


A review of the research progress on co-cultivation of symbiotic methanotroph bacteria with algae

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ABSTRACT

This review provides an in-depth analysis of recent advancements in co-cultivation strategies involving methanotroph and algal symbiotic bacteria, while exploring their biotechnological applications. The study specifically highlights the consortium's potential for simultaneous methane and carbon dioxide utilisation in producing value-added bioproducts, including biofuels, single-cell proteins, single-cell oils, and biodegradable plastics. A comprehensive examination is presented regarding the underlying mechanisms of microbial cross-feeding, critical influencing factors (e.g., nutrient exchange dynamics, redox balance maintenance, and quorum sensing regulation), and system optimisation approaches for enhanced symbiotic efficiency. Current technical bottlenecks in industrial scalability are critically addressed, particularly focusing on reactor type, metabolic flux imbalances, and ecological stability maintenance in open systems. Future research directions are proposed to emphasise multi-omics-guided consortia design, synthetic biology-enabled metabolic pathway engineering, and AI-driven cultivation parameter optimisation. By synthesising cutting-edge findings from recent studies, this review establishes a systematic framework for understanding and engineering symbiotic microbial systems, ultimately contributing to the development of carbon-negative biomanufacturing platforms that align with circular economy principles.

KEYWORDS

methanotroph, algal, co-culture, methane, carbon dioxide

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1. INTRODUCTION

With the escalating global demand for sustainable energy and environmental remediation technologies, co-cultivation strategies integrating methanotrophs and algae-associated symbiotic bacteria have emerged as a prominent research focus. Methane – a potent greenhouse gas – has garnered significant attention due to its substantial climate impact when released into the atmosphere (van der Ha et al., 2011). Concurrently, biogas – a renewable energy source generated through anaerobic digestion – presents methane and carbon dioxide as its principal components, whose efficient utilisation has become a critical research priority (Rasouli et al., 2018). The symbiotic co-culture system not only enhances conversion efficiency of both methane and carbon dioxide but also enables dual environmental benefits through greenhouse gas mitigation and simultaneous biosynthesis of value-added bioproducts (Ruiz-Ruiz et al., 2020). These include biodiesel precursors, polyhydroxyalkanoate-based bioplastics, and nutritionally enriched single-cell proteins, thereby establishing a paradigm for carbon-neutral circular bioeconomy.

Symbiotic co-culture technology emulates natural microbial interactions to establish synergistic metabolic partnerships, thereby enhancing methane and carbon dioxide conversion efficiencies while minimising exogenous energy input requirements. This approach not only optimises substrate utilisation efficiency and reduces environmental contaminants but also drives bioproduct portfolio diversification, accelerating the transition toward a sustainable bioeconomy (van der Ha et al., 2011). Furthermore, symbiotic co-cultivation systems demonstrate exceptional promise in environmental remediation and waste valorisation applications, offering innovative biotechnological pathways to address dual challenges of green energy production and ecological preservation.

This study synthesises recent transformative advancements in symbiotic co-cultivation research. Regarding microbial interplay mechanisms, investigators have elucidated the sophisticated network of metabolic cross-feeding, quorum-mediated signalling cascades, and horizontal gene transfer between methanotrophs and algal partners, establishing theoretical frameworks for consortium optimisation (Hill et al., 2017). In terms of bioreactor design and operation, researchers have developed various bioreactor designs to improve the conversion efficiency of methane and carbon dioxide, and optimised operating conditions such as light, temperature, and pH to enhance the biotransformation ability of symbiotic bacteria (Li et al., 2022). Bioproduct innovation has achieved milestone developments through advanced extraction protocols for lipid fractions (C16–C22 profiles), high-purity polyhydroxybutyrate recovery, and single-cell protein harvest from consortium biomass, alongside pioneering biosynthesis of extremolyte compounds like ectoine through stress-induction strategies (Cantera et al., 2016). These multidimensional advances fundamentally expand our mechanistic understanding of microbial syntrophy while pioneering carbon-negative biomanufacturing paradigms that integrate greenhouse gas valorisation with circular bioresource utilisation.

2. MECHANISM OF SYMBIOTIC BACTERIAL INTERACTION

2.1. Material exchange between methanotroph and algae

The co-cultivation system of methanotrophs and algae exemplifies a unique microbial symbiosis pivotal for enhancing methane and CO₂ conversion efficiencies. Within this syntrophic

consortium, methanotrophs utilise methane as an electron donor to drive enzymatic cascades for energy generation and biomass synthesis, concurrently releasing metabolic CO₂ as a substrate for algal assimilation (Manefield and Turner, 2002). Reciprocally, algae employ photosynthetic processes to convert CO₂ into organic biomass while generating oxygen byproducts that sustain methanotrophic activity. This closed-loop interaction not only elevates methane bioconversion rates but also eliminates dependency on external oxygen supplementation, thereby reducing operational costs while improving system safety profiles and long-term sustainability (Veraart et al., 2018). Research has delineated multifaceted interaction mechanisms underpinning this partnership. Central to this symbiosis is bidirectional metabolite exchange: methanotroph-derived CO₂ and algal oxygen reciprocally optimise niche-specific growth conditions. Methanotrophs exhibit upregulated particulate methane monooxygenase expression under algal oxygen gradients, while algae demonstrate enhanced RuBisCO activity through CO₂ enrichment. Auxiliary interactions include pH stabilisation *via* algal bicarbonate assimilation and redox balancing through cross-species electron shuttle systems. Such metabolic integration creates a self-regulating microenvironment that sustains robust consortium performance under fluctuating substrate availabilities, establishing foundational principles for designing next-generation gas-to-bioproduct platforms.

2.2. Signal communication between symbiotic bacteria

Intercellular signalling among symbiotic consortia constitutes a critical regulatory mechanism for maintaining microecological homeostasis and facilitating microbial cooperation in syntrophic systems. N-acyl homoserine lactones (AHLs), serving as quorum sensing (QS) mediators, modulate bacterial gene expression to influence metabolic activity and biofilm formation, thereby potentially governing algal growth dynamics and metabolic reprogramming (Manefield and Turner, 2002). Experimental evidence demonstrates that AHLs induce auto-aggregation behaviour in algae, significantly enhancing their flocculation efficiency – a breakthrough advancement for microalgal harvesting and biomass recovery (Zhou et al., 2017). Beyond AHLs, volatile organic compounds (VOCs) participate in cross-kingdom signalling networks. Specific bacterial strains emit volatile metabolites (e.g., dimethyl sulphide (DMS), dimethyl disulphide (DMDS), and bicyclic sesquiterpenoids) that modulate methanotrophic growth kinetics and catalytic activity (Veraart et al., 2018). These compounds exhibit multifaceted roles in microbial cross-talk, though their precise mechanistic contributions to algal-bacterial symbiosis require systematic elucidation. Emerging research reveals microalgal responsiveness to bacterial signalling molecules like AHLs, with reciprocal modulation through specialised metabolite secretion. Such trans-kingdom signalling potentially governs nutrient cycling efficiency and energy flux redistribution within consortia, thereby fine-tuning ecosystem-level functionality (Hom et al., 2015). Decrypting these signalling paradigms is paramount for rational consortium engineering, as targeted manipulation could amplify greenhouse gas conversion rates while upgrading bioproduct titer and quality metrics.

These mechanistic insights establish theoretical foundations for consortium optimisation. Strategic modulation of microbial interactions enables: 1) enhanced CH₄/CO₂ conversion efficiency through synchronised metabolic coupling, 2) improved bioproduct yield *via* pathway coordination, and 3) reinforced system robustness through ecological network stabilisation. Practical implementation involves environmental parameter optimisation (e.g., photon flux

density tuning, thermal cycling protocols, pH-stat regulation) to maximise cross-feeding synergies between methanotrophs and algae. These scientific advancements not only deepen our understanding of microbial syntrophy but also pioneer novel biotechnological frameworks for sustainable carbon valorisation, bridging fundamental microbial ecology with industrial bioengineering applications.

3. FACTORS AFFECTING CO-CULTIVATION OF METHANOTROPH AND ALGAE SYMBIOTIC BACTERIA

The methanotroph-algae co-cultivation system, through coupled carbon-nitrogen cycling and reciprocal utilisation of oxygen and organic substrates, demonstrates superior carbon sequestration efficiency and pollutant degradation potential. However, consortium stability and performance are governed by multifaceted environmental parameters, including metal ion bioavailability, pH gradients, temperature fluctuations, photoperiod modulation, and dissolved oxygen tension. This review critically examines current literature to decipher their mechanistic impacts on syntrophic interactions, thereby establishing theoretical frameworks for engineered system optimisation.

3.1. Regulation of metal ions and trace elements

Metal ions serve as essential cofactors in the metabolic pathways of methanotrophs and algae, where their homeostatic regulation directly governs enzymatic activity and cellular proliferation. Methanotrophs rely on methane monooxygenases (MMOs) for methane activation, with copper-dependent particulate MMO (pMMO) dominating under copper sufficiency, while iron-coordinated soluble MMO (sMMO) operates during copper limitation (Vorobev et al., 2011; van der Ha et al., 2013). Elevated Cu^{2+} concentrations induce chloroplast structural disintegration and photosynthetic inhibition in algae, whereas low levels impair methanotrophic metabolic efficiency (Stauber and Florence, 1987). Optimal Cu^{2+} supplementation achieves maximum methane oxidation rates concurrent with algal biomass accumulation, attributed to pMMO upregulation and algal copper tolerance mechanisms (Yu et al., 2003; El Ghazouani et al., 2012).

Trace elements including iron (Fe), manganese (Mn), and zinc (Zn) exhibit critical synergistic interactions. $\text{Fe}^{2+}/\text{Fe}^{3+}$ mediates chlorophyll biosynthesis and photosynthetic electron transport in algae, with deficiency causing photosystem II degradation and subsequent oxygen depletion that compromises methanotrophic aerobic metabolism (Jiang et al., 2020). Mn^{2+} modulates algal antioxidant defences *via* superoxide dismutase activation, mitigating oxidative stress and maintaining redox homeostasis in syntrophic systems (Li et al., 2007). Zn^{2+} acts as a metalloenzyme cofactor for carbonic anhydrase, promotes the fixation of CO_2 by algae and enhances the efficiency of carbon source supply (Escudero-Almanza et al., 2012). Crucially, inter-metal antagonisms (e.g., Fe^{3+} - mediated Mn^{2+} uptake inhibition) necessitate combinatorial optimisation using response surface methodology to achieve consortium productivity improvements, balancing metal speciation and bioavailability (Pasiczna, 2017).

3.2. The influence of pH value and temperature

The pH parameter exerts critical regulatory control over symbiotic consortia by modulating cell membrane permeability, enzymatic catalysis efficiency, and metabolite partitioning, thereby governing microbial physiological functionality. Methanotrophs thrive in neutral to slightly alkaline conditions (pH 6.5–8.0), while algal species (e.g., *Chlorella* spp.) exhibit optimal growth at pH 6.0–7.5, resulting in an overlapping operational range of pH 6.5–7.5 for synergistic performance (Gong et al., 2014; Reddy et al., 2020). Within this zone, both algal photosynthetic rates and methanotrophic MMO activity demonstrate peak performance. Acidic stress (pH < 6.0) induces algal cell wall lysis with subsequent release of inhibitory metabolites (e.g., acetate), while alkaline conditions (pH > 8.5) trigger methanotrophic proton motive force collapse through membrane potential dissipation. Thermally, mesophilic regimes (25–30 °C) accommodate most consortium configurations: maximal algal photosynthetic efficiency occurs at 25 °C, whereas methanotrophic metabolic flux peaks at 30 °C (Singh and Singh, 2015; Reddy et al., 2019). Temperature fluctuations exceeding ± 5 °C cause aberrant starch accumulation in algae coupled with ATP depletion in methanotrophs, disrupting energy metabolism coupling.

3.3. The influence of light and oxygen concentration

Light irradiation serves as the fundamental energy source for algal photosynthesis, with its intensity and periodicity directly governing oxygen evolution and organic carbon export. Sub-optimal irradiance induces photosynthetic insufficiency in algae, resulting in oxygen evolution rates below methanotrophic oxygen demand, thereby creating anaerobic microniches that suppress methane oxidation. Conversely, excessive irradiance enhances algal biomass accumulation but generates photoinhibitory reactive oxygen species (ROS) that peroxidise methanotrophic membrane lipids, necessitating antioxidant supplementation (e.g., cysteine) for oxidative stress mitigation (Gong et al., 2014; Sakimoto et al., 2016). Photoperiod modulation synchronises algal metabolic rhythms (Grobbelaar, 1991), enabling glycogen reserves accumulated during photophase to sustain carbon supply for methanotrophs in scotophase, thereby reducing diurnal methane removal fluctuations by 25% through metabolic buffering.

Oxygen tension constitutes a critical determinant of methanotrophic metabolism (Tan et al., 2024). The algal-methanotrophic symbiosis achieves dynamic oxygen regulation through photosynthetic dissolved oxygen (DO) modulation: photophase DO surges to 8–10 mg L⁻¹ (supersaturation state) requiring gas sparging or turbulence control to maintain optimal O₂ levels, whereas scotophase DO declines to 2–3 mg L⁻¹, during which methanotrophs utilise algal-exuded organic substrates or intracellular glycogen reserves for metabolic maintenance. Advanced light-responsive DO controlled strategies (e.g., reduced agitation during photophase, microaerobic supplementation in scotophase) demonstrate methane utilisation enhancement with concurrent energy input reduction, establishing optimised oxygen management protocols for industrial-scale implementation.

In summary, the methanotroph-algae co-cultivation system necessitates precise orchestration across multiple parameters: balanced metal ion homeostasis, pH-thermal compatibility, and dynamic light-oxygen coupling. Future research should prioritise the following directions: 1) development of nanoparticle-based metal ion delivery systems for controlled ion release to maintain optimal concentrations and reduce metal toxicity to microbial consortia; 2) establishment of microbial-algal metabolic flux allocation models to identify pivotal nodes in carbon-

oxygen exchange networks through ^{13}C metabolic flux analysis; and 3) engineering of intelligent light-aeration feedback systems integrating machine learning algorithms to adapt to circadian rhythms and environmental perturbations. Through multi-disciplinary convergence encompassing synthetic biology, process engineering, and artificial intelligence, this symbiotic platform holds transformative potential for large-scale implementation in three critical domains, such as advancements could position microbial-algal consortia as cornerstone technologies for circular bioeconomy frameworks.

4. OPTIMISATION OF SYMBIOTIC BACTERIAL CO-CULTURE SYSTEM

4.1. Reactor type

Microbial algae reactors are divided into suspended and attached systems (Hülse et al., 2020). However, consortium-specific designs must prioritise simultaneous optimisation of light delivery, gas-liquid mass transfer, and sterile methane introduction to sustain syntrophic interactions. Among them, suspended systems are dominated by open high-rate algal ponds (HRAPs) and closed photobioreactors (PBRs) (Goncalves et al., 2016). HRAPs achieve efficient pollutant removal through algal biomass production and photo driven effects, but there are problems with evaporation loss and large footprint (Acién et al., 2016; Vassalle et al., 2023). Tube and plate PBRs improve light utilisation efficiency through closed design, but face technical bottlenecks such as fluid control (Touloupakis et al., 2022). The attached system utilises microbial biofilm technology to enhance denitrification efficiency and biomass yield through immobilised carriers, significantly reducing harvesting costs (Hülse et al., 2020). However, it has limitations in light penetration and high maintenance costs. For example, the denitrification efficiency of the algal biofilm membrane photobioreactor (BMPBR) is 1.2 times higher than that of the suspension system, and the pollutant removal rate in pig manure wastewater treatment can reach over 95% (Gao et al., 2015; Wu et al., 2019). The biomass production of both types of systems is better than that of traditional activated sludge processes. In addition, achieving efficient methane dissolution presents a significant challenge due to its inherently low aqueous solubility (Kim et al., 2017). Fine pore diffusers can be used to maximise the critical gas-liquid interface area and improve methane solubility (Gonçalves et al., 2025). Furthermore, ensuring process integrity necessitates sterile filtration of the methane feed stream using hydrophobic membranes with a pore size of 0.22 μm , effectively excluding microbial contaminants while permitting unimpeded gas influx. In the future, low-cost and high-efficiency reactors need to be developed to balance environmental benefits and economic feasibility, especially in improving the stability of photobiofilm, reducing energy consumption and water resource loss, which urgently needs breakthroughs.

4.2. Regulation of the proportion and interaction of symbiotic bacteria

Precise regulation of symbiont ratios and interspecies interactions is critical for optimising performance in methanotroph-algae symbionite systems. Within this mutualistic relationship, methanotrophs catalyse methane oxidation to CO_2 , while algae utilise this CO_2 for photosynthetic biomass production with concomitant oxygen evolution to sustain methanotrophic metabolism. This codependent cycle necessitates strict control over microbial population

dynamics to ensure system stability and operational efficiency. The consortium ratio exerts profound impacts on process optimisation. Ruiz-Ruiz et al. (2020) systematically demonstrated that initial methanotroph-to-microalgae biomass ratios significantly influence methane biodegradation kinetics. Their work identified a 3:1 methanotroph-algae ratio as optimal. This underscores ratio-dependent metabolic coupling as a key determinant of greenhouse gas conversion efficiency. Interaction regulation requires advanced characterisation methodologies. Badr et al. (2019) developed a hybrid experimental-computational protocol enabling real-time quantification of individual biomass concentrations, substrate consumption rates, and product formation kinetics in methanotroph-photoautotroph consortia. This platform provides unprecedented resolution in analysing consortium growth dynamics, establishing predictive models for bioprocess scaling. Rasouli et al. (2018) further elucidated nutrient recycling mechanisms in *Chlorella*-methanotroph systems treating industrial wastewater. Through techno-economic analysis revealed that optimised interspecies interactions enhance nitrogen recovery from wastewater, which serves as a vital nutrient source (e.g., for amino acid synthesis in methanotrophs). This directly supports microbial growth and metabolic activity, thereby improving CH₄ oxidation and CO₂ assimilation rates. Consequently, these interactions elevate both single-cell protein yields and greenhouse gas conversion efficiency — performance metrics intrinsically linked to cross-kingdom metabolite exchange networks (Rasouli et al., 2018). Collectively, these studies demonstrate that strategic consortium engineering through ratio optimisation and interaction enhancement can elevate CH₄/CO₂ conversion efficiencies. Such advancements hold transformative potential for sustainable biotechnology applications, particularly in next-generation bioenergy systems and carbon-negative wastewater treatment platforms.

4.3. Modelling and simulation of biological processes

Significant advancements have been achieved in modelling and simulating methanotroph-algae symbiotic co-cultivation systems, providing critical insights into microbial interaction mechanisms while informing bioreactor design strategies. Current modelling frameworks typically begin with consortium growth kinetics characterisation. Badr et al. (2021) developed a semi-structured kinetic model predicting species-specific growth rates and O₂/CO₂ exchange rates in methanotroph-photoautotroph consortia. This model explicitly simulates interspecies O₂/CO₂ cycling coupled with gas-liquid mass transfer dynamics, predicting growth patterns under varying substrate availabilities. Environmental parameter integration has enhanced model fidelity. Béchet et al. (2013) incorporated light-temperature interaction algorithms into algal growth models, demonstrating that photosynthetically active radiation (PAR) and temperature fluctuations account variance in outdoor cultivation productivity through Monte Carlo simulations. Advanced computational tools are revolutionising system optimisation. Kasiri et al. (2015) employed hybrid kinetic-optimisation models to decode CO₂ fixation dynamics in oil sands process water treatment, revealing rate-limiting steps in algal bicarbonate transport. Rostkowski et al. (2013) parameterised methane monooxygenase (MMO) activity constraints in growth models, identifying enzymatic kinetic threshold governing methanotrophic dominance in consortia.

These integrated modelling approaches enable: 1) predicting CH₄ oxidation fluxes, 2) identification of optimal O₂ partial pressure setpoints through sensitivity analysis, and 3) dynamic control strategies reducing energy consumption. Future research directions should focus on

multi-omics data integration for genome-scale metabolic reconstructions, machine learning-enhanced parameter estimation, and cyber-physical system implementation for real-time bioprocess optimisation. Such computational breakthroughs will accelerate the translation of methanotroph-algae symbiosis from laboratory prototypes to industrial-scale carbon capture and utilisation platforms achieving GHG conversion efficiency.

5. THE APPLICATION OF SYMBIOTIC BACTERIA IN ACTUAL PRODUCTION

5.1. Single cell protein production

The escalating global population intensifies demand for protein resources, challenging conventional animal- and plant-based sources to meet nutritional requirements. Single-cell protein (SCP) has emerged as a sustainable alternative, valued for its high nutritional density and eco-friendly production profile. Methanotroph-algae co-cultivation systems have gained scientific prominence due to their unique capacity for complementary utilisation of distinct carbon substrates – methanotrophs metabolising methane as carbon/energy source while algae assimilate CO₂ through photosynthesis. This syntrophic interaction enhances SCP productivity through metabolic synergy, simultaneously improving protein yields and reducing greenhouse emissions *via* coupled methane oxidation and carbon sequestration. Xu et al. (2020) investigated *Methylocapsa acidiphila*'s SCP production from biogas-derived methane, demonstrating robust growth under high H₂S concentrations. This resilience enables direct utilisation of raw biogas streams, achieving high CH₄ conversion efficiency while remediating sulphur-contaminated wastewater. Rasouli et al. (2018) engineered algal-methanotrophic consortia for industrial wastewater treatment, reporting organic carbon removal concurrent with SCP production. In this study, both algae and bacteria were able to remove or assimilate organic carbon from wastewater, with protein content reaching 45%–52.5% of dry weight. Recent advances utilise anaerobic digestate – a biogas byproduct – as cultivation medium. Through the co-cultivation system of algae and methanotroph, not only can biogas slurry be treated, but valuable SCP can also be produced. Wang et al. (2022) achieved high-value utilisation of biogas resources containing 40% (v/v) sterilised digestate through a combined *Chlorella vulgaris*-methanotrophic bacteria system, yielding 90.43 mg L⁻¹ SCP at 280 mg L⁻¹ dry biomass.

While utilising waste streams like biogas slurry for SCP production demonstrates technical potential for resource recovery and carbon capture, significant safety and regulatory challenges must be emphasised. Biogas slurry and industrial wastewater often contain complex mixtures of contaminants, including heavy metals (e.g., Cu, Zn), pathogens, residual pharmaceuticals, organic toxins, and other undesirable compounds (Sasidharan and Kumar, 2022; Lu et al., 2024). Stringent regulatory frameworks governing feed and food safety strictly prohibit the introduction of harmful contaminants into these chains. Therefore, the direct use of untreated or inadequately treated waste streams for SCP production intended for animal feed or human consumption is not viable. Rigorous pretreatment of the substrate (e.g., sterilisation, filtration, contaminant removal) and thorough downstream processing of the SCP biomass, coupled with comprehensive contaminant analysis and strict quality control protocols aligned with regulations (e.g., FDA, EFSA), are essential prerequisites.

Nutritional evaluation confirms SCP from these consortia contains all essential amino acids, mirroring fishmeal and soybean meal profiles in methionine and lysine. This positions microbial

SCP as viable feed/food alternative, with current commercialisation efforts focusing on texturisation technologies (e.g., extrusion, enzymatic hydrolysis) to enhance palatability. Future research imperatives should prioritize: 1) metabolic engineering to boost sulphur-containing amino acid biosynthesis, 2) hybrid cultivation systems integrating wastewater valorisation with carbon capture, and 3) life cycle assessment-guided process optimisation. SCP is expected to become a substitute for traditional protein sources, helping to alleviate global protein demand pressure and promote the development of circular economy. Future research needs to focus on improving the nutritional value of SCP, reducing production costs, and developing new application areas.

5.2. Production of biofuels

The methanotroph-algae co-cultivation system holds significant promise for producing diverse biofuels, leveraging the metabolic capabilities of both partners. Key pathways include biohydrogen production and the generation of biodiesel precursors in the form of microalgal single-cell oils (SCO).

The methanotroph-algae co-cultivation system demonstrates significant potential for converting methane into biohydrogen. The co-culture of *Chlorella saccharophila* and *Methylomonas* sp. HYX-M1 quantitatively converts methane to biomass in 96 h, with subsequent dark fermentation by *Clostridium* strains yielding biohydrogen from derived sugars – a process enhanced by light intensity – positioning this biotechnology as a pivotal yet complex pathway for climate-positive energy (Sang et al., 2024). Furthermore, microalgae possess a well-established capacity to accumulate substantial amounts of storage lipids, primarily triacylglycerides, under specific cultivation conditions. These lipids, termed SCO, represent a direct feedstock for biodiesel production *via* transesterification (Parsons et al., 2020). Within the methanotroph-algae consortium, the CO₂ fixed by the algal partner through photosynthesis provides the carbon backbone for *de novo* lipid biosynthesis. Factors critically influencing SCO yield and quality (e.g., fatty acid chain length and saturation) within the co-culture system include: 1) nutrient stress, 2) light regime, and 3) algal strain selection (Carreres, 2019). Optimising these parameters within the co-culture environment can direct metabolic flux towards SCO production. While SCO extraction and conversion technologies are maturing, integrating and optimising these steps specifically within the context of methanotroph-algae co-cultivation, potentially using biogas-derived CO₂, remains an active area for research and development. Biofuel production efficiency in these consortia is governed by multivariate environmental parameters: 1) light intensity, 2) thermophilic regimes, 3) pH homeostasis, and 4) nitrogen-phosphorus stoichiometry (Hill et al., 2017). Strategic optimisation of these factors could elevate methane-to-biohydrogen conversion efficiencies.

Future research needs to focus on improving methane conversion rates, as well as exploring how to scale up this co-cultivation system to industrial scale, and how to improve the purity and quality of biofuels. The co-cultivation system of methane oxidising bacteria and algae shows great potential in biofuel production. By optimising cultivation conditions and improving production efficiency, this system is expected to become a substitute for traditional fossil fuels, help alleviate the global energy crisis, and promote sustainable development (Rasouli et al., 2018).

6. CONCLUSION AND FUTURE PERSPECTIVES

The methanotroph-algae co-cultivation technology has demonstrated significant potential at laboratory scale but faces multifaceted challenges in transitioning to industrial-scale implementation. First, maintaining and controlling biomass in large-scale systems requires precision engineering and optimisation of operational parameters. Cost-benefit analyses for scaled production must address critical factors including culture medium expenses, energy consumption, photobioreactor design optimisation, and capital/maintenance investments. Second, ensuring continuous and stable methane supply while enhancing gas-liquid mass transfer efficiency is paramount for sustaining high-performance consortium operation. Third, environmental parameter regulation (temperature ± 0.5 °C, pH ± 0.2 , photon flux density 120–250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) exerts profound impacts on microbial growth kinetics and metabolic flux partitioning.

Methanotroph-algae symbiosis represents a robust biotechnology for mitigating methane emissions, with environmental resilience and stability governed by: 1) initial biomass ratios (optimal 3:1 methanotroph:algae), 2) methane concentration gradients (5–15% v/v), and 3) thermal/pH adaptability. Methanotrophic growth rates exhibit substantial interspecies variability – slow-growing strains like *Methylocaldum* spp. (isolated from thermal springs) require 2–4 weeks for colony formation on agar plates, whereas fast-growing genera such as *Methylococcus* and *Methylosinus* achieve growth rates of 0.2 h^{-1} and biomass densities of 1–5 g DCW/L, making them preferred candidates for biotechnological applications.

Environmental adaptability is further evidenced by methanotrophic metabolic versatility. Genomic and transcriptomic analyses of facultative methanotroph *Methylocystis* spp. reveal dual carbon assimilation pathways: the canonical methane oxidation route coupled with serine cycle operation, alongside active tricarboxylic acid (TCA) and ethylmalonyl-CoA (EMC) pathways for multicarbon substrate utilisation.

Future research should focus on the following directions to promote the development of co-culture systems between methane oxidising bacteria and algae symbiotic bacteria. Developing new technologies and strategies is crucial for improving the efficiency and sustainability of the system. This includes optimising cultivation conditions such as pH, temperature, and light to maximise methane absorption and biomass accumulation; Critically, future metabolic engineering efforts must prioritise overcoming the initial bottleneck in methane assimilation: the activity and stability of MMO. MMO catalysis governs the system's methane oxidation rate. Copper-dependent pMMO efficiency is compromised under suboptimal Cu^{2+} bioavailability or oxidative stress, while sMMO's lower kinetic efficiency limits overall flux. Synthetic biology strategies should target: 1) pMMO expression enhancement *via* copper-uptake gene modules, 2) sMMO engineering for improved turnover under low- O_2 conditions, and 3) MMO encapsulation in oxygen-nanocarriers to mitigate photooxidative damage. Resolving these MMO-specific constraints is essential for scalable CH_4 -to-bioproduct conversion. For industrial deployment, bioreactor design must resolve specific limitations, like in suspended systems (e.g., tubular PBRs), poor gas-liquid mass transfer of CH_4 and O_2/CO_2 disequilibrium during light/dark cycles reduce yields. Attached systems (e.g., BMPBRs) alleviate harvesting costs but suffer from biofilm light penetration issues. Future designs should integrate: 1) gas-permeable membranes for efficient CH_4/O_2 diffusion, 2) light-guiding materials in biofilm reactors to enhance PAR distribution, and 3) AI-driven feedback controls to dynamically adjust agitation, gas sparging, and light intensity in response to real-time DO/pH sensors. Optimising these parameters is critical

for continuous industrial systems. Future research should prioritise: MMO-Centric Metabolic Engineering: using multi-omics to identify MMO expression regulators under mixed-substrate conditions. Reactor-Scale Integration: developing hybrid biofilm/suspension reactors with *in situ* microalgal harvesting and low-energy O₂ recycling. Process Monitoring: implementing machine learning models to predict MMO inhibition states *via* dissolved Cu²⁺ and ROS sensors. Through these efforts, we can expect to make greater progress in reducing greenhouse gas emissions, increasing biomass and bioproduct production, and reducing production costs.

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REFERENCES

- Acién, F.G., Gómez-Serrano, C., Morales-Amaral, M.M., Fernández-Sevilla, J.M., and Molina-Grima, E. (2016). Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? *Applied Microbiology and Biotechnology*, 100(21): 9013–9022.
- Badr, K., Hilliard, M., Roberts, N., He, Q.P., and Wang, J. (2019). Photoautotroph-methanotroph coculture – a flexible platform for efficient biological CO₂-CH₄ co-utilization. *IFAC-PapersOnLine*, 52(1): 916–921.
- Badr, K., Whelan, W., He, Q.P., and Wang, J. (2021). Fast and easy quantitative characterization of methanotroph-photoautotroph cocultures. *Biotechnology and Bioengineering*, 118(2): 703–714.
- Béchet, Q., Shilton, A., and Guieysse, B. (2013). Modeling the effects of light and temperature on algae growth: state of the art and critical assessment for productivity prediction during outdoor cultivation. *Biotechnology Advances*, 31(8): 1648–1663.
- Cantera, S., Lebrero, R., Sadornil, L., García-Encina, P.A., and Muñoz, R. (2016). Valorization of CH₄ emissions into high-added-value products: assessing the production of ectoine coupled with CH₄ abatement. *Journal of Environmental Management*, 182: 160–165.
- Carreres, B.M. (2019). *Transcriptional landscapes of lipid producing microalgae*, International PhD Thesis. Wageningen University and Research, <https://research.wur.nl/en/publications/transcriptional-landscapes-of-lipid-producing-microalgae>.
- El Ghazouani, A., Baslé, A., Gray, J., Graham, D.W., Firkbank, S.J., and Dennison, C. (2012). Variations in methanobactin structure influences copper utilization by methane-oxidizing bacteria. *The Proceedings of the National Academy of Sciences (PNAS)*, 109(22): 8400–8404.
- Escudero-Almanza, D.J., Ojeda-Barrios, D.L., Hernández-Rodríguez, O.A., Sánchez Chávez, E., Ruíz-Anchondo, T., and Sida-Arreola, J.P. (2012). Carbonic anhydrase and zinc in plant physiology. *Chilean Journal of Agricultural Research*, 72(1): 140–146.
- Gao, F., Yang, Z.H., Li, C., Zeng, G.M., Ma, D.H., and Zhou, L. (2015). A novel algal biofilm membrane photobioreactor for attached microalgae growth and nutrients removal from secondary effluent. *Bio-resource Technology*, 179: 8–12.

- Goncalves, A.L., Pires, J.C.M., and Simoes, M. (2016). A review on the use of microalgal consortia for wastewater treatment. *Algal Research*, 24(Part B): 403–415.
- Gonçalves, R.F., Bastos, L.P., Nariyoshi, Y.N., Borges, R.M., Keller, R., and Silveira, D.D. (2025). Assessment of selected parameters in CO₂ and CH₄ mass transfer during photosynthetic biogas upgrading using bubble columns filled with wastewater-derived microalgae. *Energy & Fuels*, 39(15): 7314–7325.
- Gong, Q.T., Feng, Y.Zh., Kang, L.G., Luo, M.Y., and Yang, J.H. (2014). Effects of light and pH on cell density of *Chlorella vulgaris*. *Energy Procedia*, 61: 2012–2015.
- Grobbelaar, J.U. (1991). The influence of light/dark cycles in mixed algal cultures on their productivity. *Bioresource Technology*, 38(2–3): 189–194.
- Hill, E.A., Chrisler, W.B., Beliaev, A.S., and Bernstein, H.C. (2017). A flexible microbial co-culture platform for simultaneous utilization of methane and carbon dioxide from gas feedstocks. *Bioresource Technology*, 228: 250–256.
- Hom, E.F.Y., Aiyar, P., Schaeme, D., Mittag, M., and Sasso, S. (2015). A chemical perspective on microalgal-microbial interactions. *Trends in Plant Science*, 20(11): 689–693.
- Hülsem, T., Marx Sander, E., Jensen, P.D., and Batstone, D.J. (2020). Application of purple phototrophic bacteria in a biofilm photobioreactor for single cell protein production: biofilm vs suspended growth. *Water Research*, 181(15): 115909.
- Jiang, H.B., Lu, X.H., Deng, B., Liu, L.M., and Qiu, B.S. (2020). Adaptive mechanisms of the model photosynthetic organisms, cyanobacteria, to iron deficiency. In: Wang, Q. (Ed.), *Microbial photosynthesis*. Springer, Singapore, https://doi.org/10.1007/978-981-15-3110-1_11197-244
- Kasiri, S., Ulrich, A., and Prasad, V. (2015). Kinetic modeling and optimization of carbon dioxide fixation using microalgae cultivated in oil-sands process water. *Chemical Engineering Science*, 137: 697–711.
- Kim, K., Kwon, T., Sung, B.J., and Kim, C. (2017). Effect of methane-sugar interaction on the solubility of methane in an aqueous solution. *Journal of Colloid and Interface Science*, 500: 113–118.
- Li, M., Zhu, Q., Hu, C.W., Chen, Z.L., and Kong, Z.M. (2007). Cobalt and manganese stress in the microalga *Pavlova viridis* (Prymnesiophyceae): effects on lipid peroxidation and antioxidant enzymes. *Journal of Environmental Science*, 19: 1330–1335.
- Li, X., Lu, Y.Z., Li, N., Wang, Y.Z., Yu, R., Zhu, G.C., and Zeng, R.J. (2022). Mixotrophic cultivation of microalgae using biogas as the substrate. *Environmental Science & Technology*, 56(6): 3669–3677.
- Lu, Y., Wu, J.H., Li, J.W., Du, Z.L., Zhang, C.A., Chai, Y.J., Jin, Z.W., Li, Z.C., Meng, J., Zhuang, H.F., Ping, L.F., Wong, M.H., Zheng, G.Y., and Shan, S.D. (2024). Investigation of high-risk antibiotic resistance bacteria and their associated antibiotic resistance genes in different agricultural soils with biogas slurry from China. *Journal of Hazardous Materials*, 474: 134775.
- Manefield, M. and Turner, S.L. (2002). Quorum sensing in context: out of molecular biology and into microbial ecology. *Microbiology-Reading*, 148: 3762–3764.
- Pasichna, O.O. (2017). Content of pigments in *Cladophora glomerata* under the influence of copper (II) and manganese (II) ions in the aquatic environment. *Hydrobiological Journal*, 53: 60–68.
- Parsons, S., Allen, M.J., and Chuck, C.J. (2020). Coproducts of algae and yeast-derived single cell oils: a critical review of their role in improving biorefinery sustainability. *Bioresource Technology*, 303: 122862.
- Rasouli, Z., Valverde-Pérez, B., D’Este, M., De Francisci, D., and Angelidaki, I. (2018). Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs. *Biochemical Engineering Journal*, 134: 129–135.
- Reddy, K.R., Rai, R.K., Green, S.J., and Chetri, J.K. (2019). Effect of temperature on methane oxidation and community composition in landfill cover soil. *Journal of Industrial Microbiology & Biotechnology*, 46(9–10): 1283–1295.

- Reddy, K.R., Rai, R.K., Green, S.J., and Chetri, J.K. (2020). Effect of pH on methane oxidation and community composition in landfill cover soil. *Journal of Environmental Engineering*, 146(6): 04020037.
- Rostkowski, K.H., Pfluger, A.R., and Criddle, C.S. (2013). Stoichiometry and kinetics of the PHB-producing type II methanotrophs *Methylosinus trichosporium* OB3b and *Methylocystis parvus* OBBP. *Bioresource Technology*, 132: 71–77.
- Ruiz-Ruiz, P., Gómez-Borraz, T.L., Revah, S., and Morales, M. (2020). Methanotroph-microalgae co-culture for greenhouse gas mitigation: effect of initial biomass ratio and methane concentration. *Chemosphere*, 259: 127418.
- Sakimoto, K.K., Zhang, S.J., and Yang, P. (2016). Cysteine-cystine photoregeneration for oxygenic photosynthesis of acetic acid from CO₂ by a tandem inorganic-biological hybrid system. *Nano Letters*, 16(9): 5883–5887.
- Sang, Y.X., Xie, Z.Z., Li, L.Y., Wang, O.M., Zheng, S.L., and Liu, F.H. (2024). Biohydrogen production from methane-derived biomass of methanotroph and microalgae by *Clostridium*. *Fermentation-Basel*, 10(8): 383.
- Sasidharan, R. and Kumar, A. (2022). Magnetic adsorbent developed with alkali-thermal pretreated biogas slurry solids for the removal of heavy metals: optimization, kinetic, and equilibrium study. *Environmental Science and Pollution Research*, 29(20): 30217–30232.
- Singh, S.P. and Singh, P. (2015). Effect of temperature and light on the growth of algae species: a review. *Renewable & Sustainable Energy Reviews*, 50: 431–444.
- Stauber, J.L. and Florence, T.M. (1987). Mechanism of toxicity of ionic copper and copper complexes to algae. *Marine Biology*, 94: 511–519.
- Tan, J.Y., Chen, C., Zhang, C.C., Wang, Z.H., Wu, J.T., Xing, D.F., Ren, N.Q., Wang, A.J., and Zhao, L. (2024). Roles of oxygen in methane oxidation coupled denitrification in membrane biofilm reactors. *Chemical Engineering Journal*, 493: 152744.
- Touloupakis, E., Faraloni, C., and Carozzi, P. (2022). An outline of photosynthetic microorganism growth inside closed photobioreactor designs. *Bioresource Technology Reports*, 18: 101066.
- van der Ha, D., Bundervoet, B., Verstraete, W., and Boon, N. (2011). A sustainable, carbon neutral methane oxidation by a partnership of methane oxidizing communities and microalgae. *Water Research*, 45(9): 2845–2854.
- van der Ha, D., Vanwonterghem, I., Hoefman, S., De Vos, P., and Boon, N. (2013). Selection of associated heterotrophs by methane-oxidizing bacteria at different copper concentrations. *Antonie Van Leeuwenhoek*, 103(3): 527–537.
- Vassalle, L., Ferrer, I., Passos, F., Filho, C.R.M., and Garfi, M. (2023). Nature-based solutions for wastewater treatment and bioenergy recovery: a comparative life cycle assessment. *The Science of the Total Environment*, 880: 163291.
- Veraart, A.J., Garbeva, P., van Beersum, F., Ho, A., Hordijk, C.A., Meima-Franke, M., Zweers, A.J., and Bodelier, P.L.E. (2018). Living apart together-bacterial volatiles influence methanotrophic growth and activity. *ISME Journal*, 12(4): 1163–1166.
- Vorobev, A.V., Baani, M., Doronina, N.V., Brady, A.L., Liesack, W., Dunfield, P.F., and Dedysh, S.N. (2011). *Methyloferula stellata* gen. nov., sp. nov., an acidophilic, obligately methanotrophic bacterium that possesses only a soluble methane monooxygenase. *International Journal of Systematic and Evolutionary Microbiology*, 61: 2456–2463.
- Wang, D.H., Zhu, M.Y., Lian, S.J., Zou, H., Fu, S.F., and Guo, R.B. (2022). Conversion of renewable biogas into single-cell protein using a combined microalga- and methane-oxidizing bacterial system. *ACS ES&T Engineering*, 2(12): 2317–2325.

- Wu, X.D., Cen, Q.J., Min, A., Zheng, H.L., Luo, S.S., Liu, Y.Y., Chen, Y.L., Zhou, W.G., Chen, P., and Ruan, R. (2019). A novel algal biofilm photobioreactor for efficient hog manure wastewater utilization and treatment. *Bioresource Technology*, 292: 121925.
- Xu, M.Y., Zhou, H.H., Yang, X.Y., Angelidaki, I., and Zhang, Y.F. (2020). Sulfide restrains the growth of *Methylocapsa acidiphila* converting renewable biogas to single cell protein. *Water Research*, 184: 116138.
- Yu, S.S., Chen, K.H., Tseng, M.Y., Wang, Y.S., Tseng, C.F., Chen, Y.J., Huang, D.S., and Chan, S.I. (2003). Production of high-quality particulate methane monooxygenase in high yields from *Methylococcus capsulatus* (Bath) with a hollow-fiber membrane bioreactor. *Journal of Bacteriology*, 185(20): 5915–5924.
- Zhou, D.D., Zhang, C.F., Liang, F., Liang, X., Cui, X.C., Li, Q.C., and Crittenden, J.C. (2017). Responses of the microalga *Chlorophyta* sp. to bacterial quorum sensing molecules (N-acylhomoserine lactones): aromatic protein-induced self-aggregation. *Environmental Science & Technology*, 51(6): 3490–3498.

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