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Review

Valorization of methane from environmental engineering applications: A critical review

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ABSTRACT

Wastewater and waste management sectors alone account for 18% of the anthropogenic methane (CH_4) emissions. This study presents a critical overview of methanotrophs ("methane oxidizing microorganisms") for valorizing typically discarded CH_4 from environmental engineering applications, focusing on wastewater treatment plants. Methanotrophs can convert CH_4 into valuable bioproducts including chemicals, biodiesel, DC electricity, polymers, and S-layers, all under ambient conditions. As discarded CH_4 and its oxidation products can also be used as a carbon source in nitrification and annamox processes. Here we discuss modes of CH_4 assimilation by methanotrophs in both natural and engineered systems. We also highlight the technical challenges and technological breakthroughs needed to enable targeted CH_4 oxidation in wastewater treatment plants.

1. Introduction

Methane (CH_4), a key component of natural gas can be used for generating heat and power. However, the the undesirable emissions of CH_4 into atmosphere resulted in a 34-fold increase in global warming over 100 years (EPA, 2010). Agriculture, energy, wastewater and solid waste sectors account for ~60% of the global anthropogenic CH_4 emissions (~359 Tg CH_4 per year) (Saunois et al., 2020). Nearly 9% of these emissions occur during collection, treatment and disposal of wastewater (~500 Tg of CH_4; 512 $MtCO_2$ equivalent) (Ho et al., 2013), (EPA, 2013). A gram of soluble chemical oxygen demand (sCOD) removed by a wastewater treatment plant (WWTP) can yield ~0.35 L of CH_4 (Tchobanoglous et al., 2003). In a typical WWTP with $sCOD_{influent}$ of 200 mg/L and COD removal efficiency of 90%, 45% of the as produced CH_4 remains dissolved in the WWTP effluent (Liu et al., 2014), eventually being released into the atmosphere.

Anaerobic digestor (AD), thickener, buffering tank, sludge dewatering, combined heat and power plant and flare unit (Yoshida et al. 2014) of a WWTP contribute to CH_4 emissions. The biogas emissions from the AD units account for 26% of a WWTP's carbon (C) footprint (~36 kg CO_2e/PE/a) (Parravicini et al. 2016), 0.4% of which turns into fugitive CH_4 emissions. Although biogas can be used to fuel gas turbines, steam boilers and residential cooking and heating systems, a complex collection and purification infrastructure is needed to purify biogas. The conversion efficiency of combustion engines fed with biogas is also quite low (~33%), explaining why biogas at many WWTPs and landfills continues to be flared. The purpose of AD units is often being limited to control odor and generate carbon credits.

Oil and gas production along with mining activities account for ~86% of the CH_4 emissions from the energy sector. Nearly 467.2 billion cubic feet of natural gas is being flared annually (U.S. Energy Information Administration., 2019), primarily due to lack of direct market access, transportation and refinery infrastructure for the gas, along with reasons related to safety, economics, and operational expediency. For example, ~35% of the as producednatural gas (485 million ft^3/d; 58% CH_4) from the fractured Bakken shale in North Dakota, United States is being flared due to the lack of adequate pipelines, ports and refineries (Shrestha et al., 2017).

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With a goal of reducing the carbon footprint due to CH_4 emissions from energy and waste sectors, this study explores feasibility of biogas oxidation by methanotrophs for generating chemicals (e.g., ethanol, volatile fatty acids), extracellular polymeric substances, biopolymers, and single cell protein. Such an initiative would open new avenues for reusing CH_4 in environmental engineering applications. The remaining part of this study will focus on aerobic and anaerobic metabolic pathways of methanotrophs (Section 2), point sources of CH_4 (Section 3), bioproducts and bioprocesses driven by CH_4 oxidation (Section 4), and notable challenges and needed technical breakthroughs to sustain CH_4-fed biotechnology applications (Section 5), and conclusions (Section 6). We provide a case study on potential uses of methanotrophs for valorizing typically discarded natural gas (CH_4) from hydraulically fractured oil fields.

2. Legacy of methanotrophs

From a biology perspective, methanotrophs use CH_4 as a C source to meet their own metabolic needs or sustain syntrophic bioprocesses within their ecosystem. From an environmental engineering perspective, they turn CH_4 fraction of biogas into valuable bioproducts. CH_4 and its oxidation products (volatile fatty acids and methanol) can be used as C substrates in denitrification, annamox and biological phosphorus removal (BPR) bioprocesses in WWTPs.

While methylotrophs use single-C compounds including CH_4, alcohols, aldehydes, and acids as carbon substrates, methanotrophs prefer CH_4 substrates. Methanotrophs fall under *γ-proteobacteria* (Group I), *α- proteobacteria* (Group II), or *Verrucomicrobia* class (Group III), based on their formaldehyde (HCHO) assimilation pathways, morphological properties and biochemical characteristics. Group I methanotrophs use a ribulose monophosphate pathway for assimilating HCHO while Group II members use serine pathway.

Group II members account for 90% of the known methanotrophs, which have been typically isolated from the accessible regions of the earth's crust (Bender and Conrad, 1992). Recently psychrophiles (Islam et al., 2020), thermophiles (Tsubota et al., 2005), acidophiles (Dunfield et al., 2007), halophiles (Heyer et al., 2005), and alkaliphiles (Kaluzhnaya et al., 2001) have been reported to display CH_4 oxidation capabilities. Most of these extremophiles belong to Group-III. Although the *Verrucomicrobia* members mostly live under aerobic conditions, their ability to grow under O_2 limiting conditions justify their ability to survive harsh acidic (pH = 1.0), thermophilic (~80 °C) and salty conditions (Sharp et al., 2014). For example, both Group I and II methanotrophs were found in Lake Constance, migmatite rock bed (Olkiluoto site, Finland) (Pester et al., 2004) and Evander (South Africa) (Ward et al., 2004), which are characterized by anoxic and volcanic conditions, respectively.

2.1. C substrates and a methane monooxygenase (MMO) enzyme

Methanotrophs use the MMO enzyme to assimilate CH_4 as well as to oxidize ammonia using CH_4 as a co-metabolic substrate (Carlsen et al., 1991; Hoefman et al., 2014). The MMO enzyme occurs in a soluble form (sMMO) within the cytoplasm as well as in particulate form (pMMO) within an intracellular membrane. Compared to pMMO, sMMO uses a wide range of C substrates (e.g., propene, butane, cyclohexane, chlorotrifluoroethylene, toluene, naphthalene, chloroform, diethyl ether, and CO) (Jiang et al., 2010). However, pMMO can oxidize complex C substrates including C1-C5 n-alkanes and terminal alkanes (Sirajuddin and Rosenzweig, 2015). While pMMO is expressed by both the Group I and II members, sMMO is expressed only by specific members including *Methylocella* spp. (Theisen et al., 2005). From the environmental engineering perspective, *Methylomicrobium album, Methylomicrobium alcaliphilum* and *Methylomonas methanica* (Group I) and *Methylosinus sporium, Methylocella tundrae, Methyloferula stellate* (Group II) are important as they can use biogas as the C source under aerobic conditions (Knief, 2015; Patel et al., 2018, Patel et al., 2016, 2016b).

2.2. Terminal electron acceptors (TEAs) and other growth conditions

Methanotrophs can couple CH_4 oxidation with reduction of dissolved oxygen (aerobic), nitrite and nitrate (anoxic), sulfate (anaerobic), insoluble metals (ferrihydrite), selenite (metal bioremediation) and solid conductive electrodes (microbial fuel cells). Table 1a and 1b provides examples of methanotrophs, their C source, TEA, and associated bioproducts under aerobic and anaerobic conditions, respectively. Each of these bioproducts have been discussed in detail in Section 3.2.

The physiological requirements of methanotrophs can be understood by reviewing the composition of nitrate mineral salts (NMS) medium, a preferable growth media that has been used for methanotroph cultivation in the last five decades (Table S1). Methanotrophs grow in a temperature (T) range of 25 – 35 °C, pH of 7.0 - 7.65, and 0.1 g phosphorus (P) per kg soil and 0.1 g nitrogen (N) per kg soil. While Group I methanotrophs require 21% O_2 (v/v), Group II methanotrophs prefer 1% O_2 (v/v) (Hanson and Hanson, 1996; Nikiema et al., 2007). Adequate levels of copper (~5 μM, Cu^{2+}) are needed to stimulate sMMO expression, and both $Cu^{2+}$and di-iron for pMMO expression (Prior and Dalton, 1985; Stanley et al., 1983). Extensive details of the growth requirements have been described in earlier studies (Prior and Dalton, 1985; Stanley et al., 1983).

2.3. Metabolic pathways of methanotrophs

Under aerobic conditions, methanotrophs yield methanol, formaldehyde, pyruvate, formate, lactate, and polyhydroxybutyrate, which can be used in environmental engineering applications. For example, methanol, the first intermediate product of CH_4 oxidation (Fig. 1) is a preferred C source for denitrification. Methanotrophs, preferably along with its consortia members can carry out denitrification via anaerobic oxidation of CH_4 (AOM) (Wang et al., 2017) (Bennett et al., 2018) via a reverse methanogenesis process (Wang et al., 2014). Archaeal species that display AOM pathways possess homologous enzymes typical to those involved in methanogenesis, except for the N^5, N^{10}-methylene-tetrahydromethanopterin (H_4MPT) reductase (Mer). Anaerobic methanotrophs (ANME), the archaeal partners of syntrophic AOM use a homolog of nickel-containing methyl-coenzyme M reductase (MCR) as an enzyme for activating CH_4 (Scheller et al., 2010).

3. Point sources of discarded methane in environmental engineering applications

Agriculture (53%), energy (28%), and waste (19%) sectors jointly emit 7000 Mt CO_2 equivalent of CH_4 (Yusuf et al., 2012). Fig. 2 shows multifarious sources of CH_4 in waste (landfills, manure management), wastewater sectors (rising main sewers and WWTPs) and energy sectors (mining, oil and gas industry) (Fig. 2). Many of these infrastructure components including landfills already contain a well-engineered gas collection system. Their typical life ranges from 20 to 50 years, representing them as a consistent source of CH_4 for subsequent use by methanotrophs. For instance, landfills generate CH_4 throughout its lifetime (~30 years) as well as during the post closure period (30–50 years) [67]. Al-

Table 1a
The potential uses of aerobic methanotrophs in wastewater treatment processes.

Bacteria/archaea	Electron donor	Electron acceptor	Potential product	WWTP	References
Heterotrophic consortia	CH_4	Oxygen	PHB	CH_4 oxidation	(Carrillo et al., 2018)
Methylosinus trichosporium OB3b	CH_4	Oxygen	PHB	CH_4 oxidation	(Zhang et al., 2019)
Heterotrophic consortia	CH_4 and ammonium	Oxygen	PHB	CH_4 oxidation and NH_4 denitrification	(Fergala et al., 2018a, 2018b)
Candidatus Methylomirabilis sinica	CH_4	Oxygen	Methanol	CH_4 oxidation and nitrite denitrification	(He et al., 2016)
Methylacidiphilum fumariolicum	CH_4 and ammonium	Oxygen	Methanol	CH_4 oxidation and ammonia denitrification	(Mohammadi et al., 2017)
Methylomicrobium album and *Methylocystis* sp.	CH_4 and ammonium	Oxygen	Methanol	CH_4 oxidation and ammonia denitrification	(Nyerges et al., 2010)
Methylomicrobium alcaliphilum	CH_4	Oxygen	Ectoine	CH_4 oxidation and nitrate denitrification	(Cantera et al., 2017; Cyplik et al., 2012a)
Halomonas sp	CH_4	Oxygen	Ectoine	CH_4 oxidation and desulfurized wastewater treatment	(Wang et al., 2016)
Heterotrophic consortia	CH_4	Oxygen	Methanol	CH_4 oxidation, nitrate denitrification, and phosphate removal	(Kim et al., 2019)

Table 1b
The potential uses of anaerobic methanotrophs in wastewater treatment processes.

Bacteria/archaea	Electron donor	Electron acceptor	Potential product	WWTP	References
Candidatus 'Methanoperedens nitroreducens'	CH_4	Nitrite	Methanol	CH_4 oxidation and nitrite denitrification	(Cui et al., 2015)
Candidatus 'Methylomirabilis oxyfera'	CH_4	Nitrite	Methanol	CH_4 oxidation	(Cui et al., 2015)
ANME1, ANME-2abc, and ANME-3	CH_4	Sulphate	Methanol	CH_4 oxidation and sulfate reduction along with heavy metal removal	(Cassarini et al., 2019; Timmers et al., 2017)
ANME-2d	CH_4	Nitrate	Methanol	CH_4 oxidation and nitrate denitrification	(Timmers et al., 2017)
Candidatus 'Methanoperedens'	CH_4	Chromate	Methanol	CH_4 oxidation and chromate reduction	(Luo et al., 2019)
Candidatus 'Methanoperedens nitroreducens'	CH_4	Iron and manganese	Methanol	CH_4 oxidation and iron/manganese removal	(Ettwig et al., 2016)
Candidatus 'Methanoperedens' and *Candidatus* 'Methylomirabilis'	CH_4	Selenate	Methanol	CH_4 oxidation and selenate reduction	(Luo et al., 2018)
Candidatus 'Methanoperedens ferrireducens'	CH_4	Ferrihydrite (Iron)	Methanol	CH_4 oxidation and iron removal	(Cai et al., 2018)
Denitrifying anaerobic methane oxidizers and anammox consortia	CH_4 and ammonium	Nitrate	Methanol	CH_4 oxidation, ammonium oxidation, and nitrate denitrification	(Ding et al., 2014)
Anaerobic methane oxidizing consortia	CH_4	Iron oxide	Methanol	CH_4 oxidation and iron removal	(Sivan et al., 2011)
Candidatus 'Methanoperedens nitroreducens'	CH_4	Nitrate/nitrite	Methanol, acetate, PHB and glycogen	CH_4 oxidation and denitrification	(Cai et al., 2019)
Nitrite-dependent anaerobic methane-oxidizing consortium	CH_4	Nitrite	Methanol	CH_4 oxidation and nitrite denitrification	(Li et al., 2018)
Co-culture of engineered archaeal strain, *Paracoccus denitrificans* and *Geobacter sulfurreducens*	CH_4 (reverse methanogenesis)	Electrode	Methanol and electricity	CH_4 oxidation	(McAnulty et al., 2017)
Geobacter spp. and *Methanobacterium* spp.	CH_4	Electrode	Methanol and electricity	CH_4 oxidation	(Gao et al., 2017)
Co-culture of *Methanosarcina acetivorans, Geobacter sulfurreducens*	CH_4 (reverse methanogenesis)	Electrode	Methanol and electricity	CH_4 oxidation	(Yamasaki et al., 2018)
Geobacter and methanotroph consortia	CH_4	Electrode	Methanol and electricity	CH_4 oxidation and anaerobic membrane bioreactors for wastewater organics conversion	(Chen and Smith, 2018)

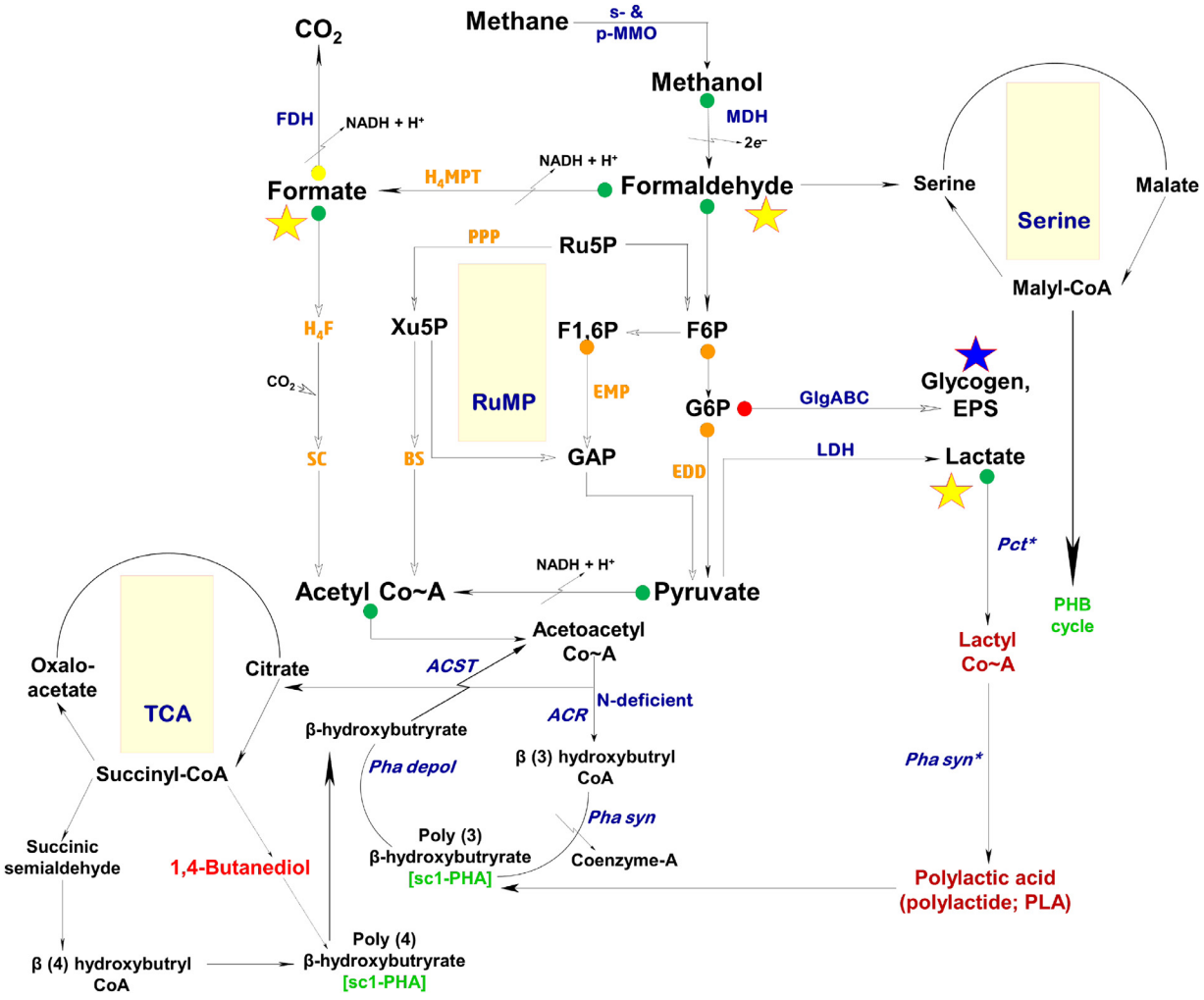

Fig. 1. Methane metabolism in different methanotrophs for synthesis of bioproducts including methanol, formate, formaldehyde, pyruvate, lactate and polyhydroxybutyrate.

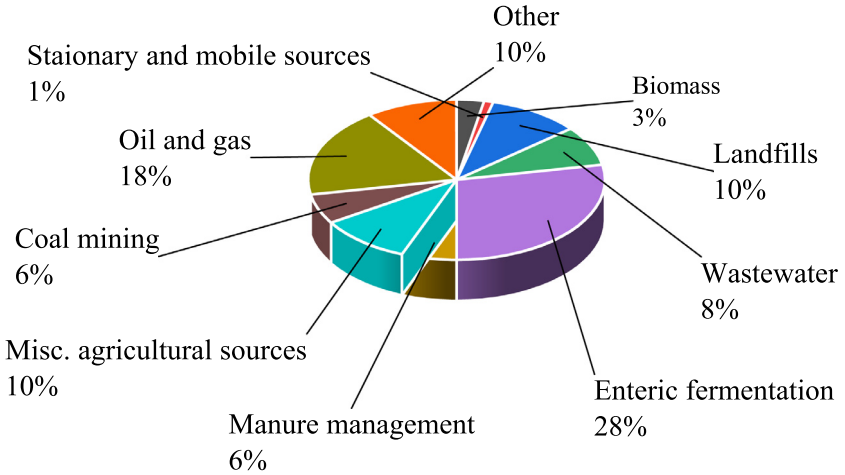

Fig. 2. Relative proportios of anthropogenic sources of CH_4 emissions from energy, waste management, and agriculture sectors (Adapted from Yusuf et al., 2012).

though CH_4 emission vary with changes in the climate and the age of landfills, engineered landfills have been reported to yield biogas consistently with CH_4 composition of 75% to 95%. Around 18–26% of the captured CH_4 can be used to meet heating and energy requirements of CH_4-fed biorefineries, and the remaining 74–82% for producing bioproducts (Chidambarampadmavathy et al., 2017).

4. Feasibility of CH_4 oxidation in WWTPs: capture, valorization or reuse of CH_4

Fig. 3 depicts different sources of a WWTP responsible for CH_4 emissions, including point sources (e.g., anaerobic digestion and primary sedimentation) and non-point sources (receiving water

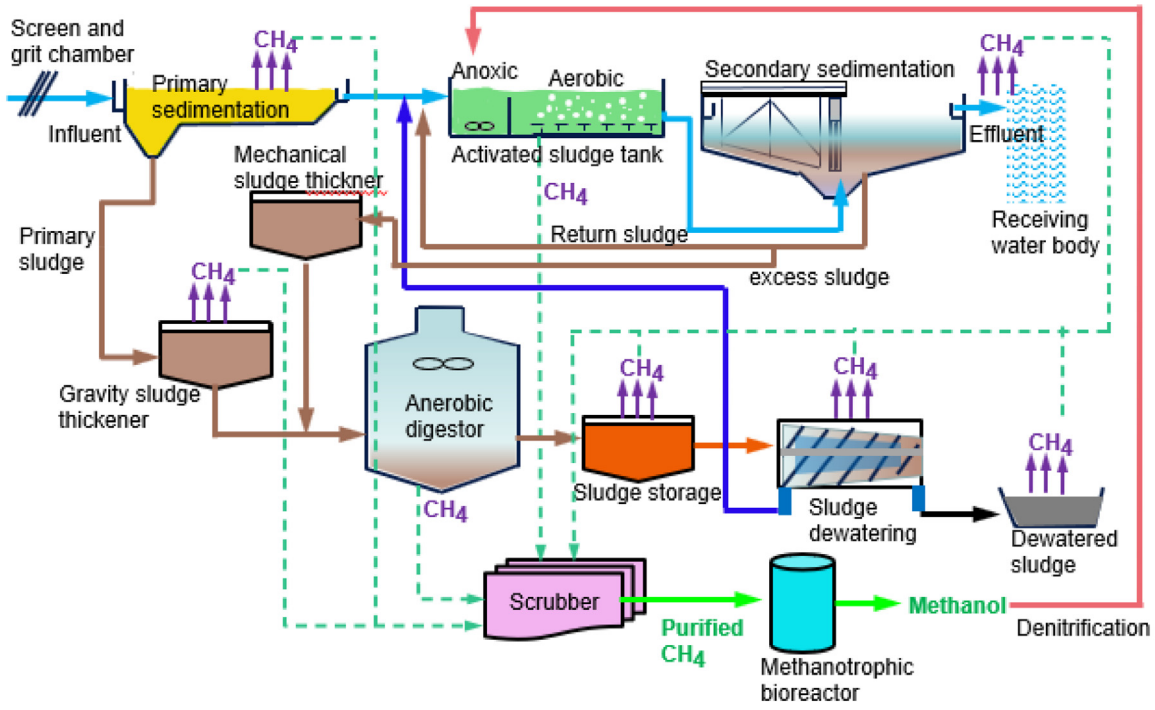

Fig. 3. Methane emissions at wastewater treatment plant with anaerobic digestor (Adapted from Daelman et al., 2012).

bodies of WWTPs). Anaerobic digestion (AD) is a key mode of CH_4 production in both sewers and WWTPs. A megagram (dry weight) of solid waste yields ~280 m^3 of CH_4 via AD process (Gossett et al., 1982). Around 72% of CH_4 production in WWTPs occurs in primary sludge thickener, sludge dewatering, and the dewatered sludge storage tanks (Campos et al., 2016), and remaining 28% due to undesirable AD of wastewater in collection systems or bioreactors of WWTPs.

CH_4 collected from different sources of WWTPs can be purified using adsorption, scrubber and membrane technologies (Luo et al., 2014). Such a pure CH_4 gas streams can be used as C sources in bioprocesses or fed into a separate methanotrophic bioreactors to yield bioproducts (Fig. 3). (Modin et al., 2007).

Both aerobic methanotrophs (e.g. *Methylacidiphilum fumariolicum, Methylomicrobium alcaliphilum* (Hanson and Hanson, 1996) (Table 1a) and anaerobic methanotrophs (e.g. *Candidatus* 'Methanoperedens nitroreducens' and *Candidatus* 'Methylomirabilis') (Table 1b) exist in WWTPs. Although Group-I methanotrophs typically dominate in WWTPs (Hatamoto et al., 2010), Group II methanotrophs including *Methylacidiphilum fumariolicum* (*Verrucomicrobia*) (Mohammadi et al., 2017) and *Methylomicrobium album* and *Methylocystis* sp. (Nyerges et al., 2010) are common in WWTPs. Although CH_4 oxidation is a dominant pathway compared to ammonium oxidation the Group II members can carry out ammonia oxidation with concurrent CH_4 co-oxidation in WWTPs (Fergala et al., 2018b; Myung et al., 2015). *Methylomicrobium alcaliphilum* (Cantera et al., 2019; Cyplik et al., 2012a) and heterotrophic consortia (Kim et al., 2019) couple CH_4 oxidation with nitrate reduction.

M. alcaliphilum (Cantera et al., 2017; Cyplik et al., 2012a) and *Halomonas* sp. (Wang et al., 2016) oxidize CH_4 while producing ectoine as a soluble metabolites. Ectoine additives can be used to neutralize the "osmosis" stress of microorganisms in bioprocesses (Vyrides and Stuckey, 2017) as well as activate key enzymes needed for denitrification (Cyplik et al., 2012a). Under saline conditions, ectoine additives have been reported to increase the performance of anammox processes by ~40% (Liu et al., 2014).

Anaerobic Methanotrophs (ANME) perform AOM via reverse methanogenesis pathways. They use the reducing equivalents generated during CH_4 oxidation to drive the reduction of sulfate, nitrate or metal oxides (Bennett et al., 2018; Timmers et al., 2017). ANME contains four phylogenetic clusters belonging to ANME-1, ANME-2, ANME-3, and GOM Arc I (formerly ANME-2d) (Bennett et al., 2018; Timmers et al., 2017). ANME-2, ANME-3, and GOM Arc I lie within *Methanosarcinales* whereas ANME-1 relate to *Methanomicrobiales* and *Methanosarcinales* (Bennett et al., 2018). Methanotrophs including *Candidatus* 'Methanoperedens nitroreducens' (Cui et al., 2015), ANME-2d (Timmers et al., 2017), denitrifying anaerobic methane oxidizers and anammox consortia can couple CH_4 oxidation with reduction of nitrate (Ding et al., 2014) chromate (Luo et al., 2019), sulfate (Cassarini et al., 2019), selenate (Luo et al., 2018), iron and manganese (Ettwig et al., 2016).

4.1. Valuable intermediate bioproducts from CH_4 oxidation

Fig. 4 shows an overview of the three groups of methanotrophs involved in bioconversion of CH_4 into bioproducts, which can be categorized under: (1) carbohydrates, (2) fuels/power, (3) biomolecules, and (4) proteins (Fig. 4). Tables 1a and 1b provide examples of aerobic, anaerobic and electrogenic forms of methanotrophic species, their preferences for electron donors and electron acceptors, all in a context of targeted bioconversion of CH_4 in WWTPs.

4.2. Thermodynamics and kinetic considerations

Many methanotrophs require a high concentration of CH_4 due to their low affinity towards gaseous substrates (Saari et al., 2004; Baani and Liesack, 2008). In general, CH_4 oxidation by methanotrophs follow Michaelis–Menten kinetics (Steenbergh et al., 2010). From a thermodynamics perspective, CH_4 oxidation entails a two-step mechanism both of which are irreversible.

$$CH_4 + O_2 \rightarrow CH_3OH \rightarrow CO_2 \quad (1)$$

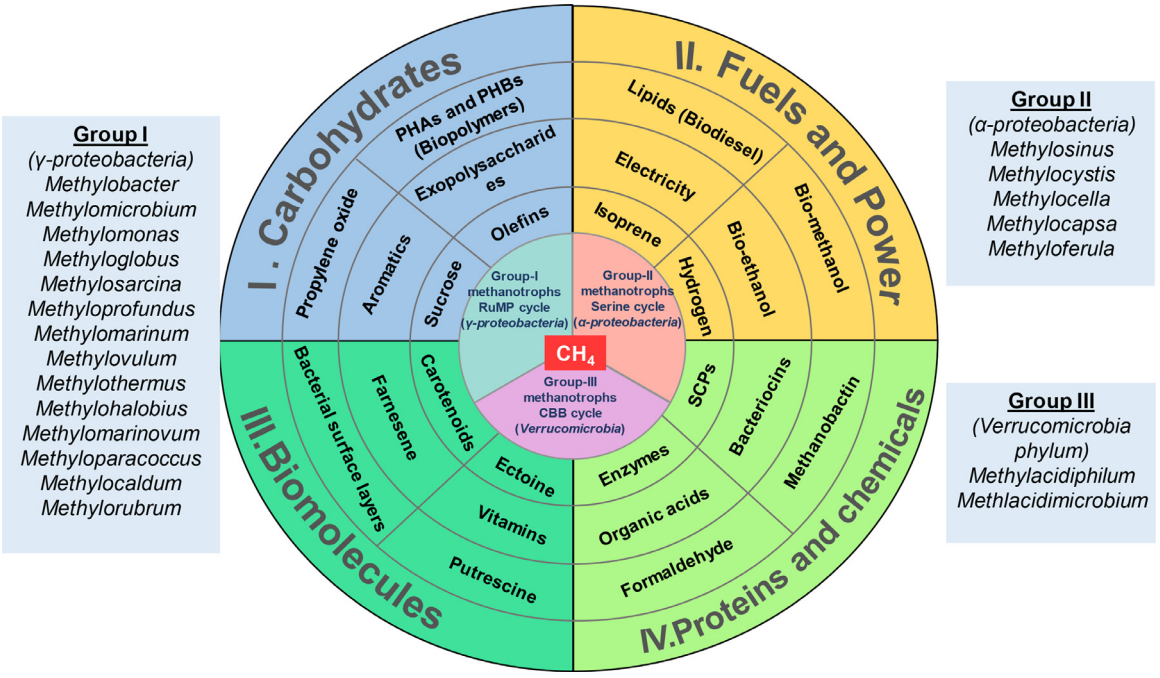

Fig. 4. Three groups of methanotrophs that enable CH_4 conversion into one of the four classes of bioproducts. The conversion can be carried out by the wild type or genetically engineered methanotrophs. The major pathways of CH_4 oxidation include Ribulose Monophosphate (RuMP) Cycle, Calvin–Benson–Bassham (CBB) cycle, and serine cycle.

Table 2
Thermodynamics of the key metabolites derived from methane oxidation.

Reaction	ΔH (KJ mole^{-1})	ΔS (KJK^{-1} mol^{-1})	ΔG (KJ mole^{-1})
$CH_4 \rightarrow CH_3OH$	−802.3	−0.005	−800.8
Acetate	−137.6	−0.001	−36
PHA*	−332.4	−0.002	−282.6

The rate constants k_1 and k_2 represent the conversion of CH_4 to CH_3OH (Step I) and subsequently into CO_2 (Step II), respectively. CH_3OH, a desired bioproduct for environmental engineering applications is thus a transient intermediate. Hydrogen abstraction from CH_4 to CH_3OH has often been observed to be a rate-limiting step for both the processes. Also, the rate constant ratio (k_2/k_1) can be rewritten as

$$\frac{k_2}{k_1} = e^{\Delta G/T} \quad (2)$$

$$\Delta G = \Delta G_{CH4} - \Delta G_{CH3OH} \quad (3)$$

Table 2 provides an overview of thermodynamics parameters for methanol and other typical bioproducts from CH_4 oxidation. Owing to numerous pathways of dehydrogenation of CH_4 activation in biochemical system via radical formation, a best-case scenario where CH_4 is fully converted into CH_3OH was assumed. Likewise, bond energies were also considered for the calculations of the thermodynamics of the reaction. In case of a complete CH_4 oxidation reaction, 170 kcal/mol excess energy is released than is consumed, resulting in production of the heat (i.e., exothermic reaction). A similar strategy was adopted while accounting for formulation of other bioproducts derived from CH_4.

4.3. Direct current (DC) electricity from methane

Biogas can be used as a sole source of C and electron donor by certain methanotrophs (e.g. *Methanobacterium* spp., *Methanosarcina acetivorans, Paracoccus denitrificans, Methylomicrobium* sp. *Methylobacillus* sp. and *Methylophilus* sp.) for generating electric current in bioelectrochemical systems (BESs). Such methanotrophs use an extracellular electron transfer (EET) capabilities to transfer electrons to solid conducting electrodes in microbial fuel cells (MFCs) and microbial capacitive deionization (MCDCs) (Shrestha et al., 2018) (Monzon et al., 2016). Additional products from CH_4 oxidation in BESs include methanol, formic acid, acetate, and lactate (Table 5).

Fig. S1 depicts an overview of AOM by methanotrophs (ANME-1, ANME-2, or jointly by these archaeal groups) along with sulfate-reducing bacteria (SRB) in BESs These archaeal members oxidize CH_4 and shuttle the reduced compounds to SRB (Valentine and Reeburgh, 2000). The electricity production (10.5 mA/m^2) from CH_4 by methanotrophs (*M. methanica)*, albeit under aerobic conditions (CH_4-air = 10:1 v/v) was first reported by (Girguis and Reimers, 2011). Several other follow-up studies have been focused on morphological features, characterization, and taxonomic frameworks of methanotrophs involved in CH_4 oxidation under MFC conditions (Soo et al., 2016) (Ding et al., 2017). Sediment MFCs have been used to generate electricity from CH_4 generated by soil in paddy fields (Rizzo et al., 2013).

Low electrochemical power output due to low exoelectrogenic capabilities of methanotrophs is a major drawback of CH_4-fed MFCs (Ding et al., 2017). This challenge can be addressed by using a two-step conversion process, entailing CH_4 oxidation into CH_3OH (Step I) under aerobic conditions (Myung et al., 2018), and subsequently into electricity (Step II) in MFCs (Myung et al., 2018). As demonstrated by McAnulty et al. (McAnulty et al., 2017), CH_4 can be converted into acetate using ANME Mcr-producing *M. acetivorans* and subsequently into electricity using *G. sulfurreducens*. The electrons generated by *M. acetivorans* were consumed by *G. sulfurreducens* using multi-haem cytochromes. The redox shuttles produced by *P. denitrificans* were used to transfer the electrons to the conductive electrode (Fig. S1) (McAnulty et al., 2017).

A study by Ding et al. demonstrated the feasibility of using denitrifying anaerobic methane oxidizers (DAMO) in MFCs (Ding et al., 2017). They used enrichment procedures to facilitate the prolifer-

ation of DAMO bacteria (initial: 24.4%; final: 2.07%) and archaea (initial: 24.4%; final: 65.77%).

A purple non sulfur bacteria (*Rhodobacter sphaeroides* spp.) has been reported to generate electricity from methane oxidation products (e.g., methanol) in MFCs (Islam et al., 2020; Jawaharraj et al., 2020). Under a photoheterotrophic mode, *Rhodobacter Sphaeroides* was found to oxidize pyrroloquinoline quinone dependent dehydrogenase and periplasmic c-type cytochrome metabolic pathways and enable methanol oxidation under anaerobic conditions that prevail in MFCs (Jawaharraj et al., 2020; Wilson et al., 2008). Table S2 provides an overview of experimental conditions and key outcomes for CH_4-fed BESs. Readers are encouraged to review relevant literature for understanding the techno-economic benefits of abiotic CH_4-to-electricity compared to flare gas recovery methods (de Klerk, 2015). Such studies are needed for microbially catalyzed CH_4-to-electricity conversion. In summary, bioelectricity generation from CH_4 in absence of oxygen is a less explored topic.

4.4. Biodiesel from methane

Typically discarded C substrates including sludge and CH_4 at WWTPs can serve as precursors for biodiesel production (Balasubramanian et al., 2018). Wastewater sludge contains lipids including monoglycerides, diglycerides, triglycerides, phospholipids and free fatty acids (Kargbo, 2010) which can be assimilated by cyanobacteria (Jawaharraj et al., 2017), microalgae, (Karpagam et al., 2015) as well as oleaginous methanotrophs (Choi et al., 2014; Mondala et al., 2009; Muller et al., 2014). Methanotrophs can assimilate lipids that contain C18:1 characterized by nearly 50% fatty acid methyl esters (FAME) (Cea et al., 2015), suitable for producing biodiesel.

Both Group I and Group II methanotrophs yield phospholipids including phosphatidyl-ethanolamine (phosphatidyl dimethyl ethanolamine and phosphatidyl methyl ethanolamine) or phosphatidyl-glycerol (Bowman et al., 1991), both of which are suitable for biodiesel production (Brennan and Owende, 2010a). There is an adequate literature that confirm that lipids and associated fatty acids of methanotrophs can be used for biodiesel production (Demidenko et al., 2017). *Methylomicrobium buryatense, Methylosinus, Methylocystis, Methylocella* and *Methylocapsa* can accumulate mono-unsaturated fatty acids (MUFAs) as well as saturated fatty acid (SFAs), both of which are characterized by C_{14}–C_{18} chain lengths (Dedysh et al., 2007; Fei et al., 2014; Fei et al., 2018).

Calysta Energy Inc., reported that methanotrophs solely fed with CH_4 can generate microbial lipids at half the cost compared to algae and bacteria that require pure sugar substrates (Calysta Energy., 2013). The MMO enzymes in methanotrophs can effectively generate energy from CH_4 compared to chemical catalysts (Balasubramanian et al., 2010). *Methylomicrobium buryatense* has been reported to accumulate 10% fatty acid content (dry cell wt) using a gas-sparged fermentation system (Dong et al., 2017). The composition of fatty acids in the lipids of methanotrophs determines the quality of resulting biodiesel and its use in engines. Unlike polyunsaturated fatty acids (PUFA), both MUFA and SFA which can be accumulated by methanotrophs display excellent oxidative stability, minimizing clogging issues (Jawaharraj et al., 2016), rendering their use in both the modified and unmodified engines.

Lipid accumulation in methanotrophs can be enhanced by optimizing physiological conditions (e.g., N/P ratio and O2 levels) and process engineering strategies (e.g., use of a two-stage fed batch cultivation strategy (Fei et al., 2014). Irrespective of these strategies, the lipid accumulation in methanotrophs including *Methylomicrobium buryatense* which display robust growth characteristics is limited to 10.7%, even under continuous cultivation and O_2 limited conditions (Gilman et al., 2015). Engineered methanotrophs developed using gene knockout/knock-in techniques could potentially convert CH_4 into intracellular membrane-lipid-fraction and free fatty acids at a higher efficiency (Fei et al., 2014).

4.5. Biodegradable polymers from CH_4

Biopolymers such as polyhydroxyalkanoates (PHAs) including poly (3-hydroxybutyrate) (PHB) are biodegradable, nontoxic, and thermoplastic molecules which can be used in multifarious energy and environmental applications. The use of inexpensive C substrates including CH_4 and sludge would reduce greatly the manufacturing costs. The recent reports suggest that indigenous microorganisms in WWTP sludge can accumulate raw biopolymers (Kumar et al., 2004), at a cost of ~ US$ 1.26 and 2.26/kg for the large and small WWTP respectively (Crutchik et al., 2020). Other preferable C substrates include acetyl Co-A, acetoacetyl Co-A, 1,4-butanedione, organic acids, starch wastewater (Nielsen et al., 2017). Certain methanotrophic symbionts can use *Sphagnum* peat moss mixed with activated sludge to accumulate biopolymers under P limiting conditions (Pérez et al., 2019).

Methanotrophs accumulate PHAs intracellularly to store energy reserves in response to a physiologically stressful conditions (Zhang et al., 2018) (Wendlandt et al., 2001). Although both Group I and II methanotrophs can assimilate PHA, Group II members have been reported assimilate C into biopolymers (Hanson and Hanson, 1996;) at a higher efficiency (~50%) compared to the Group I counterparts (5–15%) (Higgins et al., 1981; Whittenbury and Dalton, 1983). Table 3 provides an overview of key enzymes and enzymatic reactions involved in PHB production.

Indigenous methanotrophs in landfilling and composting sites have been reported to assimilate biopolymers (Chidambarampadmavathy et al., 2017). A review article by Heimann and coworkers provided extensive details of biopolymer production from landfill CH_4 (Chidambarampadmavathy et al., 2017). They reported that 162 t of CH_4 can be recovered annually from smaller landfills (~5000 tons waste/year) to produce 71 t of PHB, and 7480 t of CH_4 from larger landfills (~230,000 tons waste/year) to produce 3252 t of PHB. The cost of PHB production can be reduced by increasing production volumes via a centralized extraction and refinement facility, especially in large metropolitan cities (Chidambarampadmavathy et al., 2017)..

LanzaTech (USA) and Mango Materials (USA) are key industrial players involved in PHA manufacturing from CH_4. Although feasibility of CH_4-derived biopolymers has been demonstrated via experimental, computational, and life cycle assessment approaches, operational parameters and logistic issues are yet to be streamlined for practical applications (Levett et al., 2016). Process engineering strategies are needed to address issues of low CH_4 solubility, slow biokinetics, challenges of capturing, storing, and delivering CH_4 (a flammable gas) along with O_2 into aerobic methanotrophic bioreactors (see Section 4 and Section 5). Purging techniques can be used to enhance CH_4 oxidation rate and increase biopolymer yield to ~51% (w/w) (Zúñiga et al., 2011).

Effective operational strategies can promote one of the existing metabolic pathways of PHB synthesis (Fig. 1). Artificial metabolic pathways of PHB synthesis can be introduced into model methanotrophs, for example those based on 1, 4-butanediol (Fig. 1). Enzymes involved in the assimilation of malyl-CoA (from serine cycle), lactate and lactyl-CoA could be genetically engineered to improve their turn-over rate kinetics and enhance PHB production (Fig. 1). Table 3 depicts the role of several enzymes involved in PHB biosynthesis and regulation. From environmental engineering perspective, both process engineering and genetic engineering strategies should consider the field scale conditions (e.g., impurities in biogas, ability to use macronutrients, micronutrients and trace elements available in typical wastewaters and leachates)

Table 3
Role of different enzymes involved in the regulation of polyhydroxybutyrate.

Enzyme	Gene	Role	Note	Reference
β-ketothiolase	*PhaA*	synthesis of PHB	Linked to same chromosome having *PhaB* & gene for granule associated protein *orf3*	(Song et al., 2012)
Ketoacyl-CoA thiolase	*FadA*	condensation of two acetyl-coenzyme A (acetyl-CoA) molecules into acetoacetyl-CoA	Aerobic and anaerobic degradation of long-chain fatty acids	(Kalyuzhnaya et al., 2013)
3HA-ACP dehydratase[a]	*FabA*	introduction of cis u*nsa*turation into fatty acids	In case of diol, 3-hydroxypropionyl-coenzyme A (CoA) get synthesized via an oxidative cycle	(Nomura et al., 2004)
PHB depolymerase	*depA*	encode intracellular PHB depolymerases	Highly identical to *depB* and unlinked with other genes on chromosome	(Myung et al., 2017)
Crotonase	*croA*	fatty acid degradation	Typical works on acyl esters of coenzyme A	(O. . O. Lee et al., 2019)
AC–CoA reductase[b]	*PhaB*	synthesis of PHB	Linked to same chromosome having *PhaA* & gene for granule associated protein *orf3*	(Chohan and Copeland, 1998)
3HA-CoA dehydro.[c]	*FadB*	aerobic and anaerobic degradation of long-chain fatty acids via beta-oxidation cycle	Forms 3-oxoacyl-CoA from enoyl-CoA via L-3-hydroxyacyl-CoA	(Ren et al., 2000)
β-K ACP Synthase I[d]	*FabB*	Catalyzes the condensation reaction of fatty acid synthesis	Condensation by the addition to an acyl acceptor of two carbons from malonyl-ACP	(Zhang et al., 2017)
PHB depolymerase	*depB*	encode intracellular PHB depolymerases	Highly identical to *depA* and unlinked with other genes on chromosome	(Korotkova and Lidstrom, 2001)
PHB synthase	*PhaC*	catalyzes the PHA polymerization reaction	Unlinked to *PhaA* and *PhaB*	(Valentine and Steinbuechel, 1993)
Acyl-CoA synthase	*FadD*	activates fatty acid by adding Co-A; coverts hydroxybutyrate to hydroxybutyrate Co-A	Change the "*R*" form of hydroxybutyrate Co-A to "*S*" form	(Kocharin et al., 2012)
M-CoA ACP trans.[e]	*FabD*	involved in fatty acid synthesis pathway	Separates Malonyl Co-A in to Co-A & acyl-carrier protein bound malonyl molecule	(Zhang et al., 2007)
β-hydroxybutyrate dehy.[f]	*hbd*	oxidation of 3-hydroxybutyrate to acetoacetate	Catalyzes the first step of β-hydroxybutyrate utilization	(Dedkova and Blatter, 2014)
Acyl-CoA dehydrogenase	*FadE*	catalyzes the initial step of fatty acid oxidation and resulting in "*trans*" conformation product	Oxidizes acyl Co-A into enoyl Co-A; introduces double between 2nd and 3rd carbon of fatty acid	(Eggers and Steinbüchel, 2013)
β-K ACP Synthase II[g]	*FabF*	Catalyzes the condensation reaction of fatty acid synthesis by the addition to an acyl acceptor of two carbons from malonyl-ACP	Prefers short chain acid substrates and may supply the octanoic substrates for lipoic acid biosynthesis	(Amara and Moawad, 2011)
CoA transacylase	*PhaG*	a functional replacement of *FabB*; functional role in PHB synthesis is not clear	Structure is similar to acetoacetyl-CoA reductase, a crucial enzyme for fatty acid synthesis	(Hoffmann et al., 2002)
β-K ACP reductase[h]	*FabG*	enhances production of PHB copolymer; catalyzes the reduction of 3-ketoacyl-CoA to (*R*)−3-hydroxyacyl-CoA	Provides monomers for PHA production from the fatty acid biosynthesis pathway in transformants	(Nomura et al., 2004)
β-K ACP Synthase III[i]	*FabH*	catalyzes the condensation of malonyl-ACP and acetyl-CoA to form acetoacetyl-ACP; mediates the transacylase reaction	Possesses acetoacetyl-ACP synthase and acetyl transacylase activities. Its substrate specificity determines the biosynthesis of straight-chain of fatty acids instead of branched-chain	(Choi et al., 2000)
E-ACP reductase[j]	*FabI*	Catalyzes the reduction of a carbon-carbon double bond in an enoyl moiety that is covalently linked to an acyl carrier protein	Elongation cycle of fatty acid used in the lipid metabolism	(Massengo-Tiassé and Cronan, 2009)
Phasin like protein	*PhaI*	PHA granule associated protein	Encodes for phasin but transcribed divergently to the other *pha* genes	(De Eugenio et al., 2010)
Enoyl-CoA hydratase	*PhaJ*	catalyzes the conversion of enoyl-CoA to (*R*)−3-hydroxyacyl-CoA during fatty acid metabolism	Catalyzes the hydration of trans-2-enoyl-CoA with a chain-length of 4–6 carbon atoms	(Flüchter et al., 2019)
Phasin like proteins	*phbP*	PHA granule associated protein	Encodes for phasin but transcribed divergently to other *pha* genes	(De Eugenio et al., 2010)
PHA depolymerase	*PhaZ*	mobilization of PHB within microbial cell	Mediates depolymerization of PHB into 3 hydroxybutyrate	(Wang et al., 2009)

[a] 3-Hydroxypropionyl-Coenzyme A dehydratase.
[b] NADPH dependent acetoacetyl-CoA reductase.
[c] 3-Hydroxyacyl Coenzyme-A dehydrogenase.
[d] β-Keto acyl carrier protein synthase I.
[e] Malonyl CoA-acyl carrier protein transacylase.
[f] β-Hydroxybutyrate dehydrogenase.
[g] β-Keto acyl carrier protein synthase II.
[h] β-Ketoacyl-acyl carrier protein reductase.
[i] β-Keto acyl carrier protein synthase III.
[j] Enoyl-(acyl carrier protein) reductase.

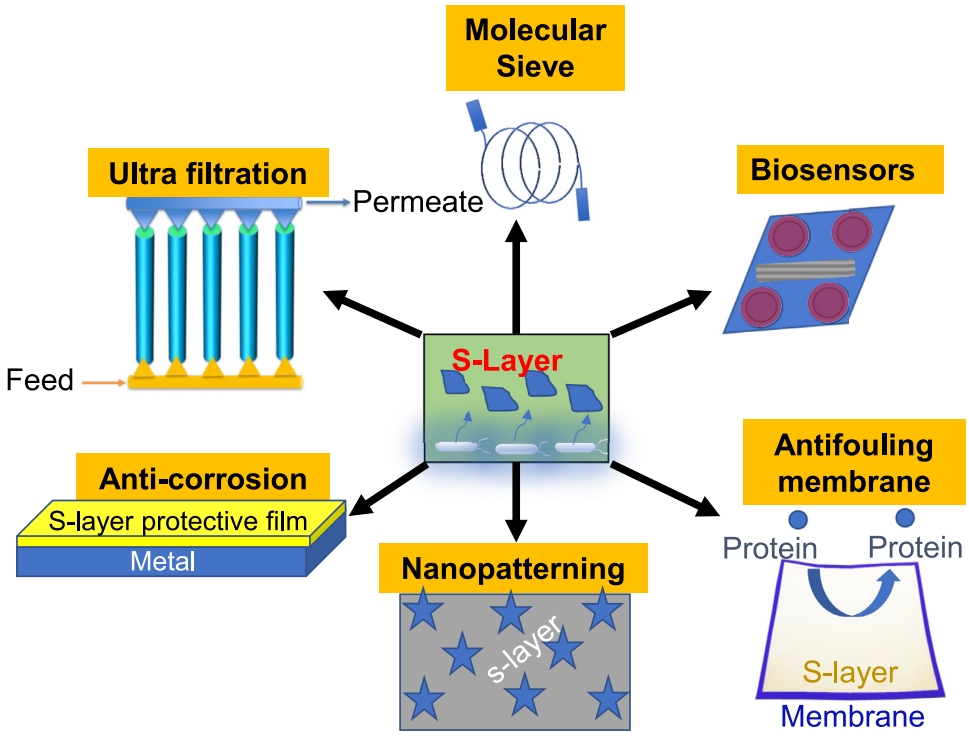

Fig. 5. Uses of CH_4-derived S-layers in nanotechnology and biotechnology applications.

4.6. Surface layers from methane

Methanotrophs (*Methylococcus capsulatus, Methylomicrobium album, Methylothermus thermalis, Methylomicrobium alcaliphilum)* (Khmelenina et al., 2013) and denitrifying anaerobic methane oxidizers (DAMOs) exposed to stressful physiological conditions induced by heavy metals have been reported to develop a defensive strategies by forming surface layer (S-layer) proteins as their outermost cell envelope (Lederer et al., 2013). These S-layer proteins resist harsh conditions including high temperatures, ionic strength, and high levels of protons (Claus et al., 2002). These S-layer proteins can be isolated from extracellular polymeric substance (EPS) components of biofilms (Boleij et al., 2018; Ding et al., 2014). Thus, process engineering strategies can be used to optimize biofilm growth, accumulation of EPS and S-layer proteins (Wong et al., 2019).

S-layer displays unique properties including low molecular weight (40 to 170 kDa), sub-nanometer thickness (5–25 nm), a small and uniform pore size (2–8 nm), tunable porosity (20 to 70%), and ability to form a periodic self-assembled monomolecular lattices with a structurally diverse symmetry (oblique, square and hexagonal lattices) (Sleytr et al., 2014). S-layer proteins from *Methylomicrobium album* BG8, *Methylomicrobium alcaliphilum* 20Z, and *Methylococcus capsulatus* bind Cu^{2+}ions (Khmelenina et al., 2013), while those from *Methylococcus, Methylomicrobium* or *Methylothermus* species display immunoglobulin G antibodies that bind pathogens and toxins.

Fig. 5 illustrates the applications of CH_4-derived S-layer proteins on nanotechnology and biotechnology. S-layer protein can self-assemble into a large monolayers on technologically relevant metals, polymers, and semiconductors (Sleytr et al., 2014), rendering their use in semiconductor (Pum and Sleytr, 1999), nanotechnology (Douglas et al., 1986), membrane filtration (Sara and Sleytr, 1987), corrosion (Sara and Sleytr, 1987), biosensor (Ferraz et al., 2011; Neubauer et al., 1993) and nanopatterning applications (Sobel and Hess, 2015). Despite several reports of S-layer from methanotrophs, their commercial prospects remain unattractive due to low yields. Metabolic engineering strategies could be used to develop viable routes for using methanotrophic S-layer in nanotechnology, vaccines and biotechnology applications.

4.7. Other value-added compounds

Methanotrophs cultivated in WWTPs can generate products such as cosmetics, diesel, plastics, lubricants and rubber industries. They can generate these products intracellularly (ectoine) as a part of their metabolic pathways or as part of their survival mechanism in response to external stressors (e.g. thermophilic, saline and toxic conditions). Methanotrophs can also produce products (isoprene) extracellularly, especially in a case of engineered strains..

4.7.1. Ectoine

Hyperosmotic conditions allow halo-tolerant methanotrophs to accumulate ecotine (Czech et al., 2018; Strong et al., 2015), a cyclic imino acid used in counteracting osmosis and environmental stresses in bioreactors (e.g., denitrification) (Cyplik et al., 2012b; Czech et al., 2018). Ectoine is an effective stabilizer for nucleic acids, enzymes, and DNA-protein complexes (Pastor et al., 2010). They protect bacteria from stressful conditions of high temperature, drying, freezing and thawing (Strong et al., 2015). Ectoine is widely used in cosmetics and moisturizers, with a global consumption rate of 15,000 tons/year and with a putative retail value of ~1000–1300 US dollars/kg (Cantera et al., 2017).

Methylomicrobium alcaliphilum, Methylobacter marinus, Methylomicrobium kenyens, Methylophaga thalassica, Methylophaga alcalica, Methylarcula marina are typical methanotrophs that produce ectoine. Their key ectoine biosynthetic genes include diaminobutyric acid (DABA) acetyltransferase (*EctA*), DABA aminotransferase (*EctB*) and ectoine synthase (*EctC*) (Cantera et al., 2017; Reshetnikov et al., 2011). Moderately halotolerant methanotrophs accumulate 12–20% ectoine on a dry cell weight basis (Khmelenina et al., 1999).

Halomonas elongata, a halotolerant heterotrophic proteobacteria has been commercially employed to produce ectoine us-

ing hyperosmotic shock techniques (Nakayama et al., 2000; Strong et al., 2015). A bioprocess using *H. elongata* was developed by reusing microbial biomass for nine times, increasing ectoine yield (15.5% g g^{-1} biomass) (Sauer and Galinski, 1998). Nearly 37.4 mg of ectoine (per gram biomass) has been reported to be accumulated by *Methylomicrobium alcaliphilum* biomass (Cantera et al., 2017).

Halomonas sp. strain, a haloalkaliphilic methanotroph has been reported to yield nearly 70–92 mg of ectoine per gram of biomass, highest among the currently known methanotrophs (Cantera et al., 2019). Using CH_4 as a feedstock hydroxyectoine, another osmotolerant and heat stress resistant, ectoine-like compound has been reported to be biosynthesed by methanotrophs with a yield of 5 mg/g biomass (Cantera et al., 2019). Alternatively, mixed methanotrophic bacterial consortia can be used to further increase the ectoine production to 1.33–0.42 mg g DW^{-1} (Stępniewska et al., 2014).

4.7.2. Isoprene, farnesene, and olefins

A genetically modified *Methanosarcina acetivorans* strain has been reported to synthesize isoprene using methanol as a sole carbon source. This strain is capable of growing in wastewater anaerobic digesters (Buan and Weber, 2014). The annual global market for isoprene is 4 billion US dollars, primarily used in the synthetic rubber industry, adhesives, building blocks of the chemical industry and advanced fuels (Matos et al., 2013).

Microbial isoprene biosynthesis involves 1-deoxy-D-xylulose-5-phosphate (DXP) pathway (non-mevalonate or mevalonate-independent pathway) and mevalonate (MVA) pathway (Kuzuyama, 2002). Methanotrophs contain the genes for the upper steps of DXP pathway and not necessarily for isoprene synthase (*IspS*) (Lee et al., 2016). Metabolic engineering approaches can facilitate heterologous expression of codon-optimized genes of isoprene synthase and DXP pathway along with error-prone PCR based random mutations. Metabolic engineering approaches have been used to facilitate isoprene biosynthesis by *Methylococcus capsulatus* (Leonard et al., 2016). To express a foreign gene (e.g., isoprene synthase) in methanotrophs, heterologous expression of codon-optimized gene fragments involved in DXP pathway can be performed. Low product yield is a primary bottleneck to commercialization of isoprenoids.

Farnesene, a widely used bioproduct in cosmetics, diesel, plastics, lubricants and rubber industries (Global Market Insights., 2016.) can also be produced by methanotrophs. Although there is a paucity of scientific literature on farnesene production by methanotrophs, Intrexon Co. developed a genetically modified methanotroph with a biosynthetic pathway to yield farnesene.

Olefins are alkene compounds that occur either as cyclic/ acyclic compounds (based on the location of a double bond between carbon atoms) or monoolefins/diolefins/triolefins (classified based on several double bonds per molecule). Olefins such as ethylene, benzene, and propylene are building blocks for a variety of industrial products including polymers, cosmetics, detergents and lubricants (Schwach et al., 2017).

4.7.3. Organic chemicals from methane

Methanotrophs can produce aldehydes, organic acids, keto acids, and carboxylic acids from biogas (Henard et al., 2018) under ambient conditions (Fei and Pienkos, 2018). (Strong et al., 2015). Methanol, the initial oxidation product (Fei and Pienkos, 2018) can be converted to formaldehyde and formic acid. *Methylomicrobium alcaliphilum* (Henard et al., 2018), *Methylomonas* (Lee et al., 2019) and *Methylocystis* can produce lactic acid and formic acid from CH_4 (Tays et al., 2018).

Research is underway to develop genetic engineering pathways to overproduce targeted metabolic intermediates including lactic acid (Calysta Energy., 2013), 1-butanol, fatty alcohols, fatty acid esters, and 2,3-butanediol (Coleman et al., 2016), succinic acid, acetic acid, ectoine, vitamins, and astaxanthin (Strong et al., 2015). Bioconversion of CH_4 to lactate has been reported in *M. buryatense cultures* through heterologous expression of *Lactobacillus helveticus* L-lactate dehydrogenase to result in a yield of 0.05 g lactate/g of CH_4 at a rate of 0.008 g lactate/L/h (Henard et al., 2016). The low yield of lactate has been reported to be a major bottleneck, which can be addressed via genetic engineering strategies.

A novel fermentation approach for synthesizing formic acid, acetic acid, formate, acetate, succinate, and lactate by *M. alcaliphilum* 20Z at low oxygen levels was recently reported (Kalyuzhnaya et al., 2013). Although methanotrophs can theoretically yield a suite of organic chemicals, their yields are quite low. The maximum titer levels by genetically modified strains did not exceed 1 g/L. New synthetic biology approaches may be needed to meet the commercial scale production (Lee et al., 2019).

5. Technical challenges to CH_4 valorization in WWTPs - Potential solutions

Table 4 provides an overview of technical challenges and potential solutions for enabling the use of biogas as a C substrate by methanotrophs. CH_4 is a non-polar substance which does not interact with other compounds. Its C–H links are the strongest among the alkanes. Thus, CH_4-fed bioreactors can be expected to be challenged by mass transfer limitations, low volumetric productivity, and slow biomass growth rates (Latimer et al., 2018). Here we discuss three emerging strategies for enabling CH_4 oxidation by methanotrophs; they include: (1) Biotechnological interventions for manipulating competing pathways of CH_4 oxidation including denitrification and anammox processes, (2) plasma technologies for CH_4 oxidation, (3) material surface modification approaches for enhancing biofilm growth.

5.1. Metabolic engineering

Biotechnological interventions can activate the key gene regulatory networks needed by methanotrophs to produce desired bioproduct (Haynes and Gonzalez, 2014). OMICS approaches can be used to enhance CH_4 uptake, alter metabolic fluxes and suppress competing pathways (Fei et al., 2014, Fei et al., 2018). Other intervening strategies to maximize CH_4 oxidation include: activation of acetyl CoA pathway/enzymes; blocking of genes/proteins involved in competing pathways; and adaptive evolution-based strain engineering. Activating key precursors including acetyl CoA and pyruvate via genetic engineering approaches (Kalyuzhnaya et al., 2015) can yield high efficiency methanotrophs. Table 5 shows an overview of key gene regulatory targets and desired bioproducts.

A triple knock-out of *Methylomicrobium buryatense* has been reported to enhance the lipid content by 90% via blocking of two glycogen synthase genes and a sucrose synthase genes (Fei et al., 2018). Altered phenotype of increased CH_4/oxygen uptake has also been observed, suggesting an increased C assimilation by the mutant strains. Recently an adaptive evolution based engineered mutants were developed using *Methylomicrobium alcaliphilum* for putrescine biosynthesis (from CH_4) and tolerance by eliminating putrescine utilization pathway and expressing ornithine decarboxylase (Nguyen and Lee, 2019). Also, the plasmid-free production strain of *Methylomonas* sp. was developed by integrating a cassette encoding gene for astaxanthin and hemoglobin, enhancing astaxanthin production by 80% of total carotenoids (Tao et al., 2007). The secondary carotenoid production in *Methylomonas* sp. has been reported to be imparied due to reduced O_2 availability. Moreover, the colorless *Methylomonas* sp. was conjugated with carotenogenic biosynthetic genes viz., beta-carotene ketolase and beta-carotene

Table 4
Methane upcycling in wastewater treatment systems.

Challenge	Solution	Potential outcome	Section #
Sulphide impurities in biogas	Add scrubber or AOM coupled sulfate reduction	Minimal hydrogen sulphide emission	5.4
Low methane oxidation rates	Metabolic engineering approaches	Enhanced bioproduct accumulation and unveiling novel microbial pathways	5.1
Natural gas	Burn it to heat fermenters in the cold months	Reduced natural gas emission to the environment	5.2
Oxygen limitation and excessive CO_2 accumulation	"METHALGAE" – methanotroph - algae coculture	Efficient methane oxidation with minimal CO_2 emissions and limited O_2 supply	5.1
Low methane solubility	Heterogenous-catalyst-activated plasma technology	Enhanced methane conversion efficiency	5.3
High levels of COD	Pretreat using MCDC	Optimal COD for MOB	5.4
Produced water disposal	Treatment techniques for desalination	reuse in oil fields/crop irrigation	5.4
Low solubility	Use paraffins	Enhanced solubility	5.4
High overpotential	Use catalysts/methane oxidation to methanol	Optimal overpotential	5.3
High overpotential in MFCs	Surface modification techniques	Optimal overpotential	5.3
Lower growth due to lower mass transfer rate of methane	Paraffins to improve the transfer rates and cell yield	Enhanced microbial growth	5.1

Table 5
Key products from engineered strains of methanotrophs and their gene regulatory targets (deletion targets in brackets).

Methanotroph	Bioproducts	Gene targets	References
Methylomicrobium buryatense	Lipid	Sucrose-phosphate synthase (deletion)	(Fei et al., 2018)
Methylomonas sp.	Canthaxanthin, and astaxanthin	β -carotenoid ketolase, β -carotenoid hydroxylase	(Sharpe et al., 2007; Ye and Kelly, 2012)
Methylococcus capsulatus	Succinic acid	Malate dehydrogenase, pyruvate carboxylase	(Subbian, 2015b)
Methylomicrobium buryatense	Lactic acid	Lactate dehydrogenase	(Henard et al., 2016)
Methylococcus capsulatus	Isobutanol	Keto acid decarboxylase, acetolactate synthase, ketol-acid reductoisomerase, dihydroxyacid dehydratase, alcohol dehydrogenase	(Coleman et al., 2016)
Recombinant methylotroph	1,4-Butanediol	CoA-dependent succinate semi-aldehyde dehydrogenase, 4-hydroxybutyrate dehydrogenase and alcohol dehydrogenase	(Furutani et al., 2014)
Methylococcus capsulatus	Isoprene	Isoprene synthase	(Leonard et al., 2016)
Methylomonas sp.	Astaxanthin	β-carotene ketolase, β-carotene hydroxylase, truncated hemoglobin (co-expression cassette)	(Tao et al., 2007)
Recombinant Methanotroph	Lactic acid	Lactate dehydrogenase	(Silverman et al., 2017)
Methylomicrobium alcaliphilum	Putrescine	Ornithine decarboxylase (overexpression), acetate kinase, spermidine synthase, lactate dehydrogenase (deletion)	(Nguyen and Lee, 2019)

hydrolase 95% of carotenoid as astaxanthin with the sufficient oxygen supply (Ye and Kelly, 2012). These results corroborate that the low O_2 levels would hamper astaxanthin accumulation and instead promotes the formation of intermediates. A US patent application on metabolic engineering of methanotrophs for lactic acid production from methane has been reported recently (Subbian, 2015).

For synthesis of PHB, by inserting artificial metabolic pathways in methanotrophic cells, a precursor molecule viz. 1,4-butanediol could be synthesized which in turn gets converted into the short-chain PHA molecules (Fig. 1). In another approach, lactate, a bio-product that has been reported to be a suitable C substrate for PHB synthesis. Lactate could easily be converted into PHA via a two-step reaction using two different recombinant enzymes, i.e., *Pct* and PHA synthase (Fig. 1). A similar strategy could also be applied to produce organic acid, i.e. malate, synthesized by Type-II methanotrophs possessing a complete Serine cycle. To increase the PHB via CH_4 oxidation, a continuous stir tank reactor configurations have been used, increasing the PHB yield to 60% (w/w) (Chidambarampadmavathy et al., 2015).

Optimizing the C-to-microbe ratio while increasing the N-to-microbe ratio has been reported to support the growth of Type-II methanotrophs used for PHB synthesis. Adequate attention should be given towards the N source as any accumulation of nitrate (as N-source) will support the growth of Type-I methanotrophs, degrading the quality of produced PHB. The x-ome technologies including metabolome, transcriptome, proteome, and fluxome approaches along with genome-scale metabolic modeling could be used to rewire the genetic mechanism of methanotrophs. Since, the essential biotechnology parameters including genetic tractability, growth, and nutrient uptake have been reported for well-characterized methanotrophs like *Methylocystis* sp., *Methylosinus trichosporium* OB3b, and *Methylococcus capsulatus* Bath, the genomic tools could be established widely for other non-model methanotrophs. However, with the recent explorations of novel halophilic, psychrophilic, acidophilic, alkaliphilic, and extremely thermophilic methanotrophs could further augment the genomic insights for their biotechnological potential.

5.2. Plasma technology

Plasma, referred to as the "fourth state of matter" which includes negative ions, positive ions, neutral species, and electrons can be used to oxidize CH_4 effectively (Zakaria and Kamarudin, 2016) under ambient conditions, and with low specific input energy (electrical input energy per unit mass of the material gas) (Okumoto and Mizuno, 2001). To address the issue of low CH_4 solubility, methane hydrates can be used to enhance CH_4 solubility during the plasma-assisted bioconversion process (Zakaria and Kamarudin, 2016). The synergistic effect of heterogenous-catalyst-activated plasma technology using Pt, Fe_2O_3, and CeO_2 is another

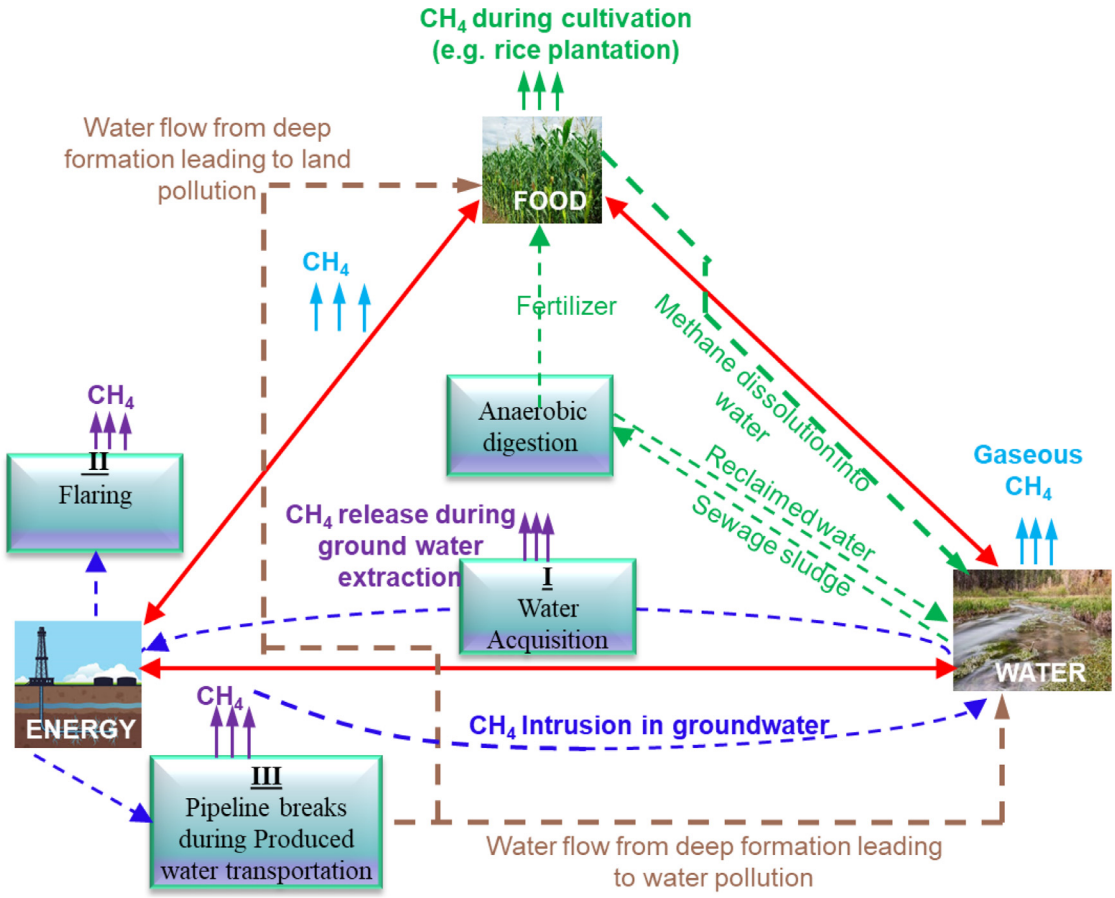

Fig. 6. A schematic of FEW nexus challenges in the upper Great Plains region. (1) Excessive freshwater use for UOP decreases water availability for agriculture and drinking water; (2) flaring of natural gas; (3) Pipeline breaks during produced water transportation contaminates crop and water resources;Oil pipeline failures causes crop contamination.

effective way to enhance CH_4 conversion efficiency (Chen et al., 2009; Holmen, 2009; Reddy et al., 2013).

5.3. Surface modification techniques

Surface modification techniques could be used to promote dissociation of C–H bonds in CH_4 and render its amenability to subsequent conversion into bioproducts. Here we focus on the use of surface modification techniques to enhance bio-electrochemical oxidation of CH_4. Instead of relying upon expensive catalysts (e.g., Ni, Pt, Al_2O_3, ZrO_2, etc.) that require high temperatures, methanotrophs can be used to convert CH_4 into methanol and subsequently into other bioproducts. Chemical functionalization techniques can be used to tune material surface properties and render them suitable for the growth of electrogenic methanotrophs in BESs. The bio-electrocatalytic activity of the working electrode (WE) in BESs largely depends upon its surface properties. The biofilm growth, interactions between the biofilm and WE surface, and the resulting biofilm phenotypes are influenced by the chemical composition of electrode, surface charge, surface area, surface roughness, wettability, and porosity. Surfactants immobilized on the WE surfaces can enhance its effective surface area, reduce the impedance, and improve the overall kinetics pertinent to bioelectrochemical oxidation of CH_4.

Table S3 provides an overview of surface modification methods for enhancing catalytic activity and biofilm growth. Recently, we demonstrated the use of a multilayered reduced graphene oxide (rGO) to achieve super hydrophilic nickel electrode surfaces which were found to yield electrogenic biofilms of *Rhodobacter Sphaeroides spp* in MFCs (Islam et al., 2020). Similarly glassy carbon electrode surfaces modified with $-OH$, $-CH_3$, $-SO_3$, and $-N^+(CH_3)_3$ groups via electrochemical reduction of aryl diazonium salts have been found to yield suitable surface properties for promoting the growth of *Geobacter* biofilms (Guo et al., 2013). Positively charged, hydrophilic surfaces promote the biofilm growth when compared to negatively charged, hydrophobic surfaces. Surface roughness and porosity of the electrode also influences bio-electrochemical impedance in MFCs (Ye et al., 2013). The rougher the electrode surface the lower the overall bio-electrochemical impedance of the BES systems.

5.4. A case study on the use of valorization of stranded methane in the oil industry

Horizontal drilling and hydraulic fracturing techniques have enabled oil production from previously inaccessible tight shale oil deposits including the Mississippian-age Bakken Shale which extends into Montana and the Canadian provinces of Saskatchewan and Manitoba.

While the oil production from the Bakken shale has been a major economic driver, it has attracted a significant number of foods, energy, and water (FEW) nexus challenges, as discussed in (Shrestha et al., 2017), and depicted in Fig. 6. Natural gas, a byproduct of the crude oil production is flared in open atmosphere, due to lack of transportation and refinery infrastructure (Fig. 6). Hydraulic fracturing also generates a hypersaline oil and gas wastewater ("produced water") that is currently being piped and permanently injected into class II injection wells, causing undesir-

Table 6
Overview of bioproducts derived from CH_4, their unit operations and wastewater treatment levels.

Constituent	Unit operation or process	Treatment level	Description	References
CH_4	Denitrification	3°	Potential external electron donors	(dos Santos et al., 2004)
S-layer	Membrane modification	Advanced	Methylotrophs can convert CH_4 into S-layers	(Sara and Sleytr, 1987)
Acetic acid	Phosphorus removal	3°/Advanced	Carbon source for production of polyhydroxyalkanoates (PHA). Internally stored PHAs are oxidized and used for microorganism growth and phosphorus uptake.	(Chen et al., 2004)
Propionic acid	Phosphorus removal	3°/Advanced	Higher phosphorus release to short-chain fatty acids uptake	(Li et al., 2008)
Bicarbonate	Nitrogen removal	3°	Can be used as base during anaerobic digestion for maintaining alkalinity	(Jetten et al., 1997)
Electricity	Offset against the power required for the treatment process	1/2/3°	Sufficient to power the aerobic processes as well as the mixing etc. of the anaerobic stages	(Keller and Hartley, 2003)
Biodegradable polymer	–	–	Methanotrophs can convert a part of the unused CH_4 to biodegradable polymers	(Wendlandt et al., 2001)
Biodiesel	–	–	Intracellular and membrane lipids of methanotrophs could be used as a renewable feedstock for biodiesel production	(Brennan and Owende, 2010b).

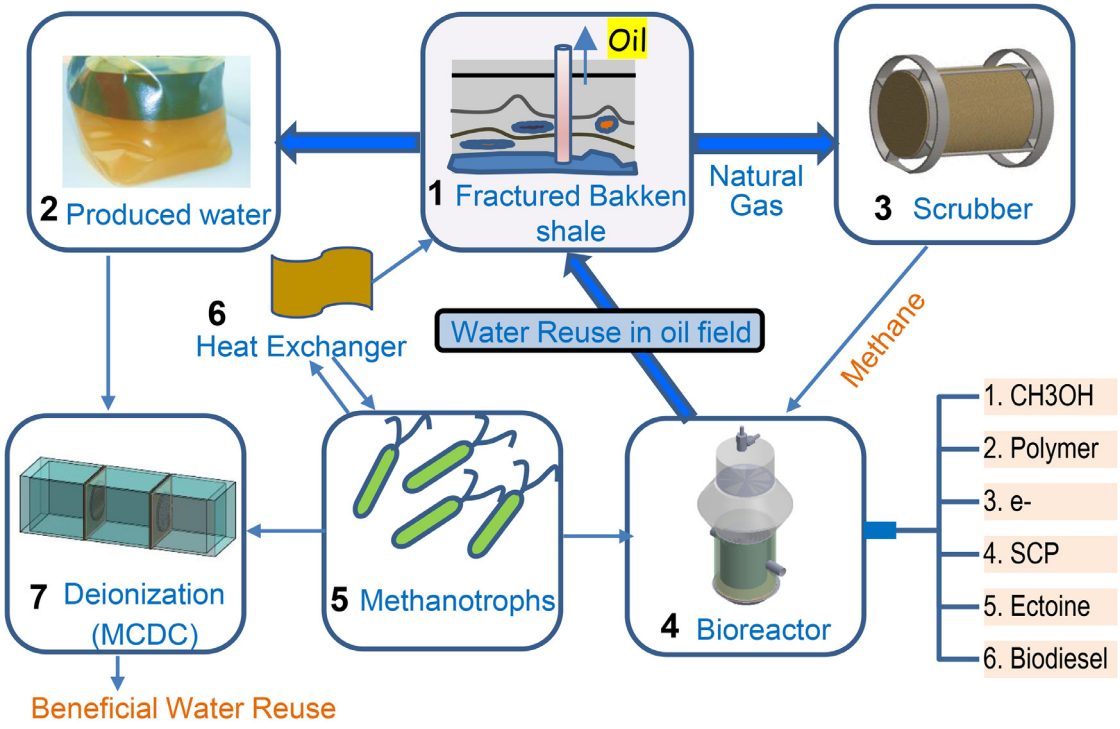

Fig 7. A case study on valorization of previously stranded CH_4 in energy industry. (1) hydraulic fracturing in Bakken shale generates natural gas (a byproduct) and produced water (a waste product); (2) produced water is treated in a microbial capacitive deionization (MCDC) unit; (3) natural gas is purified in scrubber (4) a bioreactor is fed with CH_4 (from scrubber), water (from MCDC), and methanotrophs, and CH_4 is upcycled into bioproducts (methanol, polymer, electrons, SCP, ectoine, biodiesel); (5) pure culture or indigenous mixed cultures of methanotrophs for use in bioreactor and/or MCDC; (6) waste heat recovered from the stack is used to maintain bioreactor and/or MCDC at a desired temperature, especially in cold winters (7) MCDC generates wastewater effluent (in anode) and a pure water stream (in CDI unit), both of which can be recycled for different uses.

able spills and contamination of nearby agricultural and water resources. Here we will discuss the potential use of methanotrophs for valorizing a commonly stranded natural gas in the oil fields.

As shown in Fig. 6, methanotrophs can use methane fraction of the stranded gas as a C source. Produced water can be desalinated and treated in a microbial capacitive deionization (MCDC) unit (Shrestha et al., 2018) and used to provide macro-, micro-nutrients and trace elements to support physiological growth of methanotrophs. The average volume of produced water in the Bakken shale alone is ~ 2.31 Mgal/year (Shrestha et al., 2017), which will produce an adequate amount of electrolyte to sustain methane-based bioreactors throughout the year.

Indigenous methanotrophs present in drilling mud, geological formation, fluid amendments, handling water infrastructure, and produced water can be used as inoculum in methanotrophic bioreactors. Thus, the development of methanotrophic processes for in-situ reuse and recycling of produced water, and the simultaneous treatment of stranded methane is beneficial. In our earlier study, we have demonstrated the proof-of-concept for MFCs and MCDC technologies to treat and desalinate produced water (Shrestha et al., 2018). Thus, it is also plausible to enrich methanotrophs for treating dissolved methane in produced water, while accomplishing the typical COD removal (Fig. 7). As discussed in the subsequent paragraphs, the natural gas stream that is currently be-

ing openly flared could be used to supersaturate the produced water with dissolved methane.

As the primary chemical constituent of natural gas (70–90% by volume raw natural gas from the well) methane is a powerful greenhouse gas that can significantly impact global atmospheric chemistry. Increasing crude oil production has also resulted in the increasing air pollution, primarily due to the open flaring of natural gas which results in emissions of air pollutants including VOCs, PM, CO, etc., The problem with flaring is that it doesn't burn all the VOCs and some escape into the atmosphere.

We could recycle some of the CH_4 from the natural gas and treat it in the methanotroph-based bioprocesses. The desalinated water from MCDC can be used as the electrolyte for CH_4-fed bioreactors. Methanotrophs can use stranded CH_4 as the sole carbon source. The indigenous methanotrophic consortia can turn the produced water treatment system into a biorefinery platform for generating a range of value products shown in Fig. 4.

For instance, it is viable to set up bioreactor systems to convert CH_4 into methanol. Using methanotrophs (*Methylomicrobium album and Methylomicrobium alcaliphilum)* (1.5 mg of dry cell mass/ml and 20% of CH_4 as a feed), Patel et al. (2016) was able to produce methanol (3.43 and 3.73 mM) under optimum conditions (pH 7.0, 30 °C, and 175 rpm) with the efficiency of 51–61%. Production of methanol in batch cultures was evaluated in 120 mL serum bottles containing 20 mL of phosphate buffer (100 mM) with 10 μM of Fe (II) and 5 μM of Cu (II). Hence a 10,000 L fermenter could be designed to generate ~7000 L of methanol. Methanotrophs could be enriched using the CH_4 gas and accumulated cells could be used as a protein source. If all the currently flared CH_4 is efficiently used in a fermenting (~ 10,000 L), 2282 t of methanotrophic cells could be generated that could be used as cattle feed. CH_4-derived bioproducts can be used at various unit operations at different treatment levels of the wastewater treatment systems as shown in Table 6. Also, detailed investigations on each bioproduct derived from various methanotrophs and their technical challenges were described in Section 4.

6. Conclusions

We conclude that methanotrophs and their recombinant enzymes provide excellent opportunities for treating commonly discarded CH_4. A series of further studies are warranted to unlock their potential for treating and recycling unprocessed CH_4 in real-time systems, especially at wastewater treatment plants. From a biology standpoint, there is a need to decode key mechanisms of CH_4 assimilation under semi-aerobic or anaerobic conditions that may be beneficial for treating CH_4 collected from rising sewers, anaerobic digesters, landfills, and other point sources in environmental engineering applications. Advanced immune labeling techniques are required to enable precise characterization of the intracytoplasmic membranes of methanotrophs. From a modeling standpoint, computational models describing the active sites of pMMO, sMMO and AMO genes will enable the development of robust microbes via genome editing techniques. These models could also be used to trace electron transfer routes during CH_4 oxidation within wastewater treatment systems integrated with microbial fuel cells (MFCs) (versus using methanotrophs to treat CH_4 in separate MFC units). A random in-vitro protein engineering techniques could improve the biotransformation efficiency of large multi-subunit proteins viz. MMOs. From an engineering standpoint, novel approaches based on a combination of advanced gas-phase bioreactor technology, novel materials, and robust methanotrophs are required to address challenges with low solubility, thermodynamic stability, and inertness of CH_4.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2020.116400.

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