



Microbial electrolysis cells for electromethanogenesis: Materials, configurations and operations

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ABSTRACT

Anaerobic digestion is a traditional method of producing methane-containing biogas by utilizing the methanogenic conversion of organic matter like agricultural waste and animal excreta. Recently, the application of microbial electrolysis cell (MECs) technology to a traditional anaerobic digestion system has been extensively studied to find new opportunities in increasing wastewater treatability and methane yield and producing valuable chemicals. The finding that both anodic and cathodic bacteria can synthesize methane has led to the efforts of optimizing multiple aspects like microbial species, formation of biofilms, substrate sources and electrode surface for higher production of the combustible compound. MECs are very fascinating because of its ability to uptake a wide variety of raw materials including untreated wastewater (and its microbial content as biocatalysts). Extensive work in this field has established different systems of MECs for hydrogen production and biodegradation of organic compounds. This review is dedicated to explaining the operating principles and mechanism of the MECs for electromethanogenesis using different biochemical pathways. Emphasis on single- and double-chambered MECs along with reactor components is provided for a comprehensive description of the technology. Methane production using hydrogen evolution reaction and nanocatalysts has also been discussed.

Keywords: Electromethanogenesis, Hydrogen evolution reaction, Microbial electrolysis cells, Nanocatalysts, Process improvement, Renewable energy production

1. Introduction

Since the last century, the entire world energy requirements have been powered by the use of fossil fuels such as coal, natural gas, petroleum and its products [1, 2]. These non-renewable and fossil resources have a major share in the global economy. Fossil fuels are exhaustible and are currently depleting at the most rapid rate ever seen due to their increasing applications and continual usage. They are at the verge of depletion in roughly around 35 years [3]. Moreover, serious environmental threats and concerns are raised due to the emission of greenhouse gases into the atmosphere [4-6]. Increased levels of carbon dioxide (CO₂) and other toxic gases get released due to the burning of fuels which contribute to global

warming and ocean acidification [7]. To overcome this alarming situation, there is a quest to explore carbon-free energy sources to provide energy most cleanly and sustainably.

To create energy in a sustainable and environmentally friendly manner, microbial electrochemical systems (MES) proves to be an efficient way [8, 9]. In simple terms, MES develop electrical energy by transforming the chemical energy obtained from waste lignocellulosic biomass and wastewater through the reduction-oxidation process by using the biological catalysts [10]. MES technology is a multidisciplinary field combining subjects of electrochemistry, material sciences, microbiology and chemical engineering [11]. In MES the oxidation of water takes place at the anode giving rise to electrons and protons which are further transported



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to the cathode in presence of (external) electric potential [12]. In the cathode region, the redox reactions take place under the presence of electroactive microbes [13]. MESs are further classified into microbial fuel cells (MFCs) [14], microbial electrolysis cells (MECs) [15], microbial desalination cells (MDCs) [16] and microbial solar cells (MSCs) [17] among others based on the configuration of the reactor, environmental conditions and products desired [18]. These types of MESs are operated by the principles of electro-microbiology used to explore the varied potential of electroactive bacteria (EAB) [19]. MFCs are known to generate electricity from organic waste streams whereas MECs requires a supply of electricity for producing hydrogen from organic waste streams [20]. MFCs are one of the important MESs to produce economically and environmentally friendly electricity, but it fails to compete with other energy sources such as hydrogen, methane, ethanol and other value-added chemical products. MECs are a capable technology to produce fuel and energy sourced from organic matter including wastewater and renewable resources (like an agricultural waste) [21]. MECs not only promises the production of renewable hydrogen and value-added products but also helps in the removal of organic compounds from wastewater [22]. Low energy input, self-sustaining microbial biocatalysts, high conversion efficiency, low cost and pollution inhibition are the remarkable characteristics of MECs [23-25]. Hydrogen developed from the MECs was the important metabolic gas product, however, in recent times, methane (CH₄) has gained attention from the scientific community [26-29].

Methane is a renewable fuel which was conventionally produced from anaerobic digestion of bio-waste [30]. However, the process takes many days to complete. The presence of methanogens in MES microbial community developed the process of collecting CH₄ from cathode portion of MECs utilizing CO₂ electromethanogenesis [31]. Methane is generally detected in MECs during the hydrogen production stage due to the growth of methanogens [32]. Chae *et al.* noted that the generation of methane varies with shift in inoculum, substrate, and reactor design [33]. Methanogens appear in the production phase of hydrogen, which lessens the hydrogen yield. Various approaches have been tried to cut down the development of methanogens microorganisms in MECs, but most of the approaches have turned out to be energy exhaustive and ineffective [34, 35]. Instead of inhibiting the methanogens, producing methane directly through MECs have various advantages when compared

to the anaerobic digestion process. Methane production and oxidation of organic matter are two different processes in MECs which provide a high content of methane in biogas [36]. Another advantage of MECs includes the production of methane in presence of ambient temperature which means heating is not required, thus MECs proves to be energy efficient. An added advantage of using MECs includes the acceptance of electrons directly from cathodes, making the process tolerant to toxic substances like ammonia [37]. Anaerobic digestion requires high organic content to make methane, while MECs develops CH₄ even in a lower concentration of organic compounds [23, 38].

In this review, we highlight the growth of MECs in the methane production and how it works as a MES with help of other technologies like hydrogen evolution reaction (HER) and nanoparticles. A generalized pathway for electromethanogenesis along with its electron transfer method is delineated. Moreover, the contents like microbial species, reactor components, configuration along with the operating conditions which make up MECs are discussed intricately.

2. Principle of MECs for Methane Production and Microbial Pathway

MECs are the technology which is derived from MES where the external voltage is applied to overcome the thermodynamic energy barrier to drive biochemical reactions [39]. Electromethanogenesis synthesizes methane in two ways, either by direct uptake of electrons from electrode called direct electromethanogenesis (Eq. (1)), or mediated by hydrogen and other compounds such as acetate, formate which are produced and combined with carbon dioxide to form methane called as mediated or indirect electromethanogenesis (Eq. (2)) and (Eq. (3)) [40, 41].

Direct electromethanogenesis:



$$E = -0.244 \text{ V vs. Normal hydrogen electrode (NHE)}$$

Indirect electromethanogenesis:

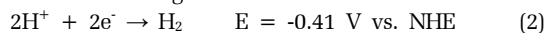


Table 1. Electroactive Bacteria (EAB) Used in MECs for Methane Production

Electrogenic microorganisms	Substrate	References
<i>Methanobacterium</i> sp.	Municipal wastewater	[227]
<i>Clostridium</i> sp.	Waste activated sludge	[112]
<i>Methanocorpusculum</i> sp.	Waste activated sludge	[170]
<i>Methanosaeta</i> sp.	Food waste and sewage sludge	[228]
<i>Geobacter sulfurreducens</i>	Acetate	[209]
<i>Methanobrevibacter</i> sp.	Anaerobic digester sludge	[189]
<i>Methanosaeta</i> spp.	Alkaline pretreated sludge	[229]
<i>Shewanella oneidensis</i> MR-1	Acetate	[230]
<i>Geobacter</i> sp., <i>Methanosarcina</i> sp.	Waste activated sludge	[231]
<i>Desulfuromonadales</i> sp., <i>Pseudomonas</i> sp.	Leachates of municipal solid waste	[149]
<i>Petrimonas</i> sp., <i>Methanocorpusculum</i> sp.	Alkaline pretreated waste activated sludge	[170]

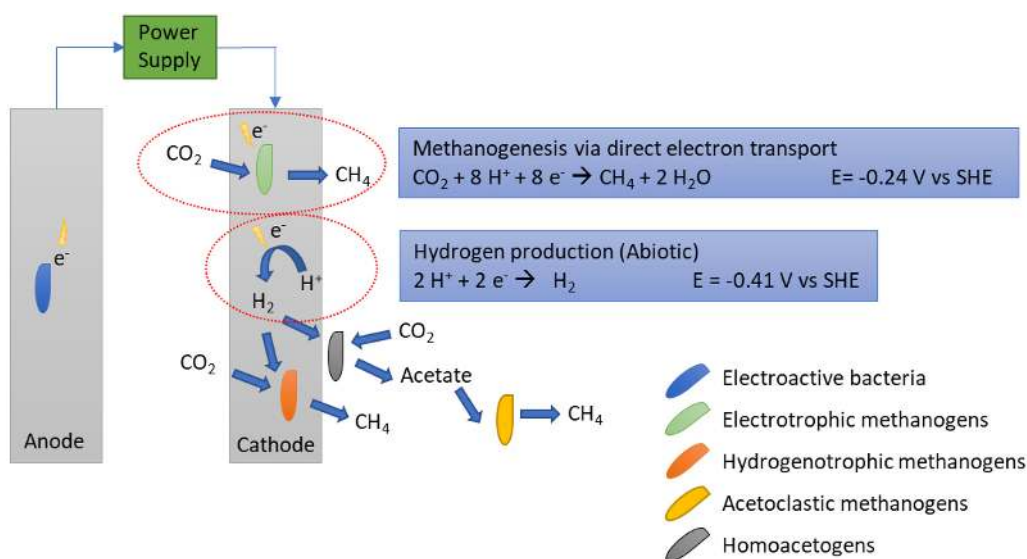
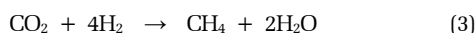


Fig. 1. Schematics of a typical MEC for electromethanogenesis.



The hydrogenotrophic archaea have the potential to accept electrons directly from cathode catalysing only direct electromethanogenesis while, methanogenic archaea which are present in the biocathode play a crucial role in both direct and indirect electromethanogenesis, [42]. Methane producing MECs is composed of four major components: anode, biocathode, separator, electricity source (Fig. 1). In anode, oxidation reaction takes place which is necessary to provide electrons for reduction of CO_2 in the biocathode region [43]. Secondly, biocathode is the important component where methane is produced by the help of microorganisms using the electrons supplied from oxidation reaction taking place in anode [44]. A separator or ion exchange membrane is necessary for migration of positively charged ions such as Na^+ , K^+ , H^+ from anodic chamber to cathodic chamber to keep the solution electroneutral. Lastly, externally provided electrical energy is needed to drive the reaction thermodynamically [45]. Low voltage supply (0.2 - 0.8 V) is sufficient for the bioelectrochemical reaction to produce methane [42].

3. Hydrogen Evolution Reaction (HER)

Due to the increasing global energy requirements, the traditional ways of power generation can lead to the degradation of the environment. The environment-friendly and sustainable production of energy is a challenge in upcoming generations [46]. The environmental concerns caused because of the fossil fuels have gained attention towards the sustainable, low-cost and less carbon emitting sources [47]. Hydrogen (H_2) is one of the striking alternative for fossil fuels in the approaching future [48]. Hydrogen is a green and clean source of energy in replacement of the traditional fossil fuels because of its recyclability and non-polluting by-products [49]. In today's scenario hydrogen production is done through

the transformation of fossil fuels which is not sustainable and lead by high carbon dioxide (CO_2) emissions [50]. Hydrogen gas could be produced by electrochemical HER in the presence of low-cost catalyst coupling renewable energy sources such as wind, solar energy, geothermal heat and biomass which enhances the rate of hydrogen production [51]. It is also the most efficient and feasible method for production of hydrogen with high purity and a large quantity [52]. HER is one of the most studied electrochemical processes since the 18th century [53]. Despite the high cost of the water electrolysis, it offers an effective method to produce highly pure hydrogen [54]. HER ($2\text{H}^+ + 2\text{e}^- \rightarrow \text{H}_2$) is a type of electrochemical cathodic reaction in which also has the potential to produce H_2 [55, 56]. HER is a prime example of two-electron transfer reaction having single catalytic intermediate which produces H_2 [57]. HER may lead to the sustainability of hydrogen fuel, which is transportable, storable and applicable in the zero-emission fuel cell of combustion engines [58, 59]. Hydrogen production from the MECs is a renewable technique to produce H_2 from organic materials like wastewater in presence of electric current [60, 61]. External energy in the form of applied potential is required to propel free energy of reaction negative, further producing hydrogen at cathode [62]. In this MEC process the substrate undergoes oxidation through microbes which then further produces electrons, protons and CO_2 [63]. Electrons get transferred by the external circuit to the cathode, while the protons (H^+) travels to the cathode through proton exchange membrane. The protons diffuse to the cathode and combine with electrons to develop hydrogen [64, 65].

4. The Cathode and the HER

Selection of appropriate cathode material is crucial for the MECs to produce hydrogen as it acts as the main site for HER [66]. The HER is the rate-limiting step for the MECs as it requires the transfer of mass between the gas and solid-liquid interfaces [67]. The cathode

electrode of MECs has a structured layer which consists of a catalyst layer situated on a conductive substrate [68, 69]. The catalyst layer is composed of a fusion of catalyst, binder, substrate (e.g., made up of carbon or metal) and conductive powder such as activated carbon and carbon black. The substrate act as a support for the shape of the cathode as well as gathers the current. These cathodes are usually connected to the power supply through an external wire [70]. Functional catalysts not only reduce the activation barrier of the HER process but also determines the efficiency of H₂ generation in the MECs [71]. Significant factors like production rate of hydrogen, the overall recovery of hydrogen, long-term durability and Coulombic efficiency needs to be contemplated when a cathode is being assessed [72, 73]. To achieve high energy efficiency for the water-splitting electrochemical reaction use of specific catalyst is much required for minimization of overpotential and enhancement of the HER rate [74]. There is an energy barrier or resistance in the electrochemical processes for producing hydrogen from water called overpotential of the system [75]. For decreasing the energy input as well as to increase hydrogen production rate, catalyst is placed at the cathode where hydrogen is formed [76, 77]. An appropriate catalyst is required for the minimization of activation energy to reduce overpotential of the hydrogen production system, which also helps in charge transfer reaction at the surface of the cathode electrode [78, 79]. Various studies have shown the use of catalysts such as transition metals: platinum (Pt), palladium (Pd), iridium (Ir) and rhodium (Rh) in MECs [80, 81]. Pt is a famous noble metal which efficiently catalyses hydrogen reaction [82]. Platinum-based electrodes such as brushes, rods, graphite, titanium (Ti) mesh, plates and Pt coated carbon felt are commercially available [83, 84]. Also, the rare and lustrous metal like palladium has been used in catalysing the generation of hydrogen in hydrogen fuel cells [85]. However, using such catalytic cathodes made up of platinum and palladium have disadvantages including its high cost, negative effects on the environment, and its inactivation by chemicals like sulphides [15]. In their place, cheap metals such as iron (Fe), nickel (Ni), molybdenum (Mo), tungsten (W) can be used as cathodes in MECs [68, 86]. For improving the performance, metals could be combined with non-metal species like nitrogen, carbon, phosphides, sulphides and oxides [87]. This could also reduce the cost to produce an effective catalyst. Non-metals not only act as supportive structures which improve the charge transfer but also enhances the reduction and adsorption of hydrogen [69]. Metal alloys are also known to reduce HER activation potential such as Ni alloys and tungsten carbide [88-90].

Alternatively, biocathodes were adopted as a viable solution to catalyse HER. A biocathode is more or less an 'electron sink' which receives protons and electrons from the anodic chamber via the oxidation of organic substrates. Hence, for this purpose, electrogenic microbes are adsorbed on the cathode surface [91]. Biocathodes does not essentially require metals for the biofilm formation and their electrochemical activity [92, 93]. The species like Firmicutes, *Desulfovibrio* spp. and Proteobacteria phyla can be found naturally present and are reported for hydrogen production [94-96]. Sediment microbial fuel cells were shown to be converted into hydrogen- and methane-producing MES easily by reversing the potentials [97]. Hydrogen can be then recovered immediately, or it can be easily transformed into methane in presence of carbon

dioxide (CO₂ + H₂ → CH₄) by methanogenic bacteria or archaea after some days, producing up to 86% methane enriched biogas [98].

However, electrochemical systems and bioelectrochemical systems have differences. The catalyst which works well in the slightly alkaline or acidic conditions may not optimally produce H₂ in near-neutral solutions [99]. This is attributed to the pH-dependency of the metals for catalytic activity. Thus, catalysts suitable for MECs was a focus of research for HER and methanogenesis. Nanocatalysts was explored for this purpose. Many studies have been conducted using MoS₂ as nanocatalyst owing to its outstanding activity due to the increased number of catalytic active edge sites [100, 101]. A better activity was observed when MoS₂ was conjugated with graphene sheets for hydrogen evolution catalysis [102]. Another high catalytic activity was reported by Liu and others stating a 'nanoroll' like structure formed by MoS₂/Ti₃C₂T_x hybrid [103]. A different Mo based 3D-open nanorod arrays was shown to be boosting the catalytic activity of HER drastically with a mere overpotential of 17 mV [104]. Hence, nanocatalysts should be more explored for the production of hydrogen and methane.

5. Electroactive Bacteria and its Extracellular Electron Transfer (EET) Mechanism

5.1. EET at Anode

Microbial electrolysis cells are those bioelectrochemical devices which transform the chemical form of energy stored in the feedstock to high value-added chemicals such as hydrogen, methane, acetate and many more. This biological conversion takes place using the metabolic activity of few microbial species which can produce electrons or reduce of CO₂. These microorganisms are called electroactive bacteria (EAB) [105]. In MECs, the interaction of microbes with other components plays a crucial role in the production of combustible products like methane. For the smooth operation of MECs, transfer of electrons from organic matter (or substrate) to the electrodes is essential [106]. Understanding this microbial extracellular electron transfer (EET) gives added advantage to develop newer strategies to engineer MECs in an effective manner [107, 108]. Microbes proficient of accepting electrons are known as electrotrophs, while exoelectrogens refers to the microbes which has the ability to transfer electrons extracellularly [109]. EAB has been found in various environments such as ocean and marine sediments, domestic wastewater and anaerobic sewage sludge [110-112]. In MECs different microbes can be developed both on the anode or cathode [113]. Gram-negative species such as *Geobacter* and *Shewanella* are usually present on the anode which oxidizes organic matter generating a flow of electrons (Fig. 2(a)) [114].

5.1.1. EET for Gram-negative bacteria

Two types of electron transfer take place- outside the membrane to inside and vice-versa. The initial system is MtrAB porin cytochrome complex that exists in electroactive bacteria such as *Shewanella* and *Geobacter* [115]. The second system comprises Cyc2 fused with porin cytochrome which primarily is present in iron oxidizing bacteria like *A. ferrooxidans* under acidic conditions [116]. In general two pathways are studied as model for exocellular

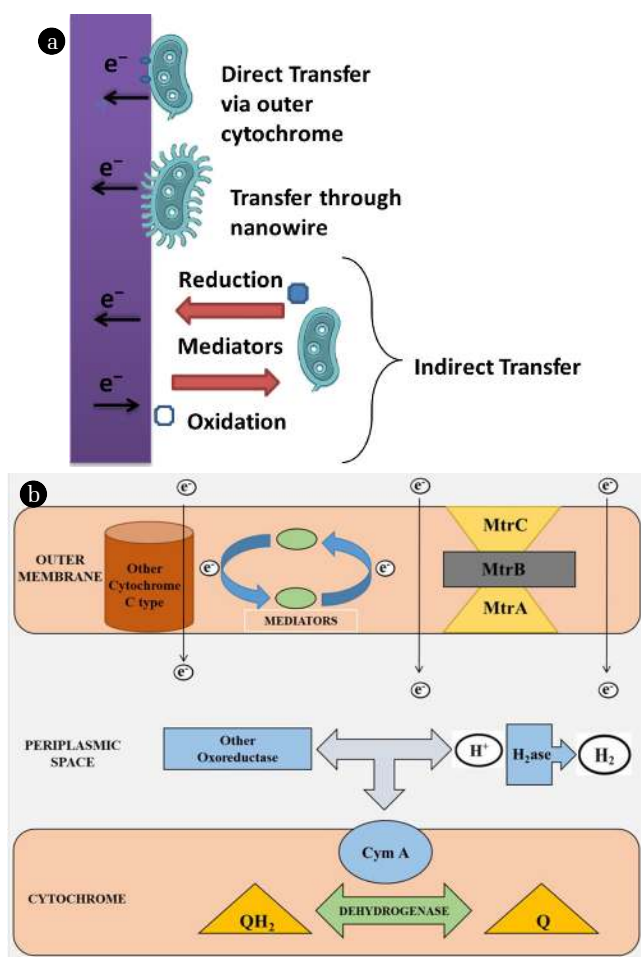


Fig. 2. (a) A schematic on different electron transfer from exoelectrode to anode. (b) MTR pathway in *Shewanella*.

electron transfer that is Mtr *Shewanella oneidensis* which depicts MTrAB porin cytochrome system and another one is nanowire by *Geobacter sulfurreducens*.

5.1.2. MTR pathway in *Shewanella*

S. oneidensis MR-1- most commonly studied MTR pathway it uses Fe(III), Mn(III), Mn(IV) as a terminal electron acceptor [117]. C type cytochrome consists of an element like Cym A, FccA, Mtr A, OmcA, MtrB, MtrC, small tetraheme cytochrome and porin like MtrB. Cym A oxidation of quinol present in quinol pool in cytoplasmic membrane then it passes to the periplasmic membrane with the help of Fcc A and (small tetraheme cytochrome) STC [118, 119]. Mtr A, B, C Cym A present in outer membrane helps in transfer of an electron to outside insoluble electron acceptors [120]. Primarily, Mtr CAB protein complex present at the outer membrane (Fig. 2(b)). Mtr c decaheme cytochrome and OmcA is a lipoprotein. Mtr B porins exist in outer membrane it helps in fixing Mtr A and Mtr C also Mtr c and OmcA. A lipoprotein use as an extracellular protein present in nanowires. *S. oneidensis* MR-1 contains flavin redox mediator for extracellular electron process. Anode side reactions are more elaborately studied than cathode reaction.

5.1.3. Nanowires - *Geobacter sulfurreducens*

Geobacter sulfurreducens is a gram-negative, obligate anaerobic bacteria which comes under class of Delta Proteobacteria [121]. In *G. sulfurreducens* PCA, multiheme c type cytochrome component helps in the electron transport system [122]. C type cytochrome contains ImcH, CbcL, PpcA, PpcD, and omc, it comprises of B, C, S, and Z components [123]. ImcH and CbcL are present in the cytoplasmic side of the cell. PpcA and PpcD exist at periplasmic space and help in electron transfer further into Omcs (B, S, C, S, Z). This element assists in donating an electron to external electrode. Especially Omcz facilitates the transfer of an electron to an electrode [124]. Besides OmcB and OmcS component also involve largely in a reduction of iron and minerals. But it provides less output. Most electroactive bacteria like *Geobacter sulfurreducens* forms tremendous biofilm and has outstanding EET capacity. Approximately 40-50 micrometre thick biofilm produces 5 mA of current [125]. Nanowires attach physically to the electrodes using pili like organelle.

Apart from mesophiles, extremophiles also play important role in MES.

5.1.4. Extremophilic bacteria

Extremophiles are extraordinary, living and growing microorganisms under the most drastic conditions, Extremophiles can be divided into acidophiles, thermophiles, alkaliphiles, halophiles, psychrophiles, etc. based on stress conditions in which they reside [126].

Alkaliphiles: Alkaliphiles are extremophiles that are tolerant to high alkalinity (pH 8.5-11) [127]. *Geotalkalibacter* sp., alkaliphilic *Bacillus* and *S. oneidensis* are the diverse alkaliphilic strains have demonstrated good current density production in MFCs as a biocatalyst [128, 129]. Increased cell voltages were achieved in alkaline conditions in the anode and acidic conditions in a cathode chamber. The riboflavin synthesis was increased in alkalinity for *S. oneidensis* MR-1 that leads to higher current output [128].

Thermophiles: There are certain advantages while using thermophile in BES, which include the capacity to regulate the BES under hot environment which leads to an increase in catalytic activity [130, 131]. High temperatures also provide high mass and ion transfer and high solubility of the substrate. A MFC was stable for 100 days with strong Coulombic productivity when mixed culture was used (55°C). The study in the 16S rRNA clone library showed that 80 percent of Firmicutes are endospore-forming materials and electricity-producing. In MFC operated under thermophilic conditions, higher output power and coulombic efficiency were recorded as well as reduction of sulphates were seen [132].

Halophiles: Halophiles can reproduce in hypersaline conditions. The halophiles occur in Eubacteria in various phyla, namely, Proteobacteria, Firmicutes and Actinobacteria [133]. The application of high salt levels in MFCs has been advantageous due to improved proton transfer and increased conduction that also assists in improving the resultant energy. Therefore, halophilic EAB are useful in generating electricity and treating saline wastewater [134].

5.1.5. Immobilization strategies on the anode: Biochemical enrichment strategies

Often MES is operated with mixed consortia. Difficulty faced in

MEC is the overgrowth of unwanted non-electrogenic population and substrate deficiency in the anode chamber. Various techniques are invented to diminish the non-electrogenic population. Pre-treatment is a convenient and the most applicable method both in anodic (MFC, MEC) and cathodic (MES) chambers [135]. Pre-treatment is done for increasing performance of electrochemical efficiency and utilization of substrate by electroactive bacteria for the production of energy and by-product [136]. There are two ways to gain product- one is the enrichment of electroactive bacteria and the second approach is to suppress the unwanted non-electrogenic microbial population [137]. Enrichment of electroactive bacteria is done by fixed anode potential, bioaugmentation mechanism and supplement of electron acceptors to increase the rate of electrochemical oxidation in anode chamber [35].

Bioaugmentation: A promising alternative approach is bioaugmentation by utilizing pure electrogen culture in mixed seeds of electroactive bacteria. The presence of non-electrogenic bacteria imposes resistance by utilising substrate and they are unable to send their extracellular electrons to the anode. On a contrary the electrogenic bacteria donates their electron directly to the anode and can be used for bioaugmentation. These electrons can be collected more effectively by anode for the current production using the simultaneous effect of pure culture and mixed inoculum by a binary electron transfer method. The pure culture of *Shewanella* secretes redox shuttles like flavins, which transfer electrons from bacteria to electron acceptors [138]. This helps improve the electron transfer in the electrode by the synergistic interaction of the two cultures. Through adding different substrates or alternate electron receivers to support the growth of particular bacteria, organic growth could be accomplished by increasing efficiency by introducing pure bacterial monoculture.

Regulating anodic half-cell potential: Anode potential is controlled by microbial diversity in the anodic chamber. Anode potential is correlated with electrogen biofilm, which affects microbial diversity, and the net current generation [139]. Lower anodic half-cell potential and higher cathode half-cell potential are usually crucial to yield a higher voltage in MFCs. The applied potential of + 0.2 V (vs.

Ag/ AgCl) may be ideal for the growth of *Geobacter sulfurreducens* on anode, resulting in higher coulombic yields. Thus, the application of external voltage may be a substitute way of enriching the electrogens and reducing the start-up time of the MFC [140].

5.2. EET at Cathode

Electrons and protons generated during anodic oxidation is utilized at the cathode side for CO₂ reduction directly or indirectly. The direct electromethanogenesis in the biocathode is carried out through redox external membrane proteins in the form of cytochromes that are in contact with the cathode [141]. In addition to cytochrome, different outer membrane proteins like the ferredoxin, rubredoxin, hydrogenase and/or formate dehydrogenase are involved in electron transfer [106]. The conductive pili (nanowires) also take part in EET of electromethanogenesis like it did in bioanode. The indirect electromethanogenesis can also be performed in three sources - i. the electrochemically or bioelectrochemically produced hydrogen; ii. formate or iii. acetate [141]. The soluble external electron shuttler or mediators in the form of riboflavins, quinones and phenazines secreted by microbes also facilitate electron transfer, consequently indirect electromethanogenesis [142].

Exoelectrogenic species like *Methanosarcina* and *Methanosaeta* which reduces CO₂ to CH₄ can transfer electrons directly from elemental iron [143]. A greater portion of methane is developed through the carbon dioxide reduction pathway similar to that shown in (Fig. 3) [141]. The hydrogenotrophic methanogenic archaea are found to grow on both electrodes (anode and cathode) but are mainly found at the cathode. Hydrogenotrophic methanogens such as *Methanobacterium*, *Methanospirillum*, *Methanobrevibacter*, *Methanosarcina*, *Methanoculleus* and *Methanocorpusculum* have been reported to grow on the anode suggesting the formation possibility of methane at anodes [144-149]. While on the other hand, methane can be synthesized by *Methanosarcina* through hydrogenotrophic and acetoclastic pathway. *Methanobrevibacter*, *Methanoregula* and *Methanospirillum* have been observed growing on cathode [147, 150, 151]. Species such as *Methanobacterium*

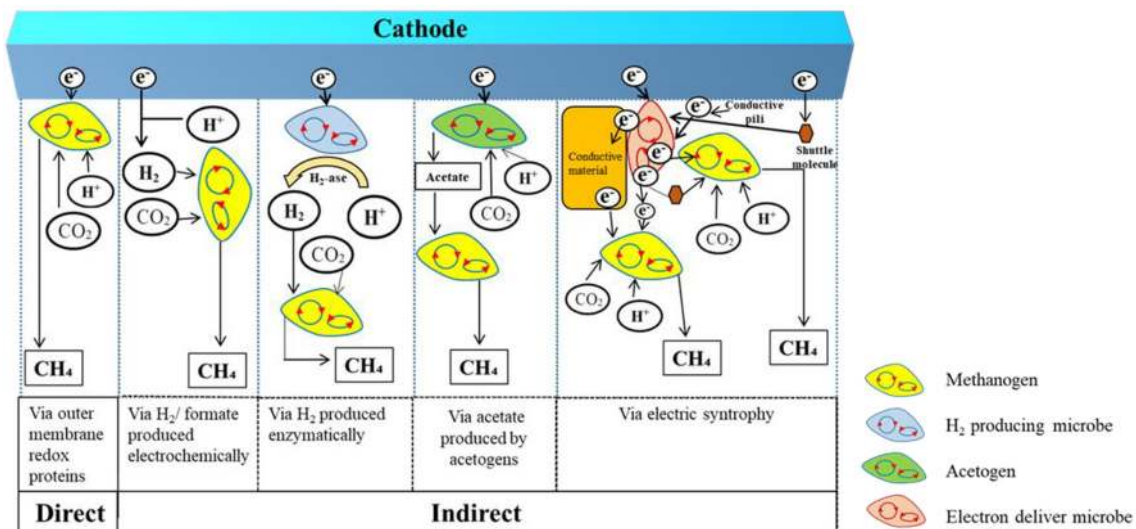


Fig. 3. A schematic diagram on microbial electron transfer scheme for production of methane at cathode.

and *Methanosaeta* uses electrons to produce methane directly through the reduction of CO_2 [152, 153]. Excessive methane production rate is reported when diverse phylotypes of methanogens come together to a definite extent and get associated with the electrode. Syntrophic relationships of the electromethanogens play a vital role in CH_4 production by developing on the surface of cathode [154]. For example, methanogens like *Methanocorpusculum* are inefficient to arrest the electrons of a cathode and are dependent on the interspecies electron transport carried by EAB such as *Acetobacterium* or *Geobacter* [42]. The electrical syntrophy of various microbial species for producing methane depends on the type of electrode material and microbes present in culture [155]. Understanding the principle of electron transfer between EAB and an electrode is necessary to optimize the methane generation in MECs. Till date, the exact mechanism for electron transfer has not been completely proved. However, there are three known pathways which explain the generation of CH_4 by MECs. The first mechanism for EET is based on direct electron transfer from bacteria to the solid electrode via outer membrane proteins such as cytochrome [156]. Another mechanism takes place in presence of soluble electron shuttle (compounds like phenazines, quinones, melanin and flavins) that carries electrons amid bacteria and the electrode through diffusive transport [157-159]. While the third mechanism states a solid component which is part of the extracellular biofilm matrix (termed as nanowires) and is conductive for electron transfer from the bacteria to the electrode as electron acceptor [160, 161].

6. Microbial Pathways Used for Methane Production

Any product synthesized inside an organism is a result of a biochemical reaction or a pathway. This may involve many metabolic steps involving breaking, modifying or building up of biochemical structures and compounds. Some end product of a metabolic process can be a reactant for another pathway. Here we elaborate the dedicated pathways for methane anabolism i.e. methanogenesis. There are three metabolic routes of methane production: acetoclastic, methylotrophic and hydrogenotrophic/ CO_2 reduction pathway (Fig. 4). Among them, carbon dioxide reduction pathway is believed to be the major driver of the methane biosynthesis. However, other pathways gain importance when working with mixed cultures [141].

Almost, all methanogens used in methane-producing bio-electrochemical systems employ the CO_2 reduction pathway except *M. barkeri*, which can use all 3 pathways for instance. Whereas species like *M. thermophila* and *M. hollandica* are capable of using acetoclastic and methylotrophic pathways only, respectively [162].

CO_2 reduction pathway is around 4 times more widely observed than both methylotrophic and acetoclastic pathways combined. This is because CO_2 and hydrogen are readily available during hydrogenotrophic methanogenesis. CO_2 gets reduced and activated to formylmethanofuran where reduced ferredoxin (Fd_{red}) is the electron donor. The second step involves the transfer of the formyl group to tetrahydromethanopterin (H_4MTP). Dehydration reaction

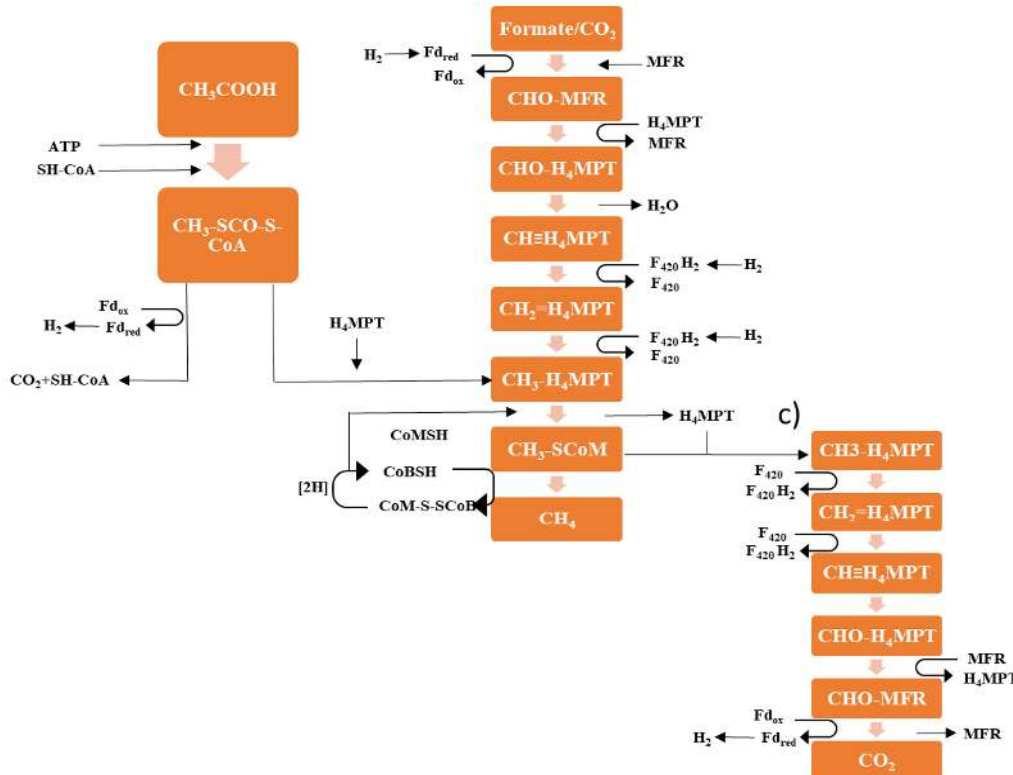


Fig. 4. Three metabolic routes implemented by microbes for electromethanogenesis. (a) Acetoclastic, (b) Hydrogenotrophic and (c) Methylotrophic methanogenesis.

produces methylene- H_4MTP subsequently reduced to methyl- H_4MTP with reduced F_{420} as an electron donor. After the transfer of the methyl group to coenzyme M (HS-CoM), finally, methyl-CoM is reduced to CH_4 with coenzyme B (HS-CoB) as the electron donor. The resulting CoM-S-S-CoB is reduced with H_2 to recycle the coenzymes. It should be noted that some methanogens can use formate instead of hydrogen as an electron source for carbon dioxide reduction.

Methanotrix and *Methanosarcina* genera use acetate for methane synthesis. For acetoclastic methanogenesis to occur, acetate must be activated. This is achieved by ATP and coenzyme A by its transformation into acetyl-CoA which is further split by CODH/acetyl-CoA synthase complex. The CH_3 group is transferred to tetrahydrosarcinapterin (H_4SPT) and further converted into methane similar to the hydrogenotrophic methane production pathway.

The third route of biological methane production utilizes methyl containing substrates like methylamines, methanol, dimethyl sulphide or methanethiol. Many of methylotrophic microbes belong to *Methanosarcinales*. In the first step, the methyl-group from the substrate is transferred to a corrinoid protein by suitable methyl-transferases and subsequently to HS-CoM by another methyl-transferase, to form methyl-CoM. Simultaneously one methyl-CoM is oxidized to CO_2 via the reverse hydrogenotrophic pathway thereby generating enough hydrogen to reduce three methyl-CoM to CH_4 (with a by-product of proton motive force) [163].

7. Materials Used in Constructing MECs

The materials used to construct the MECs system are the key to determine the performance of the system. By analysing the type of materials one can know its economic value for applications in industry. While setting up a MEC system the total cost of the electrodes and membranes are also considered. Optimization of the materials used in a MEC is critical and needs to be considered earnestly for an efficient system.

7.1. Anode

The anode in a MECs system should have the features such as high electrical conductivity, chemical stability, anti-corrosiveness, good biocompatibility, low resistance, large surface area, strong mechanical strength, fouling resistance and scalability preferably with ease of construction and low cost [164]. These parameters are considered to be necessary for electrogenic bacteria to make effective use of anode to implement anaerobic respiration. The carbon-based anode materials are most commonly used due to its excellent biocompatibility, high electric conductivity, low overpotentials, stability and cheapness [165]. Various carbon-based anodes such as graphite fibre brush [166], graphite granules [167], graphite felt [168], carbon paper [169], carbon cloth [170], carbon fibre [171] and vitreous carbon [172] are used for the production of methane.

MFC often generates low operating voltage (V_{op}) in comparison to the electromotive force (E_{themo}) of the cell, often termed as thermodynamically predicted potentials which are irreversible in nature. Energy loss may occur because of several different ways such as

activation loss, bacterial metabolism loss, mass transfer loss, and ohmic losses caused because of various reasons but the most common reason is excess biofilm and the organic compounds produced by the inoculum may cause biofouling of the anode thereby reducing the electron transfer from the organism to the anodic material. One such strategy is utilization of nanomaterials in anodic modification [173]. The nanomaterials facilitate the formation of electroactive bacteria and thereby promote electron transfer. Various forms of nano-metal or oxide metals, such as manganese oxide, iron oxides and titanium oxide, are used for modifications to the anode surface to improve the holding capabilities of the inoculum and to boost the electricity transport rate of the electron. Iron oxide can promote EET through two mechanism - In the form of an electrical conduit within biofilm or interface by accumulating in the cell surface [174]. Previous studies indicated the enhanced expression of c-type cytochromes responsible for improved electricity generation due to the anode surface modification with iron oxide nanoparticles. Iron oxide nanoparticles promoted the biofilm of dissimilatory iron reducing microbes like iron oxide (Fe_2O_3). Iron oxides can be used at the anode interface to speed up the EET phase as a redox couple between Fe (II) and Fe (III). A study shows that conductive iron oxide can be used by encouraging EET in substrate degradation [175]. Titanium oxide (TiO_2) nanoparticles, due to their features like stability, abundance and low cost are used to dope carbon nanotubes. These have resulted in double the electricity generation. Au-NPs, the multi-layered gold nanoparticles, are the electron receivers providing better biofilm formation and enhanced current output with reduced electron transfer resistance [176].

Another strategy to reduce electrode overpotential in MES is to utilize conductive carbon materials like carbon nanotubes (CNT), graphene etc. Carbon nanotube has been proved to be a better alternative for anode material because of its good conductivity, biocompatibility, large surface area, and increased catalytic ability. CNTs possess some properties which are found to be strongly beneficial to improve the performance of MFCs, such as large surface area (usually up to $1,300\text{ m}^2\text{ g}^{-1}$) and improve catalytic properties. Furthermore, spaces between the CNTs provide more space for microbes to grow [177]. Presently, use of graphene as anode material enormously attracted the researchers worldwide. Its honeycomb like network contains higher mechanical strength, excellent electron conductivity, increased surface area for biofilm adhesion and stable chemical ability. Its long range of π -conjugation plate shaped crumpled structure provides wide range of applications for electricity production and its storage [178]. Carbon nanotubes are known for its prominent electrical and structural properties like extensive surface area, molecule-like size, and easy functionalization with various groups which simplifies the reactions [179]. They are notably known for its hardness (comparable to diamond) and lightweight property. But carbon-based materials have a disadvantage as its intrinsic Ohmic resistance is a little high, which can cause Ohmic energy losses at a large scale. The durability of the carbon-based anode can also be a matter of concern [180]. Therefore, to further improve its performance research has been conducted to modify it. Feng *et al.* [181] demonstrated a modification of graphite fibre fabric with multiwalled carbon nanotube to act as a support for nickel deposition which increased the methane production rate

by 52%. Transition metals like alloys of Ti/Ru [112] and Ti/RuO₂ [182] and Fe [26] were also used for methane production which also leads to decrease in operating cost of MECs. Nowadays, application of composite containing metal oxide and conductive carbon (CNT/graphene) is applied as electrode materials. For the details, readers are referred to elsewhere [183].

7.2. Cathode

Cathode materials play pivotal role in electromethanogenesis as well as electrohydrogenesis. The electromethanogenesis is need less energy input compared to electrohydrogenesis (-0.23 V vs SHE to -0.41 V vs SHE). However, extra energy input is always required to reduce cathodic overpotential. Henceforth, cathode material properties, its surface area, catalytic activity, biocompatibility play significant part in terms of MEC performance output [184]. Rozendal et al. [185] reported that the cathode accounts for 47% of the total capital expenditure for developing MECs. The overall performance of the MECs depends upon the electrodes and the materials from which they are developed. With the help of direct electron transfer or hydrogen evolution, production of methane takes place by CO₂ reduction carried on both electrodes: anode and cathode [186]. Nonspontaneous reactions usually take place at the cathode region which requires specific material to catalyse reduction reaction. Expensive metals such as platinum proved its catalytic property by speeding up the reduction reaction. It is a precious transition metal having good stability. But there are many disadvantages including adverse environmental impacts and high cost [165]. Materials such as stainless steel alloys and nickel are identified

as an effective alternative due to ease of availability, low cost, stability in alkaline solutions and low overpotentials. Stainless steel has been employed in developing anaerobic reactors combined with single-chamber MECs to enhance the production of methane [187]. Along with stainless steel, electrode materials developed based on alloys such as iron-graphite [26] and Ti/Ru [112] have evolved to amplify CH₄ production in the presence of sewage sludge substrate. Carbon-based cathode consisting of reticular vitreous carbon [188] and graphite [187] can also be especially beneficial for methane production. Siegert et al. [189] compared the methane production amongst precious metals (platinum, stainless steel and nickel) and nonprecious carbon-based materials (carbon brush, carbon black and plain graphite). He found that the plain graphite cathode produced higher amount of methane compared to a precious metal-based cathode. The highest yield of methane and cathode capture efficiency was observed in cathodic materials which constitute of graphite felts and carbon stick [189]. Multiwalled carbon nanotubes doped with catalytic materials such as iron phthalocyanine, ammonia, platinum, manganese oxide and nickel have also been used as alternatives of cathodic material to enhance the rate of methanogenesis [190]. Zhen et al. [191] demonstrate that plain carbon stick with a layer of graphite felt is effective in increasing methane production rate, the felt on carbon stick eventually reduced overpotential and act as 'artificial pili' for electron transfer between methanogen and cathode. Another study by Liu et al. [192] suggested granular activated carbon (GAC) as promising biocathode for promoting methanogenic biofilm which was responsible for improved methane production at cathode potential of -0.52 V vs. Ag/AgCl

Table 2. Types of Membrane Used in Two-Chambered MECs

Cathode	Anode	Membrane	Voltage	Methane Yield	References
Graphite granules	Graphite granules	Nafion 117 proton exchange membrane	-0.2 V	47.7 ± 4.8 milli equivalent/d (meq/d)	[232]
Carbon stick	Carbon sticks	CSO monovalent-cation-selective exchange membrane	-0.9 V	2.30 ± 0.34 mL	[150]
Carbon stick	Platinum	Nafion 117 proton exchange membrane	-1.4 V	80.9 mL/L	[191]
Carbon felt	Carbon felt	Proton exchange membrane	-0.8 V	62.8 mL	[210]
Granular graphite	Carbon felt	Ultrex CMI-7000 cation exchange membrane	-800 mV	0.45 ± 0.06 m ³ m ⁻³ d ⁻¹	[226]
Graphite carbon mesh	Graphite carbon mesh	Non-woven fabric	0.3 V	17.0 ± 1.6 L/d	[233]
Graphite granules	Graphite granules	Tubular anion exchange membrane	0.2 V	300 meq/d	[234]
Nickel steel	Reticulated vitreous carbon	Nafion 117 proton exchange membrane	2.0 V	-	[197]
Porous graphite felt	Porous graphite felt	CMI-7000 cation exchange membrane	0.2 V	0.113 mol/mol	[235]
Carbon doped with metals	Carbon fibre brush electrodes with titanium wire cores	Nafion membrane	-600 mV	247 ± 87 nmol cm ⁻³ d ⁻¹	[236]
Graphite granules	Graphite granules	Nafion 117 proton exchange membrane	+500 mV	6.4 meq L ⁻¹ d ⁻¹	[209]
Carbon felt	Carbon felt	Proton exchange membrane	0.8 V	62.8 mL	[210]
Graphite blocks	Carbon fibre brushes	Nafion membrane	-600 mV	250 ± 30 nmol cm ⁻³ d ⁻¹	[151]
Graphite felt	Graphite felt	Cation exchange membrane	-0.7 V	5.1 L/m ² projected cathode per day	[237]

(-0.32 Vs. SHE). Guo *et al.* [193], demonstrated that 3D iron oxide nanoparticles impregnated stainless-steel cathode promoted robust biofilm provided due to high surface area and improved biocompatibility, consequently methane production increased significantly. Guo *et al.* [194] showed that at lower applied potential (till 0.5 V), there is negligible increase in methane production rate with increase in cathode surface area to anode spatial volume (1, 2.5 and 4 cm²/cm³), nevertheless, production rate increased with higher applied voltage (0.7-0.9 V). De Vrieze *et al.* [195] showed that retention of methanogenic biofilm on the cathode played pivotal role in increasing methane production rate via electromethanogenesis; their study also indicated the little or null effect of applied potential on the methane production rate. Park *et al.* [196] found a sharp drop of around 51% in methane generation with increasing the electrode spacing between the anode and cathode electrodes from 1 cm to 5 cm at a fixed applied voltage of 0.3 V. Nonetheless, all these studies emphasized that high cathode properties are critical to methane production via electromethanogenesis.

7.3. Membrane or Separator

Membranes are the component of MECs which is used to physically divide the cathode and anode into chambers. This separator is very important to design any two-chambered MECs. Membranes prevent the mass diffusion of substrate, methane gas, hydrogen gas and microorganisms between the anodic and cathodic chambers. They only allow the conveyance of protons between electrodes. They also act as a separator to avoid short circuit in the MECs. Numerous types of membranes are recruited in MECs such as the commonly used proton exchange membrane called Nafion [197]. Anion exchange membranes for example AMI-7001 [147], CSO monovalent-cation-selective exchange membranes [150], Ultrex CMI-17000 [198] and Ultrex CMI-7000 [199] have been tested so far in the MEC reactors. It is crucial to consider the changes in pH gradient across the membrane due to the exchange of cations rather than the exchange of protons which lead to lower pH at the anode and higher pH at the cathode. This pH change can negatively affect the bacterial activity in both, the anodic and cathodic chamber [200].

8. Nanocatalysts for Methane Production

Nanoparticles have a very high surface area to volume ratio. This increases the surface space available for both catalytic activity and microbial interaction [201]. As it is reported that the size of the external cellular components and the pili are in the range of nanometres, nanoparticle modified electrodes can improve electron transfer and enhance chemical production rate [161]. Many properties like surface conductivity, affinity, porosity and roughness of the electrode materials can be altered with the addition of functional groups to the nanoparticles for improving cellular adherence [193, 202]. Similar to every other field where nanotechnology has helped to improve results in processes and applications it has been instrumental in bioelectrochemical systems for energy production [203].

Kim *et al.* [204] synthesized nickel-granular activated carbon

(Ni-GAC) nanocomposite using solution plasma and microwave-assisted methods and studied their effects on methane production in a single-chamber MEC. Along with the media, they were suspended freely in the solution. Highest methane yield (20.7 mL) was obtained by Ni-GAC prepared using plasma method followed by microwave method (19.6 mL) whereas bare GAC MECs and control produced only 15.6 and 9.6 mL, respectively. Bacterial adhesion and excellent adsorptivity is attributed to increased CH₄ production. Furthermore, they reported that CH₄ can be produced even in the absence of external power supply possibly through direct interspecies electron transfer (DIET) and other non-electrode reactions like the acetoclastic pathway [204].

Recently, magnetite (Fe₃O₄) nanoparticles (NPs) were recognized to be promoting DIET in mixed culture anaerobic digestion systems. A single chamber MES was constructed with suspended synthetic medium and magnetite nanoparticles to enhance methanogenesis. With this around 215 mL of methane volume was obtained whereas non-nanoparticulate systems gave relatively poorer yields. The addition of the NPs to anaerobic digestion also improved the CH₄ production by almost 26%. The reason was discussed to be the promotion of the charge transfer process amid electrogens by magnetite NPs as an electrical conduit [205].

9. MEC Reactor Configuration

The configuration of the chamber(s) in MECs plays a vital character in the selection of the process to produce methane. The MEC reactor design directly influences the methane production, the energy efficiency of system and capital costs. Different reactor configurations have been propounded which consists of single-chamber and double-chamber configurations. Various chamber configurations depend upon the presence or absence of ionic exchange membranes in the system, which usually tends to divide the system into single or two chambers.

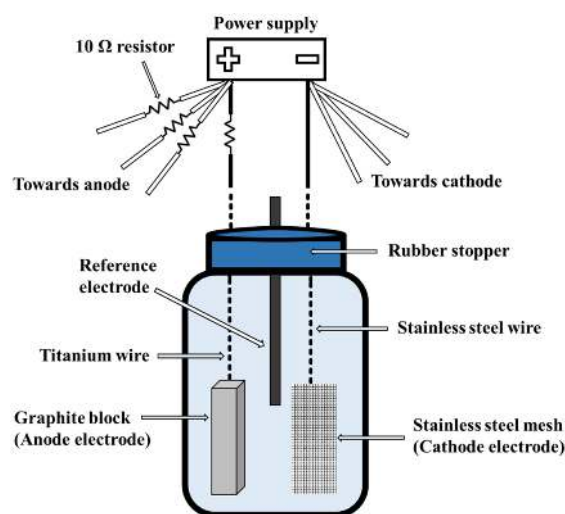


Fig. 5. A small simple single chamber membraneless MEC with a provision of parallel operation where multiple anodes and cathodes can be connected to a single power source.

9.1. Single-Chamber Configuration

Single-chambered MECs are developed by detaching membranes from the double-chambered systems. The anode and the cathode, therefore, remain in the same solution of the single-chamber which helps to minimize both the Ohmic voltage loss and pH gradient. This reduces the potential loss caused due to the membrane resistance. It also decreases the high capital cost and simplifies the design of the reactor. Due to this advantage, fabrication and sterilization become easy. Single chamber reactor is easy to fabricate and maintain because it does not involve membrane related complications like fouling and resistance [24]. In addition to that, the single-chamber MEC configuration encourages the multiplication of hydrogenotrophic methanogens which produces methane by consumption of hydrogen. Single chamber reactors have been reported to be constructed from materials such as stainless steel, acrylic and polypropylene. Many such designs of MECs having single-chamber configuration have been employed to produce methane. One such design includes single chamber MEC having a brush anode and flat carbon cathode. Liu et al. [206] designed a novel microbial electrolysis anaerobic digestion reactor to produce methane from waste activated sludge. It was a single chamber MEC made up of polycarbonate having a total volume of 130 mL. The chamber consisted of an anode made from graphite brush (80 mm length, 40 mm diameter; 1.01 m² surface area) and cathode developed from carbon cloth of 40 mm diameter. The cathode was covered with platinum catalyst layer. Methane production under this single chamber MEC configuration at an applied voltage of 0.8 V reached 91.8 g/m³ reactor/d after 12 days of continuous batch operation. A simple small scale MEC system was developed by Siegert and his group [189]. They developed mini MECs using clear glass serum bottles having a total volume of 5 mL. Both cathode and anode electrodes were made up of graphite plates (1.5 cm long, 1 cm wide and 0.32 cm thick, 1.5 cm² projected surface area). A voltage of 0.7 V was applied to the reactor which was operated in fed-batch resulting in methane production of 0.27 mL/mL cm². Another design of single-chamber configuration comprises of tubular shaped glass reactor and non-precious metal cathode. One such reactor was developed by Hou et al. [198] made of plexiglass having a total volume of 577 mL (height of 15 cm and an inner diameter of 7 cm). The anode was made up of carbon cloth while the cathode was developed from cost-effective metal material called nickel foam. The size of the anode and cathode electrode was accounted of 30 cm × 10 cm, while the membrane size was 30 cm × 10.4 cm. An optimal applied voltage of 0.95 V was applied to the reactor increasing methane production rate to 0.17 m³/m³ d. Still another type of single-chamber configuration was proposed by Guo et al. [112] who investigated the bio-electrochemical enhancement of methane production from anaerobic digestion of sewage sludge. The single-chamber membrane-free MEC reactor was manufactured using plexiglass having a total volume of 300 mL. The anode and cathode both were made up of Ti/Ru alloy mesh plates having a distance of 2 cm between them. Methane productivity at applied voltages of 1.4 and 1.8 V was enhanced by 11.4-13.6 fold. A vertical electrode configuration employed by Katuri et al. [207] consisted of a tubular-shaped plexiglass MECs reactor of 23 cm length and 4.5 cm internal diameter

(working volume of 350 mL). The cathode was constructed of a nickel-based hollow-fibre membrane which was placed vertically at top of reactor and anode made up of graphite fibre brush which was positioned at the bottom of the reactor vertically. At an applied voltage of 0.7 V, the reactor achieved methane production of around 0.028 m³/m³/d. While Guo and team [194] configured horizontal electrode configuration of a single-chamber MECs reactor having a reactor volume of 700 mL made up of glass. The anode was made up of graphite fibre brushes (volume of 78.5 cm³). Using the different layers of circular stainless-steel mesh, stacked cathodes (diameter of 5 cm and 40 mesh) was constructed and installed in series by titanium wires having 5 mm interspace. The high surface area was fabricated, and the ratio of cathode surface area/anode volume was efficient in improving the methane production when the ratio was higher than 2.5. Concentric electrodes placed in a tubular configuration has also been an alternative MEC setup for the improvement of methane production through anaerobic digestion. Feng et al. [26] used a pair of Fe tube electrode as anode and graphite pillar electrode as the cathode. The graphite pillar cathode electrode was located inside the axes of anode electrode Fe tube, which were connected to direct current (DC) power source by an electric wire. This configuration increased methane production by 22.4% at an applied voltage of 0.3 V.

9.2. Two-Chamber Configuration

Two-chamber configuration is the long-established type of MECs in which the cathode and anode work individually in separate chambers which are usually divided by a membrane. In this configuration, cathode and anode are coupled by the external circuit, to which electrical energy is supplied using a power source. Various membranes have been used in two-chamber configuration MECs, in which the most common membrane used is a proton exchange membrane constructed using functional groups which only allows free protons (H⁺) to pass from the membrane [73]. Other membranes used in two-chamber MECs include anion-exchange membranes like AMI-7001, charge-mosaic membranes and bipolar membranes [208].

A two-chamber H-shaped reactor configuration was constructed by Cheng et al. [147] which was made up of two glass bottles of 300 mL each. These glass bottles were separated by the anion exchange membrane called AMI-7001 (diameter 2.9 cm). The cathode and anode electrodes were made of carbon cloth (14 cm²) and graphite brush (diameter of 5 and 7 cm long) sequentially. Methane production was observed up to 656 mmol/m²/d at an applied voltage of -0.7 V to -1.2 V. A new type of two-chamber MEC reactor was designed by Villano et al. [209] to generate methane from acetate using *Geobacter sulfurreducens* as a microbial bioanode coupled to methanogenic microbial biocathode. The reactor setup consisted of two plexiglass frames (internal dimensions of 17 cm × 17 cm × 3 cm) and a proton exchange membrane Nafion 117 was placed in between the two frames. In this reactor configuration, the methane production rate was observed to be 0.018 L/L/d when the anode electrode was poised at +0.5 V. Zhen et al. [191] developed a two-chamber MEC (200 mL volume) having an anode made of platinum (23 cm length). The tubular-shaped hybridized cathode was used in the MECs which comprises of plain carbon stick having

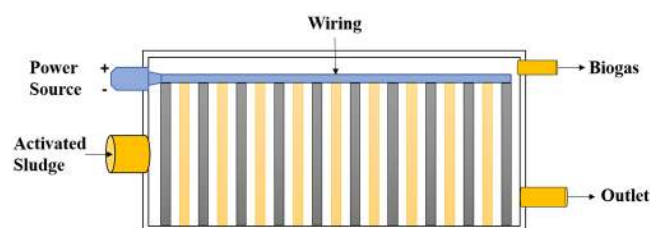


Fig. 6. 10 anodes and 9 cathodes stacked in a 33 L MEC tank as used by [154]. Each electrode compartment can be modified to contain a polyethersulfone or Nafion 117 membrane for a dual chambered configuration. Orange = Cathode, Grey = Anode.

a working surface area of 11 cm². To this carbon stick, a thin layer of graphite felt was coated in a tube shape to encourage the growth of methanogens. This led to the formation of concentric biocathode two-chamber MEC configuration. Nafion 117 (working surface area of 4 cm²) was used as a separator between the cathode and anode electrode. At an applied potential of -1.4 V, 80.9 mL/L methane was produced and Coulombic efficiency of 194.4% was obtained after 24 hours of the incubation period. When compared to the single-chamber reactor, two-chamber MEC configuration is more complex. Nonetheless, drawbacks include high prices of the membrane, membrane resistance and fouling and pH gradient caused due to the presence of the membrane.

10. Challenges and Factors Affecting MECs Performance

10.1. Applied Potential

The applied voltage or external voltage is one of the requisite physical parameters for the performance of the MECs to manufacture methane. Adjustment in the applied voltage has a significant influence on the growth and distribution of EAB and further impacts methane generation [167, 210]. It is important to consider that the high values of applied electric potential could have adverse effects on the microorganism. Wang *et al.* reported a decrease in cell metabolism and its rupture in presence of high potentials [211]. This study proposed the need to investigate applied potentials to optimize the modifications happening in microbial activity and community. In various researches, Gram-positive bacteria were found to be mostly used as EAB in MECs combined with anaerobic digestion process for the production of methane. Due to their characteristic thick peptidoglycan cell wall having three-dimensional structure provides significant resistance to external disturbances [212]. Thus there is a need to consider optimal external voltage which should be specific for various substrates to achieve high rate production of methane. The method of voltage supply and proper consumption is needed to determine the cost of the process. Mostly in a lab-scale reactor, the direct current power supply is used, but they fail in scaling up of the methane production process [213]. Proper control of the voltage and real-time monitoring is essential for the industrialization of the process. Thus the optimal applied voltage in MECs is influenced by various factors like the

type of substrate, cell configuration, electrode material and microorganisms. This dependence on different factors reveals the need to carry out voltage optimization for every MECs or BESs for better energy efficiency of the system.

10.2. Temperature

Temperature has a significant effect on the performance of MECs. The varying temperature in the course of MEC operation brings about the changes in microbial activity and community [214]. This changes subsequently affects the performance of the reactor. Most of the electromethanogenesis studies have been carried under two ranges of temperature, one is in ambient temperature (22-25°C) while others are in mesophilic conditions (30-35°C) [209, 215]. The temperature change affects the growth and mass transfer rate of EAB [216]. Proper understanding of the temperature parameter role is still not known completely, so there is less information by which we can know the exact effect of temperature in cell activity and microbial community. Understanding the accurate role of temperature will help in achieving high yield of methane.

10.3. pH

Along with applied potential and temperature, pH too affects the production of methane in MECs. Most of the methane-producing MECs operate at pH 7 due to the neutral behaviour of microorganisms [170]. This is because the EAB is sensitive to its surrounding pH, even the slight fluctuation in the pH would cause changes in the metabolism of microbes [217]. Several other factors like ion transfer, substrate oxidation and solution conductivity too are associated with the change in pH directly or indirectly. Anaerobic digestion when coupled with MECs overcomes the acidification process offered by anaerobic digestion and also allows the treatment of substrates at high organic load [42].

10.4. Substrate

EAB oxidizes the substrate and transfers the electrons to the cathode through the anode to produce methane. Choosing the appropriate substrate is necessary to determine the final yield of methane. Organic concentration and feeding rate of the substrate are the two parameters which have a crucial role in MECs for high CH₄ yield. Any organic substrate ranging from simple carbohydrates to complex fermentable substrates like wastewater and biomass can be employed in MECs [208]. The most commonly used substrate in MECs is acetate, having higher Coulombic efficiency of 91% [218]. Various types of wastewaters such as industrial (dairy, soybean and beer), domestic and municipal have been evaluated for methane production [42]. Using such waste materials as a substrate for producing methane also helps in cleaning of environment proving the MECs process to produce CH₄ as environmentally friendly.

10.5. Current Density

Rousseau *et al.* [219] reviewed MECs in an electrochemical perspective. Applied current density is underutilized in the process design of MEC reactors which can largely contribute to the methane production. Stacked MECs can overcome the performance limited due to low cathode/anode ratio by increasing the current density

[194]. Another study showed that multiple electrode configurations can both increase current generation and wastewater treatment [220]. Call and Logan developed a parallel operating single chamber MEC system as shown in (Fig. 5) which overcame many internal resistances for better conductivity and completely produced methane [61]. Sugnaux et al. [197] employed a multiple electrode reactor in both membraneless and dual-chambered configuration (Fig. 6). They reported significantly higher methane content in the produced biogas and reduced chemical oxygen demand in the outlet sludge. Therefore, focusing on the current density instead of volumetric parameters can boost methane production.

10.6. Scale

Latest reviews on MECs as of today discuss wastewater treatment [221], hydrogen production, valuable chemical production [222], remediation [223], electrochemical aspects of the reactor [219] and other challenges [224]. Although, widespread research has been conducted in this field, 'scale-up' of MECs to produce methane is not extensively explored. One study which used a pilot-scale 50 L bioelectrochemical reactor for methane synthesis concluded that instead of one big reactor, joining multiple smaller reactors will avoid large internal resistances and improve efficiency [225]. Another medium-scale (32 L) study could produce a maximum of 4.4 L CH₄ per metre square per day by stacking 45 cells. While integrating anaerobic digestion and MECs can improve methane production rate many manifolds with the feed of CO₂ [209]. Recently, aspects related to scale-up of MECs such as electrode materials and their properties were discussed by Zakaria and Dhar [184].

11. Conclusions and Outlook

Due to the increased global warming around the world, the development and implementation of new green technologies for the production of methane must be environmentally friendly and sustainable. MECs have already emerged as a promising technology in the field of methane, hydrogen and wastewater treatment. Last two decades of research in the field of MECs have been noteworthy towards the practical application of MECs to produce methane. The ability to produce methane in single-stage having the capacity to work at room temperature using low-cost inputs makes it an excellent alternative for treating wastewater which can yield methane as a significant value-added product. The indirect method of methane production through highly efficient HER was elaborated. Excellent interactions like direct interspecies electron transfer by EAB have been shown in the presence of conductive carbon electrodes within the MECs reactor which further enhances the substrate digestion and yield of methane. We also discussed how the nanotechnological intervention as a catalyst has improved the methane yields and HER efficiency.

There is a need for better understanding of the electron release and transfer mechanisms between the EAB and the reactor components such as electrode and membrane. To minimize the losses caused due to the electron transfer, research and development of novel configurations in anode-cathode electrode and membrane architecture are needed to intensify the product yield. Using waste-

water as the substrate for the working of MECs not only helps in producing methane but also reduces environmental pollution. The future of methane-producing MECs looks promising. Few pilot-scale reactors have been functional since last decade but still a long way is left to develop MECs for commercial applications. Besides, challenges such as large capital costs are the main barrier for the commercialization of such value-added products.

There is a need for steps of improvement for the high performance of the biofuel production. Few complications which need further studies are design and evaluation of novel and nanomaterials to improve the catalytic efficiency. Also, research and validation will be required to stimulate the adhesion of the microbes which can increase quality and quantity of biomethane. Nanoparticles in this field are promising.

In spite of having such an advantage, MECs are not a full substitute to anaerobic digestion (AD) process largely due to its scale and far better advancement. However, MESs as a wastewater purification technology is beneficial. Therefore, MEC-AD combined MES is a more desirable technology which will be successful. Because of high cost of capital and long payback, small and medium scale AD are considered as economically unattractive. The electro-methanogenesis in MEC-AD hybrid has the possibility to deliver high-quality biogas by reducing CO₂ to gas CH₄ that can lower the cost of biogas improvement.

In this review, we have provided the evaluation of the current applications and the future perspectives of using the MECs for methane production. Several conclusions were drawn regarding the production mechanism of methane, nature of electrodes, usage of EAB and reactor configurations.

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Author Contributions

A.A.P. (Postgraduate student) wrote the manuscript. A.K. (Postgraduate student) collected, analysed related literatures and wrote the manuscript. S.P. (Assistant Professor) wrote, reviewed and edited the manuscript and conceived the study and led the project. S.L. (Professor) reviewed and edited the manuscript. S.P.J. (Associate Professor) reviewed and edited the manuscript.

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