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Engineering rheology of electrolytes using agar for improving the performance of bioelectrochemical systems

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ABSTRACT

The present study is focused on enhancing the rheological properties of the electrolyte and eliminating sedimentation of microorganisms/flocs without affecting the electron transfer kinetics for improved bioelectricity generation. Agar derived from polysaccharide agarose (0.05–0.2%, w/v) was chosen as a rheology modifying agent. Electroanalytical investigations showed that electrolytes modified with 0.15% agar display a nine-fold increase in current density (1.2 mA/cm^2) by a thermophilic strain (*Geobacillus* sp. 44C, 60 °C) when compared with the control. Sodium phosphate buffer (0.1 M, pH 7) electrolyte with riboflavin (0.1 mM) was used as the control. Electrolytes modified with 0.15% agar significantly improved chemical oxygen demand removal rates. This developed electrolyte will aid in improving bioelectricity generation in Bioelectrochemical Systems (BES). The developed strategy avoids the use of peristaltic pumps and magnetic stirrers, thereby improving the energy efficiency of the process.

1. Introduction

The energy crisis is one of the most burgeoning issue worldwide. It raises serious concerns and there is a constant search for renewable and green sources of energy. Among the different renewable energy sources, bioelectrochemical energy sources have been realized as promising options for several applications. Recent decades evidenced tremendous advancements in bioelectrochemical systems (BES) including microbial fuel cell (MFC) (Yeruva et al., 2015), microbial electrolysis (Pasupuleti et al., 2015), microbial desalination (Nikhil et al., 2016), implantable fuel cells (Zebda et al., 2013), and bioelectrosynthesis (Roy et al., 2016). They have been realised as promising technologies for a wide range of applications such as bioremediation (Nancharaiah et al., 2016), desalination (Nikhil et al., 2016), sensing/biomonitoring (He et al., 2016), production of biofuels (bioelectricity, biohydrogen, methane, methanol) (Navanietha Krishnaraj et al., 2013; Selvaraj et al., 2016), and value-added products (Navanietha Krishnaraj et al., 2014). BES have also been developed for conversion of greenhouse gases such as methane and carbon dioxide into bioelectricity (McAnultyet al., 2017). These devices harness the electrocatalytic activity of microorganisms and enzymes to convert chemical energy into electricity. BES make use of microorganisms as electrocatalysts and operate at a broad range of operating conditions (pH and temperature), unlike chemical fuel cells. BES help in converting wastes to bioenergy and generating value-added products, thereby making these processes more economical and environmentally benign. However, low rates of electrocatalysis and product yields limit practical applications of BES for bioelectricity generation and other energy applications. Unlike other bioprocesses, bioelectrocatalysis is driven by both mass and electron transfer reactions in electrochemical systems (Babauta and Beyenal, 2014; Bonanni et al., 2013).

Although several reports have documented improved electron transfer rates in BES, the approaches to improve mass transfer rates across electroactive microorganisms-electrolyte interfaces are limited. Improving mass transfer rates will greatly increase the performance of BES for bioelectricity production, as well as disposal of wastes in an environmentally benign manner at accelerated rates. Several approaches such as genetic engineering of microorganisms and surface engineering of electrodes have been used to improve electron transfer rates from microorganisms to the electrode through direct and mediated electron transfer routes for improved bioelectricity generation (Alfonta, 2010; Karthikeyan et al., 2016). Reports have documented the

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identification of new electroactive microorganisms (Bhuvaneswari et al., 2013; Shrestha et al., 2018), elucidating their electron transfer mechanisms (Huang et al., 2011) and engineering microorganisms to improve electron transfer rates at electrode-electrolyte interfaces in BES (Yong et al., 2014).

Metabolic/genetic engineering approaches have been carried out to improve the expression of conductive proteins that are involved in direct electron transfer reactions and production of electron shuttling compounds that are involved in mediated electron transfer reactions (Kim et al., 2016). Approaches such as new electrode materials, electrode functionalization strategies, and biofilm engineering strategies have also been carried out to improve the direct electron transfer reactions at the electrode-electrolyte interface and in bioelectrocatalysis (Liang et al., 2016; An et al., 2016). Wen et al. (2009) demonstrated that reaction kinetic loss and mass transport loss greatly affect the performance of MFC operated with beer brewery wastewater.

Sedimentation of microorganisms and heterogeneous substrates remains a major challenge to mass transfer in the electrochemical system. Sedimentation of the microorganisms decreases the residence time of the microorganisms to adhere to the electrode surface. Herein, we report a facile and unique strategy to overcome this obstacle by modifying the rheology of electrolytes. Microorganisms are prone to aggregate with each other which further increases the rate of sedimentation. The aggregation of microorganisms decreases the number of catalytic sites on microorganisms and increases the mass transfer resistance for the solute to reach catalytic sites within the aggregates (Li and Yuan, 2002). The use of heterogeneous and insoluble feedstocks also suffers from settling issues and requires constant stirring. However, application of stirring in bioelectrochemical systems is challenging because of increased operation cost, decreased system efficiency, and significantly decreased electron transfer rates (Tang et al., 2017). The rates of sedimentation of microorganisms can be increased by increasing the viscosity of the electrolyte which inturn increases the rate of adherence of the microorganisms to the electrode surface. This will also help in improving the direct electron transfer of the electroactive microroganisms at electrode-electrolyte interface (Cui et al., 2016). Alfonta (2010) reported yeast surface display technology for the expression of electrocatalytic enzymes on the surface of the microorganism, thereby decreasing the mass transport limitations across the cell walls and improving bioelectricity yields. Furthermore, magnetic stirrers have been used to prevent sedimentation of microbial cells and improve mass transfer rates with minimal effects on biofilms and electron transfer rates (Foad Marashi and Kariminia, 2015). Flow cells and peristaltic pumps were also reported to minimize mass transfer resistance in MFCs. However, they cannot be used for large scale systems due to high cost and decreased efficiency (Michie et al., 2014; Kim et al., 2012).

The rate of sedimentation in microbial cells/flocs depends on their sizes, density and viscosity, and the electrolyte density (Atkinson and Daoud, 1976). Rate of settling is inversely proportional to the viscosity of the electrolyte. Increasing the viscosity will decrease the rate of settling. Herein, we report the development of electrolytes with enhanced rheological properties using agar as the rheology modifying agent that decreases the rate of sedimentation. Agar is a biopolymer derived from polysaccharide agarose produced from algal sources. It has been used for centuries as a solidifying agent in culture media for growth of microorganisms. Gelation mechanism clearly describes the effect of agar on the rheological properties of the solution including viscosity, viscoelastic properties, and flow rates (Ko and Van Gundy, 1988). This is the first work reporting the use of rheology modifiers to significantly increase the viscosity of the electrolyte in BES without a major increase in density, while helping to decrease the rate of sedimentation of microbial cells and heterogeneous feedstocks. An additional advantage of the developed strategy is that the rheology modifier (agar) that is used also helps to support growth and proliferation of microorganisms in the electrolyte or at the electrode surface, contributing to enhanced electron transfer at the electrode-electrolyte interface. This novel approach helps to accelerate electrocatalysis of substrates and significantly increases bioelectricity generation. In addition, this strategy helps decrease the external energy source for operating stirring systems, thereby decreasing mass transfer resistance.

2. Materials and methods

2.1. Fabrication of electrodes

Carbon felt (procured from Fuel Cell Earth, LLC) with an area of 1.0 cm^2 was used for electrode fabrication. The electrical contact was made with a brass rod. The fabricated carbon felt electrodes were cleaned thoroughly and were used as electrodes for bioelectrocatalytic activity evaluation (Navanietha Krishnaraj et al., 2015).

2.2. Engineering electrolytes

Sodium phosphate buffer solutions (0.1 M, pH 7) with different concentrations of agar (e.g. 0.05, 0.10, 0.15, and 0.20% w/v) were used as electrolytes. Agar was used as the rheology modifying agent and riboflavin (0.1 mM) was used as an electron mediator in the electrolyte. Electrolytes with four different concentrations of agar were compared to study the effect of increasing viscosity on microbial electrocatalysis of glucose. Phosphate buffer (0.1 M, pH 7) without agar was used as the control sample.

2.3. Growth of microorganisms

Thermophilic strain of *Geobacillus* sp. 44C *(G-44C)* isolated from the deep Sanford Underground Research Facility (4850 ft. deep level, Yates Shaft) were used as electrocatalysts for electrocatalysis of glucose in the experiment. *G-44C* was inoculated in Luria Bertani broth and grown in anaerobic conditions at 60 °C. After 24 h, the broth containing the cells was centrifuged at 10000 rpm for seven minutes, and the pellets were thoroughly washed with sodium phosphate buffer (0.1 M, pH 7) to remove cell debris and broth constitutes. The cells were then dispersed in sodium phosphate buffer and used for the experiments.

2.4. Electrochemical analysis

Cyclic voltammograms (CVs) of *G-44C* were recorded in phosphate buffer (0.1 M, pH = 7) using Ag/AgCl and Pt as a reference and counter electrode, respectively. The temperature was maintained at 60 °C. Phosphate buffer solutions with different agar concentrations were used as the electrolyte. Aseptic conditions were maintained throughout the experiments. The effect of different electrolytes on microbial electrocatalysis was analysed with oxidation currents recorded at different concentrations of glucose as electron donors and riboflavin as an electron mediator. CV plots of the electroactive microorganisms in different electrolytes were recorded at different scan rates from 10 to 100 mV/s with increments of 10 mV/s. Further, long-term chronoamperometry was carried out for the bioelectrochemical systems with electrolytes at different concentrations of agar (Navanietha Krishnaraj et al., 2018). Electrochemical Impedance Spectroscopy (EIS) was performed in a three-electrode electrochemical cell at the frequency range of 20 Hz to 10 mHz with an AC amplitude of 10 mV for five different electrolytes.

2.5. SEM analysis of microbial growth

Biofilms of *G-44C* formed on the carbon felt grown in different electrolytes were characterized by SEM analysis. A piece of carbon felt with *G-44C* biofilm (0.5 × 0.5 cm) was cut under aseptic conditions and dried in a desiccator. Analyses of the biofilms were carried out using a Zeiss Supra40 variable-pressure field-emission SEM.

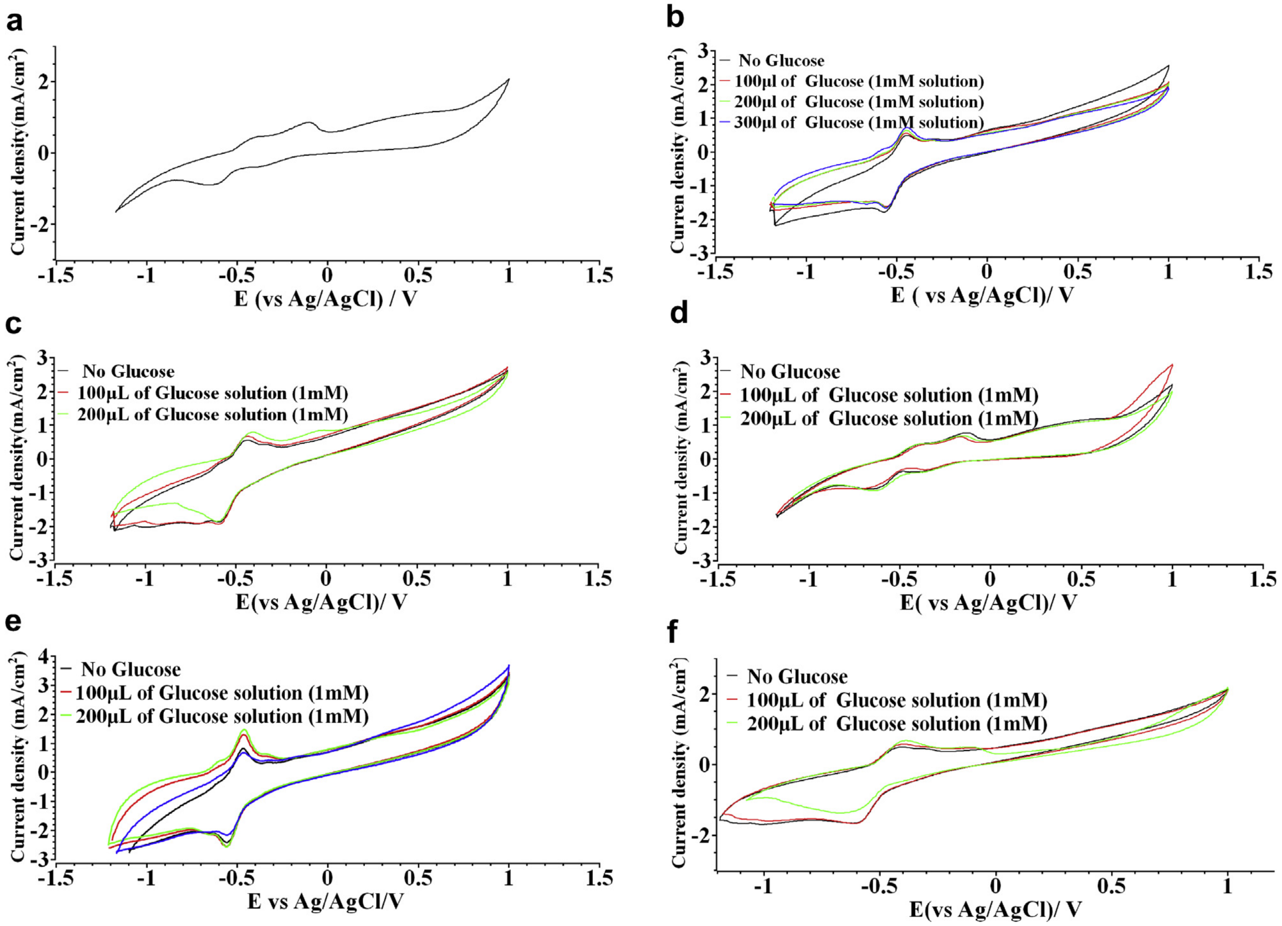

Fig. 1. Cyclic voltammograms of thermophilic G-44C in: (a) Phosphate buffer electrolyte (0.1 M, pH = 7) at 5 mV/s; (b) Same electrolyte with glucose and without agar (control sample); and phosphate buffer electrolyte with glucose and at different agar concentrations: (c) 0.05%; (d) 0.1%; (e) 0.15%, and (f) 0.2%.

2.6. Kinetics of fuel consumption

Kinetics of the fuel consumption of the biofilms in a phosphate buffer electrolyte were analysed by measuring the change in the COD levels. Analyte samples (0.2 mL) were taken every day and the values of COD were measured using a COD digester (Spectroquant 320, Merck).

3. Results and discussion

3.1. Effect of rheology modifiers in electrolytes on microbial electrocatalysis

Initially, CV plots of G-44C in the phosphate buffer were recorded at a scan rate of 5 mV/s to elucidate the G-44C bioelectrocatalytic activity. The CV plots (Fig. 1a) demonstrate two reversible peaks at −0.37 V and 0.16 V, suggesting that G-44C is an electroactive microorganism. This result indicates the presence of redox proteins that are involved in the electron transfer from G-44C to the electrode.

The observed biocatalytic activity of G-44C correlates with CV tests for *Geobacter sulfurreducens* in the range of −0.488 to −0.188 V (Marsili et al., 2008a,b). Furthermore, the presence of OmcZ in *G. sulfurreducens* was confirmed in the range of −0.708 to −0.348 V with a midpoint potential of −0.508 V (Inoue et al., 2010). *Shewanella oneidensis* MR-1 demonstrated a peak at −0.498 V related to the redox-active flavins involved in the mediated electron transfer (Marsili et al., 2008a,b). The midpoint redox potential of −0.238 V was shown to be related to OmcA-MtrCAB outer membrane complexes in *S. oneidensis* MR-1. These results confirm that CV analysis allows identification of direct electron transfer mechanisms initiated by electroactive microorganisms such as G-44C (Okamoto et al., 2011).

In the first set of experiments the bioelectrocatalytic activity of *G-44C* in phosphate buffer in the presence of a mediator and increasing concentration of glucose without agar was evaluated (Fig. 1b). CV plots displayed an oxidation peak at −0.45 mV. Upon addition of 100 μL of 5 mM of glucose, the anodic peak current increased from 0.46 mA to 0.52 mA. On subsequent incremental addition of 5 mM of glucose (100 μL), the current increased to 0.697 mA. These results confirm that glucose can be used to evaluate *G-44C* biocatalytic activity in phosphate buffer electrolytes modified with agar.

The second set of experiments involved *G-44C* in phosphate buffer with glucose (riboflavin (0.1 mM)), but different agar concentrations in the range of 0.05–2.00% (Fig. 1c–f). The corresponding CVs of *G-44C* for 0.05% agar in solution (Fig. 1c) demonstrate the oxidation peak at −0.4369 mV. Addition of glucose increased the oxidation current from 0.4953 mA to 0.6449 mA and 0.777 mA upon the first and second addition, respectively. Addition of 0.05% agar increased the bioelectrocatalytic activity of *G-44C* towards glucose oxidation by two-fold when compared with the control sample without agar. By increasing the agar concentration to 0.1%, the oxidation current increased from 0.629 mA to 0.6786 mA on the first addition of 100 μL of 5 mM of glucose. However, upon the second addition, the current increased to 0.7679 mV (Fig. 1d). When compared with the control sample, the electrolyte with 0.1% agar increased the bioelectrocatalysis of glucose by 130%. The control sample had only an 0.06 mA increase with the addition of glucose, whereas the electrolyte with 0.15% had an 0.139 mA increase with addition of glucose. The electroactive microorganisms in the phosphate buffer with 0.15% agar (shown in Fig. 1e) displayed a characteristic peak at −0.468 mV. The first addition of 100 μL of 5 mM of glucose increased the oxidation current from

0.6595 mA to 0.8023 mA. Upon second and third additions, the oxidation current increased to 1.269 mA and 1.451 mA, respectively. Interestingly, the electrolyte with 0.15% agar demonstrated an increase in current by 0.600 mA which is a nine-fold increase when compared with the control sample without agar.

Cyclic voltammogram of the electroactive microorganisms in the electrolyte with 0.2% of agar (Fig. 1f) had the oxidation peak at −0.4079 mV. On addition of 100 μL of 5 mM of glucose, the current increased from 0.4702 mA to 0.565 mA. Further addition of 100 μL of 5 mM of glucose, increased the current to 0.6603 mA. It had nearly one and one-half-fold increase in oxidation current when compared with a control sample without agar. However, the current was 83% lower when compared with the electrolyte with 0.15% agar.

Overall this study clearly showed that the oxidation current has increased on addition of agar up to 0.15% (w/v), and thereafter oxidation rates decreased. The results showed that 0.15% of agar in the electrolyte is the optimal concentration at which bioelectrocatalytic activity rates are improved. The use of agar increased viscosity, while decreasing the sedimentation rate of thermophilic *G-44C,* leading to increased rates of the bioelectrocatalysis.

Further, amperometric investigations were carried out to study the bioelectrocatalytic rates of glucose with *G-44C* with different electrolytes. The specific oxidation potential applied for amperometric analysis was obtained from the corresponding CV plots. The amperometric analysis was carried out in the presence of electrolyte without agar by applying an oxidation potential of 0.4 mV. The recorded amperogram showed that the current increased by 0.3 mA initially, and thereafter the current increase was around 0.1 mA with the addition of 100 μL of 5 mM of glucose. The addition of glucose was made initially when the current was stable at 0.94 mA at 0.4619 h, and the oxidation current increases to 1.342 mA. Upon the second addition of glucose, the current increased from 0.4579 mA to 0.489 mA at 3.4 h. Subsequent addition of glucose was made at 10.07 and 17.26 h. The oxidation current increased from 0.2257 mA to 0.3072 mA with addition of glucose at 10.07 h and from 0.1818 mA to 0.2445 mA with the addition of glucose at 17.26 h. The cyclic voltammetry and amperometry experiments confirm the good electrocatalytic activity of *Geobacillus* sp. 44C. This 44C strain oxidized glucose at very high rates, and produced bioelectricity (up to 1.2 mA/cm^2) compared with the yields of well-known electroactive microorganisms such as *Geobacter sulfurreducens* (Xing et al., 2009), *Shewanella oneidensis* (Rosenbaum et al., 2011), *Enterobacter cloacae* (Feng et al., 2014), and *Rhodopseudomonas palustris* (Wrighton et al., 2008). This thermophilic strain also displayed much higher rates (up to 1.2 mA/cm^2) of bioelectrocatalysis of glucose when compared with other thermophiles such as *Thermincola sp.* strain JR (0.01 mA/cm^2) and Anaerobic thermophilic consortium TC60 (0.001 mA/cm^2) that have been reported (Jong et al., 2006; Carver et al., 2011).

Amperometric studies of *G-44C* in electrolytes with 0.15% agar (shown in Fig. 2) showed that the oxidation current significantly increased when compared with the control sample. Initially, current is stabilized at 0.41 h. Upon addition of glucose, the current increased from 0.36 mA to 0.619 mA. The second addition was made after ∼4 h, causing the current to increase from 0.1296 mA to 0.3791 mA. The next glucose addition made after ∼6 h had a similar effect, resulting in current increase from 0.1296 mA to 0.3791 mA. However, the glucose addition after a longer period (∼20 h) increased the current from 0.297 mA to 1.248 mA. The addition made at 21.5 h increased the current from 0.806 mA to 2.068 mA. Similar responses were also observed at 23.83 h and 24.81 h, wherein the current increased from 0.6334 mA to 1.91 mA and 0.657 mA to 2.399 mA, respectively. These results corroborate well with electrocatalytic studies using the CV approach and confirm that the increase in viscosity enhances the performance of microbial electrocatalyst.

Amperometric results of the system with electrolyte containing 0.2% agar were not smooth and displayed lots of noise in the electrochemical signals. Low signal to noise ratio indicates that agar at high concentrations affect electron transfer reactions from *G-44C* to the electrodes. The amperogram was recorded for ∼24 h by applying the oxidation potential of 0.4 V. Once the current reached a stable state of 0.023 mA at 0.4888 h, the addition of glucose was made which increased the current from 0.238 mA to 0.060 mA. The next addition of glucose increased the current from 0.015 mA to 0.018 mA. A further addition at around 4.46 h increased the current from 0.01 mA to 0.015 mA. This indicates that at a higher viscosity, electron transfer characteristics are greatly affected. Overall, the amperometric investigations showed that the electrocatalysis of glucose was higher in the case of electrolytes with 0.15% agar.

These findings corroborate well with previous studies on electrocatalysis of sugars using cyclic voltammetry. Reports are available for improving the rates of microbial bioelectrocatalysis by modifying the type of the buffer, for example, using saline catholytes and bicarbonate buffers (Ahn and Logan, 2013; Fan et al., 2007). Phosphate buffer solutions that are commonly used in bioelectrocatalytic systems aid in maintaining the optimal pH for microbial electrocatalysis and increasing the solution conductivity. Bicarbonate buffers act as pH buffers and are known to improve proton conductivity. Reports are also available on the effect of the pH of electrolytes on improving the rates of microbial electrocatalysis in MFCs (He et al., 2008). Attempts have also been made to improve the oxidation of electron donors for bioelectricity generation by supplementing electrolytes with mediators that are involved in shuttling electrons from microorganisms to the

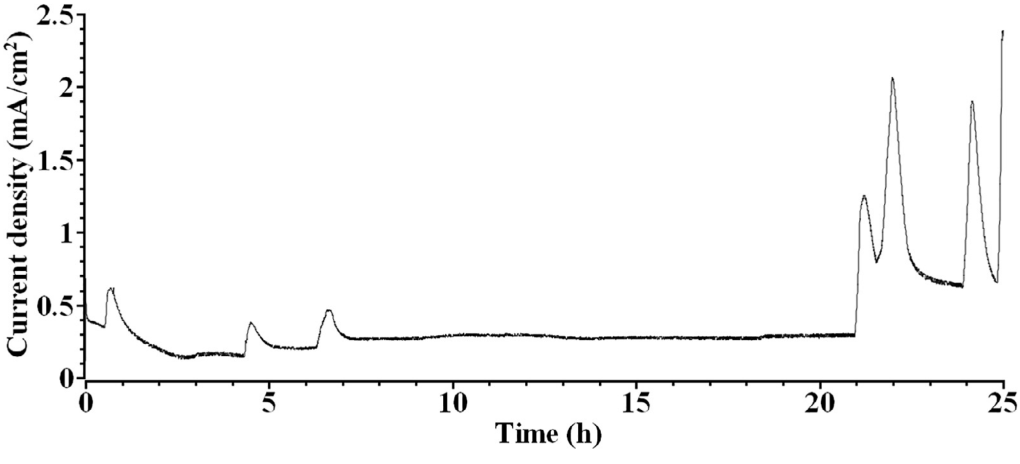

Fig. 2. Amperograms of G-44C in phosphate buffer electrolyte (0.1 M, pH = 7) with 0.15% agar.

electrodes (Sund et al., 2007; Thygesen et al., 2009). These approaches aid in modifying the electrolyte for improving electron or proton transfer rates in bioelectrochemical systems. However, the use of a rheology modifying agent is based on improving mass transfer rates between the solute and the microorganisms, thereby improving the rates of electrocatalysis of the substrates.

3.2. Effect of agar in electrolytes on electron transfer kinetics

To study the effect of the rheology modifying agar on electron transfer kinetics, CVs of *G-44C* in electrolyte concentrations of agar (0, 0.15, and 0.2%) were recorded at different scan rates (10–100 mV/s) (Fig. 3a and b). As expected, oxidation currents increased with increase in scan rate from 10 mV/s to 100 mV/s. The measured current is the sum of faradaic current that comes from the redox reaction at the electrode-electrolyte interfaces and the capacitive current that comes from double layer charging. Fig. 3B clearly confirmed that the anodic peak current is linearly proportional to the scan rate in electrolyte without agar, indicating that it is a surface confined process. From the scan rate dependence plots, the linear regression equations, $y = 0.078 + 0.0032x$ and $y = 0.138 - 0.0032x$, and the regression coefficients, 0.9920 and 0.9929, were obtained for oxidation and reduction peaks, respectively. With increase in scan rates from 10 mV/s to 100 mV/s, the values of anodic peak potential (Epa) and cathodic peak potential (Epc) displayed very minimal shifts in the positive or negative direction. As a result, the value of ΔEp (difference in peak potentials) also increased with the increase in scan rate. ΔEp had a minimum value of 0.38 mV at 10 mV/s and a maximum value of 0.54 mV at 100 mV/s. This suggests that this bioelectrode in electrolyte without agar displayed more quasi reversible characteristics. The ratio of anodic and cathodic peak currents (Ipa/Ipc) was found to be 0.6 at 10 mV/s and −0.866 at 100 mV/s. The peak current is proportional to the square root of the scan rate in cyclic voltammogram which indicates the redox reaction. The magnitude of increase in anodic current and decrease in cathodic current depends on the electrocalytic activity of the electroactive microorganisms/immobilized enzymes.

The square root dependence of scan rate with I_{pa} and I_{pc} values in the CVs of *G-44C* bioelectrodes in electrolyte with 0.15% agar recorded at different scan rates (10–100 mV/s) confirmed that this is a diffusion-controlled process. The shifts in E_{pa} and E_{pc} were found to be slightly higher when compared with the control sample. For instance, at 10 mV/s the ΔE_p value was 0.38 in the control sample and 0.324 in the electrolyte with 0.15% agar. The ratio of the anodic to cathodic peak currents increased when compared with the control with the value of 0.86 at 10 mV/s and it approaches the value of 0.9 at 100 mV/s.

High solution resistance is evident from these voltammograms recorded at different scan rates in the electrolyte with 0.2% agar. The voltammograms displayed an abrupt signal indicating poor electron transfer from microorganisms to the electrode across the *G-44C* electrolyte-electrode interface. The values of Epa and Epc had a significant shift in the positive and negative direction with an increase in scan rates (when compared with the control and electrolyte with 0.15% agar). ΔEp values were found to be 0.69 at 10 mV/s and 1 at 100 mV/s. The ratio of anodic to catholic peak currents had a maximum value of 0.68 indicating a lower electron transfer reaction when compared with electrolyte with 0.15% agar. Among the three electrolytes with different concentrations of agar that were analysed, the electrocatalytic system with 0.15% electrolyte had positive Eo values (0.46 at 10 mV/s and 1.27 at 100 mV/s) which is favourable for enhanced bioelectrocatalytic rates. In the absence of stirring, the microorganisms in the electrolyte will flocculate and increase the drag force and sedimentation rate of microorganisms. This decreased the microbial electrocatalysis especially in the case of BES with mediated electron transfer. The use of agar increased the viscosity of the electrolyte and is prone to decrease the electron transfer rates of the electrolyte. However, at a lower concentration of 0.15%, it prevents the settling of

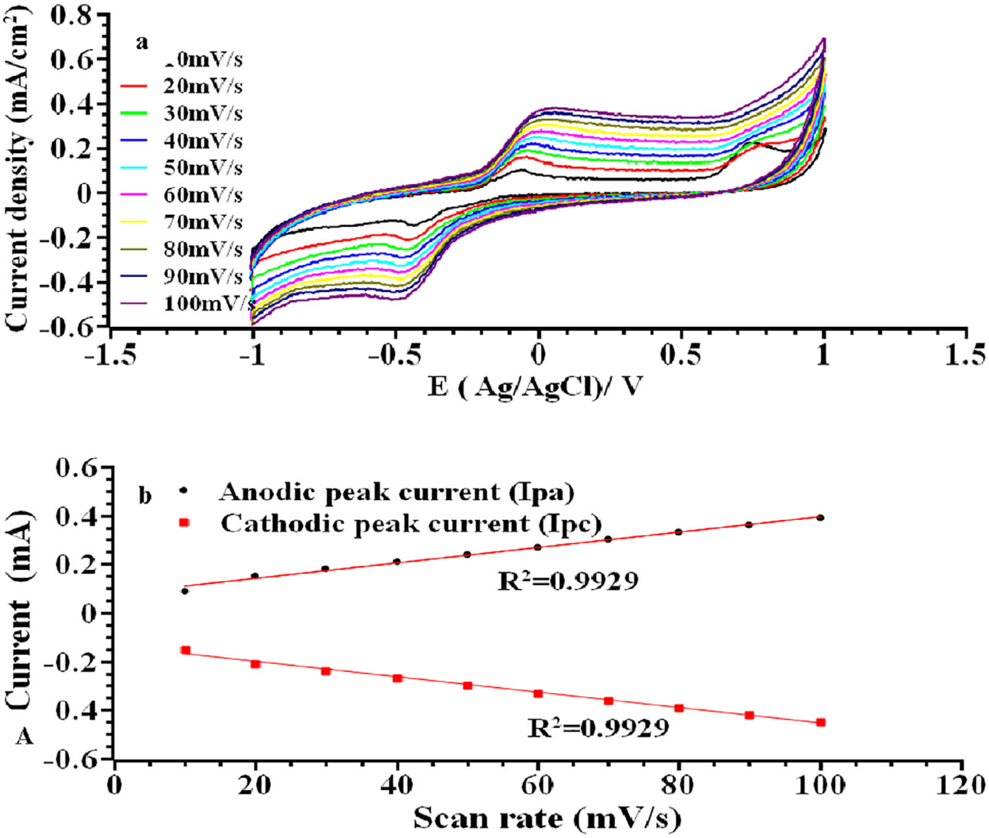

Fig. 3. (a) CVs showing the dependence of scan rate in control sample; and (b) Peak currents vs. scan rate in control.

microorganisms leading to enhanced oxidation of electron donors. This increased the electron transfer rates at electrode-electrolyte interfaces. In addition, the use of electrolytes with agar increased the rate of adherence of the microorganisms to the electrode surfaces and biofilm formation. This contributes to direct electron transfer from microorganisms to the electrodes and increased overall rates of electrocatalysis.

The Laviron equation was used for calculating the value of the apparent charge transfer rate constant (k_s) for the electron transfer between *G-44C* and the electrode at the electrode–electrolyte interface (Laviron, 1979):

$$\log Ks = \alpha\log(1-\alpha) + (1-\alpha)log\alpha - \log\left(\frac{RT}{nFv}\right) - \alpha(1-\alpha)nF\Delta Ep/2.3RT$$

where K_s is apparent charge transfer rate constant for electron transfer, α is the transfer coefficient, n is the number of electrons transferred in the rate determining step, ΔE_p is peak potential separation, v is scan rate, $R = 8.314\,J\,mol^{-1}\,K^{-1}$, $T = 298\,K$, and $F = 96{,}485\,C\,mol^{-1}$. The values of α and n were obtained from the slope of E_{pa} vs. lnv, and used for calculating K_s. Using the Laviron equation, the value of the apparent charge transfer rate constant (K_s) in electrolyte with 0.15% agar in the *G-44C* bioelectrocatalytic system was found to be $0.44\,s^{-1}$. Ks is a measure of the electronic transfer, and it clearly shows that the *G-44C* system with 0.15% agar displayed a fast electron transfer kinetics process.

3.3. Electrochemical Impedance Spectroscopy (EIS)

Fig. 4 is Nyquist plots of the microbial electrocatalytic system in the presence of electrolyte with different agar concentrations. The low frequency of the real axis of the Nyquist plot corresponds to the sum of solution resistance (Rs) and charge transfer resistance (Rct) of the microbial electrocatalytic system. Rct indirectly provides information about the effect of the modified electrolytes on microbial electrolysis (Manohar et al., 2008). Better Rct values shows that the modified electrolyte contributes to a thicker biofilm formation. Better the biofilm formation, higher is the electrocatalytic activity. Bioelectrodes in the electrolyte without agar (control) have an Rs of 4 Ω and Rct of 129 Ω. Upon increasing the agar concentration in the electrolyte to 0.05%, Rs and Rct slightly increased to 4 Ω and 146 Ω, respectively. However, Rs and Rct increases to 5 Ω and 160 Ω, respectively, with electrolyte at 0.1% agar.

An increase in the concentration of agar to 0.15% increased Rs and Rct to 6 Ω and 160 Ω, respectively. The increase in Rs is due to the increase in viscosity of the solution and the presence of agar in the electrolyte. The increase in Rct indicates the resistance offered by the biofilm on the electrode-electrolyte interfaces. Increasing the viscosity of the solution with agar, decreases the settling/sedimentation rate, thereby improving biofilm formation by providing stability to the biofilm. Further increasing the concentration of agar to 0.20% increased Rs to 10 Ω which is nearly 30 times higher than the control. Similarly, Rct also increased to 160 Ω which is two times higher than the control. These results corroborate well with other electrochemical experiments (voltammetry). However, the effects of rheology modifiers on the solution resistance and charge transfer resistance can be minimized by increasing the conductivity of the electrolytes, pH of the solution, or using conducting polymers in electrolyte/electrode surfaces. Conducting polymers will greatly aid in improving the conductivity of the electrolyte, but it should be noted that it does not affect the growth of the microorganisms or its electron transfer characteristics.

3.4. Kinetics of fuel consumption

The effect of different electrolytes on electrocatalysis rates was performed using COD analysis. COD values measure the amount of substrate (including polymer) lost with other oxidation processes via microbial electrocatalysis. Though glucose is used as the substrate, glucose estimation assays (reducing sugar assay using Dinitrosalicylic acid method) or the Total carbohydrate test (Phenol sulphuric acid method) could not be used for assessing the kinetics of fuel consumption because agar in the electrolytes interferes with the reaction, leading to erroneous results. Therefore, COD values were recorded for every day, and are shown in Fig. 5. COD removal was slow at the initial phase while the microorganisms adapted to the electrochemical environment. After 24 h, the rate of oxidation of substrate increased rapidly and thereafter slowed down. This clearly depicts bacterial growth lag, log, stationary, and death/decline phases.

COD studies were carried out for several days and showed that in all cases, COD levels decreased. In most cases, COD levels have been shown to decrease at the end of seven days. However, COD increased in some cases due to metabolites produced by microorganisms or the presence of microbial cells/debris in analytes. It was difficult to remove microbial cells/cell debris from analytes taken for COD analysis because of the viscosity of the electrolyte. Electrolytes without agar had initial and final COD values of 4010 mg/L (Day 0) and 3460 mg/L (Day 5), respectively, and had a minimal COD removal rate of 13%. The microbial electrocatalytic system with electrolytes at 0.05, 0.1, and 0.15% agar had COD removal rates of 77, 80, and 79%, respectively, which indicated that mass transfer was not affected significantly with an addition of agar up to 0.15%. However, the bioelectrocatalytic system with electrolyte containing 0.2% agar had a very low COD removal rate of 63% when compared with the control (COD removal rate of 88%).

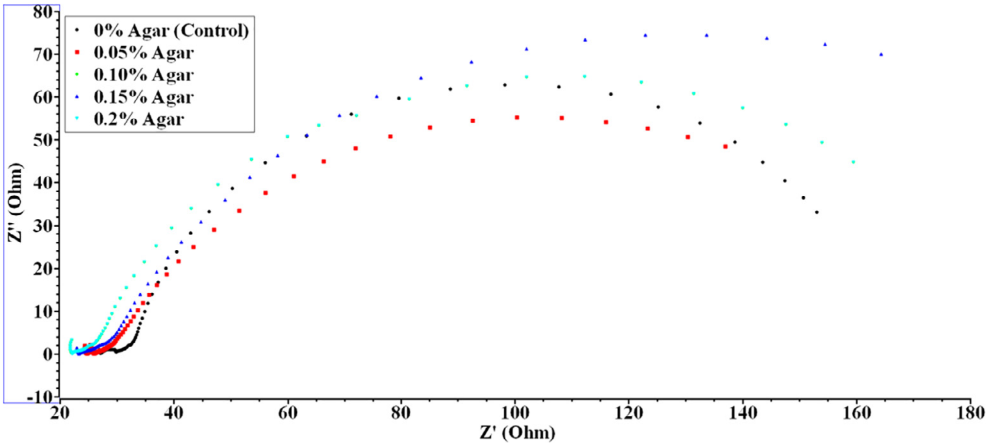

Fig. 4. Nyquist Plots for Microbial Electrochemical System (containing thermophilic *Geobacillus* sp. 44C) in phosphate buffer electrolyte (0.1 M, pH 7) with different concentrations of agar (0–0.15% agar).

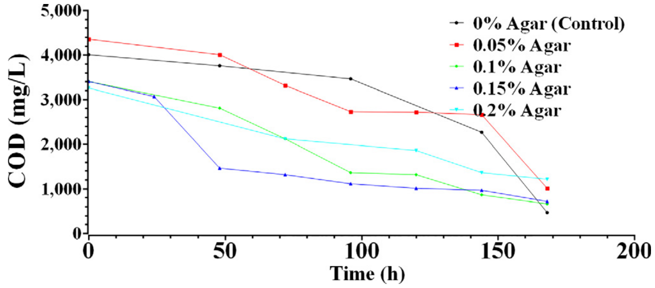

Fig. 5. Kinetics of COD in BES with different electrolytes.

3.5. Morphological characterization of bioelectrodes

SEM of carbon felt electrodes with biofilms of *G-44C* after the bioelectrocatalytic experiments were analyzed to study the effect of agar on biofilm formation. SEM of the electrodes without agar showed that the biofilm were scarcely distributed. Thicker biofilms of *G-44C* were formed on the electrodes collected after seven days of the electrocatalytic process in the presence of the electrolyte with 0.05% agar. At an even higher agar concentration (0.10%), the effect of agar improved *G-44C* colonization onto the surface of the electrodes was visible. At the highest agar concentration (0.15%) the formation of a denser and thicker biofilm was observed.

These results indicate that the use of phosphate buffer electrolyte with agar provides a suitable sustaining environment for the adherence of *G-44C*onto the surface of carbon felt. Furthermore, it showed that unlike the control sample, the electrolyte with agar provides suitable conditions for the adherence, growth, and proliferation of *G-44C* film on the electrode surface. As a result, decreased settling of *G-44C* in the presence of agar improved mass transfer rates, leading to improved biofilm quality.

4. Conclusion

This is the first study reporting the use of the rheology modifying agent agar in electrolytes for improving mass and electron transfer rates of microbial electrocatalytic systems under thermophilic conditions. This strategy can be further improved by the use of conducting polymers and nanomaterials, along with rheology modifiers in the electrolyte to decrease the solution and charge transfer resistance in BES. This approach can be extended to the operation of different BES such as microbial electrolysis cells, microbial desalination cells, and microbial electrosynthesis.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.biortech.2018.04.089.

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