

Manuscript Number: JCLEPRO-D-15-00202

Title: Bioelectrochemical treatment of municipal waste liquor in microbial fuel cells for energetic valorization

Article Type: Original Research Paper

Corresponding Author: Dr. Peter Bakonyi, Ph.D.

Corresponding Author's Institution: University of Pannonia

First Author: László Koók, B.Sc.

Order of Authors: László Koók, B.Sc.; Tamás Rózsenberszki, M.Sc.; Nándor Nemestóthy, Ph.D.; Katalin Bélafi-Bakó, Professor; Peter Bakonyi, Ph.D.

Abstract: Microbial fuel cells (MFCs) are recognized as promising applications to produce bioelectricity by utilizing various waste materials. In this study, dual-chamber microbial fuel cells were employed for energetic valorization of an untested substrate, liquid fraction of pressed municipal solid waste (LPW). This by-product is potentially applicable for MFCs because of its high organic matter content. In the course of the experiments, the anodic biofilm response and energy production efficiency have been investigated by experimental design approach, taking substrate and fresh inoculum - mesophilic anaerobic sludge (MAS) - addition as factors into account. It was observed that reinoculation could demonstrate a negative effect on the energy production, especially at low substrate (LPW) dosing levels. However, when the LPW to fresh MAS ratio in the anode chamber exceeded a particular value, the biofilm-associated electrical utilization dominated against the degradation in the bulk phase. Furthermore, the results indicated that the highest energy yields ($8-9 \text{ J g}^{-1} \text{ COD d}^{-1}$) could be attained at the lowest input COD concentrations. The maximal and average COD removal efficiency were 94% and 87 %, respectively, which indicate the excellent biodegradability of LPW. As for COD removal rate, $1.2-1.9 \text{ kg COD m}^{-3} \text{ d}^{-1}$ could be reached.

Suggested Reviewers: Gopalakrishnan Kumar PhD
Department of Environmental Engineering, Daegu University, Republic of Korea
gopalakrishnanchml@gmail.com
Well-established on biotechnological applications for waste management

Germán Buitrón Professor
Laboratory for Research on Advanced Processes for Water Treatment, Universidad Nacional Autónoma de México
gbuitronm@iingen.unam.mx
Qualified scientist on the field of bio-electrochemical systems

Chiu-Yue Lin Professor
Department of Environmental Engineering and Science, Feng Chia University, Taiwan
cylin@fcu.edu.tw
Expert of anaerobic biodegradation processes

Zbynek Pientka PhD

Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic
pientka@imc.cas.cz
Recognized researcher of membrane-associated bioprocesses

Dear Prof. Lozano,

Attached to this letter, please find the manuscript of an intended “Original research article” entitled “Bioelectrochemical treatment of municipal waste liquor in microbial fuel cells for energetic valorization” for your consideration for publication.

The current work presents the experiences with an untested substrate, the liquid fraction of municipal solid waste in microbial fuel cells (MFCs) for simultaneous treatment and energy recovery purposes. Since communications on the biotechnological applications for waste management have been considerably extended in number, the data in this manuscript can be considered quite important for the current researches in this area. Based on our results, it has been demonstrated that the substrate – originated from communal landfill – with high organic material content could be successfully biodegraded by the MFCs. However, depending on substrate concentration and reinoculation of MFCs with mesophilic anaerobic sludge, energy production efficiencies were strongly influenced by the competing phenomena taking place between anodic biofilm and bulk phase. To our knowledge, assessment of MFC (“feasibility study”) for the utilization of a particular waste generated in large quantities is scantily reported and claims novelty to the content of the paper.

Our group has been dealing with various biomethods e.g. biohydrogen and biolubricant technology and as a result of this progress, a significant number of papers (both review and research studies) have been published in highly reputed, quality journals including your journal, the *Journal of Cleaner Production* as well as the *International Journal of Hydrogen Energy*, *Bioresource Technology*, *Renewable & Sustainable Energy Reviews*, etc. For your reference, a sorted list of recent, relevant publications is as follows:

Madarász J, Németh D, Bakos J, Gubicza L, Bakonyi P. Solvent-free enzymatic process for biolubricant production in continuous microfluidic reactor. *J Cleaner Prod* 2015, doi: 10.1016/j.jclepro.2015.01.028

Bakonyi P, Nemestóthy N, Simon V, Bélafi-Bakó K. Review on the start-up experiences of fermentative hydrogen producing bioreactors. *Renew Sust Energ Rev* 2014;40:806-613

Bakonyi P, Nemestóthy N, Simon V, Bélafi-Bakó K. Fermentative hydrogen production in anaerobic membrane bioreactors: A review. *Bioresour Technol* 2014;156:357-63

Bakonyi P, Borza B, Orlovits K, Simon V, Nemestóthy N, Bélafi-Bakó K.. Fermentative hydrogen production by conventionally and unconventionally heat pretreated seed cultures: a comparative assessment. *Int J Hydrogen Energy* 2014;39:5589-96

Bakonyi P, Kumar G, Nemestóthy N, Lin CY, Bélafi-Bakó K.. Biohydrogen purification using a commercial polyimide membrane module: studying the effects of some process variables. *Int J Hydrogen Energy* 2013;38:15092-9

Ramírez-Morales JE, Tapia-Venegas E, Nemestóthy N, Bakonyi P, Bélafi-Bakó K, Ruiz-Filippi G. Evaluation of two gas membrane modules for fermentative hydrogen separation. *Int J Hydrogen Energy* 2013;38:14042-52

Bakonyi P, Nemestóthy N, Bélafi-Bakó K.. Biohydrogen purification by membranes: An overview on the operational conditions affecting the performance of non-porous, polymeric and ionic liquid based gas separation membranes. *Int J Hydrogen Energy* 2013;38:9673-87

This work is in our research sequence and we feel that the *Journal of Cleaner Production* is the most appropriate place for this contribution. The manuscript being submitted now is original, has not been communicated with your journal earlier and is not

currently sent for review to any other journal and will not be submitted elsewhere before a decision is made. All the authors agreed with the submission and confirm that all elements of the submission are in compliance with the journal publishing ethics policy.

Thanking you in advance for the time spent in dealing with this manuscript, we are looking forward to hearing from you in the near future.

Sincerely yours,

Dr. Peter Bakonyi, Ph.D.

Corresponding Author
Research Institute on Bioengineering, Membrane Technology and Energetics
University of Pannonia
10. Egyetem str., 8200 Veszprém, Hungary
Email: bakonyip@almos.uni-pannon.hu
Tel.: +36 (88) 624385

- municipal liquid waste was successfully degraded in microbial fuel cell
- reinoculation and substrate concentration affected energy recovery
- competition between anodic biofilm and bulk phase determined process efficiency
- average chemical oxygen demand removal exceeded 87 %

(Word count: 4138)

**Bioelectrochemical treatment of municipal waste liquor in microbial fuel cells for
energetic valorization**

L. Koók, T. Rózsenszki, N. Nemestóthy, K. Bélafi-Bakó, P. Bakonyi*

Research Institute on Bioengineering, Membrane Technology and Energetics
University of Pannonia, Egyetem ut 10, 8200 Veszprém, Hungary

*Corresponding Author: Péter Bakonyi

Tel: +36 88 624385

Fax: +36 88 624292

E-mail: bakonyip@almos.uni-pannon.hu

Abstract

Microbial fuel cells (MFCs) are recognized as promising applications to produce bioelectricity by utilizing various waste materials. In this study, dual-chamber microbial fuel cells were employed for energetic valorization of an untested substrate, liquid fraction of pressed municipal solid waste (LPW). This by-product is potentially applicable for MFCs because of its high organic matter content. In the course of the experiments, the anodic biofilm response and energy production efficiency have been investigated by experimental design approach, taking substrate and fresh inoculum – mesophilic anaerobic sludge (MAS) – addition as factors into account. It was observed that reinoculation could demonstrate a negative effect on the energy production, especially at low substrate (LPW) dosing levels. However, when the LPW to fresh MAS ratio in the anode chamber exceeded a particular value, the biofilm-associated electrical utilization dominated against the degradation in the bulk phase. Furthermore, the results indicated that the highest energy yields ($8-9 \text{ J g}^{-1} \Delta\text{COD d}^{-1}$) could be attained at the lowest input COD concentrations. The maximal and average COD removal efficiency were 94% and 87 %, respectively, which indicate the excellent biodegradability of LPW. As for COD removal rate, $1.2-1.9 \text{ kg COD m}^{-3} \text{ d}^{-1}$ could be reached.

Keywords: microbial fuel cell, organic waste, power generation, experimental design

1. Introduction

Nowadays, microbial fuel cells (MFCs) are increasingly mentioned in the joint subject of waste treatment and energy generation since MFCs are able to directly convert waste-bound chemical energy into electricity via bioprocesses catalyzed by exoelectrogenic microorganisms (Logan, 2008; Logan et al., 2006; Lovley, 2006, Wrana et al., 2010). A general, laboratory-scale MFC system consists of three main structural elements, (i) an anode and (ii) a cathode chamber and (iii) a proton selective membrane separating the two compartments and ensuring the proton transport between them (Huang et al., 2015). As a result of substrate decomposition by anode-living whole cell biocatalysts, electrons are liberated, captured by the anode and subsequently transferred to the cathode via external wiring. In the cathode, electrons and protons are combine with oxidative agent e.g. oxygen and in turn, water is formed.

A variety of substrates can be used for bioelectricity generation in MFCs such as saccharides (Bélafi-Bakó et al., 2011; Chaudhuri and Lovley, 2003; Kim et al., 2000; Rabey et al., 2003; Vajda et al., 2014), organic acids (Bond and Lovley, 2005; Liu et al., 2005; Min and Logan, 2004), alcohols (Kim et al., 2007; Vajda et al., 2011) as well as inorganic substances e. g. sulfate (Rabey et al., 2006). In addition, there is a significant research interest towards complex materials i.e. industrial and municipal waste streams (Leaño and Babel, 2012), which, because of their high organic matter content, are potential starting materials of power generation in MFCs (Angenent et al., 2004; Pant et al., 2010; Liu et al., 2004). Therefore, MFCs – due to the possibility of simultaneous waste management and energy production – are considered as prosperous applications facilitating sustainability. In the wide range of microbial fuel cells, the ones relying on mixed cultures have been demonstrated as feasible solutions to accomplish efficient “waste to energy” conversion (Jung and Ragen, 2007).

In this work, an untested substrate, the liquid fraction of pressed solid municipal waste (LPW) was applied in dual-chamber microbial fuel cells to reveal its viability for electricity generation. In parallel with the feasibility study, it was aimed to investigate the response of the anodic biofilm – formed during preliminary adaptation processes – to reinoculation of MFC anode compartment by mesophilic anaerobic sludge (MAS). Therefore, an experimental design was purposefully carried out by combined feeding of LPW and MAS as seeding

source. To our knowledge, no research has been conducted with LPW in microbial fuel cells, which claims novelty for the work.

2. Materials and Methods

2.1. Inoculum and substrate

MAS – the inoculum of fuel cells – was collected from a domestic biogas plant. The biogas fermenter processes kitchen wastes and cattle manure and its effluent is suitable seed source providing a good microbial community. The initial total COD and pH values of MAS were 30 g L⁻¹ and 7.8, respectively.

LPW is originated from a regional solid waste treatment plant (located in Kiralyszentistvan, Hungary) and was obtained by pressing from the biofraction of municipal solid garbage. Its most important characteristics are summarized in Table 1.

2.2. MFC design and start-up

The laboratory-scale, dual-chamber MFCs were made of plexiglass. The cells were constructed with a volume of 60 mL for anode and cathode compartments. The electrode chambers were divided by Nafion N 115 proton-selective membrane (Sigma-Aldrich). The surface area and thickness of the membrane were 7 cm² and 127 μm, respectively. Carbon cloth fixed on a graphite rod was used as electrode material. The surface area was 25 cm² for both electrodes. Five MFCs were operated in parallel with 100 Ω external resistor.

At a start-up phase, anode chamber was filled with MAS (55 mL), purged with N₂ to ensure anaerobic conditions and fed with Na-acetate (2 mL, 25 g L⁻¹ stock solution) after the initial voltage has decreased. Na-acetate was injected in two steps to promote the colonization of the electrode surface by anode-respiring, exoelectrogene microorganisms. After ~15 days long adaptation period and gradual voltage decrease back to the initial value of inoculation, the experiments with LPW and MAS feeding were carried out. In the course of these measurements, LPW and fresh MAS were loaded in various ratios (Table 2) and simultaneously, equal volume of anolyte was removed.

The cathode compartment contained distilled water (60 mL) and was continuously sparged with air. The anode and cathode sides were connected by copper wire and the

potential difference was on-line followed using a data logger device (National Instruments USB-6008) and LabView software. The MFCs were incubated at 37 °C.

2.3. Analysis and calculation

According to Ohm's law, current data (I) and thus electrical power (P) could be calculated based on the voltage measured. Cumulative energy data (E) were the products of integrating the time-dependent power curve. Specific values, namely current density (jI), power density (jP) were derived by taking into consideration the electrode surface area. The energy yield (jE) was a product of the amount of substrate eliminated (ΔCOD), the time of operation (designated by t in Fig. 4) and the cumulative energy generated. The results presented throughout in this work are the mathematical averages of triplicates and the standard deviations were below 5 %. COD was determined following APHA (APHA, 1995). The most particular traits of LPW substrate are given in Table 1.

3. Results and Discussion

3.1. The effect of LPW and MAS ratio on MFC performance

Quality feature of the adaptation substrate supporting the biofilm formation have pronounced effect in the start-up phase as well as in further operation. The electrodes coated with sufficiently stable biofilm layers – and thus the complete MFC – might be used for long-terms. Na-acetate is a simple, readily accessible substance with fair biodegradability and excellent electron donor capacity (Dulon et al., 2007). Hence, it is recognized as a potential substrate candidate for MFC start-up and was applied in this study to promote the development of biofilm on the anode surface. After MFCs start-up, an experimental design (Table 2) was carried out to study the biofilm response in terms of energetic valorization of LPW.

Table 2 represents a complete 2^2 experimental design, having 2-2 low and high values and one central point, as illustrated by Fig. 1. The two-level designs are particularly useful to obtain reliable information and make firm conclusions about the impacts of factors of primary importance (Bakonyi et al., 2011). In this study, as it can be seen in Table 2 and Fig.1, the anodic concentrations of LPW substrate and that of fresh MAS (Table 2) were chosen as

independent variables to get a feedback about their influence on waste utilization efficiency, which can be correlated with the biofilm response.

Substrate – in our case LPW – concentration has a defining role on any bioprocess e.g. MFC since it affects the growth rate of microorganisms (in the biofilm) as well as their metabolic activity, which determines the formation of product (in this work, bioelectric potential). Moreover, the capability of the anode-living, exo-electrogenic bacteria to manage higher organic loadings is a well-known and desired process indicator. However, LPW, due to its origin (landfill), is a complex mixture and potentially contains inhibitory components. Thus, viability of MFC under higher LPW additions is uncertain. Therefore, assessing the response of MFC to various substrate doses can reveal the robustness and feasibility of the application.

The electrochemically active bacteria that power MFC are the hearth and soul of the technology. Among them, organisms such as *Shewanella*, *Geobacter*, etc. species have been identified as strains capable of converting chemically-bound energy into bioelectricity (Wrana et al., 2010). However, when their pure cultures are employed as seeding source to colonize MFC anode, maintaining sterility is a strict requirement. Such applications, which are started-up and operated under sterile circumstances, suffer from considerable economic as well as technological drawbacks especially when industrial waste streams or bioreactor effluents are to be utilized. This is attributed to the threat of contamination by indigenous microflora carried in the influents which presumably cut the overall MFC performance. Due to these limits of pure cultures, mixed communities are suggested to start-up MFC systems, as performed in this study using MAS.

When non-sterile MFCs are seeded with microbial consortia, e.g. anaerobic digester sludge, waste water sludge, etc. exo-electrogenic strains will be enriched on the anode surface and form a bioactive layer. The profile of the active microbial population present is a crucial issue of biotechnological applications which can be changed by competing bacteria present in the feed streams, leading possibly to altered process performance (Bakonyi et al., 2014). Direct methods (electron microscopic investigation, identification of strains based on genetic information, cyclic voltammetry, etc.) to characterize anode biofilm are attractive but mostly require the deconstruction of the MFC. Therefore, in this work, an indirect method (via in-situ bioelectricity measurements, when the whole MFC played the role of a “biosensor”) was chosen to evaluate the effect of fresh MAS addition (“reinoculation” with untreated sludge) on energy production.

The assessment was carried out based on the assumption that the fresh microbial consortia fed into the anode chamber might disturb the biofilm and simultaneously, the electricity generation. Furthermore, it was hypothesized that the nutrients available in the feed can be consumed by microbes (supplemented with the fresh MAS) that are not localized on the electrode surface, which does not contribute to useful bioelectricity production.

A typical voltage output is demonstrated in Fig 2, where the response to each steps are seen. In the first stage, the MFC unit was inoculated with raw MAS and initial voltage has increased but shortly, it turned into a strong decrease. At that point, in the second stage, sodium acetate was added to help the establishment of the bioactive layer on the anode which resulted in prompt and sharp jump of voltage, followed by a gradual reduction in the cell potential. Subsequently, during the third step, the MFC was supplemented again with Na-acetate. As it can be noticed, it has induced an observable increment of bioelectric potential, which has continuously fallen presumably due to the depletion of adequate substrates. It would also appear that the second adaptation resulted in a more stable voltage signal as compared to the first one. This was a positive feedback and may be correlated with biofilm formation. In the fourth step, after two-times acclimatization with acetate, the MFCs were loaded with the mixture of LPW and fresh MAS in accordance with the experimental design matrix (Table 2). As it can be concluded from Fig. 2, there was a growing cell potential after the injection of LPW:MAS mixture, which has reached a definite peak range where quite steady voltage (roughly 40 mV) could be measured (approximately between the 650th and 950th hour of MFC operation). Voltage fluctuations may be associated with the complexity of the feed since various fractions of the raw materials could become accessible and biodegradable at different periods of times. Based on the voltage data collected, the main energetic characteristics were calculated and to be found in Table 3. The outcomes are comparable with other literature studies. For instance, Cercado-Quezada et al. (2010) tested various food industry wastes as well as compost leachate to extract energy in microbial fuel cells. Using the latter substrate (that shows certain relation with landfill-derived LPW), 232 mA m⁻² current density was reported applying MFC seeded with anaerobic sludge, meanwhile in this research 152-218 mA m⁻² could be achieved. However, power densities obtained were notably different, since the 54 mW m⁻² value by Cercado-Quezada et al. (2010) exceeded our power densities ranging between 5.8 and 11.9 mW m⁻². In another paper, Ganesh and Jambeck (2013) operated MFCs fed with leachate and attained 114 mA m⁻² current density which was slightly lower than our ones as indicated in Table 3. Tugtas and co-workers (2013)

employed MFC to biodegrade pre-digested landfill leachate. It has turned out that the steady current densities of 418-548 mA m⁻² could be obtained, meaning a salient system performance.

The contradicting MFC efficiencies can be attributed to a number of biological (e.g. the source of inoculum) and architectural (e.g. the external resistance used, the distance of electrodes, the conductivity of anolyte, etc.) reasons.

Fig. 3 shows the response of MFC performance to various LPW and fresh MAS additions. According to the results, it can be pointed out that the MFCs were able to generate bioelectricity and microbes responded positively to the presence of LPW since higher substrate concentrations allowed to gain more (cumulative) energy. However, it would appear that there could be a joint effect of simultaneous LPW and MAS supplementation, as follows. On one hand, the analysis of the response surface in Fig. 3 implies that at low LPW concentrations the addition of MAS in larger quantities should be avoided. It may be explained by the competition between bulk phase and anodic biofilm, when degradation of organic matter in bulk phase is more pronounced and therefore only a significantly reduced amount of substrate is able to reach the bioactive layer on the anode surface. In other words, most of the chemical energy bound in the feed material is lost via an undesired bioprocess. On the other hand, when LPW concentration increases in the anode cell (higher substrate loading), the phenomena taking place due to fresh inoculum injection (MAS content) has less negative effect on the anode-surface reaction and hence bioelectricity generation will not be limited so strongly. Overall, it is to conclude that outsider microbes entering the MFC anode chamber can influence the substrate utilization and related bioelectricity production, although it seems to be dependent on substrate to inoculum ratio.

3.2. Effect of LPW and MAS concentrations on energy yield and COD removal

In Fig. 4, the dependency of energy yield on LPW and MAS concentrations is plotted. The results show that significantly higher energy yields (up to 8-9 J g⁻¹ ΔCOD d⁻¹) were achieved in case of low COD loadings that occur with low LPW and MAS concentrations. As elucidated above, the addition of MAS (“reinoculation”) could pose a threat on reliable MFC operation since microorganisms in the bulk phase compete for the nutrients and hence, reduce their amount being available for the electrochemically active anode-surface consortia, inherently causing depressed energy yields. Moreover, it can be observed that improved

energetic valorization of LPW favors its low concentrations. LPW is a complex organic matter and therefore, its bioelectrochemical decomposition has significant time-demand, which, in addition, is likely influenced by the diffusion rate of substances into the biofilm, as well. Besides, the biofilm can be characterized by limited substrate processing capacity. Thus, the phenomenon of substrate saturation may occur, when the ability of microbes in the biofilm is fully exploited, they are unable to take up more compounds and thereby different bulky phase processes could take place in parallel that do not support electricity generation. From an energetic point of view, it means an unbeneficial utilization of the substrate. Therefore, if the substrate level is maintained sufficiently low or in other word, when the influent is properly diluted (Cercado-Quezada et al., 2010) then the anode-living strains can have the possibility to more efficiently convert bigger portions of LPW into desired electricity.

The COD removal capacity of MFC is an important parameter to evaluate the attractiveness of MFC technology from environmental perspective. COD removal data are summarized in Table 4, where one can see that remarkable COD degradation was possible from a complex substrate such as LPW, which is a notable advantage of the MFCs. According to Table 4, the average COD removal was 87.3 % and in case of MFC 1 it exceeded even 94 %. These values can be considered high in the view of other literature data (Gálvez et al., 2009; Mohan et al., 2007; Rengasamy and Berchmans, 2012) as well as in comparison with our previous accomplishments on sugar industry wastewater (Bélafi-Bakó et al., 2014). The organic-removal efficiency of the cells can be described well by the average COD removal rates, which varied between 1.2 and 1.9 kg COD d⁻¹ values, assuming 1 m³ anode chamber volume.

4. Conclusions

Based on the results, it was concluded that the wastewater (LPW)-based microbial fuel cell system operated successfully and was shown suitable for power generation with simultaneous COD removal. It was found that reinoculation of MFC with mesophilic sludge influenced the effectiveness of the preliminary adapted anode-surface biofilm. In addition, it has turned out that energy recovery was strongly dependent on LPW (substrate) concentration in the anode chamber and improved MFC performance favored low organic material loadings.

Acknowledgements

This work was supported by the European Union and co-financed by the European Social Fund in the frame of the TAMOP-4.2.2.A-11/1/KONV-2012-0071 and TAMOP-4.2.2/A-11/1/KONV-2012-0038 projects. Nándor Nemestóthy acknowledges the János Bolyai Research Scholarship of the Hungarian Academy of Sciences.

References

- Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domínguez-Espinosa, R., 2004). Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol.* 22, 477-485.
- APHA, 1995. Standard methods for the examination of water and wastewater. 19th ed. New York, USA: American Public Health Association.
- Bakonyi, P., Nemestóthy, N., Lövitusz, É., Bélafe-Bakó, K., 2011. Application of Plackett-Burman experimental design to optimize biohydrogen fermentation by *E. coli* (XL1-BLUE). *Int. J. Hydrog. Energ.* 36, 13949-13954.
- Bakonyi, P., Nemestóthy, N., Simon, V., Bélafe-Bakó, K., 2014. Review on the start-up experiences of continuous fermentive hydrogen producing bioreactors. *Renew. Sust. Energ. Rev.* 40, 806-813.
- Bélafe-Bakó, K., Vajda, B., Bakonyi, P., Nemestóthy, N., 2014. Removal of COD by Two-Chamber Microbial Fuel Cells. In: Wang CT (ed) *Technology and application of microbial fuel cells*, InTech, Rijeka, pp. 77-87.
- Bélafe-Bakó, K., Vajda, B., Nemestóthy, N., 2011. Study on operation of a microbial fuel cell using mesophilic anaerobic sludge. *Desalin Water Treat.* 35, 222-226.
- Bond, D.R., Lovley, D.R., 2005. Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans*. *Appl. Environ. Microbiol.* 71, 2186-2189.
- Cercado-Quezada, B., Delia, M.L., Bergel, A., 2010. Testing various food-industry wastes for electricity production in microbial fuel cell. *Bioresour. Technol.* 101, 2748-2754.
- Cercado-Quezada, B., Delia, M.L., Bergel, A., 2010. Treatment of dairy wastes with a microbial anode formed from garden compost. *J. Appl. Electrochem.* 40, 225-232.
- Chaudhuri, S.K., Lovley, D.R., 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nature Biotechnol.* 21, 1229-1232.
- Dulon, S., Parot, S., Delia, M.L., Bergel, A., 2007. Electroactive biofilms: new means for electrochemistry. *J. Appl. Electrochem.* 37, 173-179.
- Gálvez, A., Greenman, J., Ieropoulos, I., 2009. Landfill leachate treatment with microbial fuel cells; scale-up through plurality. *Bioresour. Technol.* 100, 5085-5091.
- Ganesh, K., Jambeck, J.R., 2013. Treatment of landfill leachate using microbial fuel cells: Alternative anodes and semi-continuous operation. *Bioresour. Technol.* 139, 383-387.

Huang, L., Liu, Y., Yu, L., Quan, X., Chen, G., 2015. A new clean approach for production of cobalt dihydroxide from aqueous Co(II) using oxygen-reducing biocathode microbial fuel cells. *J. Cleaner Prod.* 86, 441-446.

Jung, S., Regan, J.M., 2007. Comparison of anode bacterial communities and performance in microbial fuel cells with different electron donors. *Appl. Microbiol. Biotechnol.* 77, 393-402.

Kim, J.R., Jung, S.H., Regan, J.M., Logan, B.E., 2007. Electricity generation and microbial community analysis of alcohol powered microbial fuel cells. *Bioresour Technol* 98, 2568-2577.

Kim, N., Choi, Y., Jung, S., Kim, S., 2000. Effect of initial carbon sources on the performance of microbial fuel cells containing *Proteus vulgaris*. *Biotechnol. Bioeng.* 70, 109-114.

Leaño, E.P., Babel, S., 2012. The influence of enzyme and surfactant on biohydrogen production and electricity generation using Palm Oil Mill Effluent. *J. Cleaner Prod.* 31, 91-99.

Liu, H., Cheng, S., Logan, B.E., 2005. Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* 39, 658-662.

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. *Environ. Sci. Technol.* 38, 4040-4046.

Liu, H., Ramnarayanan, R., Logan, B.E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.* 38, 2281-2285.

Logan, B.E., 2008. *Microbial fuel cells*. John Wiley & Sons, New York

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. *Environ. Sci. Technol.* 40, 5181-5192.

Lovley, D.R., 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. *Curr. Opin. Biotechnol.* 17, 327-332.

Min, B., Logan, B.E., 2004. Continuous electricity generation from domestic wastewater and organic substrates in a flat plate microbial fuel cell. *Environ. Sci. Technol.* 38, 5809-5814.

Mohan, S.V., Raghavulu, S.V., Srikanth, S., Sarma, P.N., 2007. Bioelectricity production by mediatorless microbial fuel cell under acidophilic condition using wastewater as substrate: Influence of substrate loading rate. *Curr. Sci.* 92, 1720-1726.

Pant, D., Van Bogaert, G., Diels, L., Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. *Bioresour. Technol.* 101, 1533-1543.

Rabaey, K., Lissens, G., Siciliano, S.D., Verstraete, W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol. Lett.* 25, 1531-1535.

Rabaey, K., Van de Sompel, K., Maignien, L., Boon, N., Aelterman, P., Clauwaert, P., Schampelaire, L.D., Pham, H.T., Vermeulen, J., Verhaege, M., Lens, P., Verstraete, W., 2006. Microbial fuel cells for sulfide removal. *Environ. Sci. Technol.* 40, 5218-5224.

Rengasamy, K., Berchmans, S., 2012. Simultaneous degradation of bad wine and electricity generation with the aid of the coexisting biocatalysts *Acetobacter aceti* and *Gluconobacter roseus*. *Bioresour. Technol.* 104, 388-393.

Tugtas, A.E., Cavdar, P., Calli, B., 2013. Bio-electrochemical post-treatment of anaerobically treated landfill leachate. *Bioresour. Technol.* 139, 266-272.

Vajda, B., Bélaŕi-Bakó, K., Nemestóthy, N., 2011. The role of methanol in microbial fuel cells. *Hung. J. Ind. Chem.* 39, 387-390.

Vajda, B., Nemestóthy, N., Bakonyi, P., Bélaŕi-Bakó, K., 2014. Xylose substrate as the only nutrient in the operation of microbial fuel cells. *Environ. Prot. Eng.* 40, 131-141.

Wrana, N., Sparlin, R., Cicek, N., Levin, D.B., 2010. Hydrogen gas production in a microbial electrolysis cell by electrohydrogenesis. *J. Cleaner Prod.* 18, 105-111.

Figure and Table Captions

Fig 1: Layout of the experimental design

Fig 2: Voltage output as a function of time (in case of 5 mL:9 mL LPW:MAS ratio)

Fig. 3: Effect of MAS-LPW mixture feeding on energy production

Fig 4: Changes in energy yield to initial MAS and LPW concentrations

Table 1: The main characteristics of LPW

Table 2: Parameters of the experimental design

Table 3: Values of energetic parameters obtained from the results of the experimental design

Table 4: COD parameters of each MFCs

Fig.1

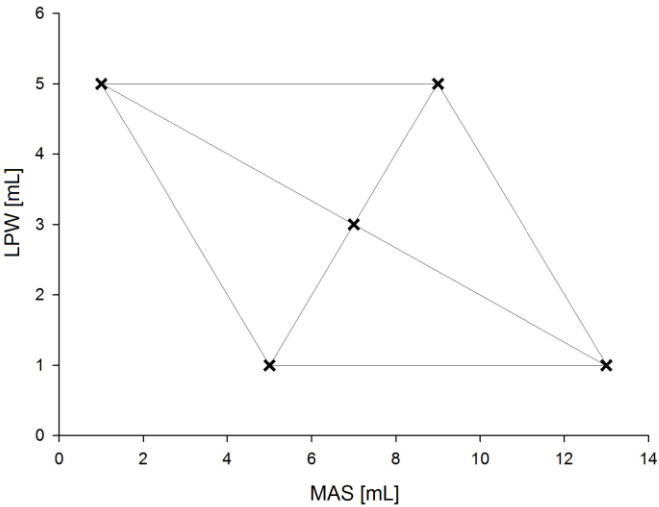


Fig. 2

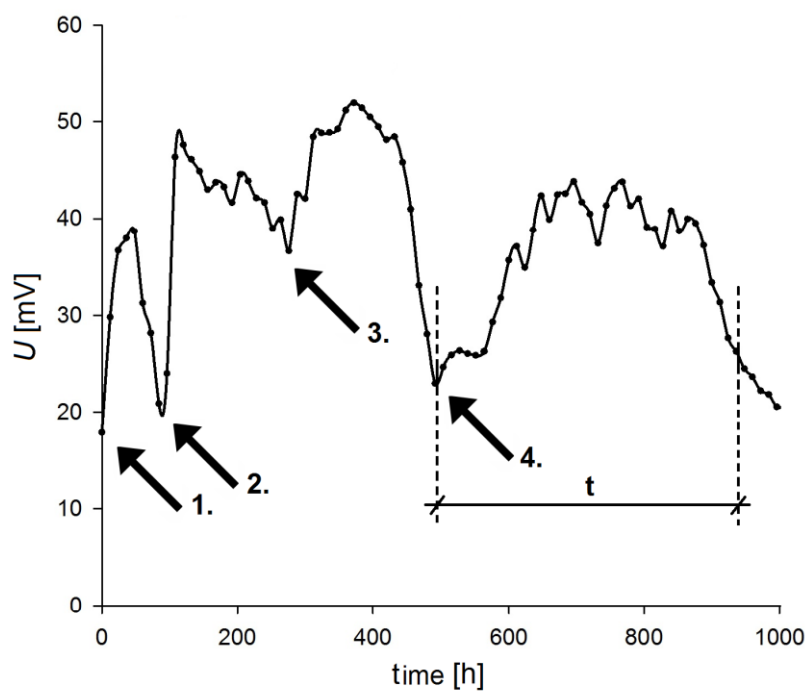


Fig. 3

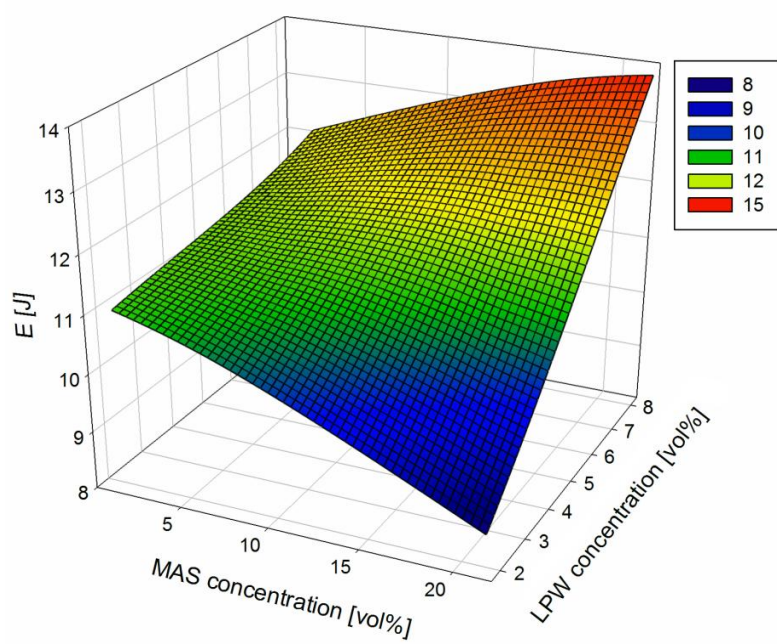


Fig.4

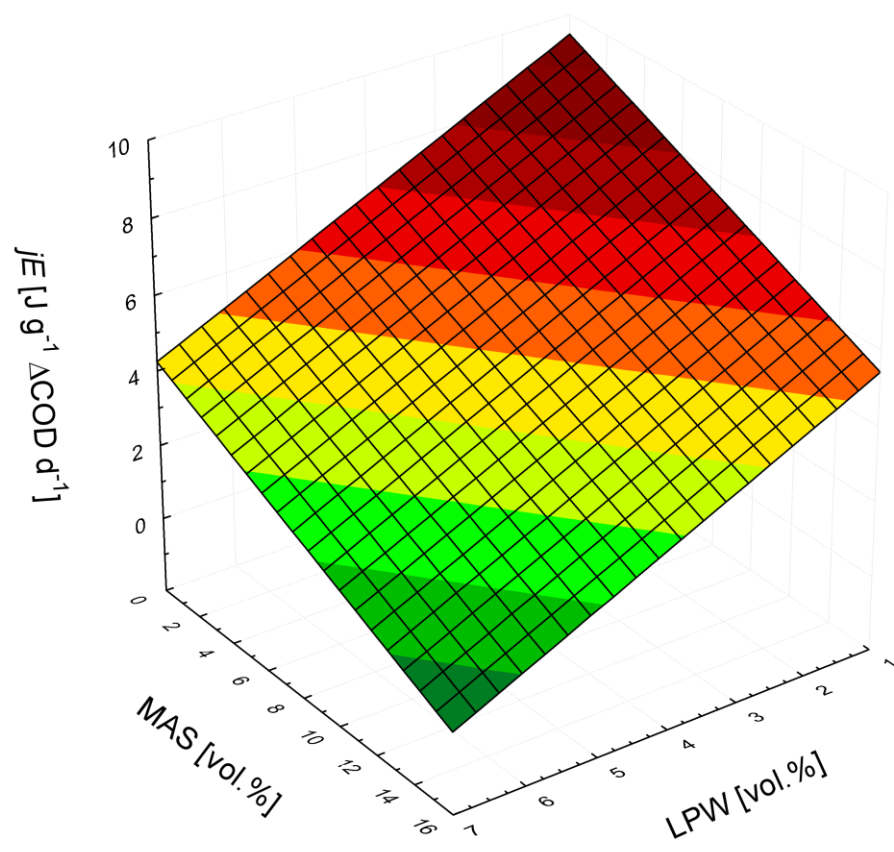


Table 1

Parameter	Value
total COD	111.6 g L ⁻¹
BOI ₅	50.8 g L ⁻¹
pH	4.7
Protein content	42.5 g L ⁻¹
TS	7.3 %
Reducing sugar content	1.6 g L ⁻¹
TOC	35.9 g L ⁻¹

Table 2

MFC ID	Added LPW (mL)	Added MAS (mL)	LPW concentration (vol.%)	MAS concentration (vol.%)
1.	5.0	9.0	8.33	15.00
2.	1.0	5.0	1.67	8.33
3.	1.0	13.0	1.67	21.67
4.	3.0	7.0	5.00	11.67
5.	5.0	1.0	8.33	1.67

Table 3

MFC	LPW:MAS	$U_{\max}$	$jI_{\max}$	$jP_{\max}$	E
ID	(mL:mL)	(mV)	(mA m ⁻²)	(mW m ⁻²)	(J)
1.	5:9	43.8	175	7.7	13.4
2.	1:5	54.6	218	11.9	10.6
3.	1:13	47.8	191	9.2	8.8
4.	3:7	49.9	200	10.0	11.6
5.	5:1	38.0	152	5.8	12.0

Table 4

MFC ID	COD input (g)	Initial COD (g L ⁻¹)	Final COD (g L ⁻¹)	COD removal (%)
1.	0.855	44.67	2.31	94.8
2.	0.268	34.88	5.11	85.4
3.	0.512	38.94	4.11	89.5
4.	0.562	39.77	8.52	78.6
5.	0.612	40.61	4.84	88.1