

Article

Heterotrophic Anodic Denitrification in Microbial Fuel Cells

Jakub Drewnowski¹ and Francisco Jesus Fernandez-Morales^{2,*}

¹ Faculty of Civil and Environmental Engineering, Gdansk University of Technology, ul. Narutowicza 11/12, 80-233 Gdansk, Poland; jdrewnow@pg.gda.pl

² Department of Chemical Engineering, University of Castilla-La Mancha, Avda. Camilo José Cela S/N, 13071 Ciudad Real, Spain

* Correspondence: fcojesus.fmorales@uclm.es; Tel.: +34-926-295-300 (ext. 6350)

Academic Editor: Andrew Kusiak

Received: 31 January 2016; Accepted: 13 June 2016; Published: 17 June 2016

Abstract: Nowadays, pollution caused by energy production systems is a major environmental concern. Therefore, the development of sustainable energy sources is required. Amongst others, the microbial fuel cell (MFC) seems to be a possible solution because it can produce clean energy at the same time that waste is stabilized. Unfortunately, mainly due to industrial discharges, the wastes could contain nitrates, or nitrates precursors such ammonia, which could lead to lower performance in terms of electricity production. In this work, the feasibility of coupling anodic denitrification process with electricity production in MFC and the effect of the nitrates over the MFC performance were studied. During the experiments, it was observed that the culture developed in the anodic chamber of the MFC presented a significant amount of denitrificative microorganisms. The MFC developed was able to denitrify up to 4 ppm, without affecting the current density exerted, of about 1 mA/cm². Regarding the denitrification process, it must be highlighted that the maximum denitrification rate achieved with the culture was about 60 mg·NO₃⁻·L⁻¹·h⁻¹. Based on these results, it can be stated that it is possible to remove nitrates and to produce energy, without negatively affecting the electrical performance, when the nitrate concentration is low.

Keywords: Bio-electrochemistry; nitrogen removal; electricity generation; microbial fuel cell; electron acceptor

1. Introduction

Microbial fuel cells (MFC) are electrochemical devices that exploit the metabolic abilities of the microorganisms to convert the chemical energy contained in organic and inorganic substrates into electricity [1]. In an MFC, substrates are oxidized at the anodic chamber, whereby the electrons are released, directly or by means of mediators, to the solid electrode. The electrons flow through an external circuit, finally reaching the cathodic chamber. At the cathode, the electrons are used to reduce an oxidizing agent, usually oxygen [2]. In this way substrates are oxidized at the same time that an electrical current is produced [3–6].

The microorganisms that power MFC can oxidize a diverse range of “dirty” fuels that are often of little perceived value. Amongst these dirty fuels, organic waste, organic matter contained in soils and sediments, compost, *etc.*, can be found [7,8]. Because of that, one of the most attractive energy sources for MFCs is wastewater, mainly because electricity production could be combined with wastewater treatment [9,10]. The use of MFCs for wastewater treatment presents several advantages, such as savings in aeration and solids handling. The aeration process account for half of the operation costs at a typical treatment plant [11] but it is not necessary in MFC. Moreover, the MFC process is inherently an anaerobic process, the sludge yields for an anaerobic process are approximately one-fifth of that for

an aerobic process [12]. Thus, using MFCs could drastically reduce solids production at a wastewater treatment plant, substantially reducing operating costs for solids handling [13].

Nowadays, the excessive application of fertilizers, intensive exploitation of farms and the significant contribution from industry have increased the nitrogen load discharged to receiving waterways [14–16]. This has led to a decrease in water quality and has caused health problems related to oxidized forms of nitrogen. When dealing with wastewaters, the power output of MFC is sometimes reduced by the presence of oxidizing agents, such as nitrates. The reduction in the power density output is caused because these nitrates could be used to oxidize, under anoxic conditions, the substrates contained in the wastewater without net electricity generation. For these reasons, when working with wastewaters containing high concentrations of nitrogen species, the heterotrophic denitrification process could consume significant amounts of chemical oxygen demand (COD) in MFC [17], therefore reducing the Coulombic efficiency (CE) of the system.

The overall rate and extension of the denitrification process depends mainly on the biodegradability characteristics of the electron donor used [18,19], N/COD ratio in the bioreactor [5,20] and on the metabolic abilities of the culture. Because of that, in order to obtain the highest electricity yields, the anodic chamber should operate under anaerobic conditions. Only under anaerobic conditions do the electron acceptors avoid limiting the associated COD consumption.

Unfortunately, the absence of oxidized nitrogen compounds cannot be guaranteed when domestic or industrial wastewater is fed to the MFC. This can be explained because on one hand the ammonia nitrogen could be oxidized by nitrificative microorganisms contained in the biofilm formed in the anodic chamber, therefore generating nitrates. On the other hand, industrial wastewater discharges could happen in the sewage net. Amongst other factors, wastewaters generated by several industrial activities, such as synthetic fibers, mineral processing, fertilizers, metal finishing, and ammunitions and explosives industries are characterized by a very high concentration of nitrates, even higher than 1500 ppm [21]. In these cases the MFC should deal with nitrates, and therefore the influence on the MFC performance must be studied. In the literature, the electricity generation using MFCs has been studied and described using simple organic compounds, such as acetate, butyrate, glucose, *etc.* [3,22,23] and complex wastewaters such as sewage [24] or industrial wastewaters [25–27]. The nitrate removal in the cathodic chamber has been also studied [28–31]. However, hardly any study dealing with the denitrification ability of the anodic MFC consortia and the effects of the NO_3^- over the performance of the MFC has been published. In these situations it is important to define whether the bacteria will use the solid electrode in presence of an alternate electron acceptor. The use of the external electron acceptor could reduce or even stop the electricity production which is very important because that would limit the potential MFC potential applications. Because of that, nitrate removal is a key aspect for achieving an optimum performance in MFC.

In this context, the aim of this paper was to study the denitrification ability of a MFC consortia when treating wastewater. In this study, the effect of different nitrate concentrations over the denitrification rate, electricity production, Coulombic efficiency and COD removal was evaluated.

2. Materials and Methods

2.1. MFC Configuration

The experimental set-up used in this work consisted of a two-chambered micro-scale MFC separated by a Sterion[®] membrane (Alfa Aesar, Heysham, UK). Both chambers were built on a graphite plate and their dimensions were: 0.95 cm³ of volume for the anodic chamber and 0.5 cm³ of volume for the cathodic chamber, which had serpentine channels. Toray carbon papers TGPH-120 (Fuel Cell Store, Texas, USA) were used as electrodes in both chambers, the anodic electrode with 20% Teflon content and the cathodic one with 10%. Furthermore, a microporous catalytic layer with 0.5 mg·Pt·cm⁻² loading was deposited on the cathode because of the advantages that this additional layer offers. The active areas of the anodic and cathodic electrodes were 4.65 cm² and 2.85 cm²,

respectively. An air breathing cathode was used. This system uses free convection airflow to supply oxygen to the cathode [2]. A schematic view of the set-up is shown in Figure 1.

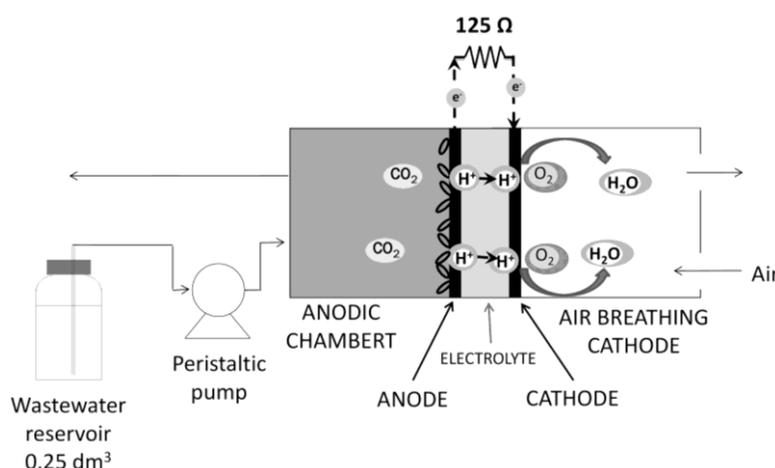


Figure 1. Schematic view of the set-up.

The MFC was based on a membrane-electrode assembly, it was performed applying a load of 1 ton for 15 minutes. The electrodes and membrane were introduced between two stainless steel blocks equipped with heating surfaces and the temperature was controlled by a control system at 130 °C [32]. Working in this way, the distance between the electrodes is minimized, reducing the internal resistance of the MFC.

During operation of the MFC, the anode and the cathode were connected by means of an external load of 125 Ω. The potentials between the edges of this resistance were continuously monitored by using a high-impedance electrometer Keithley 2000. These potentials were directly related to the exerted electrical current according to the Ohms law.

$$I_{MFC} = \frac{E_{125\Omega}}{125} \quad (1)$$

A external load of 125 Ω was used in order to facilitate the development of an electrogenic culture in the MFC.

2.2. Microbial Fuel Cell Inoculation and Operation

The anodic compartment was seed with electrogenic organisms from a working MFC [32]. The MFC was continuously operated by feeding synthetic wastewater through the anodic chamber at a flow rate of 0.2 cm³·min⁻¹. To do that, the anodic compartment of the MFC was connected to a wastewater reservoir of 250 mL, in which the wastewater was stored. In order to avoid the degradation of the wastewater during its storage, it was sterilized for 20 min at 120 °C [33].

The composition of the synthetic wastewater used in the experiments is presented in Table 1.

Table 1. Composition of the synthetic wastewater fed to the microbial fuel cell (MFC).

Component	Concentration (mg/L)	Component	Concentration (mg/L)
Glucose	161	KH ₂ PO ₄	44.5
Fructose	161	MgCl ₂	37.1
NaHCO ₃	111	CaCl ₂	30.7
(NH ₄) ₂ SO ₄	74.2	(NH ₄) ₂ Fe(SO ₄) ₂	3.1

In order to evaluate the influence of the nitrate presence on the performance of the MFC, nitrate loads ranging from 0 to 15 ppm were added to the fed influent. These modifications were maintained during 10 h and the influence over the MFC performance was evaluated.

In the MFC, the electrical current was generated due to the organic matter oxidation. This oxidation was monitored by measuring the COD removal rate (rCOD). The denitrification rate was determined by measuring nitrates and nitrites. It is important to measure not only nitrates but also nitrites because nitrite accumulation has been reported in numerous denitrification studies [34,35]. The evaluation of the nitrite accumulation is important for two reasons; firstly, neglecting it would increase the real denitrification rate obtained if nitrates are supposed to be completely reduced and, secondly, the real denitrification rate will be decreased if oxidized nitrogen were measured. The corrected value for the amount of nitrate-nitrogen that is completely reduced during the anoxic experiment can be evaluated by taking into account the variations in nitrates and nitrites, Equation (2) [36].

$$\Delta_{N-corrected} = (\Delta_{N-NO_3} - 0.6 \cdot \Delta_{N-NO_2}) \quad (2)$$

The relationship between the COD removal rate, the denitrification rate and the electricity generation would inform us about the possibility of coupling the denitrification process with the electrogenic one without affecting the latter. In this sense, the determination of the exerted current and the CE would serve as accurate indicators of the electrogenic performance of the MFC.

The CE, can be calculated using Equation (3).

$$CE = \frac{M \cdot \int_0^t I \cdot dt}{n \cdot F \cdot V_{anodic} \cdot r_{COD}} \quad (3)$$

where:

M is the molecular weight of oxygen I is the electrical current

n is the stoichiometry of the oxidation of the COD, (Mol COD mol⁻¹ · e⁻)

V is the volume

rCOD is the COD removal rate

Faraday constant (96485 C mol⁻¹ · e⁻)

2.3. Sampling Procedure and Analytical Methods

Samples were taken by syringe and were immediately filtered through a Millipore glass fiber filter. After filtration, samples were analyzed for the following parameters: COD, NO₂⁻ and NO₃⁻. The COD and the oxidized nitrogen compounds were measured according to Standard Methods [37].

3. Results and Discussion

In order to establish reference values for electricity generation in the MFC, the set-up was inoculated and operated for several weeks without oxidized nitrogen addition. Once the steady-state was reached, the system was operated for four weeks in order to evaluate the stability of the system. During these weeks, the current density exerted was about 0.9 ± 0.05 mA · m⁻² (see Figure 2).

In Figure 2 it can be seen that, after several weeks of lag phase, an exponential growth appears. Once the exponential stage had finished, a very stable current density was maintained. Once the steady-state was verified, the influent wastewater that fed to the MFC was supplemented with different nitrate loads in order to evaluate its effect on the overall performance of the system. The nitrate concentrations in these solutions ranged from 0 to 15 ppm. In order to evaluate the existence of hysteresis in the behavior of the system, the concentrations of the solutions were first step-wise increased and then step-wise decreased.

The experiment with each nitrate solution was maintained for 10 h. During each test, the exerted voltage was continuously determined and liquid samples were taken. The exerted current density for

each nitrate concentration fed to the MFC and its corresponding standard deviation is presented in Figure 3.

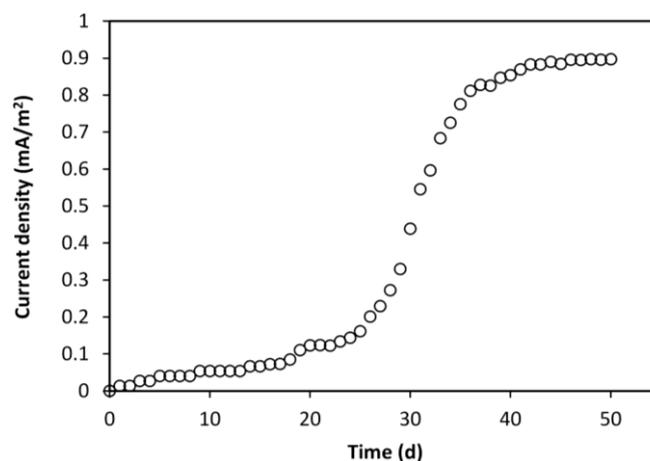


Figure 2. Current density *vs.* time along the start-up process.

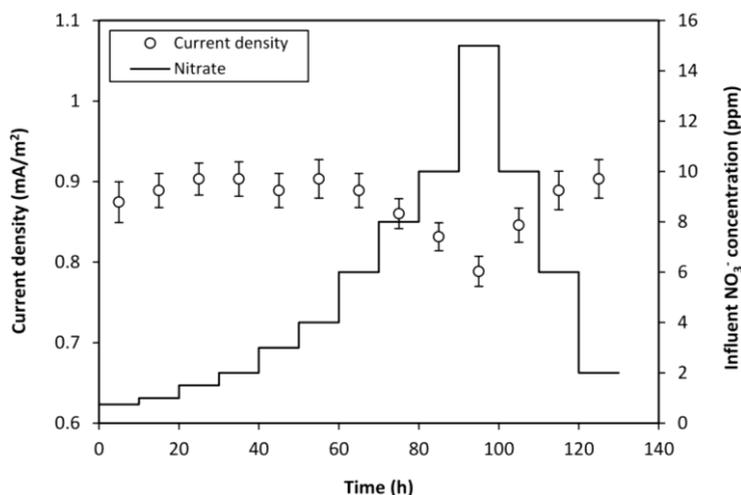


Figure 3. Effect of the nitrate concentration over the exerted voltage.

From the obtained results, it must be stated that no hysteresis was observed in the behavior of the system. As can be seen in Figure 3, the exerted current density for the same influent nitrate concentration was the same in the forward and in the reverse scan. This result indicates that short-term exposition to external electron acceptors does not modify the response of the system once the perturbation is finished.

Regarding the nitrate effect on the performance of the MFC, it was observed that low influent nitrate concentrations, ranging from 0 to 4.0 ppm, do not reduce the current density exerted. This result could be related to a negligible effect of the nitrate concentration over the electricity production of the MFC. However, concentrations higher than 6.0 ppm reduced the current density exerted by the MFC. This change in the behavior of the MFC could be explained because of the negative effects caused by the nitrate concentration over the electricity production or because of the competence between the electrogenic and denitrificative microorganisms for the COD available in the MFC. In this research, the latter statement was refused because all the tests were performed with an excess of COD.

In order to get a better knowledge of the denitrification process and the effects of the nitrates over the MFC performance, the effluent nitrate and nitrite concentrations were determined in each test. The effluent nitrite concentration was negligible in all the experiments performed, without any relationship

with the influent nitrate fed to the MFC. However, the effluent nitrate concentration directly depended on the influent concentration. These results are presented in Figure 4.

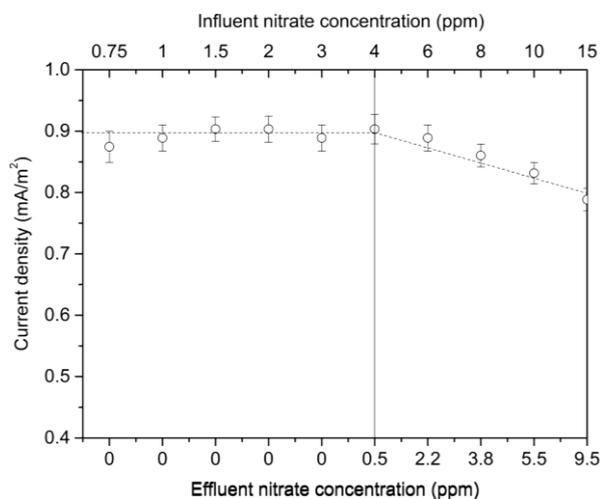


Figure 4. Exerted voltage as function of the influent and effluent nitrate concentration.

As can be seen in Figure 4, when the effluent nitrate concentration was negligible the exerted current density remained unaffected. This corresponds to influent nitrate solutions ranging from 0 to 4 ppm as was previously indicated in Figure 3. However, when the influent nitrate concentration was higher than 4 ppm, the availability of the nitrates in the anodic chamber generated a pure anoxic environment characterized by full availability of the electron acceptor. In these conditions, the nitrate was always present, which affected the electrical performance of the MFC. The reduction of the voltage exerted was about $0.013 \text{ mA} \cdot \text{m}^{-2}$ per ppm of NO_3^- present in the MFC.

Regarding the denitrification process, it must be highlighted that the maximum denitrification rate achieved with the MFC culture was about $60 \text{ mg} \cdot \text{NO}_3^- \cdot \text{L}^{-1} \cdot \text{h}^{-1}$, which is a similar value to those obtained in conventional nitrification-denitrification processes for nitrogen removal [5,17,36,38].

In order to study the substrate removal coupled to the denitrification process, the COD consumption due to the denitrification process was calculated according to the equation proposed in the literature [36].

$$\frac{dS_{DQO}}{dt} = \frac{2.86}{1 - Y_M} \times \frac{dS_{N-NO_3}}{dt} \quad (4)$$

In Figure 5 we present the COD consumption due to the denitrification process and the total COD removal due to all the processes taking place in the system.

As can be seen in Figure 5, the denitrificative COD removal was proportional to the nitrate availability in the MFC in both the first and the second stages. It is also important to remark that no saturation was observed, which could be explained by the low nitrate concentration applied in these tests. Regarding the total COD removal in the MFC, it must be highlighted that two different trends were observed. On one hand, during the first stage, the increase in the influent nitrate concentration leads to an almost proportional COD removal. This indicates that the system behaves in a very similar way, the only difference being that a higher extension of the denitrification process was taking place, therefore consuming the stoichiometric COD. Apart from the higher COD consumption due to the stoichiometry of the denitrification process, no significant influence was observed in the performance of the MFC. It is also important to remark that no saturation was observed, which could be explained by the low nitrate concentration applied in these tests. However, when working with influent nitrate concentrations higher than 4 ppm, the COD removal trend shifted to a stepped descendent one. This response was the opposite to the expected one, because the higher the nitrate load the higher the COD consumption for denitrification. Moreover, the decrease in the COD removal was significantly higher

than the decrease in the voltage exerted. This behavior could be explained because of the inhibition of anaerobic side reaction taking place at the MFC. In the literature it has been described that several non-electrogenic anaerobic side reactions, such as acidogenic fermentation, methanogenesis, *etc.*, take place in the anodic chamber of MFC [39]. In the literature, it has been also stated that high nitrate concentrations could inhibit the anaerobic microorganisms [12], reducing its COD consumption, and therefore reducing the COD removal from the system. Fitting the trend observed in both stages, it was observed that in stage I the denitrification process removed about 7.5 mg COD per mg NO_3^- added. However, in stage II, the negative effect of the nitrates reduced the COD removal in the system by about 5.5 mg COD per mg NO_3^- added. These trends corroborate the possible inhibition caused by the high influent nitrate concentration.

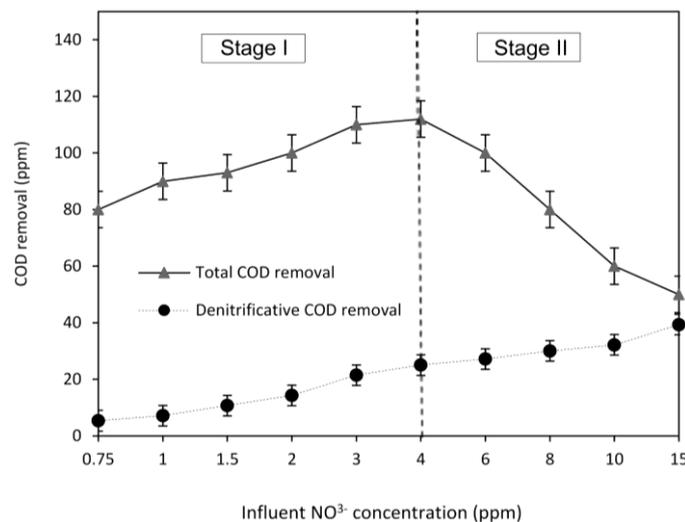


Figure 5. Effect of the influent nitrate concentration over the COD removal.

In addition to the effect of the influent nitrates over the conventional anaerobic processes, a possible effect on the electrogenic processes could also be possible. In order to study that, the coulombic efficiency was determined for each influent nitrate concentration. The Coulombic efficiency obtained is presented in Figure 6.

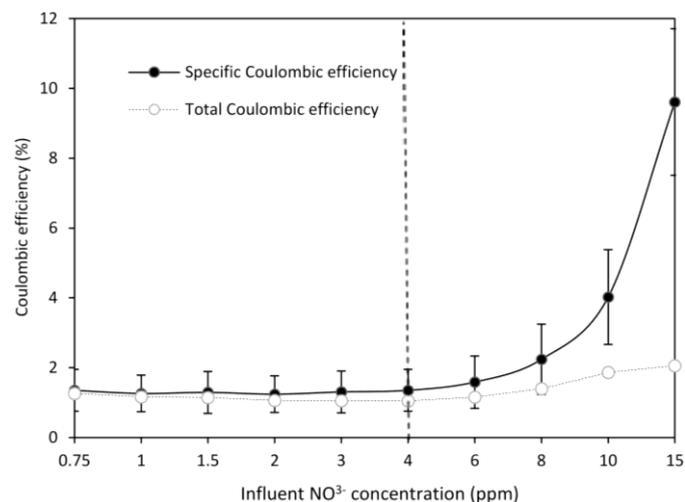


Figure 6. Coulombic efficiency vs. influent nitrate concentration.

In this figure it can be seen that, the higher the influent nitrate the lower the Coulombic efficiency observed. In order to isolate the effect of the influent nitrate, two coulombic efficiencies were determined. The total coulombic efficiency was determined taking into account the total COD removal and the electricity exerted, whereas the Specific Coulombic efficiency was determined by taking into account only the COD removed by different processes than the denitrification process. In this Figure it can be observed that the presence of nitrate enhanced the Coulombic efficiency, this could be explained because of the inhibition of the side reactions taking place at the anodic chamber of the MFC. Of course, some limitation of the electrogenic process could occur due to the full availability of electron acceptors at the anodic chamber. However, this effect was lower than that experienced by the side reactions, leading the system to a better Coulombic efficiency.

4. Conclusions

From the investigation described in this paper, the following conclusions were obtained:

- Influent concentrations lower than 4 ppm allow electricity generation because of the full exhaustion of the oxidant inside the anodic chamber of the MFC.
- When the nitrate is not quickly consumed, the presence of nitrate would decrease electricity generation due to the competition of the denitrification process with the electrogenic process. The reduction of the voltage exerted was about 0.1 mV per ppm of NO_3^- present in the MFC.
- The presence of nitrate mainly affects the non-electrogenic processes, increasing the Coulombic efficiency of the MFC.
- The maximum nitrate removal rate reached in the MFC was about $60.0 \text{ mg} \cdot \text{L}^{-1} \cdot \text{h}^{-1}$.

Acknowledgments: Authors thanks the JCCM for the financial support thorough the Project POII10-0329-5194.

Author Contributions: Francisco Jesus Fernandez Morales designed and perform the experiments; Francisco Jesus Fernandez Morales and Jakub Conrad Drewnosky analyzed the data and wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

CE	Coulombic Efficiency
COD	Chemical Oxygen Demand
rCOD	COD removal rate

References

1. Ren, H.; Pyo, S.; Lee, J.I.; Park, T.J.; Gittleson, F.S.; Leung, F.C.C.; Kim, J.; Taylor, A.D.; Lee, H.S.; Chae, J. A high power density miniaturized microbial fuel cell having carbon nanotube anodes. *J. Power Sour.* **2015**, *273*, 823–830. [[CrossRef](#)]
2. Mateo, S.; Rodrigo, M.; Fonseca, L.P.; Cañizares, P.; Fernandez-Morales, F.J. Oxygen availability effect on the performance of air-breathing cathode microbial fuel cell. *Biotechnol. Prog.* **2015**, *31*, 900–907. [[CrossRef](#)] [[PubMed](#)]
3. Chaudhuri, S.K.; Lovley, D.R. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol.* **2003**, *21*, 1229–1232. [[CrossRef](#)] [[PubMed](#)]
4. Logan, B.E.; Regan, J.M. Electricity-producing bacterial communities in microbial fuel cells. *Trends Microbiol.* **2006**, *14*, 512–518. [[CrossRef](#)] [[PubMed](#)]
5. Chiu, Y.C.; Chung, M.S. Determination of optimal COD/nitrate ratio for biological denitrification. *Int. Biodeterior. Biodegrad.* **2003**, *51*, 43–49. [[CrossRef](#)]
6. Ren, H.; Lee, H.S.; Chae, J. Miniaturizing microbial fuel cells for potential portable power sources: Promises and challenges. *Microfluid. Nanofluid.* **2012**, *13*, 353–381. [[CrossRef](#)]
7. Lovley, D.R. Bug juice: Harvesting electricity with microorganisms. *Nature Rev. Microbiol.* **2006**, *4*, 497–508. [[CrossRef](#)] [[PubMed](#)]

8. Wang, C.T.; Lee, Y.C.; Liao, F.Y. Effect of composting parameters on the power performance of solid microbial fuel cells. *Sustainability* **2015**, *7*, 12634–12643. [[CrossRef](#)]
9. Pinto, R.P.; Srinivasan, B.; Manuel, M.F.; Tartakovsky, B. A two-population bio-electrochemical model of a microbial fuel cell. *Bioresour. Technol.* **2010**, *101*, 5256–5265. [[CrossRef](#)] [[PubMed](#)]
10. Ren, H.; Torres, C.I.; Parameswaran, P.; Rittmann, B.E.; Chae, J. Improved current and power density with a micro-scale microbial fuel cell due to a small characteristic length. *Biosens. Bioelectron.* **2004**, *61*, 587–592. [[CrossRef](#)] [[PubMed](#)]
11. Fernandez, F.J.; Seco, A.; Ferrer, J.; Rodrigo, M.A. Use of neurofuzzy networks to improve wastewater flow-rate forecasting. *Environ. Model. Softw.* **2009**, *24*, 686–693. [[CrossRef](#)]
12. Buendía, I.M.; Fernández, F.J.; Villaseñor, J.; Rodríguez, L. Biodegradability of meat industry wastes under anaerobic and aerobic conditions. *Water Res.* **2008**, *42*, 3767–3774. [[CrossRef](#)] [[PubMed](#)]
13. Logan, B.E.; Hamelers, B.; Rozendal, R.; Schröder, U.; Keller, J.; Freguia, S.; Aelterman, P.; Verstraete, W.; Rabaey, K. Microbial fuel cells: Methodology and technology. *Environ. Sci. Technol.* **2006**, *40*, 5181–5192. [[CrossRef](#)] [[PubMed](#)]
14. Vitousek, P.M.; Mooney, H.A.; Lubchenco, J.; Melillo, J.M. Human domination of Earth's ecosystems. In *Urban Ecology: An International Perspective on the Interaction Between Humans and Nature*, 2008 ed.; Springer: Berlin, Germany, 2008; pp. 3–13.
15. Swinarski, M.; Makinia, J.; Czerwionka, K.; Chrzanowska, M.; Drewnowski, J. Comparison of the effects of conventional and alternative external carbon sources on enhancing the denitrification process. *Water Environ. Res.* **2009**, *81*, 896–906. [[CrossRef](#)] [[PubMed](#)]
16. Drewnowski, J.; Makinia, J. The role of colloidal and particulate organic compounds in denitrification and EBPR occurring in a full-scale activated sludge system. *Water Sci. Technol.* **2011**, *63*, 318–324. [[CrossRef](#)] [[PubMed](#)]
17. Pant, D.; Mohanakrishna, G.; Srikanth, S. Bioelectrochemical systems (Bes) for microbial electroremediation: An advanced wastewater treatment technology. In *Applied Environmental Biotechnology: Present Scenario and Future Trends*; Springer India: New Delhi, India, 2015; pp. 145–167.
18. Bolzonella, D.; Innocenti, L.; Pavan, P.; Cecchi, F. Denitrification potential enhancement by addition of anaerobic fermentation products from the organic fraction of municipal solid waste. *Water Sci. Technol.* **2001**, *44*, 187–194. [[PubMed](#)]
19. Swinarski, M.; Makinia, J.; Czerwionka, K.; Chrzanowska, M. Industrial wastewater as an external carbon source for optimization of nitrogen removal at the “Wschod” WWTP in Gdansk (Poland). *Water Sci. Technol.* **2009**, *59*, 57–64. [[CrossRef](#)] [[PubMed](#)]
20. Czerwionka, K.; Makinia, J.; Pagilla, K.R.; Stensel, H.D. Characteristics and fate of organic nitrogen in municipal biological nutrient removal wastewater treatment plants. *Water Res.* **2012**, *46*, 2057–2066. [[CrossRef](#)] [[PubMed](#)]
21. Cyplik, P.; Marecik, R.; Piotrowska-Cyplik, A.; Olejnik, A.; Drozdzyńska, A.; Chrzanowski, Ł. Biological denitrification of high nitrate processing wastewaters from explosives production plant. *Water Air Soil Pollut.* **2012**, *223*, 1791–1800. [[CrossRef](#)] [[PubMed](#)]
22. Min, B.; Logan, B.E. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environ. Sci. Technol.* **2004**, *38*, 5809–5814. [[CrossRef](#)] [[PubMed](#)]
23. Liu, H.; Cheng, S.; Logan, B.E. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* **2005**, *39*, 658–662. [[CrossRef](#)] [[PubMed](#)]
24. Liu, H.; Ramnarayanan, R.; Logan, B.E. Production of Electricity during Wastewater Treatment Using a Single Chamber Microbial Fuel Cell. *Environ. Sci. Technol.* **2004**, *38*, 2281–2285. [[CrossRef](#)] [[PubMed](#)]
25. González Del Campo, A.; Fernández, F.J.; Cañizares, P.; Rodrigo, M.A.; Pinar, F.J.; Lobato, J. Energy recovery of biogas from juice wastewater through a short high temperature PEMFC stack. *Int. J. Hydrog. Energy* **2014**, *39*, 6937–6943. [[CrossRef](#)]
26. Gonzalez del Campo, A.; Perez, J.F.; Cañizares, P.; Rodrigo, M.A.; Fernandez, F.J.; Lobato, J. Study of a photosynthetic MFC for energy recovery from synthetic industrial fruit juice wastewater. *Int. J. Hydrog. Energy* **2014**, *39*, 21828–21836. [[CrossRef](#)]
27. Zhang, F.; Ahn, Y.; Logan, B.E. Treating refinery wastewaters in microbial fuel cells using separator electrode assembly or spaced electrode configurations. *Bioresour. Technol.* **2014**, *152*, 46–52. [[CrossRef](#)] [[PubMed](#)]

28. Virdis, B.; Rabaey, K.; Yuan, Z.; Keller, J. Microbial fuel cells for simultaneous carbon and nitrogen removal. *Water Res.* **2008**, *42*, 3013–3024. [[CrossRef](#)] [[PubMed](#)]
29. Virdis, B.; Rabaey, K.; Rozendal, R.A.; Yuan, Z.; Keller, J. Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells. *Water Res.* **2010**, *44*, 2970–2980. [[CrossRef](#)] [[PubMed](#)]
30. Zhang, F.; He, Z. Integrated organic and nitrogen removal with electricity generation in a tubular dual-cathode microbial fuel cell. *Process Biochem.* **2012**, *47*, 2146–2151. [[CrossRef](#)]
31. Li, J.T.; Zhang, S.H.; Hua, Y.M. Performance of denitrifying microbial fuel cell subjected to variation in pH, COD concentration and external resistance. *Water Sci. Technol.* **2013**, *68*, 250–256. [[CrossRef](#)] [[PubMed](#)]
32. Gonzalez del Campo, A.; Lobato, J.; Cañizares, P.; Rodrigo, M.A.; Fernandez Morales, F.J. Short-term effects of temperature and COD in a microbial fuel cell. *Appl. Energy* **2013**, *101*, 213–217. [[CrossRef](#)]
33. Fernández, F.J.; Villaseñor, J.; Infantes, D. Kinetic and stoichiometric modelling of acidogenic fermentation of glucose and fructose. *Biomass Bioenergy* **2011**, *35*, 3877–3883. [[CrossRef](#)]
34. Henze, M. Nitrate versus oxygen utilization rates in wastewater and activated sludge systems. *Water Sci. Technol.* **1986**, *18*, 115–122.
35. Sözen, S.; Orhon, D. The effect of nitrite correction on the evaluation of the rate of nitrate utilization under anoxic conditions. *J. Chem. Technol. Biotechnol.* **1999**, *74*, 790–800. [[CrossRef](#)]
36. De Lucas, A.; Rodríguez, L.; Villaseñor, J.; Fernández, F.J. Denitrification potential of industrial wastewaters. *Water Res.* **2005**, *39*, 3715–3726. [[CrossRef](#)] [[PubMed](#)]
37. Eaton, A.D.; Clesceri, L.S.; Rice, E.W.; Greenberg, A.E.; Franson, M.A.H.; American Public Health Association; American Water Works Association; Water Environment Federation. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
38. Rodríguez, L.; Villaseñor, J.; Fernández, F.J. Use of agro-food wastewaters for the optimisation of the denitrification process. *Water Sci. Technol.* **2007**, *55*, 63–70. [[CrossRef](#)] [[PubMed](#)]
39. De los Ángeles Fernandez, M.; de los Ángeles Sanromán, M.; Marks, S.; Makinia, J.; Gonzalez del Campo, A.; Rodrigo, M.; Fernandez, F.J. A grey box model of glucose fermentation and syntrophic oxidation in microbial fuel cells. *Bioresour. Technol.* **2016**, *200*, 396–404. [[CrossRef](#)] [[PubMed](#)]



© 2016 by the authors; licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC-BY) license (<http://creativecommons.org/licenses/by/4.0/>).