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Review Paper

Methanotroph biotransformation for nutrient recovery: a review of current strategies and future opportunities

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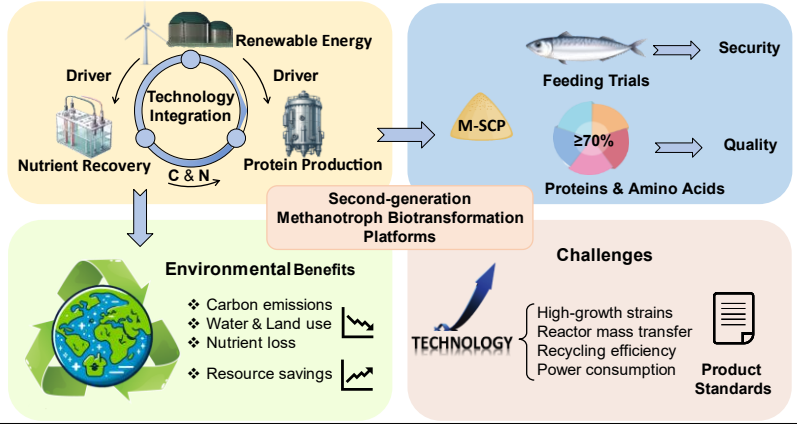
HIGHLIGHTS

- Renewable energy is a crucial driver in promoting methanotroph biotransformation platforms.
- Environmental benefits are the core competitiveness of the second-generation methanotroph single-cell protein (M-SCP).
- M-SCP from waste should be tested in extensive feeding trials to verify safety.
- Combining biotransformation and nutrient recovery from a circular economy perspective.

GRAPHICAL ABSTRACT



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ABSTRACT

The escalating global demand for protein and the imperative to meet sustainable development goals have driven the emergence of biotransformation platforms, with methanotrophs showing significant potential in this field. In this paper, the metabolism, nutritional requirements, cultivation strategies, and bioreactors of methanotrophs are reviewed. Integrating upstream and downstream technologies is also advocated to advance the development of methanotroph biotransformation platforms toward a circular economy model. The advancements in utilizing biogas as a viable carbon source and wastewater as a nitrogen source are discussed, emphasizing the need for detailed quality control and safety assessments to ensure the suitability of single-cell protein as animal feed. In general, by integrating advanced nutrient recovery technologies to define new process routes, methanotroph biotransformation platforms can bring better environmental benefits by reducing carbon emissions and saving resources. Shifting to renewable energy is crucial for achieving environmental sustainability. By using renewable energy to power microbial fermentation, biomass dehydration, and waste recycling, the platform can offset high energy consumption and attain significant market competitiveness with traditional protein sources.

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Contents

Abbreviations

AMS	Ammonium mineral salt
AD	Anaerobic digestion
BESs	Bioelectrochemical systems
BPM	Bacterial protein meal
CDW	Cell dry weight
CHP	Combined heat and power
FM	Fish meal
HOB	Hydrogen oxidizing bacteria
k_La	Gas mass transfer efficiency
LCA	Life cycle assessment
MOB	Methane oxidizing bacteria
M-SCP	Methanotroph single-cell protein
MMO	Methane monooxygenase
MFC	Microbial fuel cells
MEC	Microbial electrolysis cells
NMS	Nitrate mineral salt
pMMO	Particulate methane monooxygenase
RuMP	Ribulose monophosphate pathway
SCP	Single-cell protein
sMMO	Soluble methane monooxygenase
SBM	Soybean meal
WRRFs	Water resource recovery facilities
WWTPs	Wastewater treatment plants

1. Introduction

With economic development and improved living standards, the structure of human diets has significantly changed, leading to an increased demand for high-quality protein. This change has prompted the expansion of farming and cultivation to meet the demand for feed. However, protein production based on traditional agriculture is increasingly acknowledged as a principal contributor to global environmental pollution. Problems like nutrient loss, greenhouse gas emissions, land use, water usage, and wastewater production are linked to traditional agricultural methods of protein production (Willett et al., 2019). According to research, the global demand for animal-derived food is projected to increase by approximately 40% by 2050 compared to 2020 (Komarek et al., 2021). Thus, there is a need to produce protein more sustainably and to rebalance the contributions between animal and plant proteins or explore other alternatives like single-cell protein (SCP).

Methanotroph single-cell protein (M-SCP) is considered a viable alternative protein source due to its wide range of essential amino acids and unsaturated fatty acids, comparable to traditional feed proteins such as soybean meal and fish meal (Biswas et al., 2020). Notably, methanotrophs, also known as methane-oxidizing bacteria (MOB), are a group of microorganisms that use methane as their sole source of carbon and energy (Jiang et al., 2021). M-SCP began in the 1980s but stalled due to the high cost of natural gas and synthetic nitrogen, along with competition from other feed products (Marcellin et al., 2022). Nevertheless, the recent rapid development of the circular economy has revitalized M-SCP production. It has shifted towards a second-generation route utilizing carbon and nitrogen sources reclaimed from waste streams, showing a promising trajectory (Khoshnevisan et al., 2022).

Biogas, derived from various anaerobic facilities like biogas plants and landfills, serves as a promising carbon source, offering substantial yields and considerable developmental potential. Previous studies have shown that methanotrophs cultured on biogas can attain production rates ranging from 0.66–0.87 grams per gram of methane (Khoshnevisan et al., 2019; Zha et al., 2021). Assuming that 0.7 $g \cdot g^{-1}$ of M-SCP can be produced, renewable methane in the US alone could meet up to 14% of the global fishmeal market, and the price was equal to or lower than that of the current cost of fishmeal (about USD 1,600 per metric ton) (El Abbadi et al., 2021).

For the nitrogen source, wastewater has attracted significant attention due to the high amount of organic matter and elements such as nitrogen and phosphorus (Vethathirri et al., 2021). Currently, the field of wastewater management is transitioning towards nutrient recovery in water resource recovery facilities (WRRFs) rather than pollutant removal in wastewater treatment plants (WWTPs) (Wu and Vaneeckhaute, 2022). This shift is more in line with the principles of a sustainable and circular economy concept. The use of nutrients from effluent to cultivate methanotrophs has demonstrated as feasible in previous studies, and an increasing range of new water treatment technologies are being applied to this process as auxiliary technologies, such as membrane technology, electrochemical technology, and bioelectrochemical technology (Khoshnevisan et al., 2020a; Yang et al., 2022b; Wan et al., 2023), to obtain better recovery and safer products. Although SCP production based on resource recovery has many advantages, it also presents great challenges regarding its security. The accumulation of pollutants such as antibiotics, heavy metals, and pathogens may affect the quality of SCP, thus posing a potential risk to animals or humans.

In summary, biotransformation platforms for recovering methane and nitrogen from the waste stream to cultivate methanotrophs for protein production are currently being proposed and investigated. However, the absence of thorough analysis regarding the recovery of nutrients from biogas and wastewater, as well as the environmental impact of these

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technologies, has limited the development of the platform. To further enhance the market competitiveness of M-SCP, proposing a recycling route that aligns with sustainable development and offers high returns is essential. Therefore, this paper summarizes the key upstream and downstream technologies of the second-generation methanotroph biotransformation platform and analyzes feasible recycling strategies. The unique advantage of the second-generation methanotroph biotransformation platform lies in its sustainability. The paper also examines the environmental impact of the platform to promote its development towards a circular economy model, thereby contributing to future development planning and industrial application. Additionally, it analyzes the quality and safety of M-SCP as feed protein to bolster public confidence in M-SCP products. **Table 1** provides an overview of the key studies on in M-SCP biotransformation platform and the main parameters investigated, emphasizing the novelty of the current study. This study comprehensively examines all the mentioned parameters.

2. Classification and metabolism of methanotrophs

Most methanotrophs belong to Alphaproteobacteria and Gammaproteobacteria of the Gram-negative bacteria (Ahmadi and Lackner, 2024); the optimum pH is between 4 and 7, and the temperature is between 20 and 35°C (Kambara et al., 2022). They can convert methane to carbon dioxide through two carbon assimilation pathways: the Ribulose Monophosphate (RuMP) pathway and the Serine pathway. Methanotrophs using the RuMP pathway are divided into Type I and Type X, and those using the serine pathway are Type II. Type I methanotrophs are more suited for growth under high oxygen and low methane concentrations, while Type II is the opposite (Xu et al., 2023). In addition to the three types mentioned above, a family of extremely acidophilic and thermophilic methanotrophs has been discovered recently, belonging to the Methylacidiphilaceae family of the Verrucomicrobia, which utilizes the Calvin-Benson-Bassham cycle and grows best at pH 2–3 (Schmitz et al., 2021). This group is enriched in geothermal environments, and their optimal growth temperature (55–60°C) is much higher than other methanotrophs (Picone et al., 2021). Currently, research on this group is limited.

As shown in **Figure 1**, the process of methane oxidation catalyzed by methanotrophs involves a series of electron transfer reactions that oxidize methane into methanol, formaldehyde, and formic acid, ultimately converting it into carbon dioxide and water. The initial step involves the oxidation of methane to methanol catalyzed by methane monooxygenase (MMO). MMO, the key enzyme in methanotrophs, occurs in a particulate form (pMMO) on the intracellular membrane or a soluble form (sMMO) within the cytoplasm (Le and Lee, 2023). Although both enzymes can oxidize methane, their structures, active sites, and catalytic mechanisms differ. All methanotrophs can produce pMMO, and only some methanotrophs can produce sMMO (Khider et al., 2021).

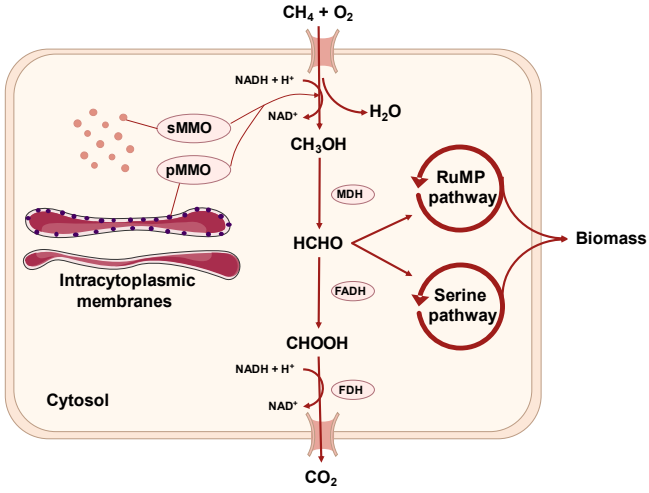

Fig. 1. Metabolic pathway of methanotrophs (sMMO: soluble methane monooxygenase; pMMO: particulate methane monooxygenase; MDH: methanol dehydrogenase; FADH: formaldehyde dehydrogenase; FDH: formate dehydrogenase).

Copper is considered a key factor in regulating the activity of two types of MMO (Manesis et al., 2021). This particular regulation mechanism is known as the "copper switch". pMMO is expressed at a high copper-to-biomass ratio, while sMMO is expressed under low conditions; however, the mechanism of this switch has not been elucidated. In experiments where copper was a variable, biomass yield at 100 $\mu g \cdot L^{-1}$ Cu^{2+} increased by 41% compared to that at less than 5 $\mu g \cdot L^{-1}$ Cu^{2+} (Tsapekos et al., 2020). Although the copper switching mechanism has not been clearly defined, some key aspects are being investigated thoroughly. It has been discovered that methanotrophs can secrete a copper-binding natural product known as methanobactin to collect copper (Kenney et al., 2016). The latest study has proposed a modified model of copper switching, which aids in understanding how microbes collect and compete for copper and how methanobactin uptake coordinates the expression of different forms of methane monooxygenases (Peng et al., 2022).

Methanol dehydrogenase is the second essential enzyme in the methane metabolism process; it catalyzes the conversion of methanol to formaldehyde (Kang et al., 2024). Then, a portion of formaldehyde is converted to formic acid by formaldehyde dehydrogenase, and formic acid is converted to carbon dioxide by formate dehydrogenase; another portion

Table 1.
Comparing this review with previously published reviews of Methylotroph single cell protein biotransformation platform.

Metabolism	Culture				Protein		Methanotroph biotransformation platform				Reference
	Nitrogen Source	Carbon Source	Strain	Reactor	Quality	Safety	Up/Down Stream Technology	Environmental Benefit	Economic Benefit	Policy	
√	×	√	√	×	√	√	×	×	√	√	Gundupalli et al. (2024)
×	√	√	√	√	√	×	√	×	√	×	Shahzad et al. (2024)
√	×	√	×	×	×	×	√	√	√	×	Li et al. (2023)
×	×	√	√	×	√	×	×	×	×	×	Sakarika et al. (2022)
×	√	√	√	√	√	×	√	√	√	×	Khoshnevisan et al. (2022)
√	×	√	√	×	×	×	×	×	×	×	Gesicka et al. (2021)
√	√	√	√	√	√	√	√	√	√	×	**This Review**

√: Included
×: Not included

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of formaldehyde is assimilated into cells through the RuMP pathway or the Serine pathway (**Fig. 1**) (Khan et al., 2023). In the serine pathway, formaldehyde undergoes a series of assimilations and finally condenses with glycine to form serine. In the RuMP pathway, formaldehyde is condensed with ribulose monophosphate to create hexulose phosphate, which is then sequentially converted to fructose-6-phosphate and pyruvate. Both serine and pyruvate are eventually used to synthesize biomass.

The use of C1 compounds, including methane, as substrates to replace carbohydrate-based metabolism has emerged as a research hotspot in recent years. However, compared with other industrial microorganisms such as *Escherichia coli*, some key enzymes and mechanisms of action of methanotrophs are still unclear, which limits its industrial development. In addition, some researchers have attempted to integrate the metabolic pathways of methanotrophs with those established in industrial biotechnology; however, the lack of synthetic biology tools has impeded this development (Gregory et al., 2022). Therefore, further elucidation of the key mechanisms of methanotrophs and the development of usable synthetic biology toolkits remain the focus of future research.

3. Culture of methanotrophs

Carbon and nitrogen sources are two indispensable components in microbial culture. Additionally, differences in strains and bioreactors can significantly affect the culture of methanotrophs. In **Figure 2**, the key issues associated with these factors are summarized and discussed in this section.

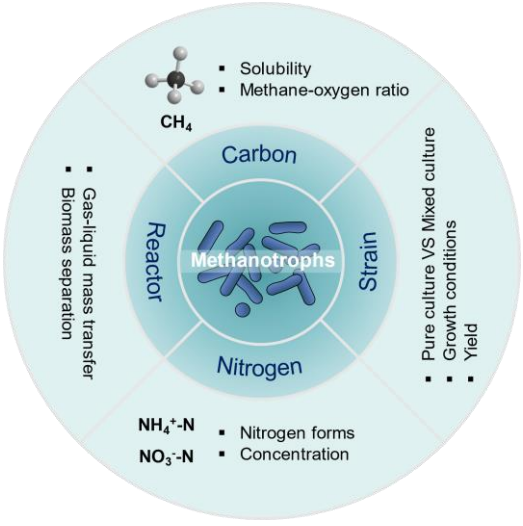

Fig. 2. Influencing factors and key issues in methanotroph culture.

3.1. Nitrogen

Methanotrophs promote cell growth through assimilation, detoxification of ammonium and hydroxylamine, and reduction of nitrate and nitrite (direct or indirect) (Bishoff et al., 2021). The commonly used nitrogen forms in the medium are nitrate or ammonia, which are referred to as nitrate mineral salt (NMS) medium and ammonium mineral salt (AMS) medium, respectively (Kim et al., 2021). Nitrogen sources can influence pH changes. Compared to the NMS medium, pH declines more rapidly in the AMS medium because protons are typically released when ammonium is consumed (Khoshnevisan et al., 2019). However, from the perspective of protein synthesis, ammonium is more advantageous because it can be used directly for amino acid synthesis, while nitrate must be reduced to ammonium (Bordel et al., 2019). Four predicted ammonium transporters were identified in the *Methylococcus capsulatus* genome, confirming that ammonium is a crucial nitrogen source (Wood et al., 2004).

Besides the form of nitrogen, concentration is also a critical issue. It was reported that the inhibitory concentrations of NH_4Cl or KNO_3 by *Methylosinus sporium* were 71 and 142 mmol·L^{-1}, respectively (He et al., 2017). The inhibition by nitrate may be attributed to an altered osmotic balance of cells due to salt effects, while inhibition by ammonium is more complex. Firstly, ammonia and methane compete for access in methanotrophs, as MMO not only oxidizes methane but also converts ammonium to nitrite; thus, ammonium concentration may influence methane consumption (Bodelier and Laanbroek, 2004). Secondly, the effect is due to metabolites. The intermediate and final products of ammonia oxidation, namely hydroxylamine and nitrite, are toxic to methanotrophs and inhibit their growth (He et al., 2017). Differences in the affinity of MMO for ammonia or the ability of methanotrophs to resist the toxicity of ammonia oxidation metabolites may contribute to the variation in their tolerance to ammonium (Nyerges and Stein, 2009). Therefore, the nitrogen concentration in the actual methanotroph cultures should be maintained within a reasonable range, and the nitrogen tolerance of various types of methanotrophs should be evaluated to optimize biomass production.

3.2. Carbon

Carbon composes the cells and metabolites and provides the energy needed for the vital activities of methanotrophs. Unlike the diversity of nitrogen sources, methanotrophs use only methane as their sole carbon source (Kwon et al., 2019). It is important to note that methane, a gaseous carbon source, has very low solubility in water, only 2.37%—which is a disadvantage (Han et al., 2009). Methane is typically combined with oxygen or air in the culture environment. However, to achieve greater biomass yield, a more precise gas ratio is essential for optimizing the production process. Methane ventilation ratios in previous studies, summarized in **Table 2**, typically range from 1:0.5 to 1:2 for methane to oxygen. It has been reported that the optimal ratio of methane to oxygen in experiments is 1:1.5, mirroring the calculated stoichiometry of *M. capsulatus* (Tsapekos et al., 2019). The stoichiometric equations for *M. capsulatus* are as follows (**Eq.1**):

$$CH_4 + 0.104NH_3 + 1.45O_2 \rightarrow 0.52CH_{1.8}O_{0.5}N_{0.2} + 0.48CO_2 + 1.69H_2O \quad \text{Eq.1}$$

For pure cultures or those dominated by methanotrophs, this value is indicative. However, for complex microbial communities, this value is not reliable because other microorganisms may consume the intermediate metabolites and indirectly affect the methane oxidation process of methanotrophs. Therefore, for microbial communities, it is crucial to identify each microorganism and its carbon metabolic flux and to appropriately supply the required concentration of methane.

3.3. Strain

Several potential products can be produced by methanotrophs, including SCP, biopolymers, methanol, ectoine, and extracellular polysaccharides (Wang et al., 2021). Strains within the same class may exhibit biases in certain aspects, such as metabolism and function, thus making strain selection critical for the target product due to its impact on maximum productivity and yield. *M. capsulatus* is a well-established commercial strain for producing SCP, characterized by a growth rate of 0.3–0.4 h^{-1} and a protein content of up to 70% or more. Furthermore, Type I methanotrophs, particularly *Methylomonas*, are widely used for SCP production. **Table 2** summarizes several strains and communities that have been utilized in previous attempts to produce SCP.

Methanotrophic communities, enriched from a methane-rich environment, are widely applied in existing research. Within a community, different species of microorganisms exhibit various types of interactions, which can be beneficial. Co-cultures offer advantages over pure cultures in terms of improved cell growth, biocatalytic potential, stability, and environmental adaptability (Singh et al., 2019). These characteristics enable methanotrophic communities to thrive in wastewater streams. In previous

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Table 2.
Summary of previous studies comparing culture parameters for single-cell protein production in methanotrophs culture.

Cultivation Mode	Cultivation Type	Strain	Temperature (°C)	pH	Biomass Yield ($g \cdot L^{-1}$)	Unit Methane Yield (g/g)	Gas Ratio (v/v)	Nitrogen	Reference
Continuous	MOB [a]	*Methylocapsa acidiphila*	19–25	5.70	0.119	N.A. [b]	—	NO_3^-	Xu et al. (2021b)
Batch	MOB	Type I methanotrophs	25-28	7	N.A.	0.592	O_2:CH_4 (1:1)	NO_3^-	AlSayed et al. (2018)
Batch	MOB	Type II methanotrophs	25	N.A.	N.A.	0.82	O_2:CH_4 (1:2)	NH_4^+	Fergala et al. (2018)
Batch	MOB	Mixed (enriched in *Methylococcales* and *Methylophilales*)	—	6.8	—	0.59 - 0.76	O_2:CH_4 (2:1)	NH_4^+	Khoshnevisan et al. (2019)
Batch	MOB	Mixed (enriched in *Methylophilus sp. (44%), Methylomonas sp. (14%), and Comamonadaceae sp. (13%)*)	25	6.8	0.49	0.88	O_2:CH_4 (2:1), CH_4 from Bio-methane (98% purity)	NH_4^+ (extracted nitrogen)	Khoshnevisan et al. (2020a)
Batch	MOB	Mixed (dominated by *Methylomonas* and *Methylophilus* spp.)	25	N.A.	—	0.66	O_2:CH_4 (2:1), CH_4 from Biogas	AD [c] supernatant	Zha et al. (2021)
Batch	MOB+ HOB [d]	Mixed	28	N.A.	0.585	—	H_2:O_2:CH_4 (35:15:26)	NH_4^+	Acosta et al. (2020)
Batch	MOB+ Microalgae	Mixed (dominated by *Scenedesmus* sp.)	—	6.9	N.A.	—	193 mL of O_2, 235 mL of CH_4, 793 mL of N_2 and 0.07 mL of CO_2 per L of gas phase	NH_4^+ NO_3^-	van der Ha et al. (2011)
Batch	MOB+ Microalgae	*Methylococcus capsulatus* and *Chlorella sorokiniana*	37	7.0	1.488	N.A.	CO_2:CH_4 (2:3)	NH_4^+ (Industrial wastewater from potato processing plant)	Rasouli et al. (2018)
Continuous	MOB	*Methylomonas* sp. and *Methylocystis* sp.	22.5	6.56	—	0.43	CH_4: 15.78~31.57 $ml \cdot h^{-1}$ Air: 16.89~29.01 $ml \cdot h^{-1}$ (period 1) O_2: 45.78–91.55 $ml \cdot h^{-1}$ (period 2)	NH_4^+	Valverde-Pérez et al. (2020b)
Continuous	MOB	*Methylomonas* sp.	—	—	—	0.79	O_2:CH_4 (2:3)	centrifuged-filtered AD digestate	Tsapekos et al. (2019)
Continuous	MOB	*Methylomonas* sp.	—	—	—	0.37	N.A.	NH_4^+ (Extract from wastewater)	Tsapekos et al. (2020)

[a] MOB: methane-oxidizing bacteria (Methanotrophs); [b] N.A. = not available; [c] AD: anaerobic digestion; [d] HOB: hydrogen oxidizing bacteria.

studies, pasteurized anaerobic digestion (AD) supernatant and pretreated wastewater facilitated substantial growth of methanotrophic communities and product accumulation (Zha et al., 2021). Additionally, they typically exhibit a more comprehensive amino acid composition compared to pure cultures.

Co-cultivation of methanotrophs and microalgae is regarded as a promising method due to its ability to simultaneously convert methane and carbon dioxide into valuable biomass (Ruiz-Ruiz et al., 2020). The presence of microalgae helps reduce the external dependence on oxygen for the methanotrophic community, while it consumes carbon dioxide produced by methane oxidation. Moreover, the exchange of O_2 and CO_2 produced *in situ* by microalgae and methanotrophs significantly reduces the mass transfer resistance of the two gaseous substrates and improves the conversion efficiency (Roberts et al., 2020). Bacterial and microalgal co-cultures are reported to readily form flocs, which represent a mutually beneficial co-trophic relationship and make it easier to separate the biomass from the liquid phase (van der Ha et al., 2011).

However, there are still some issues that require attention. Regulating co-cultures is more challenging than pure cultures. Competition among microorganisms for nitrogen and trace elements may occur, resulting in reduced protein content. The co-cultured biomass of *M. capsulatus* and *Chlorella sorokiniana* has reportedly reached up to 1488.2 $mg \cdot L^{-1}$. However, the protein content was significantly lower, comprising only 28% of the cell dry weight (CDW), which is well below the typical protein content of 67% to 81% for *M. capsulatus* (Rasouli et al., 2018). Although co-cultivation enhances the environmental adaptability of methanotrophs in complex substrates, it does not necessarily imply an improvement in protein quality. Essentially, this boils down to clarifying the interaction and metabolic network between microorganisms in a community to determine a suitable nutrient supply strategy, potentially a pressing issue for future research.

3.4. Bioreactor

Bioreactors are indispensable for microbial fermentation to achieve high-density culture and mass production. In contrast to other aerobic microbial fermentations, methanotrophs impose greater demands on the reactor due to the simultaneous transfer of methane and oxygen. Presently, a primary

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technical challenge restricting the scalability of methanotroph culture is the effective dissolution of gaseous substrates (methane and oxygen) into the liquid medium. In **Figure 3**, several types of bioreactors currently applied to methanotroph culture are compared.

Stirred tank reactors have long been favored in the biotechnology industry (Safaric et al., 2023). The most common configuration of stirred tank reactors includes a vertical shaft with one or more impellers driven by a gearbox. However, the presence of impellers can frequently subject methanotrophs to mechanical stress, resulting in cell damage and affecting biomass accumulation (Myung et al., 2016). Bubble column reactors enable stirring without inducing mechanical stress. In this design, the gas phase is introduced at the bottom, and gas diffusion occurs through convection driven by density variations. Derived from the bubble column reactor, the airlift reactor was developed, employing a high-speed nozzle for gas injection. However, there are certain risks associated with the use of bubble aeration. If the methane and oxygen bubbles generated by aeration become mixed, or if the aeration rate is excessively rapid, some gases may remain unconsumed, accumulating at the top of the reactor or within the pipeline. Consequently, there is a risk of explosion, particularly in large-scale or industrial cultivation, if the methane content in the gas phase surpasses the lower explosion limit of 5%.

The U-loop reactor is a multi-phase forced circulation tubular reactor where gases are pumped along with liquids. Static mixers are strategically placed at multiple locations along the loop to redisperse the gas and enhance interphase contact (Petersen et al., 2020). The U-loop reactor effectively meets the process demand for a substantial volumetric gas fraction (Petersen et al., 2017). Dansk Bioprotein A/S (Odense, Denmark) employs a U-loop reactor, achieving a productivity rate of 4 $kg \cdot m^{-3} \cdot h^{-1}$ (Ritala et al., 2017). Currently, this reactor has only undergone testing with natural gas and synthetic nitrogen as feedstock, and its applicability to waste streams remains to be validated. However, a pretreatment step is certainly necessary to prevent abrasion and clogging resulting from particulate matter or grit.

Hollow fiber membrane reactors are increasingly employed in cultivating gas-based microorganisms due to their ability to offer a high specific surface area. Moreover, as a type of membrane reactor, they can facilitate microbial growth and biofilm formation. Pressurized gas diffuses into the water through the hollow fiber membrane without forming bubbles, requiring lower gas supply pressure compared to bubbling aeration (Valverde-Pérez et al., 2020b). Indeed, hydrophobic membranes have emerged as an efficient gas transport in recent years. The gas-liquid transfer process is bidirectional; while mass transfer from the gas phase to the liquid phase occurs at high partial pressure, conversely, reducing the gas phase partial pressure through the vacuum or stripping gas can facilitate the diffusion of dissolved gas from the liquid phase to the gas phase (Hou et al., 2019).

While hollow fiber membranes offer significant advantages in gas transportation, the persistent issues of increased filtration resistance and energy consumption due to membrane fouling (Daud et al., 2023) remain challenging in membrane reactor applications. Moreover, the gas mass transfer efficiency (k_La) is not optimal due to the reactor being at the laboratory research stage. Future research endeavors may focus on enhancing k_La to meet the industrial production demands through increasing membrane surface area and reactor volume ratio. Notably, it has been reported that the addition of paraffin oil to the medium can enhance the growth of methanotrophs by mitigating mass transfer limitations. The addition of 5% paraffin oil yields a cell density of approximately 14 g of dry weight. L^{-1}, seven times higher than the control (Han et al., 2009). While this finding could alleviate the mass transfer issue in bioreactors to some extent, it complicates biomass harvesting and is deemed unsustainable from both environmental and food application perspectives.

Reactor cooling is an overlooked critical in prior research. The metabolic activity of microorganisms generates substantial heat, which is frequently underutilized. In methanotroph biotransformation platforms, significant energy inputs are reported within the cooling chain, with electricity costs comprising 45% of the total cost, 60% of which is allocated to reactor cooling (El Abbadi et al., 2021). For methanotroph biotransformation platforms, the next aspects to address and optimize are the energy requirements, as well as the recovery and circulation of the heat released from the reactor.

4. Methanotroph biotransformation platform

M-SCP production, based on natural gas and synthetic nitrogen, remains a microbial fermentation model in the traditional sense. To attain lower production costs and improved product competitiveness, contemporary M-SCP production is progressively shifting towards a second-generation route for protein recovery from the waste stream, entailing the integration of more

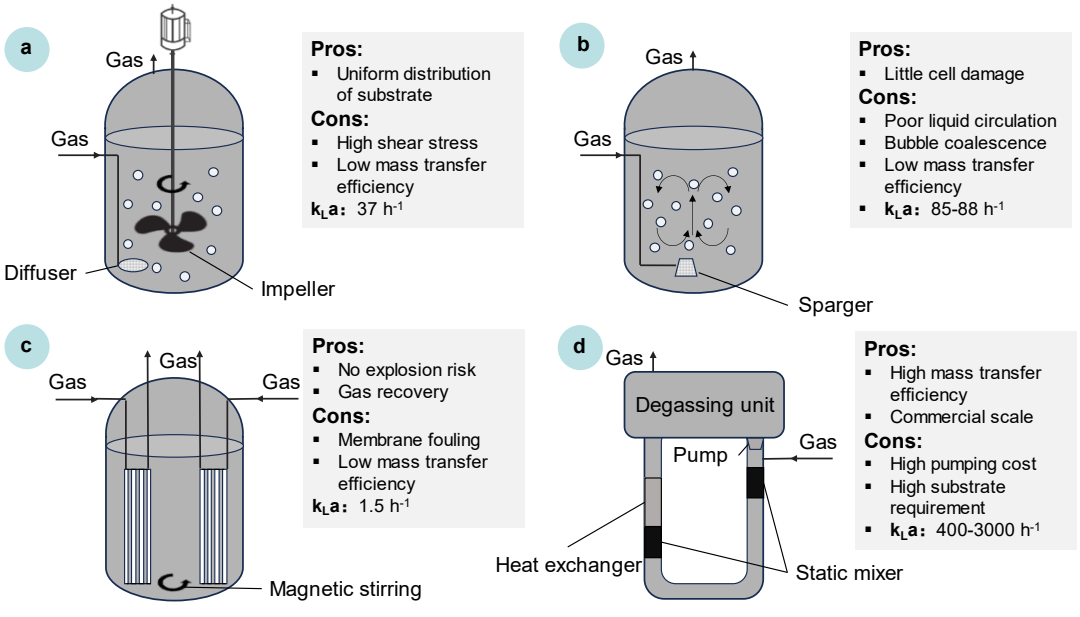

Fig. 3. Bioreactors for culturing methanotrophs: their pros, cons, and methane gas mass transfer efficiency (k_La). **a)** Stirred tank reactor, **b)** Bubble column/ Airlift reactor, **c)** U-loop reactor, **d)** Hollow fiber membrane reactor. The k_La values of the relevant reactors are referred to (Rocha-Rios et al., 2010 and 2011; Petersen et al., 2017; Valverde-Pérez et al., 2020b; Sahoo et al., 2023).

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technologies into the process (**Fig. 4**). Apart from the traditional fermentation unit, the methanotroph biotransformation platform incorporates upstream technologies that include nutrient and methane recovery, as well as downstream technologies focused on biomass dehydration and drying.

4.1. Upstream technologies

4.1.1. Nitrogen recovery

Typical nitrogenous wastewater, such as municipal, industrial (including food processing), aquaculture, livestock and poultry wastewater, and landfill leachate, could be viable alternatives to replace synthetic medium as a nitrogen source (Khoshnevisan et al., 2019; Xiang et al., 2020). Nitrogenous waste effluent can be utilized directly or following pretreatment as a culture medium for methanotrophs to provide nitrogen sources and trace elements (Zha et al., 2021). However, beyond nutritional requirements, it is also necessary to ensure that the wastewater is safe, being either free or low in heavy metals and antibiotics.

Some food processing wastewater sources, such as those from breweries, sugar factories, and soybean processing plants, may be safer. However, complex livestock wastewater is an adventure, but it is undoubtedly challenging and interesting. Additives and antibiotics are widely used in livestock and poultry production, and these substances frequently enter wastewater, affecting methanotroph growth and the quality of the final product, thereby complicating the recycling process. From a consumer perspective, products derived from wastewater heavily contaminated with heavy metals and antibiotics are unacceptable, particularly as protein feeds enter the animal and human food chain. Therefore, current research favors extracting nitrogen from wastewater for recycling with rigorous sterilization before use.

Struvite precipitation and ammonia stripping represent common nitrogen recovery technologies. Struvite can recover ammonium and orthophosphate. However, this process is constrained by the phosphorus concentration in wastewater and does not permit the recovery of ammonium nitrogen isolation, while ammonia stripping can recover more than 90% of ammonium nitrogen (Wu and Vaneeckhaute, 2022). The recovered nitrogen can be obtained in the form of ammonium sulfate and ammonium bicarbonate or condensed and collected as liquid ammonia. Notably, nitrogen recovery technologies are typically associated with fertilizer production, particularly for obtaining struvite and ammonium sulfate (Yang, et al., 2022a). Given that reintroducing the recovered nitrogen into liquid form (suitable for methanotroph utilization) entails additional costs, direct access to dissolved nitrogen may represent a more viable method for nitrogen recovery.

Emerging technologies, including electrochemical and bioelectrochemical systems (BESs), are creating additional opportunities for nutrient recovery. These technologies, targeting specific nutrient ions, exhibit enhanced selectivity and can yield nutritional products of higher quality (Xie et al., 2016). The widespread use of BESs enables their integration with existing nitrogen recovery technologies to reduce the usage of chemical reagents (Du et al., 2023). It is noteworthy that BESs not only facilitate nitrogen recovery but also support biogas upgrading, hydrogen and oxygen generation, and electrical energy recovery through the design of microbial fuel cells (MFC) and microbial electrolytic cells (MEC) (**Fig. 5**).

The concurrent recovery of multiple products renders this strategy particularly promising (Rodríguez Arredondo et al., 2015). The recovered ammonia nitrogen is utilized for microbial cultures and the energy is harnessed for reactor operations or the synthesis of other substances. Reports indicate that MFCs can recover 32–42% of nitrogen from wastewater (**Fig. 5b**) (Yang et al., 2021). Although the nitrogen recovery

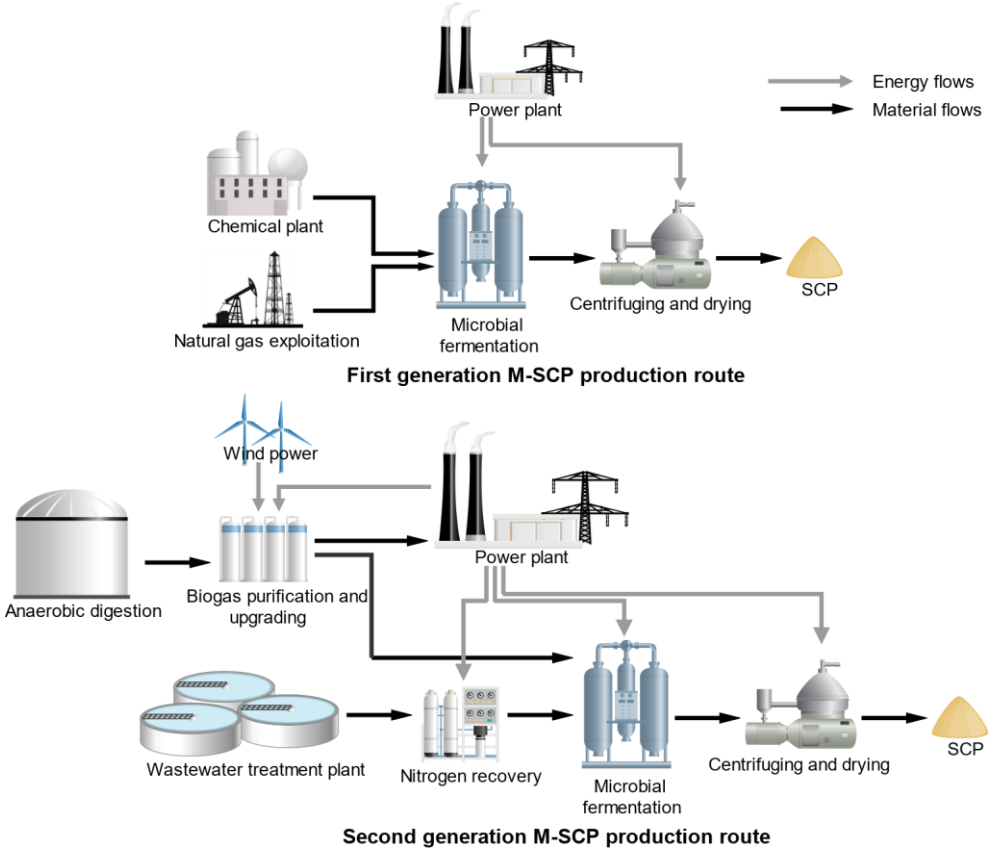

Fig. 4. Comparison of first- and second-generation methanotroph single-cell protein production routes (M-SCP: Methanotroph single- cell protein, SCP: single- cell protein).

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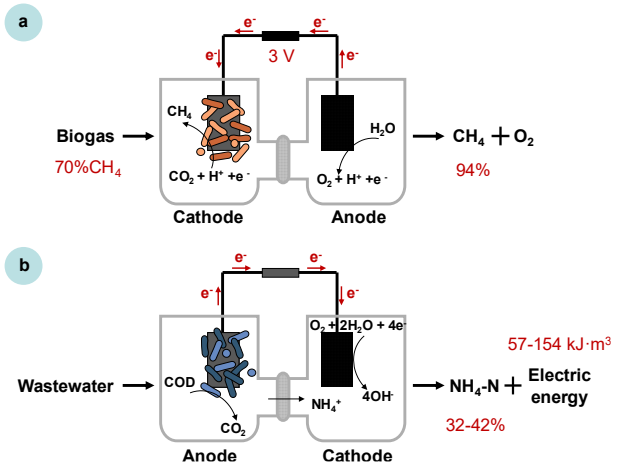

Fig. 5. Nutrient recovery strategies based on bioelectrochemical systems (BESs). **a)** Methane upgrading technology, **b)** Nitrogen recovery technology. Recycling performance parameters referenced by Xu et al. 2021a and Yang et al. 2021.

rate is not high, the microorganisms utilize the nitrogen to achieve robust growth, demonstrating the feasibility of this method. Additionally, electrochemical systems support oxygen to methanotrophs *via* water electrolysis, replacing inefficient air. Therefore, integrating an electrochemical system with a methanotroph biotransformation platform is highly promising, as it can simultaneously meet the carbon, nitrogen, and oxygen demands of the platform while fostering favorable production conditions.

One of the principal challenges in recovering nitrogen from wastewater using BESs is the accumulation of solid particles. These accumulated solids can impede the nitrogen recovery process from digestate in BESs. In fact, Bolognesi et al. (2021) reported that solid particle accumulation in the anodic compartment of an MFC filled with granular graphite can alter the interactions between substrates and electrodes. This alteration leads to significant changes in the influent distribution within the cell compartment, ultimately decreasing the overall performance of the nitrogen recovery process. To render BESs suitable for nitrogen recovery, a pretreatment step aimed at removing solid particles and precipitates has been proposed (Rodríguez Arredondo et al., 2015). Consequently, it is crucial to separate digestate into solid and liquid fractions at the initial stage of most treatment processes for digestate (O'Shea et al., 2022).

Decanter centrifuges and screw presses represent two widely used mechanical separation approaches (Cathcart et al., 2023). **Table 3** lists the reported separation efficiency and the distribution of main constituents following solid-liquid separation. The separated solid fraction with low water content can be directly applied in agriculture, providing the benefit of significantly reduced transport costs (Drosg et al., 2015). The significance of separation for environmental performance is well established. A recent study conducted a life cycle assessment (LCA) to evaluate and compare the environmental performance of digestate solid-liquid separation. The results indicated that solid-liquid separation of digestate exhibited a lower environmental impact than directly spreading digestate on soil (Angouria-Tsorochidou et al., 2022).

4.1.2. Methane recovery

In the past, methane purified from natural gas has been extensively utilized as a carbon source (Banks et al., 2022). However, the primary use of natural gas as a high-quality fuel and chemical feedstock limits its application in M-SCP production. Additionally, its non-renewable nature renders it unsuitable as a long-term carbon source. The most promising alternative involves purified methane sourced from biogas plants, landfills, wastewater treatment plants, as well as oil and gas extraction facilities.

Among these renewable methane sources, biogas produced by biogas plants using agricultural waste as feedstock is the most readily achievable (Angelidaki et al., 2018), with a high methane content of 50–70% (Khoshnevisan et al., 2021). Additionally, even higher methane content can be achieved by regulating organic components (Zhou et al., 2022; Zhou et al., 2024). The composition of biogas varies among biogas plants, influenced by parameters like feedstocks and temperature (Salehi and Chaiprapat, 2021). If the methane content in biogas is too low, it reduces the efficiency of the culture, and an excess of carbon dioxide in biogas is detrimental to methanotroph growth (Dizon et al., 2023). Impurities in biogas, such as H_2S, have inhibitory effects on the growth of methanotrophs (Xu et al., 2020; Pei et al., 2022). Therefore, desulfurization and purification of biogas prior to use is essential (Tsapekos et al., 2019; Xu et al., 2020).

Various biogas purification and upgrading technologies, including physical absorption, chemical absorption, low-temperature separation, variable pressure adsorption, membrane separation, and biotechnology, have been developed (Gkotsis et al., 2023). All these technologies can recover methane with a purity exceeding 90% and recovery efficiency of at least 95% (Sun et al., 2022). Chemical and physical absorption techniques, particularly water scrubbing, are more commonly employed in practice. While water is a cheap and effective absorbent, it is energy intensive and typically has a negative environmental impact (Awe et al., 2017), which contradicts the sustainable philosophy underlying the methanotrophs platform.

Most upgrading technologies separate the feed gas into methane and carbon dioxide streams, the carbon dioxide should be fully utilized in subsequent applications. Biological biogas upgrading employing hydrogenotrophic archaea offers advantages in converting CO_2 to methane, as illustrated in **Figure 5a**. However, this technology depends on electrolytic hydrogen production, which is powered by renewable sources such as solar and wind power (Luo and Angelidaki, 2012); otherwise, it proves more expensive than physicochemical upgrading technology

Table 3.
Contributions of the constituents after solid-liquid separation in different studies.

Separator Type	Solid Fraction (%)					Liquid Fraction (%)					Reference
	Input Mass (%)	TS [a]	TN [b]	NH_4-N [c]	TP [d]	Input Mass (%)	TS	TN	NH_4-N	TP	
Screw press	-	30.3	8.6	-	9.7	-	69.7	91.4	-	90.3	Cathcart et al. (2023)
	-	32.5	13.1	-	28.4	-	67.5	86.9	-	71.6	Tambone et al. (2017)
	10.4	35.4	14.1	9.7	34.3	89.6	64.6	85.9	90.3	65.8	O'Shea et al. (2022)
	15	55	30	25	60	85	45	70	75	40	Drosg et al. (2015)
	10	48.1	17	9.2	21.8	90	51.9	83	82	78	Drosg et al. (2015)
	11	37	15	-	17	89	63	85	-	83	Hjorth et al. (2010)
Centrifuge	-	21.6	43.9	-	61.3	-	78.4	56.1	-	38.7	Cathcart et al. (2023)
	17.9	71.9	34.2	15.5	74.5	82.1	28.1	65.8	84.5	25.5	O'Shea et al. (2022)
	14	61	28	16	71	86	39	72	84	29	Hjorth et al. (2010)

[a] TS: Total Solid; [b] TN: Total Nitrogen; [c] NH4-N: Ammonium Nitrogen; [d] TP: Total Phosphorus.

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(Khoshnevisan et al., 2022). Preliminary attempts to employ this technology have been made by researchers (Xu et al., 2021a and b), achieving a methane concentration of 94% and utilizing it to accumulate 472 mg·L^{-1} biomass. However, current yields remain low, and process parameters need to be optimized to further enhance electrolysis efficiency and microbial growth rates.

It is worth noting that upgraded high-purity methane is extensively used to generate heat and electricity (Tsapekos et al., 2021), including in cogeneration and as vehicle fuel. However, this does not mean that M-SCP production competes with prevalent downstream technologies of AD. Some LCA studies have demonstrated that using coupled technologies yields superior environmental benefits (Khoshnevisan et al., 2020b; Marami et al., 2022a). Only a portion of the methane is required for M-SCP production, with the remainder being usable for heat and power generation. A viable initiative involves redirecting the generated heat and electricity towards M-SCP production, although this may reduce the profitability of the combined heat and power (CHP) segment, it significantly lowers the production costs and enhances the competitiveness of M-SCP. Compared to access to heat and power, protein represents a higher-value product.

Another option for recovering methane is from more dispersed sources of methane emissions like landfills, wastewater treatment plants, and oil and gas extraction facilities, where the gases usually need to be captured, cleaned, and upgraded to obtain methane. By 2030, global landfills are projected to produce 43.34 $TgCH_4$, wastewater treatment plants will produce 25.30 $TgCH_4$, and fugitive methane from natural gas, oil, and coal extraction will reach up to 131.52 $TgCH_4$ (Abbasi et al., 2012). The potential for capturing methane from these sources is substantial. However, this approach is more expensive than recovering methane from biogas plants. Thus, realizing this approach requires assessing whether the value of the product surpasses the costs of methane capture and production to justify converting smaller or waste methane sources.

4.2. Downstream technologies

A significant challenge in microbial fermentation is the high water content of the output (Qin et al., 2023); thus, the downstream process of the platform focuses on dewatering, centrifuging, and drying the biomass. The two principal dewatering methods are centrifugation and filtration. Centrifugation is superior in terms of its dewatering effect and facilitates the subsequent drying process, but it is energy-intensive. Filtration separates water from biomass using filters, primarily membranes. This method is straightforward in principle, but its economic viability is debatable due to potential filter contamination, which often necessitates frequent membrane replacement or cleaning. A recent demonstrated that methanotroph cultures could be effectively dewatered *via* forward osmosis using a biomimetic aquaporin membrane without significant impact on water flux despite biological contamination (Valverde-Pérez et al., 2020a). Advancements in membrane technology, including new materials with anti-pollution properties and reduced membrane costs, promise even greater potential.

After the supernatant is separated from the biomass, the concentrated cell slurry is transferred to the drying unit. Drying biomass is essential to achieve an end product with the same moisture content as existing protein products (10–25% dry matter) (Fasaei et al., 2018). Freeze drying and spray drying are common methods, achieving recovery rates above 90%. Freeze drying consumes less heat than other methods, yet large-scale operations necessitate significant investment and high operating costs. Spray drying is a faster drying method; however, it presents challenges such as potential volatile losses and low efficiency. In drying processes, the heat consumption for evaporating water is high, at 2260 kJ·kg^{-1} (0.628 kWh·kg^{-1}), with dryers typically exhibiting low thermal efficiencies ranging from 40% to 85% (de Carvalho et al., 2020). Consequently, regardless of the chosen technology, external energy is required to operate it. For the downstream process of the platform, the aim is to produce more dry biomass with reduced energy consumption. Energy recycling within the biorefinery platform, such as utilizing the exothermic heat from the fermentation process and cogeneration to supply the necessary heat and power for dewatering and drying, is a viable option.

5. Quality and security

As a novel source of protein, the quality and safety of M-SCP are critical factors limiting its market entry. Similar to other SCPs, M-SCP can be processed into bacterial protein meal (BPM) suitable for aquafeed or livestock feed. The amino acid profile of BPM is superior to that of soybean meal (SBM) and comparable to fish meal (FM). As depicted in **Figure 6**, the essential amino acid content of BPM can constitute up to 30% of the dry weight, covering most of the essential amino acids necessary for animal growth, particularly rich in arginine and leucine. However, BPM contains lower levels of histidine and lysine compared to FM, necessitating a balanced feed formulation to mitigate its impact when utilized. Among non-essential amino acids, BPM has the highest glutamate content, although it remains lower than that in FM; the levels of other amino acids are similar to those in FM. Despite their high protein content, SCP derived from bacteria features a high nucleic acid concentration, reaching up to 16% of the dry weight (Kumar et al., 2023). A high nucleic acid content may increase serum uric acid levels, potentially leading to the formation of kidney stones (Sharif et al., 2021; Graham and Ledesma-Amaro, 2023). Therefore, compared to traditional feed, protein feeds derived from methanotrophs require comprehensive detoxification, including nucleic acid removal.

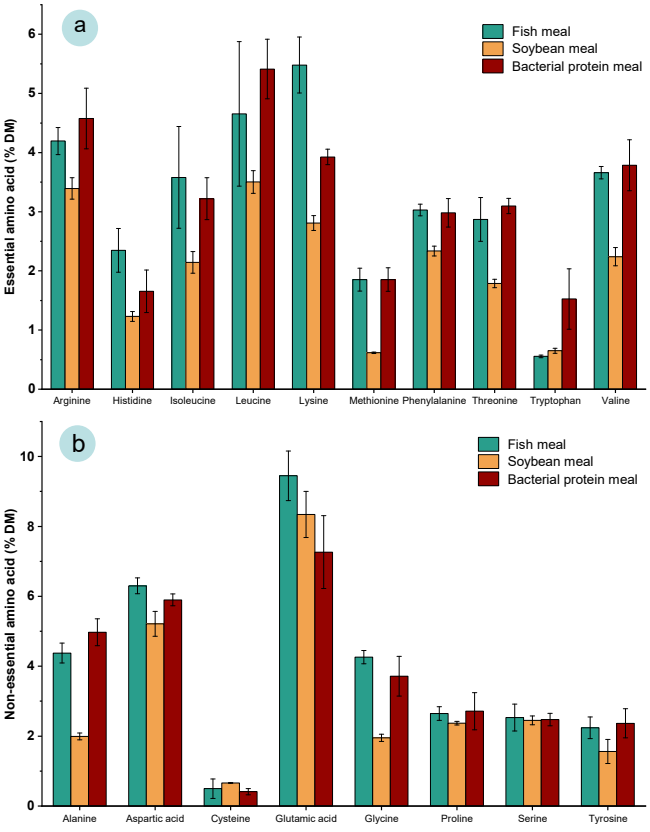

Fig. 6. The **(a)** essential amino acid composition and **(b)** non-essential amino acid composition of bacterial protein meal and traditional protein meal (Data from (Overland et al., 2001; Schoyen et al., 2007; Biswas et al., 2020; Rajesh et al., 2022; Yu et al., 2023; Zhang et al., 2023; Zheng et al., 2023)).

The safety of BPM as a feed protein has been a significant concern among researchers. The BPM from the first-generation M-SCP route, using natural gas as the raw material, has reached the stage of practical

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verification. **Table 4** summarizes recent feed trials where BPM has been to replace traditional protein sources. Attempts have been made to replace FM and SBM with BPM in the diets of aquatic species (Biswas et al., 2020; Zhang et al., 2023), broilers (Schoyen et al., 2007), and pigs (Overland et al., 2001). Studies indicate that BPM can partially substitute for protein components in feed without affecting growth performance; however, it impacts various aspects, such as animal fat, gut microbial communities, immunity, and feed digestibility, to varying degrees. Additionally, acceptance of BPM varies among different animal species. For instance, in fish, halibut exhibits greater sensitivity to BPM compared to Atlantic salmon (Biswas et al., 2020).

Currently, BPM from the second-generation M-SCP route primarily focuses on protein quality, though extensive safety experiments have not yet been conducted; however, such testing is crucial. Obtaining proteins from wastewater significantly increases the risk of protein contamination, especially considering that some contaminants may not be completely removed. Previous studies have primarily focused on analyzing whether the protein content and amino acid composition of SCP from waste streams meet the criteria for replacing conventional feed proteins. However, limited research has been conducted on the accumulation of contaminants, such as heavy metals, in microorganisms or the effects of substances secreted by the microorganisms themselves. Therefore, long-term and comprehensive feeding trials are necessary to ascertain whether prolonged feeding has adverse effects on animal physiology and livestock products. Although this approach of obtaining proteins from wastewater remains in the research phase, its potential is undeniable. It is foreseeable that, with population growth and the escalating demand for high-quality protein, utilizing protein from microorganisms to meet these needs is undoubtedly a viable solution.

Table 4.
Attempts at substituting feed protein with bacterial protein meal produced by methanotrophs.

Source	Feeding species	Original feed protein content	BPM [a] replacement content (%)	Effect	Reference
Production of FeedKind®	Japanese yellowtail	68% FM [b]	20%, 25%, 30%, 50%, 75%, 100%	Substitution with a high proportion of FM resulted in a significant reduction in growth performance, and substituting 30% did not affect growth performance and feed efficiency.	Biswas et al. (2020)
Production of FeedKind®	Pacific white shrimp	25% FM	15%, 30%, 45%	No significant effect on the growth performance; it increased the height of the mucosal folds, improved the structure of the intestinal flora, and increased the resistance to disease.	Chen et al. (2021)
Production of String Pro®	Rainbow trout	50% FM	25%, 50%, 75%, 100%	Growth rates and feed efficiency were higher in all diet groups, with no difference in feed efficiency and protein efficiency ratios.	Rajesh et al. (2022)
produced and supplied by Norferm AS (Stavanger, Norway)	Broiler Chickens	34.5%SBM [c], 46.5% wheat	6%	No differences in growth or feed intake throughout the experimental period; feed conversion efficiency improved.	Schøyen et al. (2007)
Produced and supplied by Dansk Bioprotein A:S (Odense, Denmark).	Pigs	7.5% SBM	50% or 100% of the lysine in SBM	Replacing SBM with BPM in diets for growing-finishing pigs has no adverse effect on growth performance or feed efficiency but increases carcass fat content.	Øverland et al. (2001)

[a] BPM: bacterial protein meal, [b] FM: fish meal, [c] SBM: soybean meal.

6. Environmental and economic implications

The impact of the methanotroph biotransformation platform on the environment and economy represents the comprehensive effect following technology integration. Specifically, this platform can be viewed as a system comprising three complementary components: M-SCP production, nutrient recovery, and renewable energy supply. In the best-case scenario, renewable energy could offset the negative environmental impacts of high energy consumption involved in M-SCP production and nutrient recovery, while M-SCP production enhances product value by transforming low-value energy and nutrient substrates into high-value proteins, thus improving overall economic benefits. Nutrient recovery and renewable energy ensure the sustainability of M-SCP production, meaning no additional carbon or nitrogen sources are added to the system, no extra fossil fuels are used, and a "perpetual cycle" of matter and energy is achieved. In recent years, economic assessments and LCA analyses of methanotroph biotransformation platforms have been extensively conducted. Specific recycling scenarios have been evaluated, including farm-scale biogas plants, wastewater treatment plants, and landfills (**Table 5**), demonstrating significant environmental benefits (**Fig. 7**).

6.1. Environmental implications

Compared to conventional fermentation platforms, the impact of the methanotroph biotransformation platform on climate change is significant. Methane is a gas with significant economic value; however, it is also the second most important greenhouse gas globally, following carbon dioxide. According to the latest Sixth Assessment Report by the Intergovernmental Panel on Climate Change, the global warming potential ranges of methane from 27.2 to 29.8 over a 100-year period (IPCC AR6, 2021), significantly higher than that of carbon dioxide. Therefore, controlling methane emissions is undoubtedly crucial for reducing carbon emissions and mitigating climate change in the short term (Ngoc Pham et al., 2023). For the second-generation M-SCP route, methane is sourced from biogas plants, sewage treatment plants, and landfills, thereby avoiding the direct release of these gases into the atmosphere and significantly benefiting the environment. It has been reported that the environmental impact of producing 1 ton of M-SCP, soybean meal, and fishmeal corresponds to −711, 7060, and 3000 kg of CO_2, respectively, in terms of climate change (Marami et al., 2022b), showing M-SCP with negative carbon emissions.

The environmental impact of background emissions from energy supply is substantial. The largest contributions typically arise from upstream technologies, such as methane production using anaerobic facilities, biomethane upgrading, and wastewater treatment, to render it suitable for microbial cultivation. All the processes above require significant inputs of heat and electricity, generally supplied by traditional fossil fuels, thereby proving detrimental to resources and ecology (Khoshnevisan et al., 2020b). Renewable energy sources, such as wind power and methane CHP, have been extensively studied in the existing assessments. CHP can achieve heat and electricity savings on the biorefinery platform, and while biogas combustion may produce carbon dioxide and result in methane emissions, it is considered biogenic, renewable, and environmentally superior to non-renewable sources (Marami et al., 2022b). It has been reported that heat substitution from CHP contributed the most to resource savings, accounting for 65% of the total savings (Marami et al., 2022a).

Unlike CHP, which is applicable to all segments, wind power was utilized solely for biomethane upgrading. Specifically, this electricity

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Table 5.
A summary of previous studies conducted to scrutinize the economic and environmental benefits of methanotrophs biotransformation platforms.

Year	Assessment Type	Country	Basis of Calculation	Scenes		Main Technologies	Reference
2020	Economic Viability	Belgium	A model mesophilic farm-based digester	Sc1	Protein production based on the methane in the biogas by MOB [a]	Anaerobic digestion, Ammonia stripping, M-SCP [b] production	Verbeeck et al. (2021)
				Sc2	Protein production based on the CO_2 from biogas upgrading or from biogas combustion by extra energy, input in the form of hydrogen gas, and using HOB [c]	Anaerobic digestion, Ammonia stripping, Biogas upgrading, CHP [d], H-SCP [e] production	
2022	Economic Viability	United States	Assuming the use of the current technically mature M-SCP production facility	Sc1	Methane from wastewater treatment plants, reactor sized to match methane production facility	Methane cleanup, M-SCP production	El Abbadi et al. (2021)
				Sc2	Methane from landfills		
				Sc3	Methane from oil and gas facilities		
				Sc4	Purchasing natural gas from the grid on a scale consistent with the landfill		
2022	Life Cycle Assessment	Denmark	A wastewater treatment plant	Sc1	Protein production based on pasteurized wastewater and purchased biomethane by MOB.	Wastewater treatment [f], M-SCP production	Marami et al. (2022a)
				Sc2	Change to biogas supply from the WWTP [g] itself based on Sc1, Residual biogas for CHP.	Wastewater treatment, M-SCP production, CHP	
				Sc3	Sc3 has used biogas upgrading compared to Sc2	Wastewater treatment, Biogas upgrading, M-SCP production, CHP	
				Sc4	Unlike Sc3, the recovery of ammonium using ES [h] and methane is preferentially supplied to CHP	ES, Biogas upgrading, M-SCP production, CHP	
				Sc5	Unlike Sc4, the recovery of ammonium using BES [i]	BES, Biogas upgrading, M-SCP production, CHP	
2022	Economic and Life Cycle Assessment	Denmark	A wastewater treatment plant		The scenario covers all processes within the wastewater treatment plant, with the addition of nutrient recovery and M-SCP production facilities, as well as electrolytic hydrogen production using the off-peak surplus power required for biomethane upgrading.	Wastewater treatment, M-SCP production, Biogas upgrading, CHP	Marami et al. (2022b)

[a] MOB: methane-oxidizing bacteria; [b] M-SCP: methanotroph single cell protein; [c] HOB: hydrogen-oxidizing bacteria; [d] CHP: Combined heat and power; [e] H-SCP: single cell protein produced by HOB; [f] Wastewater treatment: the wastewater undergoes "Centrifugation + filtration + pasteurization" before being used to culture methanotrophs; [g] WWTP: wastewater treatment plant; [h] ES: Electrochemical system; [i] BES: Bioelectrochemical system.

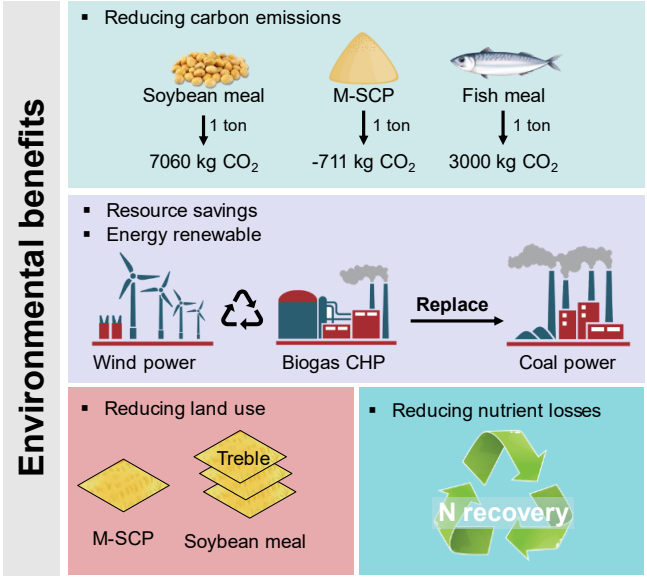

Fig. 7. Environmental benefits of methanotroph biotransformation platform (M-SCP: Methanotroph single-cell protein; CHP: Combined heat and power).

powers a bioelectrochemical system that converts carbon dioxide into biomethane *via* hydrogen generated from the electrolysis of water. This technology is highly energy-intensive yet offers more substantial environmental benefits compared to the widely used water scrubbing method. For a biogas plant processing 240,000 tonnes of agro-industrial wastes annually, this biomethane upgrading method demonstrated superior environmental performance compared to water scrubbing, with net environmental savings scores of -5.46 and -3.64 kPt, respectively (Elyasi et al., 2021). In the relevant assessment, geographic location plays a crucial role; for example, in Denmark, abundant wind power supports the implementation of the method. However, in other regions, suitable biogas upgrading methods must be identified, or alternative renewable energy sources like solar power must be considered. The equivalence of these solutions' environmental benefits requires further evaluation.

In studies assessing the environmental effects of different protein sources, significant variations in land use were observed among protein products, with soybeans contributing substantially more to land use than M-SCP (Kobayashi et al., 2023). It has been found that the contribution to agricultural land occupation of soybean meal is three times higher, and its impact on natural land transformation is nearly double that of SCP (Spiller et al., 2020). As soybean production for livestock feed continues to rise, the expansion of cultivation areas exacerbates the issue of greenhouse gas emissions resulting from land use changes (Castanheira and Freire, 2013; Garofalo et al., 2022). Therefore, the adverse environmental impacts

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associated with soybean meal production provide a strong incentive for the adoption of microbial protein production (Spiller et al., 2020).

While carbon and nitrogen sources for the methanotroph growth are recovered from waste streams, damage to the ecosystem cannot be entirely prevented. Chemicals used in the fermentation process to balance nutrients, such as some trace elements, along with background emissions from their production and transport, also contribute to ecosystem damage (Marami et al., 2022a). Although wastewater may contain these trace elements, the presence of other hazardous pollutants restricts the recovery of these nutrient fractions. Moreover, in nutrient recovery facilities, the use of chemical materials and power consumption contribute to the eutrophication of water bodies, negatively impacting the ecological environment (Farago et al., 2021). Despite the mentioned challenges, this approach still results in less environmental damage compared to the production of soybean meal reliant on agricultural cultivation.

Overall, the environmental benefits of the methanotroph biotransformation platform can be assessed from two perspectives: those arising from the production of protein from waste within the platform and those resulting from substituting traditional protein production pathways. The latter requires a broader analysis due to the complex nature of agricultural systems. In environmental assessments, various options all have positive benefits; however, the development potential varies significantly. For instance, biogas production capacities differ between biogas plants and sewage treatment plants, affecting the scale of M-SCP production and, consequently, the environmental impacts. Furthermore, the technologies and processes across different options vary, necessitating an understanding of how these choices influence life cycle assessment outcomes and which approaches minimize environmental impacts.

In future studies, better measures should be investigated, especially in the upstream process chain, to evaluate the suitability of each recycling technology, to further enhance the environmental benefits and sustainability of the platform. It should be emphasized that many studies employ simplified models, such as lab-scale processes scaled up to simulate actual engineering, which may omit certain production steps and cost inputs. Furthermore, the prices of chemicals and electricity may vary greatly from one period to another due to market fluctuations, potentially seriously affecting the assessment results. Therefore, more sophisticated models should be developed and subjected to more extensive and longitudinal evaluations.

6.2. Economic implications

Similar to the environmental assessment, the economic assessment was calculated based on various models. It is widely recognized that the production costs for converting recovered methane and nitrogen to SCP are comparable to fishmeal and that there are microeconomic benefits (Verbeeck et al., 2021). While the benefits continue to be enhanced, the production of M-SCP broadens the methane utilization pathway compared to other downstream pathways of AD, generating a higher value product than merely heat and power. This enhancement will improve the ability of biogas plants to be self-sustaining and reduce reliance on financial incentives from the government (Verbeeck et al., 2021).

It has been reported that 71% of the total cost for SCP production is allocated to the production and recovery of feedstock, of which 46% is allocated to methane, 20% to the recovery of ammonia, 5% to O_2, while 19% is attributed to capital expenditure and operational expenditure for the fermentation unit (USD 314 ton SCP^{-1}) and 10% to the dewatering and drying of the wet biomass (USD 172 ton SCP^{-1}) (Verbeeck et al., 2021). Different production scales and processes influence the share of each segment in the total cost and ultimately result in variations in the total cost. Based on sensitivity analysis, several more favorable cost-reduction segments have been identified in the past. It has been demonstrated that increasing the yield of pure protein from ammonium nitrogen, replacing conventional reactors with U-loop reactors, improving power efficiency, and reducing labor requirements can potentially increase the gross profit and significantly reduce the investment cost (El Abbadi et al., 2021; Marami et al., 2022b).

In the long term, the methanotroph biotransformation platform can achieve cost reduction through a unit-volume economic model (Clomburg et al., 2017). The concept of "unit-volume economy" entails a shift from a small number of high-capacity facilities to a large number of facilities operating on a smaller scale. This strategy enables the construction of small-scale operating facilities based on multiple decentralized methane point sources. By increasing the number of operating units and facilities, process experience can be leveraged to improve design, materials, and production methods (Dahlgren et al., 2013), thereby further reducing production costs.

7. Policy and practical implications of the present review

The safety of alternative proteins, including M-SCP, represents the main issue that countries around the world should consider while creating their policies. For this reason, the EU has regulated the materials and microbiologically acceptable substrates that can be used for feed production, paying special attention to avoiding contamination by pathogens or heavy metals in waste (Areniello et al., 2023). While stringent safety checks are understandable and are favorable for contributing to consumer confidence, strict regulation of substrates may limit the development and application of the second-generation SCP route.

Additionally, with their continuous development and promising prospects, alternative proteins have received widespread support from governments. According to the Good Food Institute, governments invested USD 635 million in the alternative protein ecosystem in 2022 (The Good Food Institute, 2023). Although governments have increased their financial, political, and regulatory support for alternative proteins, this support remains insufficient. These inputs depend on government policies on carbon reduction and other sustainability issues (Gundupalli et al., 2024), which will inevitably shape the price and competitiveness of alternative proteins in the protein market.

Based on this review, several conclusions can inform the next stages of policy development. Firstly, effective nutrient recovery strategies can mitigate the impact of contaminants on SCP products. And regulatory standards for substrates and processes can be relaxed by specifying prohibited components in the product, based on a detailed understanding of the protein composition of the SCP and extensive feeding experiments. Secondly, the environmental benefits of SCP outweigh its economic benefits. Considering the additional environmental impacts of alternative proteins in financial policy development is essential. This approach will enhance their market competitiveness and promote development within a circular economy model.

8. Conclusions and perspectives

Collectively, these recent studies and major successes demonstrate that the field of methanotrophs biotransformation platform is on a new path. Further efforts, building on the success of the first-generation M-SCP and identifying new process routes through the integration of advanced nutrient recovery technologies, are likely to achieve greater market competitiveness and provide substantial environmental benefits. Key conclusions include:

1. *Technological advancements and challenges:* Advancements in nutrient recovery from biogas and wastewater have propelled the development of methanotroph biotransformation platform; however, challenges such as high costs, significant energy consumption, efficient cultivation strategies, bioreactor mass transfer, and the impacts of pollutants and impurities continue.
2. *Safety and market adaptation:* Ensuring SCP is free from contaminants such as heavy metals and antibiotics is crucial for market acceptance, requiring stringent quality control.
3. *Energy and sustainability:* For the second-generation M-SCP production route, transitioning to renewable energy inputs is essential for environmental sustainability. This transition enables the

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offsetting of the high energy demands inherent in the fermentation and nutrient recovery processes.

4. *Environmental and economic benefits:* M-SCP production significantly reduces greenhouse gas emissions, decreases reliance on non-renewable resources, and avoids ecological destruction associated with traditional protein production, potentially offering a viable alternative protein source.

Although the methanotroph biotransformation platform shows promising prospects, there are still many challenges that need to be addressed within this platform. Primarily, the safety of SCP should be a key consideration to ensure that it is safe for animals or humans when used as a feed or a protein supplement within the biological chain. On this basis, detailed product standards should be formulated, and extensive FM and SBM replacement feeding trials should be conducted to enhance farmers' confidence.

Secondly, critical technological breakthroughs are necessary. For culturing methanotrophs, it is essential to select strains with high growth rates, construct mixed microbial communities, identify interactions between different organisms to optimize the culture strategy, as well as develop novel bioreactors to adapt to waste stream substrates and enhance gas-liquid mass transfer. For upstream technology, it is imperative to implement nutrient recovery technologies that are highly efficient and consume low energy through technological innovation and integration. For downstream technology, it is crucial to urgently reduce the energy consumption of the drying process to further decrease costs. Furthermore, conducting more detailed economic and environmental assessments based on typical waste recovery facilities is essential for optimizing the production chain for commercialization and scaling in various scenarios.

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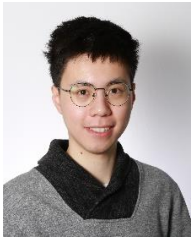

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