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Microalgae and cyanobacteria as biological agents of biocathodes in biofuel cells

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Abstract

Biofuel cells (BFCs) are an environmental friendly technology that can simultaneously perform wastewater treatment and generate electricity. Peculiarities that hinder the widespread introduction of this technology are the need to use artificial aeration and chemical catalysts, which make the technology expensive and cause secondary pollution. A possible solution to this issue is the use of biocathodes with microalgae and cyanobacteria. Microalgae in the biocathodic chamber produce oxygen as the terminal electron acceptor. Various BFC technologies with algal biocathode (microbial fuel cells, microbial desalination cells, and plant microbial fuel cells) can address a variety of issues such as wastewater treatment, desalination, and CO_2 capture. The main technological parameters that influence the performance of the biocathode are light, pH, and temperature. These technological parameters affect photosynthetic production of oxygen and organic compounds by microalgae or cyanobacteria, and hence affect the efficiency of electricity production, wastewater treatment and production of added-value compounds in microalgae biomass like lutein, violaxanthin, astaxanthin. The ability to remove carbon, nitrogen, and phosphorus compounds; antibiotics; and heavy metals by pure cultures of microalgae and cyanobacteria and by mixed cultures with bacteria in the cathode chamber can be used for wastewater treatment.

Key words: biofuel cell, bioelectricity, biocathode, algae, added-value products

Introduction

Water and energy deficiency is currently a very serious global issue (Ashwaniy and Perumalsamy, 2017). Growing demand for fossil fuel energy may intensify global warming (Zhang et al., 2019). Therefore, search for alternative sustainable technologies of energy production and wastewater treatment, especially by energy-dependent countries, is a necessary and urgent task today (Kuzminskiy and Shchurska, 2018). Biofuel cells (BFCs) are an environmental friendly and promising technology that may facilitate to resolve the above-mentioned issues (Kokabian and Gude, 2015), because they can be applied in both wastewater treatment and electricity generation or to obtain energy carriers (Kuzminskiy and Shchurska, 2018). The major reasons that prevent the introduction of this technology on an industrial scale is the use of expensive catalysts such as platinum and toxic chemical agents such as ferricyanide (Gude, 2016), which can be overcome by using microalgae as biocathodes. Microalgae and cyanobacteria can perform aeration at the cathode, which is advantageous for reducing the aeration cost (Huang et al., 2011; Arun et al., 2020). The purpose of this review was to investigate the possibility of using microalgae as a biological agent for biocathodes in microbial fuel cells.

Microalgae in the biocathodic chamber produce oxygen as the terminal electron acceptor. Electrons pass through an external electrical circuit from the anode to the cathode (Mohan et al., 2014). Electrons transmitted to the anode are released through the bioelectrochemical decomposition of organic compounds from wastewater by exoelectrogenic bacteria. Microalgae- and cyanobacteria-based BFCs can address various issues such as desalination, wastewater treatment, bioremediation, bioenergy production, CO_2 capture, and synthesis of high added-value products (Saratale et al., 2017; Enamala et al., 2020).

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Microalgae and cyanobacteria as biological agents for biocathodes

BFCs with biocathodes, in which the biological agent is a mixture of bacteria and microalgae, can be used for treating wastewater (Wu et al., 2014) and for removing nitrogen compounds from it (Sun et al., 2019). There are two stages of nitrogen removal: the light stage during which oxygen is produced and ammonium compounds are oxidized to nitrites and nitrates, and the dark stage during which the oxygen concentration is reduced and facultative anaerobic denitrifiers reduce nitrates to nitrogen gas (Sun et al., 2019). Microalgae can also assimilate nitrogen compounds (Giordano and Raven, 2014). Sun et al. (2019) reported that the removal of nitrogen compounds using microalgae does not require additional aeration and the addition of carbon compounds in contrast to traditional nitrification and denitrification methods of biological wastewater treatment. Based on the concentration of dissolved oxygen in the biofilm, Sun et al. (2019) noted that nitrifiers are located outside the biofilm and denitrifiers are located closer to the electrode.

Another solution for removing nitrogen compounds is the addition of undiluted wastewater to the biocathodic chamber for nitrification. On the one hand, this addition may cause consumption of oxygen for the oxidation of organic compounds, but not as a terminal electron acceptor due to the high content of organic compounds in wastewater (Huang et al., 2011). However, on the other hand, the addition of an anolyte to the biocathodic chamber (after pretreatment of wastewater) can increase the efficiency of wastewater treatment. Commault et al. (2017) showed an increase in wastewater treatment efficiency by chemical oxygen demand (COD) from 34% after the first stage of purification in the anode chamber to 49% after the second stage of purification in the cathode chamber, with the initial COD being 2922 ± 66 mg/l. Wastewater post-treatment in the cathode chamber after the anode chamber also reduces the level of CO_2 (Jadhav et al., 2017).

Although the addition of undiluted wastewater can cause competition for oxygen as the terminal electron acceptor between electrons and organic compounds, Zhang et al. (2019) found that the addition of diluted wastewater for three times to the full BFC in the cathode chamber enabled to obtain the efficiency of NH_{4+} extraction of 85.6 ± 4.28%, with the initial concentration being 320 ± 16.0 mg/l. The addition of a catholyte from the biocathode to the anode chamber for denitrification is also undesirable, because the catholyte contains oxygen as the terminal electron acceptor (Sun et al., 2019). Microalgae such as *Chlorella vulgaris* can also remove phosphorus compounds (Zhang et al., 2019), and this ability can be used for the treatment of anolyte in the cathode chamber.

Concentration of dissolved oxygen in catholyte affects electricity generation. Technological parameters that affect photosynthetic production of oxygen by microalgae or cyanobacteria are temperature, pH, and light (illuminance and photoperiod) (Wu et al., 2013; Yadav et al., 2020). The optimal temperature of cultivation primarily depends on the metabolic characteristics of the biological agent. Temperature affects the content of chlorophyll in algae (Yadav et al., 2020); hence, its fluctuation can reduce the efficiency of photosynthesis. Oxygen production increases with the increase in illuminance, and consequently, the current density of the BFC increases. The phenomenon of photoinhibition and kinetic and mass resistance of oxygen transport to the cathode requires the selection of optimal illuminance of the biocathode chamber. Reddy et al. (2019) noted that optimal illuminance depends on the species of microalgae; for example, 5000 lx is the optimal illuminance for *C. vulgaris*, while 7000 lx is the optimal illuminance for a mixed algal culture.

The efficiency of photosynthesis and hence the power of BFC are influenced by seasons of the year. Mohan et al. (2014) showed that in summer, the efficiency of BFC with a biocathode is lower due to photoinactivation of RuBisCo in strong light. On the other hand, insufficient light in winter and autumn can cause photo-limitation and reduce the production of oxygen (Kokabian and Gude, 2015).

The growth rate of microalgae depends on the photoperiod and the species of algae (Wu et al. 2013). For example, for *Thalassiosira nordenskiöldii* Cleve (Durbin, 1974), the maximum growth is observed during the light : dark cycle of 15:9 h, while for *C. vulgaris* (Kendirlioglu et al., 2015) and *Chlorella pyrenoidosa*, the cycle is 16 : 8 h (Gunawan et al., 2018). The optimal ratio of light/dark also depends on temperature. Thus, with the increase in temperature from 10 to 15 °C, the optimal photoperiod for *T. nordenskiöldii* Cleve (Durbin, 1974) changed from 15 : 9 to 9 : 15.

Wu et al. (2013) noted that the dark stage was required because high levels of illumination and high oxygen concentration can inhibit photosynthesis and cause photooxidation. In the dark stage, less electricity is produced because oxygen is not produced and is consumed by algae for respiration. For sustainable current generation, 50% of electrode immersion is proposed (Ling et al., 2019). Thus, in the dark condition, oxygen from air become an electron acceptor at the cathode, while oxygen from the catholyte will be used by microalgae for respiration.

According to the Nernst equations, the pH affects the metabolism of microalgae and the potential of cathode (1) (Reddy et al., 2019).

$$EORR = 1.22 - 0.058\ pH$$

The pH value in microbial carbon capture cells (MCCs) may decrease due to the addition of CO_2 and other flue gases such as SO_2 and NO_2 as flue gas dissolves in the catholyte (Yadav et al., 2020). The pH in the double chamber microbial fuel cell (MFC) may change due to proton transporting from anode chamber to cathode chamber through the proton exchange membrane. In microbial desalination cell (MDC) due to translocation of positive ions into the cathode chamber the pH may also change. Lowering of pH causes partial inhibition of the enzyme RuBisCo (Laterre et al., 2017); thus, the pH value must be maintained with buffer solutions. The use of phosphate, carbonate, borax, and saline catholyte (Ahn and Logan, 2013) and zwitterionic buffers such as PIPES (piperazine-N, N-bis [2-ethane sulfonate]) is proposed for this purpose (Shukla and Kumar, 2018; Reddy et al., 2019). In the catholyte, carbon in the form of CO_2 or HCO_3^- can be supplied using *ex situ* and *in situ* methods. In the *ex situ* method, CO_2 is externally purged or bicarbonate is added to the catholyte. In the *in situ* method, the pre-treated wastewater in the anode chamber, which contains products of metabolism of exoelectrogens, particularly CO_2, is supplied to the cathode chamber (Reddy et al., 2019). The *in situ* method is more sustainable and environmental friendly, but difficult to control, and it depends on the metabolism of exoelectrogenic microorganisms. In addition, it is necessary to consider the carbon fixation limit of various algal species (González et al., 2013).

Microalgae in the cathode chamber can be used to produce biomass with a high content of lipids (Elakkiya and Niju, 2020) for the production of biodiesel (Golub and Levtun, 2016) and carotenoids (Gouveia et al., 2014). Rodolfi et al. (2009) showed that the accumulation of lipids can be caused by nitrogen starvation. But the need for nitrogen starvation hinders the use of the biocathode with algae to remove nitrogen compounds. Production of added-value products such lutein, violaxanthin, astaxanthin, and cantaxanthin in *Scenedesmus acutus* was obtained in plant microbial fuel cells (PMFCs). The accumulation of these xanthophylls was stimulated by the stress conditions in PMFCs and protects the microalgae photosystem against oxidative stress (Angioni et al., 2018). Added-value compounds such as carotenoids identified in algae in the cathode chamber have important applications in food, feed, and pharmaceutical industries (Gouveia et al., 2014). Light stress and nutrient stress influence the carotenogenesis process and stimulates carotenoid production (Gouveia et al., 2014).

Algal biomass can be used for biodiesel production (Nayak and Ghosh, 2019); alternatively, the dead microalgae biomass may be used as a substrate at the anode in BFCs (Cui et al., 2014). A previous study demonstrated that temperature affects the lipid content in algae (Ma et al. 2014). Yang et al. (2018) obtained a lipid production rate of 6.26 $mg \cdot l^{-1} \cdot d^{-1}$ in the full BFC with *Scenedesmus quadricauda* SDEC-8 at the biocathode. For comparison, the lipid productivity of *Scenedesmus* sp. was 41–54 $mg \cdot l^{-1} \cdot d^{-1}$ (Schnurr and Allen, 2015). The production of lipids can make the BFC technology more attractive due to simultaneous wastewater treatment and generation of bioelectricity and biodiesel (Nayak and Ghosh, 2019).

Microalgae and cyanobacteria in the cathode chamber are present in the catholyte and in the biofilm on the biocathode. Because of the attachment of microalgae to the cathode, a decrease in their concentration may be observed initially, and an exponential increase in growth occurs thereafter (Wu et al., 2013). It is important to consider this aspect when starting the BFC. The rate and nature of biofilm formation depend on the type of cathode material; more porous surfaces such as carbon felt show faster adsorption than carbon paper (Zhou et al., 2011; Wu et al., 2013). To reduce the mass transfer resistance, the catholyte and anolyte should be mixed (Jadhav et al., 2017), but mixing leads to additional operating costs. Reddy et al. (2019) identified inefficient design of the BFC, the source of inoculum, and the materials used to prepare electrodes as some of the reasons for the relatively low capacity of the BFC. Platinum catalysts can increase power density, but can also increase the cost of technology. Therefore, more attention has been given to carbon materials such as carbon paper, carbon cloth, graphite rod, and graphite fiber brush (Zhou et al., 2011). Recently, stainless steel has received increasing interest as a material for cathode. The modification of steel mesh by granular activated carbon allowed to achieve the power density of 55 mW/m^2 (Li et al., 2019). Furthermore, although the BFC battery can increase power output, Zhuang et al. (2012) indicated that a parallel circuit provides more output power due to lesser energy loss.

It is known that exoelectrogenic biofilms are not uniform in terms of homogeneous distribution of microbial cells, and microorganisms are localized in the biofilm according to the nutrient gradient (Prakasam et al., 2018). Leading biopolymers such as polyaniline can improve the performance of BFC and enhance the adhesion of bacteria on the anode or cathode (Li et al., 2012). To the best of our knowledge, very few studies have investigated the influence of polymers on the formation of microalgae biofilms; moreover, abiotic modification of electrodes with polymers for better adhesion of microorganisms and biofilm formation could negatively affect the conductivity of electrodes (Yaqoob et al., 2020).

Another approach to use biocathodes is microbial desalination cell (MDC). This technology is especially promising for desalination of seawater with simultaneous wastewater treatment in the anode chamber (Koltysheva et al., 2020). It was shown that the biocathode with microalgae is more sustainable than the abiotic cathode in the MDC (Zamanpour et al., 2017). High salt concentrations are a stressor for microalgae; hence, high lipid production by microalgae is expected under such conditions (Shetty et al., 2019). However, the disadvantages of this technology include the need to use cation exchange membranes and anion exchange membranes. The possibilities of application of various types of microalgae, cyanobacteria, and algae-bacteria mixes in different BFC technologies are given in Table 1.

Biocathodes can be used in a PMFC. It has been shown that power density obtained in a PMFC with a biocathode containing a mix of microorganisms (bacteria, fungi, algae, and protozoa) was 240 mW/m^2 (Wetser et al., 2015). *Chlorella* sp. can eliminate toxic metals (Jaroo et al., 2019) and antibiotics from wastewater. Extraction of chlorinated and nitroaromatic, oxytetracycline antibiotics with a biocathode used as an electron donor was investigated. The power density of 54 mW/m^2 was obtained at 5 mg/l oxytetracycline, which corresponded to a 1.8-fold increase as compared to BFC without the addition of oxytetracycline; however, nitrogen removal and antibiotic degradation were inhibited at 50 mg/l oxytetracycline concentration (Sun et al., 2019). The power generation of a photo-bioelectrochemical fuel cell was significantly enhanced with oxytetracycline stress by adding the antibiotic into the biocathode; thus, this technology has potential to be used as as environmental friendly technology for pharmaceutical wastewater treatment and electricity production (Zhou et al., 2018; Sun et al., 2019). Biofilm consisting of a mix of microalgae and bacteria has potential for wastewater treatment from azo dyes due to the adsorption process facilitated by the extracellular polymeric substances from the membrane of microalgae (Wang et al., 2016). Because of their higher potential, reactive oxygen species (ROS) are more competitive electron receptors than oxygen (Cai et al., 2013). Liu et al. (2020) showed that an increase in the ROS produced by algae photosynthesis caused a subseuquent increase in the output voltage of the PMFC. The possibility of using ROS in addition to oxygen is being considered, for example, the cyanobacteria *Microcystis aeruginosa* IPP in the bioelectrochemical system can produce ROS (Cai et al., 2013); moreover, the addition of 25 mM mannitol (which inhibits H_2O_2) caused a decline in current from 240 to 160 μA. It was also demonstrated that carbon felt cathodes modified with NiO and ZnO due to enhanced ROS adsorption provided increased output voltage in PMFC. For example, an output voltage of 0.3 V was obtained in PMFC with spongy $ZnO_{0,2}$–NiOArGO carbon felt cathode, which corresponded to a 3-fold increase than that achieved by a pre-fabricated carbon felt cathode (Liu et al., 2020).

The performance of MFC with the biocathode containing bacteria and microalgae as compared to that of MFC without the biocathode increased by 55% in power density (54.48 mW/m^2) (Yadav et al., 2020). On the other hand, some MFC with biocathode compared to abiotic cathode had low current density (13.45 mA/m^2 in compare to 53.5 mA/m^2), but the COD removal efficiency from wastewater improved to 96% (Elakkiya and Niju, 2020).

Table 1. Use of microalgae and cyanobacteria as biological agents of biocathodes in biofuel cells

Biological agent	Technology	Electrode material	Immobilization of microorganisms	Functions	References
Mixed microalgae	biofuel cell	graphite plates	not given	wastewater treatment, electricity generation	Mohan et al., 2014
Mixture of anaerobic sludge, aerobic sludge, and *Chlorella vulgaris*	photo-bioelectrochemical system	nickel foam	biofilm	wastewater treatment from nitrates, ammonium	Sun et al., 2019
Chlorella vulgaris	sediment microbial fuel cell	graphite felt – multiwalled carbon nanotubes	biofilm	electricity generation	Wang et al., 2014
Desmodesmus sp.	microbial fuel cell	plain graphite felt	biofilm	wastewater treatment, electricity generation	Wu et al., 2014
Mixed microalgae	microbial fuel cell	plain graphite plates	not given	wastewater treatment, electricity generation, biodiesel production from algae lipids	Elakkiya and Niju, 2020
Scenedesmus abundan	microbial desalination cell	graphite rods	biofilm	desalination of water, wastewater treatment	Ashwaniy and Perumalsamy, 2017
Bacteria, fungi, algae, and protozoa	plant microbial fuel cell	graphite felt	biofilm	wastewater treatment, electricity generation	Wetser et al., 2015
Chlorella vulgaris	microbial fuel cell	steel mesh	biofilm	wastewater treatment, electricity generation, biodiesel production from algae lipids	Bazdar et al., 2018
Chlorella sp.	microbial fuel cell	nickel foam/ graphene oxide	biofilm	Cd reduction $Cd^{2+} + 2OH^{-} = Cd(OH)_2$	Zhang et al., 2018
Scenedesmus acutus PVUW12	microbial carbon capture cells	carbon cloth	biofilm	wastewater treatment, electricity generation, CO_2 sequestration	Angioni et al., 2018
Chlorella vulgaris	microbial fuel cell	platinum-coated carbon paper	not given	wastewater treatment, electricity generation	Wu et al., 2013
Chlorella sp. and anaerobic sludge	photo-bioelectrochemical system	graphite felt	biofilm	wastewater treatment from nitrates, ammonium, and antibiotics	Li et al., 2020
Anabaena ambigua	microbial carbon capture cells	carbon felt	biofilm	wastewater treatment, electricity generation, CO_2 sequestration	Jadhav et al., 2017
Microcystis aeruginosa	bioelectrochemical system	carbon paper	biofilm	electricity generation	Cai et al., 2013
Scenedismus obliquus	microbial fuel cell	platinum-coated carbon paper	biofilm	electricity generation	Kakarla and Min, 2014
Chlorella vulgaris	microbial fuel cell	carbon fiber cloth	biofilm	wastewater treatment from nitrates, ammonium, CO_2 sequestration	Zhang et al., 2019
Scenedesmus quadricauda SDEC-8	microbial fuel cell	carbon cloth cathode with titanium	biofilm	wastewater treatment from nitrates, ammonium, nitrites, phosphates	Yang et al., 2018
Oscillatoria sp.	microbial fuel cell	graphite plate	biofilm	wastewater treatment, CO_2 sequestration, electricity generation	Naina Mohamed et al., 2019

Conclusions

Microalgae and cyanobacteria as pure cultures and in mixed cultures with bacteria can be used in biocathode to remove carbon compounds, nitrogen compounds, phosphorus compounds, antibiotics, and heavy metals. Morover, biocathodes with microalgae can be used for desalination of water, electricity generation, and wastewater treatment after passing through the anode chamber along with removal of carbon dioxide. The development of BFCs with microalgae and cyanobacteria as biological agents of biocathodes could enable energy-efficient wastewater treatment and production of added-value compounds in microalgae biomass. Furthermore, the obtained biomass of microalgae can be used as a substrate at the anode of BFC or to produce added-value products such lutein, violaxanthin, astaxanthin, and cantaxanthin and biodiesel, which could confirm that BFC is economically viable. Modification of electrodes by polymers can improve the performance of BFC and enhance the adhesion of bacteria on the anode or cathode. Further studies on the synergistic effect of light (photoperiod and illuminance), pH, temperature, modification of electrodes, etc. are necessary to determine the optimal technological parameters of the full BFC. It should be noted that estimation their effect on technology (in order to increase power density or carbon capture and nitrogen compounds removal) will depend on the target product, namely the accumulation of biomass, oxygen production.

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