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4. Selection and modification of microorganisms and substrates for acquiring and separation of energy carriers

Mikroorganismu un substrātu atlase un modifikācija energonesēju ieguvei un atdalīšanai

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4.1. Biological hydrogen production and storage research

Hydrogen is regarded as a sustainable energy carrier for various reasons:

1) high energy conversion efficiencies;
2) production from water with no emissions;
3) abundance;
4) different forms of storage (e. g. gaseous, liquid, or in together with metal hydrides);
5) long distance transportation;
6) ease of conversion to other forms of energy;
7) higher heating value (HHV) and lower heating value (LHV) than most of the conventional fossil fuels.

Hydrogen usage as an energy carrier is the most important stone in hydrogen economy as it can store the energy from fossil domestic resources (natural gas, coal, oil), biomass and intermittently available renewables such as wind and Sun for use in stationary and mobile applications [1]. The information in this chapter is obtained from PhD thesis of Ilze Dimanta, defended in June, 2016, at the University of Latvia [2], MSc thesis of Sintija Valucka, defended in June 2015, Faculty of Biology, the University of Latvia [3], Bachelor's thesis of Zane Kleinmane, defended in June 2016, Faculty of Biology, the University of Latvia [4], Bachelor's thesis of Matīss Paiders defended in June 2017, Faculty of Biology, the University of Latvia [5], which to some extent is reflected in publications [6–11].

4.1.1. Hydrogen gas as renewable energy carrier

Hydrogen is a flammable, colourless and odourless gas with low solubility in water, discovered in 1766 by Henry Cavendish. Its melting point is –259.2 °C and its boiling point is –252.8 °C. The density of hydrogen gas at conditions of 0 °C and 101.3 kPa is 0.08987 kg/m^3, which is about 14 times less than air under same conditions [12]. Hydrogen is the most abundant element in the Universe, making up about three quarters of all matter, though scarcely available on the Earth in a molecular (dihydrogen, H_2) form. The atmosphere contains trace amounts of it (0.07%), while the Earth's surface contains about 0.14% [13]. Hydrogen can be found in many substances in nature, for example, in sea/fresh water and biomass. Nevertheless, hydrogen can be produced from many different sources (oil, natural gas, water, biomass rich in carbohydrates and more). Currently, hydrogen covers about 2% of the world energy demand [14] and worldwide production exceeds 1 billion m^3/day of which 48% is produced from natural gas, 30% from oil, 18% from coal, and the remaining 4% is produced from water electrolysis, currently the most basic industrial process for almost pure hydrogen generation [15]. Most commonly used and the least expensive hydrogen production process is natural gas steam reforming, but it produces heavy greenhouse gas emissions [16]. Water electrolysis is more environmentally friendly. Technologies of most significance for electrochemical hydrogen production are:

1) polymer membrane [voltage efficiency (VE) 67–82%, with current density (CD) 0.6-2.0 A/cm^2];
2) alkaline membrane (VE 62-82%, CD 0.2–0.4 A/cm^2); and
3) solid oxide electrolysers (VE 81–86%, CD 0.3–1.0 A/cm^2) [17].

Apart from electrolysis, different hydrogen production methods have been developed, including photolysis, thermolysis, biophotolysis, photoelectrochemical process, photocatalysis and thermochemical water splitting, as well as thermochemical conversion of biomass, gasification, biofuel reforming and others [13]. Every physical or chemical method of hydrogen production requires energy input, which is not always environment-friendly.

On the contrary, biological processes of hydrogen recovery and collection from organic resources such as municipal wastewater and sludge facilitate recycling of sewage and are environmentally benign [18].

4.1.2. Biohydrogen production processes

Bacteria have their own hydrogen fuel cycle. Hydrogen production by dark fermentation is restricted by the incomplete degradation of organic matter into volatile fatty acids, hydrogen and carbon dioxide. In nature, low partial pressure of hydrogen is maintained by the presence of hydrogen consumers in syntrophic association with hydrogen producers

[19]. Estimates are that microorganisms annually form approximately 150 million tons of hydrogen. The combustion of 150 million tons of hydrogen yields 18×10^{18} J of energy that is equivalent to 3.75% of the primary energy consumed by world population [20].

Hydrogen production using microbial fermentation process is acknowledged from the 1920s (photosynthesis in microalgae). Biological processes for hydrogen production and collection from organic resources such as municipal wastewater and sludge facilitate recycling of sewage are environmentally benign [21]. Biohydrogen generation via dark fermentation process is widely studied due to higher production rates and treatment capacity for organic waste products. Many institutions and universities worldwide are involved in the research of hydrogen production using bacteria or/and algae [22].

Biohydrogen production is divided in five broader groups: direct photolysis, indirect photolysis, photofermentation, dark fermentation and coupled or hybrid systems and there are vast group of organisms that can produce hydrogen in various environmental conditions [23]. A more detailed description is provided in Table 4.1.

Table 4.1. **Processes of biohydrogen production (modified from [24–27])**

Process	Description	Organisms
Direct biophotolysis	Hydrogen from water and the sun through photosynthesis, genetic modifications necessary for maintaining higher efficiency	Green algae (*Chlamydomonas reinhardtii)*
Indirect biophotolysis	$6H_2O + 6CO_2 + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2$ $C_6H_{12}O_6 + 6H_2O + \text{light} \rightarrow 12H_2 + 6CO_2$	Cyanobacteria *(Anabaena variabilis)*
Photo-fermentation	Conversion of organic substrate by using light energy with photosynthetic purple non-sulphur bacteria in anaerobic environment	Photosynthetic bacteria (*Rhodobacter* spp.)
Dark fermentation	By using different substrates, including organic waste in anaerobic environment produces hydrogen	Anaerobic and facultative anaerobic bacteria *(Enterobacter* spp.)
Hybrid systems	Firstly, via fermentation process acetate, CO_2 and H_2 are produced. Secondly, in a photo-bio-reactor, acetate is turned into H_2 and CO_2	Association of photosynthetic and fermentative bacteria

All biohydrogen production ways depend on either a hydrogenase or nitrogenase enzyme for hydrogen evolution and derive energy either directly from light energy or indirectly by consuming photosynthetically derived carbon compounds. Each approach has positive and negative aspects, and each has serious technical barriers that need to be overcome before it could become practical [28]. There is a natural consequence of the fact that fermentations have been optimized by evolution to produce cell biomass and not hydrogen. Thus, a portion of the substrate is used in both cases to produce ATP giving a product that is excreted. In many anaerobic microorganisms, the actual hydrogen generation yields are reduced by hydrogen recycling due to the presence of one or more uptake hydrogenases, which consume the hydrogen that is produced [29].

Hydrogen production by fermentation of carbohydrate-containing substrates (glucose, cellulose, starch and organic waste materials) is frequently preferred to photolysis, because it does not rely on the availability of light sources [24]. Hydrogenases are enzymes that catalyse the oxidation of hydrogen to protons and the reduction of protons to hydrogen. They can be distributed into three classes: the [Fe]-H_2ases, the [NiFe]-H_2ases, and the metal-free hydrogenases. Hydrogenases may interact with membrane-bound electron transport systems in order to maintain redox balance, particularly in some photosynthetic microorganisms such as cyanobacteria [30]. One of the pyruvate oxidation products, alongside with acetyl-CoA, is a formate, which is produced by pyruvate formate lyase and is the sole source of hydrogen in these bacteria. The formate is split into CO_2 and H_2 by formate hydrogen lyase (FHL) complex, which comprises seven proteins, six of them being encoded *hyc* operon. Five *hyc* operon encoded proteins are membrane-embedded electron transporters. The HycE protein is one of the three *E. coli* NiFe hydrogenases (also referred to as Hyd-3) [27].

The HycE and FDH-H components of FHL complex are soluble periplasmic proteins. The hydrogen evolved from FHL is consumed by *E. coli* uptake hydrogenases Hyd-1 and Hyd-2. In contrast to enterobacteria, strictly anaerobic fermenters, e.g. clostridia, use a reduced ferredoxin (required to oxidize pyruvate to acetyl-CoA) for H_2 production by the hydrogenase that generates ferredoxin in the oxidized form and releases electrons as molecular hydrogen [24]. Glucose fermentation by enteric bacteria yields the maximum of 2 mol H_2/ mol glucose [31]. To enhance the hydrogen production and utilize the substrate in the full measure for complete conversion, synergy of biological processes (two-stage/ hybrid ones) should be applied. Anaerobic fermentations with *Escherichia coli* and *Clostridium* spp. are the most thoroughly investigated hydrogen bioprocesses, which offer hydrogen yields from glucose up to 2 or 4 moles per mol of glucose [32]. The end products of fermentation process are mainly volatile organic acids and spirits that are produced depending on microbial metabolic pathways and substrate used.

4.1.3. Microbial strains and optimal substrates for hydrogen production

Various microorganisms have the ability to produce hydrogen under certain conditions – starting from microalgae that use light energy, cyanobacteria, but the major hydrogen yielding biocatalysts are heterotrophs in the fermentation process – facultative and obligate anaerobes. Here are listed some of active hydrogen producers: green algae (*Scenedesmus obliquus, Chlamydomonas reinhardtii, C. moewusii),* cyanobacteria heterocystous (*Anabaena azollae, A. variabilis, A. cylindrica, Nostoc muscorum, N. spongiaeforme, Westiellopsis prolifica),* cyanobacteria non-heterocystous (*Plectonema boryanum, Oscillatoria* sp. Miami BG7*, O. limnetica, Synechococcus* sp., *Aphanothece halophytico, Mastidocladus laminosus, Phormidium valderianum),* photosynthetic bacteria (*Rhodobater sphaeroides, R. capsulatus, Rhodopseudomonas palustris, Rhodospirillum rubnum, Rhodovulum sulfidophylum, Chromatium* sp. Miami PSB 1071, *Chlorobium limicola, Chloroflexus aurantiacus, Thiocapsa roseopersicina, Halobacterium halobium)* and fermentative bacteria [*Klebsiella* (*Enterobacter) aerogenes, E. cloacae, Clostridium butyricum, C. pasteurianum, Desulfovibrio vulgaris, Megasphaera elsdenii, Citrobacter intermedius, Escherichia coli*] [24, 33, 34].

Thermophiles are the closest for hydrogen production to the theoretical yield by overwhelming thermodynamic barrier, but these strains have to be cultivated at elevated temperature with high energy requirements [33]. Hydrogen research strategies organism-wise

stated that productive pure cultures were used with defined substrate as the carbon source, but mixed cultures are preferred for operational ease and process stability [35]. When wastewater or agricultural waste is used as the substrate, a mixed microbial population is more favorable and practical [36]. Regardless of the research goal, the key requirement for every biological hydrogen production research is a proper choice of hydrogen production system and understanding of the biochemical and biophysical characteristics of this system [37, 38].

The substrate plays an important role in the H_2 yield, H_2 production rate and the overall economy of the process. Carbohydrate rich substrates have been extensively used in biohydrogen production studies [39]. Glucose and sucrose are the most commonly used pure substrates in both batch and continuous processes due to their simple structures and ease of biodegradability [40]. Renewable biohydrogen production requires the substrate or feedstock to come from renewable resources. Second generation biomass sources, such as waste biomass, are abundant and thus can support the supply of renewable substrates and fermentation process offers biological treatment of the organic waste [39]. In order to reach sustainable production and also waste management, various agriculture and industrial waste materials as feedstock may be used [41]. Substantial factors like availability and cost are highly important in the selection of waste materials to be used in hydrogen production with fermentative bacteria [40].

4.1.4. Crude glycerol fermentation process

One of the substrates that can be effectively used for microbial hydrogen production is crude glycerol, which is a by-product from the process of biodiesel production. Because large quantities of crude glycerol are available and the highly reduced nature of carbon in glycerol per se, microbial conversion of it in hydrogen is an economically and environmentally viable possibility, especially because over the last several years the demand and production of biodiesel has remarkably increased [42]. Glycerol ($C_3H_8O_3$) is a trihydroxyl (sugar alcohol) compound, the energy content of pure glycerol is 19.0 MJ/kg, but for crude glycerol the energy content is 25.30 MJ/kg, that most probably is due to presence of methanol and traces of biodiesel. Energy content of crude glycerol indicates its high potential to be an effective substrate for biological hydrogen production, especially because it does not require additional pre-treatment to make it available for the hydrogen-producing microorganisms [43]. About 10 billion litres (L) of crude glycerol per year are a by-product of biodiesel generation from plant oils, giving 1 L of glycerol for every 10 L of biodiesel [44, 45]. Biodiesel is mainly produced from the transesterification of vegetable oils or animal fats with methanol catalysed by alkalis, such as NaOH and KOH. After the transesterification process, two layers are formed: the top layer is the desired product, i. e., biodiesel, and the bottom layer is the raw/unrefined crude glycerol [46, 47]. One of the major challenges for the utilization of crude glycerol is the inconsistency of its composition since it varies with the feedstock, production processes, and post-treatments involved in the biodiesel production. Upgrading or refining crude glycerol to technical grade glycerol (>98% glycerol content) makes its composition more consistent, but currently this is not economically viable, especially for small and/or medium-sized biodiesel plants [48].

Reungsang et al. [49] for hydrogen production with *Enterobacter aerogenes* KKU-S1 used crude glycerol with glycerol concentration of 441.3 g/L, methanol 230 g/L, NaCl 10 g/L. Mangayil et al. (2012) [50] used Savon Siemen Oy manufactory glycerol, that contained 45% (v/v) glycerol and 30% (v/v) methanol. Valuable compounds can be

produced from crude glycerol such as hydrogen, and 1,3-propanediol as main products, methane, ethanol and succinic acid in smaller amounts [50]. Hydrogen production comparing to production of 1,3-propanediol is more valuable, hydrogen has a higher energy content (142.9 kJ/g) and it results in higher yield and productivity [43]. Various bacterial strains are considered promising for glycerol utilization because of possibility to ferment crude glycerol, and H_2 is one of the end-products of this process [51]. The most productive microorganisms that grow anaerobically on glycerol as the sole carbon and energy source are *Rhodopseudomonas palustris*, *Enterobacter aerogenes*, *Escherichia coli*, *Thermotoga neapolitana* and others [43, 51].

There are two ways of glycerol metabolism – oxidative and reductive. In the reducing pathway glycerol is converted in 1,3-propanediol. In the oxidative pathway glycerol is firstly converted in dihydroxyacetone using catalytic activity by glycerol dehydrogenase, then dihydroxyacetone is phosphorylated by the glycolytic enzyme dihydroxyacetone kinase and the phosphorylated product is metabolized through glycolysis. Pyruvate may be further converted to various end-products depending on microorganism. In most of glycerol bioconversion pathways hydrogen is produced along with other metabolites (e. g. butyrate, ethanol, butanol, acetate, acetone, lactate) [43, 50].

Optimal conditions are pH 6.5; 40 °C for hydrogen production using crude glycerol with microbial consortium mainly dominated by *Clostridium* species. Environmental conditions like medium pH and temperature are the major parameters to be controlled in the hydrogen production, because they affect qualitative and quantitative content and hydrogen yield and rate of bacterial produced gas. Hydrogen production using glycerol is 1.5-fold higher at pH 5.5 than at pH 6.5 [52] using *E. coli*. Anaerobic conditions have to be maintained during the hydrogen production process, which are ensured by barbotage media with reducing agent or inert gas such as nitrogen or argon [40, 53].

Guillaume and Patrick [53] have reported hydrogen production using photo-fermentation of pure and crude glycerol by *Rhodopseudomonas palustris* with maximum of 6 mol of hydrogen per mole of glycerol, that is 75% of theoretical maximum 8 mol hydrogen production per mole glycerol, meaning that intermediate products such as acetic acid, ethanol and butyric acid were further metabolized to hydrogen, which is otherwise accumulated during dark fermentation [43]. *Enterobacter aerogenes* have an ability to convert glycerol in a fermentative process to yield hydrogen and ethanol as the main by-products. Yields of 0.86 mol H_2/mol glycerol and 0.75 mol ethanol/mol glycerol were reported by Jitrwung and Yargeau (2015) [54]. Dharmadi et al. (2006) [55] reported that glycerol can be fermented also by *E. coli* at slightly acidic pH (pH 6.3). [56] have reported production of hydrogen from crude glycerol with anaerobic sludge.

4.1.5. Feasibility of biohydrogen production processes

In order to achieve higher overall volumetric hydrogen production co-culture employing gives a possibility to maintain anaerobic conditions for strict anaerobes and eliminate the need for a reducing agent – facultative anaerobes consume oxygen in medium [57]. Biohydrogen price could compete with current fuel prices, when fermentation process outcome is 10 H_2 moles per glucose mol (it is close to maximally theoretically possible outcome – 12 moles) and glucose price is approximately 0.05 USD for one glucose dry matter pound. Dark fermentation – 2.4 $/kg. Comparatively, more developed method costs for hydrogen gas – fossil fuel reforming (0.75 $/kg), plasma arc decomposition (0.85 $/kg) and coal gasification (0.92 $/kg) [17, 58].

4.1.6. Metal hydride alloy usage in storing hydrogen

Research activity in hydrogen storage field has increased substantially due to practical need for hydrogen storage possibilities. Hydrogen is a low density gas in atmospheric pressure and room temperature, for example, 1 kg of hydrogen occupies a volume of 11 m^3. Various methods are used to increase hydrogen density:

1) Hydrogen storage in high-pressure gas cylinders with a maximum pressure up to 80 MPa and therefore the hydrogen gas can reach a volumetric density of 36 kg/m^3; current state of art storage tanks in electric/hydrogen passenger vehicles (Toyota, Hyundai) hold hydrogen at pressures up to 70 MPa, which allows the storage of 5.4 kg of hydrogen in a 260 L tank and travel 600 km without refilling;
2) Liquid hydrogen storage in cryogenic tanks at –252 °C. The volumetric density of liquid hydrogen is 70.8 kg/m^3;
3) Adsorbed hydrogen on materials with a large specific surface area [59], e.g. carbon nanotubes and nanofibers [60];
4) Hydrogen absorption on interstitial sites in a host metal (metal hydrides);
5) Chemically bonded hydrogen in covalent and ionic compounds (such as sodium tetrahydroborate ($NaBH_4$) and ammonia borane (NH_3BH_3), which stores 19.6 wt% or 145 kg/m^3.

Hydrogen storage in solid-state materials is an alternative way to store hydrogen at low pressure and ambient temperatures, and may be a very promising potential solution. Reversible hydrogen storage materials tend to be hydrides or microporous materials. The most extensively known metal forming hydride is palladium – white, silver like metal that soaks up hydrogen like a sponge – at a room temperature and atmospheric pressure, palladium can absorb up to 900 times its own volume of hydrogen; it was first noted by T. Graham in 1866. Material that can reversibly absorb or adsorb hydrogen in atomic (H) or molecular (H_2) form is used to compress hydrogen (chemically or physically) to high storage densities (Fig. 4.1).

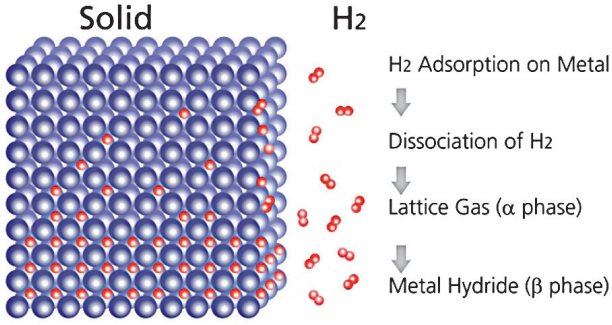

Figure 4.1. **Metal hydride formation [61]**

In standard practices, biohydrogen is synthesized in the fermentation medium, but collected from the headspace of the flask or fermenter after diffusion into gaseous phase. Theoretically, dissolved hydrogen is in equilibration with the gas phase and tends to release from the liquid phase, if partial pressure of H_2 decreases [62]. Nevertheless, during dark fermentation, biohydrogen oversaturation in the liquid phase takes place [6]

and inhibits further synthesis of the gas. To optimize biohydrogen production, it must be removed from the liquid phase by, e. g. barbotage N_2 or Ar through the fermentation medium, and afterwards purified, collected and stored from gaseous phase. Absorption of biohydrogen from gaseous phase by alloys and metals that can form hydrides is a safe and efficient method to collect the gas, but will it work also in liquid phase?

Metals and alloys can be "charged" with hydrogen from gas phase under pressure, they release hydrogen when heated or subjected to evacuation [63]. It is reported that the presence of moisture in the hydrogen gas decreases the efficiency of its sorption by metals and alloys, e.g. $LaNi_5$ in 300 ppm of water vapour loses its hydrogen absorption capacity [64]. $LaNi_5$ is one of the most widely used intermetallic alloys for hydrogen storage, due to its sorption capacity, reversibility of absorption and desorption and quick charge–discharge kinetics under room temperature and atmospheric pressure.

Metals and alloys can be "charged" with hydrogen also from liquid (electrolyte). Already for decades hydride-forming alloys are used as the cathode material in alkaline Ni/Me hydride batteries (see, for example, [65]). Hydrogen ion (proton) is inserted in metal hydride cathode through overvoltage applied to the battery in the process of charging and is ejected due to the difference of electrode potentials in the battery.

4.1.7. Biohydrogen isolation experiments in hydride-forming metals and alloys

Our innovative idea in this research is that absorption of the biohydrogen directly from the medium of cultivation would increase the efficiency of the gas production due to

1) removing of its inhibitory over-saturated concentration from the medium;
2) more simple construction of the fermenter since the inert gas barbotage appliances will become superfluous;
3) collection of the gas would occur directly during the fermentation process, not afterwards with additional treatment of the gas phase.

The purpose of the study was to explore if the hydride-forming metals and alloys are able to adsorb and desorb biohydrogen directly from the fermentation solution. The mass-spectrometry was used to characterize the composition of the headspace gases in fermentation process and the thermogravimetric weight loss (TG) method was used to estimate the amount of gases and volatile substances absorbed in powdered Pd and $LaNi_5$, AB_5 and AB_2 alloys. Nobuyuki Nishimiya et al. [66] found that ZrVFe encapsulated in polymer is an effective material to enhance hydrogen produced by *Anabaena* in argon atmosphere: the total amounts of hydrogen increased 6-7 times compared to normal hydrogen production in the absence of the alloy.

Klebsiella (Enterobacter) aerogenes MSCL 758 and *Escherichia coli* BW25113 *hyaB hybC hycA fdoG frdC aceE ldhA:kan* (kindly provided by Prof. T.K. Wood, Texas A&M University, USA) were used for fermentation experiments. Strains were grown on modified anaerobic growth medium (AM, g/L): tryptone 1.0, yeast extract 2.5, cysteine 0.5. Growth medium components were suspended in sterile water and supplemented with glycerol source 6 g/L of analytical grade glycerol (Stanlab LLC, Poland) or crude glycerol (Delta Riga Ltd., Latvia and the biofuel factory in Naukšēni, Latvia). The nutritional medium was sterilized by autoclaving for 15 minutes at 121 °C. The bacterial strains were cultivated overnight aerobically in Petri dishes on solid Luria-Bertani (LB) medium at 37 °C. The overnight cultures were adjusted to 1 OD (600 nm) in sterile distilled water and the OD-adjusted cultures used as inoculum for further cultivation anaerobically in 50 mL serum bottles (Supelco Analytical, USA) containing 35 mL AM medium and 1 mL

bacteria suspension. Inoculated serum bottles were closed with butyl rubber stoppers sealed with aluminium caps and flushed with argon gas to obtain anaerobic environment. The serum bottles were incubated in Biosan RS-24 programmable rotator in a thermostat at 37±2 °C.

Pd and $LaNi_5$, AB_5, AB_2 alloy powders that were tested for the capacity to remove biohydrogen from the liquid phase in fermentation are listed in Table 4.2.

Table 4.2. **Materials tested for hydrogen absorption capacity from the liquid phase of fermentation**

No.	Metal or alloy	Origin	Composition, grain size
1	Pd	Aldrich	Microcrystalline powder, <10 μm
2	$LaNi_5$	Treibacher Industrie AG, Austria	Crystalline powder, <500 μm
3	AB_5	Gesellschaft fur Elektrometallurgie; Nurnberg; Germany	Microcrystalline powder <75 μm; A = Mm = La, Ce, Nd, Pr; B = Co, Mn, Al; $Mm(Co, Mn, Al)_5$
4	AB_2	Gesellschaft fur Elektrometallurgie; Nurnberg; Germany	Microcrystalline powder <75 μm; A = Zr, Y; B = Ni, Al, Fe, Mn $(Zr, Y)(Ni, Al, Fe, Mn)_2$

10–12 g of powders were activated before use: simultaneously heated up to 170 °C and evacuated up to ~0.01 bar in ca 8 cm^3 cylindrical stainless steel hydrogen adsorption/desorption reactor built in the Institute of Solid State Physics (University of Latvia), then exposed to hydrogen gas (2 bar) for 30 min and cooled down to room temperature in hydrogen atmosphere in the reactor; this process was repeated three times until rapid hydrogen absorption in alloy powder was observed. Finally, the absorbed hydrogen was removed by simultaneous heating and evacuation process. Argon gas was used to preserve activated alloys until their contact with the fermentation medium. One g of each alloy and 0.5 g of Pd powder was used for 30 mL of fermentation medium. Serum bottles with microorganisms, medium and metal hydride powder were rotated for 18, 24 and 36 hours in 37 °C temperature. Metal hydride powders were washed with 96% ethanol and dried in 4 °C temperature for at least 12 hours.

Gas analysis and thermogravimetry was done using equipment from Institute of Solid State Physics (University of Latvia). Qualitative analysis of evolved gases was performed with RGA Pro-100 mass spectrometer. Data was assessed by SR Residual Gas Analyzer with RGA 3.0 software. The volume of produced gas was measured by puncturing rubber stoppers of the serum bottles with a 20 mL syringe. If there was an elevated pressure in the serum bottle, the gas entered the syringe, making it possible to read the volume of produced gas.

By measuring the composition of gaseous phase of growth media using mass spectrometry after 24-hour incubation (Fig. 4.2), highest proportion of hydrogen was found in

sample 4, which consisted of growth medium, glycerol, *K. aerogenes* and multicomponent AB_5 alloy – maximum hydrogen concentration measured was 23.1%. Control samples without added bacteria showed insignificant hydrogen concentration (0.0–0.2%).

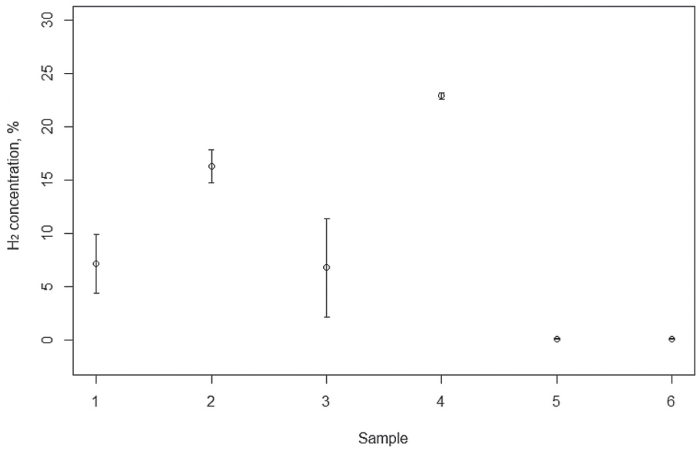

Figure 4.2. **Average amount of hydrogen (%) in the gas phase sample of growth media after 24 h of cultivation. Components of samples: 1 – *K. aerogenes*, growth medium; 2 – *K. aerogenes*, growth medium, glycerol; 3 – *K. aerogenes*, growth medium, glycerol, $LaNi_5$; 4 – *K. aerogenes*, growth medium, glycerol, multicomponent AB_5 alloy; 5 – growth medium, glycerol; 6 – growth medium, glycerol, $LaNi_5$ [5]**

By comparing samples 1 and 2 in Fig. 4.2, statistically significant effect for addition of glycerol on the proportion of hydrogen in gas phase of growth media was determined ($p = 0.0195$). The sample, which did not contain glycerol, showed an average hydrogen concentration of 7.1%, whereas for the sample containing glycerol it was 16.3%. By comparing the samples 2 and 3, it was determined that addition of $LaNi_5$ alloy statistically significantly decreased the proportion of hydrogen in gas phase of growth media ($p = 0.00016$). The sample containing $LaNi_5$ showed an average hydrogen concentration of 6.7%. Multicomponent AB_5 alloy statistically significantly increased the proportion of hydrogen ($p = 4.7 \times 10^{-6}$) by comparison with the sample 2, which did not contain multicomponent AB_5 alloy. The average hydrogen concentration in the sample with multicomponent AB_5 alloy was 22.9%. The sample with $LaNi_5$ powder showed a reduced concentration of H_2 and CO_2, which suggests inhibitory effect of $LaNi_5$ for the growth of bacteria (Ni is known as an inhibitor of metabolic pathway, in which H_2 and CO_2 is produced [67]). Conversely, the addition of multicomponent AB_5 alloy showed a positive effect on H_2 and CO_2 production.

Differential TG of the Pd or alloy grains upon heating after the incubation in the fermentation media was performed using DTG-60 device (Shimadzu). The amount of gas absorbed by the hydrogen-absorbing materials was tested by removing 1.5–2.0 cm^3 medium containing 5–15 mg of the Pd or alloy grains from the bottle with the large gauge syringe. Before the TG measurements, the grains were washed with 98% ethanol and dried at room temperature for 24 hours in argon atmosphere. The temperature of the crucible in TG experiments was increased with a constant rate –10 °C per minute. Selected hydride-forming metals and alloys were compared for their H_2 absorption capacity from

fermentation broth with H_2-producing *E. coli*. TG measurements disclosed that H_2 was absorbed most efficiently by powdered Pd, followed by AB_5, AB_2 and $LaNi_5$ alloys (Fig. 4.3).

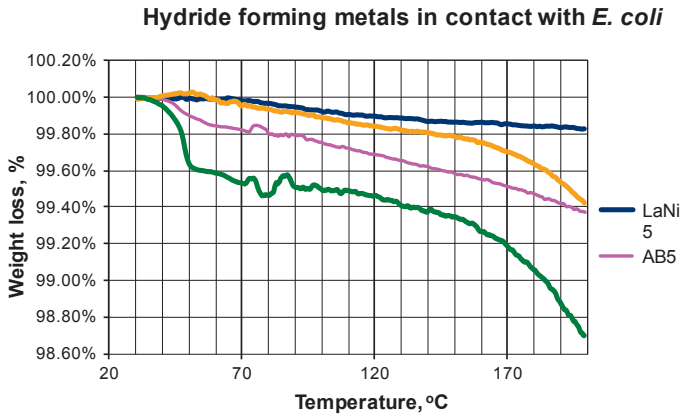

Figure 4.3. **TG assessment of H_2 release from different hydrides formed during 72-h incubation in *E. coli* fermentation medium containing crude glycerol [2]**

H_2 absorption by alloys during fermentation was analysed by TG in separate experiments by removing 5–15 mg of granulated material from the serum flask at regular intervals. Three parallel tests were conducted. Table 4.3 illustrates that the highest amount of H_2 was absorbed after 18 h of fermentation.

Table 4.3. **H_2 absorption by AB_5 alloy during 42 h *K. aerogenes* fermentation of crude glycerol**

Time after the beginning of fermentation, h	Weight loss (30–100 °C), wt%
6	0.41 ± 0.07
18	1.61 ± 0.72
24	0.38 ± 0.16
36	0.12 ± 0.01

The utilization of glycerol at bacterial fermentation was measured by HPLC on Agilent *1290 Infinity* device (Agilent Technologies, Germany) using the *Atlantis dC18 3 µm, 2.1x150mm* (Waters) column. The aliquots from the fermentation media were passed through 0.45 µm filters (Nonpyrogenic Sterile-R, Sarstedt) and 1 µL of the solution was injected into the liquid chromatography system. All the experiments were performed in triplicate. The relative standard deviation was determined to be less than 1%. Data analyses were performed using MassHunter version B05.00 software (Agilent Technologies). Glycerol utilization by bacteria was not impaired by alloy grains in the medium. HPLC-MS demonstrated that glycerol was degraded completely within 24 h of fermentation with or without alloy grains in the medium (Fig. 4.4).

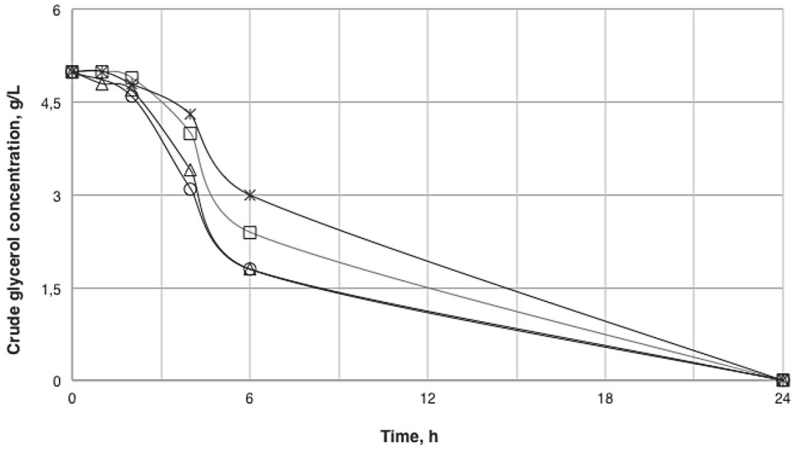

Figure 4.4. **Crude glycerol utilization by *E. coli* during fermentation in 35 mL of cultivation medium containing 1 g of hydride-forming material alloys $LaNi_5$ (✳), AB_5 (Δ), AB_2 (☐) with 7 g/L crude glycerol and control samples without hydride-forming material (O). Glycerol concentration was measured directly by HPLC combined with electrospray-ionization high-resolution MS [2]**

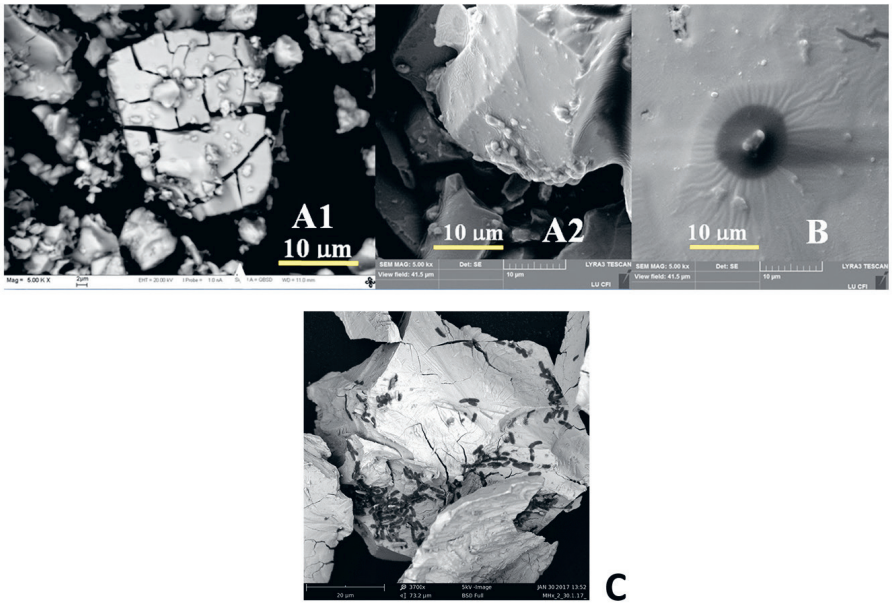

Figure 4.5. **SEM of $LaNi_5$ alloy surface: A1 – before contact with bacteria; A2 – after contact with absorbed bacteria: B – contact with *E. coli* cell [2]. C – *K. aerogenes* MSCL 758 on the surface of $LaNi_5$ alloy [5]**

The surfaces of alloy grains after incubation in fermentation medium were examined by SEM. Fig. 4.5 shows that bacteria cells are present on the surfaces of alloy grains, thus being in close contact with H_2-absorbing material. It also appeared that contact with alloy grains stimulates bacteria growth. More colony-forming units (CFU 10^7/mL medium) of *K. aerogenes* were found on AB_5 alloy (Fig. 4.5 C). Although this field with metal hydride usage for bacteria produced hydrogen storage is novelty, it has been investigated that ability to grow on special carrier or substrate stimulates hydrogen production [68].

The first attempts were made for hydrogen collection from bacteria fermentation broth using metal hydrides [2, 3, 5]. Various powdered metals and alloys (Pd, $LaNi_5$, AB_5, AB_2) forming hydrides were investigated to collect hydrogen directly from liquid phase. Vacuum extraction measurements were accomplished to ascertain the measured gas weight changes in thermogravimetry experiments were regarding to hydrogen and not water from broth or thermal decomposition changes of organic materials. As per $LaNi_5$, a similar behaviour was seen with metal hydrides immersed in fermentation medium and hydrated metal hydride: similar weight losses occurred according to temperature changes. This could be considered as evidence that hydride-forming metals absorb H_2 not only from the gas phase but also from dissolved gas. Hydrated metal hydride weight losses with $LaNi_5$ are accordingly reported elsewhere [69]. All the materials in these experiments are known to release H_2 at low temperature: Pd at 70 °C, AB_2 at 50 °C, $LaNi_5$ and AB_5 at 25 °C [69, 70]. Weight loss of material at heating within the 30–100 °C range approximates the amount of adsorbed H_2. At temperatures exceeding 150 °C, the organic substances from microorganisms and fermentation broth begin to decompose and can elicit additional weight losses not related to H_2 desorption. Thermal decomposition of organic materials is most active at around 200 °C [71].

In comparing weight loss, Pd and the 3 hydride alloys tested could be listed according to the amount of stored H_2 in the following order (starting with the highest amount): Pd, AB_5, AB_2 and $LaNi_5$. This could be explained by Pd's greater stability in corroding environments, as all the experiments were carried out in fermentation broth and with smaller size particles. It appears that, if the samples contained metals that absorbed H_2 released in fermentation, H_2 concentration in the gaseous phase at the end of the process would be lower. This was also confirmed by MS analysis. Each molecule that is produced inside the bottle, increases H_2 temperature release in metal hydrides [65] with 1.4% being the maximum (Inspection Certificate from GfE Gesellschaft für Elektrometallurgie mbH).

Our findings showed that less H_2 was detected in the gaseous phase owing to the presence of metal hydrides in all samples, except for AB_2. Vacuum extraction measurements concurred with our hypothesis that weight changes in the TG experiments were due to H_2 and not organic decomposition. Also, if some H_2 was produced by organic decomposition, CO_2 and other carbon (C)-containing gas levels would increase.

The analysis of H_2 absorption dynamics in alloys during fermentation determined that the largest amount of soak-up H_2 occurred after 18 h from the beginning of fermentation. It coincides well with the analysis of consumed glycerol – all the results indicated that crude glycerol was completely expended within 24 h of fermentation. Incidentally, this may also explain the results of the first fermentation experiments with relatively small, bonded H_2 in hydride metals – because they were removed from the fermentation bottles after 72 h.

Conclusions

1. The studies show that hydride-forming materials – Pd, AB_5, AB_2 – can be successfully exploited for H_2 collection from the liquid phase in crude glycerol fermentation by *E. coli* and *Klebsiella aerogenes* (previously known as *Enterobacter aerogenes*) MSCL 758. The inhibitory effect of $LaNi_5$ was established for the growth of bacteria or inhibition of metabolic pathway, in which H_2 and CO_2 is produced. Conversely, Pd and multicomponent alloys AB_2 and AB_5 showed a positive effect on H_2 and CO_2 production. Attachment of bacteria to the metal alloys was demonstrated and hydrogen accumulation from fermentation media containing AB_5 alloy powder was proved by vacuum extraction and MS analysis.
2. H_2 concentration in the gaseous phase after 72 h of fermentation was lower in cultivation medium loaded with hydride-forming materials than in unloaded controls, while total calculated amount of H_2 absorbed in hydrides and the gas phase exceeded the control level.
3. TG data ranked the tested hydride-forming materials by their H_2 sorption capacity in the fermentation broth in the following order (highest to lowest): Pd, AB_5, AB_2 and $LaNi_5$.
4. The presence of powdered Pd or hydride-forming alloys in cultivation medium did not impede glycerol consumption: it was fully degraded during 24 h of fermentation. Concomitantly, the highest amount of absorbed H_2 was measured by the weight loss of hydride-forming materials in TG experiments after 18 h of glycerol fermentation.

4.2. Microbial fuel cells for production of energy carriers

4.2.1. Introduction

Microbial electrolysis cells (MBECs) are a technique used to produce H_2 from a wide variety of substrates. MBECs are adapted microbial fuel cells (MBFCs). Using the MBECs as an alternative electrically driven hydrogen production process results in the conversion of a wide range of organic substrates into hydrogen under applied external potential [15]. The MBEC technology resembles an MBFC in which the primary difference is the necessity of a small input of external voltage. Based on thermodynamics, a potential higher than 0.110 V, in addition to that generated by microorganisms (−0.300 V), will produce H_2. The MBECs are capable of more than 90% efficiency in the production of H_2. The performance of MBECs is determined by the type of microorganism, electrode materials, type of the membrane used, applied potential range, composition and concentration of the substrate, and design of the MBEC [72]. Babu et al. [73] reported usage of two-stage process integrating the MBEC process with the dark fermentation in order to convert the acid-rich dark fermentation effluents into substrates for additional hydrogen gas production. MBECs were operated with a small range of varying applied potential (0.2–1.0 V) using an anaerobic mixed consortium as a biocatalyst. The process produced hydrogen with rate 0.53 mmol/h and high substrate conversion efficiency (90%) (Fig. 4.6).

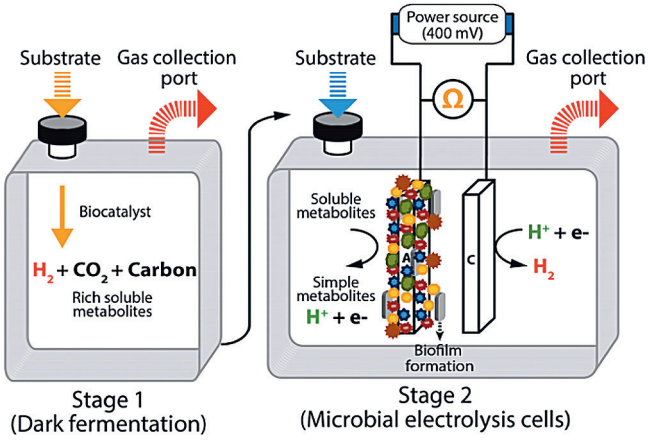

Figure 4.6. **Two-stage process integrating the MBEC process with the dark fermentation. Green, orange, brown, and blue symbols represent a mixed microbial population. In stage 1, complex substrates are used for H_2 production in dark fermentation, and in stage 2, acid-rich effluents were used as substrates in MBECs for further H_2 production (adapted from [15, 73])**

Microbial fuel cells (MBFCs) are devices that convert chemical energy of organic compounds to electrical energy by the catalytic reactions of microorganisms under anaerobic conditions (reviewed in [74]). MBFCs generate electricity directly from biodegradable materials [75]. Almost any biodegradable organic substance can be used to operate an MBFC device and generate electricity. Examples include domestic and industrial wastewater and agricultural pollution, as well as sugars, proteins, starch and cellulose [76]. MBFCs are promising future technology for alternative renewable power sources. Simultaneous wastewater treatment and energy production are one example of the effective operation of MBFCs [77]. Bacteria obtain energy in two steps. The first step involves the removal of electrons from an organic material (oxidation), and the second step is to give these electrons to the final electron acceptor (reduction), such as oxygen or nitrate or sulphate ions. If bacteria grow under anaerobic conditions, they can return electrons to a carbon electrode (anode). The electrons travel through the external resistance (the consumer) to the cathode, where they are combined with protons and oxygen. When electrons move from anode to a cathode, they generate current and voltage [78]. For most of the microorganisms, the outer surface consists of electricity non-conductive substances, lipid membranes, peptidoglycan and lipopolysaccharides, which prevent direct transport of electrons to anode [74]. The activity of MBFC is provided by microorganisms capable of carrying out electron transport outside the cell. They are called exoelectrogenic microorganisms, because they have the ability to directly transport electrons outside the cell [76] with conductive pili or nanowires [79]. Some of the bacterial species found in biofilms improve the function of MBFC and can produce and excrete soluble compounds acting as mediators to shuttle electrons to the surfaces [80].

Two different types of MBFC technologies are available – single and dual chamber MBFC [75]. A traditional MBFC consists of anode and cathode compartments that connects the proton exchange membrane (PEM) or the salt bridge [74, 81]. A single-chamber MBFC eliminates the need for a separate cathode chamber, putting the cathode

outside the cell in contact with airborne oxygen. Dual-chamber MBFCs are typically chosen to avoid mixing electron acceptors and electron donors that would lead to electron losses [75]. Nafion membranes (Dupont Co., USA) are the most commonly used PEM, but the agar-salt bridge is the simplest and cheapest solution [82]. Increased voltages or power can be achieved by connecting some MBFC circuits, or in parallel. Power generation using MBFC is influenced by several factors such as the type of microorganisms, the type and concentration of fuel biomass, ion strength, pH, temperature and reactor configuration [74]. In today's research, mixed cultures and microbial isolates are used. Often the use of mixed cultures shows better results than experiments with pure cultures [76]. However, there is a lack of information about MBFC potential in natural environments and usage of natural sludge as an inoculum.

4.2.2. Applicability of 3D-printed graphene electrodes in MBFC

The choice of anode material is very important for MBFC's efficient operation. Graphite electrodes are most often applied, but their hydrophobic and antimicrobial properties reduce biocompatibility. Graphene materials are less hydrophobic and have good conductivity, thus exhibiting potentially better biocompatibility [83]. Providing favourable conditions for microorganisms it is possible to improve the functioning of the MBFC.

The aim of this study was to test the applicability of the graphene-PLA composite for the production of 3D-printed electrodes for MBFC. The pure culture of *Pseudomonas aeruginosa* and natural mixed inoculum was used to evaluate wild habitat response to MBFC technology. *P. aeruginosa* is a wide distributed Gram-negative rod-shaped bacterium which produces pyocyanin, a compound which acts as an electrochemical extracellular mediator and promotes electron transport to the anode [84].

Sandwich-type dual-chamber MBFCs were built in our laboratory. Each MBFC consisted of two hermetically sealed polycarbonate containers, which were interconnected with a bridge (Fig. 4.7). Anode chamber (100 mL) was separated from cathode chamber (100 mL) with Nafion® 212 membrane or agar-salt bridge. The active surface of Nafion membrane was 25 cm^2. The conductive part of agar-salt bridge (4% agar, 2% NaCl) has diameter 18 mm and length 40 mm. Four different anodes (5x5 cm, Fig. 4.8) were used:

1) graphite paper (Sidrabe JSC, Latvia);
2) plain 3D-printed graphene-PLA composite (BlackMagic3D, Graphene 3D Lab, USA);
3) sponge-like 3D-printed graphene-PLA composite;
4) self-made pressed graphene powder electrode.

Electrochemically (0.00384 A/cm^2) platinum-coated stainless steel wire mesh, 5 × 5 cm, was used as a cathode.

In total, 16 MBFCs were constructed, of which eight operated with natural substratum and inoculum, and eight operated with pure culture of *Pseudomonas aeruginosa* MSCL 331 in MH medium (glucose 5 g/L). The anode chamber was filled with a natural lake sediment (particle size less than 5 × 5 mm) or suspension of *P. aeruginosa* in medium. After filling with an inoculum/ substrate, an MBFC anode chamber was flushed with argon gas for 5 minutes (2 L/min). The cathode chamber was filled with tap water. MBFC operated at a room temperature of 20±2 °C. The measurements were taken with potentiostat Voltalab PGZ301 (Radiometer Analytical) and software VoltaMaster4 after a 96-hours adaptation period in the case of sediment and after 12 hours in the case of *P. aeruginosa*.

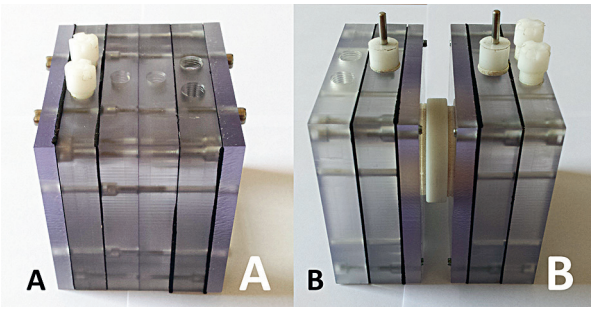

Figure 4.7. **Two-chamber MBFCs. A – with Nafion 212 membrane; B – with agar-salt bridge**

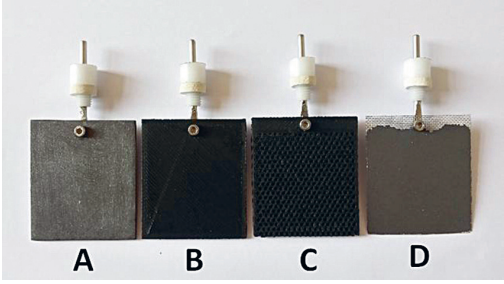

Figure 4.8. **Anodes made from A – graphite paper, B – plain 3D-printed graphene-PLA composite, C – sponge-like 3D-printed graphene-PLA composite, and D – pressed graphene powder**

MBFCs with agar bridges showed three times lower power densities than MBFCs with Nafion membranes because the bridge had a higher internal resistance. MBFCs with a lake sludge and plain printed graphene-PLA electrode showed a similar maximum power density (11.6 mW/m^2) as a graphite paper (12.4 mW/m^2) (Fig. 4.9).

However, the MBFCs with *P. aeruginosa* culture (Fig. 4.10) and plain printed graphene-PLA electrodes showed a significantly higher power density (35.8 mW/m^2) than graphite electrodes (6.5 mW/m^2). The best result showed printed sponge-like electrodes, which had three times larger surface area than plain graphene-PLA electrodes. The highest power densities obtained by *P. aeruginosa* point to a much more efficient MBFC performance than a natural system. However, such MBFCs proved to be unstable as they did not work after 36 h. The pressed graphene powder electrodes also showed good power density (15.7–28.5 mW/m^2) however, the electrodes were mechanically unstable. The open circuit potential increased from 0.45 V to 0.75 V in MBFCs with graphene-PLA electrodes (Fig. 4.11, 4.12), which could indicate a better biocompatibility however the output power was limited by increased material resistance. The obtained maximum power density was 10 times smaller than for MBFCs with graphene electrodes mentioned in the literature [85, 86], which could be explained by the limiting function of the cathode. The properties of the platinum-coated stainless steel cathode were significantly different from the catalytic properties of pure platinum (Fig. 4.13).

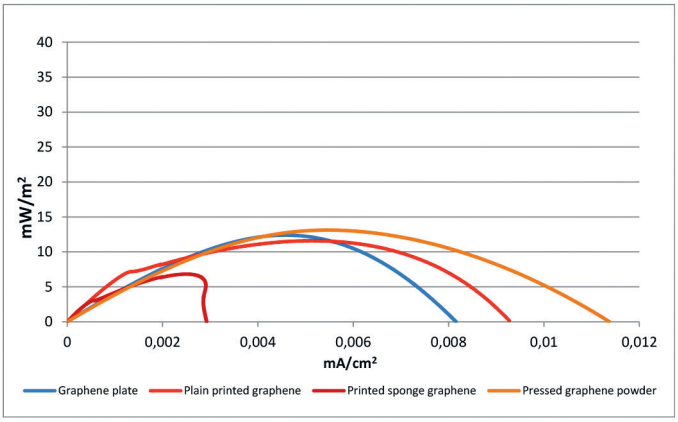

Figure 4.9. **Power curves of MBFC after 96 hours' operation with lake sediment**

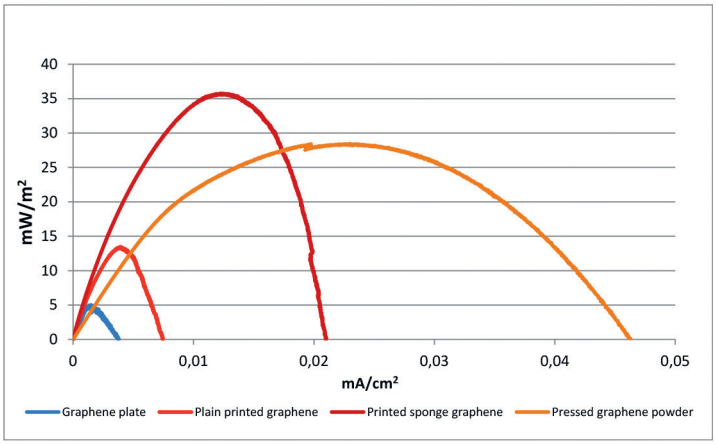

Figure 4.10. **Power curves of MBFC after 12 hours' operation with P. *aeruginosa***

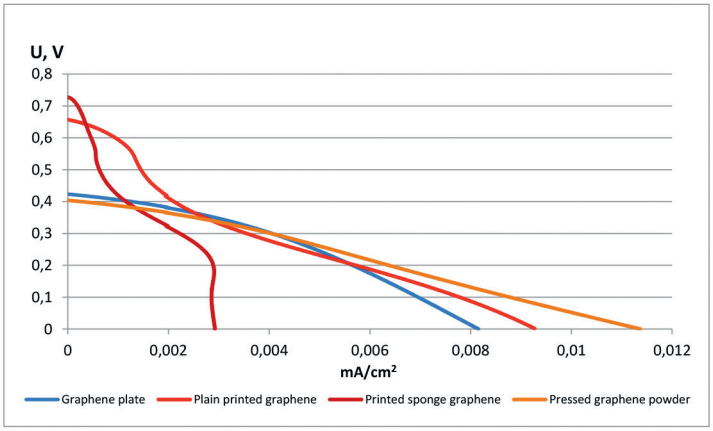

Figure 4.11. **Polarization curves of MBFC after 96 hours operation with lake sediment**

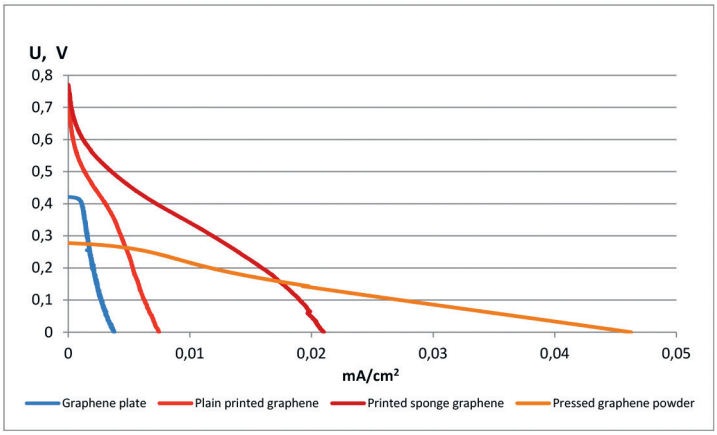

Figure 4.12. **Polarization curves of MBFC after 12 hours operation with *P. aeruginosa***

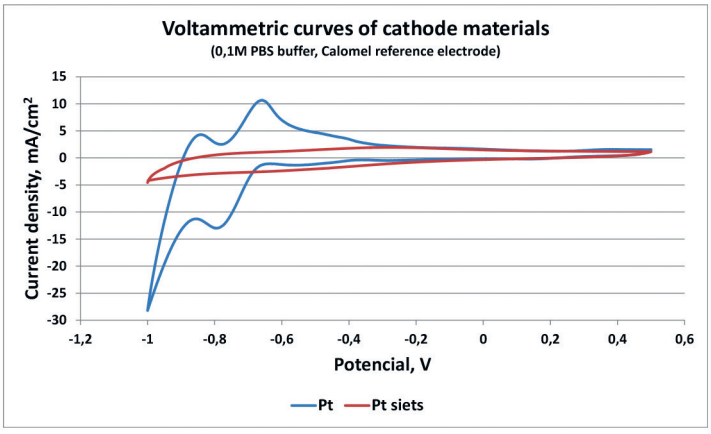

Figure 4.13. **Voltammetric curves of cathode materials**

The voltammetry of graphene-PLA composite electrodes showed worse electrochemical performance than graphite, but electrodes worked equally well or better than graphite, indicating better biocompatibility. However, the increased MBFC potential may also indicate a better biodegradability of the electrode. With 3D printable material, it is possible to produce any form of MBFC electrodes quickly. An innovative anode configuration is required to reduce the spatial conductivity resistance of the graphene-PLA composite. The long-term operation of MBFC was more efficient with lake sediment as a substrate/ inoculum than with a bacterial pure culture.

4.2.3. Anodic biofilm vitality during operation of MBFC

Freshwater and marine sediments, landfill leachate, wastewaters, activated sludge and other substrates serve as organic-rich nutrients and sources of microorganisms for sustainable energy production in MBFCs (reviewed in [87]). Bacteria are looking for electron

acceptors such as nitrate or sulphate ions during decomposition of organic materials under anaerobic conditions. They can return electrons also to an anode which usually contains carbon-based materials. It has been discovered that it does many bacteria using several electron transfer mechanisms. Exoelectrogenic bacteria transfer the electrons either directly to the anode surface via specialized protein-based nanowires and/or mediate them by secreting different soluble electron shuttles (reviewed by [88]). Bacteria form biofilms on the anode (reviewed by [89]). Correlation between increasing bacterial biomass on the anode and current is proven. There is no decrease in cell viability as current increases. Reguera et al. [90] studied biofilm development and energy production by pilin-deficient *Geobacter sulfurreducens* mutant and showed that the live population was preferentially located in direct contact with the anode surface, and that the dead cells were present primarily in the upper biofilm layers. Situation with mixed-species biofilms is more complicated because it is not known which species or strains may be conductive and contribute to the transfer of electrons [91]. Read et al. [92] demonstrated that biofilm viability was maintained highest nearer the anode during closed circuit operation, but viability was highest on the top of the biofilms in open circuit. Other authors have noted that development of biofilms is influenced by startup performance of MBFC under different external resistances and that compact biofilm resulted in a poor mass transfer [93].

The aim of this study was to determine the vitality and succession of microorganisms in MBFCs fed with organic-rich lake sediment during operation at various load conditions.

Three sandwich-type dual-chamber MBFC was built in our laboratory. Each MBFC consisted of two hermetically sealed 1 L polycarbonate containers (Cambro, USA), which were interconnected with a bridge. The conductive part of agar-salt bridge (4% agar, 2% NaCl), made from POM plastic (Industriplasts Ltd., Latvia) cylinder with threads at both ends, has the diameter of 18 mm and the length of 40 mm. The anode chamber was filled with 0.7 L of natural organic-rich sediment obtained from watercourse near lake Jugla in Riga (Latvia). After insertion of sediment, anode was placed into the anode chamber. The anode was made from graphite paper (Sidrabe JSC, Latvia), 13 × 6 cm, and it was fixed in a stainless steel mesh frame (Severstallat JSC, Latvia). The cathode chamber was filled with 0.7 L of cold tap water. Platinum-coated stainless steel wire mesh, 13 × 6 cm, was used as a cathode. After filling, an MBFC anode chamber was flushed with argon gas for 10 minutes (2 L/min).

After providing an anaerobic environment, the open circuit potential was measured and MBFC1 was connected to a supercapacitor system (Kamcap, 2.6 V 10 F) with a total capacity of 30 farads (F) and a portative pH-meter PH-208 (Lutron, USA). Supercapacitor system consisted of three parallel-connected 10 F supercapacitors. A 500 kΩ external resistance and a portative pH-meter were connected between the MBFC2 outputs and the MBFC2 was operating at this low load. The third MBFC (MBFC3) was load-free, without external consumer. Voltage readings were taken with a multimeter DPM (Solid Polska, Poland) every day. Super capacitor charging was monitored in time with Arduino based data acquisition system. When the MBFC1 supercapacitor system voltage approached the open circuit voltage, the supercapacitor system was discharged and reconnected. MBFCs operated at a room temperature of 20 ± 2 °C.

Samples for chemical and microbiological analyses were taken at the beginning and after 14 and 42 days since the beginning of the experiment. Liquid (anolyte) was mixed to obtain homogeneous structure. 25 mL and 50 mL of liquid was removed from each MBFC with

a sterile spoon and placed in 50 mL polypropylene tubes for determination of BOD. One mL of liquid was taken for the determination of COD and one mL for the determination of bacterial colony-forming units (CFU). Measurements of biochemical oxygen demand (BOD_5) were made, using OxiDirect (Lovibond, Germany). Measurements of chemical oxygen demand (COD) were conducted using Lovibond thermoreactor RD125 and COD VARIO photometer MD200.

Biofilm was removed from the anodes by scraping of one cm^2 surface area of each anode under sterile conditions. Biofilm was suspended in one mL of sterile water in 1.5 mL tube for the determination of bacterial CFU. For microscopy, the piece of each anode in size of 0.5 × 0.5 cm was cut and placed in sealed 1.5 mL tubes. After sampling, MBFC was hermetically closed and argon gas was blown with a flow rate 2 L/min for 10 min.

The amount of culturable bacteria was analysed in the samples of liquid and anode biofilms. Serial dilutions of each suspension were plated in duplicate in the medium R2A (Bio-Rad, France). Equal amount of plates was incubated aerobically and anaerobically at 21°C for 3–10 days. Anaerobic growth was provided in GasPak™ EZ Anaerobe Pouch System (BD, USA). The number of CFU was estimated and expressed as CFU per mL of liquid or per cm^2 of anode surface. Based on colony and cell morphology, predominant bacterial species were isolated from the highest dilutions of samples. These bacteria were purified and identified with BBL® Crystal™ ID kits (BD, USA). Viability of bacteria was determined with LIVE/DEAD BacLight Bacterial Viability kit L7007 (Molecular Probes, USA).

Performance indicators of MBFCs. Electrical performance of MBFCs was confirmed by monitoring of open circuit voltage and supercapacitor charging. Results of measurements showed that lake sediment used as inoculum and feed for the fuel cells had an initial COD and BOD_5 value of 51720 mg/L and 223 mg/L, respectively. The ratio of BOD_5/COD, which characterizes the biological stability of substrate, was low, i. e. 0.43% which points to the fact that the sediment contains many difficult to break down or non-biodegradable organic compounds, or toxic substances. High COD and low BOD/COD-ratio is a common feature of landfill leachate [94] and indicates that there is a low concentration of volatile fatty acids and relatively higher amount of recalcitrant humic substances [95]. During operation of MBFCs, BOD_5 decreased by 28.3–42.2% (Fig. 4.14 A). COD decreased by 539 mg/L, 171 mg/L and 34 mg/L per day in MBFC1, MBFC2 and MBFC3, respectively, or by 43.8% (MBFC1), 13.9% (MBFC2) and 2.8% (MBFC3) after 42 days (Fig. 4.14 B). COD removal showed that MBFC should operate under maximally high load conditions for optimal performance in bioremediation. MBFCs open circuit voltage measurements showed that 24-hours adaptation period is needed for natural sediment based MBFC to gain highest voltage. After this period, voltage stayed stable at about 0.57 V. Supercapacitor charging results showed that under high load conditions MBFC adapts and works better with each next cycle. However, results from 4th week showed worse but it can be explained with detected air-tightness problems of particular MBFC.

Characteristics of microbial communities. The amount of culturable microorganisms in the anolyte decreased from 6.7 log CFU/mL for aerobic growing bacteria and 7.9 log CFU/mL for anaerobic growing bacteria in the beginning of the experiment to 5.1 log CFU/mL (MBFC1) for aerobic and anaerobic bacteria after six weeks. The biggest decrease was detected in MBFC1 while the bacterial concentration on the anode surface increased in all MBFCs and reached 4.8–5.6 log CFU/cm^2 for aerobic growing bacteria and 4.3–4.9 log CFU/cm^2 for anaerobic growing bacteria after six weeks without significant difference between MBFCs.

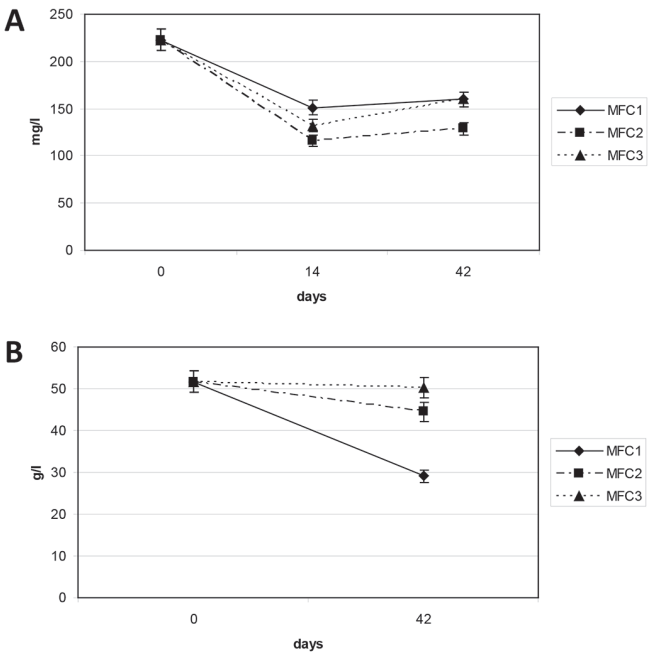

Figure 4.14. **Changes of BOD_5 (A) in mg/L and COD (B) in g/L during operation of MBFCs**

From 15 isolated predominant species of culturable bacteria, seven belonged to phylum *Firmicutes* (*Bacillus, Clostridium* species), five belonged to *Bacteroidetes* (*Bacteroides, Empedobacter, Prevotella* species), and one species belonged to *Actinobacteria (Leifsonia aquatica)*, *Fusobacteria (Fusobacterium necrophorum)* and *Proteobacteria (Sphingomonas paucimobilis*) (Table 4.5). *Bacteroidetes (Bacteroides eggerthii, B. uniformis, Prevotella buccae*), *Firmicutes (Clostridium clostridioforme, C. perfringens)*, as well as *Actinobacteria (Leifsonia aquatica)* were isolated directly from the anode biofilms. *Clostridium* spp. did not appear between the predominant at the beginning (day 0) but were enriched in MBFCs. Electrochemical activity of *Clostridium butyricum* has been recognized [96]. Taxonomic analysis provided by Kiseleva et al. [97] also showed that three bacterial phyla, *Proteobacteria, Firmicutes* and *Bacteroidetes* constitute the core of microbiomes in MBFCs in distinct climatic zones. Study provided by Read et al. [92] indicated a synergistic or mutualistic relationship between Gram-positive bacterium *Enterococcus faecium* and Gram-negative bacteria in current generation.

After 42 days, biofilms were formed around anodes of all three MBFCs. Graphite paper of MBFC1 had the most durable and best immobilized biofilm. This can be explained by the positive effect of the increased load on bacterial metabolism due to the availability of a solid final electron acceptor, i.e. anode. The MBFC2 electrode biofilm was thinner than the biofilm of MBFC1 electrode. The MBFC3 biofilm was the weakest and it was easily rinsed with water. This indicates that bacteria did not have favourable growth conditions on the surface of the electrode, if the MBFC was not connected to an electricity consumer. Bacteria have different respiration strategies in environments containing multiple electron acceptors [98]. It is possible that there was a lack of suitable chemical electron acceptors on the anaerobic anode of MBFC3.

Table 4.5. **Predominant culturable bacteria isolated from anolyte and anode biofilms and identified biochemically with BBL Crystal ID kits**

Day	Substratum	Aerobic/ anaerobic cultivation	Species		
			Phylum	Name	Confidence
0	Anolyte	Aerobic	*Firmicutes*	*Bacillus pumilus*	0.9500
			Bacteroidetes	*Empedobacter brevis*	0.7121
			Proteobacteria	*Sphingomonas paucimobilis*	0.9999
		Anaerobic	*Firmicutes*	*Bacillus mycoides*	0.9500
			Bacteroidetes	*Bacteroides uniformis*	0.9170
42	Anolyte	Aerobic	*Firmicutes*	*Bacillus subtilis*	0.9930
		Anaerobic	*Firmicutes*	*Clostridium butyricum*	0.9246
			Firmicutes	*Clostridium clostridioforme*	0.9940
			Fusobacteria	*Fusobacterium necrophorum*	0.7878
	Anodes	Aerobic	*Actinobacteria*	*Leifsonia aquatica*	0.8976
		Anaerobic	*Bacteroidetes*	*Bacteroides eggerthii*	0.8048
			Bacteroidetes	*Bacteroides uniformis*	0.6845
			Firmicutes	*Clostridium clostridioforme*	0.9927
			Firmicutes	*Clostridium perfringens*	0.8704
			Bacteroidetes	*Prevotella buccae*	0.8771

Fluorescent staining results showed that after 42 days most of all live bacteria are visible in MBFC1 anodic biofilm which operated under high load conditions. Lake sediment COD removal also indicated the highest biological activity in MBFC1 in comparison with MBFC2 and MBFC3 (Fig. 4.14 B). It means that a 500 kΩ external resistance was too small to provide biofilm bacteria with sufficient electron removal. Without the load, MBFC3 acted as a storage vessel and bacterial viability on the surface of the electrode should have been unaffected. However, the possible lack of a solid electron acceptor and/or the difficulty in getting nutrients in the depth of biofilm led to cell death. It coincides with results obtained by Ieropoulos et al. (2005) that MBFC biofilms can store electrons when the device was left in open circuit for an extended period of time [99]. Also, Wrighton et al. [100] revealed a greater percentage of viable cells and a higher cell density associated with electrode surface. Right bacterial cytochromes are those that act as electron sinks [100]. No differences were observed between investigated MBFCs in viability of planktonic cells (in the liquid).

Conclusions. Dual-chamber MBFCs were operated under different external load conditions with high COD and low BOD/COD-ratio lake sediment as a substrate and inoculum. Fluorescent staining and count of CFU were used for evaluation of anodic biofilm vitality. 24-hours' adaptation period was needed to gain the highest voltage. COD removal showed that MBFC should operate under maximally high load conditions for optimal performance in bioremediation. Number of culturable bacteria on the anode surface increased in all MBFCs and after six weeks reached 4.8-5.6 log CFU/cm^2 without significant difference between MBFCs. Predominant species of culturable bacteria

belonging to phyla *Bacteroidetes*, *Firmicutes* and *Actinobacteria* were isolated directly from the anode biofilms. No differences were observed between MBFCs in viability of bacterial cells in anolyte but there was better viability in anode biofilms in case of high load conditions than without external resistance. COD removal also indicated the highest biological activity under high load conditions. This can be explained by the availability of anode as additional electron acceptor.

4.2.4. Benthic MBFC

Sediment or benthic MBFC is a special type of MBFC that that can be deployed in a natural water body for energy production [102]. It means that anode is embedded, for example in a pond, lake or marine sediment but the cathode is placed in the upper layer of water to get access to atmospheric oxygen. Power generation results from oxidation of sedimentary organic carbon catalysed by microorganisms [103].

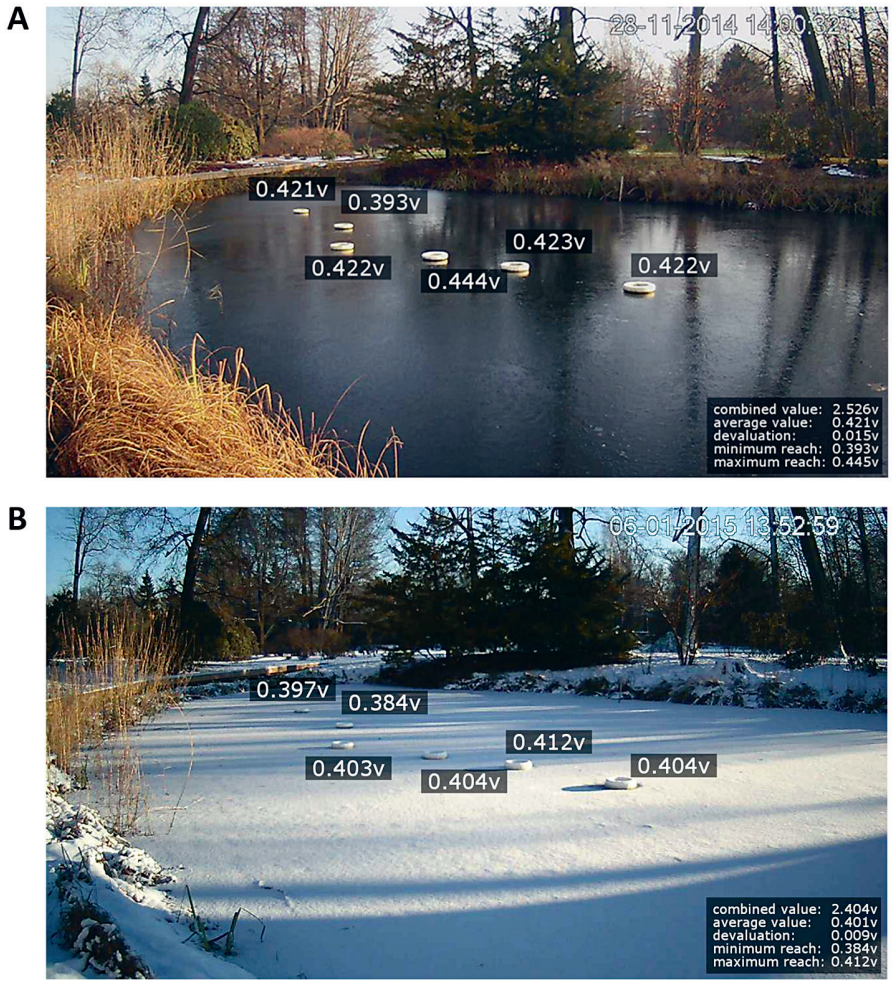

Figure 4.15. **"Pond Battery" installation in the Botanical Garden of the University of Latvia: A – screen-shot from November 11, 2014; B – screen-shot from January 6, 2015**

Sediment MBFCs are particularly promising for the bioremediation purposes. Our team, Artūrs Gruduls et al. in cooperation with artists Rasa Šmite and Raitis Šmits (RIXC) has developed a "Pond Battery" installation in the Botanical Garden of the University of Latvia in 2014/2015 (Figure 4.15). This battery consisted of six MBFCs and was installed in August 2014 and operated up to April, 2015, both in the summer and in the winter seasons. It has been found that benthic MBFCs have been operated for several years with no remarkable decrease in power output [104].

4.3. Summary

Researchers of the Faculty of Biology in close cooperation with Institute of Solid State Physics of University of Latvia (Laboratory of Materials for Energy Harvesting and Storage) studied the microbiological production of hydrogen (H_2) in dark fermentation reactions and storage possibilities in hydride-forming materials, constructed and developed laboratory-scale bioreactors, and researched possibilities to generate electrical energy with microbial fuel cells (MBFC) or bacterial batteries.

The studies show that hydride-forming materials – Pd, AB_5, AB_2 – can be successfully exploited for H2 collection from the liquid phase in crude glycerol fermentation by *E. coli* and *Klebsiella aerogenes* (previously known as *Enterobacter aerogenes*) MSCL 758. The inhibitory effect of $LaNi_5$ was established for the growth of bacteria or inhibition of metabolic pathway, in which H_2 and CO_2 is produced. Conversely, Pd and multicomponent alloys AB_2 and AB_5 showed positive effect on H_2 and CO_2 production.

Dual-chamber MBFCs were operated under different external load conditions with high COD and low BOD/COD-ratio lake sediment as a substrate and inoculum. Fluorescent staining and count of CFU were used for evaluation of anodic biofilm vitality. 24-hours adaptation period was needed to gain the highest voltage. COD removal showed that MBFC should operate under maximally high load conditions for optimal performance in bioremediation. Number of culturable bacteria on the anode surface increased in all MBFCs and after six weeks reached 4.8–5.6 log CFU/cm^2 without significant difference between MBFCs. Predominant species of culturable bacteria belonging to phyla *Bacteroidetes*, *Firmicutes* and *Actinobacteria* were isolated directly from the anode biofilms. No differences were observed between MBFCs in viability of bacterial cells in anolyte but there was better viability in anode biofilms in case of high load conditions than without external resistance. COD removal also indicated the highest biological activity under high load conditions. This can be explained by the availability of anode as additional electron acceptor.

It has been found that benthic MBFCs have been operated for several years with no remarkable decrease in power output.

4.4. References

1. Hydrogen Tech Roadmap (2015). Available at https://www1.eere.energy.gov/hydrogenandfuelcells/pdfs/h2_tech_roadmap.pdf (accessed on December 4, 2018).
2. Dimanta, I. (2016) Biohydrogen Production from Crude Glycerol, Lactose and Hydrogen Storage Possibilities with Metalhydrides from Fermentation Broth. PhD thesis. Available at https://dspace.lu.lv/dspace/handle/7/31856 (accessed on December 4, 2018).
3. Valucka, S. (2015) Ūdeņraža mikrobioloģiska producēšana tumsas fermentācijā un uzkrāšana metālhidrīdos (Microbial hydrogen production by dark fermentation and its storage in metal

hydrides). MSc thesis. Available at https://dspace.lu.lv/dspace/handle/7/29440 (accessed on December 4, 2018).
4. Kleinmane, Z. (2016) Dažādu oglekļa bāzētu materiālu elektrodu pielietojamība mikroorganismu degvielas šūnās (Application of different carbon-based electrodes in microbial fuel cells). Bch thesis. Available at https://dspace.lu.lv/dspace/handle/7/32186 (accessed on December 4, 2018).
5. Paiders, M. (2017) Ūdeņraža mikrobioloģiska producēšana, izmantojot *Enterobacter aerogenes*, un uzkrāšana AB_5 tipa sakausējumā (Microbial hydrogen production using *Enterobacter aerogenes* and its storage in AB_5 type alloy). Bacherlor's thesis. Available at https://dspace.lu.lv/dspace/handle/7/35181 (accessed on December 4, 2018).
6. Klepere, I., Muiznieks, I., Kleperis, J. (2010). A bacterial hydrogen production test system for measuring H2 concentrations in liquids and gases. *Latvian Journal of Physics and Technical Sciences,* 2, 60–68.
7. Dimanta, I., Nikolajeva, V., Gruduls, A., Muižnieks, I. (2013). Assessment of bio-hydrogen production from glycerol and glucose by fermentative bacteria. *Energetika*, 59, 124–128.
8. Nikolajeva, V., Neibergs, M., Valucka, S., Dimanta, I., Kleperis, J. (2015) Application of pretreatment, bioaugmentation and biostimulation for fermentative hydrogen production from maize silage. *The Open Biotechnology Journal*, 9, 39–48.
9. Dimanta, I., Kleperis, J., Nakurte, I., Valucka, S., Nikolajeva, V., Rutkovska, Z., Muiznieks, I. (2016) Metal hydride alloys for storing hydrogen produced by anaerobic bacterial fermentation. *International Journal of Hydrogen Energy*, 41, 9394–9401.
10. Kleinmane, Z., Gruduls, A., Nikolajeva, V., Kleperis, J. (2016) Anodic biofilm vitality during operation of microbial fuel cells at various load conditions. *Journal of Biotechnology*, 231, S105–S106.
11. Paiders, M., Gruduls, A., Kalnina, L., Valucka, S., Kleperis, J., Dimanta, I., Nikolajeva, V. (2017) Biogas and hydrogen production from glycerol by *Enterobacter aerogenes* and anaerobic microbial communities. *Agronomy Research*, 15, 256–269.
12. Wiberg, E., Wiberg, N., Holleman, A.F. (2001) Inorganic Chemistry. San Diego, CA: Academic Press, 1884 pp.
13. Goswami, D. Y., Kreith, F. (2007). Handbook of Energy Efficiency and Renewable Energy. CRC Press.
14. Suleman, F., Dincer, I., Agelin-Chaab, M. (2016) Comparative impact assessment study of various hydrogen production methods in terms of emissions. *International Journal of Hydrogen Energy*, 41, 8364–8375.
15. Chandrasekhar, K., Lee, Y. J., Lee, D. W. (2015) Biohydrogen production: Strategies to improve process efficiency through microbial routes. *International Journal of Molecular Sciences*, 16, 8266–8293.
16. Abánades, A. (2012) The challenge of hydrogen production for the transition to a CO_2-free economy. *Agronomy Research*, 10, 11–16.
17. Dincer, I., Acar, C. (2015). Review and evaluation of hydrogen production methods for better sustainability. *International Journal of Hydrogen Energy*, 40, 11094–11111.
18. Das, D., Khanna N., Dasgupta, C. (2008) Biohydrogen Production: Fundamentals and Technology Advances. CRC Press, 408 pp., Boca Raton, Florida.
19. Madigan, M. T., Martinko, J. M. (2006). Brock Biology of Microorganisms. 11th ed. Pearson Prentice Hall (USA), 118–119.
20. Thauer, R. K., Kaster, A. K., Goenrich, M., Schick, M., Hiromoto, T., Shima, S. (2010) Hydrogenases from methanogenic archaea, nickel, a novel cofactor, and H2 storage. *Annual Review of Biochemistry*, 79, 507–536.
21. Das, D., Veziroglu, T. (2008) Advances in biological hydrogen processes. *International Journal of Hydrogen Energy*, 33, 6046–6057.
22. Ghimire, A., Frunzo, L., Pirozzi, P., Trably, E., Escudie, R., Lens, P. N. L., Esposito, G. (2015) A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. *Applied Energy*, 144, 73–95.

23. Manish, S., Banerjee, R. (2008) Comparison of biohydrogen production processes. *International Journal of Hydrogen Energy*, 33, 279–286.
24. Das, D., Veziroglu, T. N. (2001) Hydrogen production by biological processes: a survey of literature. *International Journal of Hydrogen Energy*, 26, 13–28.
25. Hallenbeck, P. C., Benemann, J. R. (2002) Biological hydrogen production: fundamentals and limiting processes. *International Journal of Hydrogen Energy*, 27, 1185–1193.
26. Levin, D. B., Pitt, L., Love, M. (2004) Biohydrogen production: prospects and limitations to practical application. *International Journal of Hydrogen Energy*, 29, 173–185.
27. Maeda, T., Sanchez-Torres, V., Wood, T. K. (2008) Enhanced hydrogen production from glucose by metabolically engineered *Escherichia coli*. *Applied Microbiology and Biotechnology*, 77, 879–890.
28. Hallenbeck, P. C. (2009) Fermentative hydrogen production: Principles, progress, and prognosis. *International Journal of Hydrogen Energy*, 34, 7379–7389.
29. Rogner, M., Igarashi, Y., Asada, Y., Miyake, J. (2002) Biohydrogen III: Renewable Energy System by Biological Solar Energy Conversion. Elsevier Science. 1 ed. AIST/METI, 187 pp.
30. Vignais, P. M., Colbeau, A. (2004) Molecular biology of microbial hydrogenases. *Current Issues in Molecular Biology*, 6, 159–188.
31. Nath, K., Muthukumar, M., Kumar, A., Debabrata, D. (2008) Kinetics of two-stage fermentation process for the production of hydrogen. *International Journal of Hydrogen Energy*, 33, 1195–1203.
32. Nath, K., Das, D. (2004) Improvement of fermentative hydrogen production: various approaches. *Applied Microbiology and Biotechnology*, 65, 520–529.
33. Hallenbeck, P. C. (2012) Hydrogen production by cyanobacteria. Microbial Technologies in Advanced Biofuels Production. Springer: New York, NY, USA, 15–28.
34. Boichenko, V. A., Greenbaum, E., Seibert, M. (2004) Hydrogen production by photosynthetic microorganisms. In: Molecular to Global Photosynthesis. Imperial College Press: London, UK, 397–451.
35. Pachapur, V. L., Sarma, S. J., Brar, S. K., Bihan, Y. E., Buelna, G., Verma, M. (2015) Biological hydrogen production using co-culture versus mono-culture system. *Environmental Technology Reviews*, 4, 55–70.
36. Mohan V. S., Chandrasekhar, K., Chiranjeevi, P., Babu, P. S. (2013) Biohydrogen production from wastewater. In: Biohydrogen. Pandey, A., Chang, J.-S., Hallenbeck, P. C., Larroche, C. (eds.), Elsevier: Amsterdam, The Netherlands, 223–257.
37. Angenent, L. T., Karim, K., Al-Dahhan, M. H., Domiguez-Espinosa, R. (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends in Biotechnology*, 22, 477–485.
38. Wang, J., Wan, W. (2009) Factors influencing fermentative hydrogen production: a review. *International Journal of Hydrogen Energy*, 34, 799–811.
39. Zhang, Y.-H. P. (2010) Renewable carbohydrates are a potential high-density hydrogen carrier. *International Journal of Hydrogen Energy*, 35, 10334–10342.
40. Kapdan, I. K., Kargi, F. (2006) Bio-hydrogen production from waste materials. *Enzyme and Microbial Technology*, 38, 569–582.
41. Hawkes, F. R., Dinsdale, R., Hawkes, D. L, Hussy, I. (2002) Sustainable fermentative hydrogen production: challenges for process optimisation. *International Journal of Hydrogen Energy*, 27, 1339–1347.
42. Raghunandan, K., McHunu, S., Kumar, A., Kumar, K. S., Govender, A., Permaul, K., Singh, S. (2014) Biodegradation of glycerol using bacterial isolates from soil under aerobic conditions. *Journal of Environmental Science and Health, Part A, Toxic/Hazardous Substances & Environmental Engineering*, 49, 85–92.
43. Sarma, S. J., Brar, S. K., Sydney, E. B., Bihan, Y. L., Buelna, G., Soccol, C. R. (2012) Microbial hydrogen production by bioconversion of crude glycerol: a review. *International Journal of Hydrogen Energy*, 37, 6473–6490.

44. Adhikari, S., Fernando, S. D., Haryanto, A. (2009) Kinetics and reactor modelling of hydrogen production from glycerol via steam reforming process over Ni/Ceo2 catalysts. *Chemical Engineering & Technology*, 32, 541–547.
45. Mane, R. B., Rode, C. V. (2013) Catalytic recycling of glycerol formed in biodiesel production. In: Grohens, Y., Kumar, S.K., Boudenne, A., Weimin Y. (eds.), Recycling and reuse of materials and their products, Advances in materials science. Oakville, Apple Academic Press, 9–24.
46. Hu, S., Luo, X., Wan, C., Li, Y. (2012) Characterization of crude glycerol from biodiesel plants. *Journal of Agricultural and Food Chemistry*, 60, 5915–5921.
47. Hajek, M., Skopal, F. (2010) Treatment of glycerol phase formed by biodiesel production. *Bioresource Technology*, 101, 3242–3245.
48. Manosak, R., Limpattayanate, S., Hunsom, M. (2011) Sequential-refining of crude glycerol derived from waste used-oil methyl ester plant via a combined process of chemical and adsorption. *Fuel Processing Technology*, 92, 92–99.
49. Reungsang, A., Sittijunda, S., Angelidaki, I. (2013) Simultaneous production of hydrogen and ethanol from waste glycerol by *Enterobacter aerogenes* KKU-S1. *International Journal of Hydrogen Energy*, 38, 1813–1825.
50. Mangayil, R., Aho, T., Karp, M., Santala, V. (2012) Bioconversion of crude glycerol from biodiesel production to hydrogen. *International Journal of Hydrogen Energy*, 37, 12198–12204.
51. Ito, T., Nakashimada, Y., Senba, K., Matsui, T., Nishio, N. (2005) Hydrogen and ethanol production from glycerol-containing wastes discharged after biodiesel manufacturing process. *Journal of Bioscience and Bioengineering*, 100, 260–265.
52. Trchounian, K., Sanchez-Torres, V., Wood, T. K., Trchounian, A. (2011) *Escherichia coli* hydrogenase activity and H_2 production under glycerol fermentation at a low pH. *International Journal of Hydrogen Energy*, 36, 4323–433.
53. Guillaume, S. P., Patrick, C. H. (2009) High yield conversion of a crude glycerol fraction from biodiesel production to hydrogen by photofermentation. *Bioresource Technology*, 100, 3513–3517.
54. Jitrwung, R., Yargeau, V. (2015) Biohydrogen and bioethanol production from biodiesel-based glycerol by *Enterobacter aerogenes* in a continuous stir tank reactor. *International Journal of Molecular Sciences*, 16, 10650–10664.
55. Dharmadi, Y., Murarka, A., Gonzalez, R. (2006) Anaerobic fermentation of glycerol by *Escherichia coli*: a new platform for metabolic engineering. *Biotechnology and Bioengineering*, 94, 821–829.
56. Bruna, S. F., Guilherme, P., Fernanda, R. A., Nora, K., Saavedra, A., Marcelo, Z. (2010) Potential to produce biohydrogen from various wastewaters. *Energy for Sustainable Development*, 14, 143–148.
57. Elsharnouby, O., Hafez, H., Nakhla, G., El Naggar M. H. (2013) A critical literature review on biohydrogen production by pure cultures. *International Journal of Hydrogen Energy*, 38, 4945–4966.
58. Kotay, S. M., Das, D. (2008) Biohydrogen as a renewable energy resource – prospects and potentials. *International Journal of Hydrogen Energy*, 76, 258–263.
59. Zuttel, A. (2004) Hydrogen storage methods. *Naturwissenschaften*, 91, 152–157.
60. Sakintuna, B., Lamari-Darkrim, F., Hirscher, M. (2007) Metal hydride materials for solid hydrogen storage: a review. *International Journal of Hydrogen Energy*, 32, 1121–1140.
61. Fraunhofer Institute for Manufacturing Technology and Advanced Materials IFAM, Department Hydrogen Technology, Dresden, Germany (2018) Available at https://www.ifam.fraunhofer.de/en/Profile/Locations/Dresden/HydrogenTechnology/hydrides.html (accessed on December 22, 2018).
62. Pray, H. A., Schweickert, C. E., Minnic, B. H. (1952) Solubility of hydrogen, oxygen, nitrogen, and helium in water at elevated temperatures. *Industrial and Engineering Chemistry*, 44, 1146–1151.

63. Kleperis, J., Grīnberga, L. (2008) Laboratorijas darba materiāli (Materials of Laboratory Work), Rīga, LU CFI.
64. Sandrock, G. D., Goodell, P. D. (1980) Surface poisoning of $LaNi_5$, FeTi and (Fe, Mn) Ti by O_2, CO and H_2O. *Journal of the Less Common Metals*, 73, 161–168.
65. Kleperis, J., Wójcik, G., Czerwinski, A., Skowronski, J., Kopczyk, M., Beltowska-Brzezinska, M. (2001) Electrochemical behavior of metal hydrides. *Journal of Solid State Electrochemistry*, 5, 229–224.
66. Nishimiya, N., Kojima, Y., Toyama, T. (2014) Encapsulation of Hydrogen Storage Alloys by Softened Sol-Gel Method and Recovery of Hydrogen from Gas Mixtures, IDHE meeting, Nantes, France.
67. Macomber, L., Hausinger, R. P. (2011) Mechanisms of nickel toxicity in microorganisms. *Metallomics*, 3, 1153–1162.
68. Wongthanate, J., Polprasert, C. (2015) Immobilized biofilm in thermophilic biohydrogen production using synthetic versus biological materials. *Brazilian Archives of Biology and Technology*, 58, 124–130.
69. Broom, D. P. (2011) Hydrogen Storage Materials: The Characterization of Their Storage Properties, Springer, 260 pp.
70. Chen, P., Zhu, M. (2008) Recent progress in hydrogen storage. *Materials Today*, 11, 36–43.
71. Wiecinska, P. (2016) Thermal degradation of organic additives used in colloidal shaping of ceramics investigated by the coupled DTA/TG/MS analysis. *Journal of Thermal Analysis and Calorimetry*, 123, 1419–1430.
72. Cheng, S., Logan, B. E. (2007) Sustainable and efficient biohydrogen production via electrohydrogenesis. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 18871–18873.
73. Babu, M. L., Subhash, G. V., Sarma, P. N., Mohan, S. V. (2013) Bio-electrolytic conversion of acidogenic effluents to biohydrogen: An integration strategy for higher substrate conversion and product recovery. *Bioresource Technology*, 133, 322–331.
74. 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.
75. Deval, A., Dikshit, A. K. (2013) Construction, Working and Standardization of Microbial Fuel Cell. *APCBEE Procedia*, 5, 59–63.
76. Logan, B. E. (2008) Microbial Fuel Cells. Hoboken, NJ: John Wiley & Sons, 213 pp.
77. Liu, H., Cheng, S., Logan, B. E. (2005) Production of electricity from acetate or butyrate using a single-chamber microbial fuel cell. *Environmental Science & Technology*, 39, 658–662.
78. Rabaey, K., Boon, N., Siciliano, S. D., Verhaege, M., Verstraete, W. (2004) Biofuel cells select for microbial consortia that self-mediate electron transfer. *Applied and Environmental Microbiology*, 70, 5373–5382
79. Reguera, G., McCarthy, K. D., Mehta, T., Nicoll, J. S., Tuominen, M. T., Lovley, D. R. (2005) Extracellular electron transfer via microbial nanowires. *Nature*, 435, 1098–1101.
80. Picioreanu, C., van Loosdrecht, M. C., Katuri, K. P., Scott, K., Head, I. M. (2008) Mathematical model for microbial fuel cells with anodic biofilms and anaerobic digestion. *Water Science & Technology*, 57, 965–971.
81. Mohan, Y., Kumar, S. M. M., Das, D. (2008) Electricity generation using microbial fuel cells. *International Journal of Hydrogen Energy*, 33, 423–426.
82. Min, B., Cheng, S., Logan, B. E. (2005) Electricity generation using membrane and salt bridge microbial fuel cells. *Water Research*, 39, 1675–1686.
83. El Mekawy, A., Hegab, H. M., Losic, D., Saint, C. P., Pant, D. (2017) Applications of graphene in microbial fuel cells: the gap between promise and reality. *Renewable and Sustainable Energy Reviews*, 72, 1389–1403.
84. Gomes, A. S., La Rotta, C. E., Nitschke, M., González, E. R. (2011) Evaluation of current output in *Pseudomonas aeruginosa* microbial fuel cells using glycerol as substrate and Nafion 117 as proton exchange membrane. *ECS Transactions*, 41, 2011–2017.

85. Hou, J., Liu, Z., Li, Y., Yang, S., Zhou, Y. (2015) A comparative study of graphene-coated stainless steel fiber felt and carbon cloth as anodes in MBFCs. *Bioprocess & Biosystems Engineering*, 38, 881–888.
86. Zhang, Y., Mo, G., Li, X., Zhang, W., Zhang, J., Ye, J., Huang, X., Yu, C. (2011) A graphene modified anode to improve the performance of microbial fuel cells. *Journal of Power Sources*, 196), 5402–5407.
87. Pant, D., Van Bogaert, G., Diels, L., Vanbroekhoven, K. (2009) A review of the substrates used in microbial fuel cells (MBFCs) for sustainable energy production. *Bioresource Technology*, 101, 1533–1543.
88. Kumar, R., Singh, L., Zularisam, A. W. (2016) Exoelectrogens: recent advances in molecular drivers involved in extracellular electron transfer and strategies used to improve it for microbial fuel cell applications. *Renewable and Sustainable Energy Reviews*, 56, 1322–1336.
89. Schechter, M., Schechter, A., Rozenfeld, S., Efrat, E., Cahan, R. (2014) Anode Biofilm. In: Wang, C.T. (Ed.), Technology and Application of Microbial Fuel Cells. InTech: 57–74.
90. Reguera, G., Nevin, K. P., Nicoll, J. S., Covalla, S. F., Woodard, T. L., Lovley, D. R. (2006) Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Applied and Environmental Microbiology*, 72, 7345–7348.
91. Malvankar, N. S., Lau, J., Nevin, K. P., Franks, A. E., Tuominen, M. T., Lovley, D. R. (2012) Electrical conductivity in a mixed-species biofilm. *Applied and Environmental Microbiology*, 78, 5967–5971.
92. Read, S. T., Dutta, P., Bond, P. L., Keller, J., Rabaey, K. (2010) Initial development and structure of biofilms on microbial fuel cell anodes. *BMC Microbiology*, 10, 98.
93. Zhang, L., Li, J., Zhu, X., Ye, D., Fu, Q., Liao, Q. (2017) Startup performance and anodic biofilm distribution in continuous-flow microbial fuel cells with serpentine flow fields: effects of external resistance. *Industrial & Engineering Chemistry Research*, 56, 3767–3774.
94. Greenman, J., Gálvez, A., Giusti, L., Ieropoulos, L. (2009) Electricity from landfill leachate using microbial fuel cells: comparison with a biological aerated filter. *Enzyme and Microbial Technology*, 44, 112–119.
95. Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Braun, A., Ledin, A., Christensen, T. H. (2002) Present and long-term composition of MSW landfill leachate: a review. *Critical Reviews in Environmental Science and Technology*, 32, 297–336.
96. Park, H. S., Kim, B. H., Kim, H. S., Kim, H. J., Kim, G. T., Kim, M., Chang, I. S., Park, Y. K., Chang, H.I. (2001) A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to *Clostridium butyricum* isolated from a microbial fuel cell. *Anaerobe*, 7, 297–306.
97. Kiseleva, L., Garushyants, S. K., Ma, H., Simpson, D. J., Fedorovich, V., Cohen, M. F., Goryanin, I. (2015) Taxonomic and functional metagenomic analysis of anodic communities in two pilot-scale microbial fuel cells treating different industrial wastewaters. *Journal of Integrative Bioinformatics*, 12, 273.
98. Yang, Y., Xiang, Y., Sun, G., Wu, W.-M., Xu, M. (2015) Electron acceptor-dependent respiratory and physiological stratifications in biofilms. *Environmental Science & Technology*, 49, 196–202.
99. Ieropoulos, I., Greenman, J., Melhuish, C., Hart, J. (2005) Energy accumulation and improved performance in microbial fuel cells. *Journal of Power Sources*, 145, 253–256.
100. Wrighton, K. C., Thrash, J. C., Melnyk, R. A., Bigi, J. P., Byrne-Bailey, K. G., Remis, J. P., Schichnes, D., Auer, M., Chang, C. J., Coates, J. D. (2011) Evidence for direct electron transfer by a Gram-positive bacterium isolated from a microbial fuel cell. *Applied and Environmental Microbiology*, 77, 7633–7639.
101. Esteve-Núñez, A., Sosnik, J., Visconti, P., Lovley, D. R. (2008) Fluorescent properties of c-type cytochromes reveal their potential role as an extracytoplasmic electron sink in *Geobacter sulfurreducens*. *Environmental Microbiology*, 10, 497–505.
102. Hong, S. W., Kim, H. J., Choi, Y. C., Chung, T. H. (2008) Field experiments on bioelectricity production from lake sediment using microbial fuel cell technology. *Bulletin of the Korean Chemical Society*, 29, 2189–2194.

103. Tender, L. M., Reimers, C.E., Stecher III, H. A., Holmes, D. E., Bond, D. R., Lowy, D. A., Pilobello, K., Fertig, S. J., Lovley, D. R. (2002) Harnessing microbially generated power on the seafloor. *Nature Biotechnology*, 20, 821–825.
104. Tender, L. M., Gray, S. A., Groveman, E., Lowy, D. A., Kauffman, P., Melhado, J., Tyce, R. C., Flynn, D., Petrecca, R., Dobarro, J. (2008) The first demonstration of a microbial fuel cell as a viable power supply: powering a meteorological buoy. *Journal of Power Sources*, 179, 571–575.

4.4. Kopsavilkums

Bioloģijas fakultātes pētnieki ciešā sadarbībā ar LU CFI *Dr. phys.* Jāni Kleperi un viņa grupu pētīja ūdeņraža (H_2) mikrobioloģisku producēšanu tumsas fermentācijas reakcijās un uzkrāšanas iespējas hidrīdus veidojošos materiālos, konstruēja un pilnveidoja laboratorijas mēroga bioreaktorus, kā arī pētīja elektriskās enerģijas ieguvi, strādājot ar mikroorganismu degvielas šūnām (MBFC) jeb baktēriju baterijām.

Pētījumi parādīja, ka hidrīdus veidojoši materiāli – pallādijs (Pd) un sakausējumi AB_2, AB_5 un $LaNi_5$ – ir izmantojami H_2 saistīšanai baktēriju *Escherichia coli* un *Klebsiella (Enterobacter) aerogenes* fermentācijas šķidrajā fāzē. Kultivēšanas laikā novērota barotnes sastāvdaļu un baktēriju adsorbcija uz materiālu daļiņām. $LaNi_5$ inhibējoši iedarbojās uz baktēriju augšanu un/vai metabolisma ceļiem, kuros veidojas H_2 un ogļskābā gāze (CO_2), turpretim jaukta sastāva AB_5 sakausējuma pulvera (La, Ce, Nd un Pr pozīcijā A un Co, Mn un Al pozīcijā B) pievienošana barotnei ievērojami palielināja H_2 un CO_2 daudzumu. Vakuuma ekstrakcija un masspektrometrijas analīze pierādīja H_2 uzkrāšanos AB_5 pulverī. Pēc termogravimetrijas datiem, hidrīdus veidojošie materiāli pēc to H_2 sorbcijas kapacitātes fermentācijas šķidrumā ierindojās šādā secībā (no lielākās uz mazāko): Pd, AB_5, AB_2 un $LaNi_5$.

Pētīta baktēriju tīrkultūru un asociāciju spēja izmantot dažādus oglekļa barības avotus, tai skaitā ražošanas blakusproduktus, piemēram, jēlglicerīnu, lai producētu ūdeņradi. Izsekota dominējošo kultivējamo aerobo un anaerobo mikroorganismu sugu un grupu un metabolītu dinamika dažādos fermentācijas procesa režīmos. Hidrīdus veidojošu materiālu pievienošana kultivēšanas barotnei netraucēja glicerīna izmantošanu. Vienlaikus ar glicerīna koncentrācijas samazināšanos palielinājās baktēriju producētā H_2 daudzums.

Tika izstrādāts divkameru MBFC prototips, izmantojot jauna oglekļa materiāla elektrodus un pētot to biosaderību un efektivitāti. Ir novērtēta grafīta, grafēna un grafēna-PLA kompozīta elektrodu pielietojamība. Par substrātu izmantotas gan mikrobioloģiskās barotnes, gan dabiskas ezeru dūņas, par mikroorganismu avotu – baktēriju tīrkultūras un dūņu mikroorganismu asociācijas. No pētītajiem materiāliem maksimālo jaudas blīvumu uzrādīja 3D printēta grafēna-PLA kompozīts, kam bija arī labāka biosaderība nekā grafītam. Dūņām bija nepieciešams 24 h adaptācijas periods. MBFC ilgtermiņa darbība bija vieglāk kontrolējama, izmantojot ezera dūņas kā mikroorganismu avotu, nevis baktēriju tīrkultūras. Asociāciju papildināšana ar eksoelektrogēno baktēriju *Shewanella* sp. tīrkultūru neietekmēja MBFC darbības rezultātus.

Tika pētīta bioplēves veidošanās un tās sastāvā esošo mikroorganismu dzīvotspēja divkameru MBFC, kas darbojās dažādos elektriskās slodzes apstākļos ar ezera dūņām kā mikroorganismu un barības avotu. Dūņas saturēja daudz organisko vielu, ko raksturo liels ķīmiskais skābekļa patēriņš jeb COD, bet tajās bija maza mikroorganismiem viegli izmantojamu substrātu koncentrācija (mazs bioķīmiskais skābekļa patēriņš jeb BOD). Organisko vielu noārdīšanās (COD samazināšanās) visātrāk noritēja MBFC, kas darbojās

vislielākās elektriskās slodzes režīmā. Uz visu MBFC anodiem izveidojās bioplēves. Visblīvākā un noturīgākā bioplēve bija MBFC ar vislielāko slodzi, bet vismazākā – MBFC, kura bija atvērtas elektriskās ķēdes režīmā (bez slodzes).

Dzīvo un bojāgājušo baktēriju fluorescentās iekrāsošanas un kolonijas veidojošo vienību (kvv) noteikšanas rezultāti ļāva novērtēt anoda bioplēves veidojušo baktēriju dzīvotspēju. Kultivējamo baktēriju daudzums līdzīgi palielinājās uz visu MBFC anodiem un pēc sešām nedēļām sasniedza 4.8–5.6 log kvv/cm^2. Anodu bioplēvēs dominējošās kultivējamās baktērijas piederēja pie *Bacteroidetes*, *Firmicutes* un *Actinobacteria* tipiem. Netika konstatētas nozīmīgas atšķirības starp baktēriju dzīvotspēju dažādu MBFC anolītos (anoda kamerās), bet anodu bioplēvēs vairāk dzīvo baktēriju bija lielas slodzes apstākļos nekā bez ārējas elektriskās slodzes. Arī COD samazināšanās norādīja uz lielāku bioloģisko aktivitāti lielas slodzes gadījumā. To var izskaidrot ar anoda kā papildu elektronu akceptora pieejamību. Rezultāti liecina, ka MBFC ir jādarbojas ar maksimālu slodzi, lai nodrošinātu optimālu bioremediāciju.

Nogulšņu jeb bentosa MBFC ir īpašs MBFC veids, ko var izmantot enerģijas ieguvei dabiskās ūdenstilpēs. Tas nozīmē, ka anods ir novietots, piemēram, dīķa, ezera vai jūras nogulumos, bet katodu novieto augšējā ūdens slānī, lai tam piekļūtu atmosfēras skābeklis. Elektroenerģija rodas no mikroorganismu katalizētās nogulsnēto organisko oglekļa savienojumu oksidēšanas. Mūsu grupa, Artūrs Gruduls un kolēģi sadarbībā ar māksliniekiem Rasu Šmiti un Raiti Šmitu (jauno mediju kultūras centrs RIXC) 2014. gadā izveidoja instalāciju "Dīķa baterija" LU Botāniskajā dārzā. Šī baterija sastāvēja no sešām MBFC un darbojās gan vasarā, gan ziemā. Ir noskaidrots, ka bentosa MBFC var darboties vairākus gadus bez jaudas samazināšanās.