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Recent trends of extremophiles application in Microbial Electrochemical Systems as Energy scavenger: A mini review

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Abstract. Application of electroactive extremophiles as a biocatalytic agent inside microbial electrochemical systems (MES) holds promises for worldwide practical implementation with improved/enhanced performance under extreme environmental conditions. The MES are microbe catalysed electrochemical platforms that can turn the chemical energy stored inside organic resources/wastewater into electrical energy or other valuable bioelectrofuels with/without an external electrical stimuli to achieve maximal oxidation of organics (by exoelectrogens) at anode and to extracellularly donating the released electrons to cathode to available electron acceptors like CO₂ (by electrotrophs) to further metabolize into bioelectrofuels /or chemical compounds. The functional versatility and diverse applicability of extremophiles is due to their ability to successfully utilize various organic sources even under extreme environmental conditions. Till date, numerous efforts have been made to unravel the hidden potential of microbes in MES operating at standard environmental conditions, but its operability at extreme conditions are often overlooked. The hidden potential of extremophilic microorganisms can be channelized in MES (which either extracted from natural extreme ecosystems or cultivated in fabricated extreme biosystems) to open unknown avenues by producing novel products. This review discusses the recent state of art of extremophilic microbes by exploring novel possibilities for its application as a catalyst in MES.

1. Introduction

Extremophilic microbes are known for its ability to withstand the hostile environmental conditions, which otherwise are intolerable to most life forms on Earth. Extremophiles encompasses three domains of life viz. archaea, bacteria, and eukarya are adaptable to extreme conditions (like high or low temperature, pH, salinity, radiation, or pressure) [1, 2]. Several sub-groups of extremophiles have been investigated for its multipurpose-electrochemical efficiencies in MES, which includes, acidophiles, alkaliphiles, psychrophiles, thermophiles, halophiles and oligotrophs [3]. Extremophile's applicability as a biocatalyst in MES has opened new opportunities to sustainably oxidize/or reduce wide range of substrates at electrode surface to yield novel bioproducts. The MES are electrochemical devices are driven by microbial catalysts to catalyse the electrochemical reactions on electrode surface by using range of organic wastes. The MES are microbe driven electrochemical devices capable to convert the chemical energy stored inside organic resources into electrical energy or other valuable bioelectrofuels (on electrode surface) with/without an external application of electricity achieved by maximal



oxidation of organics by exoelectrogens at anode, and to extracellularly donating the released electrons to cathode to be used by electroreductants to further facilitate them to reduce the organics as shown in Figure 1.

The functional versatility and diverse applicability of extremophiles is due to their ability to successfully utilize various organic sources even under extreme environmental conditions. Interestingly, direct recovery of electrons through microbial fuel cell (MFCs) or MES running on different carbon resources (like wastewaters, recalcitrant organic/or inorganic compounds, plant rhizodesites) and electron donors/or acceptors (O_2 , CO_2 , acetate /or biocarbonates, sulphates, nitrate) can be harnessed either for energy generation or energy storage application or for to produce valuable products [4]. However, operability of MES at highly saline or thermophilic conditions is still limited by the unsuitability and inefficiency of available extremophilic inoculants. Many researchers have tried to enrich extremophilic biofilm on bioanode of MFCs, operating at significantly high temperature of 55-98°C [5-7]. Also, the enrichment of extremophiles (halophile and thermophiles) in MES has been achieved under both thermophilic and hypersaline conditions [8]. Although, the fundamental and applied biological aspects of extremophile based MES has already been discussed extensively in previous review [3, 9]. Present review elaborates on recent state of art of the applicability of extremophile in bioelectrochemical technologies and how its exceptional electrocatalytic potential (based on its unique electron transfer mechanism) can be fully exploited for the bioenergy applications.

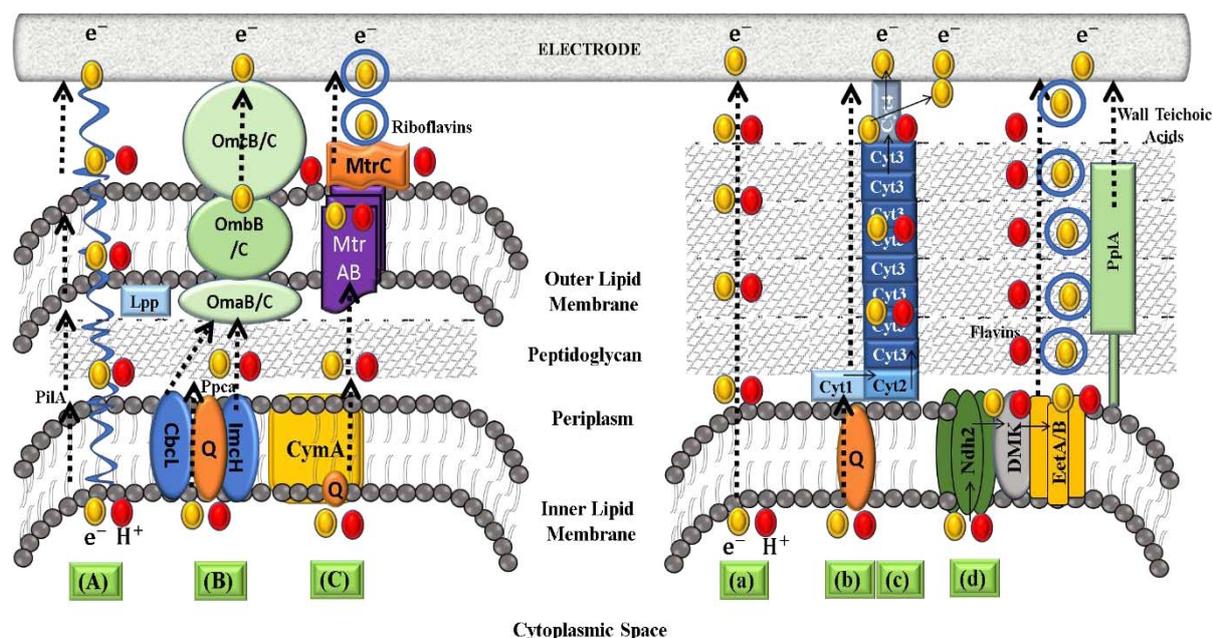


Figure 1. Schematic representation of mechanisms involved in electron transfer in MES. (A and a) Direct; (B and b; C and c) Indirect or mediator-based electron transfer; and (d) nanowire assisted transfer.

2. Extremophiles applied in microbial electrochemical applications

The MESs driven by extremophiles enable the bioelectrofuel synthesis under extreme environmental conditions either in terms of temperature (extreme high or low), pH or salinity, a feat impossible for normal standard mesophilic strains, Figure 2 [3]. Over past few years, several efforts have made to unravel the key mechanisms responsible for the extraordinary electrocatalytic capacity of extremophiles inside MES. Previously, recent advancements in extremophile based MES has been thoroughly discussed and have provided an insight into their survival mechanisms/ or specific defense strategies, which acts as its shield [3, 9]. Potential challenges implicated using the hot, cold or hypersaline electrolytes stresses the need to fill the knowledge gaps pertaining to extremo-electrocatalysis and

extracellular electron transfer mechanisms that are specifically unique to this group. Undoubtedly, MES behold boundless possibilities, which can impact the lives of the million lives, which however remained to be tapped due to our inability to fully understand/or uncover the enigmas of its unusual electrocatalytic activity under extreme conditions. Possible involvement of their unique cellular and morphological characteristics in promoting electro-catalytic activity is yet remained to be validated by conducting thorough research. Nevertheless, extremophiles present novel prospects of convert organic wastes with extreme properties into valued products, thereby offering scope of MES implementation in rural regional places/or locations with extreme environments [10].

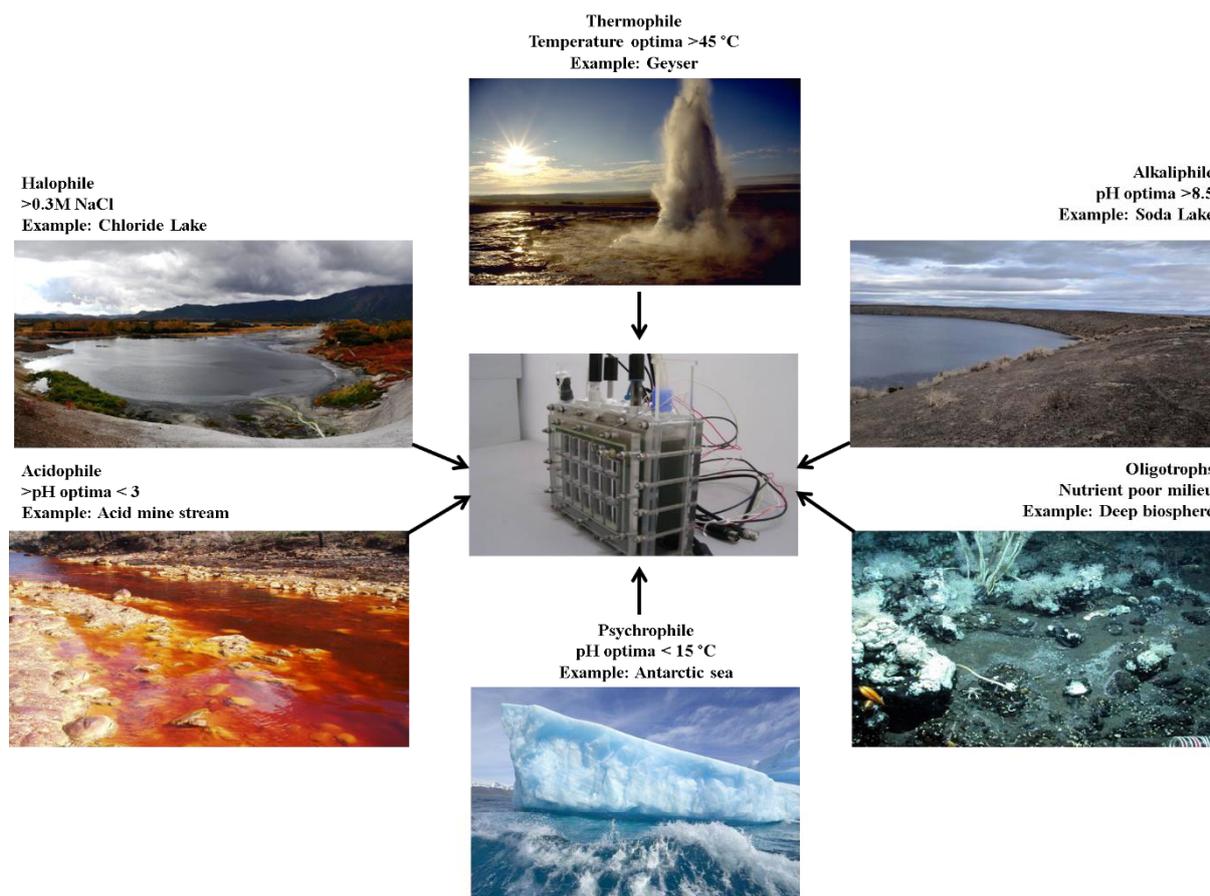


Figure 2. Different types of extremophilic microorganisms for bioelectrofuel application through MESH (adapted from [3]).

The kinetics of extremophilic electrocatalysis in MES are affected by the reactor configurations, electrode type, and mechanism of extracellular electron transport (EET) used by microbes to accept or donate the electrons to/from at biofilm-electrode interface. Understanding the EETs mechanism involved inside the extremophilic MES biosystem is yet not fully understood, which however, can be achieved by applying the electrochemical, microscopic and gene analysis techniques to pinpoint the responsible EET mechanisms such as direct electron transfer or mediated electron transfer, which allow them to withstand the harsh environmental conditions and can assist us in optimizing the power density and coulombic efficiency in MES [11]. Microbes with known EET mechanisms can help in developing wide range of unique applications such as desalination, electricity production, electrolysis, and synthesis of value-added products (Table 1).

Table 1. Performance of the different types of extremophilic microorganisms in microbial electrochemical system (MES).

Microbial System	Inoculum	Temp.	product	Production Yield	Electrode/potential	Remarks	Reference
Hybrid MES-AD system	Electro-active acetate oxidizing bacteria	10°C	CH ₄	31mgCH ₄ -COD/g VSS	Cathode potential of -0.90V (vs. Ag/AgCl),	Alternative strategy to enhance the performance of anaerobic digestion in cold areas	[12]
NFC	psychrophilic bacteria <i>Simplicispira psychrophila</i> LMG 5408(T); <i>Geobacter psychrophilus</i> P35(T)	15°C	Electricity	540mV	Anode potential of -0.5V, and cathode potential of +0.25V (vs. Ag/AgCl)	Start-up conditions of anodic biofilms could determine the cell performance	[13]
MEC (single chambered)	Psychrotolerant glucose-fermenting bacteria; <i>Geobacter</i> spp.	4°C	Hydrogen , electricity	6mol H ₂ mol ⁻¹ glucose	0.197V (vs. SHE)	Syntrophic interactions combining glucose-fermentation with oxidation of fermentation products by exoelectrogens leads to current generation	[14]
MFC	<i>Trichosporonaceae</i> , <i>Acidovorax</i> , <i>Acinetobacter</i> , <i>Blastocatella</i>	8°C	Electricity	207.31 ± 40.14 mV		Voltage generated will be higher under low temperature than under moderate temperatures.	[15]
MFC	Mixed culture (Geobacter and Pseudomonas)	5-10°C	Electricity	465.3 ± 5.8mW/m ²	Anode potential of -0.6V and cathode potential of +0.6V	Anodic biofilm with mixed culture MFC has lower overpotential and resistance than pure-culture one.	[16]

Microbial System	Inoculum	Temp.	product	Production Yield	Electrode/potential	Remarks	Reference
MFC-adsorption hybrid system	Mixed microbial consortium	15-20°C	Electricity	71.74 + 6.4Mv to 92.48 + 4.3mV		MFC-adsorption hybrid system has better pollutants removal efficiency than its standalone counterparts. Integration of adsorption with MFC helps in improving the wastewater quality	[17]
MES	<i>Moorella thermoacetica</i> , <i>Moorella thermoautotrophica</i>	60°C	Acetate	6.9±0.6mMm ⁻² d ⁻¹ to 11.6±0.9 mM m ⁻² d ⁻¹		MES operation at high temperatures Helps in minimizing the chemical recovery cost, thus making it an more economically feasible technology.	[18]
MFC	Mixed consotium	40°C	Electricity	22.9 ± 4.2A.m ⁻²	Anode potential of -0.24V and cathode potential of +0.24V (vs. SHE)	Bioanodes formed at high temperature possess three redox systems than one formed at low temperature. Also, lesser compactness of biofilm structure at high temperature contributes in enhanced MFC performance	[19]
MFC	Anaerobic electrochemically active bacteria	-60°C	Electricity	2550 ± 60mWm ⁻²		Change in temperature governs morphological and structural change of synthesised product i.e. a reaction at low temperature may result in different materials than at higher temperature due to change in thermodynamics and kinetics of nuclei formation.	[20]
MFC	<i>Arobacter</i> , <i>Pseudomonas</i> , <i>Geobacter</i>	5-10°C	Electricity	160mWm ⁻²		More stable operation at low temperatures is reported with minimal performance drop compared to higher one.	[21]

Microbial System	Inoculum	Temp.	product	Production Yield	Electrode/potential	Remarks	Reference
MFC	<i>Bacillus Licheniformis</i> , <i>Staphylococcus Hominis</i> , <i>Bacillus Sonorensis</i> , <i>Virgibacillus Salaris</i> , <i>Staphylococcus hominis</i>	1000m M NaCl	Electricity	$207.05 \pm 5 \text{ mWm}^{-2}$		MFC's can be used to treat the saline starch water.	[22]
MFC	<i>G. sulfurreducens</i> , <i>Proteobacteria</i> , <i>Enterobacteriaceae</i> , <i>Rhodocyclaceae</i> , <i>Propionivibrio</i> , <i>Escherichia-Shigella</i> <i>Bacterioidetes</i>	1.5% NaCl	Electricity	1231.1 to 1050.2 $\mu\text{A cm}^{-2}$		Salt-lake with high salinity and salt-lake soils are promising source to enrich the exoelectrogens with high salt tolerance.	[23]
MFC		55°C	Electricity	1.0 Wm^{-2}		Simultaneous electricity generation and Wastewater treatment can be achieved using a thermophilic MFC.	[5]
MFC	<i>Calditerrivibrio nitroreducens</i>	55°C	Electricity	823 mWm^{-2}	-0.14- -0.17V	-	[24]
MFC	Halotolerant extremophile bacteria	>35g L ⁻¹ NaCl		36 mWm^{-2}		Efficiency of the Isolate obtained from a salt-lake enhances the performance of hypersaline MFC than the one obtained from normal lake.	[25]

Microbial System	Inoculum	Temp.	product	Production Yield	Electrode/potential	Remarks	Reference
MEC	<i>Ferroglobus placidus</i> , <i>Geoglobus ahangari</i>	80°C	Electricity	$0.68 \pm 0.11 \text{Am}^{-2}$ to $0.57 \pm 0.10 \text{Am}^{-2}$	0.7V	Direct mode of electron transfer is found in hyperthermophilic archaea through direct contact with the electrode surface.	[26]
MFC	<i>Salinivibrio</i> spp.	100g L ⁻¹ 1.71M NaCl	Hypersaline waste treatment	87±11% of NaCl removal efficiency		Using alginate capsules to entrap the microbial cells helps in enhancing the cell density and stability, thus improves the power output by eightfold with minimum need of maintenance.	[27]
MFC	<i>Pyrococcus furiosus</i>	90°C	Electricity	225mWm ⁻²		Electroactive microbial biofilm in MFC contribute in enhancing the electricity production via direct electron transfer, pathway by which hyperthermophile harness electricity under extreme temperatures.	[28]
MEC	<i>Thermicola ferriacetica</i>	60°C	Electricity	7–8Am ⁻²	-0.06V (vs. SHE)	Thermophilic bacterium can generate electricity via anodic respiration.	[29]
MEC	<i>Geoalkalibacter</i> spp.	1.7% NaCl	Electricity	~5 to 9Am ⁻²		Research suggests the versatility in halophilic microbial family with promising scope in MEC application.	[30]
MFC	<i>Firmicutes</i> spp.	55 °C	Electricity	437 mWm ⁻²		Novel thermophilic electroactive microbial strains are yet to be isolated / or cultured which can revolutionise the MFC based synthesis of valuable bioelectrofuels/or chemicals.	[31]

3. Halophiles or halotolerant microbes

Bioelectrofuel production using MES under high salt concentration is a great challenge as its productivity adversely get affected at high level of salt concentrations. For instance, decline in electricity production using MFCs fed with wastewater with high salt conditions was proposed cause an inhibition in the electroactive microbial growth on electrode surface, however addition of an osmotic pressure compensated solute like ectoine was used to enhance the microbial tolerance towards increased salt level to restore MECs performance [32]. So, using the salt tolerant halophilic microbes is a promising strategy to treat the saline wastewater (or halophilic wastewater) using MES frameworks. The halophilic wastewater constitutes about 5% of total wastewater worldwide, which requires advanced biocatalysts to biocatalyze its efficient biological treatment [33, 34]. Researches have been done to explore the microbial diversity of halophiles in hypersaline wastewater using high through put methods like 16S rDNA phylogenetic markers and reported various halophilic and pollutant-degrading bacteria (*Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, *Chlorobi*, *Planctomycetes*, *Spirochaetes*, *Synergistes*, *Chloroflexi*, *Thermotogae*, *Verrucomicrobia*) with diverse applications [35]. The electroactive nature of the halophilic microbe and its ability to work under extreme saline conditions makes them an ideal choice for the MES running on the saline or hypersaline effluents [7, 25, 36-39]. Halophilic microbial activity and its tolerance level varies depending on variation in its inhabiting place (natural or artificial habitats/environments. Efficiency of the halophiles in terms of high catalytic activity also varies, a property which can be a promising tool broadens the horizons of the field of catalysis and bioenergy. So, researchers have remained curious to utilize the MES to explore its electroactive potential to treat the saline or hypersaline wastewater and exploring various microbial strains for its potential to generate the bioenergy. Comparing the microbial diversity potential of microbes in saline wastewater vs. non- saline one, halophilic sludge found to possess similar diversity as the conventional sludge, only presence of halophiles helps the former to catalyze efficient degradation under harsh conditions. Such halotolerant extremophile microbes were reported to act as bio-electrocatalysts to treat hypersaline wastewater using MFCs (Pt-free) along with the potential recovery of the magnesium salts at cathode [34]. In addition of hypersaline wastewater treatment, these MFCs can be applied as a biosensing tool to monitor toxic shocks, BOD, harmful chemical detection, and bio-corrosion on metal surfaces in hypersaline conditions. Its applicability in extending the life span of MES seems promising in term of supporting long-term operation [40]. This cost-effective and self-powered sensor has the potential to replace classical analytical techniques like high-performance liquid chromatography and gas chromatography due to its ability to detect minute concentration change of any chemical/or pollutant and reflecting it in terms of its power production rate. Recently, hypersaline microbial MFCs (inoculated with halotolerant extremophilic microbes from Great Salt Lake) has been applied as a self-powered monitoring device for COD detection (sensitivity one order of magnitude higher than normal air breathing cathode MFCs) with minimal environmentally-toxic waste generation [41].

Moreover, direct electrical stimulation has been proposed to improve performance of MES, along with the mechanism behind it. Electrical stimulus helps the microbial communities to quickly adapt and grow under hypersaline environment, and robustness of MES under harsh conditions can be attributed to the selective halophiles with specific functions. Biological treatment of saline wastewaters using MFCs under extreme salinity, while producing electricity, would help to counter the economic and environmental challenges especially for oil and gas industry. An exoelectrogenic halophile, *Marinobacter hydrocarbonoclasticus* with extreme saline tolerance and petroleum hydrocarbon degradation capability was found to co-colonize the anode with *H. praevalens* in MFCs. Interestingly, some exoelectrogenic- halophiles (*Propionivibrio* and *Escherichia-Shigella* spp.) has the ability to outperform the typical model exoelectrogen, *G. sulfurreducens* (where former produced current density of 1050.2-1231.1 μAcm^{-2} , which is 61.7%, -89.6% higher than latter) [23]. Techniques like quorum sensing induction can be used to successfully promote the biofilm formation in MES under saline conditions [42]. Electroactive halophilic bacteria have been successfully applied to treat saline starch water (salt concentration ranging from 0.5 M-3 M) using MFCs for simultaneous removal of starch and

nitrate [22]. They investigated the effect of increased salinity/conductivity on yielding potential of MFC system and reported maximum starch degradation ($3.2 \text{ kg/m}^3\text{-d}$ at 3 M NaCl) and significant amount of electricity production (207.05 mW/m^2 at 1 M NaCl). Increased salinity/conductivity of electrolyte solution helps in reducing the internal resistance of the system as validated by [43]. In contrary, MES run on the salt concentration of about 1.8 M was found to support the power production, but for shorter time due to reduced activity of its anodic bacteria [44]. Additionally, using microbes to catalyze the electrochemical sulfide removal using the haloalkaliphilic microorganisms in MES can effectively address the issues of high energy consumption and cost-prohibitively associated with the conventional treatment processes, thus can effectively bioremediate the sulphide maximum (2.16 mM/day) under high pH (8.9 to 9.4) and coupled with simultaneous generation of energy (maximum 3625 mA/m^2) [45]. For instance, microbial consortia (mostly chemolithoautotrophs and haloalkaliphiles) such as *Thioalkalivibrio*, *Thioalkalimicrobium*, *Thioalkalivibrio* and *Desulfurivibrio* spp. are known to efficiently metabolize the sulfur compounds with utmost ease [46-48]. The halophiles from another microbial group i.e. halophilic archaea like *H. volcanii*, *N. magadii* and *E. coli* respectively, can also be used as a biocatalyst in MFCs to improve electron transport between electroactive-halophilic microbe and electrode along, which ultimately lead to higher power generation [36]. Recently, Alginate based encapsulation of hypersaline bacteria has been suggested for the effective entrapment of bacterial cells (to increase its cell density and stability) inside alginate capsules to achieve an eightfold surge in power efficiency and COD removal efficiency (90%) of the hypersaline liquids based-MFCs and ensures biosystem's long-term stability uninterruptedly during its operation for straight five months without maintenance [27]. Profound possibilities of extreme microbial physiologies of halophiles could be a key to increase the maximum current density and power production using MES along with reducing the associated energy cost compared to previously published studies. Also, halophilic electroactive microbes can facilitate biotechnological breakthroughs (like MES) to remediate effluents with extreme saline conditions [32].

4. Thermophiles and hyperthermophiles

Exploitation of the unique metabolic properties of thermophiles (microbes which can withstand high temperature) has provided a higher temperature alternative to mesophilic MES platforms by acting as a strategic solution to the problem of the industrial high temperature effluents with improved yielding performance in terms of biotechnological products. Introduction of new strategies of metabolic engineering has helped in leveraging the thermophiles metabolically or physiologically for improved bio-based fuels and chemicals [49]. Thermophiles are a specific class of extremophiles which can operate at high temperatures which otherwise unsuitable for normal /or mesophilic microbes. Living under high temperature conditions fashions alterations in thermodynamic equilibria of thermophiles, which is not available in mesophilic organisms. Thermophiles can be classified into thermophile-microorganisms which grow at temperature range of $50\text{-}80^\circ\text{C}$ -and hyperthermophile-microorganisms which grow at temperature range of $80\text{-}125^\circ\text{C}$ [50]. Thermophiles and hyperthermophiles are said to be the new tools for water resource management and waste heat management. Thermophiles possess exceptional metabolic ability of carbon fixation and chemolithotrophic pathways, which can be makes it an ideal choice to produce biotechnological products through MES. The MES driven by microbes to reduce carbon dioxide to multi-carbon compounds using electrical current as energy source) at elevated operating temperatures has found to improve the overall performance and product recovery, for instance, acetogenic thermophilic strains i.e. *Moorella thermoacetica* and *M. thermoautotrophica* and *Thermoanaerobacter kivui* were evaluated for their ability to reduce CO_2 and produce acetate using electrical current [18]. In this study, *M. thermoautotrophica* performed better than *M. thermoacetica* in terms of electron recovery (coulombic efficiency) and acetate productivity at wider range of temperatures The thermophilic sulfate-reducing archaeon, *Archaeoglobus fulgidus* have enzyme alkylsuccinate synthase which help it to degrade long-chain alkane and thermophilic *Candidatus Syntrophoarchaeum butanivorans* anaerobically oxidizes butane via alkyl-coenzyme M formation.

In comparison to the mesophilic systems, potential of the thermophiles based MFCs are yet to be fully explored [18]. Only few years of research led research to conclude that thermophilic MFCs are potentially more superior than mesophilic MFCs in terms of its performance, reaction activity, durability, and substrate range. Screening of more biocatalysts will help in improving the performance of thermophilic MFCs, for instance, a pure culture *Calditerrivibrio nitroreducens* (thermophilic, gram-negative, nitrate-reducing bacterium) has been isolated from two-chamber MFCs fed on thermophilic anaerobic digester sludge at 55 °C, and further examined its ability to yield power using another MFCs and generated power 823 mWm⁻² after 200 h of operation (without exogenous mediator) [24]. Simultaneous electricity generation has been achieved using a thermophilic MFCs to treat the distillery wastewater and associated dominated microbiome has found to belong to the uncharacterized *Bacteroidetes* (52%) followed by *Chlostridia* (20%), unclassified class (11%), *Nitrospira* (6%), *Betaproteobacteria* (4%), *Deltaproteobacteria* (3%) and *Synergistia* (20.5%) spp. [5]. Additionally, uncharacterized *Bacteroidetes thermophiles* were found to constitute up 52% portion of its anodic biofilm, whose proliferation was found to be related to high electricity generation and high coulombic efficiency. The search for mesobiotically operating microbes capable to catalyze the electrode reduction can prove to be an asset for fuel cells. Extremophiles serves as an effective catalyst (with higher activity and stability, longer life, ability to degrade wide range of bioresources) in MFCs. Moreover, thermophilic electrode-reducing bacteria were found to be capable to generate ten times more electricity using temperate aquatic sediment fuel cells at 60°C (209-254 mA/m²) in comparison to the one operating at 10°C (22 mA/m²) and reported microbes were found to be gram-positive such as *Thermincola carboxydophila* followed by *T. carboxydophila* or uncultured *Firmicutes* and *Deferribacteres* [51].

The hyperthermophiles are microbes which can flourish even in the extremely hot environmental conditions i.e. at temperature of 100°C or even more. The simple respiratory mechanism of hyperthermophiles makes them potential candidates for its application in MFCs to harness electricity at the temperature near boiling point. The 1st model hyperthermophile to have complete genome sequenced is *Pyrococcus furiosus* sequenced [52]. Recently, extracellular electron transfer mechanism for this hyperthermophilic archaeon has been investigated to find genetic basis for its ability to generate 2 Am⁻² of power (225mWm⁻² of power density) without any external mediators referring to the direct mode of extracellular electron transfer as preferred pathway, Figure 3 [28]. Current-generating potential of hyperthermophiles using MFCs has also been examined only by few researchers. Hyperthermophilic microorganisms were reported to be capable of transferring electrons to anode by acting as biocatalysts in two-chambered MFCs anodes (operating at 95°C) during bioelectrochemical analysis Performance of this hyperthermophilic two-chambered MFCs reportedly improvised on operation temperature intensification, in which maximum power was attained (165 mWm⁻²) at 95°C (which four times higher than power produced at 75°C) [53].

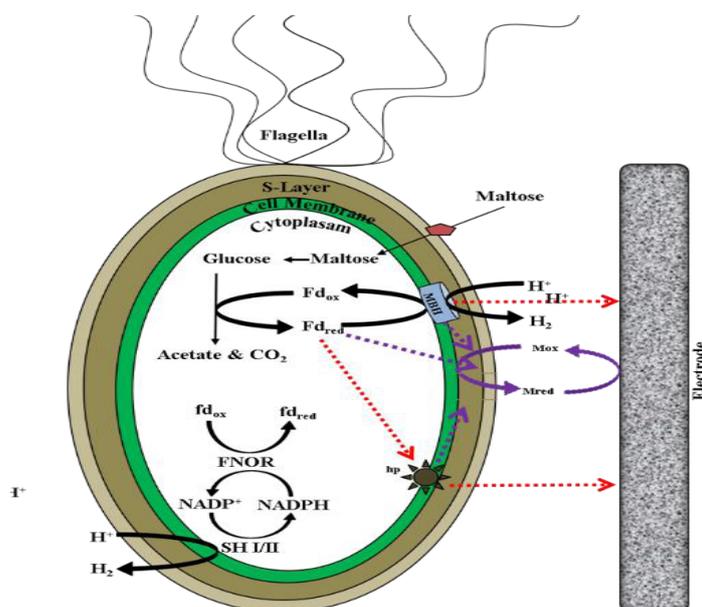


Figure 3. Hypothesized electron transfer pathways responsible for exoelectrogenicity in hyperthermophilic microbe, *Pyrococcus furiosus*. Solid black arrows indicate physiological biochemical pathways in wild type *P. furiosus*, hashed Red and Purple arrows indicate putative direct and indirect (mediated) extracellular electron transfer pathways respectively to electrode. Abbre. fd_{ox} & fd_{red}: oxidized and reduced ferredoxin; MBH: membrane bound hydrogenase; hp: hypothetical protein with ferric reductase activity; SHI/II: soluble hydrogenase I and II; FNOR: ferredoxin NADP peroxidoreductase; Mox & Mred: oxidized and reduced mediators. Figure adapted from [28].

Increase in operating temperature often increases the diffusion coefficient of substrates. further reducing the internal resistance, thereby improving MFCs performance. In this study, filamentous microbial cells reportedly found attached on anode surface, possessing limited phylogenetically diverse anodic-microbial consortium primarily consisting of hyperthermophilic bacteria like *Caldanaerobacter subterraneus* and *Thermodesulfobacterium commune* [54]. Application of this type of MFCs can be used to treat wastewaters such as chemical pulp manufacture, textile dyeing and oil/natural-gas production-based industries, arsenic containing hot-spring water and geothermal power plant wastewater effluents produced at extreme high temperatures (80-100°C), where even thriving for thermophiles become impossible. Recent study has confirmed electroactivity as one of chief metabolic pathway in environment like hydrothermal vents by being able to enrich hyperthermophilic electroactive archaeal consortium (mainly *Thermococcus* sp. and *Geoglobus* sp.) in MES (mimicking ex-situ conditions of hydrothermal vent) despite using bacterial inocula at 80°C (where these exoelectrogenic microorganisms were capable of external electron transfer to conductive support) [55]. The electricity generation by MES using the hyperthermophilic electro-active archaea, *Ferroglobus placidus* ($0.68 \pm 0.11 \text{ A/m}^2$) and *Geoglobus ahangari* ($0.57 \pm 0.10 \text{ A/m}^2$) has been achieved at 80°C of high temperature and almost similar potential of -0.39 V (vs. Ag/AgCl) [26]. Such exploitation of microbial hyperthermophilicity can further enhanced by manipulating microbial genes to produce novel industrial chemical (using hydrogen and carbon dioxide). Unconventional metabolic pathways, carbon fixation pathways and ability to metabolize diverse substrates, gives an edge to thermophiles due to its exceptional thermodynamics at higher temperatures. Although limitations which limits thermophiles performance in MES has been addressed by applying metabolic engineering tools to calculatingly structure the pathways to achieve the desired bioelectrofuel of interest, but still more deliberate studies yet to be made to explore novel avenues for its commercial application in emerging industrial biotechnology sector.

5. Psychrophiles

Antarctica, Arctic, mountainous region and glaciated ice, man-made freezers etc.) making psychrophiles the most widely diverse extremophiles and its role in many biotechnological applications has been validated [56, 57]. Successful operation of MFCs using wastewater treatment (especially in temperate regions) requires the development of cost effective and energy efficient systems that can withstand the seasonal fluctuations effectively. Psychrophiles grows at an optimum growth temperature $<15^{\circ}\text{C}$ and capable to thrive at lower temperature $<0^{\circ}\text{C}$, on the other hand psychrotolerant species also grow at low temperatures but their optimal temperature of growth is $>15^{\circ}\text{C}$ [3]. Lowest temperature for cellular activity was reported to be -20°C and -12°C for cellular reproduction [58]. Generally, MFCs performance get adversely affected under low temperatures conditions. Performance of low temperature MFCs catalyzed by mixed bacterial consortia was investigated and found to perform sustainably better than the pure-culture (*Shewanella* spp.) based MFCs at 10°C as biofilm (anodic) of the former had lower overpotential and resistance than latter, which can be attributed to presence of microbes, the *Geobacter* and *Pseudomonas*, which might have helped in mitigating the rise in resistance/ or overpotential at lower temperatures [16]. However, further reducing the temperature to 5°C adversely had adversely reduced the power output irrespective of MFCs type (mixed or pure culture). Effect of temperature on the performance of MFCs was studied using MFCs (fed with combination of wastewater from brewery and domestic waste) at different temperatures ranging from 4 to 35°C and reported maximum power yield (294.6 mW/m^3 reactor) by membrane-based cathodic MFCs configuration at 4°C in comparison to a cloth-based cathodic MFCs (174.0 mWm^3 ; YQ 1.76%) at 35°C [59].

However, opposite to its established notion of temperature sensitivity, another study pointed out little/no effect of low temperature on impacting MES performance in terms of power production and proposed that MFCs biofilms may have a self-heating mechanism that possibly help them to keep up with the lower temperature [60]. The MES biofilms grown at lower temperatures outperform the biofilms grown at higher temperatures and reported that almost all biofilms possess similar lower and higher temperature limits of 0°C and 50°C , so MES can be operated at this defined operational limit [61]. Similarly, the psychrophilic MES has been studied in terms of hydrogen production (via electrohydrogenesis), methanogen inhibition and its microbial community structures [62]. Abundance of glucose-fermentation bacteria were reportedly active at lower temperature 4°C , who syntrophically interact with electroactive bacteria to generate current from glucose. Presence of other metabolic processes (like methanogenesis and homoacetogenesis) remains negligible in bacteria at such lower temperature. The composition of electrode microbial consortia changes with the change of temperature in an MFCs, which further modify other parameters such as diffusion coefficients, activation energies, ultimately which got reflected into the form of its power outputs and COD removal efficiency. A study was conducted to better understand the adaptability of MFCs to operating at psychrophilic conditions and studied the impact of lower temperature on microbial community dynamics and MFCs performance (in terms of COD removal and coulombic efficiency). In this study, MFCs operating at psychrophilic temperature was compared with MFCs working on psychrotolerant and mesophilic conditions and reported highest coulombic efficiencies at psychrophilic temperatures, however COD removal rates ($2.98\text{ g COD L}^{-1}\text{d}^{-1}$) was higher at mesophilic conditions [63]. Many researchers have started to explore power production of psychrophilic microorganisms in MFCs against the bacteria reported at normal temperature by isolating novel cold-adapted microbes from different environments that can be used as a potential biocatalyst in MFCs. Recently, a psychrophilic *Pseudomonas fragi* DRR-2 (psychrophilic) has been isolated from goat rumen fluid (with higher growth rate, higher electron transfer and shorter lag phase) and investigated for bioelectricity production using MFCs, so it can be applied to further improves MFCs performance in remote places with low temperature [64]. This microbe was found to be more electrochemically active at 20°C , resulting more power 540 mV (using with a salt bridge) and 380 mV (using Nafion membrane) production than the system working at room temperature (30°C).

Apart from bioelectrochemical applications, relevance of psychrophiles has been widely researched for having genome with high GC; higher flexibility proteins, less thermostability and higher catalytic activity, prevalence of genes associated with the cold shock response, factors which prepare them to

physiologically adapt themselves in terms of membrane, cryoprotectants, anti-freeze proteins and production of extracellular polysaccharide. Low-temperature anaerobic digestion has also been investigated using microbial electrochemical systems (MES) a in the combined MES-AD system with highest CH₄ yield of 31 mg CH₄-COD/g VSS (at a cathode potential of 0.90 V) which was reportedly 5.3-6.6 times higher when AD reactor used alone [12]. At 10°C and below, functioning stability is retained in cold-adapted functional species due to its metabolic key electrons transfer genes (like nitroaromatics reducers) [65]. Accelerating the pollutants detoxification in colder regions calls for improving the stability of functional microbial communities or using stable cold acclimated microbial strains for wastewater treatment system using MES.

6. Conclusion

Extremophile-based MES is at its infancy. Since last decade, extremophiles-based MES platforms have come into existence to oxidize variety of substrate or industrial effluents with high temperature or salinity (using thermophiles or halophiles) or to treat wastes at low temperate regions (using psychrophiles) for bioelectrofuels recovery. This review highlights the potential applications of extremophiles in MES. Exploration of the mechanisms behind its response against stress (i.e. high or low temperature, salinity) and responsible enzymes involved, can pave way for its applicability in various biotechnological applications such as waste treatment, bioremediation, and biosensor applications. Extremophilic bacteria/archaea are considered as novel micro-factories for metal and radionuclide bioremediation and end-waste management [57]. Industrial effluent of different temperatures can be directly processed/or treated using the cold active i.e. psychrophilic microbes and heat active i.e. thermophilic microbes (for cold and hot effluent respectively), thus eliminating the cost required to bring up /or down the temperature of effluent for its processing inside MES biosystem, thus further reducing the operation cost, and thereby help in achieving practical and economical production of valuable bioelectrofuels/or chemicals under unfavorable conditions in real world scenario. Extremophiles are promising bioremediation agent over mesophilic strains inside MES platforms to effectively biotreat the industrial effluent with extreme properties/ or under in extreme conditions with simultaneous production of electricity, which cover up the operational energy cost, thus making whole operation more efficient and economical. Development of the eco-friendly bionanoelectrode coated with immobilized extremozymes (enzymes extracted from extremophiles) are considered as a promising biohybrids to be implicated for biosensor application due to their precise sensitivity and selectivity, which largely remain unexploited so far [66]. Further investigation into the extremophilic metabolism and thermodynamics help us to discover its unseen aspects that are yet to be explored and can play revolutionary role devising the novel biotechnological breakthroughs. Exploration of novel electroactive extremophilic strains has potential to take MES to next level in terms of enhanced electrochemical performance and long-term operation under extreme conditions. Nevertheless, the extremophile is a promising alternative to successfully transform the substrate/ effluents (which either are of extreme characteristics/ or obtained from extreme environments) into valuable products using microbial electrochemical technologies. Extremophile-based studies can also provide novel insights into the astrobiological mysteries about possible nature of the intergalactic-microbes thriving on different planets with extreme environmental conditions.

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