Electronic Supplementary Material (ESI) for Sustainable Energy & Fuels.

Cellulose to Electricity Conversion by an Enzymatic Biofuel Cell

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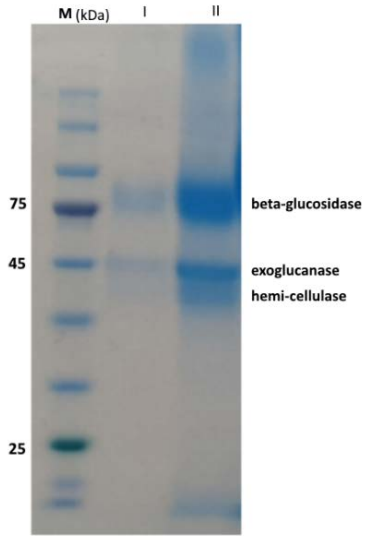
Supporting information

Materials.

Glassy carbon electrodes (GCE, 3 mm diameter) were purchased from CH-Instruments. Dopamine, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), bilirubin oxidase (Myrothecium verrucaria) and glucose were purchased from Sigma-Aldrich. Dimethylformamide was purchased from Bio-lab. Multi walled carbon nanotubes (MWCNTs) were purchased from Nanointegris (MWCNTs, 99 wt%, <20 nm OD). Flavin adenine dinucleotide dependent glucose dehydrogenase (FAD-GDH, 1150 U/mg) was purchased from Sekisui Chemicals. 2,3-dichloro-naphthoquinone (DCNQ) 98% was purchased from Acros Organics. Titanium(IV) bis(ammonium lactato) dihydroxide solution (50 wt. % in H_2O) was purchased from Sigma-Aldrich. Nafion D520 dispersion (5% in water and 1-propanol) was purchased from Alfa Aesar. Poly(ethylene glycol) diglycidyl ether (PEGDGE) was purchased from Sigma-Aldrich. Tetraethyl orthosilicate (TEOS) was purchased from Sigma-Aldrich. Cellulose- 50µm particle size (SIGMACELL cellulose- Type 50) was purchased from Sigma-Aldrich. A commercial cellulase complex- Accellerase 1500 (Endoglucanase activity 2200-2800 CMC U/g, Beta glucosidase activity 450-775 pNPG U/g) was purchased form GENECOR. The commercial Accellerase 1500 composition was analyzed using SDS-PAGE as presented below:



SDS-PAGE of cellulase complex, SDS PAGE of the cellulase complex- Accellerase 1500 diluted 10 times (lane II) and 100 times (lane I). M Protein marker in kDa. As depicts, the dominant enzyme is the beta-glucosidase.

Part 1. Glucose dehydrogenase based anode- fabrication, characterization and optimization

1.1 Glucose dehydrogenase based anode fabrication

GDH based anode was fabricated according to a previously reported procedure with several changes.[1] In order to enhance the anode stability, the mixed solution was prepared with a 5-fold higher concentration of dopamine and a pre-incubation step of the mixed solution at room temperature for 30 min was added for partial polymerization of dopamine prior to electrode surface deposition.

1.2 Anode performance optimization

GDH based anode was first prepared without pre-incubation step of the mixed solution. Cyclic voltammetry (CV) measurements were performed using a scan rate of 5mV/sec, scanning from -0.3 V to 0.5 V vs. Ag/AgCl. Measurements were performed at 50°C, in the presence of 0-30mM glucose in the cell solution. First, phosphate buffer (PB) at pH 5.8 was used. Each GDH based anode was incubated in the buffer for one minute before measurement. Results are presented in **Figure S1, A.** The anode was further tested chronoamperometrically while applying 0.1 V vs. Ag/AgCl in the presence of 30mM glucose in the cell solution. Results are presented in **Figure S1, B**.

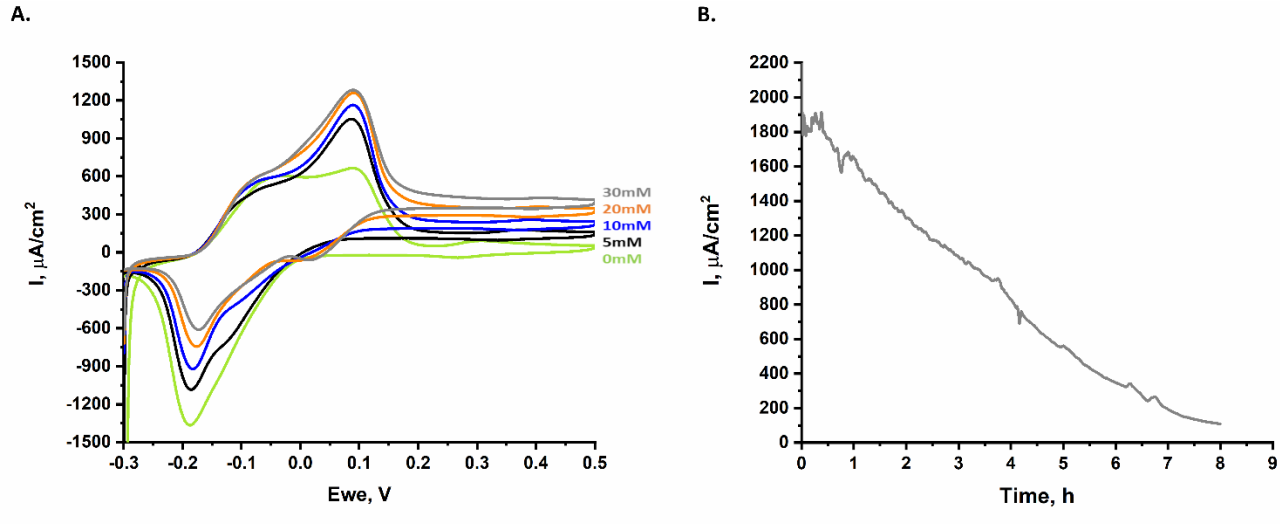


Figure S1. GDH based anode performance at pH 5.8, 50°C. **(A)** Cyclic voltammetry measurements of GDH based anode before (green) and after the addition of 5mM (black), 10mM (blue), 20mM (orange) and 30mM (grey) glucose to the cell solution, scanning from -0.3V to 0.5V vs. Ag/AgCl. **(B)** Chronoamperometry test of the GDH based anode under an applied voltage of 0.1 V vs. Ag/AgCl. Measurement was performed in PB 0.1M, pH 5.8 in the presence of 30mM glucose in the cell solution.

1.3 Enhancement of the GDH based anode stability

To enhance anode stability, a higher pH of 6.5 was used. The anodes were prepared as described above and cyclic voltammetry tests were performed at 50°C using PB 0.1M, pH 6.5 before and after the addition of 30mM glucose to the cell solution. Results are presented in **Figure S2, A**. Chronoamperometry test was then performed by applying 0 V vs. Ag/AgCl, using PB 0.1M pH 6.5 at 50°C, as presented in **Figure S2, B**.

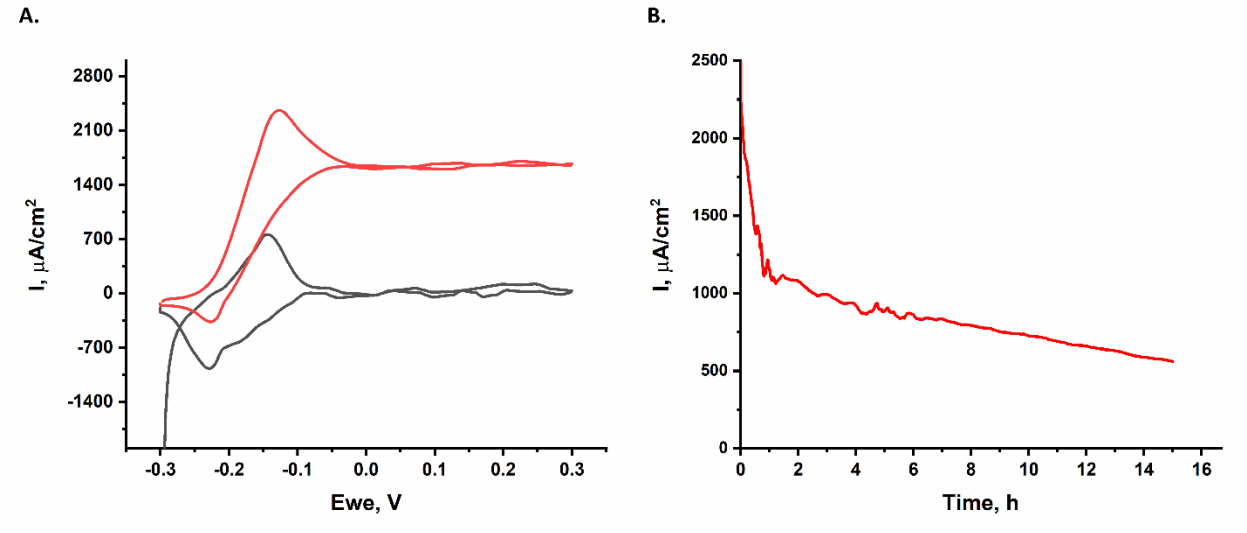


Figure S2. GDH based anode performance at pH 6.5, 50°C. **(A)** Cyclic voltammetry measurements of GDH based anode without (black) and with the addition 30mM (red) glucose to the cell solution, scanning from -0.3V to 0.3V vs. Ag/AgCl. **(B)** Chronoamperometry test of GDH based anode under an applied voltage of 0V vs. Ag/AgCl. Measurement was performed in PB 0.1M, pH 6.5 in the presence of 30mM glucose in the cell solution.

To enhance anode performance, the mixed solution was prepared with a 5-fold higher dopamine concentration. Furthermore, the mixture was incubated at room temperature for 30min before the deposition procedure. Five microliters of the mixture were deposited on each MWCNTs-DCNQ modified GCE which was then incubated for one hour under atmospheric conditions. Cyclic voltammetry measurements were performed using a scan rate of 5mV/sec, scanning from -0.3 V to 0.2 V vs. Ag/AgCl. The measurements were performed in PB 0.1M, pH 6.5 with and without 30mM glucose in the cell solution. Results are presented in **Figure S3, A**. In addition, chronoamperometry measurements were performed in PB 0.1M pH 6.5, 50°C in the presence of 30mM glucose in the cell solution while 0V vs. Ag/AgCl was applied. Results are presented in **Figure S3, B**.

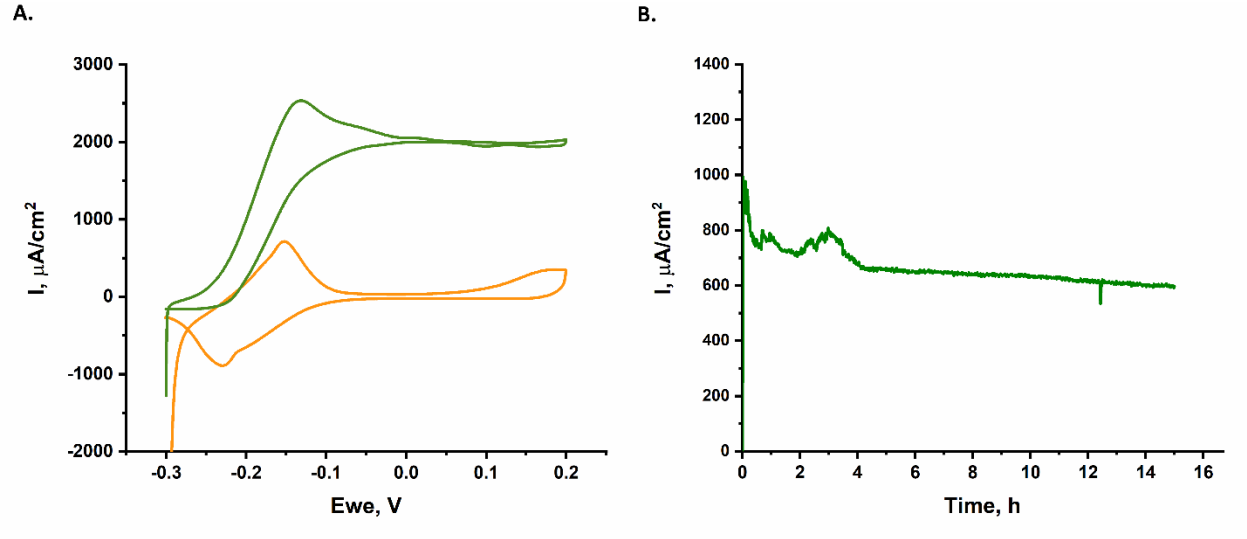


Figure S3. Performance of GDH based anode prepared with high dopamine in the mixed solution at pH 6.5, 50°C. **(A)** Cyclic voltammetry measurements without (orange) and with the addition 30mM (green) glucose to the cell solution, scanning from -0.3V to 0.2V vs. Ag/AgCl. **(B)** Chronoamperometry test of the GDH based anode under an applied voltage of 0V vs. Ag/AgCl. Measurement was performed in PB 0.1M, pH 6.5 in the presence of 30mM glucose in the cell solution.

1.4 The effect of cellulose presence in the cell solution on the GDH based anode bioelectrocatalytic performance

The effect of cellulose presence in the cell solution on the GDH- based anode performance was tested. Cyclic voltammetry measurement was performed using a scan rate of 5mV/sec, scanning from -0.3 V to 0.2 V vs. Ag/AgCl. The measurement was performed in PB 0.1M, pH 6.8, 37°C with and without 5mg/ml of cellulose in the cell solution. Additional cyclic voltammetry was performed after the addition of 30mM glucose to the cell solution. Results are presented in **Figure S4.**

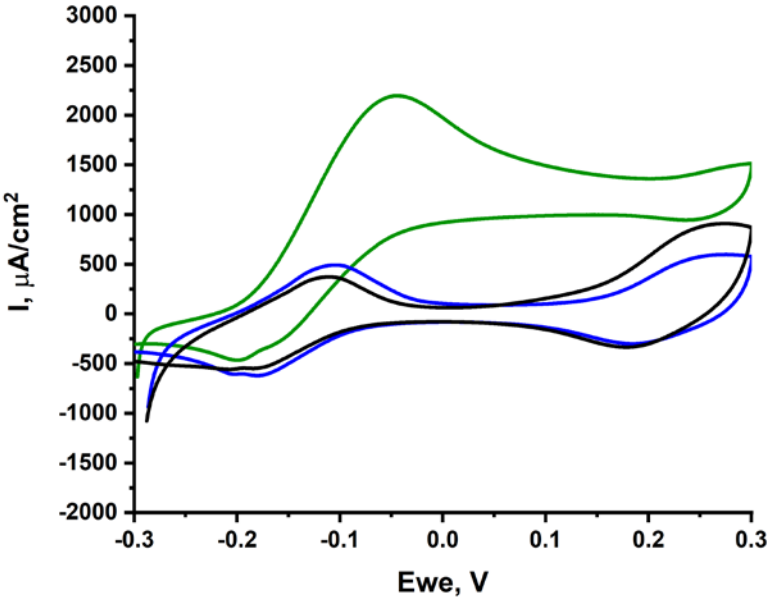


Figure S4. Effect of cellulose presence in the cell solution on the GDH- based anode (prepared with high dopamine) performance. Cyclic voltammetry measurements without (blue) and with (black) 5mg/ml cellulose dispersed in the cell solution followed by the addition of 30mM glucose to the cell solution (green). Cyclic voltammetry measurements were performed in PB 0.1M, pH 6.8, 37°C, scanning from -0.3V to 0.2V vs. Ag/AgCl.

1.5 Quantification of redox mediator deposited on the electrode.

To estimate the amount of DCNQ deposited on the electrode, the GDH based anode was measured via cyclic voltammetry in 0.1M PB, pH 6.8 under argon atmosphere. Results are presented in **Figure S5**.

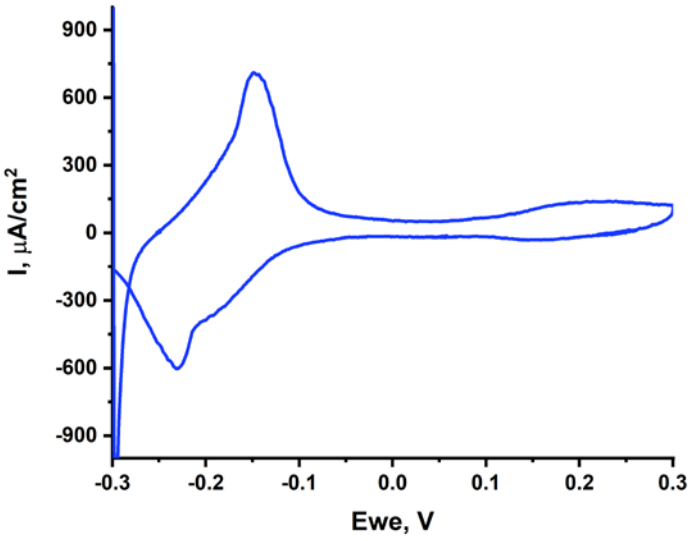


Figure S5. Cyclic voltammetry measurement of GDH based anode (prepared with high dopamine concentration) under argon atmosphere. The measurement was performed in PB, pH 6.8.

For the quantification of DCNQ deposited on the electrode, we integrated the electrochemical wave appears at -0.15V vs. Ag/AgCl, that is in order to estimate the number of coulombs attributed to the DCNQ. The number of DCNQ moles was then calculated by dividing the number of Coulombs by the Faraday constant and by 2 electrons in the reaction according to the following equation:

$$DCNQ\ [mols] = \frac{Number\ of\ Coulombs}{2\bar{e} \cdot 96485.33\ \frac{A \cdot sec}{mol}} = \frac{0.0714\frac{A \cdot sec}{cm^2}}{2\bar{e} \cdot 96485.33\ \frac{A \cdot sec}{mol}} = 3.7E^{-7}[\frac{mol}{cm^2}]$$

Part 2. Three compartment cell construction with cellulase complex and cellulose in the cell solution

2.1 Three compartment cell construction with or without physical barrier between the cellulase complex, cellulose and the bioanode

For the construction of the three-compartment cell, GDH based anode was prepared as described above. The cellulase complex was filtered (5 minutes, 4°C, 4500rpm) using a 10KDa centrifuge filter (Nanosep, Lifesciences) to discard glucose remaining within the commercial enzyme mixture, before use. Cellulase complex and cellulose (5µl and 5mg per 1 ml of cell solution, respectively) dispersed in PB pH 6.8, 0.1M, served as the cell solution. An image and a scheme of the cell design is presented in **Figure S6, A**.

In order to enhance the three-compartment cell operational life time, a physical barrier was used to separate between cellulose, the cellulase complex and the GDH based anode. Two different physical barriers were used – a dialysis bag (Spectra Por, 12-14 kD) and a polyethersulphone filter (Lifegene).

To achieve physical separation, a dialysis bag was used. The cellulose and cellulase complex were placed inside the membrane while the GDH based anode was in PB 0.1M pH 6.8 used as the cell solution. An image and a scheme of the cell design is presented in **Figure S6, B**. For the physical separation using the polyethersulphone filter, the cellulose and cellulase complex were mixed in PB pH 6.8 used as the cell solution while the GDH based anode was placed inside the upper part of the filter. An image and a scheme of the cell design is presented in **Figure S6, C.** Both of the cells contained 5µl of cellulase complex and 5mg of cellulose per 1 ml of cell solution. In order to test if the dialysis bag prevents the enzymes from reaching the GDH based anode, we followed the absorbance at 280nm during 6 hours of cell activation under an applied voltage of 0V vs. Ag/AgCl, while cellulose only or both cellulose and cellulase complex were placed in dialysis bag. The results are presented in **Figure S7**.

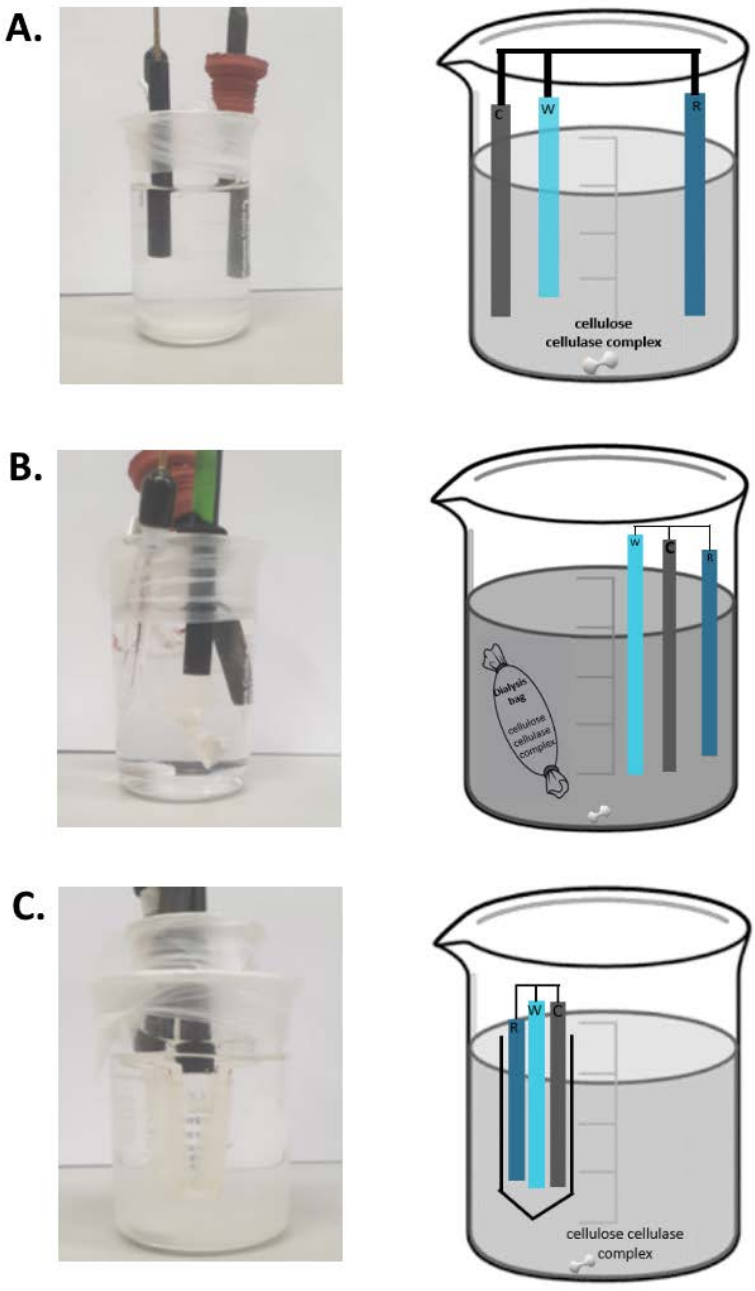


Figure S6. Images and schemes of different three-compartment cell designs without **(A)** and with the use of dialysis bag **(B)** and polyethersulphone filter **(C)** as a physical barrier.

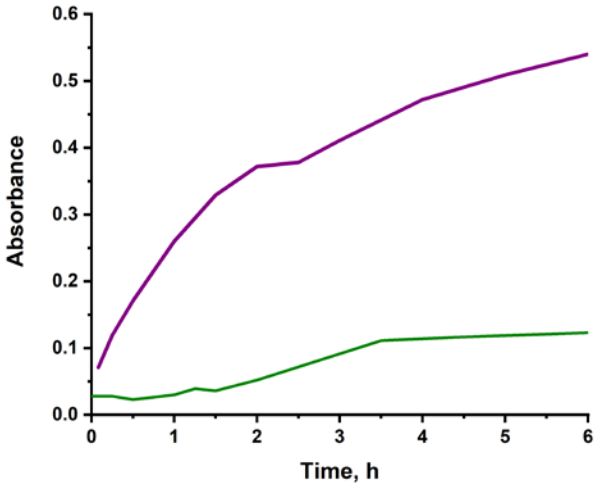


Figure S7. Absorbance measurements at 280nm of the cell solution. Measurements were performed during chronoamperometry test of GDH based anode subjected to 0V vs. Ag/AgCl at 50°C, pH 6.8 while the cellulose (green) and cellulase complex (purple) were in a dialysis bag.

2.2 The effect of each system component on the GDH based anode stability

In order to test the GDH based anode bioelectrocatalytic current loss resulting from the presence of cellulose and cellulase complex in the cell solution, we first prepared GDH based anode according to the procedure described above. The GDH based anode was then measured chronoamperometrically for 20 minutes, under an applied voltage of 0V vs. Ag/AgCl in the presence of cellulase complex (blue), cellulose (green) or both (orange) in the cell solution. After 20 minutes of current stabilization, 30mM of glucose were added to the cell solution and the bioelectrocatalytic currents were measured for 25 more hours. The current was normalized as a percent of the maximal bioelectrocatalytic current, which was measured immediately after glucose addition. Measurements were performed in PB 0.1M, pH 6.8 at 37°C. Results are presented in **Figure S8**.

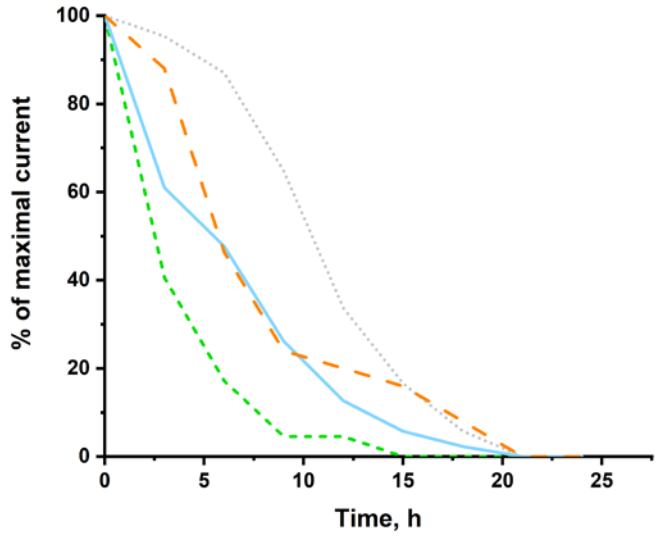


Figure S8. The anode bioelectrocatalytic current loss without (grey) and with the addition of cellulose (green), cellulase complex (blue) and both (orange). Each of the measurements was performed under an applied voltage of 0V vs. Ag/AgCl. The cell was activated in the presence of cellulase complex, cellulose or both. After 20 minutes of current stabilization, 30mM glucose were added to the cell solution. The current loss is presented as a percent of the maximal bioelectrocatalytic current achieved immediately after glucose addition. Measurements were performed in PB pH 6.8, 37°C.

2.3 The effect of cellulase concentration on the GDH based anode bioelectrocatalytic performance

In order to test the effect of the cellulase complex concentration on the GDH based anode bioelectrocatalytic performance, the three-compartment cell was tested in the presence of 0.25, 0.5, 2.5 and 5 µl cellulase complex per 1 ml of cell solution, while the cellulose concentration remained constant (5mg/ml). The chronoamperometry measurements were performed in PB 0.1M, pH 6.8, 37°C, by applying 0V vs. Ag/AgCl. The results are presented in **Figure S9**.

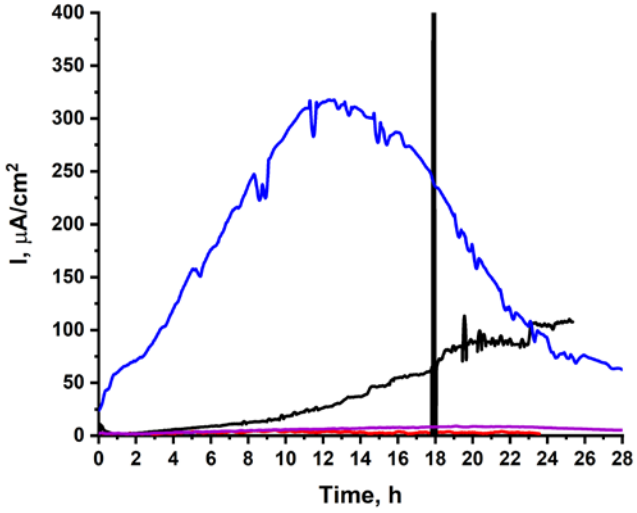


Figure S9. The effect of cellulase concentration in the cell solution on the GDH based anode bioelectrocatalytic performance. Chronoamperomtry test of the GDH based anode in the presence of 5mg/ml cellulose and 0.25 (red), 0.5 (purple), 2.5 (black) and 5 (blue) microliters of cellulase complex per 1 ml of cell solution. Measurements were performed in PB 0.1M, pH 6.8, 37°C, by applying 0V vs. Ag/AgCl.

Part 3. Cellulose degradation quantification

Cellulase complex (5µl/ml) and cellulose (5mg/ml) were suspended in PB 0.1M pH 6.8 and incubated at 37°C. Samples were taken of the suspension during 26 hours of incubation. Each 500µl sample was placed on ice to stop enzymatic activity. The samples were filtered (5 minutes, 4°C, 4000rpm) using a 10KDa filter (Nanosep, Lifesciences). The filtrate was then kept at 4°C until analyzed. The amount of glucose in each sample was quantified using a high-performance anion-exchange chromatography system equipped with a PA1 column (Dionex, Sunny-vale, CA, USA), according to a previously reported method.[2] An aqueous solution of 150mM NaOH (solution A) and an aqueous solution of 150mM NaOH and 500mM sodium acetate (solution B) were used for glucose elution using a 2 step program: 1 minute isocratic step where 100% of A was used, followed by a 50 minute linear-gradient step to reach 100% B. The flow rate was 1ml/min during the entire program. The area of the peak representing glucose during 26 hours of suspension incubation is presented in **Figure S10, A**. The peak representing the glucose typically appeared after 4 minutes, as seen in the representative chromatogram in **Figure S10, B**.

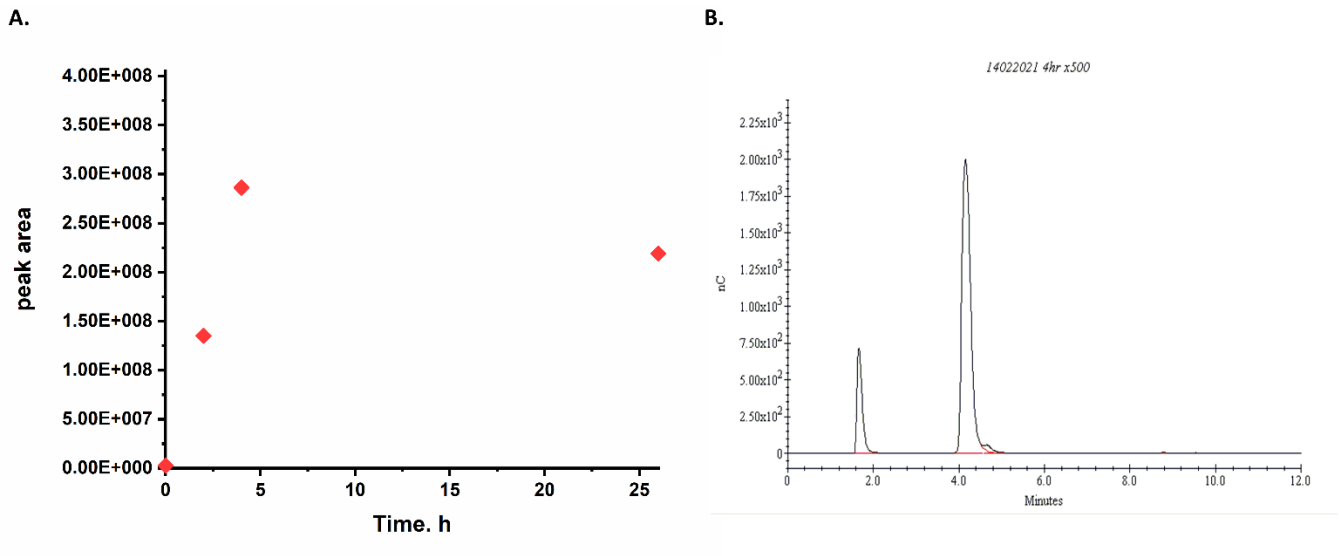


Figure S10. Cellulose degradation measured using a High performance anion-exchange chromatography system equipped with a PA1 column (Dionex). Cellulose and cellulase complex were incubated at 37°C pH 6.8 and samples were taken during 26 hours of incubation. **(A)** Peak area representing glucose during incubation time; **(B)** representative chromatogram.

<u>Part 4: GDH based anode surface morphology before and after 15 hours of half-cell activation</u>

In order to characterize the modified GDH based anode morphology during the half-cell activation, scanning electron microscopy (SEM, Phenom ProX) was used. The GDH based anode construction was performed using Toray carbon paper. Chronoamperomtry test was performed under an applied voltage of 0V vs. Ag/AgCl in the presence of cellulase complex and cellulose in the cell solution. SEM images of the GDH based anode were taken before and after 15 hours of half-cell activation. The images are presented in **Figure S11**.

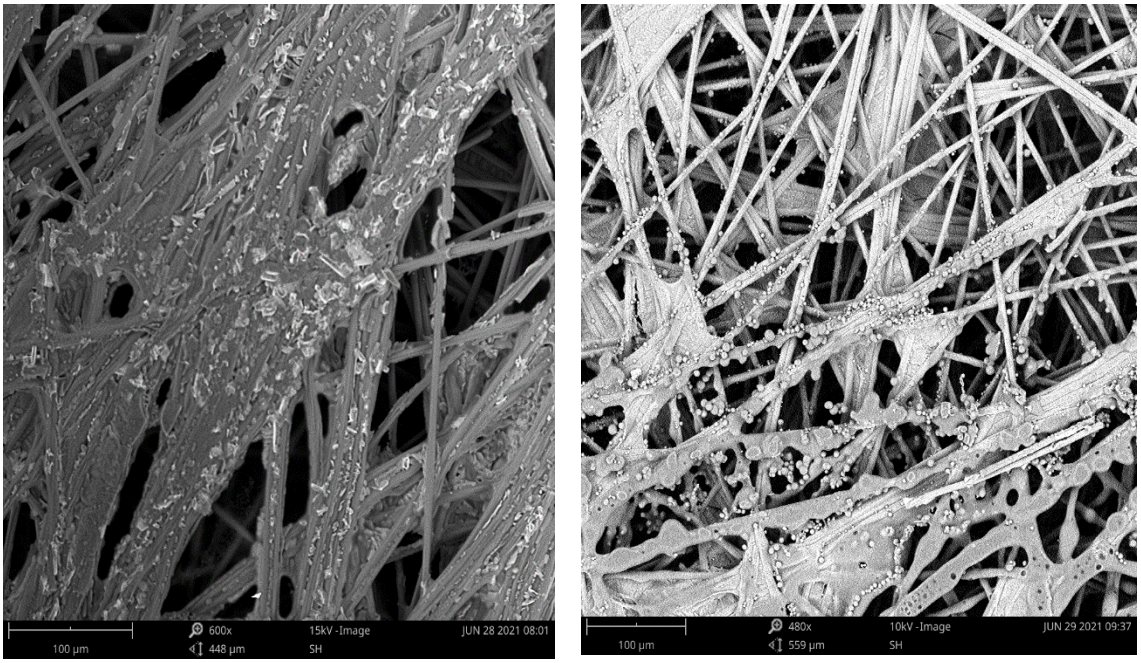


Figure S11. Scanning electron microscopy analysis of GDH based anode before (left) and after (right) 15 hours of chronoamperomtry test under an applied voltage of 0V vs. Ag/AgCl in the presence of cellulase complex and cellulose in the cell solution.

Part 5. Addition of stabilizing layer for enhanced stability of the GDH based anode

Several stabilizing layers were examined in order to enhance the GDH based anode performance. Amorphous TiO_2 has been used before as a catalyst protecting layer. Amorphous TiO_2 layer was fabricated using Titanium(IV) bis(ammonium lactato) dihydroxide solution (50 wt. % in H_2O) diluted 5-fold in PB pH 6, 0.1M. Three microliters of each of the diluted Titanium(IV) bis(ammonium lactato) dihydroxide solutions were deposited on the GDH-based anode and dried in air. The electrode was tested chronoamperometrically at 37°C, under an applied voltage of 0V vs. Ag/AgCl in PB 0.1M, pH 6.8 with cellulase complex and cellulose in the cell solution, as seen in **Figure S12**, pink curve. Furthermore, we examined the GDH based anode stability following the deposition of 2 amorphous TiO_2 layers as presented in **Figure S12**, blue curve. Sol-gels techniques were also examined for GDH based anode stabilization. Nafion dispersion (5% in water and 1-propanol) was diluted 5-fold in water, and 3µl of the solution were deposited on the electrode, which underwent drying under atmospheric conditions. The electrode was tested chronoamperometrically at 37°C, under an applied voltage of 0V vs. Ag/AgCl in PB 0.1M, pH 6.8 with cellulase complex and cellulose in the cell solution, results are presented in **Figure S12**, red curve. Pre-mix solution of Tetraethyl orthosilicate (TEOS) solution was prepared by mixing 5µl of TEOS, 6µl of H_2O and 1µl of 5mM NaOH solution. Then, aliquots of 1µl of 5mM NaOH solution were added to the pre-mix solution until white gel was seen. Then, 3µl of the solution were placed on the electrode and it was dried in air. The electrode was tested chronoamperometrically at 37°C, under an applied voltage of 0V vs. Ag/AgCl in PB 0.1M, pH 6.8 with cellulase complex and cellulose in the cell soultion, as presented in **Figure S12**, black curve. Poly(ethylene glycol) diglycidyl ether (PEGDGE, Avg. Mn 500) was examined as an enzyme cross-linker. To examine that, 6µl of the PEGDGE were mixed with 45µl of H_2O and 1 µl of the solution was deposited on the electrode during the polydopamine polymerization step. The electrode was

dried in air for additional 1 hour. Then, the electrode was tested chronoamperometrically at 37°C, under an applied voltage of 0V vs. Ag/AgCl in PB 0.1M, pH 6.8 with cellulase complex and cellulose. Results are presented in **Figure S12**, orange curve.

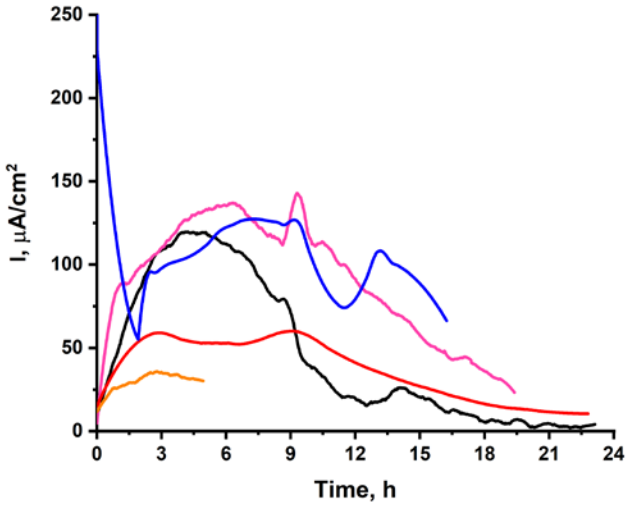


Figure S12. Chronoamperometry test of GDH based anode covered with an amorphous TiO_2 layer (pink), 2 amorphous TiO_2 layers (blue), Nafion (red), PEGDGE (orange) and TEOS (black) for enhanced stabilization. Each of the measurements was performed under an applied voltage of 0V vs. Ag/AgCl. The cell was activated in the presence of cellulase complex and cellulose in the cell solution. Measurements were performed in PB pH 6.8, 37°C.

Part 6. Bilirubin oxidase (BOD) based cathode- fabrication and characterization

6.1 BOD based cathode fabrication

BOD based cathode was prepared according to a previously reported procedure.[3] Cyclic voltammetry measurements were performed at 37°C in PB, 0.1M pH 6.8 under argon and under oxygen saturated atmosphere. Scan rate 5mV/sec was used, scanning from 0.6V to 0V vs. Ag/AgCl. Results are presented in **Figure S13**.

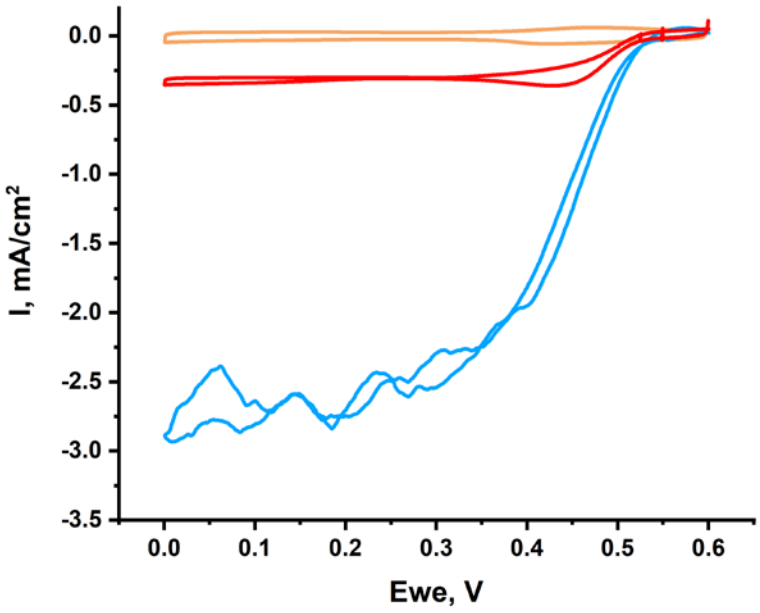


Figure S13. Bioelectrocatalytic currents of BOD based cathode under argon (orange), under atmospheric conditions (red) and under oxygen saturated atmosphere (blue). Measurements were performed in PB 0.1M pH 6.8 at 37°C using a scan rate of 5mV/s.

6.2 BOD based cathode- characterization in the presence the cellulose and cellulase complex

In order to test the BOD based cathode biolelectrocatalytic current loss resulting from the presence of cellulose and cellulase complex in the cell solution, we first prepared BOD based cathodes according to the procedure described above. The BOD based cathodes were then measured chronoamperometrically under oxygen saturated atmosphere for 20 minutes while 0.1V vs. Ag/AgCl was applied. Then, cellulase complex, cellulose or both were added to the cell solution and the bioelectrocatalytic currents were measured during additional 3 hours and 40 minutes. The currents, displayed as a percent of the maximal bioelectrocatalyic current, during four hours of chronoamperometry test is presented in **Figure S14**. Measurements were performed in PB 0.1M, pH 6.8 at 37°C.

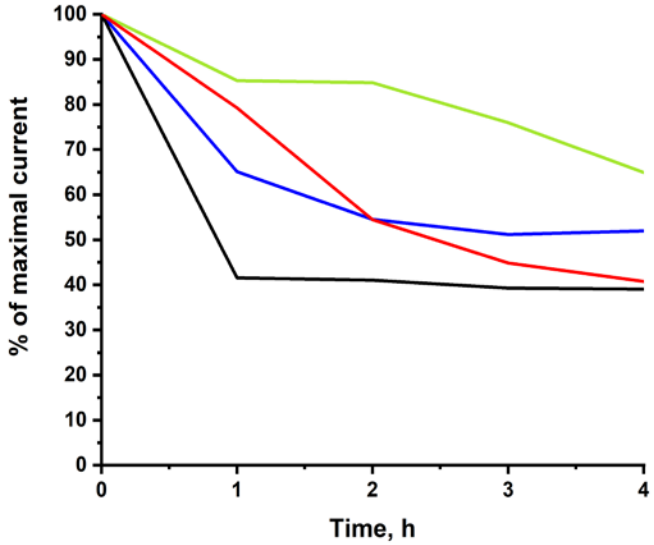


Figure S14. BOD based cathode bioelectrocatlytic current loss in the presence of cellulose, cellulase complex or both in the cell solution. The BOD based cathodes were tested chronoamperometrically under an applied voltage of 0.1V vs. Ag/AgCl in PB 0.1M pH 6.8, without (green) and with the addition of cellulose (black), cellulase complex (blue), cellulase complex and cellulose (red) to the cell solution. Measurements were performed in PB pH 6.8, 37°C under oxygen saturated atmosphere.

Part 7. Full bioelectrochemical cell construction and characterization

7.1 Full bioelectrochemical cell construction and polarization

To test full cell performance, GDH based anode and BOD based cathode were coupled for cell construction. Cellulase complex (5µl per 1 ml of cell solution) and cellulose (5mg per 1 ml of cell solution) suspended in PB 0.1M, pH 6.8 served as the cell solution. Image of the full bioelectrochemical cell is presented in **Figure S15**. The full bioelectrochemical cell was polarized after 1 min. of cellulose a cellulase complex incubation. Results are presented in **Figure S16**. Measurement was performed at 37°C.

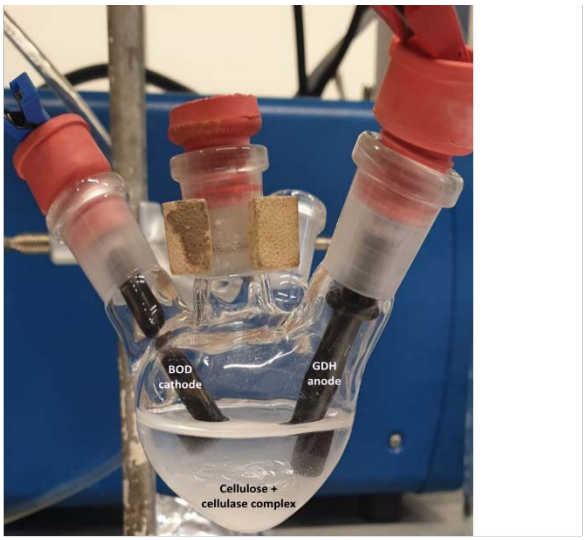


Figure S15. Image of the full bioelectrochemical cell. The cell was constructed by coupling the BOD based cathode and GDH based anode in the presence of cellulase complex and cellulose in the cell solution.

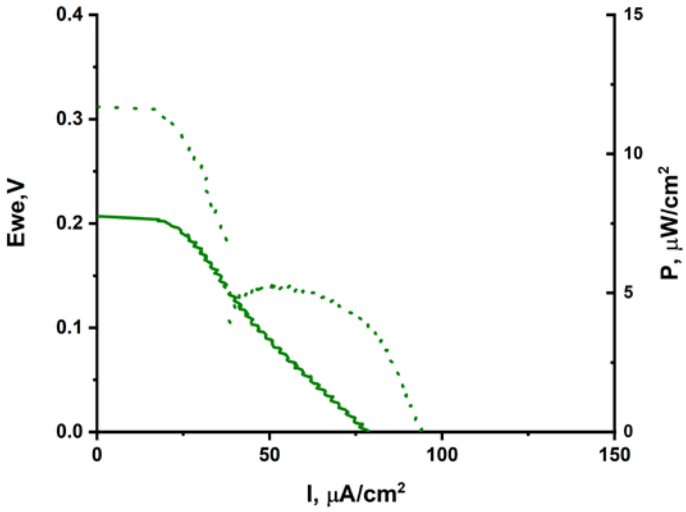


Figure S16. LSV measurements of the full bioelectrochemical cell after 1 minute of cell incubation. Cellulase complex and cellulose suspended in PB 0.1M pH 6.8 served as the cell solution. LSV measurement was performed at 37°C using a scan rate of 2mV/s.

7.2 Full cell polarization and power density curves in the presence of various glucose concentrations

To test the full cell performance in the presence of various glucose concentrations, GDH based anode and BOD based cathode were coupled for cell construction. LSV measurements were performed at 37°C using a scan rate of 2mV/s under oxygen saturated atmosphere. PB 0.1M, pH 6.8 served as the cell solution. Measurements were performed before and after the addition of 10mM, 20mM, 30mM, 40mM and 50mM

of glucose to the cell solution. The cell solution was stirred after each step of glucose addition for 10 seconds before measurement. Results are presented in **Figure S17.**

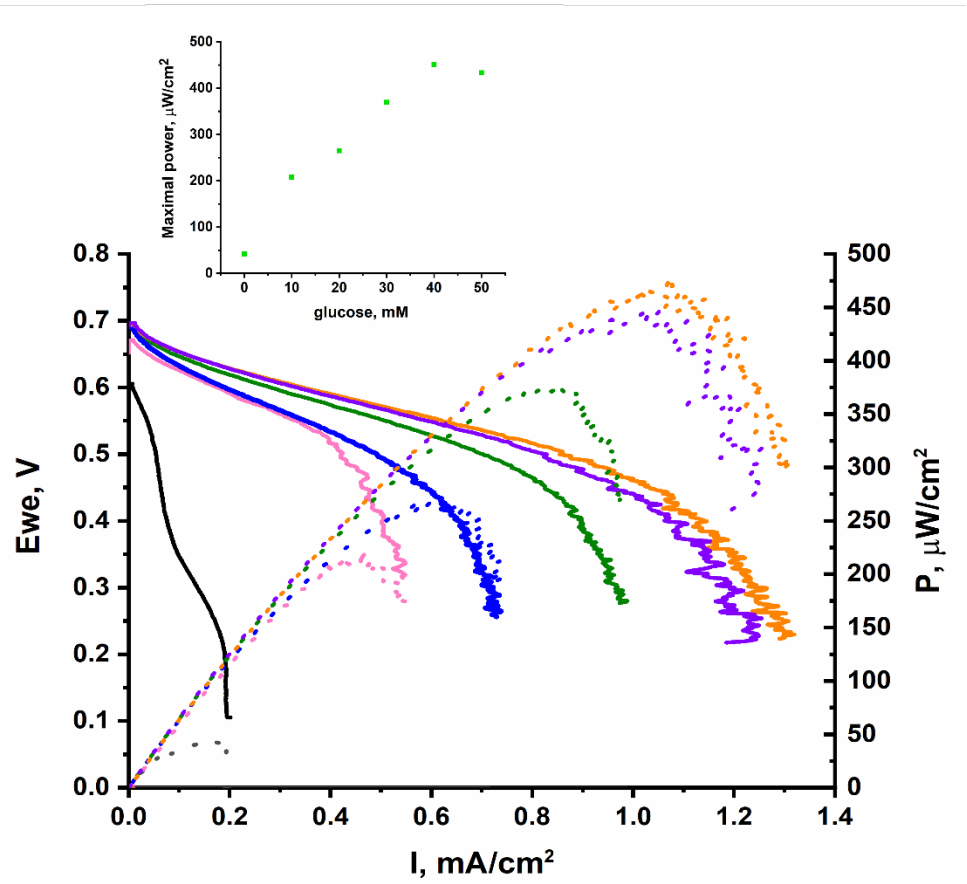


Figure S17. LSV measurements of the full bioelectrochemical cell without (black) and in the presence of 10mM (pink), 20mM (blue), 30mM (green), 40mM (orange) and 50mM (purple) of glucose in the cell solution. Measurements were performed in PB pH 6.8, 37°C under oxygen saturated atmosphere. The inset describes the maximal power output vs. glucose concentration.

7.3 Open-circuit voltage of the full-bioelectrochemical cell during time

To evaluate the full-bioelectrochemical cell stability over time, BOD based cathode and GDH based anode were coupled for cell construction. Cellulase complex (5µl per 1 ml of cell solution) and cellulose (5mg per 1 ml of cell solution) suspended in PB 0.1M, pH 6.8 served as the cell solution. The OCV potential was tested during 14 hours, at 37°C, under atmospheric conditions. The potential was recorded at a 50 sec. interval

Part 8. Comparison the obtained results with previous published work

A comparison of the obtained results with previous published work discussing energy generation of cellulosic biomass by microbial or enzyme based fuel cell technology is presented in **Table 1**.

Table S1. A comparison of the obtained results with previous published work discussing energy generation of cellulose by microbial or enzyme-based fuel cell technology

Energy source	Microbial/enzyme based cell composition	Maximal current/ power density achieved	Reference
Cellulose	*Clostridium cellulolyticum* and *Clostridium thermocellum*	130 mA per liter of bacterial suspension	4
Cellulose	Rumen bacteria consortium	55mW/m^2	5
Cellulose and carboxymethylcellulose	*Clostridium cellulolyticum* and *Geobacter sulfurreducens*	143 mW/m^2	6
Cellulose	Rice paddy field soil (*Rhizobiales* constituted the major bacteria in the MFC)	10 mW/m^2	7
Wastewater obtained from paper recycling plant	*E. cloacae ATCC 13047T* and *E. cloacae FR*	18 mW/m^2	8
Cellulose	mixed or pure cultures of *Nocardiopsis sp. KNU* and *Streptomyces enissocaesilis KNU*	188 mW/m^2	9
Carboxymethylcellulose	Cellulase, NAD-GDH, laccase	128 µW/cm^2	10
Carboxymethylcellulose	*Bacillus flexus*	267.81±1 mA/m^2	11
Cellulose	*Bacteroidetes* and *exoelectrogenic Firmicutes* (evolved during air-cathode microbial fuel cell operation. Anaerobic bacteria initially introduced to the MFC originated from cow manure)	44 mW/m^2	12
Carboxymethylcellulose	Mixed culture from waste water enriched with dialyzed cellulase	866 ± 16.6 mW/m^2	13
Carboxymethylcellulose	*Paenibacillus sp., Klebsiella sp.* and *Geobacter sulfurreducens*	1146 ±28 mW/m^2	14
Cellulosic wastewater	*LZ-P1* isolated from giant panda gut	44.05 mW/m^2	15
Cellulose	Cellulase, FAD-GDH, BOD	600 µW/cm^2	This study

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