World Journal of Chemical Education, 2022, Vol. 10, No. 4, 149-154
Available online at http://pubs.sciepub.com/wjce/10/4/4
Published by Science and Education Publishing
DOI:10.12691/wjce-10-4-4

Biological Fuel Cells with Drugstore Products

Rebecca Grandrath*, Claudia Bohrmann-Linde

Department of Chemistry Education, University of Wuppertal, Gaußstr. 20, 42119 Wuppertal, Germany
*Corresponding author: grandrath@uni-wuppertal.de

Received November 12, 2022; Revised December 19, 2022; Accepted December 27, 2022

Abstract The term fuel cell is used to describe an enormous variety of different systems. In addition to hydrogen, other fuels and various catalysts can be used, thus opening up a wide range of applications. If microorganisms or enzymes are used as catalysts, the systems are referred to as biological fuel cells [1]. So far, they are underrepresented at school [2]. To give an impulse to teachers, here we present simple hands-on experiments on biological fuel cells with baker´s yeast [3], lactic acid bacteria and lactase as biocatalysts for students in grades nine to twelve. As these three biocatalysts are drugstore products, the experimental set-ups are low-cost and harmless. Furthermore, the students may already be familiar with the metabolism of yeast or the field of application of lactic acid bacteria and lactase from their everyday lives, so that the working principle of the fuel cells can be easily understood during the experiment.

Keywords: *biological fuel cell, microbial fuel cell, enzymatic fuel cell, baker´s yeast, lactic acid bacteria, lactase, hands-on experiment, education for sustainable development, sustainability*

Cite This Article: Rebecca Grandrath, and Claudia Bohrmann-Linde, “Biological Fuel Cells with Drugstore Products.” *World Journal of Chemical Education*, vol. 10, no. 4 (2022): 149-154. doi: 10.12691/wjce-10-4-4.

1. Introduction

In the scope of the necessary energy turnaround fuel cells play an important role as “clean” energy converters. Therefore, the topic fuel cells is anchored in all of the German chemistry curricula for upper secondary schools. From the broad variety of possible fuel cell types only the hydrogen-oxygen system is specifically mentioned, giving only a very narrow insight into the technology [2]. Commercial kits exist for student experiments - unfortunately at considerable costs and often as readymade kits. Two central desiderata have emerged from these findings:

1. to address a greater variety of low-temperature fuel cell systems
2. to develop low-cost experimental set-ups for hands-on chemistry activities that allows for a stepwise construction of a fuel cell

Biological fuel cells were chosen because they have been underrepresented up to now, even though they have an enormous range of applications. The set-up of the experiments was designed to get by with only the most necessary functional components, facilitating the understanding of the underlying processes. Furthermore, the materials used should be inexpensive and the entire experiment should be safe, so that it is suitable as a students rather than a teacher demonstration experiment.

2. Biological Fuel Cells

The term biological fuel cell (or biofuel cell, BFC) describes a system, in which biocatalysts are used on at least one electrode [1]. BFC are operated at mild conditions, such as normal pressure and temperatures between 4-37°C. Their set-up is often realized as a two-chamber system (Figure 1). The fuel, referred to here as substrate, is fed to the anode side and oxidized. If an inorganic oxidant is used at the cathode side, it is also referred to as a substrate. For a continuous operation of the cell, the respective reactants have to be fed and the products removed continuously [4].

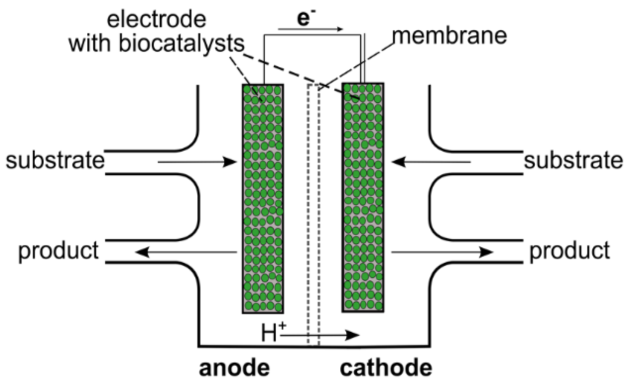

Figure 1. General set-up of a two-chamber biological fuel cell with immobilized biocatalysts.

Depending on the biocatalyst used, the biological fuel cells are classified as microbial fuel cells (MFC) or enzymatic fuel cells (EFC) [1,5]. The catalysts can either be immobilized on an electrode or be distributed in the surrounding solution. This point determines, among other aspects such as the type of catalyst, whether an artificial mediator is necessary for electron transfer to the electrode [1]. Electron transfer mechanisms are topic of current research and scientific discussion [6].

Some microbes are able to attach to surfaces spontaneously and to form a so-called “biofilm.” Some exoelectrogenic bacteria, such as *Shewanella* and *Geobacter spp*. were described transferring electrons to the electrode without the need of a synthetic mediator [6]. In microbial fuel cells a great range of substrates can be used, even waste [7]. The main area of application is therefore wastewater treatment with simultaneous generation of electricity [6,8,9].

In contrast, in enzymatic fuel cells only very specific substrates can be used in the set-up, as enzymes are very specific biocatalysts [10]. EFC represent a relatively young field of research and further developments are necessary - but they are expected to provide high potential for various applications. One focus is on the energy supply by EFC in biosensors and on-body or implantable biomedical devices such as pacemakers [11,12]. The set-ups are small, light-weighted and often even flexible [11].

Basically, many combinations of enzymes and substrates can be used for EFCs. Often glucose oxidase is used at the anode site combined with laccase at the cathode site. Since the cell voltage depends on the concentration of glucose used as substrate, this system is used for monitoring blood glucose as well as for operating incorporated applications [10,13,14,15]. A simplified explanation of the working principle of this system is shown in Table 1 [11,14]:

Table 1. Reactions in the EFC with glucose oxidase and laccase

anode	$C_6H_{12}O_6 \xrightarrow{\text{glucose oxidase}} C_6H_{10}O_6 + 2H^+ + 2e^-$
cathode	$1/2O_2 + 2H^+ + 2e^- \xrightarrow{\text{laccase}} H_2O$
overall reaction	$C_6H_{12}O_6 + \frac{1}{2}O_2 \xrightarrow{\text{enzymes}} C_6H_{10}O_6 + H_2O$

Laccase is a copper-containing oxidase that catalyzes oxidations of a wide range of aromatic compounds (phenols) through a free radical mechanism. In this reaction electrons are set free and transferred within the enzyme, so that the reduction of molecular oxygen takes place [16]. Consequently, the actual substrate is not named in the reaction equation.

3. Didactical Intention

Both hands-on experiments on biological fuel cells are suitable for students from grades nine to twelve. The experiments can be each carried out and discussed within one typical school lesson of 45 - 60 minutes. They can be dealt with in chemistry lessons following the topic galvanic cells. There are also interdisciplinary links to biology lessons through enzymology and metabolism. Intended learning aims are:

- To understand the basic working principle of both, microbial and enzymatic fuel cells.
- To get an idea of their range of applications.
- To learn that even drugstore products can be used to operate simple, yet innovative fuel cell systems.

In the lessons, the limitations of the experiments should also be addressed: They demonstrate the basic working principle, but need to be refilled with fuel after some time of operation - just like commercial fuel cell kits for schools or even real fuel cell cars. Moreover, the design of both experiments is not aimed at high performance but at clarity and simplicity, so that only essential components are used.

The experiments described in the following chapters have already been tried and tested with students within various school and extracurricular scenarios, as well as in teacher training workshops on different types of fuel cells.

4. Microbial Fuel Cell with Baker´s Yeast

In the system described below, baker´s yeast works as a biocatalyst. Baker´s yeast is suitable for school experiments because it is a harmless, inexpensive everyday product found in every local discounter or drugstore. Dry yeast is used, because it can be easily stored and weighed. The microbial fuel cell´s set-up is kept simple and reduced only to the necessary components, to be as comprehensible as possible for students: The two-pot-structure is very easy to approach as it is similar to well-known galvanic cells. Both half-cells contain glucose solution. If glucose is not available, sucrose can also be used. The anode beaker also contains baker´s yeast. A twisted toilet paper as an electrolyte bridge connects the half-cells. Iron nails are used as electrodes. The circuit is closed via cables and a multimeter.

A more compact set-up of this type of fuel cell has been published in [3].

4.1. Material and Chemicals

- two 25 mL beakers
- two alligator clips and two cables
- two iron nails / iron sheets as electrodes
- toilet paper as an electrolyte bridge
- multimeter (U, I)
- dry yeast
- distilled water
- glucose or sucrose

4.2. Procedure

Make a suspension of 1.5 g dry yeast and 25 mL distilled water in one beaker. Prepare 100 mL of a glucose or sucrose solution, w = 10 %, in the other beaker. Twist a piece of toilet paper. Add 2.5 g glucose or sucrose to the yeast suspension while stirring. Put the iron electrodes into the cell and connect the two beakers with the twisted toilet paper as shown in Figure 2. Start measuring the voltage immediately. Measure the current after ten minutes.

4.3. Observation

In the yeast suspension colourless gas bubbles are formed that rise to the surface, yielding some foam. At a temperature of 20 °C a voltage up to 400 mV is measured, that is nearly constant for a period of ten minutes. If sucrose is used instead of glucose, it takes a few seconds for the voltage to increase. The current measured after ten minutes is 30 µA.

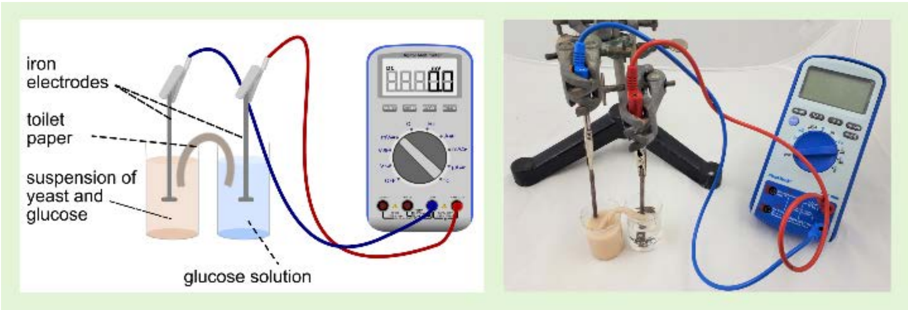

Figure 2. Two-pot set-up of the microbial fuel cell with baker´s yeast

4.4. Comment

The potential difference and hence the measurable voltage are caused by the yeast's metabolic activity at the anode compartment. Simplified reactions for glucose as a substrate can be formulated for the school context (Table 2).

Table 2. Simplified reactions in the MFC with baker´s yeast

anode	$C_6H_{12}O_6 + 6\,H_2O \xrightarrow{baker\text{´}s\ yeast} 6\,CO_2 + 24\,H^+ + 24\,e^-$
cathode	$O_2 + 24\,H^+ + 24\,e^- \rightarrow 12\,H_2O$
overall reaction	$C_6H_{12}O_6 + 6\,O_2 \xrightarrow{baker\text{´}s\ yeast} 6\,CO_2 + 6\,H_2O$

If sucrose is used, it is split into glucose and fructose. Both are oxidized, finally yielding carbon dioxide that can be observed as gas bubbles. Glucose is the yeast´s preferred substrate because no hydrolysis is needed and it can be directly converted in the metabolic pathway of glycolysis. Therefore, by using glucose the voltage can be measured immediately. If sucrose is used, it takes a moment for the voltage to increase due to the necessary formation of the monosaccharides.

Oxygen works as the final electron acceptor within the respiration chain. This is shown by the cathode reaction.

With decreasing concentration of substrate in the anode compartment and a decreasing pH the yeast´s metabolic activity decreases, so that the voltage declines and finally drops.

5. Microbial Fuel Cell with Lactic Acid Bacteria

In the system described below, lactic acid bacteria work as a biocatalyst. Thus, apart from baker´s yeast as a fungus, bacteria are also suitable for use in school experiments on microbial fuel cells. Lactic acid cultures in gastric acid-resistant capsules to improve intestinal function are sold at low costs in drugstores. The capsules can be easily opened and the powder contained can be used in the school experiment. The lactic acid bacteria can, for example, convert glucose to lactate with the release of electrons (Table 3).

Unlike the microbial fuel cell with baker's yeast, inspired by Ge, Schirhagl et al. [14] this cell is built up as a stack (Figure 4). Square pieces of filter papers coated with conductive ink based on water, activated carbon and surfactant serve as electrodes. Lactic acid bacteria are added to the anode filter paper. In between the electrodes, a third filter paper soaked with glucose solution is placed. It works as a substrate reservoir. The stack is finally fixed with a stripe of parafilm. The circuit is closed with cables and a multimeter.

5.1. Material and Chemicals

- two snap-on jars
- three Petri dishes
- two pipets
- two alligator clips and two cables
- tweezers
- parafilm
- filter paper, e.g. WINLAB filter circle
- hair clip
- multimeter (U, I)
- lactic acid cultures
- distilled water
- glucose
- activated carbon powder
- sodium dodecyl sulfate

5.2. Procedure

5.2.1. Preparation of the Components of the Cell

For each planned fuel cell, cut out three 2x2 cm squares of filter paper. The following quantities refer to three fuel cells. Open one lactic acid culture capsule. Fill 150 mg of the obtained powder in one snap-on jar. Add 15 mg sodium dodecyl sulfate, 30 mg activated carbon powder and 1.5 mL water and mix them by shaking. Prepare the same mixture of the tenside, carbon and water in the second snap-on jar, but don´t add lactic acid cultures. Place three pieces of filter paper in each of two Petri dishes (Figure 3). Drop 0.5 mL each of the conductive ink with lactic acid cultures onto the filter paper squares in one Petri dish and 0.5 mL of the bacteria-free mixture to the squares in the other Petri dish. The filter papers now have to soak for 10 minutes. In the meantime, place a previously untreated piece of filter paper in a saturated glucose solution. Cut a 1x6 cm strip of parafilm for each fuel cell.

Figure 3. Preparation of the components of the cell

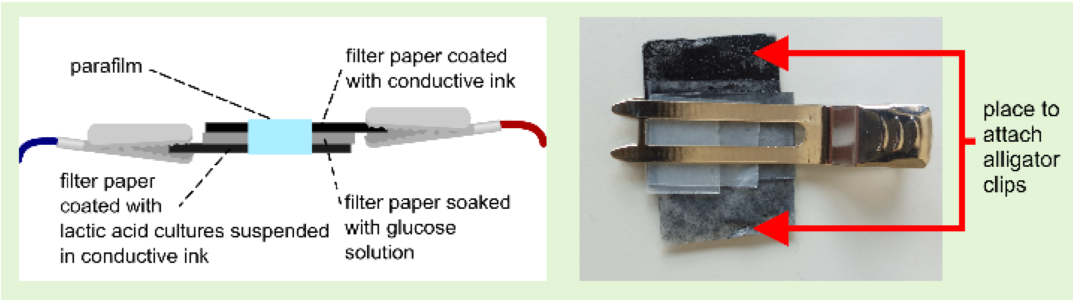

Figure 4. Stacked set-up of the enzymatic fuel cell with lactic acid bacteria and glucose

5.2.2. Building the Fuel Cell

Using tweezers place the wet components of the fuel cell on the parafilm strip with a small drift in the following order (Figure 4 left): parafilm, filter paper with conductive ink with lactic acid cultures, glucose-saturated filter paper, filter paper with conductive ink without lactic acid bacteria. Make sure that the coated sides are facing the glucose reservoir and that the ends of the filter papers stick out far enough for the alligator clips to be attached later on. Fold the parafilm strip around the layers of filter paper and fix the resulting stack with the hair clip.

5.2.3. Operation of the Fuel Cell

Connect the crocodile clips to the filter paper electrodes as shown in Figure 4. Place the prepared fuel cell on an inverted third petri dish, which serves as a base to stabilise the contacts. The voltage is measured and after ten minutes also the current.

5.3. Observation

At a temperature of 20 °C, the voltage rises to 184 mV and drops slightly within ten minutes of measurement. The current measured after ten minutes is 3.1 µA.

5.4. Comment

The potential difference and hence the measurable voltage are caused by the metabolic activity of the lactic acid bacteria at the anode compartment. For simplification, it can be assumed that only homofermentative lactic acid fermentation takes place [17]. Accordingly, the simplified reaction equations listed below can be formulated (Table 3).

Table 3. Simplified reactions in the MFC with lactic acid bacteria

anode	$C_6H_{12}O_6 \xrightarrow{lactic\ acid\ bacteria} 2\ C_3H_5O_3^- + 2\ H^+ + 2\ e^-$
cathode	$O_2 + 4\ H^+ + 4\ e^- \rightarrow 2\ H_2O$
overall reaction	$2\ C_6H_{12}O_6 + O_2 \xrightarrow{lactic\ acid\ bacteria} 4\ C_3H_5O_3^- + 2\ H_2O$

6. Enzymatic Fuel Cell with Lactase

In the system described below, the enzyme lactase works as a biocatalyst. Lactase pills are suitable for school experiments because lactase is a harmless, inexpensive everyday product found in every local pharmacy or drugstore. The unit FCC (Food Chemical Codex) refers to the enzyme activity. 1000 FCC of lactase can convert about 5 g of lactose per minute at 37 °C [18]. In Germany an increasing number of people tend to show lactose intolerance [19], so that lactase might be familiar to students from everyday life. Here, the origin of the lactase is not indicated on the packaging. Usually, lactase for medical purposes is obtained from the yeast *Kluyveromyces lactis*. This origin is also assumed here. The yeast-derived enzyme has an optimal pH range of 6.5 to 7 and a temperature optimum between 30-35 °C [20,21]. The substrate lactose can also be obtained in drugstores at low costs.

At school, the enzymatic fuel cell is operated at room temperature for simplicity. Just like the microbial fuel cell with lactic acid bacteria, the cell is built up as a stack. The differences are that lactase is added to the anode filter paper and the substrate reservoir is soaked with lactose solution.

Compared with other systems the cell proposed by us is truly low-cost: While a class set of the materials according to Ge costs about 55 euros (58 US$), the cost of our fuel cells is only about 1.50 euros (1.60 US$).

6.1. Material and Chemicals

- mortar and pestle
- two snap-on jars
- three Petri dishes
- two pipets
- two alligator clips and two cables
- tweezers
- parafilm
- filter paper, e.g. WINLAB filter circle
- hair clip
- multimeter (U, I)
- lactase pills (12 000 FCC units)
- distilled water
- lactose
- activated carbon powder
- sodium dodecyl sulfate

6.2. Procedure

6.2.1. Preparation of the Components of the Cell

With the exceptions that 300 mg ground lactase is used instead of lactic acid bacteria and an untreated piece of filter paper is soaked in lactose solution, the procedure is identical to the one described in 5.2.1.

6.2.2. Building the Fuel Cell

Here, too, the procedure is analogous to the one already described in 5.2.2, the only difference being the biocatalyst and the associated substrate, as can be seen in Figure 5 (left side).

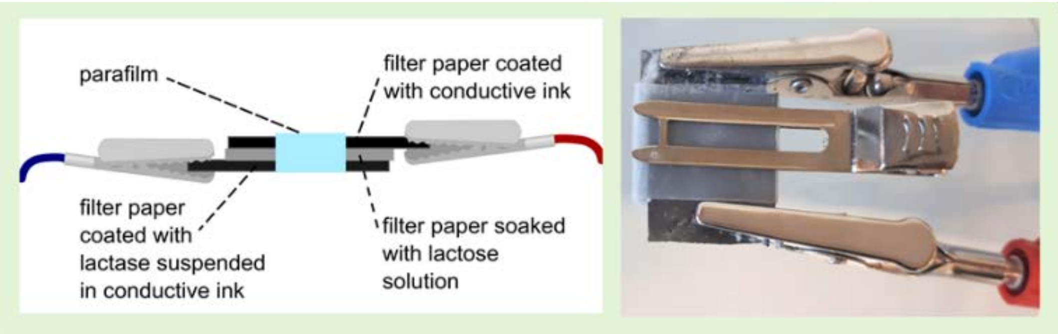

Figure 5. Stacked set-up of the enzymatic fuel cell with lactase and lactose

6.2.3. Operation of the Fuel Cell

Analogous to 5.2.3 the cell is contacted by alligator clips and connected to a multimeter. The voltage is measured and after ten minutes also the current.

6.3. Observation

At a temperature of 20 °C, the voltage rises to 229 mV and drops only slightly within ten minutes of measurement.

The current measured after ten minutes is 3.4 µA.

6.4. Comment

The pills functional ingredient is the enzyme lactase, a β-galactosidase. According to the manufacturer, the magnesium ions needed as a cofactor are contained as magnesium salts of fatty acids. Lactase functions as a catalyst for the hydrolysis from lactose in glucose and galactose and the building of allolactose. The exact mechanisms of hydrolysis and transglycolisation have not yet been clarified in detail. Up to now, it has not been possible to clearly identify the reactions that produce electricity, either by consulting specialist literature or through intensive exchange with biochemists and food chemists.

In our own studies it could be shown that during the hydrolysis of lactose by lactase, protons and electrons are released and oxygen is consumed at the counter electrode [22].

Just as in the lactose/lactase cell the enzyme-containing electrode represents the anode, while the reduction of atmospheric oxygen takes place at the cathode. For the schematic description of the processes taking place, the strongly simplified equations in Table 4 were proposed, where “S” stands for substrate, following Belitz et al [23].

Table 4. Simplified reactions in the EFC with lactase

anode	$SH_2 \xrightarrow{lactase} S + 2\,H^+ + 2e^-$
cathode	$O_2 + 4\,H^+ + 4\,e^- \rightarrow 2\,H_2O$
overall reaction	$2\,SH_2 + O_2 \xrightarrow{lactase} 2\,S + 2\,H_2O$

These considerations seem appropriate for a qualitative exploration of the working principle of EFC in the school context. If the teacher decides to take up the proposed equations in the course of the conclusion, it should be pointed out that lactase, which is known as a hydrolytic enzyme, has an oxidative function here.

7. Conclusion

Low-cost experimental set-ups on biological fuel cells with baker´s yeast, lactic acid bacteria or lactase as a biocatalyst have been presented. These three constitute a good approach to understand the basic working principle of biological fuel cells. The experiments were successfully tested by different groups of students and also introduced and discussed in teacher training workshops. Overall, biological fuel cells and corresponding hands-on experiments help to broaden the perspective on and the understanding of fuel cell systems in school.

References

[1] Katz, E., Bollella, P. (2021). Fuel cells and Biofuel Cells: From Past to Perspectives. Israel Journal of Chemistry 61, 68-84.

[2] Grandrath, R., Bohrmann-Linde, C. (2020). Fuel cells in the chemistry classroom - a brief survey among German chemistry teachers. ARiSE 1/3, 13-16.

[3] Grandrath, R., Bohrmann-Linde, C. (2019). Teaching Sustainability in the Chemistry Classroom: Exploring Fuel Cells in Simple Hands-on Experiments with Hydrogen, Sugar and Alcohol. World journal of chemical education 7/2, 172-178.

[4] Davis, F., Higson, S. P. J. (2007). Biofuel cells - recent advances and applications. Biosensors & bioelectronics 22/7, 1224-1235.

[5] Palmore, G. T. R., Whitesides, G. M. (1994). Microbial and Enzymatic Biofuel Cells. In: ACS Symposium Series. American Chemical Society, Washington, DC, 271-290.

[6] Kaiser, P., Reich, S., Leykam, D., Willert-Porada, M., Greiner, A., Freitag, R. (2017). Electrogenic single-species biocomposites as anodes for microbial fuel cells. Macromolecular Bioscience 17, 1-10.

[7] Santoro, C., Arbizzani, C., Erable, B., Ieropoulos, I. (2017). Microbial fuel cells. From fundamentals to applications. A review. Journal of power sources 356, 225-244.

[8] Logan, B. E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K. (2006). Microbial fuel cells: methodology and technology. Environmental Science & Technology 40/17, 5181-5192.

[9] Kumar, R., Singh, L., Zularisam, A. W., Hai, F. I. (2018). Microbial fuel cell is emerging as a versatile technology. A review on its possible applications, challenges and strategies to improve the performances. Int J Energy Res 42/2, 369-394.

[10] Rasmussen, M., Abdellaoui, S., Minteer, S. D. (2016). Enzymatic biofuel cells: 30 years of critical advancements. Biosensors & bioelectronics 76, 91-102.

[11] Karim, N. A., Yang, H. (2021). Mini-Review: Recent Technologies of Electrode and System in the Enzymatic Biofuel Cell (EBFC). Applied Sciences 11/11, 5197.

[12] Jeerapan, I., Sempionatto, J. R., Wang, J. (2020). On‐Body Bioelectronics: Wearable Biofuel Cells for Bioenergy Harvesting and Self‐Powered Biosensing. Adv. Funct. Mater. 30/29, 1906243.

[13] Fischer, C., Fraiwan, A., Choi, S. (2016). A 3D paper-based enzymatic fuel cell for self-powered, low-cost glucose monitoring. Biosensors & bioelectronics 79, 193-197.

[14] Ge, J., Schirhagl, R., Zare, R. N. (2011). Glucose-Driven Fuel Cell Constructed from Enzymes and Filter Paper. J. Chem. Educ. 88/9, 1283-1286.

[15] Cosnier, S., Le Goff, A., Holzinger, M. (2014). Towards glucose biofuel cells implanted in human body for powering artificial organs: Review. Electrochemistry communications. 38, 19-23.

[16] Morozova, O. V., Shumakovich, G. P., Gorbacheva, M. A., Shleev, S., Yaropolov, A. I. (2007). “Blue” Laccases. Biochemistry (Moscow) 72/10, 1396-1412.

[17] Cypionka, H. (2010). Grundlagen der Mikrobiologie, 4. Aufl. Springer, Berlin, Heidelberg.

[18] Bresson, J.-L., Flynn, A., Heinonen, M., Korhonen, H., Løvik, M., Martin, A., Przyrembel, H., Salminen, S., Strain, S., Tetens, I., van den Berg, H., van Loveren, H., Verhagen, H., Gueimonde, M., Moseley, B., Strobel, S., Szajewska, H. (2009). Scientific Opinion on the substantiation of health claims related to lactase enzyme and breaking down lactose (ID 1697, 1818) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. EFSA Panel on Dietetic Products, Nutrition and Allergies (NDA). EFSA Journal 7/9, 1-13.

[19] Niehr, H. (2017). Immer mehr Laktoseintolerante. More and more lactose intolerant. https://de.statista.com/infografik/7565/umfrage-zu-laktoseintoleranz/ (letzter Zugriff am 25.11.2022).

[20] Porciúncula González, C., Giacomini, C., Irazoqui, G. (2019). Survey of ß-Galactosidases properties: applications to transglycosylation process. In: Beta-galactosidase. Properties, structure and functions. Kras, E. (Hrsg.). Nova Science Publishers, New York, 65-116.

[21] Sedzro, D. M., Bellah, S. F., Akbar, H., Billah, S. (2018). Structure, Function, Application and Modification Strategy of ß -Galactosidase. Journal of Multidisciplinary Research and Reviews 1/1, 10-16.

[22] Grandrath, R. (2021). Brennstoffzell-Systeme mit Fokus auf biologischen Brennstoffzellen: Entwicklung und Optimierung von schulgeeigneten Experimenten, Konzepten und Medien. Dissertation. Bergische Universität Wuppertal, Wuppertal.

[23] Belitz, H.-D., Grosch, W., Schieberle, P. (2008). Lehrbuch der Lebensmittelchemie, 6. Aufl. Springer-Verlag, Berlin, Heidelberg.