



In situ microbial fuel cell-based biosensor for organic carbon

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ABSTRACT

The biological oxygen demand (BOD) may be the most used test to assess the amount of pollutant organic matter in water; however, it is time and labor consuming, and is done ex-situ. A BOD biosensor based on the microbial fuel cell principle was tested for online and in situ monitoring of biodegradable organic content of domestic wastewater. A stable current density of 282 ± 23 mA/m² was obtained with domestic wastewater containing a BOD₅ of 317 ± 15 mg O₂/L at 22 ± 2 °C, 1.53 ± 0.04 mS/cm and pH 6.9 ± 0.1 . The current density showed a linear relationship with BOD₅ concentration ranging from 17 ± 0.5 mg O₂/L to 78 ± 7.6 mg O₂/L. The current generation from the BOD biosensor was dependent on the measurement conditions such as temperature, conductivity, and pH. Thus, a correction factor should be applied to measurements done under different environmental conditions from the ones used in the calibration. These results provide useful information for the development of a biosensor for real-time in situ monitoring of wastewater quality.

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1. Introduction

Domestic wastewater commonly contains organic matter that includes nitrogenous compounds, proteins and amino acids, and non-nitrogenous compounds, carbohydrates and lipids. A detailed characterization of the organic matter in domestic wastewater is time and labor consuming; therefore, bulk parameters like biological oxygen demand (BOD) and chemical oxygen demand (COD) are routinely used as a measure of the degree of water pollution by organic carbon. The BOD is a measure of the quantity of oxygen used by microorganisms (e.g., aerobic bacteria) to oxidize the organic matter in a sample of water during a period of 5 days (BOD₅), while the COD is used as a measure of the oxygen requirement of a sample that is susceptible to oxidation by a strong chemical oxidant (e.g., potassium dichromate). COD values are always higher than BOD values. COD measurements can be made in a few hours, while BOD measurements take five days, but they do not differentiate between biologically available and inert organic matter [1–5].

An online biosensor to quantify BOD was developed using the microbial fuel cell (MFC) principle [6]. In a MFC, microorganisms growing on the surface of an anode electrode transfer electrons from the oxidation of organic matter present in the wastewater through an electrical circuit to a cathode electrode, thus generating an electrical current [7]. A biosensor based on the MFC principle does not need a transducer to read the signal and converts it to an electrical signal because the measured signal is already an electrical current [8]. This type of biosensor is an example of the successful application of an electrochemical method in the evaluation of wastewater quality. Several configurations based on MFC have been tested and optimized to be used as a BOD biosensor. The usual configuration was a mediator-less MFC, with two chambers separated by a cation exchange membrane and continuous wastewater flow in the anode chamber [6,9–14]. The main drawback of this configuration is the complexity of the setup that is not suitable for in situ applications.

A very compact MFC configuration, known as submersible microbial fuel cell (SMFC), was developed by Min and Angelidaki [15]. This configuration uses an air-cathode which simplifies considerably the MFC configuration [16]. The aim of the present study was to adapt and test the SMFC configuration as an in situ BOD biosensor. The SMFC contained an anode electrode connected to a rectangular cathode chamber and was submerged in anaerobic wastewater. The cathode chamber was continuously flushed with air. A proton exchange membrane was assembled between anode and cathode. The membrane-electrodes assembly was pulled together to reduce the internal resistance of the cell and placed on one side of the cathode

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chamber. Several experiments were carried out with domestic wastewater containing a BOD₅ concentration in the range of 17 ± 0.5 mg O₂/L to 183 ± 4.6 mg O₂/L. The effect of temperature (11 ± 0.2 °C– 33 ± 0.3 °C), wastewater conductivity (1.1 ± 0.012 mS/cm– 13.4 ± 0.013 mS/cm) and pH (6.0 ± 0.1 – 8.5 ± 0.1) were investigated.

2. Materials and methods

2.1. Wastewater collection and composition

Domestic wastewater was collected at the Lundtofte Wastewater Treatment Plant (Lyngby, Denmark), screened to remove coarse particles and, used as a source of organic matter and inoculum to start biofilm formation on the anode surface of the BOD biosensor. The composition of the wastewater was: 470 ± 79 mg O₂/L as COD, 317 ± 15 mg O₂/L as BOD₅, 61 ± 13 mg/L total nitrogen, 12 ± 2.6 mg/L total phosphorous, 6.93 ± 0.16 pH units and 1.53 ± 0.04 mS/cm conductivity. After collection, the wastewater was sparged with argon gas to establish anaerobic conditions, and kept at 4 °C for later use.

2.2. BOD biosensor description

The BOD biosensor was constructed based on a previous Submersible Microbial Fuel Cell (SMFC) configuration described by Min and Angelidaki [15] (Fig. 1a). The electrode system was very compact to minimize the internal resistance and comprised an anode electrode in direct contact with the proton exchange membrane that was hot-pressed to the cathode electrode. The anode was made of carbon paper (Toray carbon paper, E-TEK division, USA) with 9 cm². The cathode, with similar dimensions to the anode, was made of 5% wet proofed carbon paper. The cathode's surface in direct contact with the proton exchange membrane (Nafion™ 117, DuPont Co., USA) was covered by a Pt catalyst (0.5 mg/cm² with 20% Pt, E-TEK). The electrodes were connected to a resistance with 1 kΩ using copper coated wires (2 mm diameter).

The electrode system (Fig. 1b) was connected to one side of a rectangular cathode chamber made of non-conductive polycarbonate material. The other side of the cathode chamber was closed with a plate of the same material. The cathode electrode faced the inside of the chamber while the anode, in the opposite side, was in direct contact with the wastewater to be analysed. Air was continuously sparged inside the cathode chamber and functioned as the final electron acceptor. To assemble the BOD biosensor and to keep it tight, rubber gaskets and stainless steel screws were used, as depicted in Fig. 1.

2.3. BOD biosensor evaluation

Two biosensors were operated simultaneously at room temperature (22 ± 2 °C) during 3 weeks to promote biofilm formation on the surface of the anode. Subsequently, one of the two biosensors was discontinued and the biofilm was investigated by Scanning Electron Microscopy (SEM). The other was operated to evaluate current density as a function of BOD₅ concentration in wastewater. The influence of environmental parameters was also tested.

2.3.1. Biofilm formation

The BOD biosensor was immersed into 1 L domestic wastewater contained in a glass vessel tightly closed with a glass lid (1.5 L total volume), as described in Fig. 1c. The vessel was stirred at 200 rpm and the wastewater was initially sparged with argon gas for 10 min to obtain anaerobic conditions. Subsequently, the cathode chamber was continuously flushed with air (final electron acceptor) at a flow rate of 5 mL/min. The voltage difference between anode and cathode electrodes was measured across a fixed resistance with 1 kΩ every 10 min using a multimeter (Fluke), and data were collected automatically by a data acquisition program and a computer. The wastewater was replaced periodically to avoid organic carbon limitation. The biofilm formed on the anode electrode reached steady-state when similar values of voltage were obtained 3 times consecutively. Then, the polarization curve

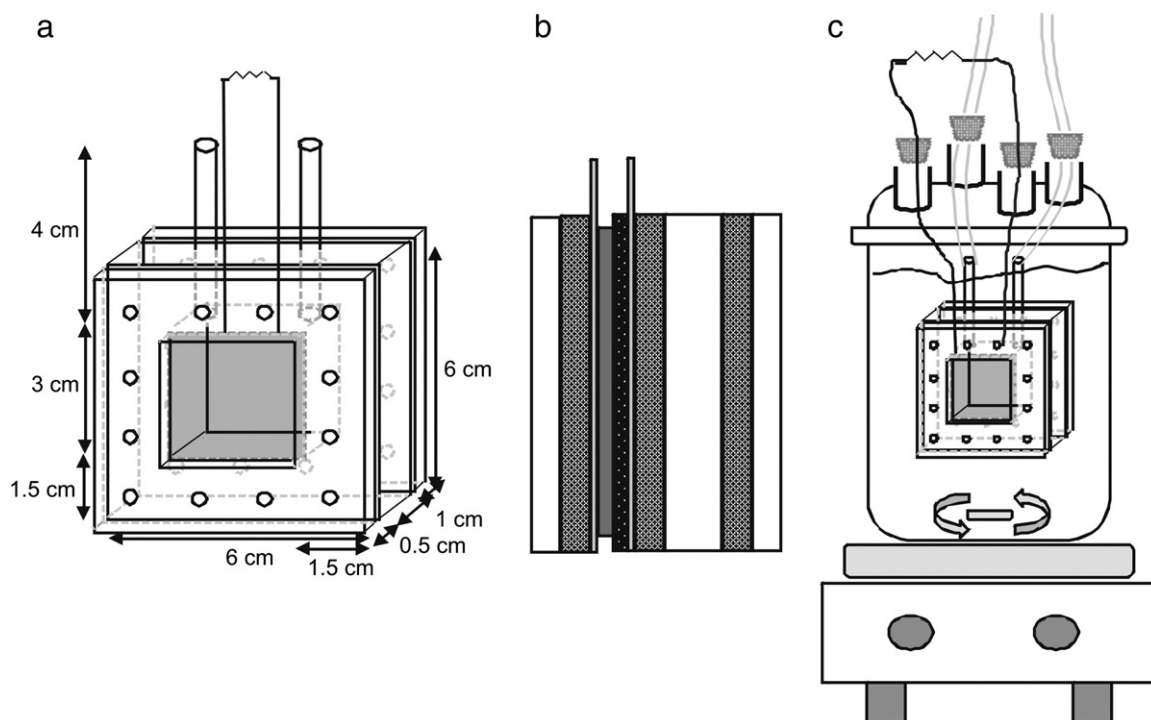


Fig. 1. Schematic diagram of the submersible microbial fuel cell type BOD biosensor (a), the electrode system (b) and the experimental set-up (c). For the electrode system: polycarbonate support (white), rubber gaskets (hatched), wires (grey), anode (dark grey) and cathode hot pressed with proton exchange membrane (dotted).

(voltage (U) versus current density (j)) and the power curve (power density (P) versus current density (j)) were recorded using a series of resistances in the range of 22 k Ω to 100 Ω (domestic wastewater with a BOD₅ of 298.2 $\pm$ 4.2 mg O₂/L) [17]. The open circuit voltage (U(i=0), where i is the current intensity) was determined at infinite resistance. At last, one of the two biosensors was discontinued and the biofilm was investigated by SEM as described previously [18].

2.3.2. Effect of BOD₅ concentration and environmental parameters on current density

To evaluate maximum current density with different concentrations of BOD₅, five wastewater dilutions were prepared with tap water (17 $\pm$ 0.5 mg O₂/L–183 $\pm$ 4.6 mg O₂/L). The maximum voltage between anode and cathode electrodes was measured 2 times consecutively for each BOD₅ concentration across a fixed resistance with 1 k Ω , at which the maximum power was obtained from the previous polarization and power curves. Temperature (T), pH, and conductivity (σ) were tested independently using diluted domestic wastewater with a BOD₅ concentration of 143.5 $\pm$ 8.7 mg O₂/L, 1.1 $\pm$ 0.012 mS/cm and pH 6.5 $\pm$ 0.2. Temperature tested varied from 11 $\pm$ 0.2 $^{\circ}$ C to 33 $\pm$ 0.3 $^{\circ}$ C. The glass vessel was immersed in a water bath and temperature was adjusted with a thermostat. The wastewater pH was adjusted with 1 M HCl or NaOH solutions to obtain pH values in the range of 6.0 $\pm$ 0.1 to 8.5 $\pm$ 0.1. The wastewater conductivity was adjusted with a KCl solution and varied between 1.1 $\pm$ 0.012 mS/cm and 13.4 $\pm$ 0.013 mS/cm.

2.4. Analytical methods and calculations

The organic content of wastewater was assessed both as COD, using chromate as the oxidant agent, and BOD₅, using the Oxitop® method, as described in Standard Methods [19]. Initially, a correlation between BOD₅ and COD was experimentally determined for the Lundtofte domestic wastewater. The following least square regression equation obtained from the results was further used to convert COD into BOD₅ values, being both expressed in mg O₂/L: BOD₅ = 0.676 COD – 1.752 (R^2 = 0.986). pH was measured with a PHM 210 pH meter (Radiometer) and conductivity with a CDM 83 conductivity meter (Radiometer).

To determine the mass of volatile suspended solids per surface area of the electrode (mass of biofilm), two electrodes (similar surface area) with and without biofilm were dried at 105 $^{\circ}$ C and weighed. Next, the electrodes were burned at 550 $^{\circ}$ C and weighed again for comparison and quantification of biofilm biomass per cm².

The current intensity (i) was calculated according to the Ohm's law, $i = U/R$, where U is the voltage and R the resistance. Current density (j) was calculated as $j = i/A_o$, where A_o is the projected surface area of the anode electrode. Power density (P) was calculated as the product of the current intensity and the voltage divided by the projected surface area of the anode electrode ($P = iU/A_o$).

3. Results and discussion

3.1. Biofilm formation

When the BOD biosensor was immersed in domestic wastewater (317 $\pm$ 15 mg O₂/L BOD₅), current kept increasing over time and, after a period of 3 weeks, it stabilized at a maximum value of 0.27 mA, corresponding to a current density of 282 $\pm$ 23 mA/m². Once the maximum current was reached, it could be maintained as long as domestic wastewater was periodically changed. The profile of current generation of a single-batch operation was characterized by a steep increase of current after addition of domestic wastewater, followed by a plateau of maximum current with a length dependent on the concentration of BOD₅, and finally a decrease of current. SEM analysis performed after the enrichment period, when the current generation

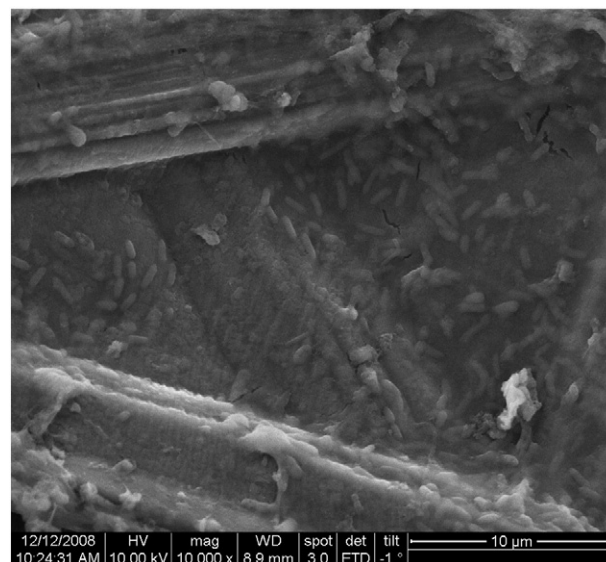


Fig. 2. Scanning electron micrograph of anode surface inoculated with domestic wastewater in the stable phase of current density generation.

was stable, showed a confluent biofilm on the surface of the anode dominated by rod shaped microorganisms (Fig. 2). The amount of biofilm formed on the anode was 5.2 $\pm$ 1.47 mg/cm², expressed as mass of volatile suspended solids per surface area of the electrode. Considering a wet biofilm density of 1.02 g/cm³, the estimated biofilm thickness was 51 μ m. The attached biomass determined in the present study was more than 10 times higher than previous reported values [20] but the thickness was of the same order of magnitude [20,21] suggesting the formation of a anode biofilm with high density.

3.2. Performance of BOD biosensor

The open circuit voltage measured in the stable phase of current generation was 414 $\pm$ 6 mV using domestic wastewater with a BOD₅ concentration of 298.2 $\pm$ 4.2 mg O₂/L. The polarization curve depicted in Fig. 3 (grey line) shows the relation between voltage (U) and current density (j) obtained by changing the resistance between electrodes from 22 k Ω to 100 Ω (current densities in the range of 20 mA/m² to 522 mA/m²). The shape of the curve confirmed the prevalence of ohmic losses generated by membrane resistance, electrolyte resistance of the wastewater, and bacterial metabolism. The initial steeper slope occasionally observed in polarization curves due to activation losses was not observed in the present study. The maximum power density (P) obtained in the power curve (Fig. 3,

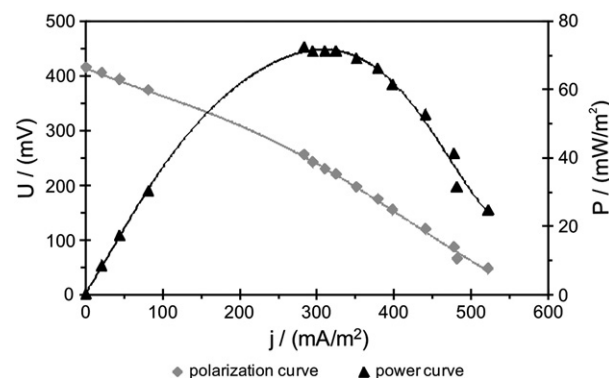


Fig. 3. Polarization and power curves obtained by varying the external resistance between the electrodes, ranging from 22 k Ω to 100 Ω (domestic wastewater with a BOD₅ concentration of 298.2 $\pm$ 4.2 mg O₂/L at 22 $^{\circ}$ C).

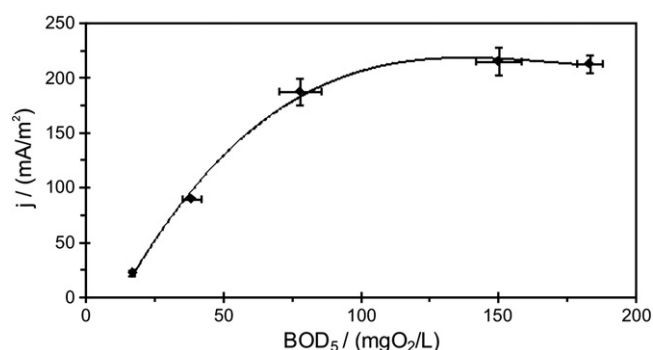


Fig. 4. Current density (j) generation in the BOD biosensor as a function of initial BOD₅ concentration of domestic wastewater at 22 °C ($R = 1 \text{ k}\Omega$).

black line) was 72 mW/m^2 , which is equivalent to a current density of 283 mA/m^2 ($1 \text{ k}\Omega$). These values are similar to the ones obtained in the stable phase of the SMFC power generation using an external resistance of $1 \text{ k}\Omega$. In effect, the variation of resistance values did not cause any change in power density.

3.3. Current generation at various BOD₅ concentrations

The maximum current density generated from domestic wastewater with a BOD₅ concentration in the range of $17 \pm 1 \text{ mg O}_2/\text{L}$ to $183 \pm 5 \text{ mg O}_2/\text{L}$ was determined (Fig. 4). The current density increased linearly with BOD₅ up to a concentration of $78 \pm 8 \text{ mg O}_2/\text{L}$ ($j = 2.68 \text{ i} - 18.78$ with $r^2 = 0.996$). The range of BOD₅ concentrations that were evaluated with the BOD biosensor is in agreement with those published in literature [6,9,11,12,20] (Table 1). The maximum current density obtained in the present study was 222 mA/m^2 , which is similar to those obtained by Gil et al. [9] and Kim et al. [6]. BOD₅ concentrations higher than $78 \pm 8 \text{ mg O}_2/\text{L}$ resulted in further slow increase in the current density until reaching a plateau value. These experiments also revealed that the higher the BOD₅ concentration, the longer the BOD biosensor took to reach a new steady-state current (i.e., response time) after changing the strength of the wastewater. The response time for a BOD₅ concentration of $17 \pm 1 \text{ mg O}_2/\text{L}$ was shorter than 30 min, however, about 10 h were needed for concentrations higher than $78 \pm 8 \text{ mg O}_2/\text{L}$. Several values are reported in literature for this parameter which encompasses the ones determined

in the present study (Table 1). The main contribution of the proposed BOD biosensor compared to others is the fact that it is very compact and can be used in situ.

3.4. Effect of environmental conditions on current generation

The effect of changes in environmental parameters in the performance of the biosensor was assessed using domestic wastewater with a BOD₅ of $144 \pm 9 \text{ mg O}_2/\text{L}$. The current density increased linearly with temperature (Fig. 5a), about $6 \text{ mA}/(\text{m}^2 \text{ } ^\circ\text{C})$, in the range of $11 \pm 0.2 \text{ } ^\circ\text{C}$ to $33 \pm 0.3 \text{ } ^\circ\text{C}$. The maximum current density (288 mA/m^2) was obtained at pH 7.0. Minimum values were observed at pH 6 ± 0.1 and pH 8 ± 0.1 , corresponding to current densities of 186 mA/m^2 and 184 mA/m^2 , respectively (Fig. 5b). These results are consistent with literature studies regardless of the MFC configuration and organic carbon sources used [8]. The current density increased with the conductivity of domestic wastewater in the range of $1.1 \pm 0.012 \text{ mS/cm}$ to $7.51 \pm 0.01 \text{ mS/cm}$, from 199 mA/m^2 to 316 mA/m^2 , respectively, the effect being more important between 1.1 ± 0.012 and $2.1 \pm 0.013 \text{ mS/cm}$ (Fig. 5c). These results suggested that a correction factor should be applied to measurements done under other environmental conditions. It is clear that further work is required to assess the long term operational stability of the BOD biosensor proposed in the present study. Also, the effect of competing electron acceptors present in the wastewater, such as oxygen, nitrate, and sulfate, on the generated current density should be determined.

4. Conclusions

A mediator-less submersible microbial fuel cell was tested as an in situ BOD biosensor. BOD₅ values of up to $78 \pm 8 \text{ mg O}_2/\text{L}$ could be measured based on a linear relation between organic carbon content of the wastewater and current density, and the biosensor response time was lower than 10 h. The current density measured with the biosensor was affected by changes in pH and temperature and the measured values need to be corrected.

The biosensor developed and tested in the present work has an application in the field of wastewater with a medium to low organic carbon concentration. Its main advantage is that no special anaerobic chamber (anode chamber) is needed because the sensor might be directly submerged in a wastewater channel or anaerobic reactor.

Table 1
MFC-type biosensors and their respective characteristics.

Configuration	Electron donor	Enrichment	Measuring range	Max concentration*	Maximum current intensity	Maximum current density	Response time	Reference
Mediator-less 1-chamber MFC (SMFC)	Wastewater	Wastewater (3 weeks)	17–183 mg O ₂ /L BOD	78 mg O ₂ /L BOD	0.2 mA (9 cm ²)	222 mA/m ²	30 min–10 h	Present study
Mediator-less 2-chamber MFC	Glucose and glutamate	Activated sludge (4 weeks)	20–200 mg O ₂ /L BOD	100 mg O ₂ /L BOD	6 mA (62 cm ²)	968 mA/m ²	60 min	Chang et al. [11]
Mediator-less 2-chamber MFC	Wastewater	Enriched culture of electrochemical active bacteria (5 weeks)	10–400 mg O ₂ /L COD	50 mg O ₂ /L COD	1.7 mA (56 cm ²)	304 mA/m ²	Not available	Gil et al. [9]
Mediator-less 2-chamber MFC	Wastewater	Enriched culture of electrochemical active bacteria	2.6–206 mg O ₂ /L BOD	25 mg O ₂ /L BOD	1.1 mA (56 cm ²)	196 mA/m ²	30 min–10 h	Kim et al. [6]
2-chamber MFC C-type	Glucose and glutamate	Activated sludge (8 weeks)	20–200 mg O ₂ /L BOD	200 mg O ₂ /L BOD	2.9 mA (16 cm ²)	1812 mA/m ²	5 min–10 h	Kim et al. [22]
2-chamber (MFC) C-type	Glucose and glutamate	Enriched culture of electrochemical active bacteria	50–100 mg O ₂ /L BOD	Not available	1.9 mA	Not available	5 min	Moon et al. [12]
2-chamber MFC Oligotrophic	Glucose and glutamate	Oligotrophic microbes	2–10 mg O ₂ /L BOD	10 mg O ₂ /L BOD	8 μA (13 cm ²)	6.15 mA/m ²	60 min	Moon et al. [13]

*Maximum concentration of the interval where a linear relation between x and y was observed.

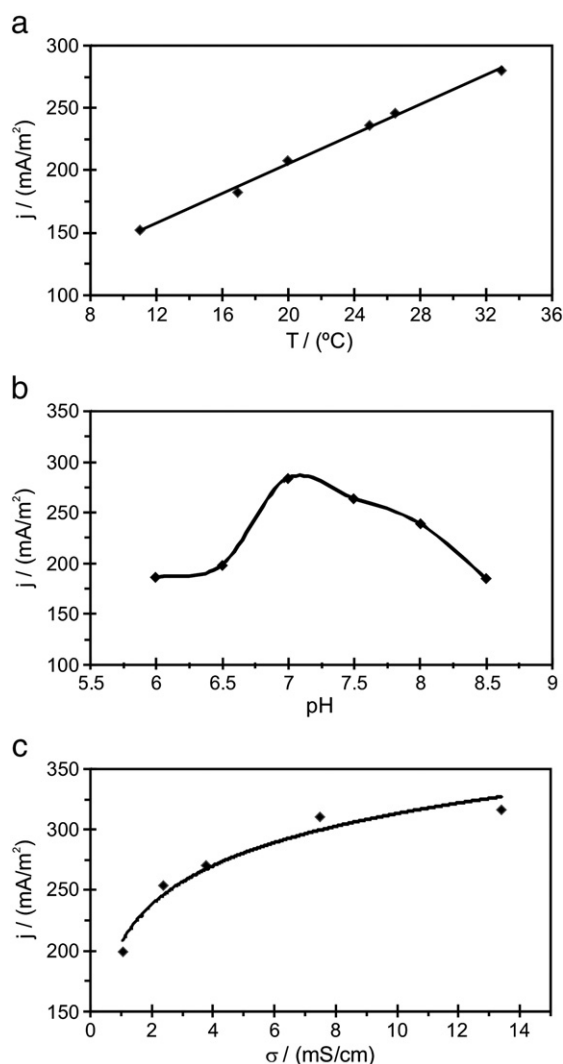


Fig. 5. Effect of temperature (T) (a), pH (b) and conductivity (σ) (c) on current density (j) generation in the BOD biosensor (143.5 ± 8.7 mg O_2 /L of BOD₅).

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