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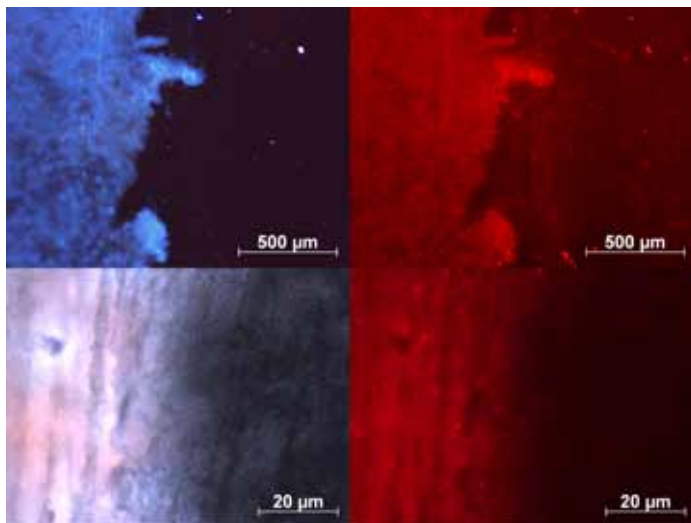
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Attachment under current –  
biofilm formation by electroactive bacteria



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**Attachment under current –  
biofilm formation by electroactive bacteria**

**Dissertation**

for attainment of the academic degree of

**Doktor der Naturwissenschaften**

**- Dr. rer. nat -**

presented by

**Markus Stöckl**

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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  $172 \pm 29 \mu\text{A cm}^{-2}$  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  $116 \text{ pg cell}^{-1}$ , the highest amount of EPS was measured for the soluble EPS fraction of *G. sulfurreducens* using anode respiration, followed by the tightly bound ( $18 \text{ pg cell}^{-1}$ ) and loosely bound ( $11 \text{ pg cell}^{-1}$ ) 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 k $\Omega$  to 120 k $\Omega$  was observed with an increased current of  $0.52 \mu\text{A cm}^{-2}$  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  $100 \mu\text{m}^{-2}$  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<sub>MAG/AC</sub>). The surface area is around  $300 \text{ m}^2 \text{ g}^{-1}$  and the particle size ranges between 20 and 200  $\mu\text{m}$ . Resting cells of *S. oneidensis* attached to a maximum concentration of  $8 \cdot 10^{10}$

$\pm 3 \cdot 10^9$  resting cells  $\text{g}^{-1}$   $\text{P}_{\text{MAG/AC}}$ . Electrochemical examination revealed that magnetically immobilized  $\text{P}_{\text{MAG/AC}}$  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 Elektronendonator 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  $172 \pm 29 \mu\text{A cm}^{-2}$  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  $116 \text{ pg Zelle}^{-1}$  in der löslichen Fraktion der EPS der elektroaktiven Kultur (Anoden-Atmung) gefunden, gefolgt von den kapsulären ( $18 \text{ pg Zelle}^{-1}$ ) und den kolloidalen ( $11 \text{ pg Zelle}^{-1}$ ) 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 k $\Omega$  auf 120 k $\Omega$  und eine maximale Stromdichte von  $0,52 \mu\text{A cm}^{-2}$  wurde nach 17 h gemessen. Mittels CLSM wurde eine einschichtige Zelllage mit einer Dichte von 26 Zellen  $100 \mu\text{m}^{-2}$  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  $300 \text{ m}^2 \text{ g}^{-1}$ , die Größenverteilung liegt zwischen 20 und 200  $\mu\text{m}$ . Ruhende *S. oneidensis* Zellen haften mit einer maximalen Zelldichte von  $8 \cdot 10^{10} \pm 3 \cdot 10^9$  ruhenden Zellen  $\text{g}^{-1}$  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 Substanzen, Impedanzspektroskopie, Konfokale Laser Scanning Mikroskopie, magnetische Elektrodenpartikel

## Preface

### **The mayor part of this study was accomplished at the**

DECHEMA-Forschungsinstitut  
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60486 Frankfurt am Main, Germany  
Under the supervision of  
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### **The biochemical EPS analysis was conducted at the**

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With the permission of  
Prof. Dr. Rainer Meckenstock  
Head of the Aquatic Microbiology, Biofilm Centre

1. Gutachter der Arbeit: Prof. Dr. Wolfgang Sand
2. Gutachter der Arbeit: Dr. Klaus-Michael Mangold
3. Gutachterin der Arbeit: Prof. Dr. Anna Gorbushina



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## Table of Contents

<b>1 General introduction.....</b>	<b>1</b>
1.1 Future energy management.....	2
1.2 Bioelectrochemical systems .....	3
1.2.1 Microbial fuel cells.....	5
1.2.2 Microbial electrosynthesis.....	7
1.3 Extracellular electron transfer mechanisms.....	8
1.3.1 Mediated electron transfer.....	10
1.3.2 Direct electron transfer mechanism .....	11
1.4 Biofilm formation.....	12
<b>2 Motivation and aims .....</b>	<b>15</b>
<b>3 EPS analysis of <i>Geobacter sulfurreducens</i> biofilms .....</b>	<b>17</b>
3.1 Introduction.....	17
3.1.1 <i>Geobacter sulfurreducens</i> .....	17
3.1.2 <i>G. sulfurreducens</i> and its biofilms in bioelectrochemical systems.....	18
3.1.3 EPS composition and analysis .....	19
3.2 Materials and Methods .....	22
3.2.1 Microorganism.....	22
3.2.1.1 Growth media .....	22
3.2.1.2 Heterotrophic cultivation of <i>G. sulfurreducens</i> .....	23
3.2.2 Laboratory electrochemical H-cell.....	23
3.2.3 Electrochemical cultivation of <i>G. sulfurreducens</i> .....	25
3.2.4 Evaluation of the extraction reagent for EPS.....	26
3.2.4.1 Total protein concentration – bicinchoninic acid assay.....	26
3.2.4.2 Correlation of total protein concentration and OD <sub>600</sub> .....	27
3.2.4.3 Extraction reagent evaluation.....	28

3.2.5 EPS harvest and extraction .....	28
3.2.6 EPS harvest of heterotrophically cultivated <i>G. sulfurreducens</i> (controls).....	31
3.2.7 Biofilm staining and electrode imaging after harvesting.....	31
3.2.7.1 Fluorescence staining and epifluorescence microscopy imaging.....	31
3.2.7.2 Scanning electron microscopy imaging.....	32
3.2.8 Biochemical EPS analysis.....	32
3.2.8.1 Protein determination.....	33
3.2.8.2 Carbohydrate determination .....	33
3.2.8.3 Uronic acid determination .....	33
3.2.8.4 Lipid determination.....	34
3.2.8.5 eDNA determination .....	34
3.3 Results.....	35
3.3.1 Growth curves for heterotrophic <i>G. sulfurreducens</i> cultivation .....	35
3.3.2 Electrochemical cultivation.....	36
3.3.3 Biofilm harvesting and EPS extraction .....	37
3.3.3.1 Evaluation of the EPS extraction reagent .....	38
3.3.3.1.1 Total protein concentration and cell lysis .....	38
3.3.3.1.2 Extraction reagent evaluation .....	39
3.3.3.2 Biofilm harvest and subsequent imaging.....	41
3.3.3.2.1 Fluorescence staining and EFM imaging of anode respiring biofilm .....	41
3.3.3.2.2 SEM images of anode respiring <i>G. sulfurreducens</i> biofilm.....	44
3.3.4 Biochemical EPS analysis.....	45
3.3.4.1 Total protein concentration in EPS .....	45
3.3.4.2 Total carbohydrate concentrations in EPS.....	46
3.3.4.3 Total uronic acid concentration in EPS .....	47
3.3.4.4 Total lipid concentration in EPS .....	48

3.3.4.5 Total eDNA concentration in EPS .....	49
3.3.4.6 Summary for analysis on EPS composition of <i>G. sulfurreducens</i> .....	50
3.4 Discussion and conclusions .....	51
3.4.1 Electrochemical cultivation in H-cells .....	51
3.4.2 Extraction reagent evaluation .....	52
3.4.3 Biofilm harvest .....	53
3.4.4 Biochemical EPS analysis .....	53
<b>4 Combination of EIS and CLSM for simultaneous biofilm monitoring .....</b>	<b>57</b>
4.1 Introduction .....	57
4.1.1 Monitoring of electroactive biofilms .....	57
4.1.2 Electrochemical impedance spectroscopy .....	59
4.1.3 Confocal laser scanning microscopy .....	60
4.1.4 <i>Shewanella oneidensis</i> .....	61
4.1.4.1 Strain description .....	61
4.1.4.2 <i>S. oneidensis</i> in bioelectrochemical systems .....	62
4.2 Materials and Methods .....	63
4.2.1 Microorganism .....	63
4.2.1.1 Growth media .....	63
4.2.1.2 Heterotrophic cultivation of <i>S. oneidensis</i> .....	64
4.2.1.3 <i>Shewanella oneidensis</i> MR-1: eGFP gene introduction .....	64
4.2.2 Flow cell construction .....	64
4.2.2.1 Final PEEK flow cell .....	65
4.2.2.2 Insertion of a Ag/AgCl reference electrode in the flow cell .....	66
4.2.3 Electrochemical flow cell characterization .....	67
4.2.3.1 Cyclic Voltammetry with $\text{Fe}(\text{CN})_6^{4-}$ .....	67
4.2.3.2 Electrochemical Impedance Spectroscopy with $\text{Fe}(\text{CN})_6^{4-}$ .....	67



4.2.4 Simultaneous Electrochemical Impedance Spectroscopy and Confocal Laser Scanning Microscopy .....	68
4.2.4.1 Current and EIS Measurements .....	68
4.2.4.2 Confocal Laser Scanning Microscopy .....	69
4.2.5 Application as MFC with inserted Ag/AgCl reference electrode .....	70
4.3 Results.....	71
4.3.1 Aerobic heterotrophic growth by <i>S. oneidensis</i> .....	71
4.3.2 Flow cell construction .....	72
4.3.2.1 Prototypes .....	72
4.3.2.2 Flow cell made from PEEK.....	74
4.3.3 Electrochemical flow cell characterization .....	75
4.3.3.1 Cyclic Voltammetry with $\text{Fe}(\text{CN})_6^{4-}$ .....	75
4.3.3.2 Electrochemical Impedance Spectroscopy .....	76
4.3.4 Simultaneous Electrochemical Impedance Spectroscopy and Confocal Laser Scanning Microscopy in Microbial Fuel Cell Mode.....	77
4.3.4.1 Current and electrochemical impedance spectroscopy measurements .....	77
4.3.4.2 Confocal laser scanning microscopy images .....	79
4.3.5 Application of flow cell as MFC with inserted Ag/AgCl reference electrode .....	82
4.4 Discussion and Conclusions .....	84
4.4.1 Flow cell construction .....	84
4.4.2 Simultaneous Electrochemical Impedance Spectroscopy and Confocal Laser Scanning Microscopy in the Microbial Fuel Cell Mode.....	85
<b>5 Development of magnetic electrode particles.....</b>	<b>89</b>
5.1 Introduction.....	89
5.1.1 Electrochemical fluidized bed reactor .....	89
5.1.2 Magnetic particles in fluidized bed reactors .....	90
5.1.3 Artificial immobilization of electroactive bacteria .....	91

5.2 Materials and Methods .....	94
5.2.1 Production of magnetic electrode particles .....	94
5.2.1.1 Electrode particles without activated carbon P <sub>MAG</sub> .....	94
5.2.1.2 Electrode particles with activated carbon P <sub>MAG/AC</sub> .....	95
5.2.2 SEM imaging of prepared electrode particles .....	96
5.2.3 Combination of <i>S. oneidensis</i> and magnetic electrode particles.....	97
5.2.3.1 Qualitative evaluation of surface attachment .....	97
5.2.3.2 Time dependent attachment of <i>S. oneidensis</i> to P <sub>MAG/AC</sub> particles.....	98
5.2.4 Surface area determination with BET .....	99
5.2.5 Electrochemical characterization.....	99
5.2.5.1 Cyclic voltammetry of magnetically immobilized P <sub>MAG/AC</sub> particles .....	99
5.2.5.2 Cathodic linear sweep voltammetry of P <sub>MAG/AC</sub> in a stirred fluidized bed reactor .....	100
5.3 Results.....	102
5.3.1 Particle preparation .....	102
5.3.1.1 SEM imaging of P <sub>MAG</sub> and P <sub>MAG/AC</sub> particles .....	102
5.3.2 Fixation of resting cells of <i>S. oneidensis</i> on magnetic electrode particles .....	105
5.3.2.1 Qualitative evaluation of the attachment by microscopy .....	105
5.3.2.2 Time dependent attachment of <i>S. oneidensis</i> to P <sub>MAG/AC</sub> particles.....	109
5.3.3 Surface determination via BET measurement.....	110
5.3.4 Electrochemical characterization.....	111
5.3.4.1 CV with particles magnetically attracted to an electrode .....	111
5.3.4.2 Cathodic LSV of P <sub>MAG/AC</sub> particles .....	113
5.4 Discussion and conclusions .....	115
5.4.1 Production of magnetic electrode particles .....	115
5.4.2 Fixation of electroactive resting cells on particles.....	117
5.4.3 Electrochemical characterization of particles.....	117

<b>6 Summary and outlook.....</b>	<b>121</b>
<b>7 References.....</b>	<b>125</b>
<b>8 Declaration .....</b>	<b>144</b>

**List of Abbreviations**

AC	activated carbon
BCA	bicinchoninic acid
BES	Bioelectrochemical system
BET	Brunauer Emmett Teller
BSA	bovine serum albumin
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
<i>G. sulfurreducens</i>	<i>Geobacter sulfurreducens</i>
GC	Glassy carbon
IET	indirect electron transfer

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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 <sub>600</sub>	optical density at $\lambda = 600$ nm
OM	outer membrane
Omc	outer membrane cytochrome
ORR	oxygen reduction reaction
PBS	phosphate buffered saline
PEEK	poly ether ether ketone
P <sub>MAG</sub>	magnetic particles without activated carbon
P <sub>MAG/AC</sub>	magnetic particles with incorporated activated carbon
PP	periplasm
R <sub>CT</sub>	charge transfer resistance [ $\Omega$ ]
RE	reference electrode
R <sub>U</sub>	solution resistance [ $\Omega$ ]
<i>S. oneidensis</i>	<i>Shewanella oneidensis</i>
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
$Z_{\text{IMAG}}$	imaginary part of the impedance [ $\Omega$ ]
$Z_{\text{REAL}}$	real part of the impedance [ $\Omega$ ]
$Z_{\text{TOT}}$	total impedance [ $\Omega$ ]