**Abstract**

The challenges concerning global energy supply originating from the rapid depletion of fossil fuels demand new solutions to produce sustainable, regenerative and clean energy. Regarding this, we aimed our project towards developing a system to generate electricity with the help of bacteria in a Microbial Fuel Cell (MFC). Based on a combined genetic and engineering approach, we designed, constructed and optimized the fuel cell, as well as investigating different genetic approaches. Using Synthetic Biology, we designed several BioBricks for bioelectricity generation. To provide an offshore infrastructural electron transfer (IET), we introduced and improved electron shutters, so-called endogenous mediators in E. coli. Furthermore, permeabilization of the cell surface by integrating large membrane pores led to an additional increase of the IET and allowed us to use larger mediators like riboflavin.

Our aim to extend our MFC-system beyond the closed domes of a laboratory required a functional and reliable biosafety concept. We developed and proved the functionality of three different double-foam-wick biofilm systems to guarantee an absolutely safe use of the MFC for small scale applications.

**GEM Bielefeld MFC evolution:**

Dissimilation of our BioBricks and devices required the design and construction of a suitable microbial fuel cell. Starting with a very simple device, the system was extended by a novel and effective exchange membrane design, which was modified by using a new and effective exchange membrane material. A detailed description of the Bielefeld MFC with four exchange membranes is depicted in the Supporting Information.

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**DIY**

We received BioBrick vectors as dried daughters for which all necessary instructions can be downloaded from our wiki page. The building, fully functional bioBrick consists of material which can be obtained for less than 200 euro per vector. Microorganisms for all species can be obtained from the European Collection of Microorganisms for Industrial Biotechnology.

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**Mediators**

**Riboflavin**

Riboflavin, or vitamin B2 is a coenzyme that, like vitamin B1, can be efficiently used in bioelectrodes for electron transfer.

We have shown that transmembrane expression of the riboflavin channel from the bacterial channel donor Geobacillus stearothermophilus in E. coli is sufficient in a cm-scale dual chamber eew device to drive the evolution of the eew cell.

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**Glycerol dehydrogenation**

Glycerol dehydrogenase (GDA) produces the endogenous mediator NADH from NADP and glycerol, useful for the fermenter culture in our experiment. We were able to reduce the NADP/NADP+ ratio from 0.5 to 0.02 in the fermenter and resulted in a more efficient electron transfer. We could show that the increased membrane and exchangeable NADP concentration leads to a 30% enhanced current production in our Biowire fuel cell. Using glycerol as the main carbon source further enhances the efficiency of NADH.

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**Porins**

Porins are integral membrane proteins, which are the first step for the generation of high currents from bacteria. We bioengineered the pores forming protein porin OmpF from Escherichia coli (E. coli). This allows transport of larger molecules through the cell membrane.

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**Project Timeline**