

**EFFECT OF *LEMNA MINOR* POPULATION DENSITY
ON BIOELECTRIC PARAMETERS OF ELECTRO-BIOSYSTEMS**

Iryna Rusyn, Vasil Dyachok

*Department of Ecology and Sustainable Environmental Management,
Viacheslav Chornovil Institute of Sustainable Development,
Lviv Polytechnic National University,
12, S. Bandery Str., Lviv, 79013, Ukraine
rib7@i.ua, dyachokvasil@gmail.com*

<https://doi.org/10.23939/ep2021.04.195>

Received: 03.08.2021

© Rusyn I., Dyachok V., 2021

Abstract. The article presents a study of the influence of *Lemna minor* population density on the bioelectric potential and current of model electro-biosystems in the laboratory conditions using 500 and 1000 Ω resistors and in the open circuit. The positive effect of increasing the density of duckweed plants populations from 60 to 120 fronds/ml on the growth of bioelectric parameters of model electro-biosystems under load conditions and without resistors was revealed. Increasing the amount of duckweed biomass is a factor of enhancing the efficiency of electro-biosystems based on *L. minor*.

Keywords: renewable energy, bioelectricity, electrode, population, electro-biosystem, plant.

1. Introduction

The plant-microbial electro-biosystem generates bioelectricity due to the microbial oxidation of photosynthetants released by plants through the roots and utilization of the components of the substrates in which they grow (Strik et al., 2008; Kaku et al., 2008; Deng et al., 2012; Timmers et al., 2012). The plant component is one of the main factors in the functioning of electro-biosystems (Nitorisavut, Regmi, 2017; Kabutey et al., 2019). The same electro-biosystems based on the same anodes and cathodes but with different plant species show different parameters of bioelectricity generation (Helder et al., 2010; Wang et al., 2017; Oodally et al., 2019). The species of plants determines the qualitative and quantitative composition of the microbial flora of the roots and also affects the electro-active microorganisms

(Berg, Smalla, 2009; Azri et al., 2018). For example, the bioelectricity of identical electro-biosystems was 10 times higher with *Spartina anglica* than with the plant *Arundinella anomala* (Helder et al., 2010). Electro-biosystems based on the *Cyperus prolifer* plant showed significantly higher voltages than the same systems, but with the plants *Wachendorfia thyrsiflora* and *Phragmites australis* (Oodally et al., 2019). The species of plants is one of the key factors in the functioning of the electro-biosystems, and the search for new plant species is of great relevance.

The genus *Lemna* L. and the species *Lemna minor* are not sufficiently studied as an object of electro-biotechnology. In 2012, experiments were conducted to generate bioelectricity with another species of duckweed *L. minuta* (Hubenova, Mitov; 2012). *Lemna minor* is a small floating plant (Landolt, 1986; Gubanov et al., 2002). The vegetative body of duckweed presents a round plate 2-4.5 mm long, 2-3 mm wide and not more than 1 mm thick with a translucent unbranched root (Landolt, 1986; Gubanov et al., 2002). Due to its structure, the plant easily holds on the surface of the water, and under favourable conditions multiplies rapidly, often covering the entire surface of contaminated standing water, cleaning it from contaminants (Cheng et al., 2002; Ziegler et al., 2014; Ceschin et al., 2018; Iqbal et al., 2019; Ceschin et al., 2020). Thus, the plant species *Lemna minor* presents a promising object for the study of electro-biotechnology, which can be used both as a source of bioelectricity and for water purification from pollution.

The electro-biosystem is considered as a complex hybrid biological-technological device, the functioning of which depends on a set of factors, among which plant development is very important (Hubenova, Mitov, 2012). While in terrestrial plants as biological components of electro-biosystems, the important characteristics are the degree of development of leaf biomass which is calculated by biometric measurement, for duckweed plants, such a parameter is the density of its population.

Therefore, taking into account the above, in order to develop the model electro-biosystems based on *L. minor* and to identify optimal conditions for their functioning, we set ourselves the task to investigate the effect of duckweed plants population density on bioelectric potential and voltage of model electro-biosystems in laboratory conditions with different loads.

2. Materials and Methods

L. minor plants were collected from contaminated ditches around Lviv. Contaminated water ditches with the addition of pure water were used as a medium for the development of duckweed plants. The studies were

performed in laboratory conditions. For model electro-biosystems, transparent plastic containers with a diameter of 120 mm and a height of 120 mm were used, in which duckweed plants were placed (Fig. 1). Monosystems with a pair of electrodes (Rusyn, Medvediev, 2016) with the following dimensions: cathode 87 mm × 28 mm × 14 mm and anode 78 mm × 36 mm × 1 mm were used as electrode systems. The electrodes were completely immersed in the water around the roots of duckweed.

Bioelectric potential parameters were recorded using a digital multimeter UT890C UNIT-T. The bioelectric potential and current were recorded with short-term connected resistors of 500 Ω and 1000 Ω and in open-circuit conditions: the open-circuit voltage (OCV) and short-circuit current. The subject of research involves the dynamic fluctuations of the bioelectric potential and current of plant-microbial associations of the model electro-biosystems.

Duckweed population density was calculated by directly counting the number of fronds in 1 ml of medium. The reported results were presented as the average of all replicate experiments and their standard errors ($x \pm SE$).

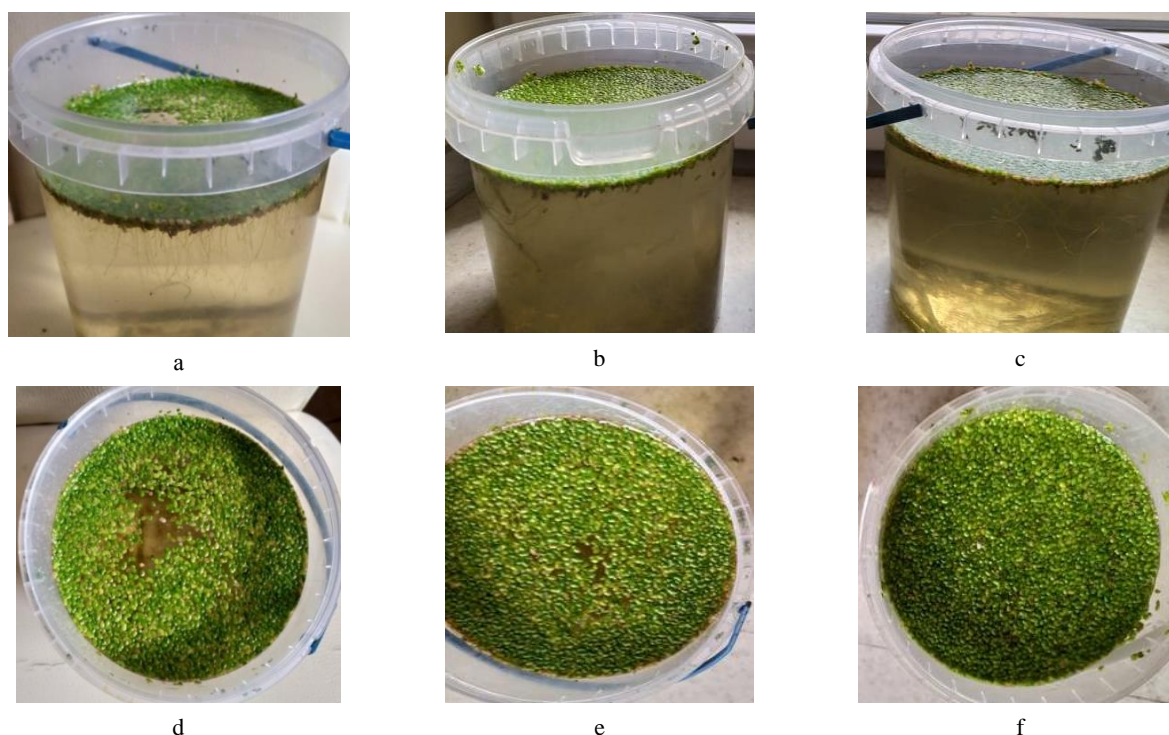


Fig. 1. Object of study *L. minor*, obtained from contaminated ditches at a concentration of 60 fronds/ml (a, d), 90 fronds/ml (b, e), 120 fronds/ml (c, f)

3. Results and Discussion

An efficient model electro-biosystem with *L. minor* was constructed. The density of duckweed populations had an important influence on the obtained parameters of bioelectricity of model electro-biosystem,

both in the open-circuit conditions and when using 500 Ω and 1000 Ω resistors. As can be seen from Figures 2 and 3, with an increase in the population density of *L. minor*, both the bioelectric potential of the open circuit and the short-circuit current grew. At a population density of 120 fronds/ml of medium, the current was higher on average

by 0.6 mA than at a density of 60 fronds/ml, and the bioelectric potential of the open circuit – by 0.056 V.

Similar trends in the positive effect of increasing the density of the plant population on the parameters of bioelectricity were recorded when using resistors of 500 Ω and 1000 Ω on the current (Fig. 4, 5) and the bioelectric potential (Fig. 6, 7). At 500 Ω with an increase in the population density of *L. minor*, the current increased by 0.067 – 0.083 mA (Fig. 4). At 1 000 Ω, the current values were higher by 0.048–0.058 mA at

a biomass density of 120 fronds/ml than at a density of 60 fronds/ml (Fig. 5).

At 500 Ω, the difference in parameters of bioelectric potential between electro-biosystems with intensive growth and less intensive growth of *L. minor* was 0.071–0.101 V depending on external environmental factors as lighting, etc. (Fig. 6). At 1000 Ω, the bioelectric potential was higher by 0.020–0.025 V at a duckweed biomass density of 120 fronds/ml than at a density of 60 fronds/ml (Fig. 7).

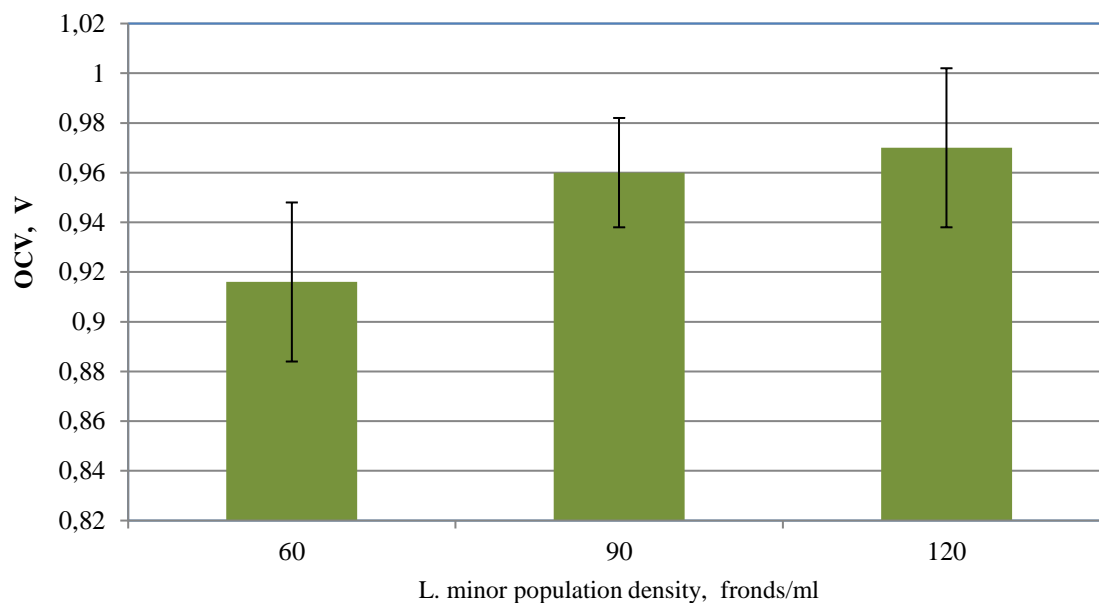


Fig. 2. Influence of duckweed population density of 60, 90 and 120 fronds/ml on bioelectric potential of the open circuit

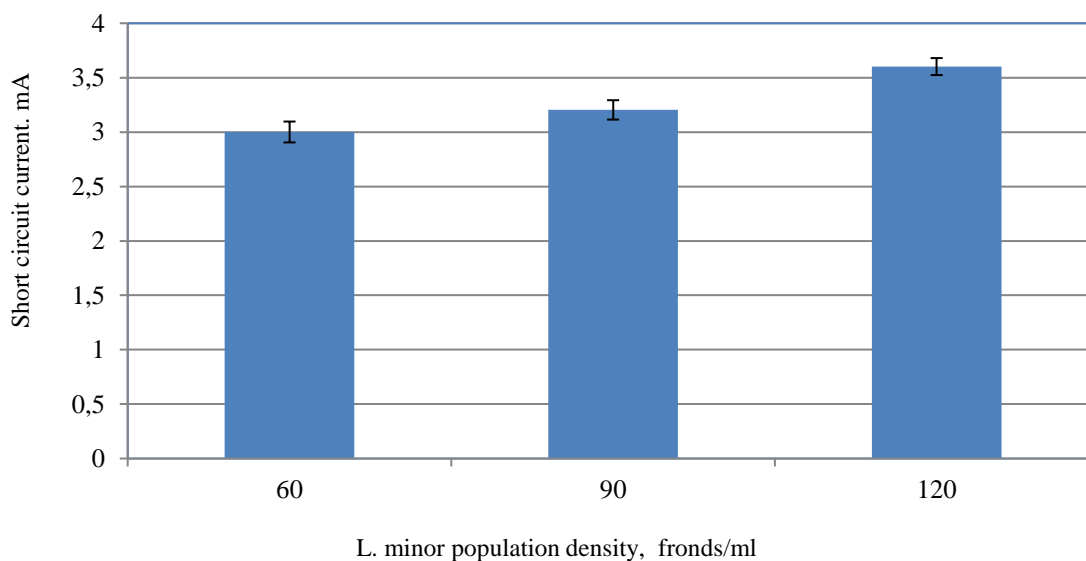


Fig. 3. Influence of duckweed population density of 60, 90 and 120 fronds/ml on short-circuit current

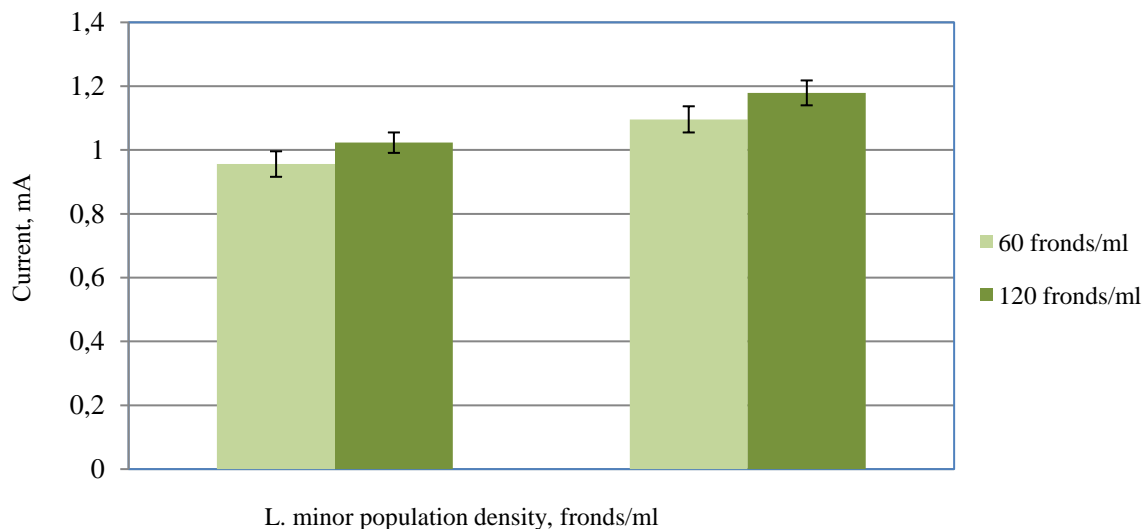


Fig. 4. Influence of the population density of duckweed on the voltage of the electro-biological system with *L. minor* at a short-term load of 500 Ω

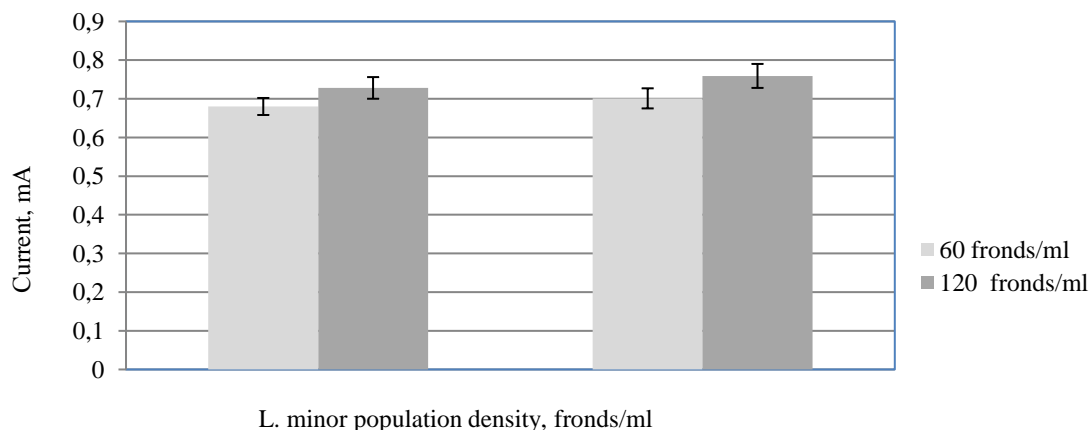


Fig. 5. Effect of the population density of duckweed on the voltage of the electro-biological system with *L. minor* at a short-term load of 1000 Ω

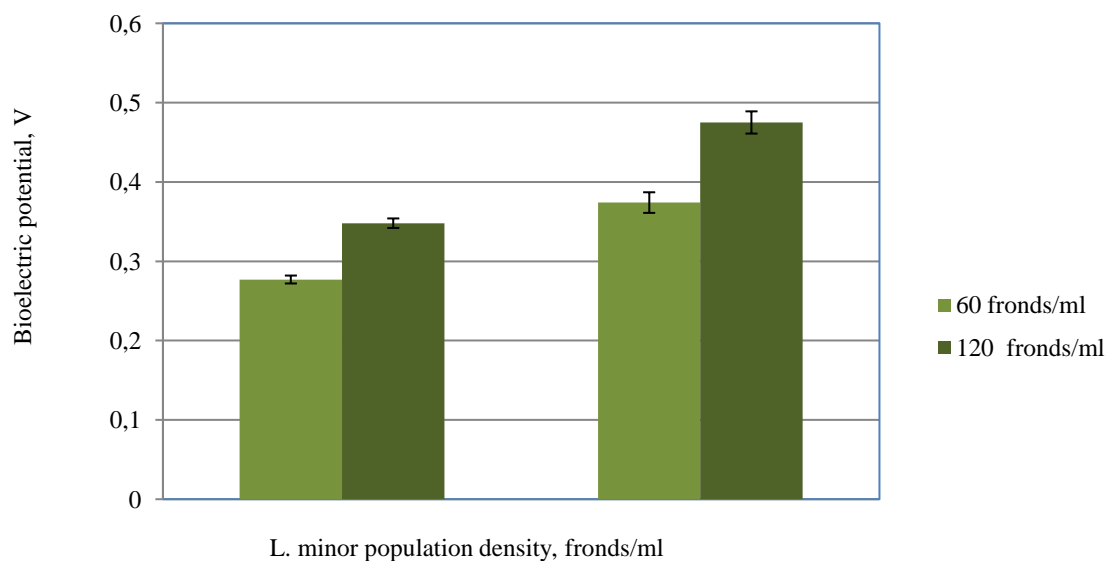


Fig. 6. Influence of duckweed population density on the bioelectric potential of an electro-biosystem with *L. minor* at a short-term load of 500 Ω

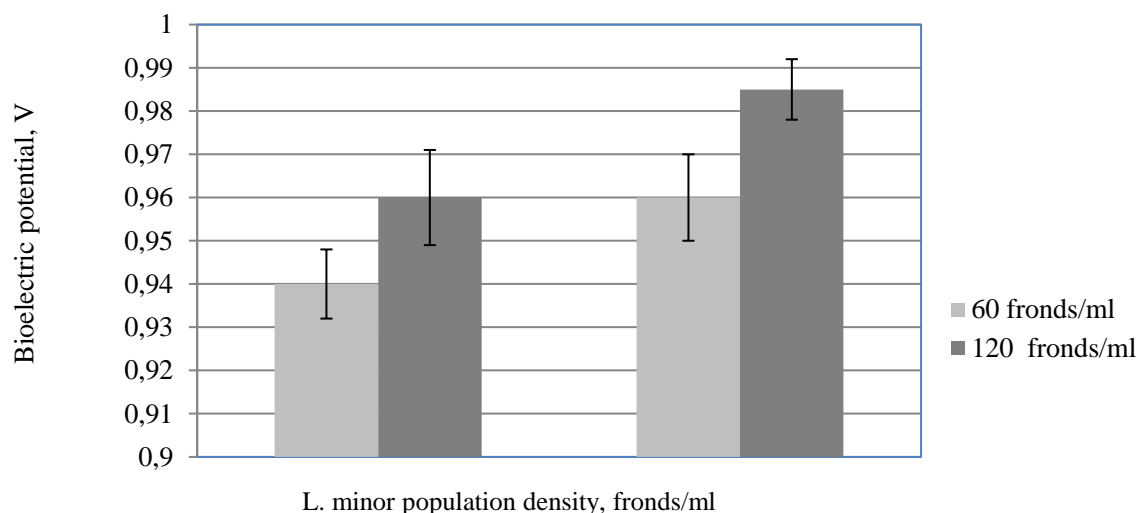


Fig. 7. Effect of duckweed population density on bioelectric potential of electro-biosystem with *L. minor* at a short-term load of 1000 Ω

With an increase in external resistance, the parameters of the current decrease, and the bioelectric potential increases. It was observed that oxidation of substrates by microorganisms was greater at lower resistances than at higher resistances, where microorganisms donate electrons to the anode electrode and the electrons migrate in a closed circuit (Sangeetha & Muthukumar, 2013). At low resistance, the electrons move more easily through the circuit than at high resistance, which explains the effect of the loads on the bioelectric parameters (Sangeetha, Muthukumar, 2013).

In the generation of bioelectricity, an important role belongs to the root secretions of the plant and excreted photosynthetants, which are substrates for the growth of electricity-generating microorganisms grow (Strik et al., 2008; Kaku et al., 2008; Deng et al., 2012; Timmers et al., 2012). The operation of plant-microbial electro-biosystems is based on the microbial conversion of photosynthesis products released by the root system with the formation of electrons and protons. With an increase in the density of the duckweed biomass, the release of photosynthetants and organic compounds correspondingly increases, which is obviously the reason for the growth of bioelectric parameters of electro-biosystems. A similar trend is also recorded in the works where the dependence of bioelectric parameters on the degree of the development of the plant bush or the stage of the plant development is demonstrated (Deng et al., 2016; Rusyn et al., 2019). The constructed electro-biosystem was characterized by the average OCV values of about 970 mV (Fig. 2) at the level recorded in the electro-biosystem with the plant *Chlorophytum comosum* (Tou et al., 2019), where the values of the average bioelectric potential were in the range from 900 mV. The electro-biosystem with duckweed plant *L. minor* showed

higher OCV values than the electro-biosystem with another species of duckweed plant, *L. minuta*, where the recorded open-circuit voltage was at a level of 700-850 mV (Hubenova, Mitov, 2012).

4. Conclusions

Model electro-biosystems with *L. minor* were constructed. Electro-biosystems based on duckweed plant with biomass of 120 fronds/ml of medium operating with resistors of 500 and 1000 Ω , have appeared to be more efficient than with a population density of duckweed of 60 and 90 fronds/ml at the same load. The bioelectric potential was 986 mV at 1000 Ω . Electro-biosystems based on *L. minor* are promising sources of renewable energy, and the increase in duckweed biomass is a factor regulating their efficiency.

References

- Azri, Y. M., Tou, I., Sadi, M. & Benhabyles, L. (2018). Bioelectricity generation from three ornamental plants: *Chlorophytum comosum*, *Chasmanthe floribunda* and *Papyrus diffusus*. *International Journal of Green Energy*, 15(4), 254–263. doi: <https://doi.org/10.1080/15435075.2018.1432487>
- Berg, G., & Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS Microbiology Ecology*, 68(1), 1–13. doi: <https://doi.org/10.1111/j.1574-6941.2009.00654.x>
- Ceschin, S., Abati, S., Ellwood, N. T. W., Zuccarello, V. (2018). Riding invasion waves: spatial and temporal patterns of the invasive *Lemna minuta* from its arrival to its spread across Europe. *Aquatic Botany*, 150, 1–8. doi: <https://doi.org/10.1016/j.aquabot.2018.06.002>
- Ceschin, S., Crescenzi, M. & Iannelli, M. A. (2020). Phytoremediation potential of the duckweeds *Lemna minuta*

- and *Lemna minor* to remove nutrients from treated waters. *Environmental Science and Pollution Research*, 27, 15806–15814. doi: <https://doi.org/10.1007/s11356-020-08045-3>
- Cheng, J., Landesman, L., Bergmann, B. A., Classen, J. J., Howard, J. W., & Yamamoto, Y. T. (2002). Nutrient removal from swine lagoon liquid by *Lemna minor* 8627. *Trans ASAE*, 45, 1003–1010.
- Deng, H., Chen Z., & Zhao F. (2012). Energy from Plants and Microorganisms: Progress in Plant–Microbial Fuel Cells. *ChemSusChem*, 5, 1006–1011. doi: <https://doi.org/10.1002/cssc.201100257>
- Deng, H., Cai, L., Jiang, Y., & Zhong, W. (2016). Application of Microbial Fuel Cells in Reducing Methane Emission from Rice Paddy. *Huan Jing Ke Xue*, 37 (1), 359–365. <https://doi.org/10.13227/j.hjhx.2016.01.046>
- Gubanov, I. A., Kiseleva, K. V., Novikov, V. S., & Tikhomirov, V. N. (2002). *Lemna minor* L. - Duckweed small. Illustrated determinant to plants of Middle Russia, Vol 1, Ferns, horsetails, moss, gymnosperms, angiosperms (monocotyledons). Moskva, Tovarishestvo nauchnykh izdaniy KMK, Institut tekhnologicheskikh issledovaniy.
- Helder, M., Strik, D. P., Hamelers, H. V. M., Kuhn, A. J., Blok, C., & Buisman, C. J. N. (2010). Concurrent bio-electricity and biomass production in three Plant-Microbial Fuel Cells using *Spartina anglica*, *Arundinella anomala* and *Arundo donax*. *Bioresource Technology*, 101(10), 3541–3547. doi: <https://doi.org/10.1016/j.biortech.2009.12.124>
- Hubenova, Y., & Mitov, M. (2012). Conversion of solar energy into electricity by using duckweed in direct photosynthetic plant fuel cell. *Bioelectrochemistry*, 87, 185–191. doi: <https://doi.org/10.1016/j.bioelechem.2012.02.008>
- Iqbal, J., Javed, A., & Baig, M. A. (2019). Growth and nutrient removal efficiency of duckweed (*lemna minor*) from synthetic and dumpsite leachate under artificial and natural conditions. *PLoS One*, 14(8), e0221755. doi: <https://doi.org/10.1371/journal.pone.0221755>
- Kabutey, F. T., Zhao, Q., Wei, L., Ding, J., Antwi, P., Quashie, F. K. & Wang, W. (2019). An overview of plant microbial fuel cells (PMFCs): Configurations and applications. *Renewable and Sustainable Energy Reviews*, 110 (C), 402–414. doi: <https://doi.org/10.1016/j.rser.2019.05.016>
- Kaku, N., Yonezawa, N., Kodama, Y., & Watanabe, K. (2008). Plant/microbe cooperation for electricity generation in a rice paddy field. *Applied Microbiology & Biotechnology*, 79(1), 43–49. doi: <https://doi.org/10.1007/s00253-008-1410-9>
- Landolt, E. (1986). *Biosystematic investigation on the family of duckweeds: The family of Lemnaceae*. A monograph study. Zurich, Switzerland, Geobotanischen Institute.
- Nitorisavut, R., & Regmi, R. (2017). Plant microbial fuel cells: A promising biosystems engineering. *Renewable and Sustainable Energy Reviews*, 76, 81–89. doi: <https://doi.org/10.1016/j.rser.2017.03.064>
- Oodally, A., Gulamhussein, M., & Randall, D. G. (2019). Investigating the performance of constructed wetland microbial fuel cells using three indigenous South African wetland plants. *Journal of Water Process Engineering*, 32, 100930, 1–8. doi: <https://doi.org/10.1016/j.jwpe.2019.100930>
- Rusyn, I. B., & Medvediev, O. V. (2016). *UA Patent No.112093*. Ukrainskyi instytut intelektualnoi vlasnosti (Ukrpatent).
- Rusyn, I. B., Vakuliuk, V. V., & Burian, O. V. (2019). Prospects of use of *Caltha palustris* in soil plant-microbial electrochemical biotechnology. *Regulatory Mechanisms in Biosystems*, 10(2), 233–238. doi: <https://doi.org/10.15421/021935>
- Sangeetha, T., & Muthukumar, M. (2013). Influence of electrode material and electrode distance on bioelectricity production from sago-processing wastewater using microbial fuel cell. *Environmental Progress & Sustainable Energy*, 32 (2), 390–395. doi: <https://doi.org/10.1002/ep.11603>
- Strik, D. P. B. T. B., Hamelers, H. V. M., Snel, J. F. H., & Buisman, C. J. (2008). Green electricity production with living plants and bacteria in a fuel cell. *International Journal of Energy Research*, 32(9), 870–876. doi: <https://doi.org/10.1002/er.1397>
- Timmers, R. A., Rothballer, M., Strik, D. P. B. T. B., Engel, M., Schulz, S., Schloter, M., Hartmann, A., Hamelers, B., & Buisman, C. (2012). Microbial community structure elucidates performance of *Glyceria maxima* plant microbial fuel cell. *Applied Microbiology & Biotechnology*, 94(2), 537–548. doi: <https://doi.org/10.1007/s00253-012-3894-6>
- Tou, I., Azri, Y. M., Sadi, M. H., Lounici, H., & Kebbouche-Gana, S. (2019). Chlorophytum microbial fuel cell characterization. *International Journal of Green Energy*, 16(12), 1–13. doi: <https://doi.org/10.1080/15435075.2019.1650049>
- Wang, J., Song, X., Wang, Y., Bai, J., Li, M., Dong, G., Lin, F., Lv, Y., & Yan, D. (2017). Bioenergy generation and rhizodegradation as affected by microbial community distribution in a coupled constructed wetland-microbial fuel cell system associated with three macrophytes. *Science of the Total Environment*, 607–608, 53–62. doi: <https://doi.org/10.1016/j.scitotenv.2017.06.243>
- Ziegler, P., Adelmann, K., Zimmer, S., Schmidt, C., Appenroth, K. J. (2014) Relative in vitro growth rates of duckweeds (Lemnaceae) - the most rapidly growing higher plants. *Plant Biol*, 17, 33–41. doi: <https://doi.org/10.1111/plb.12184>