the full potential of *M. capsulatus* is accompanied by some challenges. For instance, the viability of the use of the bacteria for methane capture and utilization requires its enhancement or adaptation to optimize the conversion of methane into methanol. The second challenge is the need for the scalability of the use of the bacteria to harness the benefits of methane from laboratory to industrial levels. Third, scaling the use of the bacteria for the purpose under consideration requires its enhancement to improve its long-term stability under industrial conditions, necessitating innovative solutions such as genetic engineering to enhance its adaptation for industrial use. Additionally, economic feasibility and adherence to regulatory frameworks are critical for the large-scale implementation of *M. capsulatus*-based systems. This review aims to comprehensively explore potential solutions to these challenges by exploring the potential of *M. capsulatus* in methane capture and utilization. Through a comprehensive synthesis of existing research, this review seeks to elucidate the characteristics, metabolic capabilities, mechanisms, challenges, and potential solutions associated with the use and further adaptation of *M. capsulatus* for methane capture and utilization. Furthermore, it will go into the benefits of value-added products and discuss future directions for research in sustainable energy production and climate change mitigation strategies. Through this exploration, the review aims to contribute to the broader understanding and advancement of environmentally conscious practices and the contributions of synthetic biology.

This review also highlights the innovative potential of *M. capsulatus* towards methane metabolism. By presenting the bacterium and its capability to make a major contribution to sustainable energy practices, this review connects biotechnology with environmental concerns, with a view of presenting a dual solution for capturing methane, one of the most effective greenhouse gases, while producing bioenergy at the same time. This originality is further expanded with a presentation of genetic and metabolic engineering processes for *M. capsulatus* for applications in industries, which offers a view on elevating methane conversion efficiency. Further, the integration of real case studies about the practical use and possibility of scale of this strategy presents a new perspective, addressing real-world problems and options. The review is supported by microbiology, and environmental biology, as well as an energy policy approach that adds to this direction, which, by providing a comprehensive solution, considers the technical side of the problem and the environmental repercussions of methane capture. In addition, a comparative analysis with the other methane utilization technologies allows for the positioning of this approach within the context of the more comprehensive category of sustainable energy solutions, with the potential to revolutionize the field.

This literature review adopts a comprehensive approach to synthesize and analyze existing research on the methane capture and utilization capabilities of *M. capsulatus*. The methodology involves the following steps:

2. Methodology

2.1. Literature Search and Selection Criteria

A systematic search was conducted across reputable academic databases, including PubMed, ScienceDirect, and Google Scholar. The search terms included, but were not limited to, "*Methylococcus capsulatus*", "methane capture", "methane utilization", and related keywords. The inclusion criteria involved articles and reviews published in peer-reviewed academic journals, research papers from conferences, and postgraduate theses, that were all released in the last 10 years. The literature chosen for this study concentrates on the features, metabolic versatility, working principles, limitations, and viable applications of *M. capsulatus* in methane capture and exploitation.

2.2. Data Extraction and Synthesis

The information drawn from the articles pertinent to *M. capsulatus* was extracted and compiled according to themes regarding the organism's specifics and its metabolic function in the process of CH_4 capture and its utilization, limitations, and possibilities.

Focus was made on the latest advancement in genetic and metabolic engineering along with the lower-value-added steps that contributed to the maximization of the conversion of methane to methanol.

3. Characteristics and Metabolic Capabilities

M. capsulatus is a methane-oxidizing bacterium in the *Methylococcaceae* family. It is an aerobic methanotroph that exhibits mainly Gram-negative properties. The organism is versatile in habitat, and has been found in terrestrial habitats, freshwater bodies, and aquatic environments [16,17]. Given the unique features of *M. capsulatus*, it is well suitable for the capture and use of methane [16,17]. *M. capsulatus* has an advanced methane capture mechanism that makes it capable of detaching methane rather selectively from its environment. This capability is coupled with the particulate methane monooxygenase (pMMO) gene expression, which has higher affinities than the soluble methane monooxygenase (sMMO) found in the other strains. This helps in enhancing the efficiency of the bacteria's uptake of methane even in low concentrations, particularly in methane-rich niches [4,18,19]. Essentially, *M. capsulatus* can convert methane to methanol by its use of the MMO enzymes. Hence, it plays a role in the provision of a cheap source of manufacturing chemicals and biofuels while adequately supporting the increasing demand for energy [20–22]. Further, the bacteria have metabolic versatility in terms of operations under different methane concentrations, temperature regimes, and nutrient availability; specifically, for industrial processes it can operate optimally under varying concentrations of methane, changing temperatures and fluctuations in nutrient supplies. Furthermore, recent studies have identified genetic and metabolic engineering strategies to boost *M. capsulatus'* performance by improving methane uptake rates and optimizing methanol production pathways while promising to further increase its efficiency and scalability as a methane capture and utilization technology [23,24].

3.1. A Comparison of Methylococcus capsulatus with Other Organisms

A study by Indrelid et al. [25] characterized the immune modulatory properties of *M. capsulatus* and compared its immunological properties to those of another Gram-negative gammaproteobacterium, the commensal *Escherichia coli* K12, and the immune modulatory Gram-positive probiotic bacterium, *Lactobacillus rhamnosus* GG, in vitro. *M. capsulatus* induces intermediate phenotypic and functional DC maturation. In a mixed lymphocyte reaction, *M. capsulatus*-primed monocyte-derived dendritic cells (MoDCs) enhance T cell expression of CD25, the γ-chain of the high affinity IL-2 receptor, support cell proliferation, and induce a T cell cytokine profile different from both *E.coli* K12 and *L. rhamnosus* GG. *M. capsulatus.* Bath thus interacts specifically with MoDCs, affecting MoDC maturation, cytokine profile, and subsequent MoDC-directed T cell polarization. *M. capsulatus* and other methanotrophs are not the only microorganisms that can oxidize methane and have applications in the production of biofuels through the mechanism. Archaea, too, have the capacity for methane oxidation. However, the two microorganisms differ fundamentally in their approach to methane oxidation. While the bacteria oxidize methane aerobically, the archaea do so anaerobically [26]. Notably, most efforts to oxidize methane and adapt the mechanisms involved in the process for the production of renewable energy focus primarily on methanotrophs such as *M. capsulatus.* This is due to the limitations of the use and genetic engineering of the microorganisms involved in the anaerobic oxidation of methane, particularly the inherent inability to obtain pure cultures of the archaea needed for the process.

3.2. Mechanism of Methane Capture and Utilization by Methylococcus capsulatus

The mechanisms through which *M. capsulatus* captures and utilizes methane, a robust greenhouse gas, involve the following processes of enzymology: uptake (absorption), oxidation, and conversion to usable products [27,28]. This process starts with the uptake or absorption of methane from the environment, provided for by special transport proteins

in the bacterial cell membrane, selective for methane. As the methane moves into the cell, it is oxidized through an enzymatic reaction catalyzed by methane monooxygenase [29]. This enzyme will oxidize methane to methanol, which is an important intermediate in the metabolic pathway. After oxidation, methanol is metabolized through other chemical reactions, which include methanol dehydrogenase, whereby it is converted to formaldehyde and eventually utilized as biomass and energy [30]. These enzymatic transformations not only assist in the efficient usage of methane but are essential in the regulation of atmospheric methane concentrations within the atmosphere of the Earth, thereby reducing climatic change. These enzymatic processes are quite efficient and specific [31,32]. They establish the bacterium's potential to exist in environments with high methane contents.

3.2.1. Methane Capture

M. capsulatus is a methanotrophic bacterium that has an intricate and efficient system for the uptake of methane in the form of an enzyme complex called particulate methane monooxygenase (pMMO). This complex is crucial to the activity within the bacterium regarding the required processing of methane from the environment. The pMMO enzyme complex is made up of several subunits, such as PmoA and PmoB, involved in the first steps of methane capture and conversion [33]. The capture process begins with the reaction of methane (CH_4) with ferrous iron (Fe^{2+}) incorporated in the pMMO complex, forming a transient methane–iron complex (CH_4-Fe (II)) [34]. This complex is represented as:

$$CH_4 + Fe\ (II) \rightarrow CH_4\text{-}Fe\ (II).$$

This intermediate state also involves the binding of methane to the ferrous iron, as this element is very important in the activation of methane molecules. Ferrous iron serves to enhance the catalytic activity by bringing about the necessary transformation of methane to enable it to reach an active state [35]. This interaction precedes the phase of methane oxidation in which methane is converted into methanol (CH_3OH). The enzyme pMMO plays this oxidative role by inserting oxygen into the methane molecule, thereby converting it to methanol. This conversion is critical for the bacterium, because methanol directly participates in other metabolic pathways that produce cell biomass and energy [36].

3.2.2. Methane Oxidation

After methane is captured, it is oxidized by *M. capsulatus* using two main enzyme systems, which are particulate methane monooxygenase (pMMO) and soluble methane monooxygenase (sMMO). These systems all work in fundamentally different ways to convert methane into useful products, in response to a range of conditions and biochemical contexts [28,37].

a. The pMMO Route: The pathway involving particulate methane monooxygenase (pMMO) is a primary process of methane utilization in *M. capsulatus*. pMMO is an integral membrane enzyme complex that is selectively localized in the bacterial cell membrane. It comprises several copper ions incorporated in the active site of the enzyme, which has central importance for its enzymatic function. In this pathway, methane (CH_4) is oxidized in a copper-dependent process [14,38]. During this oxidation process, there is oxidation of ferrous iron (Fe^{2+}) which is incorporated within the enzyme into ferric iron (Fe^{3+}) which is also involved in the reaction cycle of the enzyme [39]. This process commences when methane molecules are bound to the copper centers within the active site of the pMMO enzyme. The copper centers are necessary for the binding of molecular oxygen (O_2) and for the subsequent oxidation of methane. This reaction oxidizes methane to methanol (CH_3OH) and water (H_2O) [40]. The overall reaction for this pathway is:

$$\text{Complex } CH_4\text{-Fe (II)} + O_2 \rightarrow CH_3OH + H_2O + \text{Fe (III) Species}$$

The general efficiency of the pMMO system is regulated by the accumulated amount of copper ions; the configuration of the enzyme structural integrity; and the density of metabolic activity of the host bacteria. pMMO can effectively catalyze methane oxidation, and this plays a role in the bacterium's metabolic activities and provides insight into the possible uses of this bacterium in environmental methane management.

b. The sMMO Route: Soluble methane monooxygenase (sMMO) is another form of enzymatic system for the oxidation of methane found in *M. capsulatus*. sMMO on the other hand is an enzyme complex, soluble and active in the bacterial cytoplasm. It needs other co-factors to function, including iron and alpha-ketoglutarate, which are fundamental to the functioning of the enzyme [38]. The sMMO system functions through a different mechanism that uses a diiron cluster that is positioned in the active site of the enzyme. In this pathway, the diiron cluster is directly involved in the oxidation process and helps to activate molecular oxygen (O_2) to react with methane (CH_4) and produce methanol (CH_3OH) and water (H_2O) [41]. The overall reaction catalyzed by sMMO is:

$$CH_4 + O_2 \rightarrow CH_3OH + H_2O$$

The soluble properties of sMMO can be used in the cytoplasmic compartment, while the particulate pMMO is localized to the membrane fraction. Depending on the availability of cofactors such as copper or iron and some conditions affecting the environment, the bacteria using methane can selectively prefer either pMMO or sMMO.

3.3. Conversion to Value-Added Products

Methanol (CH_3OH) is one of the vital and unique chemical intermediates used in several industries, especially in the fabrication of biofuels and many other effective chemical products. In line with its function as an intermediate in several chemical transactions, its application is significant in the advancement of sustainable energy systems and incrementing of industrial performance. The use of methanol is extensive due to its conversion into various products through several key processes [42].

3.3.1. Biofuels

The conversion of methanol to biofuels has great implications for the generation of renewable energy and the decline of the use of fossil fuels. Two biofuels that can be produced from methanol are dimethyl ether (DME) and biodiesel [43]. All these biofuels have peculiar characteristics and uses in the market.

a. Dimethyl ether (DME): Methanol is converted to dimethyl ether using dehydration, where water is removed to form DME [44]. The chemical reaction for this transformation is:

$$2CH_3OH \rightarrow CH_3OCH_3 + H_2O$$

DME is known as an alternative to diesel fuel because it has low emissions and is safe for the environment. During combustion, it emits lower nitrogen oxides and particulate matter, including those normally associated with conventional diesel fuels, which contributes to an environment with improved air quality [45]. Apart from being employed in diesel engines, DME is also used as a propellant for aerosols and as a refrigerant in industrial processes. DME is synthesized using acidic catalysts like alumina or zeolites, which promote dehydration by providing the reaction conditions for removing water and forming ether [46,47].

b. Biodiesel: Methanol is also used in large quantities in the manufacture of biodiesel through the transesterification process. This reaction involves the reaction of methanol

with vegetable oil or animal fat containing triglycerides to form fatty acid methyl esters (FAMEs) and glycerol [48]. The reaction can be represented as:

$$\text{R-COOH} + CH_3OH \rightarrow \text{R-COOCH}_3 + H_2O$$

Biodiesel, which is predominantly FAME, is a renewable energy source that can be used to replace petroleum-based diesel. Transesterification principally employs catalysts including sodium hydroxide (NaOH), potassium hydroxide (KOH), or sulfuric acid (H_2SO_4). These catalysts assist in increasing the rate of reaction, thus enhancing the production of biodiesel and glycerol [14]. Biodiesel is in high demand, as it has been shown to lower the emissions of carbon monoxide, particulate matter, and unburned hydrocarbons compared to fossil fuels. Also, biodiesel is used to reduce emissions of greenhouse gases in that, for every amount of carbon dioxide that is emitted by burning biodiesel, the carbon dioxide absorbed by feedstock crops is equivalent [49].

3.3.2. Other Chemicals (Formaldehyde)

Methanol can also be converted into other chemicals. Through oxidation, it can be transformed into formaldehyde, which is used to create various polymers, drugs, and resins, such as urea and phenol formaldehyde, that are useful for adhesives and coatings. The reaction below indicates the oxidation of methanol (CH_3OH) to produce formaldehyde and water.

$$CH_3OH + O_2 - CH_2O + H_2O$$

To further understand the conversion process, Figure 1 illustrates three potential modes of electron transfer to the particulate methane monooxygenase (pMMO):

(a) Redox arm: In this mode, the methanol dehydrogenase transfers electrons to the terminal oxidase, contributing to an increase in the proton motive force (PMF). Meanwhile, the pMMO draws electrons from the quinone pool [50,51].
(b) Direct coupling: Electrons generated from the oxidation of methanol are directly transferred to the pMMO [50].
(c) Uphill electron transfer: In this mode, electrons from the methanol dehydrogenase are fed back into the ubiquinol pool [51,52].

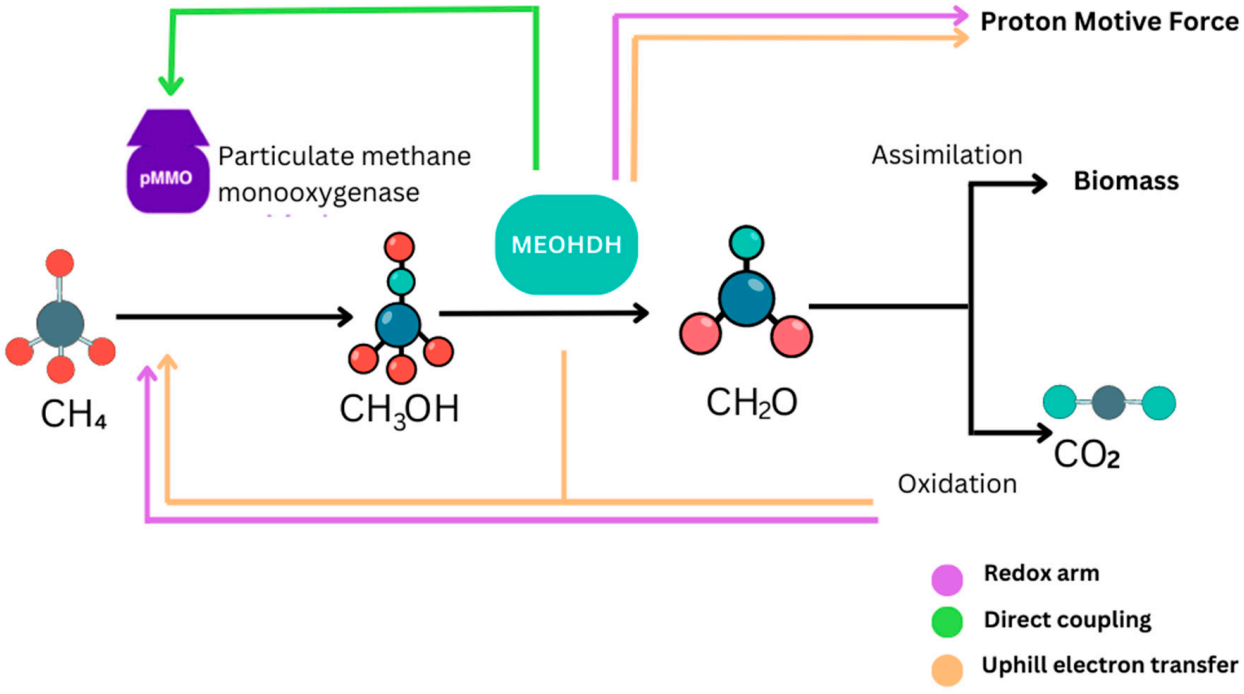

Figure 1. Modes of Electron Transfer to the Particulate Methane Monooxygenase (pMMO).

M. capsulatus can capture methane with its high-affinity uptake, and then oxidize the methane by using enzymes such as pMMO or sMMO. This is a way to produce sustainable energy.

3.4. Bioconversion of Methane to Methanol by Methylococcus capsulatus

The utilization of methane for producing methanol can be carried out using biological and chemical conversion processes. Unlike traditional techniques that utilize heat and pressure, and hence energy and funds, *M. capsulatus*, like other methanotrophic bacteria, can perform the task efficiently [52]. These bacteria oxidize methane to methanol under normal pressure and temperature using methane monooxygenase (MMO) enzymes, which gives high conversion and selectivity. This process also enables methane to serve as the only carbon and energy source and offers a non-toxic and efficient procedure for methanol synthesis [21,53].

Two groups of methanotrophs are recognized, depending on the level of methane in the surrounding media. Low-affinity methanotrophs, which are indigenous to soils with methane concentrations higher than 40 ppm, are well characterized, and all the isolated cultures belong to this category. On the other hand, affinity methanotrophs that are capable of oxidizing the ambient methane concentration (~2 ppm) have been detected by molecular biological studies from soil samples. High-affinity methanotrophs are less frequent and constitute less than 0.01% of the bacterial population of soils because of low atmospheric methane concentrations [21,22,30]. The biochemical pathway involves the oxidation of methane to methanol by MMO and then the oxidation of methanol to formaldehyde, formate, and then carbon dioxide; formaldehyde is incorporated into a biomass either by the ribulose monophosphate (RuMP) pathway or Serine pathway. MMO enzymes are capable of initiating the C–H bond of methane in a selective manner all at room temperatures and pressure. MMO-catalyzed partial oxidation of methane to methanol has the following benefits over thermochemical oxidation methods: high selectivity, better process efficiency and safety, mild conditions, and energy saving contribute to the associated economic benefits [26].

Several techniques have been studied for utilizing the strong oxidizing nature of methanotrophic bacteria, with varying applicability in industry.

a. Whole-Cell Methanotroph Cultures

Whole-cell methanotroph cultures can be a comparatively cheap pathway for the bioconversion of methane to methanol. The generation of biomass is fairly uncomplicated and relatively cheap, while more complex molecular processes, including the biogenesis of key MMO enzymes and the reduction equivalents, are regulated exclusively through bacterial activity. Whole cells are also capable of self-maintenance, and production of more 'whole cells' (replication). Further, there are downstream processing advantages because, while the biochemical reactions take place inside the cell, the methanol which builds up in the process is outside the cell, which makes it easier to recover the products. However, because whole-cell methanotroph cultures are more specialized, they are not versatile in various biotechnological processes. High cell density cultures are difficult because of the problems often encountered in gas–liquid transfer operations [22,54,55]. Also, the metabolic process of methanotrophic bacteria involves the conversion of methane in a single step to methanol, and so puts them at risk of over-oxidation to formaldehyde, which in turn interferes with normal biochemistry. Introducing a biphasic growth process for enzyme production, as well as methanol bioconversion makes the whole process even more challenging.

b. MMO Enzyme Isolates

Applying methanotrophic bacteria for the required conversion is to some extent a means to harness the potent enzyme of MMO. Literature has been published relating to the enzymatic activity of MMO, and hence there is virtual clarity regarding biochemical processes. Another approach to extracting enzymes comes from isolating them from cell

cultures. By avoiding various complex cellular interactions, and thus only performing the required reaction, it is possible to avoid over-oxidation of methanol through normal cellular metabolism. The bacteria still perform the complicated MMO synthesis, and, without other cellular components, the interactions are basic and cellular toxicity is not a problem. When working with cell-free preparations of the MMO enzyme, problems in isolation and purification arise from the fact that the purified enzyme is labile [56]. The problems connected with using an integral membrane-bound protein such as pMMO are complicating factors, although the cytoplasmic sMMO is easier for isolation. Stabilization is achieved by the fixation of enzymes on or in artificial support structures. However, evolution has not fine-tuned nature's catalysts to work under technical process conditions, and therefore problems with stability, activity, and lifetime arise [28]. Further, cofactor dependency and prerequisites for the supply of exogenous reducing equivalents support the application of whole cells. It has been found that if the energy needed for biomass production and bioconversion are combined, the quantity becomes equal to that of using a whole-cell culture without maintaining viable cells, with the associated energy cost.

c. Synthetic MMO Analogues

From the detailed characterization and biochemical analysis of the MMOs, it has been proposed that synthetic 'biomimetic' catalysts could provide the benefits of the enzyme isolates along with thermochemical process stability. Synthetic organometallic complexes have been developed to have high selectivity, yield, reaction rate, and conversion efficiency, and also to be more resistant to process conditions than purified enzymes, and be less prone to inhibited by-products [43]. Still, the realization of these objectives imposes a difficult optimization task, whose complexity is probably comparable to that of the molecular machine itself to MMO enzymes, making chemical synthesis a complex task. This is in contrast to the simplicity and rapidity with which methanotrophs synthesize strong MMO enzymes [43,44].

d. Ammonia-Oxidizing Bacteria

Another approach employs ammonia-oxidizing bacteria holding the AMO enzyme—an analogue of MMO. Similar to pMMO in both structure and function, the AMO enzyme catalyzes the oxidation of ammonia (NH_3) to hydroxylamine (NH_2OH), and is further oxidized by the hydroxylamine oxidoreductase enzyme with nitrate (NO_2^-). Ammonia being metabolized by the cell produces reducing equivalents, and carbon dioxide is used as a carbon source [45]. Owing to the similarity between the two structures, AMO like pMMO has low substrate specificity and can also oxidize methane to methanol [46,47].

3.5. Genetic and Metabolic Engineering Strategies to Boost Methylococcus capsulatus' Performance to Improve Methane Uptake Rates

a. Genome-Scale Metabolic Model

Lieven et al. [53] reconstructed a genome-scale metabolic model of *M. capsulatus* (Bath) which is an obligate methanotroph, which has been applied in several studies and used as a production organism of single-cell proteins (SCPs). It was manually constructed and covers 879 metabolites linked through 913 reactions. This is made possible by the integration of 730 genes and detailed annotations in the model, thereby making it a logical blueprint of metabolic physiology and an abridged reference center for *M. capsulatus* (Bath). The model established that methane can be oxidized by particulate methane monooxygenase via direct coupling or uphill electron transfer, both at a lower efficiency [53].

b. Transcriptional and Metabolomic Responses

A recent study carried out by Bedekar et al. [57] focuses on the ability of *M. capsulatus* Bath, a methane-oxidizing bacterium, to modulate its activity according to nitrogen supply and temperature. For ammonium, better growth was found at 37 °C than at 30 °C, and for nitrate, better growth was observed at 42 °C than at 37 °C. De novo RNA-seq also

showed that the strains displayed alterations in respiration, methane oxidation, and nitrogen metabolism relevant to nitrogen type and temperature [57]. This offers information about these bacteria at a molecular level, which is vital for comprehension of the bacterial adaptation when it comes to methane uptake and use, to design improved genetic and metabolic engineering approaches to improve the uses of these bacteria in biotechnological approaches regarding the mitigation of methane [4]. This will give an understanding of how *M. capsulatus* Bath responds at a molecular level, giving information on changes in transcription under nitrogen and temperature conditions. It is important to recognize such adaptations to best enhance the methane uptake performance of the bacterium in question. The enhancement of its methane-oxidizing capacity can be pursued using information on gene expression patterns as well as intracellular metabolomics. For this reason, researchers can identify certain great potentials for strain improvement, focusing on such pathways involved in respiration, methane oxidation, and nitrogen metabolism to enhance their efficiency as the basis for biotechnological applications such as bioreactors for methane reduction and remediation [32].

3.6. Benefits of Methylococcus capsulatus

3.6.1. Sustainable Energy Production through Methane Utilization

The above methane capture system using *M. capsulatus* is the first step in the utilization of methane for the sustainable production of energy. As the primary ingredient in the production of natural gas, the combustion of methane captured from the environment can heat buildings, produce electricity, and power machines as fuel. The production of sustainable energy using *M. capsulatus* relies on the bacterium's ability to produce methane-based biogas, a biofuel that uses methane from the atmosphere or waste, remediating the harmful greenhouse gas while producing reliable, affordable, and easily accessible green energy. Methanotrophic bacteria such as *M. capsulatus* utilize methane from the atmosphere, landfills, and wastewater as a source of energy in anaerobic digestion. In the process, methane is oxidized to produce methanol [58]. Once captured, biogas is used as a source of renewable energy that produces heat and electricity for a wide range of uses, including household heating, powering engines, and producing fuel cells. Methane-based biogas compares favorably to non-renewable sources of energy such as fossil fuels, since it is renewable, relatively easy to produce, and uses locally available materials such as biomass. From an economic perspective, however, fossil fuels are easier to produce, particularly in developing countries, following decades of infrastructure, investment, and technological advancements in the field [59]. Nevertheless, once the initial set-up costs are incurred, methane-based biogas becomes more efficient and less costly. Further, advances in synthetic biology have enabled the genetic engineering of *M. capsulatus* to enhance its production of energy from methane. For instance, Emelianov et al. [59] engineered the bacteria to produce isoprene using an economical approach that used a CRISPR-base editor to disrupt the expression of soluble methane monooxygenase (sMMO) [59]. Such advancements that enhance the ability of the methanotroph to utilize methane in the production of biofuels advance sustainability in energy production by leveraging an otherwise toxic greenhouse gas to reduce the adverse impacts of non-renewable sources of energy on the environment.

Compared to other sources of renewable energy such as geothermal energy, solar energy, and hydropower, methane is relatively efficient, affordable, and scalable. It relies on biomass from feedstocks and other waste, which are easily available, it can be produced easily at the household level for use in cooking and lighting, and is easily scalable for industrial use [60]. Despite being easily scalable, affordable after the initial cost of installation, and efficient in both domestic and industrial use, the production of sustainable energy through methane utilization faces challenges that threaten its favorable ranking against other types of renewable energy. The primary challenges that threaten its ranking include infrastructural challenges such as the unavailability of feedstock in urban households, inadequate waste management processes that could disrupt biogas production or availability, limited access to the relevant technologies, particularly in developing countries,

and the need for frequent repairs and maintenance, which is compounded by the limited availability of well-trained technical personnel, particularly in remote areas in developing countries [21]. Despite these challenges, continued research, investment, and education in methane utilization are driving the continued adoption of biogas as an alternative to non-renewable, expensive, and unreliable sources of energy across the world.

3.6.2. Applications of *Methylococcus capsulatus* beyond Energy Production

Beyond its use in the production of green energy, *M. capsulatus* is used in the production of industrial products such as biodegradable plastic, the manufacturing of drug delivery media and some medical interventions in the pharmaceutical industry, the production of cheap, environmentally friendly, and high-quality proteins, and in waste management. First, the bacterium is integral in the bioconversion of methane, the most abundant hydrocarbon, into biodegradable plastic with multiple industrial and household uses. Natural gas or biogas produced from methane using *M. capsulatus* are inexpensive and abundant sources of carbon for the production of biopolymers such as P3HB (poly-β-hydroxybutyrate), which is used to make biodegradable plastic [61]. Typically, methanotrophs such as *M. capsulatus* produce high molecular weight PHB. However, the value of this process can be enhanced through the addition of co-substrates such as valerate to produce PHAs as well, maximizing the product yield of methane [62]. The biodegradable polymers, which break down completely both aerobically and anaerobically, are affordable and renewable alternatives to chemical and fossil fuel-based plastics and have a wide range of applications, including the manufacturing of packaging materials in the food industry, hydrogels and emulsifiers in the cosmetic industry, and fabrics in the clothing industry. In addition to replacing non-biodegradable plastics, biopolymers made from methane are used in the pharmaceutical industry in drug delivery through their use in the manufacturing of microcapsules for oral, skin, and hair care through their use in the production of hydrogels, and in direct medical application through their use in the production of medical implants and antimicrobial membranes [63]. Third, methanotrophic bacteria, such as *M. capsulatus*, have the potential to produce single-cell proteins that require minimal water and land resources compared to conventional proteins. The bacterium uses methane and Nitrate or Ammonia to yield an easily digestible microbial protein that is used as an additive in animal feeds to boost their protein content [64]. Fourth, *M. capsulatus* has been integrated into waste management systems to mitigate methane emissions, which make a significant contribution to global warming and the resulting climate change [65]. The detrimental impact of methane on the environment has sparked decades of research and innovation in its management. One of the most effective ways of remediating methane in waste is the use of methanotrophs. Methane in the environment primarily comes from agricultural waste, emissions from land and aquatic animals, activities related to the extraction of oil, gas, and coal, industrial processes that emit harmful gas, and the degradation of organic waste in landfills. Methanotrophs have been used successfully in the removal of methane from waste in various terrestrial and aquatic environments, in processes that advance waste valorization by converting the methane into useful products such as biodegradable plastic, biogas, and protein. The process of the use of *M. capsulatus* and other methanotrophs in the treatment of wastewater and sewage, for instance, follows the above mechanisms. Methane, which is recovered from wastewater through anaerobic digestion, is used by the methanotrophs as a source of energy. The bacteria oxidize the methane into methanol, which undergoes further chemical reactions to produce biopolymers used in the production of biodegradable plastic, hydrocarbons used in the production of sustainable fuels, and proteins used to fortify animal and human food [65]. Beyond wastewater, *M. capsulatus* is particularly effective in the removal of methane from landfills and compost piles, breaking down the waste into organic matter while utilizing the methane.

3.6.3. Benefits of Value-Added Products

The usage of *M. capsulatus* is advantageous because it can lead to methane reduction when used as a carrier to capture and utilize methane. This helps in reducing greenhouse gases, thereby combating climate change. Methane, with its potent greenhouse gas prosperities, possesses a greater chance of causing global warming than carbon dioxide. *M. capsulatus* systems are employed in the collection of methane emitted in landfills, wastewater treatment processes, as well as in agricultural activities. This helps avoid emitting liquid into the air; this is important in curtailing greenhouse emissions [66]. *M. capsulatus* can also be manipulated to affect the conversion of methane into several useful products. At the same time, the bacterium is quite capable of synthesizing numerous and diverse valuable chemicals. It can, for instance, be enhanced to synthesize organic acids, which are useful substances in the food industry, as well as in the manufacturing of drugs and chemicals. These are organic acids which can be used for the formulation of biodegradable products, which will therefore reduce the dependence on plastics, which are from petroleum products. Consequently, however, the effects of *M. capsulatus* are multilevel since, in addition to producing organic acids, they can be applied to the creation of pharmaceutical precursors. This is a stimulus for the development of new types of pharmaceuticals based on biology. This unfortunately means reliance on power sourced from fossil fuels and is less eco-friendly when synthesizing drugs. Additional benefits are obtained when integration of the *M. capsulatus* cultivation into other sustainable practices is affected. For instance, *M. capsulatus* is capable of trapping methane and developing solutions concerning waste disposal since carbon and nutrient sources could be utilized from various industries' waste streams.

M. capsulatus can be grown to produce biogas, which is regarded as a renewable source of energy. And it may be used as a cleaner source of energy in the process of moving to a lower carbon economy. This integration of waste usage, methane extraction, and energy generation represents a circular economy where resources are utilized optimally while minimizing the wastage of resources. Another way to prevent reliance on fossil resources is the cultivation of *M. capsulatus* as a part of a bio-based economy. *M. capsulatus* systems that utilize methane, a greenhouse gas, can be another potential source of energy other than the conventional hydrocarbon-based processes. The change of energy supply to a biobased economy has the potential to decrease the emissions of greenhouse gases and stimulate employment in the bioeconomy. The use of *M. capsulatus*-based systems can be highly advantageous not only in terms of this particular aspect, that is, capturing and using methane. This is because these systems facilitate the direct mitigation of methane emissions and the generation of augmenting value-added goods as a positive contribution toward sustainable development goals. This will include measures for climate change, resource-efficient economies, and sustainable consumption and production patterns, as well as encouragement of accessible and clean energy. *M. capsulatus* has been singled out as being suitable for the production and accumulation of methane. Some of the advantages include the following: In terms of emission reduction, this process cuts out a major greenhouse gas known as methane, the production of important chemicals and pharmaceutical substances, as well as creating a link with sustainable practices. We can reduce climate change by harnessing the abilities of *M. capsulatus*. We can also reduce our dependence on fossil fuels and foster a sustainable and circular economic system.

3.6.4. Synthetic Biology Approaches for Enhanced Methane Oxidation

Synthetic biology enables the manipulation of organisms to amplify their properties and enhance the mechanisms for which they are adapted for various applications. One of the approaches of the field that have been applied to methane oxidation is the genetic engineering of methane monooxygenases. Scientists have achieved this using site-directed mutagenesis to alter the crucial amino acid residues responsible for methane oxidation [67]. This approach has the potential for industrial applications that use methanotrophs for the oxidation of methane. A second approach adapts enzyme encapsulation to enhance methane oxidation. This approach involves the use of a biopolymer or organic polymer to

embed gas-reacting enzymes and immobilize them to optimize the conditions for the oxidation of methane. A polymer material embedded with silicon has been used successfully in the development of a gas-permeable membrane to control the activity of pMMO from *M. capsulatus* in the production of methanol from methane [67]. As further research and development in the role and adaptations of methanotrophs for methane oxidation continue, scientists are bound to develop more advanced approaches to engineer the bacteria or incorporate other biotechnologies such as hydrogel technology to harness the methane oxidation properties of *M. capsulatus* and other methanotrophic bacteria.

4. Challenges and Limitations

To maximize its potential, *M. capsulatus* must overcome several challenges and limitations. Optimizing the conversion from methane into methanol is a significant challenge. Although significant progress has been made in the understanding of the enzymatic mechanism of methane Monooxygenases, further research is needed to improve their catalytic stability and activity [20,67]. MMOs that are more efficient can improve the efficiency of methane to methanol conversion. To maximize economic viability, it is also important to develop efficient downstream processes for purification and methanol recovery. Advanced separation and purification technologies can reduce energy costs and the amount of methanol extracted from complex mixtures. Scaling up *M. capsulatus*-based operations from the laboratory to the industrial scale is another challenge [20]. The majority of current research has been conducted in controlled lab environments, and little is known about the scalability. To ensure that methane utilization and capture on a large scale is efficient and economically feasible, factors such as oxygen availability, nutrients, and reactor design must be investigated thoroughly. Understanding mass transfer dynamics and optimizing bioreactor design will help to improve the system's overall performance.

In addition, *M. capsulatus* cultures must be tested for their long-term robustness and stability under industrial conditions. Various methane-capture systems should operate stably and continuously; in other words, the corresponding microbiomes must be stable. Among the limitations, *M. capsulatus* can be influenced by the invasion of other microbes, which will diminish its efficiency in capturing methane as well as utilizing it. If one wants to understand the microbial ecosystem and wishes to probe into the microbial assemblages, one would be able to sustain a stable microbial population over long time intervals. However, other issues can be taken into consideration. The regulatory permissiveness and the economic rationality of the plans to be implemented remain crucial when it comes to the application of *M. capsulatus*-based systems. Due to the need to make large-scale implementations fiscally possible, therefore, the cumulative costs of growth, operation, and sustenance of microbiological cultures must always be considered. Furthermore, the guidelines and regulatory policies must be stipulated to act as standards for the safe and prudent utilization of *M. capsulatus* in methane utilization and capture systems. *M. capsulatus* may have considerable potential for the capture and use of methane; however, there are still some problems and boundaries that cannot be ignored. These include the enhancing of the conversion of methane to methanol, scaling up the processes developed, as well as managing to develop cultures that will remain stable for a long time. Some of these challenges could be mitigated through the implementation of the strategies depicted in Table 1, and by making *M. capsulatus* an active pillar in energy generation and the tackling of methane emissions, in addition to supporting stable and sustainable microbial subcultures.

Critical Analysis and Integration

The extracted data were scrutinized to assess the patterns, trends, and shortcomings in the field's current literature. Hypotheses derived from the review connect various research to give an overall insight of the use of *M. capsulatus* for methane capture and future usage along with the benefits of the adaptation of the bacteria for the purpose and the challenges faced in its present applications.

Table 1. Challenges and Solutions for *Methylococcus capsulatus* in Methane Capture and Utilization.

Challenges and Limitations	Solutions
Optimization of Methane-to-Methanol Conversion	- Enhance catalytic stability and activity of MMOs
	- Utilize protein engineering techniques for MMO improvement
	- Explore alternative enzymes or microbial pathways
Downstream Processes and Purification	- Develop efficient separation and purification methods
	- Investigate membrane separation, adsorption, or distillation
	- Conduct techno-economic analyses for cost-effective processes
Scaling Up from Laboratory to Industrial Scale	- Conduct pilot-scale studies to assess scalability
	- Optimize reactor design, nutrient supply, and oxygen availability
	- Collaborate with engineering experts for scalable bioreactor design
Long-Term Robustness and Stability	- Implement stringent quality control measures
	- Monitor microbial communities and control contamination
	- Explore microbial consortia or co-culture systems
Regulatory and Economic Considerations	- Collaborate with regulatory bodies for guidelines
	- Perform economic assessments for cost-effectiveness
	- Seek partnerships with industry stakeholders and investors

5. Case Studies and Current Research

These applications of *M. capsulatus* have been demonstrated practically in various studies and adopted in various pilot projects that confirm their viability. In 2023, after over two decades of research, Unibio, a company in Denmark and the United Kingdom, completed the development of a technology that uses methane to produce protein for human consumption. Driven by the growing global human population, a surge in its protein needs, and the need to develop sustainable food production approaches that conserve the environment, the company adopted the use of *M. capsulatus* to produce feedstock for animals sustainably, targeting aquaculture, livestock, and pets. The end product, which is known as Uniprotein, is produced through the cultivation of *M. capsulatus* under optimal conditions that include the use of a fermenter and the supply of methane, nitrogen, oxygen, and other minerals that allow the microbe to multiply rapidly. The microbe is then harvested, dehydrated into biomass, heat treated, and turned into a granule containing 70% protein that meets international safety standards, does not contain chemicals such as pesticides, and is not genetically modified. Further, the protein contains all amino acids, unlike some common feedstocks, and is significantly less resource-intensive compared to commonly used feedstocks, since 14,000 tons of Uniprotein are produced from the same amount of land used to produce 1 ton of soy protein. Production for the pilot project is currently limited to Europe, but Unibio is in talks with countries from all over the world and hopes to increase production over the coming decade [68]. The project, which relied on research conducted in collaboration with the Technical University of Denmark (DTU) demonstrates the potential for partnerships between industry and academic institutions in the development of beneficial applications of *M. capsulatus* to promote sustainable methane utilization. In the 20 years during which research for the project was conducted, several studies have been conducted exploring similar and different applications of *M. capsulatus*, which could be adapted into beneficial projects. One such research study explored the potential of different compositions of methanotrophs in methane utilization. The study found that different methanotrophs are more suited for specific ecosystems, establishing a basis for a criterion for the optimum use of the microbes in waste management and the conversion of methane into useful products such as plastic, protein, and other products used in the pharmaceutical industry. Notable findings from the study include the suitability

of specific bacteria such as Methylothermaceae and Methylococcaceae with green compost while the methane utilization abilities of Methylocystaceae favor a relatively longer storage time [69]. This information will be instrumental in the structuring of waste management projects by guiding the determination of the optimum microbial communities and combinations for use in methane mitigation in different types of waste. Academic institutions could work with governments and industry representatives from industries that produce waste that contains methane, such as the agriculture, energy, and transport industries, to develop effective and cost-effective waste management approaches that reduce the amount of methane in their waste and transform it into useful products. Ultimately, multidisciplinary approaches involving industries, researchers, and policymakers are necessary for the utilization, valorization, and removal of methane from the environment.

A study by Emelianov et al. [59] demonstrated that *M. capsulatus* Bath produced isoprene from methane when the bacterium was modified to express the mevalonate (MVA) pathway obtained from *E. coli*. The results demonstrated that increased production of MVA pathway enzymes and isoprene synthase derived from *Populus trichocarpa*, when grown under a phenol-inducible promoter, led to a significant enhancement in isoprene accumulation. In addition, *M. capsulatus* Bath was modified one step further with a CRISPR base editor to eliminate the enzyme known as soluble methane monooxygenase, or sMMO, an enzyme that catalyzes the oxidation of isoprene leading to toxicity. Furthermore, enhancement of the metabolic flux towards the MVA pathway along with optimization of culture conditions enhanced the isoprene titer to 228.1 mg/L, which is the highest titer for methanotroph-derived isoprene production. The derived methanotroph could help initiate the cost-effective conversion of methane to isoprene and create value-added products [59].

But et al. [70] carried out a study to create glycogen-deficient mutants of *M. capsulatus* MIR for single-cell protein (SCP) production from methane. They created glycogen synthase mutants (ΔglgA1, ΔglgA2, and ΔglgA1ΔglgA2) and confirmed that the ΔglgA1ΔglgA2 mutant was glycogen-deficient, but ΔglgA1 and ΔglgA2 single mutants' strains contained glycogen, indicating redundancy. Furthermore, suppression of the expression of the glk gene decreased the levels of glycogen and at the same time increased the levels of free glucose in cells. During the batch cultivation, the protein content of the ΔglgA1ΔglgA2 mutant was considerably higher (71% dry cell weight) and glycogen content was lower (10.8 mg/g dry cell weight) as compared to the wild-type strain. However, the degree of superiority was slightly less pronounced in continuous cultivation, and the mutant still had a higher biomass in the SCP-related parameters. The glgA1-like genes were detected in methanotrophs belonging to Gammaproteobacteria and Verrucomicrobia, while glgA2-like genes were mainly present in halo- and thermotolerant Gammaproteobacteria [70].

6. Conclusions

M. capsulatus appears to be one of the most valuable organisms for use in a wide context of biotechnological applications, especially in the case of methane utilization. Due to its capability of utilizing methane selectively and further converting the gas into methanol, the organism has the potential to revolutionize several biotechnological applications. However, inquiries like how to optimize conversion efficiency, or how to make some of these processes more scalable, are still outstanding issues that need to be solved before *M. capsulatus* can be thought of as being practically implementable. By incorporating *M. capsulatus* into systems, it becomes possible to decrease methane emissions and foster the synthesis of useful chemicals simultaneously. Future works in this area should aim at understanding more aspects of *M. capsulatus*, such as the metabolic pathways of the bacteria, enhancing cross-disciplinary research, and benefiting from other technologies that may be used to further enhance the use of *M. capsulatus* in the production of sustainable energy. All these measures will be strategic in advancing our sustainability goals.

7. Future Directions

Future research that may further enhance and unlock *M. capsulatus* as a potential methane capture and utilization candidate should consider the following areas. Primarily, to improve the efficiency of methane conversion in *M. capsulatus*, it is crucial to comprehend its microbial metabolic regulation and control mechanisms. This can be performed using techniques like metabolic modelling technology and omics technologies like genomics, proteomics, and metabolomics. These approaches will help to shed light on the key biological pathways, thus creating a chance for the formation of more efficient strains.

Moreover, the collaboration of different disciplines is also critical to the success of the field. It should be noted that the integration of microbiology and biochemistry is crucial for tackling the environmental and technical difficulties in applying *M. capsulatus*-based systems. Such collaborations will be useful in advancing reactor designs, as well as improving the processes in, and achieving careful life cycle analysis aimed at, environmental stewardship.

The field of *M. capsulatus* can greatly benefit from this recent discovery in synthetic biology and metabolic engineering. This way, genetic modification of the bacteria and strain engineering allow us to develop new forms of high-value methane-based products. Scholars have believed that this approach will not only strengthen the utilization of *M. capsulatus* toward producing sustainable energy but also diversify its function towards other applications.

Author Contributions: A.A.A.: Conceptualization, writing the original draft, writing, reviewing, and editing. H.N.O.: Writing—original draft, Writing—reviewing and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare they have no known competing financial interests or personal relationships.

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