

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16

(Word count: 4964)

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

László Koók, Tamás Rózsenberszki, Nándor Nemestóthy, Katalin Bélafi-Bakó, Péter 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

1 **Abstract**

2

3 Microbial fuel cells (MFCs) are recognized as promising applications to
4 produce bioelectricity by utilizing various waste materials. In this study, dual-chamber
5 microbial fuel cells were employed for energy valorization of an untested substrate,
6 the liquid fraction of pressed municipal solid waste (LPW). This by-product is
7 potentially applicable as a substrate in MFCs because of its high organic matter
8 content. In the course of the experiments, the anodic biofilm response and energy
9 production efficiency have been investigated by experimental design approach, taking
10 substrate and fresh inoculum – mesophilic anaerobic sludge (MAS) – addition into
11 account as factors. It was observed that reinoculation could result in a negative effect
12 on the energy production, especially at low substrate (LPW) dosing levels. However,
13 when the LPW to fresh MAS ratio in the anode chamber exceeded a particular value,
14 the biofilm-associated electrical utilization dominated against the degradation in the
15 bulk phase. Furthermore, the results indicated that the highest energy yields ($8-9 \text{ J g}^{-1}$
16 $\Delta\text{COD d}^{-1}$) could be attained at the lowest input COD concentrations. The maximal
17 and average COD removal efficiencies were 94% and 87 %, respectively, which
18 indicate the excellent biodegradability of LPW. As for COD removal rate, $1.2-1.9 \text{ kg}$
19 $\text{COD m}^{-3} \text{ d}^{-1}$ could be reached.

20

21 **Keywords:** microbial fuel cell, organic waste, power generation, experimental design,
22 bioelectricity, waste utilization

23

1 **1. Introduction**

2

3 Nowadays, bioelectrochemical systems such as microbial electrohydrogenesis
4 cells (Rivera et al., 2015) and microbial fuel cells (MFCs) (Rózsensberszki et al., 2015)
5 are increasingly mentioned in the joint subject of waste treatment and energy
6 generation . MFCs are able to convert waste-bound chemical energy into electricity
7 directly via bioprocesses catalyzed by exoelectrogenic microorganisms (Logan, 2008;
8 Logan et al., 2006; Lovley, 2006). A general, laboratory-scale MFC system consists of
9 three main structural elements, (1) an anode and (2) a cathode chamber and (3) a
10 proton selective membrane separating the two compartments and ensuring the proton
11 transport between them (Huang et al., 2015). As a result of substrate decomposition by
12 anode-living whole cell biocatalysts, electrons are released, captured by the anode and
13 subsequently transferred to the cathode via an external wiring. In the cathode,
14 electrons and protons are combined with oxidative agent e.g. oxygen and in turn, water
15 is formed.

16 A variety of substrates can be used for bioelectricity generation in MFCs such
17 as saccharides (Chaudhuri and Lovley, 2003; Kim et al., 2000; Vajda et al., 2014),
18 organic acids (Bond and Lovley, 2005; Liu et al., 2005; Min and Logan, 2004),
19 alcohols (Kim et al., 2007; Vajda et al., 2011) as well as inorganic substances e. g.
20 sulphate (Rabaey et al., 2006). In addition, there is a significant research interest
21 towards complex materials i.e. industrial and municipal waste streams (Leaño and
22 Babel, 2012), which are potential starting materials of power generation in MFCs
23 because of their high organic matter content (Angenent et al., 2004; Pant et al., 2010;

1 Liu et al., 2004). Therefore, due to the possibility of simultaneous waste management
2 and energy production, MFCs are considered as prosperous applications facilitating
3 sustainability. In the wide range of microbial fuel cells, the ones relying on mixed
4 cultures have been demonstrated as feasible solutions to accomplish efficient “waste to
5 energy” conversion (Jung and Ragen, 2007).

6 MFCs are powered by electrochemically active bacteria which are the heart and
7 soul of the technology. Among them, organisms such as *Shewanella*, *Geobacter*, etc.
8 species have been identified as strains capable of converting chemically-bound energy
9 into bioelectricity (Wrana et al., 2010). However, when their pure cultures are
10 employed as seeding source to colonize MFC anodes, maintaining sterility is a strict
11 requirement. The applications, which are started-up and operated under sterile
12 circumstances, suffer from considerable economic and technological drawbacks
13 especially if industrial waste streams or bioreactor effluents are utilized. This is
14 attributed to the risk of contamination by indigenous microorganisms carried in the
15 influents which presumably reduce the overall MFC performance. Due to the
16 abovementioned limits of pure cultures, mixed communities are suggested to start-up
17 MFC systems, as performed in this study using MAS.

18 When non-sterile MFCs are seeded with microbial consortia, e.g. anaerobic
19 digester sludge, waste water sludge, etc., exoelectrogenic strains are enriched on the
20 anode surface and form a bioactive layer. The profile of the anodic microbial
21 population is a crucial issue, which can be changed by competing bacteria in the feed
22 streams or in the seed inoculum itself, possibly leading to altered process performance
23 (Bakonyi et al., 2014).

1 To assess the impact of process disturbances on MFC efficiency – related to the
2 appearance of non-electricity generating, invader microorganisms – direct methods
3 (electron microscopic investigation, identification of strains based on genetic
4 information, cyclic voltammetry, etc.) can be employed, but they mostly require the
5 deconstruction of the MFC. Nevertheless, indirect methods such as in-situ bioelectric
6 measurements – when the whole MFC plays the role of a “biosensor” – can be chosen
7 for more convenient and quick evaluation. Therefore, in this work, this latter technique
8 was used to analyze the effect of MFC reinoculation (fresh sludge addition to the
9 already developed anodic consortia) on bioenergy production. In the course of the
10 reinoculation tests, an uncommon substrate, the liquid fraction of pressed solid
11 municipal waste (LPW) was applied. Substrate concentration has a decisive role on
12 any bioprocesses, e.g. MFC, since it affects the growth rate and the metabolic activity
13 of microorganisms (in the biofilm) and hence determines the production intensity (in
14 MFC, bioelectric potential). Moreover, the capability of the anode-living,
15 exoelectrogenic bacteria to manage as high organic loadings as possible is a well-
16 known and desired process indicator. LPW, due to its origin (landfill), is a complex
17 mixture and might contain inhibitory components. Thus, its feasibility for MFCs
18 especially in higher concentrations – because of the lack of experiences – is uncertain.
19 Therefore, assessing the behaviour of MFCs to various substrate (LPW) doses was one
20 of our key-interest in order to reveal the robustness and feasibility of this application.

21 Overall, the aim of this research was twofold. Besides the feasibility study of
22 LPW, the response of the anodic biofilm – formed during preliminary adaptation
23 process – to the reinoculation of MFC anode compartment by mesophilic anaerobic

1 sludge (MAS) was also investigated. Thereby, the appropriate substrate feeding rate
2 could be estimated for scaled-up waste processing MFC systems. In addition, the
3 reactions taking place at the anode surface and in the bulk phase could be addressed to
4 obtain information about the degradation-capacity of the anodic biofilm. To our
5 knowledge, no research has been conducted with LPW in microbial fuel cells, which
6 brings the novelty of the work.

7

8 **2. Materials and Methods**

9

10 *2.1. Inoculum and substrate*

11

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

16 LPW is originated from a regional solid waste treatment plant (located in
17 Királyszentistván, Hungary) and was obtained by pressing from the biofraction of
18 municipal solid garbage. The main stages of waste processing at the plant are
19 presented in Fig. 1. The facility handles 120 000 t waste on annual basis from villages
20 and towns located in its neighbourhood. As a first step, the garbage is subjected to a
21 mechanical treatment. After shredding, iron is separated and the rest of the mass
22 stream is further sorted. Subsequently, a certain part of it is recycled, meanwhile others

1 are sent to additional techniques (Sarkady et al., 2014). The most essential
2 characteristics of LPW material are demonstrated in Table 1.

3

4 *2.2. MFC design and start-up*

5

6 The laboratory-scale, dual-chamber MFCs were made of plexiglass. The cells
7 were constructed with a volume of 60 mL for anode and cathode compartments. The
8 electrode chambers were divided by Nafion N 115 proton-selective membrane (Sigma-
9 Aldrich). The surface area and thickness of the membrane were 7 cm² and 127 μm,
10 respectively. Carbon cloth fixed on a graphite rod (1.5 mm diameter) was used as
11 electrode material. The apparent surface area (*A*) was 25 cm² for both electrodes (with
12 5.0 cm × 2.5 cm dimensions). The electrode surface specific values were calculated on
13 the basis of apparent surface area, i.e. by leaving the electrode thickness and the area
14 of the graphite rod out of account. Five MFCs were operated in parallel with 100 Ω
15 fixed external resistors (*R*). The schematic diagram of the experimental MFC and
16 occurring processes can be seen in Fig. 2.

17 In the start-up phase, the anode chamber was filled with MAS (55 mL), purged
18 by N₂ to ensure anaerobic conditions and fed with Na-acetate (2 mL, 25 g L⁻¹ stock
19 solution) after the initial voltage had decreased. Na-acetate was injected in two steps to
20 promote the colonization of the electrode surface by anode-respiring exoelectrogenic
21 microorganisms. After a ~15 days long adaptation period, when the gradual voltage
22 had decreased back to the initial value of inoculation, the experiments with LPW and
23 MAS feeding were carried out. In the course of these measurements, LPW and fresh

1 MAS were loaded in various ratios (Table 2) and simultaneously, while equal volume
2 of anolyte was removed. Besides LPW and MAS, no other materials (nutrients) were
3 added to the MFCs.

4 The cathode compartment contained water and was continuously sparged by air.
5 The anode and cathode sides were connected by copper wire and the potential
6 difference was on-line followed using a data logger device (National Instruments
7 USB-6008) and LabView software. The MFCs were incubated at 37 °C. Neither the
8 anodic nor the cathodic chamber was mechanically stirred.

9

10 *2.3. Experimental design*

11

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

21 Table 2 presents a complete 2^2 experimental design, having 2 low and 2 high
22 values and one central point, as illustrated in Fig. 3. The two-level designs are
23 particularly useful to obtain reliable information and make firm conclusions about the

1 effects of factors of primary importance (Bakonyi et al., 2011). In this study, as it can
2 be seen in Table 2 and Fig. 3, the anodic concentrations of LPW substrate and that of
3 fresh MAS were chosen as independent variables to get a feedback about their
4 influence on waste utilization efficiency, which can be correlated with the biofilm
5 response.

7 *2.4. Analysis and calculation*

9 COD (COD_0 initial and COD_t final) values were determined following APHA
10 (APHA, 1995). The COD input (in grammes) of the mixture feeding was calculated
11 based on the known COD values of the components (LPW and MAS) and their
12 injected volume. In the experimental design, the reinoculation ratio was defined as the
13 amount of the MAS injected divided by the working volume of the anode (Table2).

14 According to Ohm's law, current data (I) and thus electrical power (P) could be
15 calculated based on the voltage measured. Cumulative energy data (E) were calculated
16 from integrating the time-dependent power curve (Eq. 1).

$$18 \quad E = \int P(t)dt \quad (\text{Eq. 1})$$

19
20 Specific values, namely current density (jI), power density (jP) were derived by
21 taking into consideration the electrode surface area (Eq. 2 and Eq. 3).

$$23 \quad jI = U(t) R^{-1} A^{-1} = I(t) A^{-1} \quad (\text{Eq. 2})$$

1
$$jP = U(t) I(t) A^{-1} = P(t) A^{-1} \quad (\text{Eq. 3})$$

2
3 The energy yield (jE) was a product of the amount of substrate eliminated
4 ($\Delta\text{COD} = \text{COD}_0 - \text{COD}_t$), the time of operation (designated by τ in Fig. 4) and the
5 cumulative energy generated (Eq. 4).
6

7
$$jE = E \Delta\text{COD}^{-1} \tau^{-1} \quad (\text{Eq. 4})$$

8
9 The Coulombic efficiency (CE) of the MFCs can be calculated based on the
10 ratio of the total Coulombs obtained from the substrate and the theoretical maximum
11 of Coulombs when all of the electrons from the substrate generate electricity (Eq. 5).
12

13
$$CE = M \int I(t) dt F^{-1} b^{-1} V^{-1} \Delta\text{COD}^{-1} 100 \% \quad (\text{Eq. 5})$$

14
15 where M is the molar weight of oxygen, F is the Faraday's constant, b is the number of
16 exchanged electrons per 1 mole of O_2 (equaling to 4) and V is the volume of the liquid
17 in anodic chamber (Logan et al., 2006). The results presented throughout in this work
18 are the mathematical averages of triplicates and the standard deviations were below 5
19 %.
20
21
22

1 **3. Results and Discussion**

2

3 *3.1. The effect of LPW and MAS ratio on MFC performance*

4

5 In this work, the effect of MAS addition and LPW (substrate) loading on the
6 performance and behaviour of two-chamber microbial fuel cell was assessed. As for
7 MAS addition, it was preliminary assumed that the fresh microbial consortia fed into
8 the anode chamber might disturb the biofilm and consequently, the electricity
9 generation. Furthermore, it was hypothesized that the nutrients available in the feed
10 can be consumed by microbes (supplemented with the fresh MAS) that are not
11 localized on the electrode surface and hence does not contribute to useful bioelectricity
12 production.

13 A typical voltage output is demonstrated in Fig. 4, where it can be observed that
14 the feeding strategy in the MFCs is comprised of 4 individual stages according to the
15 recorded voltage pattern, as follows. In the first stage, the MFC unit was inoculated
16 with raw MAS and initial voltage had increased, but shortly it turned into a strong
17 decrease. At that point, in the second stage, sodium acetate was added to help the
18 establishment of the bioactive layer on the anode which resulted in prompt and sharp
19 jump of voltage followed by a gradual reduction in the cell potential. Subsequently,
20 during the third step, the MFC was supplemented again with Na-acetate. As it can be
21 noticed, it induced an observable increment of bioelectric potential, which
22 continuously fell, presumably due to the depletion of adequate substrates. It would also
23 appear that the second adaptation resulted in a more stable voltage signal as compared

1 to the first one. This was a positive feedback that may correlate with biofilm
2 formation. In the fourth step, after two-times acclimatization with acetate, the MFCs
3 were loaded with the mixture of LPW and fresh MAS in accordance with the
4 experimental design matrix (Table 2).

5 As it can be concluded from Fig. 4, there was a growing cell potential after the
6 injection of LPW-MAS mixture, which had reached a definite peak range where quite
7 steady voltage (roughly 40 mV) could be measured (approximately between the 650th
8 and 950th hour of MFC operation). Voltage fluctuations may be associated with the
9 complexity of the feed since various fractions of the raw materials could become
10 accessible and biodegradable at different periods of times.

11 In case of microbial fuel cells (and generally fuel cells) measuring current
12 density (or current) is suitable to determine the gross rate of the electrochemical
13 reaction according to Faraday's law. Thus, specific current- or power density values
14 are useful to compare the efficiency of different MFC systems, as well. Based on the
15 voltage data collected, the main characteristics were calculated which can be seen in
16 Table 3. The outcomes are comparable with other literature studies. For instance,
17 Cercado-Quezada et al. (2010) tested various food industry wastes as well as compost
18 leachate to extract energy in microbial fuel cells. Using the latter substrate (that shows
19 certain relation with landfill-derived LPW), 232 mA m⁻² current density was reported
20 applying MFC seeded by anaerobic sludge, meanwhile in this research 152-218 mA m⁻²
21 could be achieved. However, the obtained power densities were notably different,
22 since the 54 mW m⁻² value by Cercado-Quezada et al. (2010) exceeded our power
23 densities ranging between 5.8 and 11.9 mW m⁻². In another paper, Ganesh and

1 Jambeck (2013) operated MFCs fed by leachate and attained 114 mA m^{-2} current
2 density which was slightly lower than the results in our experiments as indicated in
3 Table 3. Tugtas and co-workers (2013) employed MFC to biodegrade pre-digested
4 landfill leachate. It turned out that the steady current densities of $418\text{-}548 \text{ mA m}^{-2}$
5 could be obtained, meaning a salient system performance. The compared data were
6 collected in Table 4. The contradiction in MFC efficiencies can be attributed to a
7 number of biological (e.g. the source of inoculum) and architectural (e.g. the external
8 resistance used, the distance of electrodes, the conductivity of anolyte, etc.) reasons.

9 Fig. 5 shows the response of MFC performance to various amounts of LPW and
10 fresh MAS additions. According to the results, it can be pointed out that the MFCs
11 were able to generate bioelectricity and microbes responded positively to the presence
12 of LPW since higher substrate concentrations allowed to gain more (cumulative)
13 energy. However, it would appear that there could be a joint effect of simultaneous
14 LPW and MAS supplementation, as follows. On one hand, the analysis of the
15 response surface in Fig. 5 implies that at low LPW concentrations the addition of MAS
16 in larger quantities should be avoided. It may be explained by the competition between
17 bulk phase and anodic biofilm, when the degradation of organic matter in bulk phase is
18 more notable and therefore only a significantly reduced amount of substrate is able to
19 reach the bioactive layer on the anode surface. In other words, most of the chemical
20 energy bound in the feed material is lost via an undesired bioprocess. Recently, Chae
21 et al. (2008) demonstrated that competitive degradation processes may occur in the
22 anodic side of two-chamber bioelectrochemical systems, which can cause lower
23 performance. It has turned out from the results that the gas evolved in the anode

1 compartment contained a considerable amount of methane besides carbon dioxide,
2 which implies that methanogenic strains were able to utilize the organic substrate
3 present via electrochemically-inactive reactions, leading eventually to a depressed
4 energy efficacy. On the other hand, when LPW concentration increases in the anode
5 cell (higher substrate loading), the phenomena taking place due to fresh inoculum
6 injection (MAS content) has less negative effect on the anode-surface reaction and
7 hence bioelectricity generation is not limited so strongly. Overall, it can be concluded
8 that outsider microbes entering the MFC anode chamber can influence the substrate
9 utilization and related bioelectricity production, although it seems to be dependent on
10 substrate to inoculum ratio.

11

12 *3.2. Effect of LPW and MAS concentrations on energy yield and COD removal*

13

14 In Fig. 6, the dependency of energy yield on LPW and MAS concentrations is
15 depicted. The results show that significantly higher energy yields (up to 8-9 J g⁻¹
16 Δ COD d⁻¹) were achieved in case of low COD loadings that occur with low LPW and
17 MAS concentrations. As elucidated above, the addition of MAS (“reinoculation”)
18 could pose a threat on reliable MFC operation since microorganisms in the bulk phase
19 compete for the nutrients and hence, reduce their amount being available for the
20 electrochemically active anode-surface consortia, inherently causing depressed energy
21 yields. Moreover, it can be observed that improved energy valorization of LPW
22 favours its low concentrations. LPW is a complex organic matter and therefore, its
23 bioelectrochemical decomposition has significant time-demand, which, in addition, is

1 likely influenced by the diffusion rate of substances into the biofilm, as well. Besides,
2 the biofilm can be characterized by limited substrate processing capacity. Thus, the
3 phenomenon of substrate saturation may occur, when the ability of microbes in the
4 biofilm is fully exploited, they are unable to take up more compounds and thereby
5 different bulky phase processes could take place in parallel that do not support
6 electricity generation. From the point of view of energy-realization, it means an
7 unbeneficial utilization of the substrate. Therefore, if the substrate level is maintained
8 sufficiently low or in other word, if the influent is properly diluted (Cercado-Quezada
9 et al., 2010) then the anode-living strains can have the possibility to convert bigger
10 portions of LPW into desired electricity more efficiently.

11 The COD removal capacity of an MFC is an important parameter to evaluate
12 the attractiveness of MFC technology from environmental perspective. COD removal
13 data are summarized in Table 5, where it can be seen that remarkable COD
14 degradation was possible from a complex substrate such as LPW, which is a notable
15 advantage of the MFCs. According to Table 5, the average COD removal was 87.3 %
16 and in case of MFC 1 it exceeded even 94 %. These values can be considered high in
17 the view of other literature data (Gálvez et al., 2009; Mohan et al., 2007; Rengasamy
18 and Berchmans, 2012) as well as in comparison with our previous accomplishments on
19 sugar industry wastewater (Bélafi-Bakó et al., 2014). The organic-removal efficiency
20 of the cells can be described well by the average COD removal rates, which varied
21 between 1.2 and 1.9 kg COD d⁻¹ values, assuming 1 m³ anode chamber volume.

22

23

1 **4. Conclusions**

2

3 Based on the results, it was concluded that the wastewater (LPW)-based
4 microbial fuel cell system operated successfully and it proved to be suitable for power
5 generation with simultaneous COD removal. It was found that reinoculation of MFC
6 by mesophilic sludge influenced the effectiveness of the preliminary adapted biofilm
7 on the anode surface. In addition, it was observed that energy recovery was strongly
8 dependent on LPW (substrate) concentration in the anode chamber and improved MFC
9 performance favoured low organic material loadings.

10

11 **Acknowledgements**

12

13 The research was supported by TÁMOP-4.2.2.B-15/1/KONV-2015-0004,
14 which was financed by the European Union and the European Social Fund. The János
15 Bolyai Research Scholarship of the Hungarian Academy of Sciences is also gratefully
16 acknowledged.

17

1 **References**

2

3 Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domínguez-Espinosa, R.,
4 2004). Production of bioenergy and biochemicals from industrial and agricultural
5 wastewater. *Trends Biotechnol.* 22, 477-485.

6 APHA, 1995. Standard methods for the examination of water and wastewater. 19th ed.
7 New York, USA: American Public Health Association.

8 Bakonyi, P., Nemestóthy, N., Lövitusz, É., Bélafi-Bakó, K., 2011. Application of
9 Plackett-Burman experimental design to optimize biohydrogen fermentation by *E. coli*
10 (XL1-BLUE). *Int. J. Hydrogen Energy* 36, 13949-13954.

11 Bakonyi, P., Nemestóthy, N., Simon, V., Bélafi-Bakó, K., 2014. Review on the start-
12 up experiences of continuous fermentive hydrogen producing bioreactors. *Renew.*
13 *Sustain. Energy Rev.* 40, 806-813.

14 Bélafi-Bakó, K., Vajda, B., Bakonyi, P., Nemestóthy, N., 2014. Removal of COD by
15 Two-Chamber Microbial Fuel Cells. In: Wang CT (ed) *Technology and application of*
16 *microbial fuel cells*, InTech, Rijeka, pp. 77-87.

17 Bond, D.R., Lovley, D.R., 2005. Evidence for involvement of an electron shuttle in
18 electricity generation by *Geothrix fermentans*. *Appl. Environ. Microbiol.* 71, 2186-
19 2189.

20 Cercado-Quezada, B., Delia, M.L., Bergel, A., 2010. Testing various food-industry
21 wastes for electricity production in microbial fuel cell. *Bioresour. Technol.* 101, 2748-
22 2754.

1 Cercado-Quezada, B., Delia, M.L., Bergel, A., 2010. Treatment of dairy wastes with a
2 microbial anode formed from garden compost. *J. Appl. Electrochem.* 40, 225-232.

3 Chae, K.J., Choi, M.J., Lee, J., Ajayi, F.F., Kim, I.S., 2008. Biohydrogen production
4 via biocatalyzed electrolysis in acetate-fed bioelectrochemical cells and microbial
5 community analysis. *Int. J. Hydrogen Energy* 33, 5184-5192.

6 Chaudhuri, S.K., Lovley, D.R., 2003. Electricity generation by direct oxidation of
7 glucose in mediatorless microbial fuel cells. *Nature Biotechnol.* 21, 1229-1232.

8 Dulon, S., Parot, S., Delia, M.L., Bergel, A., 2007. Electroactive biofilms: new means
9 for electrochemistry. *J. Appl. Electrochem.* 37, 173-179.

10 Gálvez, A., Greenman, J., Ieropoulos, I., 2009. Landfill leachate treatment with
11 microbial fuel cells; scale-up through plurality. *Bioresour. Technol.* 100, 5085-5091.

12 Ganesh, K., Jambeck, J.R., 2013. Treatment of landfill leachate using microbial fuel
13 cells: Alternative anodes and semi-continuous operation. *Bioresour. Technol.* 139,
14 383-387.

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

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

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

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

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

10 Liu, H., Cheng, S., Logan, B.E., 2005. Production of electricity from acetate or
11 butyrate using a single-chamber microbial fuel cell. *Environ. Sci. Technol.* 39, 658-
12 662.

13 Liu, H., Logan, B.E., 2004. Electricity generation using an air-cathode single chamber
14 microbial fuel cell in the presence and absence of a proton exchange
15 membrane. *Environ. Sci. Technol.* 38, 4040-4046.

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

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

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

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

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

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

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

12 Rabaey, K., Van de Sompel, K., Maignien, L., Boon, N., Aelterman, P., Clauwaert, P.,
13 Schamphelaire, L.D., Pham, H.T., Vermeulen, J., Verhaege, M., Lens, P., Verstraete,
14 W., 2006. Microbial fuel cells for sulfide removal. *Environ. Sci. Technol.* 40, 5218-
15 5224.

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

19 Rivera, I., Buitrón, G., Bakonyi, P., Nemestóthy, N., Bélafi-Bakó, K., 2015. Hydrogen
20 production in a microbial electrolysis cell fed with a dark fermentation effluent. *J.*
21 *Appl. Electrochem.* doi: 10.1007/s10800-015-0864-6

22

- 1 Rózsenberszki, T., Koók, L., Hutvágner, D., Nemestóthy, N., Bélafi-Bakó, K.,
2 Bakonyi, P., Kurdi, R., Sarkady, A., 2015. Comparison of anaerobic degradation
3 processes for bioenergy generation from liquid fraction of pressed solid waste. *Waste*
4 *Biomass Valor.* doi: 10.1007/s12649-015-9379-y
- 5 Sarkady, A., Kurdi, R., Rédey, A., 2014. RDF – Refuse derived fuel, possibilities in
6 the North-Balaton Regional waste management system. *Pollack Periodica.* 9, 23-30.
- 7 Tugtas, A.E., Cavdar, P., Calli, B., 2013. Bio-electrochemical post-treatment of
8 anaerobically treated landfill leachate. *Bioresour. Technol.* 139, 266-272.
- 9 Vajda, B., Bélafi-Bakó, K., Nemestóthy, N., 2011. The role of methanol in microbial
10 fuel cells. *Hung. J. Ind. Chem.* 39, 387-390.
- 11 Vajda, B., Nemestóthy, N., Bakonyi, P., Bélafi-Bakó, K., 2014. Xylose substrate as the
12 only nutrient in the operation of microbial fuel cells. *Environ. Prot. Eng.* 40, 131-141.
- 13 Wrana, N., Sparlin, R., Cicek, N., Levin, D.B., 2010. Hydrogen gas production in a
14 microbial electrolysis cell by electrohydrogenesis. *J. Cleaner Prod.* 18, 105-111.
- 15

1 **Figure Captions**

2

3 Fig. 1: The simplified overview of WTP process and LPW generation

4 Fig. 2: The scheme of the experimental dual-chamber MFC

5 Fig. 3: Layout of the experimental design

6 Fig. 4: Voltage output as a function of time (in case of 5 mL:9 mL LPW-MAS ratio).

7 1: inoculation with raw MAS; 2,3: first and second adaptation steps with Na-acetate

8 addition, respectively; 4: addition of LPW-MAS mixture

9 Fig. 5: Effect of MAS-LPW mixture feeding on energy production

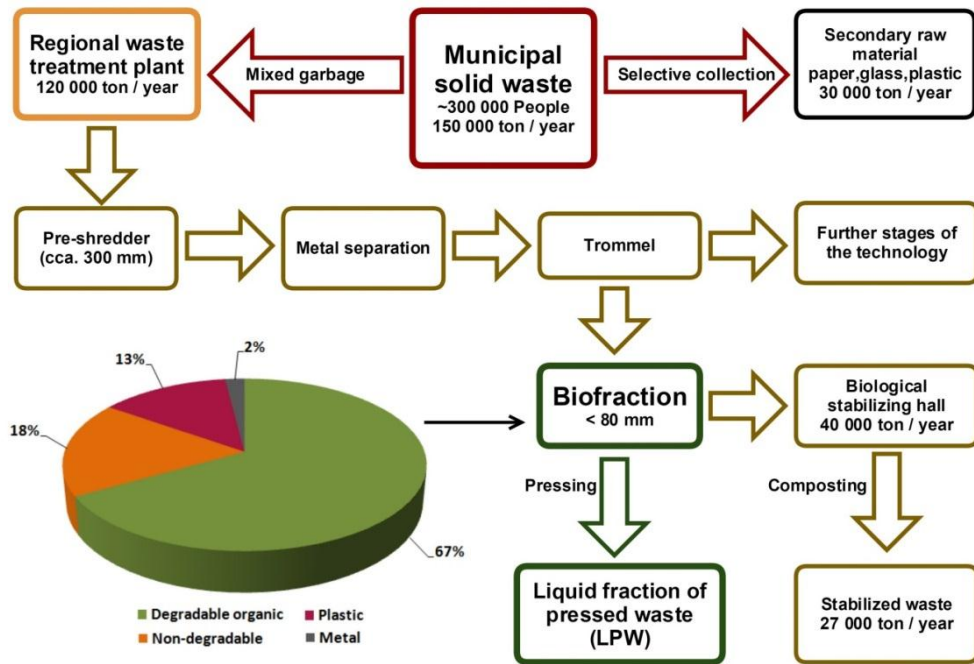
10 Fig. 6: Changes in energy yield to initial MAS and LPW concentrations

11

12

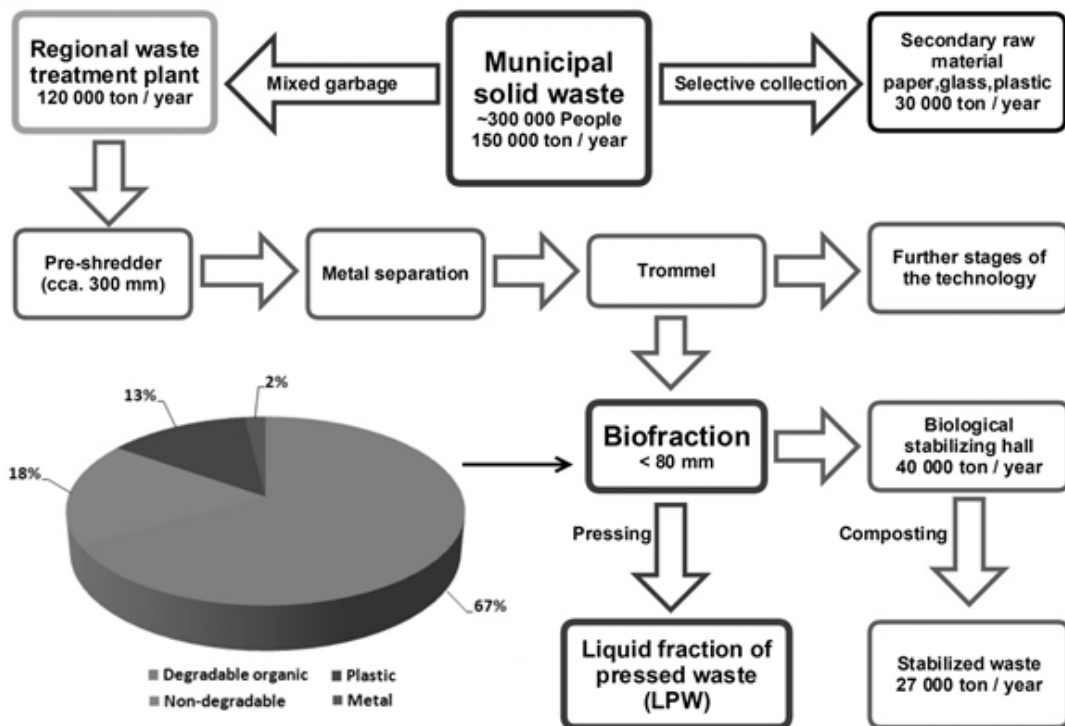
13

1 Fig.1



2

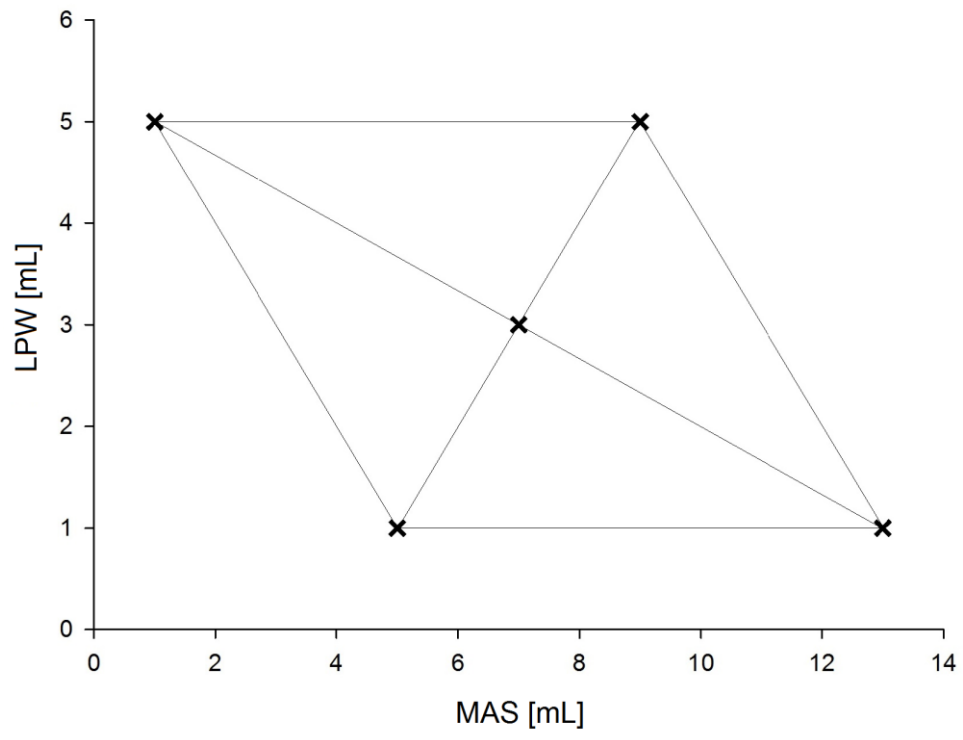
3



4

5

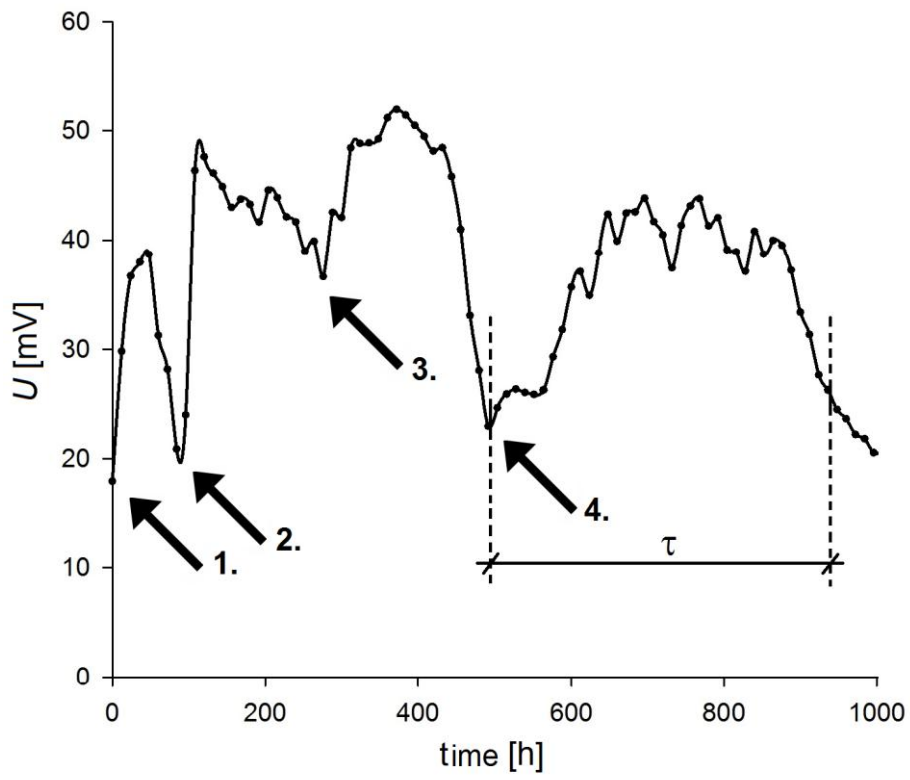
1 Fig.3



2

3

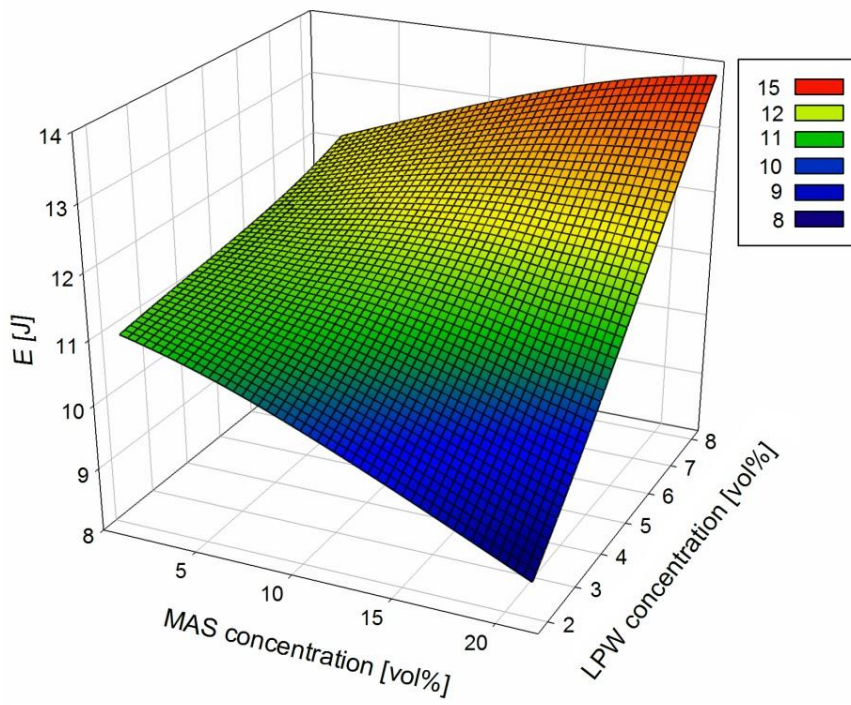
1 Fig. 4



2

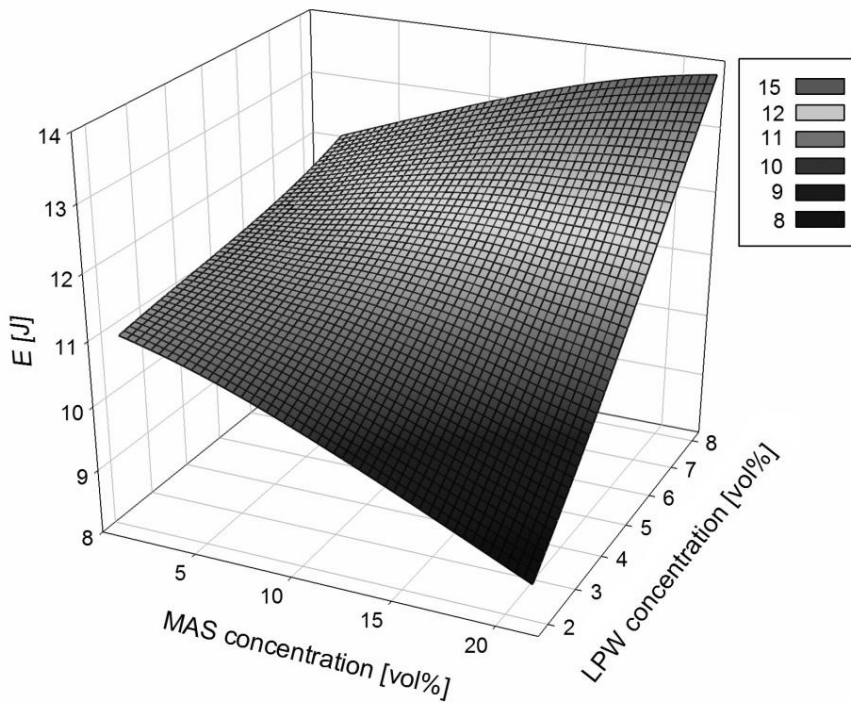
3

1 Fig. 5



2

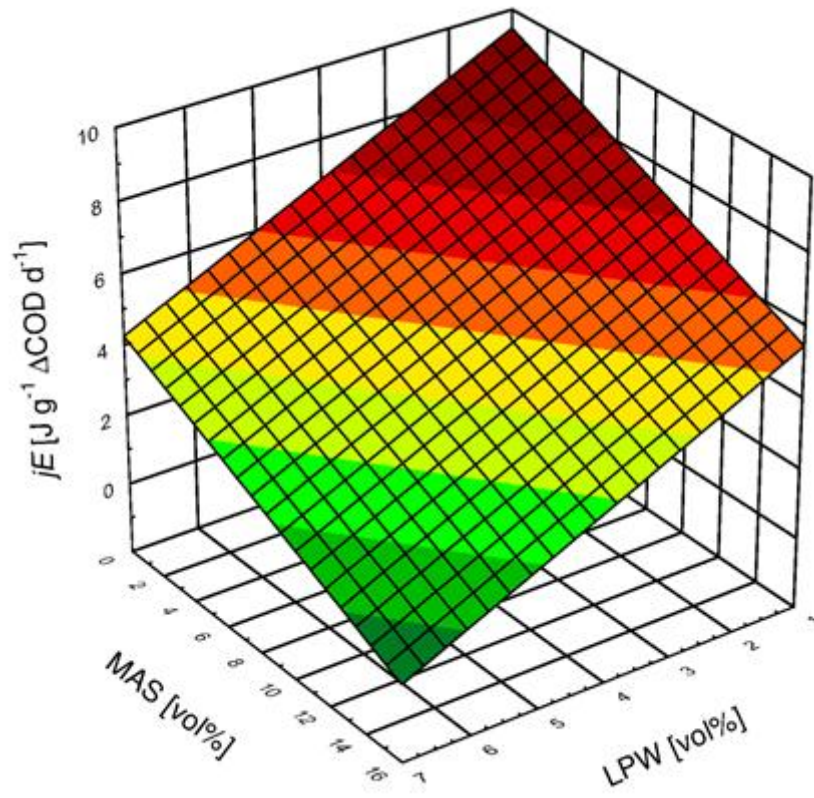
3



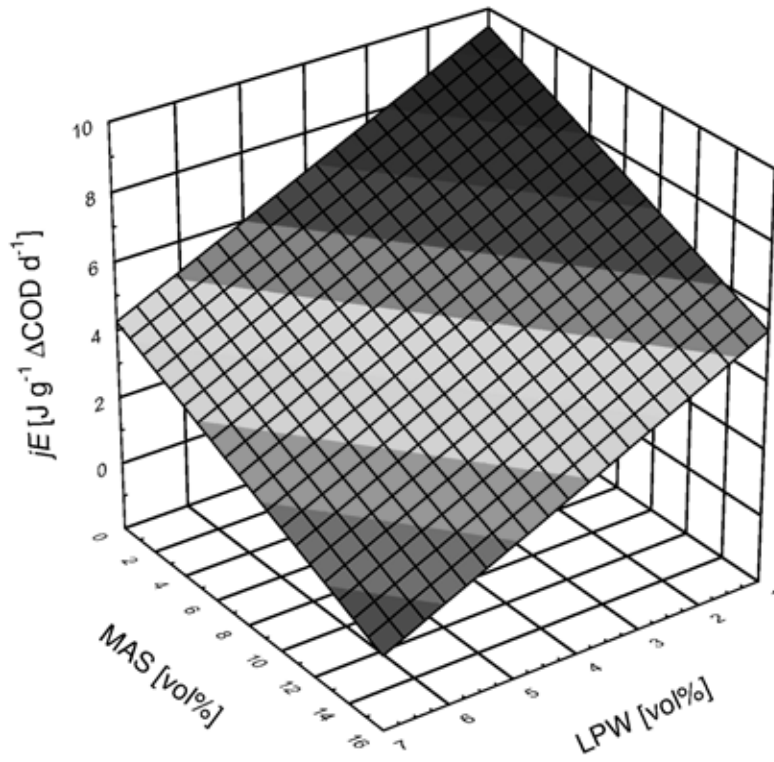
4

5

1 Fig.6



2



3

1 Table 1 – The main characteristics of LPW

2

Parameter	Value
total COD	111.6 g L ⁻¹
BOD 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

3

4

1 Table 2 – Parameters of the experimental design

2

MFC ID	Added LPW [mL]	Added MAS [mL]	LPW concentration [vol%]	MAS ^a 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

3 ^a: the term MAS concentration is identical to “reinoculation ratio”

4

1 Table 3 – Values of energy parameters obtained from the results of the experimental
2 design

3

MFC ID	LPW:MAS [mL:mL]	U_{\max} [mV]	jI_{\max} [mA m ⁻²]	jP_{\max} [mW m ⁻²]	E [J]	CE [%]
1.	5:9	43.8	175	7.7	13.4	1.95
2.	1:5	54.6	218	11.9	10.6	1.86
3.	1:13	47.8	191	9.2	8.8	1.25
4.	3:7	49.9	200	10.0	11.6	1.57
5.	5:1	38.0	152	5.8	12.0	1.41

4

5

1 Table 4 – Comparison of current density values

2

MFC type	Feed	Current density [mA m ⁻²]	Reference
Dual-chamber MFC	Food industry wastes, compost leachate	232	Cercado- Quezada et al. (2010)
Single-chamber MFC	leachate	114	Ganesh and Jambeck (2013)
Dual-chamber MFC	pre-digested landfill leachate	418-548	Tugtas et al. (2013)
Dual-chamber MFC	Liquid fraction of pressed solid waste	152-218	This work

3

4

1 Table 5 – COD parameters of each MFCs

2

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

3

4

1 List of abbreviations

Abbreviation	Full name
BOD	Biological oxygen demand
CE	Coulombic efficiency
COD	Chemical oxygen demand
LPW	Liquid fraction of pressed solid waste
MAS	Mesophilic anaerobic sludge
MFC	Microbial fuel cell
TOC	Total organic carbon
TS	Total solids

2