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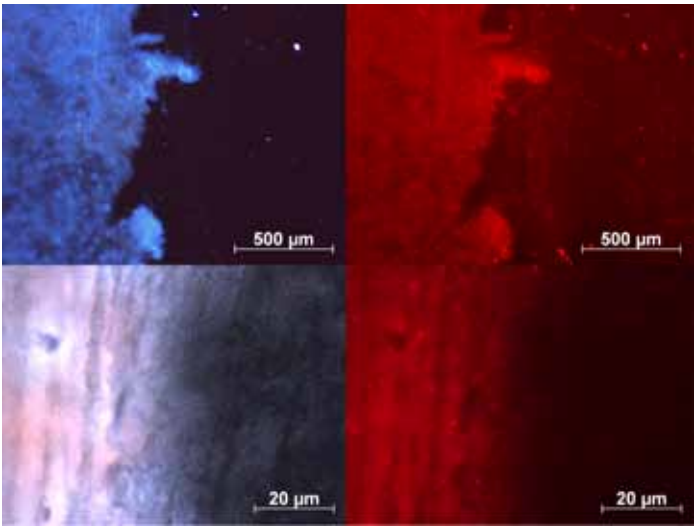
Attachment under current – biofilm formation by electroactive bacteria



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Aquatic Biotechnology in the Biofilm Centre of the Faculty of Chemistry

Attachment under current – biofilm formation by electroactive bacteria

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Abstract

Bioelectrochemical systems (BES) are hybrid systems using electroactive bacteria and electrochemical techniques. Solid electrodes serve as electron donor for or acceptor from microorganisms for the production of current and/or the generation of valuable substances. Research conducted on BES in this thesis ranged from fundamental investigations on microbial attachment to electrodes to the development of electrode materials for advanced reactor concepts.

The first part of this theses was the biochemical analysis of the extracellular polymeric substances (EPS) secreted by *G. sulfurreducens* under electroactive conditions. *G. sulfurreducens* was cultivated in MFC-mode on graphite based electrodes polarized to +400 mV vs. Ag/AgCl for 8 d. A maximum current density of 172 ± 29 µA cm^{-2} was reached after 7 d. Routine methods for the biofilm harvest and the EPS processing were established. Electroactive cultures secreted significantly more EPS compared to cells grown under standard heterotrophic conditions (fumarate respiration). With 116 pg $cell^{-1}$, the highest amount of EPS was measured for the soluble EPS fraction of *G. sulfurreducens* using anode respiration, followed by the tightly bound (18 pg $cell^{-1}$) and loosely bound (11 pg $cell^{-1}$) fractions of the EPS. Proteins were found to dominate all EPS fractions of the biofilms grown under electrochemical conditions.

The second part was the development of a membrane separated flow cell for the simultaneous electrochemical impedance spectroscopy (EIS) and confocal laser scanning microscopy (CLSM) [Stöckl et al. 2016]. A flow cell made from PEEK was constructed, using a transparent indium tin oxide electrode as working electrode. A fluorescent *S. oneidensis* was cultivated under MFC conditions. A decrease of the charge transfer (R_{CT}) from 292 kΩ to 120 kΩ was observed with an increased current of 0.52 µA cm^{-2} after 17 h of operation. The CLSM images revealed an increasing cell number of *S. oneidensis* on the WE electrode to a monolayer with 26 cells 100 μm^{-2} after 17 h under MFC conditions.

As final part a straight forward approach to synthesize magnetic electrode particles allowing the artificial fixation of electroactive bacteria was developed [Stöckl, et al. 2016, DE102014112685A, Frankfurt, Germany]. The microwave assisted synthesis of magnetite was applied for the production of the magnetic electrode particles with activated carbon ($P_{MAG/AC}$). The surface area is around 300 m^2 g^{-1} and the particle size ranges between 20 and 200 µm. Resting cells of *S. oneidensis* attached to a maximum concentration of $8 \cdot 10^{10}$

± 3 · 10^9 resting cells g^{-1} $P_{MAG/AC}$. Electrochemical examination revealed that magnetically immobilized $P_{MAG/AC}$ showed a capacitive current response during cyclic voltammetry. Linear sweep voltammetry indicated that particles were stable down to a potential of –680 mV vs. Ag/AgCl.

Key words: bioelectrochemical systems, anode respiration, extracellular polymeric substances, impedance spectroscopy, confocal laser scanning microscopy, magnetic electrode particles

Zusammenfassung

Bioelektrochemische System (BES) sind Hybridsysteme, in denen elektroaktive Bakterien und Methoden aus der Elektrochemie kombiniert werden. Elektroden dienen dabei als Elektronendonor oder Akzeptor für Mikroorganismen bei der Stromproduktion und/oder der Herstellung von Biokraft-stoffen oder Prozesschemikalien. Der Fokus dieser Arbeit erstreckt sich von den Grundlagen des mikrobiellen Anheftens bis zur Entwicklung von Elektrodenmaterialien für komplexe Reaktorkonzepte.

Im ersten Teil wurde eine biochemische Analyse der extrazellulären polymeren Substanzen (EPS) von *G. sulfurreducens* Biofilmen unter elektroaktiven Bedingungen durchgeführt. *G. sulfurreducens* wurde in mikrobiellen Brennstoffzellen bei +400 mV vs. Ag/AgCl für 8 d kultiviert. Maximale Stromdichten von 172 ± 29 µA cm^{-2} wurden nach 7 d erreicht. Die elektroaktiven Zellkulturen produzierten deutlich mehr EPS als Zellen, die bei heterotrophen Standardbedingungen (Fumarat-Atmung) wachsen. Die höchste Konzentration an EPS wurde mit 116 pg $Zelle^{-1}$ in der löslichen Fraktion der EPS der elektroaktiven Kultur (Anoden-Atmung) gefunden, gefolgt von den kapsulären (18 pg $Zelle^{-1}$) und den kolloidalen (11 pg $Zelle^{-1}$) EPS Fraktionen. Proteine stellten dabei in allen EPS Fraktionen die größte Hauptgruppe der EPS Komponenten dar.

Die Entwicklung einer durch eine Membran geteilte Durchflusszelle zur Erfassung des mikrobiellen Wachstums mittels simultaner elektrochemischer Impedanzspektroskopie (EIS) und konfokalen Laser Scanning Mikroskopie (CLSM) bildet den zweiten Teil der Arbeit [Stöckl et al. 2016]. Die Durchflusszelle wurde mit einer transparenten Indium Zinnoxid Elektrode aus PEEK hergestellt. Ein selbstfluoreszierender *S. oneidensis* Stamm wurde unter MFC Bedingungen in der Durchflusszelle kultiviert. Der Ladungsdurchtrittswiderstand (R_{CT}) verringerte sich im Laufe der Messung von 292 kΩ auf 120 kΩ und eine maximale Stromdichte von 0,52 µA cm^{-2} wurde nach 17 h gemessen. Mittels CLSM wurde eine einschichtige Zelllage mit einer Dichte von 26 Zellen 100 μm^{-2} detektiert.

Letzter Teil dieser Arbeit war die Entwicklung von magnetischen Elektrodenpartikeln zur Fixierung von elektroaktiven Bakterien [Stöckl, et al. 2016, DE102014112685A, Frankfurt, Germany]. Diese magnetischen Partikel ($P_{MAG/AC}$) wurden mittels Mikrowellen-assistierter Magnetisierung von Aktivkohle hergestellt. $P_{MAG/AC}$ Partikel haben eine Oberfläche von 300 m^2 g^{-1}, die Größenverteilung liegt zwischen 20 und 200 µm. Ruhende *S. oneidensis* Zellen haften mit einer maximalen Zelldichte von $8 \cdot 10^{10} \pm 3 \cdot 10^9$ ruhenden Zellen g^{-1} an den

Partikeln an. Elektrochemische Messungen mit zyklischer Voltammetrie zeigten hauptsächlich kapazitives Verhalten der magnetisch immobilisierten $P_{MAG/AC}$. Linear sweep Voltammetrie konnte darüber hinaus zeigen, dass die Partikel bis zu einem Potential von –680 mV vs. Ag/AgCl stabil sind.

Schlüsselwörter: Bioelektrochemische Systeme, Anodenatmung, extrazelluläre polymere Substan-zen, Impedanzspektroskopie, Konfokale Laser Scanning Mikroskopie, magnetische Elektrodenpartikel

Preface

The mayor part of this study was accomplished at the

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2. Gutachter der Arbeit: Dr. Klaus-Michael Mangold

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Table of Contents

List of Abbreviations

AC	activated carbon
BCA	bicinchoninic acid
BES	Bioelectrochemical system
BET	Brunauer Emmett Teller
BSA	bovine serum albinum
C	control
CE	counter electrode
CLSM	confocal laser scanning microscopy
CM	cellular membrane
ConA	Concanavalin A
CP	cytoplasm
CPE	constant phase element
CV	cyclic voltammetry
CW	18-crown-6-ether
DAPI	4',6-diamidino-2-phenylindole
DET	direct electron transfer
DIR	dissimilatory iron-reducing
DOW	DOWEX®
eDNA	extracellular DNA
EDTA	ethylen diamin tetra acetic acid
EET	extracellular electron transfer
EFM	epifluorescence microscopy
eGFP	enhanced green fluorescent protein
EIS	electrochemical impedance spectroscopy
EPS	extracellular polymeric substances
ER	extraction reagent
EXP	exponential
FL	flavin
G. sulfurreducens	*Geobacter sulfurreducens*
GC	Glassy carbon
IET	indirect electron transfer

ITO	indium tin oxide
LB	loosely bound
LM	lactate medium
LSV	linear sweep voltammetry
MEC	microbial electrolysis cell
MEM	membrane
MET	mediated electron transfer
MES	microbial electrosynthesis
MIC	microbiologically influenced corrosion
MFC	microbial fuel cell
Mtr	metal reducing
OCP	open circuit potential [V]
OD_{600}	optical density at λ = 600 nm
OM	outer membrane
Omc	outer membrane cytochrome
ORR	oxygen reduction reaction
PBS	phosphate buffered saline
PEEK	poly ether ether ketone
P_{MAG}	magnetic particles without activated carbon
$P_{MAG/AC}$	magnetic particles with incorporated activated carbon
PP	periplasm
R_{CT}	charge transfer resistance [Ω]
RE	reference electrode
R_U	solution resistance [Ω]
S. oneidensis	*Shewanella oneidensis*
SCB	sodium chloride buffer
SEM	scanning electron microscopy
SHE	standard hydrogen electrode
SOL	soluble
STAT	stationary
TCA	tricarboxylic acid
TB	tightly bound

US	ultra sound
WE	working electrode
W_D	Warburg diffusion
Z_{IMAG}	imaginary part of the impedance [Ω]
Z_{REAL}	real part of the impedance [Ω]
Z_{TOT}	total impedance [Ω]