An optimal and membrane-less glucose/oxygen enzymatic fuel cell based on a bioanode with a high coulombic efficiency and current density Minling Shao^a, Muhammad Nadeem Zafar^b, Magnus Falk^c, Roland Ludwig^d, Clemens K. Peterbauer^d, Dmitrii A. Guschin^a, Dónal Leech^e, Sergey Shleev^c, Wolfgang Schuhmann^a, Lo Gorton^b ^aAnalytische Chemie – Elektroanalytik & Sensorik, Ruhr-Universität Bochum, Universitätsstr. 150, D-44780 Bochum, Germany ^bDepartment of Analytical Chemistry/Biochemistry and Structural Biology, Lund University, P. O. Box 124, SE-221 00 Lund, Sweden ^cBiomedical Laboratory Science, Faculty of Health and Society, Malmö University, 20560 Malmö, Sweden ^dDepartment of Food Science & Technology, BOKU-University of Natural Resources and Applied Life Sciences, Muthgasse 18, A-1190 Vienna, Austria ^eSchool of Chemistry, National University of Ireland, University Road, Galway, Ireland An optimal and membrane-less glucose/oxygen enzymatic biofuel cell possessing a high coulombic efficiency and power output was fabricated and characterized. A mixture of two sugar oxidizing enzymes, viz. pyranose dehydrogenase from Agaricus meleagris (AmPDH) and the free dehydrogenase domain of cellobiose dehydrogenase expressed in E. coli from Corynascus thermophiles (rec DH_{CtCDH}) were entrapped in a low potential Os redox polymer hydrogel and used as the anode in combination with a cathode based on bilirubin oxidase from Myritgecium verrucaria entrapped in a high potential Os redox polymer hydrogel. Optimization showed that the current density for the mixed enzyme electrode could be further improved by using an engineered variant of *rec*DH_{CtCDH} with a high glucose turnover rate in combination with an Os redox polymer with a high concentration of Os-complexes as well as a low density graphite electrode. The optimal

enzymatic biofuel cell with the $AmPDH/recDH_{CrCDH}$ anode showed not only a similar maximum voltage as with the enzyme biofuel cell based on the $recDH_{CrCDH}$ only anode (0.55 V) but also a clear higher maximum power output (20 µW cm⁻²) at 300 mV of the cell voltage in air-saturated physiological buffer. Most importantly, the estimated half-life of the mixed enzyme biofuel cell can reach up to 12 h, which was apparently longer than that of a biofuel cell where the bioanaode was based on

only one single enzyme.