DEVELOPMENT OF IMMOBILISED CELL BASED ELECTRODE FOR USAGE IN MICROBIAL FUEL CELL

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To my beloved wife for supporting me throughout the studies and to my beloved daughter for understanding the days to finish this journey.

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ABSTRACT

Microbial fuel cell (MFC) is a kind of biological fuel cells that produce electricity through utilization of microorganisms. The efficiency of MFC performance is based on how well the electron is transferred and finally flows through an external circuit to complete electrical circuit. However, the power density produced by MFC generally is still very low and one of the major reasons is due to high internal resistance imposed by macro environment of an MFC. In the present research, the objective was to develop bio-based anode or bioanode and its usage in the MFC for power production. The bioanode was developed by mixing cells solution and activated carbon overnight before adding alginate and subjected to homogenisation. The mixture was then immobilised using entrapment method to obtained uniform beads. Initial study was conducted using glucose as fuel and both open circuit voltage (OCV) and closed circuit voltage (CCV) production were evaluated using a single chambered MFC. Results show that OCV increased gradually and still increased after 6 h of operation compared to free cells. The highest OCV was achieved after 9 h of operation (0.3 V) compared to free cells based MFC (0.2 V) and maintained for 15 h of operation. In CCV profile for free cells show a decrease in voltage generated but then rapidly increased which indicates a 'power-overshoot' phenomenon which was not observe in immobilised based bioanode MFC. The maximum OCV was 2-fold higher for immobilised based bioanode compared to free cells. In addition, the sustainability and reproducibility of power production was achieved by operating MFC in Fed-batch mode and results show that the system was stable and able to achieve in average 3.0 x 10^{-3} % of Coulombic Efficiency (CE).

ABSTRAK

Sel bahan bakar mikrob (MFC) adalah sejenis sel bahan bakar biologi yang menghasilkan tenaga elektrik menggunakan mikroorganisma. Keberkesanan MFC adalah berdasarkan kuantiti elektron dipindahkan melalui litar luaran untuk melengkapkan satu litar elektrik. Walau bagaimanapun, ketumpatan kuasa yang dihasilkan oleh MFC masih sangat rendah dan salah satu sebab utama adalah kerana rintangan dalaman yang tinggi yang dikenakan oleh persekitaran makro MFC. Objektif kajian ini adalah menghasilkan anod berasaskan bio atau bioanod dan penggunaannya dalam MFC untuk menjana kuasa. Bioanod telah dihasilkan dengan mencampurkan larutan sel dan karbon aktif semalaman dan alginat untuk tujuan homogenisasi. Campuran kemudiannya diimobilisasikan menggunakan kaedah pemerangkapan untuk mendapatkan manik seragam. Kajian awal dijalankan menggunakan glukosa sebagai bahan bakar dan penghasilan voltan litar terbuka (OCV) dan voltan litar tertutup (CCV) dinilai menggunakan satu ruang MFC. Keputusan menunjukkan peningkatan OCV secara beransur-ansur dan masih meningkat selepas 6 jam operasi berbanding dengan sel-sel bebas. OCV tertinggi dicapai selepas 9 jam operasi (0.3 V) berbanding dengan MFC sel-sel bebas (0.2 V) dan produksi dikekalkan selama 15 jam. Dalam profil CCV untuk sel-sel bebas menunjukkan pengurangan voltan yang dijana tetapi kemudian meningkat pesat yang menunjukkan fenomena kuasa-laras yang tidak diamati pada MFC bioanode yang tidak bergerak. OCV maksimum adalah 2 kali ganda lebih tinggi untuk bioanod yang telah diimmobilisasikan berbanding dengan sel-sel bebas. Di samping itu, kemampanan dan kebolehulangan semula kuasa juga dicapai dengan operasi MFC dalam mod 'Fed-batch' dan hasilnya menunjukkan bahawa sistem itu stabil dan boleh mencapai purata 3.0×10^{-3} % Coulombic Kecekapan (CE).

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LIST OF ABBREVIATIONS

MFC	-	Microbial fuel cell
SCMFC	-	Single microbial fuel cell
OCV	-	Open circuit voltage
CCV	-	Closed circuit Voltage
GAC	-	Granular activated carbon
KH_2PO_4	-	Potassium Phosphate
$MgSO_4$	-	Magnesium Sulphate
$(NH_4)_2SO_4$	-	Ammonium Sulphate
$NaC_6H_7O_6$	-	Sodium Alginate
$CaCl_2$	-	Calcium Chloride
SEM	-	Scanning Electron Microscopy
CE	-	Coulombic efficiency

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CHAPTER 1

INTRODUCTION

1.1 Background of the study

Microbial fuel cells (MFCs) is classified as a special biofuel cells types that work to produce electric power by utilizing microorganisms to assist redox reaction instead of using isolated enzyme. MFC technology had proved that it can be used to treat waste and at the same time generating electricity in which could be so called as a new form of renewable energy nowadays. For example, by utilizing the wastewater's bacteria the electricity generation is conducted along with treating the wastewater. (Lui *et al*., 2004; Min and Logan, 2004).

Microorganism was first explored to be a catalyst in fuel cells in 1970 and the MFC technology had been used as a tool of domestic wastewater treatment in 1991. This shows that the MFC technology is already known for more than 40 years back. Nowadays, researchers are more focus on enhancing the power output of MFC in which will be a promising technology to practically applied in industry. Generally, MFC will convert the energy that is contain in a bio-convertible substrate and directly

generating electricity. The generation of electricity is actually occur when the microorganism switch from the natural electron acceptor for example Oxygen, to the insoluble acceptor such as the MFC anode to transfer the electron to the cathode. A complete electron circuit transfer will generate an electricity. MFCs is classified into two different mode: one that generates electricity with the help of artificial electron shuttles (mediators) which known as Dual Chamber MFC and the other one that do not require any mediator (mediatorless) which known as Single Chamber MFC.

Furthermore, if being compared to other technologies that used organic material for generation of energy, MFC technology is having more advantages. Firstly, the direct conversion of substrate into electricity provides at some extend high conversion efficiencies. Secondly, most of the MFC operates efficiently at ambient temperature and not requires extra energy for heating purpose. Thirdly, MFC does not require the treatment of any presentable biogas generated in the cell and there is no need of additional energy to aerate the cathode, provided that it is aerated passively. Finally, MFC has the potential for application in remote areas that do not have electrical infrastructure and in turn makes MFC as an additional renewable energy option to meet global energy requirements.

Although the MFC is not use to generate power commercially due to low power production, several studies have showed that it is also possible to produce electricity from other sources such as domestic wastewater. In addition, through usage of MFC, biological wastewater treatment removal of chemical oxygen demand (COD) can be accomplished together with reducing biological oxygen demand (BOD). Furthermore, wastewater treatment using MFC system has been introduced to some extend by bigger companies such as Arbsource in US and Emefcy in Israel. Arbsouce was founded in 2011 to develop wastewater treatment system specifically for food and beverage industry.

1.2 Problem statement

As microbial fuel cell (MFC) gained much attention of its ability to generate power from organic or inorganic compounds via microoganism, researchers has focused on MFCs improvement such as the microbial processes, component of MFC, design of the construction and factors of limitations (Parkash, 2016). The electricity generated by MFC is relatively low and often the power output is fluctuated due to unstable internal resistance presence in the MFC environment. One of the problems concerned is electron transfer process from the electricigens or microbes that can directly transfer electron in the medium might be hindered by the properties of the electrolyte (medium) including the resultant internal resistance oppose by it. It is realized by several researchers that the closer the microbes or electricigens to anode electrode, more electron can be transferred efficiently. One of the alternative method to achieve high electron transfer is to immobilize microbes to the electrode. In addition, the used of immobilized microbes on the surface of electrode might reduce the internal resistance thus reduce the total resistance and will result in more power production.

Immobilization of microbes to the anodic electrodes has been established previously by Wagner *et al.* (2012). Microbial attachment was first prepared by allowing electrodes to immerse in microbial culture and incubate for several days to ensure microbes attached to the electrode base.

However, such steps are time-consuming as well as risk of non-attachment of microbes on the surface of electron is rather high. Thus, improvement of cell immobilization method with the aim to produce controlled form of circular shaped immobilized cell was proposed. This immobilized cell consists of microbes or electricigens, activated carbon and alginate. Activated carbon will act as a carrier, providing carbon support for microbial cell. Meanwhile, many type of alginate can be used for cell entrapment. The rationale for adopting cells immobilization strategy by

activated carbon and alginate was due to high cell retention which leads to more power generation. Mesran *et al.* (2014) has carried out preliminary study on usage of immobilized cells - based electrode using single chamber microbial fuel cell. The immobilized MFC system able to generate 403 mV after 200 hours operation compared to free cell system with 217 mV, proved the effectiveness of immobilization strategy. However, coulombic efficiency (CE) and stability of electricity output are not analyse. Since these evaluations are crucial for practical applications of MFCs, therefore this present research focused on developing an immobilized based MFC and evaluate the power production performance of the MFC. Meanwhile, focus also on operating the MFC in Fed-Batch mode to enhance power production and observe its sustainability.

1.3 Objective of the study

The objective of this research is to investigate the performance of MFC using immobilized cell anode electrode based on power generation and observe the sustainability of power generation by operating MFC in fed-batch mode.

1.4 Scope of the Study

i) Immobilized cell based electrode will be used in MFC trials in batch as anode electrode, and the resultant voltage will be measured using a auto logged digital multi-meter, and subsequently power and current will be calculated to analyse the power production performance.

ii) The immobilized based MFC will also be assessed in fed-batch to further prove the performance of MFC.

REFERENCE

Aehley, W. (2006). Enzymes in industry: products and applications. *John Wiley & Sons, 73*.

Ahmad, S.A, Shamaan, N. A., Noorliza, M.A., Koon, G. B., Shuko, M. Y. A., & Syed, M. A. (2012). Enhanced phenol degradation by immobilized *Acinetobacter sp.* strain AQ5NOL 1. *World Journal of Microbiology and Biotechnology*. *28*:347352.

Alferov, S. V., Minaicheva, P. R., Arlyapov, V. A., Asulyan, L. D., Alferov, V.A., Ponamoreva, O. N., & Reshetilov A.N. (2014). Bioanode for a microbial fuel cell based on *Gluconobacter oxydans* immobilised into a polymer matrix. *Applied Biochemistry and Microbiology*. *50*:637 – 643.

Arora, D. K. (2003). Handbook of fungal biotechnology. *CRC Press*, 294.

Behera, M., Jana, P. S., More, T. T., & Ghangrekar, M. M. (2010). Rice mill wastewater treatment in microbial fuel cells fabricated using proton exchange membrane and earthen pot at different pH. *Bioelectrochemistry, 79*: 228-233.

Bond, D. R., & Lovley, D. R. (2003). Electricity production by *Geobacter sulfurreducens* attached to electrodes. Applied Environmental and Microbiology. *69*(3):1548-1555.

Borole, A. P., Mielenz, J. R., Vishnivetskaya, T. A., & Hamilton, C. Y. (2009). Controlling accumulation of fermentation inhibitors in biorefinery recycle water using microbial fuel cells. *Biotechnology for Biofuels*. *2*(7). doi:10.1186/1754-6834-2-7.

Calinescu, I., Chipurici, P., Trifan, A. & Badoiu, C. (2012) Immobilisation of *Saccharomyces Cerevisiase* for the production of bioethanol. *University Politechnica of Bucharest Scientific Bulletin Series B*, *74* (1): 34 – 40.

Canbolat, M. F., Gera, N., Tang, C., Monian, B., Rao, B. M., Pourdeyhimi, B., & Khan, S. A. (2013). Preservation of cell viability and protein conformation on immobilization within nanofibers via electrospinning functionalized yeast. *American Chemical Society: Applied Material Interfaces*, *5*:9349−9354.

Cao, L. (2006) Carrier-bound immobilized enzymes: principles, applications and design. *John Wiley & Sons.* 131.

Cheng, J., Zhu, X., Ni, J. & Borthwick, A. (2010). Palm oil mill effluent treatment using a two-stage microbial fuel cells system integrated with immobilized biological aerated filters. *Bioresource Technology, 101*: 2729-2734.

Chibata, I., Tosa, T., & Fujimura, H. (1983). Immobilized living microbial cells. *Annual Reports on Fermentation Process, 6*:1-22.

Choudhury, P., Prasad-Uday, U. S., Bandyopadhyay, T. K., Ray, R. N., & Bhunia, B. (2017). Performance improvement of microbial fuel cell (MFC) using suitable electrode and Bioengineered organisms: A review. *Bioengineered. 8*(5):471-487.

Devasahayam, M., & Masih, S. (2012). Microbial fuel cells demonstrate high coulombic efficiency applicable for water remediation. *Indian journal of experimental biology.* 50(6):430-438.

Du, Z., Li, H. & Gu, T. (2007). A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy. *Biotechnology Advances*, *25*: 464-482.

Elakkiya, M., Prabhakaran, D., & Thirumarimurugan, M. (2016). Methods of cell immobilization and its applications. *International Journal of Innovative Research in Science, Engineering and Technology. 5*(4): 5429-5433.

Freeman, A., & Lilly, M. D. (1998). Effect of processing parameters on the feasibility and operational stability of immobilised viable microbial cells. *Enzyme and Microbial Technology. 23*(5):335-345.

Gude, V. G. (2016). Wastewater treatment in microbial fuel cells – An overview. *Journal of Cleaner Production,* doi: 122.10.1016/j.jclepro.2016.02.022.

Guisan, J. M. (2006). Immobilization of enzymes and cells. *Humana Press,* Totowa, 5-78

Hariharan, S., & Nambisan, P. (2013) Optimization of lignin peroxidase, manganese peroxidase and lac production from *Ganoderma lucidum* under solid state fermentation of pineapple leaf. *BioResources, 8*(1), 250-271.

Huang, L., & Logan, B. E. (2008). Electricity production from xylose in fed-batch and continuous-flow microbial fuel cells. *Applied Microbiology and Biotechnology*, *80*:655–664.

Ieropoulos, I., Melhuish, C., Greenman, J., & Horsfield, I. (2005). EcoBot-II: An artificial agent with a natural metabolism. *Journal of Advanced Robotic System. 2*(4):295-300

Jaganathan, S. K., Supriyanto, E., Murugesan, S., Balaji, A., & Asokan, M. K. (2014). Biomaterials in cardiovascular research: Applications and clinical implications: Review. *BioMedical Research International*.

Jang, K. J., Pham, T. H., Chang, I. S., Kang, K. H., Moon, H., Cho, K. S. & Kim, B. K. (2004). Construction and operation of a novel mediator and membrane-less microbial fuel cell. *Process Biochemistry, 39* : 1007–1012.

Junter G.A., Jouenne T., 2004, Immobilised viable microbial cells: from the process to the proteome em leader or the cart before the horse. *Biotechnology Advances. 22*(8):633-658.

Junyan, L., Guangfei, Q., & Ping, N. (2006). Cell immobilization technology and application. *GMSARN International Conference on Sustainable Development: Issues and Prospects for GMS.*

Karmakar. S., Kundu, K., & Kundu, S. (2010). Current Research, Technolology and Education Topics in Applied Microbiology and Microbial Biotechnology. *A. Mendez-Vilas (Ed.)*

Kassongo, J. & Togo, C.A. (2010). The potential of whey in driving microbial fuel cells: a dual prospect of energy recovery and remediation. *African Journal of Biotechnology. 9*(46):7885-7890.

Kim, B., An, J., & Chang, I. S. (2017). Elimination of power overshoot at bioanode through assistance current in microbial fuel cells, ChemiSusChem: chemistry and sustainability. *Energy and Materials. 10*(3):612-617.

Kok, F. N., Bozoglu, F., & Hasirci, V. (2001a). Immobilization of acetylcholinesterase and choline oxidase in/on pHEMA membrane for biosensor construction. *Journal of Biomaterials Science Polymer Edition, 12*(11):1161-1176.

Kok, F. N., Hasirci, V, Arica, M. Y. (2001b). Bioremediation of contaminated soils. *CRC Press*. 134-135.

Kong, H. J., Smith, M. K., and Mooney, D. J. (2003). Designing alginate hydrogels to maintain viability of immobilized cells. *Biomaterials*, *24* (22): 4023 – 4029.

Larrosa-Guerrero, A., Scott, K., Katuri, K. P. Godinez, C., Head, I. M., & Curtis, T. (2010). Open circuit versus closed circuit enrichment of anodic biofilms in MFC: effect on performance and anodic communities. *Applied Microbiology and Biotechnology. 87*:1699-1713.

Lee, C. M. (2011). Generating electricity using pre-treated lemon grass leaves as fuel with mixed microbes. Faculty of Chemical Engineering, Universiti Teknologi Malaysia.

Leenen, E., DosSantos, V. A. P., Grolle, K. C. F., Tramper, J., & Wijffels, R. H. (1996). Characteristics of and selection criteria for support materials for cell immobilization in wastewater treatment. *Water Research.* 30(12):2985-2996.

Ligler F. S., & Taitt, C. R. (2011). Optical biosensors: Today and tomorrow. *Elsevier. 2nd Ed*. pp 151.

Lim, H. C. & Shin, H. S. (2013) Fed-Batch Cultures: Principles and Applications of Semi-Batch Bioreactors. Page 1 – 10. *Cambridge Press.*

Linko, P. & Linko Y.Y. (1984). Industrial application of immobilized cells. *CRC Critical Review Biotechnology*, *4*:289—338.

Liu, H., & Logan, B. E. (2004). Electricity generation using an air-cathode single chamber microbial fuel cell in the presence and absence of a proton exchange membrane. *Environmental Science and Technology*. *38*:4040-4046.

Liu, H., Cheng, S. & Logan, B. E. (2005). Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environmental Science and Technol*ogy, *39*(2), pp 658–662.

Liu, X. W., Sun, X. F., Huang, Y. X., Sheng, G. P., Wang, S. G., Yu, H. Q. (2011). Carbon nanotube/chitosan nanocomposite as a biocompatible biocathode material to enhance the electricity generation of a microbial fuel cell. *Royal Society of Chemistry. 4*:1422-1427

Logan, B. E. (2004). Extracting hydrogen and electricity from renewable resources. *Environmental Science and Technology*, *38*:160A-167A.

Logan, B. E. (2006) Microbial fuel cells: Methodology and technology. *Environmental Science and Technology*, *40*:5181-5192.

Logan, B. E., Murano, C., Scott, K., Gray, N. D., & Head, I. M. (2005). Electricity generation from cysteine in a microbial fuel cell. *Water Research, 39*(5):942–952.

Lui, H., Ramnarayanan, R., & Logan, B. E. (2004). Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environmental Science and Technology 38*:2281-2285

Luo, H., Liu, G., Zhang, R., & Song, Jin. (2009). Phenol degradation in microbial fuel cells. *The Chemical Engineering Journal, 147*(2-3): 259-264.

Luthfi, A. A. I., Jahim, J. M., Harun, S., Tan, J. P., & Mohammad, A. W. (2017). Potential use of coconut shell activated carbon as an immobilisation carrier for high conversion of succinic acid from oil palm frond hyrolysate. *RSC Advances, 7*: 49480-49489.

Manish, S. & Banerjee, R. (2008). Comparison of biohydrogen production processes. *International Journal of Hydrogen Energy*, *3*: 276-286

Martins, S. C. S., Martins, C. M., Fiúza, L. M. C. G., and Santaella, S. T. (2013). Immobilization of microbial cells: A promising tool for treatment of toxic pollutants in industrial wastewater. *African Journal of Biotechnology*, *12*(28):4412 – 4418.

Masih, S., & Devasahayam, M., & Srivastava, R., & Gupta, S. (2012). *Enterobacter* species specific microbial fuel cells show increased power generation with high coulombic efficiency. *Trends in Biosciences. 5*:114-118.

Mesran, M. H., Mamat, S., Pang, Y. R., Hong, T. Y., Muneera Z, Ghazali, N. F. & Mahmood, N. A. N. (2014) .Preliminary studies on immobilization cell-based microbial fuel cell system on its power generation. *Journal of Asian Scientific Research*, *4*(8):428 – 435.

Min, B., Cheng, S. & Logan, B.E. (2005). Electricity generation using membrane and salt bridge microbial fuel cells. *Water Research*, *39*:1675-1686.

Mohamad, N. R., Marzuki, N. H., Buang, N. A., Huyop, F., & Wahab, R. A. (2015). An overview of technologies for immobilization of enzymes and surface analysis techniques for immobilized enzymes. *Biotechnology, biotechnological equipment*, *29*(2):205-220.

Moon, H., Chang, I.S, Kang, K.H., Jang, J.K. &Kim, B.H. (2004). Improving the dynamic response of a mediator-less microbial fuel cell as a biochemical oxygen demand (BOD) sensor. *Biotechnology Letters*, *26*:1717-1721.

Nimje, V. R., Chen, C-C., Chen, H. R., Chen, C. Y., Tseng, M. J., Cheng, K. C., Shih, R. C., & Chen, C. Y. (2012). A single-chamber microbial fuel cell without an air cathode. *International Journal of Molecular Sciences. 13*:3933-3948; doi:10.3390/ijms13033933.

Oh, S. T., Kim, J. R., Premier, G. C., Lee, T. H. & Kim, C. (2010). Sustainable wastewater treatment: How might microbial fuel cells contributes. *Biotechnology Advances*, *28*: 871-881.

Osiro, D., Filho, R. B., Assis, O. B. G., Jorge, L. A. D. C. & Colnago, L. A. (2012). Measuring bacterial size with AFM. *Brazilian Journal of Microbiology*. *43*(1).

Pandit, S., Kuppam, C., Kakarla, R., & Kadier, A. (2017). Basic principles of microbial fuel cell: Technical challenges and feasibility. Microbial Applications. *Springer International Publishing.* pp 165-188.

Panesar, P. S., Marwaha, S. S., & Chopra, H. K. (2010). Enzymes in food processing: fundamentals and potential applications. *I.K. International,* 122.

Pant, D., Van, B. G., Diels, L., & Vanbroekhoven, K. (2010). A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresoure Technology, 101*(6): 1533-1543.

Park, D. & Zeikus. (2002) Impact of electrode composition on electricity generation in a single-compartment fuel cell using *Shewanella putrefaciens*. *Applied Microbiology and Biotechnology*. 59(1):58-61

Park, D. H. & Zeikus, J. G. (1999). Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. *Journal of Bacteriology. 181*(8):2403-2410

Park, D. H., Zeikus, J. G. (2000) Electricity generation in micorbial fuel cells using neutral red as electronophore. *Applied and Environmental Microbiology. 66*(4): 1292-1297

Parkash, A. (2015). Design and fabrication of a double chamber microbial fuel cell for voltage generation from biowaste. *Bioprocessing and Biotechniques, 5*(8), doi:10.4172/2155-9821.1000246.

Parkash, A. (2016). Microbial Fuel Cells: A Source of Bioenergy. *Journal of Microbial & Biochemical Technology*. *8*(3):247-255. doi:10.4172/1948-5948.1000293.

Pham, J. K. J., Chang, T. H., Kang, I. S., Moon, K. H., Cho, H. & Kim, B. H. (2004). Construction and operation of a novel mediator and membrane-less microbial fuel cell. *Process Biochemistry, 39*:1007–1012.

Potter, M.C. (1912). Electrical effects accompanying the decomposition of organic compounds. *Proceedings of the Royal Society B: Biological Sciences*, *84*:260-276.

Ramani, V., Kunz, H. R., & Fenton, J. M. (2004). The polymer electrolyte fuel cell. *Electrochem. Soc. Interface,* 13, 17.

Ringeisen, B.R., Henderson, E., Wu, P. K., Pietron, J., Ray, R., Little, B., Biffinger, J.C., & Jones-Meehan, J.M. (2006). High power density from a miniature microbial fuel cell using *Shewanella oneidensis* DSP10. Environmental Sciece and Technology, 40(8):2629-2634.

Salleh, A. B., Abdul-Rahman, R. N. Z. R., & Basri, M. (2006). New lipases and proteases. *Nova Publishers,* 114.

Saravanan, N., & Karthikeyan, M. (2018). Study of single chamber and double chamber efficiency and losses of wastewater treatment. *International Research Journal of Engineering and Technology, 5*(3), 1225-1230.

Sassolas, A., Blum, L. J., & Leca-Bouvier, B. D., (2012). Immobilization strategies to developenzymatic biosensors. *Biotechnology Advance*, 30:489–511.

Schroder, U. (2007). Anodic electron transfer mechanisms in microbial fuel cells and their energy efficiency. *Physical Chemistry Chemical Physics*. *9*(21):2619-2629.

Spahn, C., & Minteer, S. D. (2008). Enzyme immobilization in biotechnology. *Recent Patents on Engineering. 2*(3):195–200.

Wagner, R. C., Porter-Gill, S., & Logan, B. E. (2012). Immobilization of anode-attached microbes in a microbial fuel cell. *AMB Express*, *2*(1), 1-6.

Wei, J., Liang, P., & Huang, X. (2011b). Recent progress in electrodes for microbial fuel cells. *Bioresource Technology. 102*(20): 9335–9344.

Willaert, R. G. (2016). Cell immobilization and its application in biotechnology: Current trends and future prospect. Fermentation Microbiology and Biotechnology, 3rd ed. *CRC press,* 313-367.

Winfield, J., Ieropoulos, I., Greenman, J., & Dennis, J. (2011). The overshoot phenomenon as a function of internal resistance in microbial fuel cells. *Bioelectrochemistry*. *81*(1):22-27.

Yang, X.Y., Tian. G., Jiang, N., & Su, B. L. (2012). Immobilization technology: a sustainable solution for biofuel cell design. *Energy and Environmental Science*. *5*:5540–63.

Yong, Y. C., Liao, Z. H., Sun, J. Z., Zheng, T., Jiang, R. R., & Song, H. (2013). Enhancement of coulombic efficiency and salt tolerance in microbial fuel cells by graphite/alginate granules immobilization of *Shewanella oneidensis* MR-1. *Process Biochemistry*. *48*;1947–1951.

Yuan, Y., Zhou, S., Xu, N., & Zhuang, L., (2011). Microorganism-immobilised carbon nanoparticle anode for microbial fuel cells based on direct electron transfer. *Applied Microbiolology and Biotechnology*. *89*:629-635.

Zhang, E., Zhai, W., Luo, Y., Scott, K., Wang X., & Diao, G. (2016). Acclimatisation of microbial consortia to alkaline conditions and enhanced electricity generation. *Bioresources Technology*. *211*:736 - 742.

Zhang. F., & Keasling, J., (2011).Biosensors and their applications in microbial metabolic engineering. *Trends in Microbiology*. 19(17):323-329.

Zhou, J. K., Qin, W. Q., Niu, Y. J. & Li, H. X. (2006). Ferrous ion oxidation by *Thiobacillus ferrooxidans* immobilized on activated carbon. *Transactions of Nonferrous Metals Society of China. 16*:927-930.

Zhou, M., Wang, H., Hassett, D. J., & Gu, T. (2013). Recent advances in microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) for wastewater treatment, bioenergy and bioproducts. *Journal of Chemical Technology and Biotechnology*. *8*(4):508-518.

Zhou. M., Chi. M., Luo. J., He. H., & Jin, T. (2011). An overview of electrode materials in microbial fuel cells. *Journal Power Sources 196*:4427-4435.

Zhu, X., Tokash J. C., Hong, Y. & Logan, B. E. (2013). Controlling the occurrence of power overshoot by adapting microbial fuel cells to high anode potentials. *Bioelectrochemistry.* 90: 30–35.