ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECTS OF ANTIBIOTICS AND HORMONES ON ELECTRICITY GENERATION USING MICROBIAL FUEL CELLS

Ph.D. Thesis by
Sevil AKTAN

Department : Environmental Engineering

Programme : Environmental Biotechnology

JUNE 2011

ISTANBUL TECHNICAL UNIVERSITY ★ INSTITUTE OF SCIENCE AND TECHNOLOGY

EFFECTS OF ANTIBIOTICS AND HORMONES ON ELECTRICITY GENERATION USING MICROBIAL FUEL CELLS

Ph.D. Thesis by
Sevil AKTAN
(501042802)

Date of Submision : **9 February 2011**
Date of Defence Examination : **3 June 2011**

Thesis Supervisor : **Prof. Dr. Emine UBAY ÇOKGÖR (ITU)**
Co-Supervisor : **Prof. Dr. Fahrettin GÜCİN (FU)**
Members of the Examining Committee : **Prof. Dr. Orhan İNCE (ITU)**
Prof. Dr. İzzet ÖZTÜRK (ITU)
Yrd. Doç. Dr. M. Burcu IRMAK YAZICIOĞLU (HU)
Doç. Dr. Bülent MERTOĞLU (MU)
Yrd. Doç. Dr. Mahmut ALTINBAŞ (ITU)

JUNE 2011

İSTANBUL TEKNİK ÜNİVERSİTESİ ★ FEN BİLİMLERİ ENSTİTÜSÜ

MİKROBİYAL YAKIT HÜCRELERİNDE KULLANILAN ANTİBİYOTİK VE HORMONLARIN ELEKTRİK ÜRETİMİ ÜZERİNE ETKİLERİ

DOKTORA TEZİ

Sevil AKTAN
(501042802)

Tezin Enstitüye Verildiği Tarih : **9 Şubat 2011**
Tezin Savunulduğu Tarih : **3 Haziran 2011**

Tez Danışmanı: **Prof. Dr. Emine UBAY ÇOKGÖR (İTÜ)**
Eş Danışman : **Prof. Dr. Fahrettin GÜCİN (FÜ)**
Diğer Jüri Üyeleri : **Prof. Dr. Orhan İNCE (İTÜ)**
Prof. Dr. İzzet ÖZTÜRK (İTÜ)
Yrd. Doç. Dr. M. Burcu IRMAK YAZICIOĞLU (HÜ)
Doç. Dr. Bülent MERTOĞLU (MÜ)
Yrd. Doç. Dr. Mahmut ALTINBAŞ (İTÜ)

HAZİRAN 2011

FOREWORD

I would like to express my deep appreciation to my dissertation advisor, Prof. Dr. Emine Ubay Çokgör. I would like to thank to her for the scientific guidance and encouragement. I would also like to thank to her for making me feel free to call every time. I would like to thank my co-advisor, Prof. Dr. Fahrettin Gücin. I will always grateful to him for his support, understanding and guidance during the project.

Many thanks to committee members, Prof. Dr. Orhan İnce and Prof Dr. İzzet Öztürk, Asistant Prof. Dr. Burcu Irmak Yazıcıoğlu, Associate Prof. Dr. Bülent Mertoğlu and Asistant Prof. Dr. Mahmut Altınbaş for their contributions and for their time spent to read the reports and thesis. Thanks to Prof. Dr. Ayhan Bozkurt and Associate Prof. Dr. Nurullah Arslan for their guidance and help during this study. I would like to thank Prof Dr. Ali Ata (Gebze Institute of Technology) for provide electrodes.

I would like to express my deep appreciation to Associate Prof. Dr. Barış Çallı for his scientific guidance and help. I am also grateful to Assistant Prof. Dr. İrem Uzonur and Assistant Prof. Dr. Ayşe İnci İşli for her help and friendship. Special thanks to Elif Banu Gençsoy, Cemile Ümran Ceylan, Işılay Ulusoy, Pelin Çavdar and Elif Yılmaz for their help during the thesis.

The financial support of this study by the Turkish State Planning Organization (DPT), ITU Scientific Research Project Unit and Fatih University Scientific Research Project Unit was gratefully acknowledged.

I am greateful to my all family, especially my mother and father, my brothers and their wifes and chidren, my aunts for their love, patience, pray and supporting me during this study.

I dedicate this thesis to my dear mother and my nephews Yavuz Selim Aktan, Mehmet Akif Aktan and my niece Serra Aktan.

3 JUNE 2011

Sevil AKTAN

Environmental Biotechnology

TABLE OF CONTENTS **Page**

FOREWORD .... **v**
TABLE OF CONTENTS .... **vii**
ABBREVIATIONS .... **xi**
LIST OF TABLES .... **xiii**
LIST OF FIGURE .... **xv**
1. INTRODUCTION **1**
1.1 Meaning and Significance of the Thesis 1
1.2 Aim and scope 1
2. LITERATURE SURVEY **3**
2.1 Definition of microbial fuel cell 3
2.2 Mediator Microbial Fuel Cell 4
2.3 Mediator-less Microbial Fuel Cell 5
2.4 How do Microbial Fuel Cells work? 6
2.5 Types of Microbial Fuel Cell (MFC) 8
2.5.1 Two-chambered MFC 8
2.5.2 Single Chambered MFC (SCMFC) 9
2.5.3 Stacked Microbial Fuel Cell 11
2.6 Factors of performance of Microbial Fuel Cell 12
2.6.1 Effects of electrode materials 12
2.6.1.1 Anode materials 13
2.6.1.2 Cathode materials 13
2.6.2 Effects of operating conditions 14
2.6.2.1 Anodic chamber 14
2.6.2.2 Cathodic chamber 14
2.6.2.3 Effects of PEM (Proton Exchange Membrane) 15
2.6.2.4 Effects of ionic strength, anode-cathode distance and temperature 15
2.7 Fundamentals of voltage generation 16
2.7.1 Anode potential 20
2.7.2 Cathode Potential 20
2.8 Fundamentals of power generation 21
2.8.1 Power output normalized by surface area 22
2.8.2 Power output normalized by volume 22
2.9 Coulombic Efficiency 22
2.10 Ohmic, activation, bacterial metabolic and mass trasport losses of MFCs 23
2.11 Properties of genus *Shewanella* 25
2.12 Performance of the MFCs 28
2.13 Uses of microbial fuel cells 32

2.13.1 Electricity generation 32
2.13.2 Wastewater treatment 33
2.13.3 Biosensor 34
2.14 Xenobiotics in aquatic environment 35
2.14.1 Antibiotics in Aquatic Environment 37
2.14.1.1 Source 37
2.14.1.2 Modes of Action 38
2.14.1.3 Occurence 41
2.14.1.4 Elimination (Fate) 42
2.14.1.5 Effects 44
2.14.2 Estrogens (Hormones) in Aquatic Environment 47
2.14.2.1 Sources 48
2.14.2.2 Occurence and elimination (fate) 49
2.14.2.3 Effects 56
3. MATERIALS AND METHODS 59
3.1 Pure culture (*Shewanella Putrefaciens)* and two chambered MFC experiments 59
3.1.1 Two chambered MFC 59
3.1.2 Electrode Materials For Two Chambered MFC 59
3.1.3 Medium for Aerobic and Anoxic Growth of *Shewanella Putrefaciens* 59
3.2 Mixed culture in Single Chambered Microbial Fuel Cell 63
3.2.1 Construction of Single Chambered Flat-1-Microbial Fuel Cell (SCF-1-MFC) 63
3.2.2 Construction of Single Chambered Tubular MFC (SCTMFC) 63
3.2.3 Construction of Single Chambered Flat-2-Microbial Fuel Cell (SCF-2-MFC) 64
3.3 Inoculum of SCF-1-MFC, SCTMFC and SCF-2-MFC. 65
3.4 Medium of SCF-1-MFC, SCTMFC and SCF-2-MFC 66
3.5 Antibiotics and hormones 67
3.6 Electrochemical Measurement (Voltage and Current Measurements of the MFC) 67
3.7 Calculation of current and coulombic efficiency for experiments of antibiotics and hormones. 68
3.8 Chemical Analysis 68
3.8.1 Sampling and COD analysis 68
3.8.2 pH measurement 68
3.9 Scanning electron micrograph (SEM) Analysis 68
4. RESULTS AND DISCUSSION 69
4.1 Experiments with *Shewanella Putrefaciens* 69
4.1.1 Growth curves of *Shewanella Putrefaciens* 69
4.1.2 Open circuit voltage of anoxically grown *Shewanella putrefaciens* in two chambered MFC 73
4.1.3 Open circuit voltage of aerobically grown *Shewanella Putrefaciens* 76
4.1.4 Power generation of two chambered MFC 77

4.1.5 Open circuit voltage from two chambered MFC (addition influent wastewater of brewery industry using *S.putrefaciens*) 77
4.2 Experiments with mixed culture using single chambered flat-1-MFC......................77
4.2.1 Comparison of currents using different external resistance in single chambered flat-1- MFC 77
4.3 Scanning electron micrograph (SEM) analysis..80
4.4 Voltage measurement using mixed culture microorganisms for single chambered flat-2-MFC...81
4.4.1 Carbon cloth cathode containing 1 mg/cm2 platinum 81
4.4.2 Activated carbon cloth cathode 82
4.5 Antibiotic and Hormone Experiments for SCF-2-MFC 83
4.5.1 Acetate only feeding.. 83
4.5.2 Antibiotics 83
4.5.2.1 Erytromicin (ERY) Antibiotic.. 84
4.5.2.2 Sulfamethoxazole (SMX) Antibiotic 86
4.5.2.3 Tetracycline (TC or TETRA) antibiotic.. 89
4.5.3 Estrogens (Hormones).. 93
4.5.3.1 Estrone (E1) Hormone 93
4.5.3.2 17 β-Estradiol (E2) hormone.. 96
4.5.3.3 Estriol (E3) hormone 99
4.5.3.4 17-α Ethinylestradiol(EE2) 102
5. CONCLUSION 105
REFERENCES 109
CURRICULUM VITAE 119

ABBREVIATIONS

A	: Ampere
b_e	: The number of electrons exchanged per mole of oxygen
BOD	: Biochemical Oxygen Demand (mg/L)
C	: Coulomb which is determined by the number of electron exchange in the reaction
CE	: Coulombic Efficiency (%)
CEM	: Cation Exchange Membrane
COD	: Chemical Oxygen Demand (mg/L)
DO	: Dissolved Oxygen concentration (mg/L)
E^0	: The standard cell electromotive force(Volt)
E_{cell}	: Cell Voltage(Volt)
E_{emf}:	: The maximum Electromotive Force(Volt)
E1	: Estrone
E2	: 17 β-Estradiol
E3	: Estriol
EE2	: 17-α Ethinylestradiol
ERY	: Erytromycin
F	: Faraday's constant(C/mol)
G	: Gibbs Free Energy
I	: Current (ampere)
IUPAC	: International Union of Pure and Applied Chemistry
M	: The molecular weight (g/mol)
mA	: Milliamper
mL	: Milliliter
mM	: Milimolar
MFC	: Microbial Fuel Cell
n	: The number of electrons per reaction mol
NHE	: Normal Hydrogen Electrode
OCV	: Open-Circuit Voltage (Volt)
P	: Power (Watt)
Pt	: Platinum
PC	: Polarization Curve
PEM	: Proton Exchange Membrane
R	: The universal gas constant (Joule/ rnol / K)
Rex	: External Resistance(ohm,Ω)
Rin	: Internal Resistance (ohm, Ω)
RVC	: Reticulated Vitreous Carbon
SCF-1-MFC	: Single Chamber Flat-1-Microbial Fuel Cell
SCF-2-MFC	: Single Chamber Flat-2-Microbial Fuel Cell
SCT-MFC	: Single Chamber Tubular Microbial Fuel Cell
SMX	: Sulfamethoxazole
SHE	: Standart Hydrogen Electrode
TC	: Tetracycline

TCMFC :Two chambered microbial fuel cells
V : Voltage (Volt)
μL : mikroliter

LIST OF TABLES **Page**

Table 2.1: Anode and Cathode potentials for different anodic and cathodic reactions. $E^{/}$ or $E^{0/}$ values are adjusted for pH=7 at 298 K except as indicated 19
Table 2.2: The measure of current and power generation for different pure cultures, compound, electrodes, with or without mediator in literature.................. 25
Table 2.3: Different substrates used in MFCs and the maximum current produced. 30
Table 2.4: MSDS table for erythromycin, sulfomethoxazole and tetracycline......... 40
Table 4.1: MFC performance by ERY addition... 85
Table 4.2: MFC performance by SMX addition 87
Table 4.3: MFC performance by TC addition.. 90
Table 4.4: MFC performance by E1 addition 94
Table 4.5: MFC performance by E2 addition 97
Table 4.6: MFC performance by E3 addition 100
Table 4.7: MFC performance by EE2 addition 103

LIST OF FIGURE **Page**

Figure 2.1: Schematic diagram of a typical two-chambered (A) and single-chambered (B) microbial fuel cells 4
Figure 2.2: Model for various compounds serving as electron shuttles between a bioelectrochemically active microorganism and the anode 5
Figure 2.3: Summary of components proposed to be involved in the electron transport from cells to the anode in MFCs using metal reducing microorganisms (Geobacter species) (Figure drawn with modifications after Lovley et al., 2004). 6
Figure 2.4: Respiratory chain shows how the voltage that could be recovered in a microbial fuel cell (MFC) is dependent on where electrons exit the chain of respiratory enzymes (Logan and Regan, 2006) 8
Figure 2.5: Example of an H-type microbial fuel cell 9
Figure 2.6: Schematics of a cylindrical SC-MFC containing eight graphite rods as an anode in a concentric arrangement surrounding a single cathode. ((A) drawn with modifications after Liu et al., 2004. (B) drawn to illustrate a photo in Liu et al., 2004.) (C) Photo of laboratory-scale prototype of the SCMFC used to generate electricity from wastewater 10
Figure 2.7: (a) A schematic and (b) a photograph of a single-chamber microbial fuel cell. The cathode is exposed to air on one side and the solution containing the biodegradable substrate is on the other side. The anode chamber containing the exoelectrogenic bacteria is sealed off from oxygen (Logan and Regan 2006) 11
Figure 2.8: Stacked MFCs consisting of six individual units with granular graphite anode (They are joined in one reactor block (drawn to illustrate a photo in Aelterman et al.,2006). 12
Figure 2.9: Pathway of environmental exposure to drugs consumed in human and veterinary medicine (illustrated from Diaz-Cruz et al., 2003) 37
Figure 2.10: Modes of action of some antibiotics 39
Figure 2.11: The structure of a)17 beta Estradiol b)Estrone c)17 alpha Ethynylestradiol d) Estriol 48
Figure 3.1: Sartorios Certomat IS cooling rotary shaker 60
Figure 3.2: Controlled Atmosphere Chamber(PLAS LABS USA) 60
Figure 3.3: Anoxically growth of S.putrefaciens in Atmosphere Controlled Chamber 61
Figure 3.4: Hettich Rotina 420 R Centrifuge 61
Figure 3.5: Used two chambered MFC in this study 62
Figure 3.6: Used single chambered Flat-1-MFC in this study 63
Figure 3.7: Single Chambered Tubular MFC 64
Figure 3.8: Photographs of anode side (front), cathode side (back) and side of SCF-2-MFC 65
Figure 3.9: Photograph of SCF-2-MFC connecting multimeter system 65
Figure 4.1: Aerobic Growth Curve of *S.putrefaciens* 69
Figure 4.2: Photograph of *S.putrefaciens* colonies at different dilution on LB agar 70

Figure 4.3: Viability Curve of *S.putrefaciens* 70
Figure 4.4: Comparison of *S.putrefaciens* Growth Curve in single-double-triple strenght LB(MILLER) broth 71
Figure 4.5: Comparison of *S.putrefacience* Viability Curve in single-double-triple strenght LB (MILLER) broth 72
Figure 4.6: Comparison of *S.putrefaciens* growth curve in different amount of pure culture 72
Figure 4.7: Comparison of *S.putrefacience* viability curve in single-double amount of pure culture 72
Figure 4.8: Open Circuit Voltage (OCV) from two chambered MFC containing S.putrefaciens (addition of 10 mM acetate and 1250 ml *S.putrefaciens*) 73
Figure 4.9: Open Circuit Voltage (OCV) from two chambered MFC containing *S.putrefaciens* (addition of 10 mM acetate-3750 ml *S.putrefaciens*) 73
Figure 4.10: Open Circuit Voltage (OCV) development from two chambered MFC containing S.putrefaciens (addition of 10mM glucose-2500 ml culture) 74
Figure 4.11: Open Circuit Potential (OCP) development from two chambered MFC containing S.putrefaciens (addition of 10mM glucose for 800 ml.culture 74
Figure 4.12: Open Circuit Voltage (OCV) from two chambered MFC containing S.putrefaciens (addition of 10mM glucose for 1250 ml culture). 75
Figure 4.13: Open Circuit Voltage (OCV) from two chambered MFC containing S.putrefaciens (addition of 10 mM ethanol) 75
Figure 4.14: Open Circuit Potential (OCP) development from two chambered MFC containing S.putrefaciens (addition of 1 mM propionic acid) 76
Figure 4.15: Potential development from two chambered MFC containing aerobically growth of S.putrefaciens (addition of 10mM glucose) 76
Figure 4.16: Potential development from two chambered MFC containing influent ww from brewery industry containing 1250 ml *S.putrefaciens* 77
Figure 4.17: Current versus time graphic taken from confectionery and 2000 mg/L acetate using 5100 ohm external resistance at SCF-1-MFC. 78
Figure 4.18: Current versus time graphic taken from confectionery and 2000 mg/L acetate using 2200 ohm external resistance at SCF-1-MFC 79
Figure 4.19: Current versus time graphic taken from confectionary and 2000 mg/L acetate using 100 ohm external resistance at SCF-1-MFC. Arrows indicate the replacement of the substrate with a fresh substrate. 79
Figure 4.20: A scanning electron micrograph (SEM) for anode of SCF-1-MFC 80
Figure 4.21: Voltage versus time graph for SCF-2-MFC using 300 ohm external resistance and batchly feeding with 200 mg/L acetate . Arrows indicate the replecament of the substrate with a fresh substrate. 81
Figure 4.22: Voltage versus time graph for SCF-2-MFC for activated carbon cloth cathode 82
Figure 4.23: Voltage generation from NaAc only for 12 representative cycles at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 83
Figure 4.24: Voltage generation from NaAc only and NaAc with ERY antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 84

Figure 4.25: Current for per cycle hour from NaAc only and NaAc with ERY antibiotic at 300 Ω external resistance. 85
Figure 4.26: Coulombic efficiency for per cycle hour from NaAc only and NaAc with ERY antibiotic at 300 Ω external resistance. 86
Figure 4.27: COD removal for per cycle hour from NaAc only and NaAc with ERY antibiotic at 300 Ω external resistance. 86
Figure 4.28: Voltage generation from NaAc only and NaAc with SMX antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 87
Figure 4.29: Current for per cycle hour from NaAc only and NaAc with SMX antibiotic at 300 Ω external resistance. 88
Figure 4.30: Coulombic efficiency for per cycle hour from NaAc only and NaAc with SMX antibiotic at 300 Ω external resistance. 88
Figure 4.31: COD removal for per cycle hour from NaAc only and NaAc with SMX antibiotic at 300 Ω external resistance. 89
Figure 4.32: Voltage generation from NaAc only and NaAc with TC antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 90
Figure 4.33: Current for per cycle hour from NaAc only and NaAc with TETRA antibiotic at 300 Ω external resistance. 91
Figure 4.34: Coulombic efficiency for per cycle hour from NaAc only and NaAc with TC antibiotic at 300 Ω external resistance. 91
Figure 4.35: COD efficiency for per cycle hour from NaAc only and NaAc with TC antibiotic at 300 Ω external resistance. 92
Figure 4.36: Voltage generation from NaAc only and NaAc with E1 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 94
Figure 4.37: Current for per cycle hour from NaAc only and NaAc with E1 hormone at 300 Ω external resistance. 95
Figure 4.38: Coulombic efficiency for per cycle hour from NaAc only and NaAc with E1 hormone at 300 Ω external resistance....................................... 95
Figure 4.39: COD removal for per cycle hour from NaAc only and NaAc with E1 hormone at 300 Ω external resistance. 96
Figure 4.40: Voltage generation from NaAc only and NaAc with E2 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 97
Figure 4.41: Current for per cycle hour from NaAc only and NaAc with E2 hormone at 300 Ω external resistance. 98
Figure 4.42: Coulombic efficiency for per cycle hour from NaAc only and NaAc with E2 hormone at 300 Ω external resistance....................................... 98
Figure 4.43: COD removal for per cycle hour from NaAc only and NaAc with E2 hormone at 300 Ω external resistance. 99
Figure 4.44: Voltage generation from NaAc only and NaAc with E3 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 99
Figure 4.45: Current for per cycle hour from NaAc only and NaAc with E3 hormone at 300 Ω external resistance 100
Figure 4.46: Coulombic efficiency for per cycle hour from NaAc only and NaAc with E3 hormone at 300 Ω external resistance..................................... 101

Figure 4.47: COD removal for per cycle hour from NaAc only and NaAc with E3 hormone at 300 Ω external resistance. 101
Figure 4.48: Voltage generation from NaAc only and NaAc with EE2 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution. 102
Figure 4.49: . Current for per cycle hour from NaAc only and NaAc with EE2 hormone at 300 Ω external resistance. 103
Figure 4.50: Coulombic efficiency for per cycle hour from NaAc only and NaAc with EE2 hormone at 300 Ω external resistance 103
Figure 4.51: COD removal for per cycle hour from NaAc only and NaAc with EE2 hormone at 300 Ω external resistance. 104

EFFECTS OF ANTIBIOTICS AND HORMONES ON ELECTRICITY GENERATION USING MICROBIAL FUEL CELLS

SUMMARY

A microbial fuel cell (MFC) is a bioreactor that directly converts chemical energy occurring as a result of oxidation of organic compounds to electrical energy through catalytic reactions of microorganisms under anaerobic conditions. In recent years, since electricity generation from a microbial fuel cell by using fermentation products and different wastewaters as fuel draws researchers' attention, lots of investigations have been made and well documented. Apart from electricity generation, these systems have a great potential for practical applications in the future due to wastewater treatment. The other purpose of MFC usage is a biosensor. The electricity efficiencies obtained recently in MFCs are far away from those required for commercial application and lots of fundamental works have to be done in order to develop usable technologies with low cost.

This thesis consists of two stages in general. Firstly, it is purposed to generate electricity from different organic compounds by using two chambered MFC and pure culture *Shewanella putrefaciens*. After optimization experiments, cultivated cells are transferred to the two chambered MFC. *Shewanella putrefaciens* is bioelectrochemically active and can form a biofilm on the anode surface and transfer electrons directly (without mediator) by conductance through the membrane. When they are used, the anode acts as the final electron acceptor in the dissimilatory respiratory chain of the microbes in the biofilm. Thus, it is avoided from toxicity and instability of synthetic mediators. Because of poor power density of the system (0.8 mW/m^2), it is continued with mixed culture.

In the second phase of this study, by using acclimated mixed culture microorganisms in single chambered MFC, electricity generation, current, chemical oxygen demand (COD) removal, coulombic efficiency (CE) values were measured for the system fed with sodium acetate as carbon source. In single chambered MFC, 4 different estrogens (hormones) which are estrone, 17β-estradiol, estriol ve 17α-ethinylestradiol and 3 different antibiotics (erythromycin, sulfamethoxazole, tetracycline) are used. It is investigated inhibition responses of these matters in MFC system. During antibiotic experiments, one cycle is only acetate, following cycle is antibiotic plus acetate and it continues in this way. When the values of current and CE change after antibiotic plus acetate, the system is fed with only acetate repeatedly to recover to its original value. The concentrations of antibiotics are 50, 100 ve 200 mg/L and they are given to the system together with acetate. On the other hand, the concentrations of hormones are 0,1, 0,5 ve 1 mg/L and the same procedure is carried out. Each set is compared with only the sets in which acetate is used and differences in the current, CE and COD removal values are observed. Therefore, the MFC system is used in a way as a biosensor in this study. In literature, studies that show the effects of inhibitory matters on electrogen microorganisms are too limited. Thus, making a comparison is not quite possible and also the originality of our study gains an

importance. Erythromycin (ERY), sulfamethoxazole (SMX) and tetracycline (TC) are chosen because they are widely used in Turkey and around the world. On the other hand, since it is observed by the researchers that widely usage of synthetic hormones in recent times has negative effects on fish, it is proved in this study that they show diversity in terms of electricity current of electrogen bacteria.

MİKROBİYAL YAKIT HÜCRELERİNDE KULLANILAN ANTİBİYOTİK VE HORMONLARIN ELEKTRİK ÜRETİMİ ÜZERİNE ETKİLERİ

ÖZET

Mikrobiyal yakıt hücreleri (MYH) oksijensiz ortamda mikroorganizmaları katalizör olarak kullanarak organik maddelerin oksidasyonu sonucu oluşan kimyasal enerjiyi doğrudan elektrik enerjisine çeviren sistemlerdir. Son yıllarda fermentasyon ürünlerini ve çeşitli atıksuları kullanarak elektrik üretimi araştırmacıların ilgisini çektiğinden bu konuda pek çok çalışma yapılmıştır. Elektrik üretiminin yanında bu sistemler atıksuyu arıttığından gelecekte pratik kullanımlar için potansiyel taşımaktadır. Mikrobiyal yakıt hücrelerinin diğer bir kullanım alanı ise biosensör olarak çalıştırılmalarıdır. Bu sistemlerin bugüne kadar yapılan araştırmalar sonucu elde edilen elektrik verimleri ticari olarak kullanımdan oldukça uzaktır. Kullanılabilir ve düşük maliyetli teknolojilerin geliştirilmesi için önümüzdeki yıllarda birçok temel araştırmalar yapılmalıdır.

Bu çalışma temel olarak iki kısımdan oluşmaktadır. Birincisi iki hazneli microbiyal yakıt hücresinde saf kültür *Shewanella putrefaciens* kullanılarak farklı organik maddelerden elektrik üretimi olup, optimizasyon çalışmalarından sonra kültür edilmiş hücreler iki hazneli MYH'ne transfer edilmiştir. *Shewanella putrefaciens* elektrokimyasal olarak aktif, anot yüzeyine biyofilm yapabilme özelliğine sahip olup organik maddelerden elde edilen elektronları anot yüzeyine aracı bir medyatör kullanmadan verme özelliğine sahiptir. Böylece sentetik medyatörlerin toksik etkisi ve yenilenme gereği ortadan kaldırılmıştır. Deneyler sonunda elde edilen düşük güç yoğunluğu sebebiyle (0.8 mW/m^2) çalışmaya karışık kültür bakteriler ile devam edilmiştir.

Çalışmanın ikinci kısmında, tek hazneli mikrobiyal yakıt hücresinde aklime edilmiş karışık kültür mikroorganizmalar kullanılarak sodyum asetat ile beslenen sistem için elektrik üretimi, KOİ giderimi, Colombus verimliliği bulunmuştur. Sodyum asetat ile beslenen tek hazneli microbiyal yakıt hücresi ile elektrik üretimi üzerine farklı konsantrasyonlarda dört farklı hormon (estrone, 17β-estradiol, estriol ve 17α-ethinylestradiol) ile üç farklı antibiyotik (erythromycin, sulfamethoxazole, tetracycline) maddesi eklenerek bu maddelerin olası inhibisyon etkileri araştırılmıştır. Antibiyotikler 50, 100 ve 200 mg/L konsantrasyonlarında hazırlanıp asetat ile beraber sisteme verilmiştir. Öte yandan hormonlar ise 0,1, 0,5 ve 1 mg/L konsantrasyonlarda uygulanmıştır. Bu setlerin her biri sadece asetat kullanılan setlerle karşılaştırılmış ve akım, colombus verimliliği ve KOİ gideriminde farklılıklar değerlendirilmiştir. Böylece MYH sistemi bir nevi biyosensör olarak kullanılmıştır. Literatürde bu inhibitor maddelerin elektrojen (elektrik üreten) bakteriler üzerine etkisini gösteren benzer çalışmalara rastlamak pek mümkün olmadığından kıyaslama yapılamamakla beraber çalışmamızın orjinalliği açısından önem taşımaktadır. İncelenen antibiyotikler dünyada ve ülkemizde en fazla tüketilen ana gruplardan olması nedeniyle seçilmiştir. Sentetik hormonlar da son yıllarda yoğun kullanımı ile canlılar üzerinde olumsuz etkileri araştırmacılar tarafından gözlemlendiğinden bu

çalışmada bakteriler üzerinde elektrik üretimi açısından değerlendirilmesi yapılmıştır.

1. INTRODUCTION

1.1 Meaning and Significance of the Thesis

It has been known for many years that it is possible to directly generate electricity using bacteria while accomplishing wastewater treatment in processes based on microbial fuel cell (MFC) technologies (Logan, 2008). This technology has generated significant interest among researchers in recent years (Allen and Bennetto, 1993, Moon et al., 2006). Especially, rapid advances have been occurred in MFC system and lots of journal publications has increased in a few years because of interest among academic researchers. Logan et al. (2006a) reviewed MFC designs, performances and characterization, while Rabaey and Verstraete (2005) reviewed microbial metabolisms in MFCs. Pant et al., 2010 reviewed substrates used in MFCs.

Although microbial fuel cells became of more interest, some experiments that were conducted required the use of chemical mediators which could carry electrons from inside the cell to exogenous electrodes. The breakthrough in MFCs occurred in 1999 when it was recognized that mediators did not need to be added (Kim et al. 1999c; Kim et al. 1999d). A significant amount of information has been obtained studying exoelectrogens from metal reducing genera (*Shewanella and Geobacter*). Likewise, the mechanisms of electron transfer to extracellular electron acceptors are poorly understood (Myers and Myers, 2002).

Apart from the production of electricity and wastewater treatment applications, another potential application of the MFC technology is to use it as a sensor for pollutant analysis and in situ process monitoring and control (Chang et al., 2004, 2005).

1.2 Aim and scope

The aim of this thesis is to determine and evaluate electricity generation of microbial fuel cell (MFC). It was studied both pure culture and mixed culture. Firstly, synthetic wastewater containing sodium acetate, glucose, ethanol and propionic acid mixture as a carbon source is fed in two chambered MFC with pure culture

Shewanella putrefaciens and electricity generation is observed and evaluated for initial different amount of *Shewanella putrefaciens* in MFC.

Secondly, it is studied with single chambered MFC using mixed culture and sodium acetate as a sole carbon source. Current, coulombic efficiency (CE) and chemical oxygen demand (COD) removal efficiencies are measured and evaluated with/without inhibitory matters (antibiotics and hormones)

The thesis is composed of five chapters.

- In the first chapter, introduction part and aim and scope of the thesis are presented.
- In the second chapter, it is rewieved configurations, performances, applications and important parameters of MFC in literature. It is also rewieved source, fate, effects of some antibiotics and hormones in aquatic environment and on biota.
- In the third chapter, materials and methods used in experimental studies are given.
- In the fourth chapter, experimental result and discussion parts are presented.
- In the last chapter, in conclusion, a general evaluation of the experimental studies is provided.

2. LITERATURE SURVEY

2.1 Definition of microbial fuel cell

Microbial fuel cells (MFCs) are devices which use bacteria as the catalysts to oxidize organic and inorganic matter and generate current (Logan et al., 2006). A technology using microbial fuel cells (MFCs) that convert the energy stored in chemical bonds in organic compounds to electrical energy attained through the catalytic reactions by microorganisms has produced a great deal of interest among academic researchers in recent years (Moon et al., 2006). While accomplishing the biodegradation of organic matters or wastes, bacteria can be used in MFCs to generate electricity (Oh and Logan., 2005a). Fig. 2.1 shows a schematic diagram of a typical two-chambered and single-chambered MFC for producing electricity. It consists of anodic and cathodic chambers which are divided by a proton exchange membrane (PEM). A bacterium in the anode compartment transfers electrons acquired from an electron donor (glucose) to the anode electrode. This occurs either through direct contact, nanowires, or mobile electron shuttles (small spheres represent the final membrane associated shuttle). During electron production, protons are also produced excessively. These protons move through the cation exchange membrane (CEM) into the cathode chamber. The electrons flow from the anode through an external resistance (or load) to the cathode where they react with the final electron acceptor (oxygen) and protons (Gil et al., 2003).

In a microbial fuel cell (MFC), power can be generated from the oxidation of organic matter by bacteria at the anode, with reduction of oxygen at the cathode. Proton exchange membranes used in MFCs are permeable to oxygen, leading to the diffusion of oxygen into the anode chamber (Logan et al., 2005). Electrons which are produced from these substrates by the bacteria are transferred to the anode (negative terminal) and flow to the cathode (positive terminal) linked by a conductive material including a resistor, or operated under a load (i.e., producing electricity that runs a device) (Logan et al., 2006). Electron mediators or shuttles can transfer electrons to the anode (Rabaey and Verstraete, 2005).

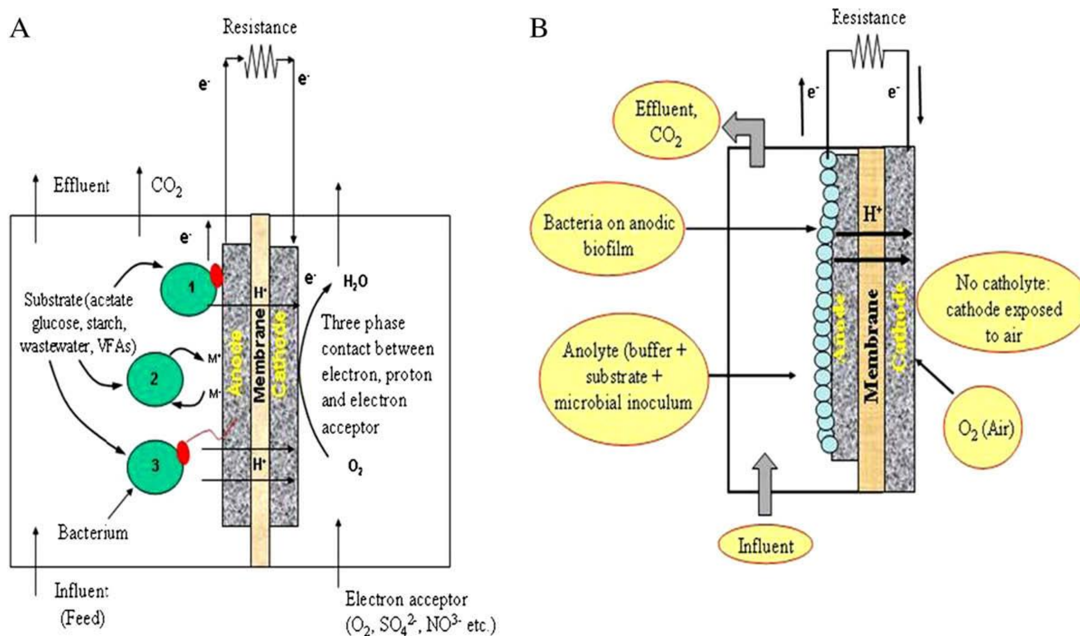


Figure 2.1: Schematic diagram of a typical two-chambered (A) and single-chambered (B) microbial fuel cells (Pant et al., 2009).

2.2 Mediator Microbial Fuel Cell

Mediators have a significant role in electron transport for microbes which are unable to transfer the electrons to the anode. Basic processes are presented as follows (Fig. 2.2) (Lovley et al., 1996). Humic acids, anthraquinone, the oxyanions of sulphur (sulphate and thiosulphate) all have the ability to transfer electrons from inside the cell membrane to the anode (Lovley, 1993). Mediators shuttle between the anode and the bacteria transferring the electrons. They take up the electrons from microbes and discharge them at the surface of the anode. *Actinobacillus succinogenes, Desulfovibrio desulfuricans, E. coli, Proteus mirabilis, Proteus vulgaris, Pseudomonas fluorescens* need extraneous mediators, while some microbes can provide their own. For instance, *Pseudomonas aeruginosa* produces pyocyanin molecules as electron shuttles (Du et al., 2007)

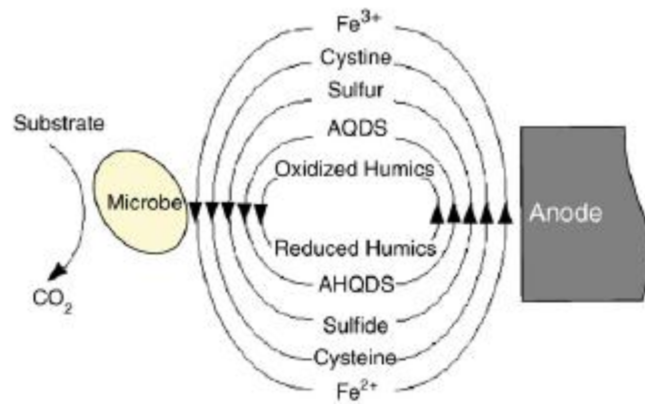


Figure 2.2: Model for various compounds serving as electron shuttles between a bioelectrochemically active bacteria and the anode (Du et al., 2007).

2.3 Mediator-less Microbial Fuel Cell

If no exogenous mediators are added to the system, the MFC is categorized as a mediator-less MFC although the mechanism of electron transfer may not be known (Logan, 2004). Applications of synthetic mediators in MFCs are limited by their toxicity and instability. Some microbes can use naturally occurring compounds including microbial metabolites (endogenous mediators) as mediators. A real breakthrough was made when some microbes were discovered to transfer electrons directly to the anode (Kim et al., 1999a, Chaudhuri and Lovley, 2003). These microbes are operationally stable and yield a high Coulombic effectiveness (Chaudhuri and Lovley, 2003; Scholz and Schroder, 2003). *Shewanella putrefaciens* (Kim et al., 2002), *Geobacteraceae sulferreducens* (Bond and Lovley, 2003), *Geobacter metallireducens* (Min et al., 2005a) and *Rhodoferax ferrireducens* (Chaudhuri and Lovley, 2003) are all bioelectrochemically active and can form a biofilm on the anode surface and transfer electrons directly by conductance through the membrane. When they are used, the anode acts as the final electron acceptor in the dissimilatory respiratory chain of the microbes in the biofilm.

Geobacter belongs to dissimilatory metal reducing microorganisms, which produce biologically useful energy in the form of ATP during the dissimilatory reduction of metal oxides under anaerobic conditions in soils and sediments. The electrons are transferred to the final electron acceptor such as Fe_2O_3 mainly by a direct contact of mineral oxides and the metal reducing microorganisms (Vargas et al., 1998). The anodic reaction in mediator-less MFCs which is constructed with metal reducing bacteria belonging primarily to the families of *Shewanella, Rhodoferax,* and

Geobacter is similar to that in this process since the anode acts as the final electron acceptor just like the solid mineral oxides. Fig. 2.3 demonstrates the chemical compounds proposed to be involved in the electron transportation from electron carriers in the intracellular matrix to the solid-state final electron acceptor (anode) in dissimilatory metal reducing microorganisms (Lovley et al., 2004; Vargas et al., 1998; Holmes et al., 2004). *S. putrefaciens, G. sulferreducens, G. metallireducens and R. Ferrireducens* transfer electrons to the solid electrode (anode) by using this system.

Since the cost of a mediator is eliminated, mediator-less MFCs are advantageous in wastewater treatment and power generation (Ieropoulos et al., 2005).

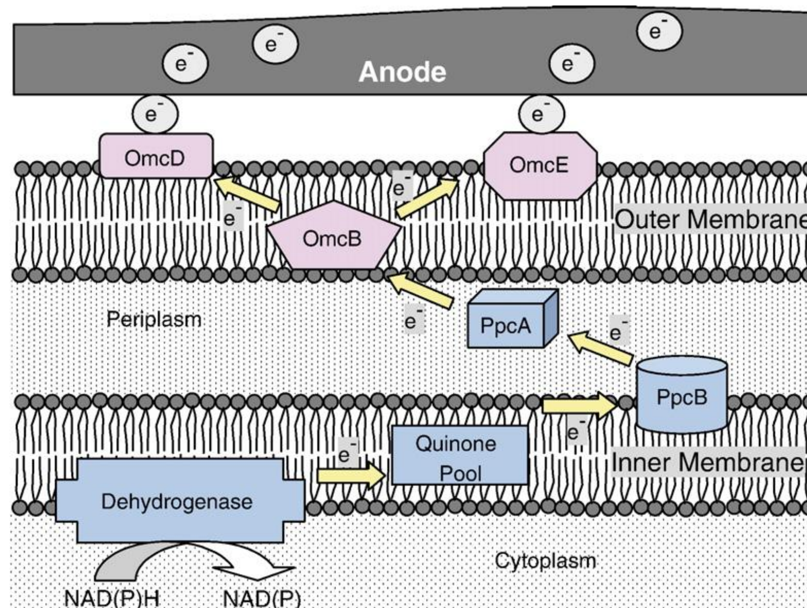


Figure 2.3: Summary of components proposed to be involved in the electron transport from cells to the anode in MFCs using metal reducing microorganisms (Geobacter species) (Figure drawn with modifications after Lovley et al., 2004).

2.4 How do Microbial Fuel Cells work?

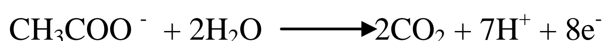
In order to understand how an MFC generates electricity, we must understand how bacteria capture and process energy. Bacteria grow by catalyzing chemical reactions and harnessing and storing energy in the form of adenosine triphosphate (ATP). In some bacteria, reduced substrates are oxidized and electrons are transferred to respiratory enzymes by NADH, the reduced form of nicotinamide adenine dinucleotide (NAD). These electrons flow down a respiratory chain (a series of enzymes that function to move protons across an internal membrane) creating a

proton gradient. The protons flow back into the cell through the enzyme ATPase, creating 1 ATP molecule from 1 adenosine diphosphate for every 3–4 protons. The electrons are finally released to a soluble terminal electron acceptor, such as nitrate, sulfate, or oxygen (Logan and Regan, 2006b).

Microbes in the anodic chamber of an MFC oxidize added substrates and generate electrons and protons in the process. Carbon dioxide is produced as an oxidation product. Nevertheless, there is no net carbon emission since the carbon dioxide in the renewable biomass originally comes from the atmosphere in the photosynthesis process. Unlike in a direct combustion process, the anode absorbs the electrons and they are transported to the cathode through an external circuit. After crossing a PEM or a salt bridge, the protons enter the cathodic chamber where they combine with oxygen to form water. Microbes in the anodic chamber extract electrons and protons in the dissimilative process of oxidizing organic substrates (Rabaey and Verstraete, 2005). Electric current generation is made possible by keeping microbes separated from oxygen or any other end terminal acceptor other than the anode and this requires an anaerobic anodic chamber. In the case presented at Figure 2.4, bacteria could derive energy from the potential between NADH (the reduced form of nicotinamide adenine dinucleotide) and cytochrome c, while the MFC could be used to recover energy from the potential between cytochrome c and oxygen. Actual potentials depend on concentrations and potentials of specific enzymes and electron acceptors (Logan and Regan, 2006b)

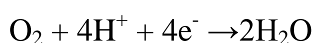
Using acetate as substrate, typical electrode reactions are demonstrated below:

Anodic reaction :



$CH_3COO^- + 2H_2O \longrightarrow 2CO_2 + 7H^+ + 8e^-$

Cathodic reaction :



$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

The overall reaction is the break down of the substrate to carbon dioxide and water with a concomitant production of electricity as a by-product. Based on the electrode reaction pair above, an MFC bioreactor can produce electricity from the electron flow from the anode to cathode in the external circuit.(Du et al., 2007)

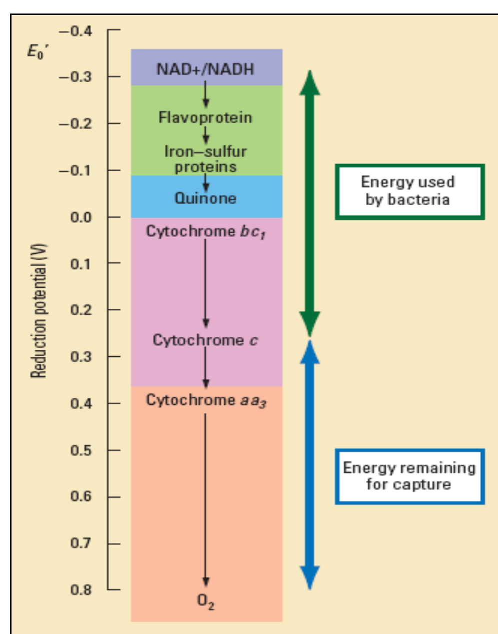


Figure 2.4: Respiratory chain shows how the voltage that could be recovered in a microbial fuel cell (MFC) is dependent on where electrons exit the chain of respiratory enzymes (Logan and Regan, 2006).

In other words, electrons are released to a terminal electron acceptor (i.e. oxygen, nitrate, sulfate etc.) and becomes reduced. The electron acceptor readily diffuse into the bacteria cell where they accept electrons forming products which can diffuse out of the cell. Some bacteria can transfer electrons exogeneously to a terminal electron acceptor such as metal anode. These bacteria called exoelectrogens. These method of electron generating process is called electrogenesis while the bacteria is called exoelectogens and in the reactor microbial fuel cell.

2.5 Types of Microbial Fuel Cell (MFC)

Many various configurations are possible for MFCs. A typical two-chambered MFC consists of an anodic chamber and a cathodic chamber. A single-compartment MFC eliminates the need for the cathodic chamber by exposing the cathode directly to the air. MFCs can be stacked with the systems that are shaped as a series of flat plates or linked together in series in order to increase the overall system voltage (Logan et al., 2006; Du et al., 2007)

2.5.1 Two-chambered MFC

A typical two compartment MFC has an anodic chamber and a cathodic chamber connected by a Proton Exchange Membrane (PEM), or sometimes a salt bridge, to let

protons move across to the cathode while blocking the diffusion of oxygen into the anode (Du et al., 2007). The anode chamber includes the bacteria, and it is tightly sealed to prevent oxygen diffusion into the chamber. The headspace can be flushed with nitrogen gas to exclude air from the chamber. The cathode is submerged in water, and the water is bubbled with air (a typical aquarium air pump works well in the laboratory for this purpose). The ionic strength of the solutions in the two chambers should be matched. The anode chamber should include nutrients (nitrogen, phosphorus and trace minerals) and biodegradable substrate (Logan, 2005). Figure 2.5 demonstrates two-chamber H-type system showing anode and cathode chambers equipped for gas sparging. Schematic demonstration of the anode where bacteria form a biofilm on the surface (with a gas sparger to remove air in the bottle) and a cathode, which is exposed to dissolved oxygen. A proton-exchange membrane (PEM), which in an ideal way allows the exchange of protons through the electrolyte (water) and not through oxygen or the substrate, separates the two chambers. Figure 2.5b demonstrates an example of a simple two-chamber system with the PEM clamped between the ends of two tubes, each joined to a bottle (Logan and Regan, 2006b).

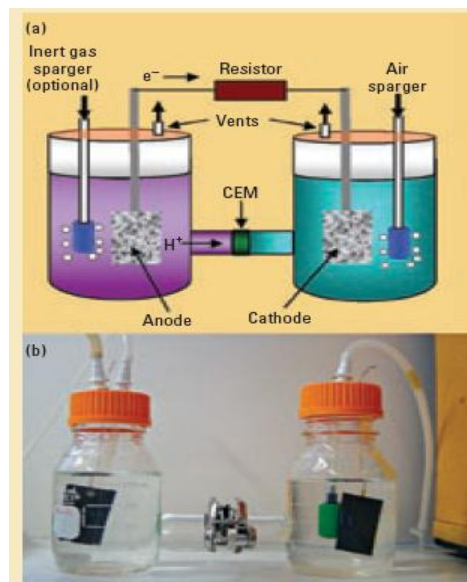


Figure 2.5: Example of an H-type microbial fuel cell (Logan and Regan, 2006).

2.5.2 Single Chambered MFC (SCMFC)

A simpler and more efficient MFC can be made by 1999 by eliminating the cathode chamber and placing the cathode electrode directly onto the PEM. This set up avoids the need to aerate water since the oxygen in air can be directly transferred to the

cathode. In the first design used in Prof. Logan's laboratory at Penn State University, used to demonstrate electricity production from wastewater, the cathode was placed in the center of a cylinder, so that the anode chamber formed a concentric cylinder around the cathode (Liu et al., 2004) Graphite rods were placed inside the anode chamber, and these rods extended outside of the anode chamber and were connected to the cathode via an external circuit containing a resistor. Air was able to passively flow through the center tube so that it could react at the cathode. The Nafion membrane was hot-pressed onto the cathode, which was wrapped around a perforated plastic tube to provide support, with the membrane in contact with the solution in the anode chamber. Scheme and photoraph of laboratory-scale prototype of the SCMFC were presented Figure 2.6.

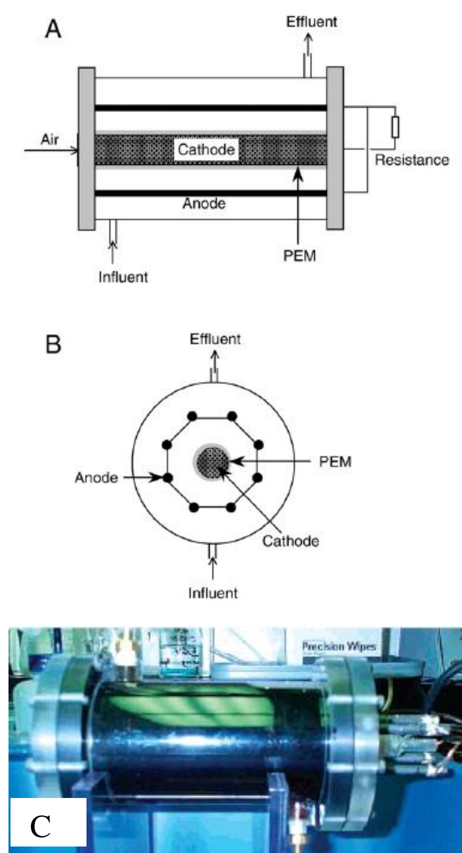


Figure 2.6: Schematics of a cylindrical SC-MFC containing eight graphite rods as an anode in a concentric arrangement surrounding a single cathode. ((A) drawn with modifications after Liu et al., (2004b). (B) drawn to illustrate a photo in Liu et al., (2004.) (C) Photo of laboratory-scale prototype of the SCMFC used to generate electricity from wastewater.

It is not necessary to place the cathode in water or in a separate chamber when using oxygen at the cathode. The cathode can be placed in direct contact with air (Liu and Logan, 2004). Much larger power densities have been attained by using oxygen as

the electron acceptor when aqueous-cathodes are replaced with air-cathodes. The second type of SCMFC was a single tube, with the two circular electrodes placed on opposite ends of the tube (small SCMFC; Liu and Logan, 2004). The end containing the anode is capped in order to prevent oxygen diffusion into the chamber, while the other end is open so that one side of the cathode faces air, while the other is bonded to the PEM and faces the solution in the anode chamber. Two platinum wires extend from the top for electrical connections (Figure 2.7).

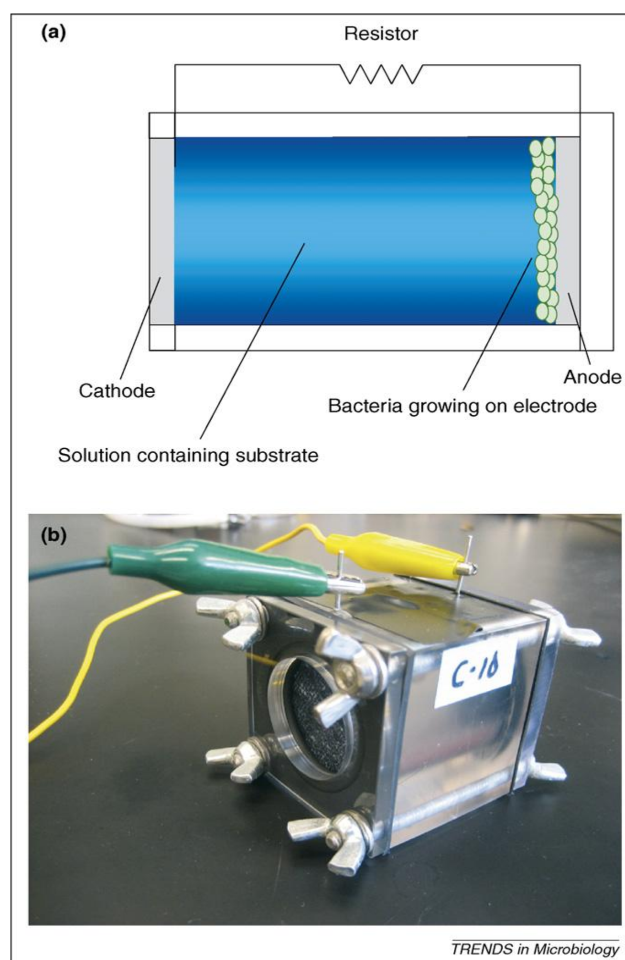


Figure 2.7: (a) A schematic and (b) a photograph of a single-chamber microbial fuel cell. The cathode is exposed to air on one side and the solution containing the biodegradable substrate is on the other side. The anode chamber containing the exoelectrogenic bacteria is sealed off from oxygen (Logan and Regan 2006).

2.5.3 Stacked Microbial Fuel Cell

A stacked MFC for the analysis of performances of several MFCs connected in series and in parallel (Aelterman et al., 2006). Enhanced voltage or current output can be attained by connecting several MFCs in series or in parallel. Stacked MFC with the systems shaped as a series of flat plates or linked together in series (Figure 2.8). No

apparent adverse effect on the maximum power output per MFC unit was noticed. Coulombic efficiencies differred greatly in the two arrangements with the parallel connection giving about an efficiency six times higher when both the series were operated at the same volumetric flow rate. The parallel-connected stack has higher short circuit current than the series connected stack, which means that higher maximum bioelectrochemical reaction rate is allowed in the connection of MFCs in parallel than in series. Thus, if the MFC units are not independently operated, a parallel connection is preferred in order to maximize chemical oxygen demand (COD) removal (Aelterman et al., 2006).

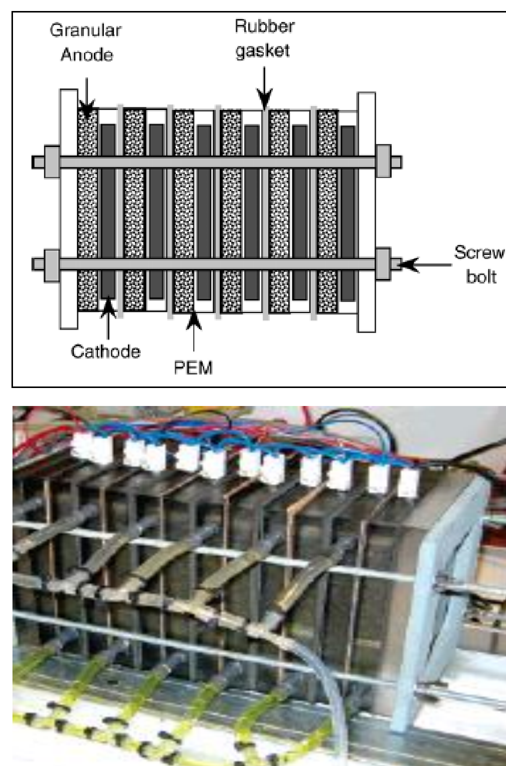


Figure 2.8: Stacked MFCs consisting of six individual units with granular graphite anode (They are joined in one reactor block (drawn to illustrate a photo in Aelterman et al.,2006).

2.6 Factors of performance of Microbial Fuel Cell

2.6.1 Effects of electrode materials

The electrodes can be made of any conducting, non-corrosive material (Logan 2005).

2.6.1.1 Anode materials

Metal anodes which consist of noncorrosive stainless steel mesh can be utilized, however, copper is not useful owing to the toxicity of even trace copper ions to bacteria. The most versatile electrode material is carbon, available as compact graphite plates, rods, or granules, as fibrous material (felt, cloth, paper, fibers, foam), reticulated vitreous carbon (RVC) and as glassy carbon (Logan et al, 2006, Du et al, 2007)

The simplest materials for anode electrodes are graphite plates or rods (inexpensive), which are easy to handle, and have a defined surface area. Much larger surface areas are achieved with graphite felt electrodes which can have high surface areas. Nevertheless, not all the indicated surface area will necessarily be available to bacteria. Carbon fiber, paper, graphite felt, reticulated vitreous carbon (RVC) and cloth (Toray) have been extensively used as electrodes(Logan et al., 2006, Du et al, 2007). Graphite fiber brush elektrodes have been also used because of the highest specific areas and porosities (Logan et al, 2007)

Using better performing electrode materials can improve the performance of an MFC since different anode materials lead to different activation polarization losses. Pt and Pt black electrodes are superior to graphite, graphite felt and carbon-cloth electrodes for both anode and cathode constructions, however, their costs are much higher (Du et al., 2007).

2.6.1.2 Cathode materials

The cathode must contain a catalyst for producing water from the protons, electrons and oxygen, and typically Pt is used and held on the carbon surface by using a binder. Oxygen is the most suitable electron acceptor for an MFC owing to its high oxidation potential, availability, low cost (it is free), sustainability, and the lack of a chemical waste product (water is formed as the only endproduct). When the cathode material is chosen, it greatly affects performance, and is varied based on application. For sediment fuel cells, plain graphite disk electrodes which are immersed in the seawater above the sediment have been used. In seawater, oxygen reduction on carbon cathodes has been shown to be microbially supported. Such microbially assisted reduction has also been observed for stainless steel cathodes which rapidly reduces oxygen when aided by a bacterial biofilm. Pt catalysts are usually used for

dissolved oxygen or open-air (gas diffusion) cathodes in order to increase the rate of oxygen reduction. The Pt load can be kept as low as 0.1 mg/cm^2 in order to decrease the costs for the MFC. The long term stability of Pt needs a more fully investigation, and there remains a need for new types of inexpensive catalysts (Logan et al., 2006).

The electrodes can be connected by any type of wire if the wire is not exposed to bacteria. Pt wire is the best choice, however, it is expensive, thus, copper wire is frequently used with all surfaces coated with a non-conductive epoxy. Even if coated in this way, copper wire can be expected to eventually fail in the system. In order to avoid wires inside the chambers, the carbon electrodes can be extended outside the chamber and then a regular wire and clip can be placed on the electrode (Logan, 2005).

2.6.2 Effects of operating conditions

2.6.2.1 Anodic chamber

Fuel type, concentration and rate are significant factors that impact the performance of an MFC. With a given microbe or microbial consortium, power density varies greatly using different fuels. Many systems have proved that electricity generation depends on fuel concentration both in batch and continuous-flow mode MFCs. Usually a higher fuel concentration yields a higher power output in a wide concentration range. A higher current level was attained with lactate (fuel) concentration increased until it was in excess at 200mM in a single-compartment MFC inoculated with *S. putrefaciens* (Park and Zeikus, 2002). Increased fuel concentration has an effect on the performance of MFC (Moon et al. 2006). The current increased with a wastewater concentration up to 50 mgCOD/L in their MFC (Gil et al, 2003). In an interesting way, the electricity generation in an MFC often peaks at a relatively low level of feed rate before heading downward. This may be because a high feed rate promoted the growth of fermentative bacteria faster than those of the electrochemically active bacteria in a mixed culture (Moon et al., 2006; Kim et al., 2004; Rabaey et al., 2003).

2.6.2.2 Cathodic chamber

Oxygen is the most commonly used electron acceptor in MFCs for the cathodic reaction. Power output of an MFC is strongly dependent on the concentration level of electron acceptors. Several studies showed that DO was a major limiting factor when

it remained below the air-saturated level (Oh et al., 2004; Pham et al., 2004;Gil et al., 2003). Surprisingly, a catholyte sparged with pure oxygen that gave 38 mg/L DO did not further increase the power output compared to that of the air-saturated water (at 7.9 mg/L DO) (Oh et al., 2004; Min and Logan, 2004; Pham et al., 2004). Rate of oxygen diffusion toward the anode chamber goes up with the DO concentration. Therefore, part of the substrate is consumed directly by the oxygen instead of transferring the electrons through the electrode and the circuit (Pham et al., 2004). Power output is much greater using ferricyanide as the electron acceptor in the cathodic chamber. Ferricyanide ($K_3[Fe(CN)_6)$ is very popular as an experimental electron acceptor in MFCs. The greatest advantage of ferricyanide is the low overpotential using a plain carbon cathode, resulting in a cathode working potential close to its open circuit potential. Reported cases with very high power outputs such as 7200 mW/m^2, 4310 mW/m^2 and 3600 mW/m^2 all used ferricyanide in the cathodic chamber (Schroder et al. 2003, Oh et al., 2004 2003; Rabaey et al., 2004), while less than 1000 mW/m2 was reported in studies using DO regardless of the electrode material. This is likely due to the greater mass transfer rate and lower activation energy for the cathodic reaction which is offered by ferricyanide (Oh et al., 2004). On the other hand, the greatest disadvantage is the insufficient reoxidation by oxygen, which requires the catholyte to be regularly replaced (Rabaey et al., 2003). Additionaly, the long term performance of the system can be affected by diffusion of ferricyanide across the PEM and into the anode chamber (Logan et al., 2006).

2.6.2.3 Effects of PEM (Proton Exchange Membrane)

Proton exchange system can affect an MFC system's internal resistance and concentration polarization loss and in turn, they influence the power output of the MFC. Nafion (DuPont Co., ABD) is the most popular due to its highly selective permeability of protons. In spite of the attempts by researchers to look for less expensive and more durable substitutes, Nafion is still the best choice. Ultrex, polyethylene.poly (styrene-co-divinylbenzene); salt bridge, porcelain septum, or solely electrolyte can be also used as PEM in MFC (Du et al, 2007).

2.6.2.4 Effects of ionic strength, anode-cathode distance and temperature

Increasing the solution ionic strength by adding NaCl increased power output. Power generation was also increased by decreasing the distance between the anode and

cathode from 4 to 2 cm (Liu et al., 2005). The power increases due to ionic strength and electrode spacing resulted from a decrease in the internal resistance. Power output was also increased by replacing the cathode with carbon cloth cathode containing the same Pt loading. The performance of conventional anaerobic treatment processes, such as anaerobic digestion, are adversely affected by temperatures below 30 °C. Nevertheless, decreasing the temperature from 32 to 20 °C reduced power output by only 9%, primarily as a result of the reduction of the cathode potential. These results, which show that power densities can be increased to over 1 W/m2 by changing the operating conditions or electrode spacing, should lead to further improvements in power generation and energy recovery in single-chamber, air-cathode MFCs. (Liu et.al. 2005)

Certainly, changing operating conditions can improve the power output level of the MFCs. Nonetheless, it is not a revolutionary method to upgrade the MFCs from low power system to an applicable energy source at the very present. The bottleneck lies in the low rate of metabolism of the microbes in the MFCs. Even at their fastest growth rate, microbes are comparatively slow transformers. The biotransformation rate of substrates to electrons has a fixed ceiling which is inherently slow. Effort should be focused on how to break the inherent metabolic limitation of the microbes for the MFC application. High temperature can accelerate almost all kinds of reactions including chemical and biological ones. Use of thermophilic species might benefit for improving rates of electron production, nevertheless, to the best of our knowledge, no such investigation is reported in the literature. Thus, this is probably another scope of improvement for the MFC technology from the laboratory research to a real applica ble energy source. (Du et al, 2007)

2.7 Fundamentals of voltage generation

MFCs commonly achieve a maximum working voltage of 0.7 V. The voltage is a function of the external resistance (Rex), or load on the circuit, and the current, I. The relationship between these variables is the well-known equation:

$$E = I \times R_{ex} \quad \textbf{(2.1)}$$

where E is used for the cell potential. V is also used for voltage, though the symbol V and the units V = Volts can lead to confusion.

The current produced from a single MFC is small, so that when a small MFC is constructed in the laboratory the current is not measured, however, instead it is calculated from the measured voltage drop across the resistor as I = E/Rex. The highest voltage produced in an MFC is the open circuit voltage, OCV, which can be measured with the circuit disconnected (infinite resistance, zero current).

When the resistances are decreased, the voltage decreases. The power at any time is calculated as

$$P = I x E \qquad \textbf{(2.2)}$$

The voltage generated by an MFC is far more complex to understand or predict than that of a chemical fuel cell. In an MFC, it takes time for the bacteria to colonize the electrode and manufacture enzymes or structures which are needed to transfer electrons outside the cell. In mixed cultures, different bacteria can grow, setting different potentials. As discussed below, the potential even for a pure culture cannot be predicted. Nonetheless, there are limits to the maximum voltages that can be generated based on thermodynamic relationships for the electron donors (substrates) and acceptors (oxidizers).

The maximum electromotive force, Eemf that can be developed in any type of battery or fuel is given by

$$E_{emf} = E^0 - \left(\frac{RT}{nF}\right)\ln \pi \qquad \textbf{(2.3)}$$

where E^0 is the standard cell electromotive force, R = 8.31447 J/mol-K the gas constant, T the absolute temperature (K), n the number of electrons transferred, and F = 96,485 C/mol is Faraday’s constant. The reaction quotient is the ratio of the activities of the products that are divided by the reactants raised to their respective stoichiometric coefficients, or

$$\pi = \frac{[\text{product}]^p}{[\text{reactants}]^r} \qquad \textbf{(2.4)}$$

By the IUPAC convention, all reactions are written in the direction of chemical reduction, so that the products are always the reduced species, and the reactants are the oxidized species (oxidized species + e^- + reduced species). At the same time, by IUPAC convention, we take as standard conditions a temperature of 298 K, and

chemical concentrations of 1 M for liquids and 1 bar for gases (1 bar = 0.9869 atm = 100 kPa). All values of E^0 are calculated with respect to that of hydrogen under standard conditions, which is defined to be E^0 (H_2)=0, referred to as the normal hydrogen electrode (NHE). Therefore, the standard potentials for all chemicals is obtained with π=1 relative to a hydrogen electrode.

In biological systems the reported potentials are usually pre-adjusted to neutral pH,

since the cytoplasm of most cells is at pH=7. For hydrogen, with $2H^+ + 2e^- \rightarrow H_2$, this means that the adjusted potential at 298 K is

$$E^{0/} = E^0 - \frac{RT}{nF}\ln\frac{H_2}{[H^+]^2} = 0 - \frac{(8.31 J/molK)(298.15K)}{(2)(9.65\times10^4 C/mol)}\ln\frac{[1bar]}{[10^{-7}M]^2} = -0.414V \quad \textbf{(2.5)}$$

where the $^/$ on E is used to denote the pH-adjusted standard condition commonly used by microbiologists. Thus, in most calculations, the hydrogen potential is not zero as a result of the assumption of all species being present in a pH = 7 solution. These potentials need to be adjusted for other temperatures or pressures, or pH if different from 7.

For hydrogen (H^+/H_2), chemicals which will be oxidized by H^+ have more negative potentials, whereas those that are reduced by H_2 have more positive potentials. For instance, H_2 is oxidized by oxygen. The half reaction for oxygen is

$\frac{1}{2}O_2 + 2H^+ + 2e^- \rightarrow H_20$ and $E^0(O_2)$= 1.229 V, so the adjusted value for oxygen at pH = 7 is

$$E^{0/} = E^0 - \frac{RT}{nF}\ln\frac{1}{[O_2]^{1/2}[H^+]^2} \quad \ldots\ldots\textbf{(2.6)}$$

$$E^{0/} = 1.229 - \frac{(8.31 J/molK)(298.15K)}{(2)(9.65\times10^4 C/mol)}\ln\frac{1}{[0.2]^{1/2}[10^{-7}M]^2} = 0.805V$$

The activity of a pure liquid or a solid is constant, so here the activity of water is unity. Because $E^{0/}(O_2)$> $E^{0/}(H_2)$ oxygen is reduced by hydrogen. When the voltage is positive, the reaction is exothermic. The calculations can also be expressed in terms of the change in Gibbs free energy, ΔG_r^0 [J], as

$$E^0 = -\frac{\Delta G_r^0}{nF} \quad \ldots\ldots\textbf{(2.7)}$$

Note here that the reaction is exothermic when ΔG_r^0; is negative.

The total potential that can be produced by any fuel cell is the difference in the anode and cathode potentials, or $E_{emf} = E_{cat} - E_{an}$. For the adjusted standard conditions of pH=7, this is

$$E_{emf} = E^{0/}_{cat} - E^{0/}_{an} \quad \text{............(2.8)}$$

For this case at the given conditions (298 K, 1 bar, pH = 7), this is

$E^{0/}_{emf} = 0.805\ V - (-0.414\ V) = 1.219\ V$.

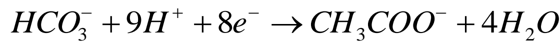
Table 2.1: Anode and Cathode potentials for different anodic and cathodic reactions. $E^{/}$ or $E^{0/}$ values are adjusted for pH=7 at 298 K except as indicated (Logan, 2008).

Anode-Cathode Reaction	E^0(V)	Conditions	$E^{/}$(V)
$2H^+ + 2e^- \rightarrow H_2$	0.000	pH=7	-0.414
$2HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$	0.187	HCO_3^- =5mM, CH_3COO^- = 16.9, pH=7	-0.300
$CO_2 + HCO_3^- + 8H^+ + 8e^- \rightarrow CH_3COO^- + 3H_2O$	0.130	pH=7	-0.284
$6CO_2 + 24H^+ + 24e^- \rightarrow C_6H_{12}O_6 + 6H_2O$	0.014	pH=7	-0.428
$O_2 + 4H^+ + 4e^- \rightarrow H_2O$	1.229	pO_2=0.2, pH=7	0.805
$O_2 + 2H^+ + 2e^- \rightarrow H_2O_2$	0.695	pO_2=0.2, H_2O_2=5mM, pH=7	0.328
$Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$	0.361	$Fe(CN)_6^{3-}$ $=Fe(CN)_6^{4-}$	0.361
$MnO_2(s) + 4H^+ + 2e^- \rightarrow Mn^{2+} + H_2O$	1.229	Mn^{2+}=5mM, pH=7	0.470
$MnO_4^- + 4H^+ + 3e^- \rightarrow MnO_2 + 2H_2O$	1.70	MnO_4^-=10mM, pH=3.5	1.385
$Fe^{3+} + e^- \rightarrow Fe^{3+}$ (low 0.77 pH)	0.77	$Fe^{3+} = Fe^{2+,}$ T= 303 K(low pH)	0.78

While it is useful to express all potentials relative to a normal hydrogen electrode (NHE) or standart hydrogen electrode (SHE), most experiments are conducted using Ag/AgCl reference electrodes. For converting voltages obtained with a Ag/AgCl electrode to NHE, it depends on the specific solution in the probe, but typically to get NHE add 0.195 V (Liu and Logan, 2004) or 0.205 V (ter Heijne et al., 2006).

2.7.1 Anode potential

If thermodynamics limits overall power production, it can be expected that the measured anode potential will approach that of the calculated maximum potential (i. e., the potential set by substrate oxidation). As noted above, the maximum voltage is produced in open-circuit mode, that’s why the maximum potential should be close to that of the open-circuit potential (OCP). Most MFCs operating on a variety of substrates produce an OCP approaching -0.3 V (vs. NHE). For acetate, we have the HCO_3^-/Ac couple expressed as a reduction as:



$$HCO_3^- + 9H^+ + 8e^- \rightarrow CH_3COO^- + 4H_2O$$

For acetate E^0= 0.187 V, with a concentration of 1 g/L (16.9 mM) and under conditions of neutral pH = 7 and an alkalinity set by the bicarbonate concentration of $HCO_3^- = 5mM$, we have

$$E_{an} = 0.187 - \frac{(8.31J/molK)(298.15K)}{(8)(9.65\times10^4 C/mol)} \ln \frac{[0.0169]}{[0.005]^2[10^{-7}M]^9} = -0.300V \qquad \textbf{(2.9)}$$

2.7.2 Cathode Potential

For an MFC using oxygen, the cathode potential is a maximum of $E^{0/}_{cat}$ = 0.805 V. Therefore, for an air-cathode MFC with 1 g/L of acetate (16.9 mM) as substrate ($HCO_3^- = 5mM$, pH = 7), the maximum cell potential is $E^{/}_{cell}$, = 0.805 V - (-0.300 V) = 1.105 V. Nevertheless, the cathode potential with oxygen is much less in practice than predicted here. Typically, the OCP of an air cathode is approximately 0.4 V, with a working potential of nearly 0.25 V even with a Pt catalyst. In one set of tests the OCP_{cat} of an MFC lacking a CEM was 0.425 V (0.230 V vs. Ag/AgCl) (Liu and Logan 2004). Hot pressing a CEM (Nafion^{TM}) to the cathode substantially reduced the cathode potential to OCP_{cat} = 0.226 V. The anode OCP was -0.275 V, with a working anode potential of ca. -0.205 V (-0.400 V vs. Ag/AgCI), either in the presence or absence of the CEM.

The most commonly used chemical catholytes in MFC fuel cells next to oxygen are ferricyanide, or hexacyanoferrate, $Fe(CN)_6^{3-}$. It has a standard potential of 0.361 V, is highly soluble in water, and it does not require a precious metal on the cathode such as Pt. Tests using ferricyanide indicate much greater power generation than those with oxygen owing to the fact that there is little polarization of the cathode so that the cathode potential achieved is quite close to that calculated for standard conditions (ter Heijne et al. 2006; You et al. 2006). Therefore, while oxygen is predicted to have a higher cathode potential than ferricyanide, in practice the potentials which are achieved by using oxygen are much lower than theoretical values. In two-chamber MFC tests, Oh and Logan (2006) discovered that replacing the aqueous cathode using oxygen with ferricyanide increased power by 1.5 to 1.8 times though power densities produced in this system were low owing to the high internal resistance of the system. Rabaey et al. (2004) achieved one of the highest power densities yet produced in an MFC using a ferricyanide catholyte (4.1 W/m2 of anode surface area), however, they did not report on power production in that system with dissolved oxygen. Nonetheless, power generation with ferricyanide is not sustainable. Ferricyanide must be externally regenerated, and can be lost over time.

2.8 Fundamentals of power generation

To make MFCs useful as a method to generate power, it is essential to optimize the system for power production. Power is calculated from a voltage and current as

P = IxE. The power output by an MFC is calculated from the measured voltage, E_{MFC}, across the load and the current as

$$P = I \times E_{MFC} \qquad \textbf{(2.10)}$$

The current produced by a laboratory-scale MFC is calculated by measuring the potential across the load (i.e. , the external resistor, R_{ext}), and using

$$I = \frac{E_{MFC}}{R_{ext}} \qquad \ldots\ldots\textbf{(2.11)}$$

Therefore, we can calculate power output as

$$P = \frac{E_{MFC}^2}{R_{ext}} \qquad \ldots\ldots\textbf{(2.12)}$$

Based on the relationship $I = \frac{E_{MFC}}{R_{ext}}$, we can alternatively express power output in terms of the calculated current as

$$P = I^2 \times R_{ext} \qquad \ldots\ldots\textbf{(2.13)}$$

2.8.1 Power output normalized by surface area

Knowing how much power is generated by an MFC does not sufficiently describe how efficiently that power is generated by the specific system architecture. For instance, the amount of anode surface area available for microbes to grow on can affect the amount of power generated. Therefore, it is common to normalize power production by the surface area of the anode, A_{an}, so that the power density which is produced by the MFC is

$$P = \frac{E_{MFC}^2}{A_{an} \times R_{ext}} \quad \text{.......}\textbf{(2.14)}$$

The surface area used for calculating A_{an} is not the same in all studies. In reactors where the anode is suspended in water, for instance, the area is defined as a projected or geometric surface area based on both sides of the electrode, i.e., $A = 2 \times l_{an} \times w_{an}$ where l_{an} and w_{an} are the length and width, respectively, of a rectangular shaped electrode (Min et al. 2005a). Nevertheless, when the anode is pressed onto a surface, only one side of the anode may be used (Liu et al. 2005; Liu and Logan 2004).

2.8.2 Power output normalized by volume

As MFCs are designed to maximize total system power, ultimately the most important factor is the power production on the basis of the total reactor volume. This is calculated as

$$P = \frac{E_{MFC}^2}{v \times R_{ext}} \quad \text{.......}\textbf{(2.15)}$$

where P_v is the volumetric power (W/m^3) and v is the total reactor volume. The reactor liquid volume can also be used, however, the convention in environmental engineering is to use the total reactor volume.

2.9 Coulombic Efficiency

While generating power is a main goal of MFC operation, we also seek to extract as much of the electrons stored in the biomass as possible as current, and to recover as much energy as possible from the system. The recovery of electrons is referred to as Coulombic efficiency, defined as the fraction (or percent) of electrons recovered as current versus that in the starting organic matter. The oxidation of a substrate occurs

with the removal of electrons, with the moles of electrons defined for each substrate (b_e) based on writing out a half reaction. For acetate, complete oxidation requires

b_e = 8 mol e^-/mol, while for glucose it is be = 24 mol e^-/mol. Coulombic efficiency, C_E, is defined as C_E =Coulombs recovered / Total coulombs in substrate. An ampere is defined as the transfer of 1 Coulomb of charge per second, or 1A = 1C/s. Therefore, if we integrate the current obtained over time, we acquire the total Coulombs transferred in our system. The C_E can therefore be calculated for a fed-batch system as (Cheng et al. 2006; Logan et al. 2006).

$$C_E = \frac{M_s \times \int_0^{tb} Idt}{F \times b_{es} \times v_{An} \times \Delta c} \quad \text{.......(2.16)}$$

Where Δc is the substrate concentration change over the batch cycle (which is usually assumed to go from c_0, the starting concentration, to completion for defined substrates such as acetate, or $\Delta c = c_0 - c = c_0 - 0 = c_0$) over a time = t_b, M_s, is the molecular weight of the substrate, F = Faraday's constant, and v_{An} is the volume of liquid in the anode compartment. For complex substrates, it is more convenient to use COD as a measure of substrate concentration, and thus, the C_E becomes

$$C_E = \frac{8 \times \int_0^{tb} Idt}{F \times v_{An} \times \Delta COD} \quad \textbf{.......(2.17)}$$

where 8 is a constant used for COD, based on M_{0_2} = 32 for the molecular weight of oxygen and b_{es} = 4 for the number of electrons exchanged per mole of oxygen.

2.10 Ohmic, activation, bacterial metabolic and mass trasport losses of MFCs

The ideal performance of an MFC is dependent on the electrochemical reactions that occur between the organic substrate at a low potential such as glucose and the final electron acceptor with a high potential, such as oxygen (Rabaey and Verstrate, 2005). Nonetheless, its ideal cell voltage is uncertain since the electrons are transferred to the anode from the organic substrate through a complex respiratory chain which varies from microbe to microbe and even for the same microbe when growth conditions differ. The actual cell potential is always lower than its equilibrium potential due to irreversible losses. These losses can be classified as ohmic losses, activation losses, bacterial metabolic losses and mass transport or concentration losses.

First of all, Logan et al. (2006) determined the ohmic losses in MFC that both the resistance to the flow of electrons through the electrodes (anode and cathode) and interconnections, and the resistance to the flow of ions through the PEM (if present) and the anodic and cathodic electrolytes. Ohmic losses can be reduced by checking thoroughly all contacts, minimizing the electrode spacing and using a membrane with a low resistivity.

Secondly, due to the activation energy needed for an oxidation/reduction reaction, activation losses (charge transfer resistance) occur during the transfer of electrons from or to a compound reacting at the electrode surface. This compound can exist at the bacterial surface, as a mediator in the solution, or as the final electron acceptor reacting at the cathode. Activation losses often demonstrate a strong increase at low currents and steadily increase when current density increases. Low activation losses can be achieved by increasing the electrode surface area, improving electrode catalysis, increasing the operating temperature, and through the establishment of an enriched biofilm on the electrode (Logan et al., 2006).

Thirdly, in order to generate metabolic energy, bacteria transport electrons from a substrate at a low potential through the electron transport chain to the final electron acceptor (such as oxygen or nitrate) at a higher potential. In an MFC, the anode is the final electron acceptor and its potential determines the energy gain for the bacteria. The higher the difference between the redox potential of the substrate and the anode potential, the higher the possible metabolic energy gain for the bacteria, but the lower the maximum reachable MFC voltage. The potential of the anode should be kept as low (negative) as possible in order to maximize the MFC voltage. Nevertheless, electron transport will be inhibited and fermentation of the substrate (if possible) may provide greater energy for the microorganisms if the anode potential becomes too low (Logan et al, 2006).

Lastly, concentration losses (or concentration polarization) occur when the rate of mass transport of a species to or from the electrode limits current production. Concentration losses occur mainly at high current densities owing to limited mass transfer of chemical species by diffusion to the electrode surface. Either a limited discharge of oxidized species from the electrode surface or a limited supply of reduced species toward the electrode causes concentration losses at the anode. This increases the ratio between the oxidized and the reduced species at the electrode

surface which can produce an increase in the electrode potential. At the cathode side, the reverse may occur, causing a drop in cathode potential (Logan et al., 2006).

It is difficult from literature to compare MFC performance, due to different operating conditions, surface area and type of electrodes and different organisms involved. Moreover, different researchers use different units to indicate the performance of a MFC (Pant et al, 2009). One of the most common unit is current density, which is either represented as the current generated per unit area of the anode surface area (mA/cm^2 or mA/m^2) or current generated per unit volume of the cell (mA/m^3).

Table 2.2 shows the measure of current and power generation for different pure cultures, substrates, materials of electrodes, and with or without mediator in MFC in literature.

Table 2.2: The measure of current and power generation for different pure cultures, compound, electrodes, with or without mediator in literature.

Pure Cultures	**Substrate**	**Anode material**	**Redox Mediated**	**I (Current, mA)**	**P (Power, mW/m2)**	**References**
Proteus vulgaris	Glucose	Glassy carbon	X	0.7	85	Choi et al. (2003)
Shewanella putrefaciens IR	Lactate	Graphite felt		0.04	0.6	Kim et al. (2002)
Shewanella putrefaciens	lactate	woven graphite		0.031	0.19	Kim et al.(2002)
Geobacter sulfurreducens	Acetate	Plain graphite		0.4	13	Bond and Lowley (2003)
Rhodoferax ferrireducens	Glucose	Plain graphite		0.2	8	Chaudhuri and Lovley(2003)
		Woven graphite		0.57	17	Chaudhuri and Lovley(2003)
		Graphite foam		0.4514	33	Chaudhuri and Lovley(2003)
Pseudomonas aeruginosa	Glucose	Plain graphite		0.1	88	Rabaey et al (2004)
Escherichia coli	Lactate	Woven graphite	X	3.3	1.2	Park and Zeikus (2003)
		Plain graphite	X	2.6	91	Park and Zeikus (2003

2.11 Properties of genus *Shewanella*

Genus *Shewanella* are a type of gram-negative, facultative anaerobic bacteria mostly found in aquatic and marine environments and can often be isolated from spoiling fish. Usually, *Shewanella* are known to be the members of the γ-subclass of the Proteobacteria that are rods, 0.4 – 0.7 µm in diameter, 2–3 µm in length, and motile by a single polar flagellum (Venkateswaran et al., 1999). Many types of *Shewanella* are grown in the laboratory with general growth media following enhancement from

environmental samples (Croal et., 2004)) with a range of salt concentrations, temperatures, and barometric pressures and have distinct roles from food spoilage organisms to symbionts, epibionts, and opportunistic pathogens. (Hau and Gralnick, 2007)

In the absence of oxygen, *Shewanella* is able to carry out anaerobic respiration by using a broad range of final electron acceptors. This feature not only enables the members of the genus *Shewanella* to survive in various environments with the capacity of growing naturally almost anywhere, but also to be utilized for bioremediation of contaminated environments by reducing some certain metals and compounds in an altered state (Myers and Myers, 2002).

The isolation of the first *Shewanella* was performed in 1931 as one of various contaminating microorganisms in charge of butter putrefaction (Nealson, 2002). In 1985, *Shewanella putrefaciens* was isolated, and no more reclassifications at the genus level have been made up to date. Approximately 40 species are assigned to the genus Shewanella based primarily on DNA at present: DNA hybridization and 16S rRNA sequences.

As a genus, *Shewanella* are the most diverse respiratory organisms described so far. There are roughly twenty inorganic and organic compounds that can be respired by Shewanella and these have several insoluble metals and toxic elements. A partial list are as follows:

Trimethylamine-*N*-oxide (TMAO) → Trimethylamine (Me_3N),
Fe (III) Chelate and Fe (III) Oxide → Soluble Fe (II),
Mn (III and IV) Chelates and Mn (III and IV) oxides → Soluble Mn (II),
Sulfur/polysulfide → H_2S, Sulfite → H_2S, Thiosulfate→ H_2S,
Dimethyl sulfoxide (DMSO) → Dimethylsulfide,
Arsenate → Arsenite, Fumarate, and Succinate.

The respiratory diversity of *Shewanella* is one of their greatest benefits in terms of survival in the environment. The fact that all isolates seem to be facultative anaerobes and the anaerobic electron acceptors are various, suggests these organisms are normally localized in both oxic and anoxic environments. Oxygen can be limiting in sediments, in intestinal tracts of higher organisms, and in organic-rich flocculates such as marine snow and fecal pellets. Some aquatic systems are permanently or

temporarily stratified, allowing the formation of large anoxic zones. The respiratory diversity of *Shewanella* allows them to breath almost anywhere. In anoxic environments, *Shewanella* are likely to respire one if not several compounds. In organic-rich flocculates the electron acceptor may be TMAO or DMSO. In sedimentary environments, the electron acceptors may be insoluble iron or manganese oxide minerals. The mechanism of anaerobic respiration in these organisms is implicit at the genetic level for some compounds (Fumarate, DMSO, TMAO, As, V, Fe, and Mn). Many of these compounds are reduced by terminal reductases located outside of the cell, and reviews have recently concentrated on the molecular details of this process (Croal et al, 2004, Weber et al., 2006). Additionally, it is likely that the list of known substrates respired by Shewanella is not yet complete. Most *Shewanella* strains are nonfermenters, but colonization with fermenters would allow them to employ products of fermentation (lactate, formate, hydrogen, and some amino acids) for anaerobic respiration (Nealson et al., 2005).

With a diverse group of electron acceptors, genus *Shewanella* have able to respire and have shown an adaption to the life in extreme and different environments. *Shewanella* can easily grow in the lab conditions and are open to genetic manipulation. So, *Shewanella* have potential to remediate environmental pollutants and in microbial fuel cells (MFCs), where their metabolism have capacity to produce electricity (Gorby et al., 2006, Kim et al., 2002 Logan and Regan 2006a, Logan and Regan 2006b)

Shewanella are superior candidates for potential use in pollutant bioremediation among dissimilatory metal-reducing bacteria due to their intrinsic ability to respire using a wide range of electron acceptors (Nealson et al, 2005). The solubility and mobility of elements in soils, sediments, and water can be influenced with their oxidation states. Microorganisms display one means by which changes in oxidation states are catalyzed so that transport into rivers and groundwater can be blocked and cleanup facilitated (Nealson et al, 2005, Tiedje, 2002). Applications might contain ex situ remediation strategies and in situ bioremediation in storage tanks or areas of environmental contamination (Nealson et al, 2005). As a result, understanding the role of Shewanella, as well as other microorganisms in the oxidation of target compounds and the results in varying these oxidation reactions are precursor factors to optimize cleanup strategies. Bioremediation strategies consisting Shewanella can

be only applied in the laboratory and have not been used outside the lab yet. The future applications of Shewanella in bioremediation strategies consist of cleanup of contaminated global environments and groundwater. Since *Shewanella* mainly lives in aqua, further studies will be focused on how *Shewanella* behave in soil and contaminated groundwater environments (Hau and Gralnick, 2007).

2.12 Performance of the MFCs

In the initial years, simple substrates were commonly used in MFC system. In this system, glucose (Park and Zeikus, 2000; Park and Zeikus, 1999; Rabaey 2004), Acetate (Pham et al. 2003; Min et al., 2005; Bond and Lovley, 2003; Bond et al., 2002), Starch, lactate, molasses (Niessen et al. (2004), Sucrose (Ieropoulos et al., 2005; Lactate, (Kim et al., 1999d), pyruvate, (Kim et al., 1999c) was used as substrate in literature.

In most of the MFC studies so far, acetate has been the substrate of choice for electricity generation. The recalcitrance of many types of wastewater makes them more difficult to be utilized as compared to acetate (Sun et al., 2009). Acetate is a simple substrate and it is widely used as carbon source to induce electroactive bacteria (Bond et al., 2002). In order to benchmark new MFC components, reactor designs or operational conditions, acetate is commonly used as a substrate owing to its inertness towards alternative microbial conversions (fermentations and methanogenesis) at room temperature (Aelterman, 2009). Furthermore, acetate is the end product of several metabolic pathways for higher order carbon sources (Biffinger et al., 2008). Using a single-chambered MFC, Liu et al. (2005) reported that the power generated with acetate (506 mW/m2, 800 mg/L) was up to 66% higher than that produced with butyrate (305 mW/m2, 1000 mg/L). Very recently, Chae et al. (2009) compared the performance of four different substrates in terms of CE and power output.

Acetate-fed MFC showed the highest CE (72.3%), followed by butyrate (43.0%), propionate (36.0%) and glucose (15.0%). At the same time, when acetate was compared with a protein-rich wastewater as substrate in MFC, the MFC based on acetate-induced consortia achieved more than 2-fold maximum electric power, and one half of optimal external load resistance compared to the MFC based on consortia induced by a protein-rich wastewater (Liu et al., 2009). Nonetheless, the protein-rich

wastewater being a complex substrate provides the possibility of enriching more diverse microbial community than acetate. Having a more diverse microbial community helps to use various substrates or to convert complex organics to simpler compounds such as acetate that is used as electron donor for current production. Pant et al., (2009) prepared a table which compares lots of different substrates used in MFC and maximum current values (Table 2.3).

The current, power density, CE and pollutants removal efficiencies differ between the various studies according to the experimental conditions (initial wastewater composition, concentration, and MFC set up conditions). Table 2.3 demonstrates the current density (mA/cm2) at maximum power density (W/m^2) achieved by using various substrates in MFCs. With similar designs of MFC, 506 mW/m^2 was produced with acetate (Liu et al., 2005), but 261 mW/m^2 with swine wastewater (Min et al., 2005) and 146 mW/m^2 with domestic wastewater (Liu and Logan, 2004). The maximum power density which is produced appears to be related to the complexity of the substrate (i.e. single compound versus several compounds). Heilmann and Logan (2006) reported that with substrates like peptone and meat processing wastewater containing many different amino acids and proteins, lower power was produced than achieved using single compound like bovine serum albumin (BSA). The power generation measured using xylose as substrate was lower than studies with other fuels such as acetate or glucose (Huang et al., 2008). Nevertheless, the fact that xylose bioconversion in MFCs takes place at room temperature and relatively low substrate concentration levels, whereas anaerobic digestion generally fails because of low reaction rates, may make the MFC a complementary technology to the anaerobic digestion for celluloses and its hydrolytes (Pham et al., 2006). Recently, while evaluating the potential of various eco-systems in harnessing bioelectricity through benthic fuel cells, Venkata Mohan et al. (2009) reported that the substrate concentration of the water body showed significant influence on the power generation as they act as carbon source (electron donor) for the benthic metabolic activity. Water bodies containing higher organic matter were able to generate higher power output.

Table 2.3: Different substrates used in MFCs and the maximum current produced

Type of substrate	Concentration	Source inoculum	Type of MFC (with electrode surface area and/or cell volume)	Current density (mA/cm2) at Maximum power	Reference
Acetate	1 g/L	Pre-acclimated bacteria from MFC	Cube shaped one-chamber MFC with graphite fiber brush anode (7170 m^2/m^3 brush volume)	0.8	Logan et al. (2007)
Arabitol	1220 mg/L	Pre-acclimated bacteria from MFC	One-chamber air-cathode MFC (12 mL) with nonwet proofed carbon cloth as anode (2 cm^2) and wet proofed carbon cloth as cathode (7 cm^2)	0.68	Catal et al. (2008)
Corn stover biomass	1 g/L COD	Domestic wastewater	One-chamber membrane-less air-cathode MFC with carbon paper anode (7.1 cm^2) and carbon cloth cathode	0.15	Zuo et al. (2006)
Farm manure	3 kg in water (20% w/v)	Self build up of anaerobic environment	One reactor vessel of manure with anode at the bottom and cathode above the manure; carbon cloth electrodes (256 cm^2)	0.004	Scott and Murano (2007)
Landfill leachate	6000 mg/L	Leachate and sludge	Two-chambered MFC with carbon veil electrode (30 cm^2)	0.0004	Greenman et al. (2009)
Phenol	400 mg/L	Mixed aerobic activated sludge and anaerobic sludge (1:1, v/v)	Two-chambered MFC with aqueous air cathode, carbon paper electrode (25 cm^2)	0.1	Luo et al. (2009)
Artificial wastewater with glucose and glutamate	300 mg/L	Anaerobic sludge	Membrane-less MFC with anode (465 cm^2) at bottom and cathode (89 cm^2) at top of cylinder; graphite felt as both electrode	0.02	Jang et al. (2004)
Brewery wastewater	2240 mg/L	Full strength brewery wastewater	One-chamber air-cathode MFC with non-wet proofed carbon cloth as anode (7 cm^2) and wet proofed carbon cloth containing Pt as cathode	0.2	Feng et al. (2008)

Table 2.4 (continued): Different substrates used in MFCs and the maximum current produced.

Chocolate industry wastewater	1459 mg/L COD	Activated sludge	Two-chambered MFC with graphite rods as electrodes (16.485 cm^2) and ferricyanide as catholyte	0.302	Patil et al. (2009)
Domestic wastewater	600 mg/L	Anaerobic sludge	Two-chambered mediator-less MFC with plain graphite electrode (50 cm^2)	0.06	Wang et al. (2009)
Food processing wastewater	1672 mg/L COD	Anaerobic sludge	Two-chambered MFC with carbon paper as electrodes (22.5 cm^2)	0.05	Oh and Logan (2005a)
Meat processing wastewater	1420 mg/L	Domestic wastewater	One-chamber (28 mL) MFC with carbon paper electrodes (25 m^2/m^3)	0.115	Heilmann and Logan (2006)
Paper recycling wastewater	2.452 g/L	Diluted paper recycling wastewater	One-chamber MFC with graphite fiber-brush anode (5418 m^2/m^3 brush volume)	0.25	Huang and Logan (2008)
Protein-rich wastewater	1.75 g/L COD	Mesophilic anaerobic sludge	Two-chambered MFC with graphite rods as electrode (65 cm^2)	0.008	Liu et al.(2009)
Real urban wastewater	330 mg/L	Domestic wastewater	Separate anolyte (1000 cm^3) and catholyte chambers (100 cm^3) connected with a salt bridge; graphite cylinder anode (20 cm^2)	0.018	Rodrigo et al. (2007)
Starch processing wastewater	4852 mg/L COD	Starch processing wastewater	One-chamber air-cathode MFC with carbon paper anode (25 cm^2)	0.09	Lu et al. (2009)
Synthetic wastewater	12.1 g/L COD	Anaerobic mixed consortia producing hydrogen	Dual chamber MFC with graphite plate electrode (83.56 cm^2)	0.086	Venkata Mohan et al. (2008)

The beginning 10 years of research on MFCs have resulted in a 10,000-fold increase in the current density acquired from MFCs (Rabaey et al., 2004). This has further improved in recent years. Nevin et al. (2008) reported that *G. sulfurreducens* grown on acetate produced 2.15 kW/m^3 anode volume, which is the highest MFC power density reported to date. In a similar way, a new axenic strain *Rhodopseudomonas palustris* DX-1, isolated from an MFC produced higher power output (2720 mW/m2) than other mixed cultures (Xing et al., 2008). Nonetheless, at present the power

generated by MFCs is low from the view of large-scale wastewater treatment. In fact, the only MFC type which has been used for practical applications is sediment MFCs that harvest power from sediment by embedding an anode in sediment and connecting it via an electrical circuit to a cathode placed in the overlying aerobic seawater, making it feasible to power on-site to sensors and telemetry devices in remote oceanic areas (Tender et al., 2008). It is expected that with time, given the continued interest and support for this research, the output will reach a usable level for other applications as well.

2.13 Uses of microbial fuel cells

2.13.1 Electricity generation

As mentioned before, MFCs are capable of converting the chemical energy which is stored in the chemical compounds in a biomass to electrical energy with the help of microorganisms. Since chemical energy from the oxidization of fuel molecules is converted directly into electricity instead of heat, the Carnot cycle with a limited thermal efficiency is avoided and theoretically a much higher conversion efficiency can be achieved (>70%) just like conventional chemical fuel cells (Du et al., 2007). Chaudhury and Lovley (2003) reported that *R. ferrireducens* could produce electricity with an electron yield as high as 80%. Rabaey et al. (2003) also reported a higher electron recovery as electricity of up to 89%. An extremely high Coulombic efficiency of 97% was reported during the oxidation of formate with the catalysis of Pt black (Rosenbaum et al., 2006). Nevertheless, MFC power generation is still very low (Tender et al., 2002; Delong and Chandler, 2002), that is the rate of electron abstraction is very low. One practicable way to solve this problem is to store the electricity in rechargeable devices and then distribute the electricity to end-users (Ieropoulos et al., 2003). Capacitors were used in their biologically inspired robots named EcoBot I to accumulate the energy generated by the MFCs and worked in a pulsed manner. MFCs are especially convenient for to power small telemetry systems and wireless sensors which have only low power requirements to transmit signals such as temperature to receivers in remote locations (Ieropoulos et al., 2005; Shantaram et al., 2005). MFCs themselves can serve as distributed power systems for local uses, especially in underdeveloped regions of the world. They are considered by some researchers as a perfect energy supply candidate for Gastrobots by self-feeding the biomass collected by themselves (Wilkinson, 2000). Realistic

energetically autonomous robots would probably be equipped with MFCs that utilize different fuels like sugar, fruit, dead insects, grass and weed. The robot EcoBot-II solely powers itself by MFCs to perform some behavior including motion, sensing, computing and communication (Ieropoulos et al., 2003; Melhuish et al., 2006). Locally supplied biomass can be used to provide renewable power for local consumption. Applications of MFCs in a spaceship are also possible as they can supply electricity while degrading wastes generated onboard. Some scientists visualize that in the future, a miniature MFC can be implanted in a human body to power an implantable medical device with the nutrients supplied by the human body (Chai, 2002). The MFC technology is particularly favored for sustainable long-term power applications. Nevertheless, only after potential health and safety issues brought by the microorganisms in the MFC are thoroughly solved, it could be applied for this purpose (Du et al, 2007).

2.13.2 Wastewater treatment

The MFCs were considered to be used for treating waste water early in 1991 (Habermann and Pommer, 1991). Municipal wastewater contains a multitude of organic compounds that can fuel MFCs. The amount of power generated by MFCs in the wastewater treatment process can potentially halve the electricity needed in a conventional treatment process which consumes a lot of electric power aerating activated sludges. MFCs yield 50–90% less solids to be disposed of (Holzman, 2005).

Moreover, organic molecules such as acetate, propionate, butyrate can be thoroughly broken down to CO_2 and H_2O. A hybrid incorporating both electrophiles and anodophiles are especially suitable for wastewater treatment since more organics can be biodegraded by a variety of organics. MFCs using certain microbes have a special ability to remove sulfides as required in wastewater treatment (Rabaey et al., 2006). MFCs can enhance the growth of bioelectrochemically active microbes during wastewater treatment, therefore, they have good operational stabilities. Continuous flow and single-compartment MFCs and membrane-less MFCs are favored for wastewater treatment due to concerns in scale-up (Jang et al., 2004; Moon et al., 2005; He et al., 2005). Sanitary wastes, food processing wastewater, swine wastewater and corn stover are all great biomass sources for MFCs since they are rich in organic matters (Liu et al., 2004; Oh and Logan, 2005a; Min et al., 2005b;

Zuo et al., 2006). Up to 80% of the COD can be removed in some cases (Liu et al., 2004; Min et al., 2005b) and a Coulombic efficiency as high as 80% has been reported (Kim et al., 2005).

2.13.3 Biosensor

A biosensor consists of two components: a bioreceptor and a transducer. The bioreceptor is a biomolecule that recognizes the target analyte, and the transducer converts the recognition event into a measurable signal.

Another potential application of the MFC technology is to use it as a sensor for pollutant analysis and in situ process monitoring and control (Chang et al., 2004, 2005). The proportional correlation between the Coulombic yield of MFCs and the strength of the wastewater make MFCs possible biological oxygen demand (BOD) sensors (Kim et al., 2003). An accurate method to measure the BOD value of a liquid stream is to calculate its Coulombic yield. A number of works (Chang et al., 2004; Kim et al., 2003) demonstrated a good linear relationship between the Coulombic yield and the strength of the wastewater in a quite wide BOD concentration range. Nonetheless, a high BOD concentration requires a long response time since the Coulombic yield can be calculated only after the BOD has been depleted unless a dilution mechanism is in place. Efforts have been made to improve the dynamic responses in MFCs used as sensors (Moon et al., 2004). A low BOD sensor can also show the BOD value based on the maximum current because the current values increase with the BOD value linearly in an oligotroph-type MFC. During this stage, the anodic reaction is limited by substrate concentration. This monitoring mode can be applied to real-time BOD determinations for either surface water, secondary effluents or diluted high BOD wastewater samples (Kang et al., 2003). MFC-type of BOD sensors are advantageous over other types of BOD sensor since they have excellent operational stability and good reproducibility and accuracy. An MFC-type BOD sensor constructed with the microbes enriched with MFC can be kept operational for over 5 years without extra maintenance (Kim et al., 2003), far longer in service life span than other types of BOD sensors reported in the literature. Kumblanghan et al. (2007) showed the development of a MFC sensor system for fast estimation of easily biodegradable organic matter. The sensor system was operated by integrating with an anaerobic bioreactor for continuous supply of stable anaerobic consortium. Replacement of the biological recognition element was carried out for

each sample analysis. The system still gives stable measurement with a good reproducibility. The sensor response time was estimated around 3–5 min and did not have to wait for the metabolic recovery of anaerobic consortium in the anodic compartment. This is considered as an advantage for this sensor system configuration. Even though glucose was used as the only preliminary substrate in the current study to evaluate the sensor performance under the well-controlled condition, the MFC sensor system used mixed consortium originated from anaerobic sludge should also be able to degrade other kinds of organic matter even in more complex form. Nevertheless, the MFC sensor needs in its present configuration maintenance in terms of regular membrane cleaning. The sensitivity and the detection limit of the sensor system can definitely be improved even further by improving the efficiency of ions transport in the electrolyte.

Furthermore, MFC-based biosensor can act as online toxicity sensor. Electrical current is a direct linear measure for metabolic activity of electrochemically active microorganisms. Microorganisms gain energy from anodic overpotential and current is strongly dependent on anodic overpotential. Thus, control of anodic overpotential is necessary to detect toxic events and prevent false positive alarms (Stein et al, 2010).

In the second part of this thesis, MFCs were used as biosensors. Some xenobiotics (antibiotics and hormones) were tried as inhibitory matters in the this system and detected of bacteria’s response.

2.14 Xenobiotics in aquatic environment

Xenobiotics (the term is derived from the Greek words xenos which means foreigner or stranger and bios which means life) are chemicals found in an organism which, however, are not normally produced (or expected to be present in it). Especially, pharmaceuticals (medicines) such as antibiotics are xenobiotics in humans since the human body does not produce them itself, nor are they part of a normal diet. Natural compounds can also become xenobiotics if they are taken up by another organism, such as the uptake of natural human hormones by fish found downstream of sewage treatment plant outfalls, or the chemical defenses produced by some organisms as protection against predators. Nonetheless, the term xenobiotics is very often used in the context of pollutants such as dioxins and their effect on the biota since

xenobiotics are interpreted as substances foreign to an entire biological system, i.e. artificial substances, which did not exist in nature before their synthesis by humans.

Less amount of human and animal medicines have been detected in many countries in groundwater, drinking water, surface water, seawater and wastewater treatment plant (WWTP) effluents. For some pharmaceuticals, effects on aquatic organisms have been investigated in acute toxicity tests.

What kind of xenobiotics and what concentrations occur in the aquatic environment? What is the fate in surface water and in Wastewater Treatment Plant (WWTP)? What are the modes of action of these compounds in humans and are there similar targets in lower animals? What acute and chronic ecotoxicological effects may be elicited by pharmaceuticals and by mixtures? What are the effect concentrations and how do they relate to environmental levels? (Fent et al, 2005)

Xenobiotics such as pharmaceticals that are not readily degraded in the wastewater treatment plant (WWTP) are being discharged in treated effluents resulting in the contamination of water sources such as rivers, lakes, estuaries, groundwater and drinking water. When wastewater sludge is applied to agricultural fields, not only contamination of soil, runoff into surface water but also drainage may occur. Figure 2.9 shows principal pathway of environmental exposure to drugs consumed in human and veterinary medicine. It is significant that environmental concern is not necessarily a high production volume of a certain pharmaceuticals by itself, but the environmental persistence and critical biological activity (e.g. high toxicity, high potency for effects on biological key functions such as reproduction) (Fent et al, 2005).

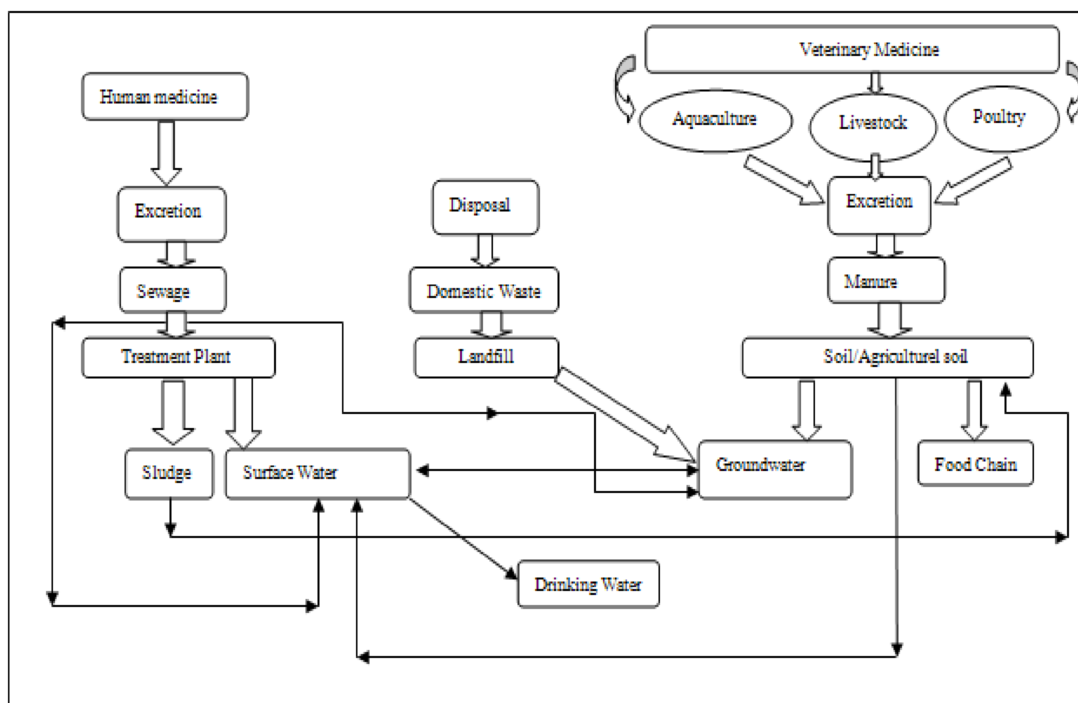


Figure 2.9: Pathway of environmental exposure to drugs consumed in human and veterinary medicine (illustrated from Diaz-Cruz et al., 2003).

2.14.1 Antibiotics in Aquatic Environment

During last decades, although antibiotics have been used lots amount, until recently the existence of these substances in the environment has received little notice. It is only in recent years that a more complex investigation of antibiotic substances in some countries has been undertaken in order to permit an assessment of the environmental risks they may pose.

2.14.1.1 Source

Pharmaceuticals are discharged after application in their native form or as metabolites and enter aquatic systems with different ways as mentioned before. The main source from humans is ingestion following excretion and disposal via wastewater. Therefore, municipal wastewater is the main route that brings human pharmaceuticals after normal use and disposal of unused medicines into the environment. Wastewaters comes from manufacturers, hospital and landfill leachates (Holm et al., 1995) and may contain significant concentrations of pharmaceuticals. Veterinary pharmaceuticals may also enter aquatic environment via manure application to fields and after runoff. Furthermore, they may enter via direct application in aquaculture such as fish farming.

An antibiotic is a chemotherapeutic agent that inhibits the growth of microorganisms, such as bacteria, fungi, or protozoa. Chemotherapeutical refers to compounds used for the treatment of disease that kill cells, specifically microorganisms. There are more than 250 different chemical entities registered for use in medicine and veterinary medicine (Kümmerer and Henninger, 2003).

2.14.1.2 Modes of Action

All antibiotics have different modes of action by which they act as therapeutic agents. Some modes of action of different antibiotics are mentioned below:

Cell Wall Synthesis Inhibitors: Bacteria contain peptidoglycan (a polymer consisting of sugars and aminoacids that forms a mesh-like layer outside the plasma membrane of bacteria) which is highly essential in maintaining the cell wall structure. Cell wall synthesis inhibitors such as beta-lactams, cephalosporins and glycopeptides block the ability of microorganisms to synthesize their cell wall by inhibiting the synthesis of peptidoglycan.

Interfering with Protein Synthesis: These classes of antibiotics inhibit the protein synthesis in the cell. For instance, tetracyclines, erythromysin and aminoglycosides. Cell Membrane Inhibitors: Antibiotics such as polymyxins disrupt the integrity and structure of cell membranes, thereby killing them. These set of antibiotics are mostly effective on gram negative bacteria since these are the bacteria that contain a definite cell membrane.

Effect on nucleic acids: DNA and RNA are extremely essential nucleic acids present in every living cell. Antibiotics such as quinolones and rifamycins bind to the proteins which are required for the processing of DNA and RNA, thus blocking their synthesis and thereby affecting the growth of the cells.

Competitive inhibitors: They are also named anti-metabolites, these are antibiotics that competitively inhibit the significant metabolic pathways occurring inside the bacterial cell. The important ones in this class are sulfonamides such as sulfamethoxazole.

Figure 2.10 shows modes of action of some antibiotics.

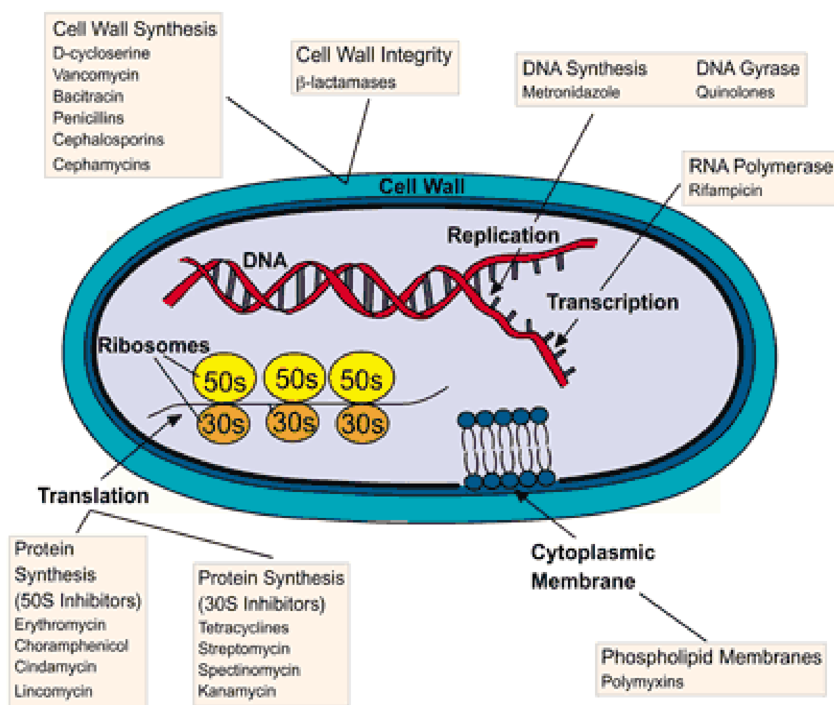


Figure 2.10: Modes of action of some antibiotics (Url-1).

Antibiotics can be grouped according to either their chemical structure or mechanism of action. They are a diverse group of chemicals that can be divided into different sub-groups such as ß-lactams (e.g. amoxicillin), quinolones (e.g. ciprofloxacin), tetracyclines (e.g. doxycycline), macrolides (e.g. erythromycin), sulphonamides (e.g. sulfamethoxazole) and others.

In this study, three different antibiotics (erythromycin, sulfomethoxazole and tetracycline) were used. Material Safety Data Sheet (MSDS) can be seen Table 2.4.

Wise (2002) estimated antibiotic consumption worldwide to lie between one hundred thousand and two hundred thousand ton annually. In 1996, about ten-two hundred ton of antibiotics were used in the EU, of which approximately 50% was applied in veterinary medicine and as growth promoters. In 1999, there were a total of thirteen thousand two hundred sixteen ton of antibiotics used in the European Union and Switzerland, 65% of which was applied in human medicine, according to European Federation of Animal Health (Kummerer, 2009). In the United States, one estimate is that 50% of the twenty two thousand seven hundred metric tons of all antimicrobials prescribed annually are for humans and 50% for use in animals, agriculture and aquaculture.

Table 2.5: MSDS table for erythromycin, sulfomethoxazole and tetracycline.

Antibiotic	Physical state	Molecular weight	M. Formula	Toxicological information (acute)
ERYTHROMYCIN (ERY)	Crystalline solid	733.9 g/mol	$C_{37}H_{67}NO_{13}$	LD50-Rat-6g/kg-oral LD50-Mouse-12g/kg-oral LD50-Hamster-3g/kg-oral
SULFAMETHOXAZOLE (SMX)	powder	253.279 g/mol	$C_{10}H_{11}N_3O_3S$	LD50-Rat-6,2g/kg-oral LD50-Mouse-2,3g/kg-oral
TETRACYCLINE (TC)	powder	444,44 g/mol	$C_{22}H_{24}N_2O_8$	LDLo-woman-0,31g/kg-oral LD50-Rat-0,807g/kg-oral LD50-Mouse-0,678g/kg-oral

Human medicine consumption in total, per capita and the individual share of each compound varies from country to country. Antibiotic prescription rates and intake without prescription vary markedly between countries (Mölstad et al., 2002).

For the EU, in total 22 g per capita and year would be due to medical use. For the USA, it is estimated to be approximately 17 g per capita and year, when calculated from the available data for use in human medicine (Kümmerer, 2004).

According to different legislation and differing degrees of importance ascribed to the use of antibiotics, reliable data providing information on the total use and the patterns of antibiotic use and per capita consumption exist for only a few countries.

As for the metabolism of active compounds in humans, there is a wide range in the degree to which these compounds are metabolized (Kümmerer and Henninger, 2003). Some compounds are metabolized by 90% or more, while others are metabolized by only 10% or even less. Nevertheless, when the amounts used for each active compound is multiplied by its excretion rate, then even some compounds with a high metabolization rate are the most important. Sometimes the formation of metabolites can result in compounds which are more toxic to humans than the parent compound.

Antibiotics are also used to promote the growth of animals in some countries where they are used at low doses in animal feeds and are considered to improve the quality

of the product, with a lower percentage of fat and higher protein content in the meat. The use of even small amounts of antibiotics is associated with the selection of resistance in pathogenic bacteria. Alternatives to improve the quality of the product with a lower percentage of fat and a higher protein content in the meat without using antibiotics are available. The use of antibiotics as growth promoters in animal farming has been banned for the last several years in the European Union and some other countries such as Sweden and Switzerland (Kummerer, 2009).

Since the 1950s, antibiotics have been used to control certain bacterial diseases of fruit, vegetable, and ornamental trees. To be a viable candidate for disease control, the antibiotic needed to: (i) be active on or inside of the plant; (ii) tolerate oxidation, UV irradiation, rainfall, and high temperatures.

Aquaculture represents that farming of some aquatic organisms including fish, molluscs, crustaceans and aquatic plants. Farming implies some sort of intervention in the rearing process to enhance production, such as regular stocking, feeding and protection from predators. In aquaculture, antibiotics have been used mainly for therapeutic purposes and as prophylactic agents.

2.14.1.3 Occurence

Antibiotics are only partially eliminated in sewage treatment plants. If they are not eliminated during the purification process, they pass through the sewage system and may end up in the environment, mainly in the water compartment. Residual amounts can reach surface waters, groundwater or sediments.

Active substances discharged with liquid manure can be washed off from the top soil after rain. Moreover, direct discharge, especially from poultry processing, meat processing, and aquaculture, as well as from pets (e.g. aquariums) is also possible and can contribute towards an increase in the total concentration of antibiotics in sewage and surface water.

Generally, concentrations were in the higher µg/Lrange in hospital effluent, in the lower µg/L range in municipal waste water, and in the higher and lower µg/L range in different surface waters, ground water and sea water in a harbour (Xu et al., 2007). Antibiotics have also rarely been found in drinking water (Ye et al., 2007).

Examples of measured concentrations of antibiotics in aquatic environment may be

given in literature. Giger et al. (2003) analyzed that the concentration of erythromycin-H_2O was 287 nanogram/L in sewage treatment plant effluent. Farber et al.(2002) found that 370 nanogram/L in sewage treatment plant effluent for sulfamethoxazol and 20 nanogram/L for tetracycline . Sulfamethoxazol was detected 40 nanogram/L in surface water and 20 nanogram/L in ground water.

2.14.1.4 Elimination (Fate)

Elimination of organic compounds in the environment is the result of different processes. These processes can be biotic ones, such as biodegradation by bacteria and fungi. Non-biotic elimination processes are sorption, photolysis, hydrolysis, oxidation and reduction.

In general, sorption of acidic pharmaceuticals to sludge is suggested to be not very important for the elimination of pharmaceuticals from wastewater and surface water. Thus, levels of pharmaceuticals in digested sludge and sediments are suggested to be relatively low, as it was demonstrated in several monitoring studies (Ternes et al., 2004; Urase and Kikuta, 2005).

The sorption behavior of antibiotics can be very complex and difficult to assess. Binding to particles or the formation of complexes may cause a loss in detectability, as well as a loss in antibacterial activity. The loss of antibacterial activity, for instance, was demonstrated for an aquaculture antimicrobial in seawater driven by the formation of complexes with the magnesium and calcium naturally present in marine water. Tetracyclines are able to form complexes with double cations, such as calcium or magnesium (Christian et al.,2003). Humic substances may alter the surface properties and sites available for sorption and reactions. They can either suppress or promote sorption of organic compounds to mineral surfaces. Limited evidence in the literature points to the reduction in sorption of the antibiotic tetracycline to clay minerals in the presence of humic substances. Gu and Karthikeyan (2008) report strong interaction between humic acids, hydrous Al oxide and the effect of this association on tetracycline sorption. The presence of humic substances, in both dissolved and mineral-bound forms, is likely to increase the environmental mobility of tetracycline compounds.

If a substance is light sensitive, photo-decomposition may be of major significance in the elimination process. Some antibiotics are light sensitive (e.g. tetracyclines,

sulphonamides). Nonetheless, not all compounds are photo-degradable (Turiel et al., 2005).

The significance and extent of direct and indirect photolysis of antibiotics in the aquatic environment are different for each compound. Studies taking into consideration indirect photolysis and interaction with dissolved organic matter such as humic acids are rare (Sukul et al., 2008). Such data would be helpful in order to comprehend better the fate of antibiotics in surface waters. Tetracyclines are susceptible to photo-degradation. Samuelsen (1989) investigated the sensitivity of oxytetracycline towards light in seawater as well as in sediments. The antibacterial substance proved to be stable in sediments rather than in seawater.

Another significant pathway for the non-biotic elimination of organic substances in the environment is hydrolysis. Some instability in water could be demonstrated for some tetracycylines (Halling-Sørensen, 2000). In general, the hydrolysis rates for oxytetracycline increase as the pH deviates from pH 7 and as temperature increases. The half-lives of oxytetracycline under investigation varied due to differences in temperature, light intensity and flow rate from one test tank to another. Nevertheless, sulphonamides and quinolones are resistant to hydrolysis.

Antibiotic formulation effluents are well known for the difficulty of their elimination by traditional bio-treatment methods and their important contribution to environmental pollution is due to their fluctuating and recalcitrant nature. For advanced effluent treatment, oxidation processes are usually applied. However, ozonation will not work well for all types of molecules. The presence of carbon–carbon double bonds, aromatic bonds or nitrogen is a necessary prerequisite. Nonetheless, the presence of these structural elements does not guarantee the fast and full degradation or even the mineralization of a molecule (Kummerer, 2009).

The effect of ozonation on the degradation of oxytetracycline in aqueous solution at different pH values (3, 7 and 11) was investigated by Li et al. (2008). The results show that ozonation as a partial step in a combined treatment concept is a potential technique for biodegradability enhancement for effluents from pharmaceutical industries containing high concentrations of oxytetracycline, provided that the appropriate ozonation period is selected.

It has been shown that COD (chemical oxygen demand) removal rates increase with

the increasing of pH as a consequence of enhanced ozone decomposition rates at elevated pH values. The results of bioluminescence data demonstrate that the initial by-products after partial ozonation (5–30 min) of oxytetracycline were more toxic than the parent compound (Li et al., 2008).

Sulfamethoxazole was efficiently degraded by ozonation (Dantas et al., 2007). The complete sulfamethoxazole removal was achieved for an in photo-Fenton process (González et al., 2007).

Most antibiotics which have been tested to date have not been biodegradable under aerobic conditions. No evidence of biodegradation for tetracycline was observed during a biodegradability test (sequence batch reactor), and sorption was found to be the principal removal mechanism for tetracycline in activated sludge (Kim et al., 2005).

2.14.1.5 Effects

If a substance is not eliminated in any way, it can reach the environment with the potential of adversely affecting aquatic and terrestrial organisms. It might reach humans again via drinking water. Until now, antibiotics have not yet been reported to be present in drinking water, only certain pharmaceuticals and diagnostics such as clofibric acid or amidotrizoic acid have been reported to date (Kümmerer, 2003).

The effects of antibiotics on human health have been reported in the medical literature.

Tetracycylines should not be applied for young children owing to the negative interaction of tetracyclines with their developing teeth. Due to their antimicrobial activity, a negative interaction within the gut can happen within therapy.

Antibiotics are of particular interest since we currently do not know whether their presence in natural waters contributes to the spread of antibiotic resistance in microorganisms (Kümmerer, 2003). Generally, the effects of antibacterial agents on bacteria and microalgae are found to be 2–3 orders of magnitude below the toxic values for higher trophic levels (Brain et al., 2004, 2008; Robinson et al., 2005; Yamashita et al., 2006). This differential sensitivity is likely dependent on differences in metabolic potential as well as uptake kinetics (Brain et al., 2008). Adverse impacts of antibiotics on higher aquatic organisms have been reported,

however, in most cases in which effects were detected the concentrations were environmentally irrelevant. Nevertheless, secondary effects due to changes in the natural balance are not negligible.

Acute tests seem to be inappropriate as a means of determining the effects of antibiotics on bacteria. Antibiotics possess specific modes of operation and impacts frequently become evident upon extending the incubation period. Toxicity tests with bacteria have demonstrated that chronic exposure to antibiotics is critical rather than acute (Backhaus and Grimme, 1999, 2000; Froehner et al., 2000; Kümmerer et al., 2004). Thomulka and McGee (1993) determined the toxicity of a number of antibiotics (e.g. novobiocin, tetracycline, chloramphenicol, ampicillin, streptomycin) on Vibrio harveyi in two bioassay methods. Almost no toxic effects were found after short incubation times when luminescence was used as an endpoint. Nonetheless, in a long term assay using reproduction as an endpoint a toxic effect in environmentally relevant concentrations could be detected for almost all the substances.

Thus, one should be aware that the results given for standard bacterial toxicity tests, such as shortterm tests with the luminescent bacterium Vibrio fisheri and others, may underestimate effects and risks.

Effectiveness may be modulated by environmental conditions e.g. if bio-availability is redsulfomeuced by sorption or activity is affected by complexation. If a substance is not eliminated in any way, it can reach the environment with the potential to adversely affect the aquatic and terrestrial organisms.

Antibiotics have the potential to affect the microbial community in sewage systems. The inhibition of waste water bacteria may seriously affect organic matter degradation; thus, effects of antibacterial agents on the microbial population are of great interest. A reduction in the number of bacteria together with alterations in microbial populations were observed in a model sewage purification system when different commonly applied antibiotics were added in concentrations that may occur in hospital waste water (Kümmerer et al., 2000).

Several antibiotics proved to have low toxicity in relation to nitrifying bacteria in acute tests. These substances showed no effects upon nitrification in concentrations even higher than what might be environmentally expected (Kummerer, 2009). Nevertheless, the time period of the test significantly influences the results

(Halling-Sørensen, 2000; Kümmerer et al., 2004). An antimicrobial was found to require high concentrations in order to inhibit the nitrification process in a short term test (2–4 h), however, a prolonged test period over 5 day showed effects one order of magnitude below the inhibitory concentrations of the acute test (Kummerer, 2009). In a study by Dokianakis et al. (2004), the effects caused by the presence of seven different pharmaceuticals on a culture of nitrite-oxidizing bacteria isolated from activated sludge were reported. For ofloxacin and sulfamathoxazole, significant inhibition was observed.

Substances which are not or are only partly eliminated in the sewage treatment plant will reach surface water where they may affect organisms of different trophic levels. In a model aquatic system using synthetic fresh water, nitrifying bacteria were remarkably affected by an aquaculture antibiotic. The disruption of the nitrification process already occurred in concentrations likely to be found in fish treatment tanks and sediments (Kummerer, 2009). The results of toxicity tests with bacteria point out that adverse toxic effects on natural bacterial communities cannot be excluded. Blue–green algae (cyanobacteria) seem to be sensitive to many antibiotics, for instance amoxicillin, benzyl penicillin, tetracycline and tiamulin (Boxall et al., 2003). As algae are the basis of the food chain, even slight decreases in the algal population may affect the balance in an aquatic system.

In a study by Kim et al. (2007) sulfamethoxazole, sulphachlorpyridazine, sulfathiazole, sulphamethazine, sulphadimethoxine, and trimethoprim were examined for their acute aquatic toxicity by employing a marine bacterium (Vibrio fischeri), a freshwater invertebrate (Daphnia magna), and the Japanese medaka fish (Oryzias latipes). In this study, Daphnia was in general the most susceptible among the test organisms. Predicted environmental concentrations (PECs) derived for the test pharmaceuticals in Korea ranged between 0.14 and 16.5µg/L.

In another study (Park and Choi, 2008), eleven commonly used antibiotics including sulphonamides, tetracyclines, aminoglycosides, fluoroquinolones, and ß-lactams were evaluated for their acute and chronic aquatic toxicities using standard test organisms e.g. Vibrio fischeri, Daphnia magna, Moina macrocopa, and Oryzias latipes. Among the antibiotics tested for acute toxicity, neomycin was the most toxic followed by trimethoprim, sulfamathoxazole and enrofloxacin. Sulphamethazine, oxytetracycline, chlortetracycline, sulphadimethoxine and sulfathiazole were of

intermediate toxicity, whereas ampicillin and amoxicillin were the least toxic for the test organisms. There were no trends in sensitivity among test organisms or among the different classes of antibiotics.

2.14.2 Estrogens (Hormones) in Aquatic Environment

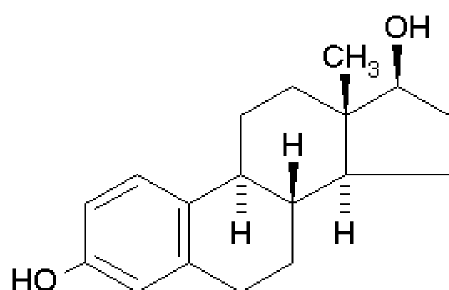
Environmental estrogens are chemical pollutants that can disrupt the endocrine system of animals by binding to and activating the estrogen receptor(s). They include both natural and synthetic steroid estrogens (Jobling et al., 2003).

Natural estrogens such as estrone (E1), 17β-estradiol (E2), estriol (E3), and the particularly recalcitrant synthetic estrogen 17α-ethinylestradiol (EE2) used as oral contraceptive accumulate in the environment and may cause health problems (Cajthaml et al., 2009).

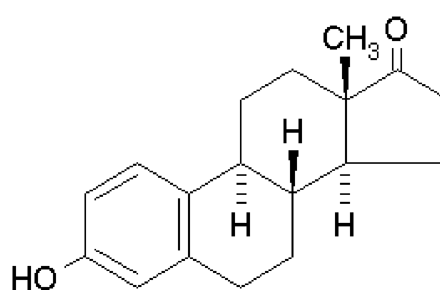
Natural estrogen is a steroid compound hormone which plays a central role in other uniquely female functions, such as reproduction and lactation. Synthetic estrogen that is hugely used as an oral contraceptive bind to the estrogen receptor and can misregulate or interfere with normal biological responses (Soto et al., 1991). A steroid is a type of organic compound which contains a specific arrangement of four cycloalkane [alkanes are types of organic hydrocarbon compounds which have only single chemical bonds in their chemical structure. Cycloalkanes consist of only carbon (C) and hydrogen (H) atoms and are saturated since there are no multiple C-C bonds to hydrogenate (add more hydrogen to).

A general chemical formula for cycloalkanes would be $C_nH_{2(n+1-g)}$ where n = number of C atoms and g = number of rings in the molecule] rings that are joined to each other.

Chemical formula of natural and synthetic estrogens can be seen figure 2.11.

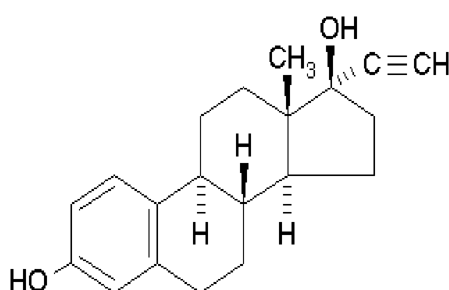


a)17β-Estradiol
Molecular Formula: $C_{18}H_{24}O_2$
Molecular Weight:272.4



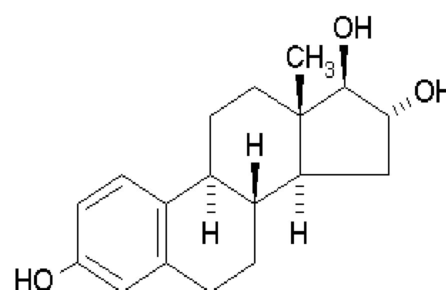
b)Estrone

Molecular Formula: $C_{18}H_{22}O_2$
Molecular Weight: 270.4



c)17α-Ethynylestradiol

Molecular Formula: $C_{20}H_{24}O_2$
Molecular Weight: 296.4



d)Estriol

Molecular Formula: $C_{18}H_{24}O_3$
Molecular Weight: 288.4

Figure 2.11: The structure of a)17 β Estradiol b)Estrone c)17 α Ethynylestradiol d) Estriol (Url-2)

2.14.2.1 Sources

Synthetic steroid hormones in contraceptive pills, the annual production lies in a couple of hundreds kilograms per year in the EU, is quite persistent in the environment and shows estrogenic activity in fish at 1–4 ng/L, or lower. Hence, pharmaceuticals having environmental relevance share the following properties: often, but not always, high production volume combined with environmental persistence and biological activity, mainly after long-term exposure (Fent et al, 2006).

Many researchers have found estrogenic compounds in streams, rivers, and lakes throughout the world, as well as in the effluent of wastewater treatment plants in the

USA, Europe, Asia, South America, and Australia. These compounds generally come from human extreta since wastewater treatment plants simply act as collectors that funnel our waste, and the hormones or hormone mimics in that waste, into the environment.

Synthetic estrogens from contraceptive pills, hormone-replacement therapy, surfactants, and plasticizers, as well as natural estrogens discharged by people in their urine, all end up in our wastewater and, from there, can be discharged into our rivers and lakes. This discharge becomes a significant problem in areas with large wastewater flows which can, in some instances, contribute the bulk of the water in a stream. Additionally, some estrogenic compounds degrade poorly and remain in the environment for long periods of time, where they can spend more time interacting with fish. In addition to studies that have shown the widespread and global presence of estrogenic compounds in lakes and rivers, other studies have reported the effects of these compounds on wildlife. For instance, many studies have identified abnormal sexual development in fish populations, which has been considered to be a result of exposure to the effluent from nearby wastewater treatment plant (Jobling et al., 1998, Vajda et., 2008). In perhaps the most striking and disturbing study to date, scientists performed a seven-year-whole-lake experiment in northwestern Ontario, Canada, in which very low levels of the active compound in contraceptive pills were added to a lake by the scientists themselves. In addition to observing abnormal sexual development of fathead minnows in the lake, investigators discovered that the minnow population decreased to near the point of extinction (Kidd et al., 2007). In the future, whole populations of fish can be at risk from estrogenic pollution, which can produce dramatic ecological effects by altering whole food webs (Lundgren M.S. and Novak, P.J., 2010).

2.14.2.2 Occurence and elimination (fate)

Steroidal hormones have been reported on in many reports. These steroids have been found in numerous studies in many countries both in wastewater and surface water. A survey in the U.S.A. demonstrated that maximal and median EE2 concentrations were as high as 831 and 73 ng/L, respectively (Kolpin et al., 2002). They were detectable in 16 and 10% of the streams sampled. Generally, median concentrations are much lower being in the range of nondetectable up to 9 ng/L in treated wastewater in several countries (Baronti et al., 2000). Typical wastewater effluent

concentrations are 0.5 ng/L and they are even lower in surface water. Nonetheless, these concentrations must put into the perspective of their high biological activity accounting for potential estrogenic effects on fish.

The processes participating in their removal from soil, wastewater, water sediments, groundwater-aquifer material, and wastewater or sewage treatment plant effluents may involve the action of bacterial and microbial consortia, and in some cases fungi and algae.

To assess the role of bacterial degradation in the fate of estrogenic steroids in the environment, the transformation of estrogens was studied under both aerobic and anaerobic conditions. Czajka and Londry (2006) investigated the potential for anaerobic biodegradation of EE2 and E2 using cultures established from lake water and sediments. No anaerobic degradation of EE2 (5 mg/L) was observed in their experiments. Even though E2 could be transformed to estrone, the complete degradation of estrogens was minimal, suggesting that they accumulate in anoxic environments. On the contrary, a rapid degradation of four estrogens, including EE2, was observed in river water-sediments and groundwateraquifer material under aerobic conditions (Sarmah and Northcott, 2008). The 90% dissipation time values for all compounds ranged from 0.9 to 2.8 days under both conditions. Nevertheless, under anaerobic conditions, the 90% dissipation time values for EE2 exceeded 1000 days. Anaerobic degradation of estrogenic compounds in this study was attributed to the sulfate-, nitrate-, and iron-reducing conditions. It was also postulated that overall degradation of the compounds was influenced by abiotic factors accounting for up to 40% degradation. The authors suggested that these abiotic factors could include hydrolysis, chemical reduction, photolysis, irreversible sorption or volatilization. Moreover, they stated that E2, EE2 can also undergo surfaced-induced abiotic transformation due to the catalytic effect of various clay minerals.

In a similar way, Ying et al. (2003) reported an aerobic degradation of E2 and 4-n-nonylphenol in the aquifer material with a half-life of the chemicals equal to 2 and 7 days. EE2 was degraded slowly with an estimated half-life of 81 days, with little or no degradation of estrogens observed within 70 days under anaerobic conditions in native groundwater. In another experiment, Ying et al. (2008a) described faster degradation of EE2 under aerobic conditions in effluent supplemented or unsupplemented aquifer material. EE2 had a half-life of 15 days compared with 26

days in the aquifer material and groundwater microcosm. Biodegradation was not important under anoxic conditions. Higher resistance of EE2 to biodegradation, when compared to E2, was proven also with microorganisms from water samples of English and Japanese rivers (Jurgens et al., 2002; Matsuoka et al., 2005). Aerobic degradation of endocrine disrupting compounds which are E2, EE2 in seawater collected from the coastal area of South Australia showed that the chemicals could be degraded by aerobes in seawater once the microbes in water became acclimated to the chemicals (Ying and Kookana, 2003). The study demonstrated that EE2 (together with bisphenol A and E2) was degraded in water within 56 days with a lag phase preceding its fastest degradation. EE2 was also found to be degraded in the marine sediment under aerobic conditions ($t1/2 > 20$ d), whereas under anaerobic conditions no degradation was noted. In comparison with aquifer and marine environments, relatively fast EE2 degradation was reported in agricultural soils and nitrifying activated sludge (Vader et al., 2000; Colucci and Topp, 2001a,b). Shi et al. (2004) showed the link between the ammonia oxidizing bacteria (AOB) from nitrifying activated sludge and biodegradability of natural and synthetic estrogens, including EE2. The addition of allylthiourea, an ammonia oxidation inhibitor, significantly reduced the estrogen-degrading activity of activated sludge and inhibited the growth of AOB. These results suggested that other microorganisms aside from AOB are also involved in estrogen degradation by nitrifying activated sludge. The estrogen degradation potential of AOB was further demonstrated with an isolated strain of *Nitrosomonas europaea* when the strain was able to decompose 1 mg/L of EE2 within 96 h during a batch experiment. Its ability of estrogen degradation was also inhibited by allylthiourea (Shi et al., 2004).

Recently, several studies characterized the estrogen degradation potential of various isolated bacterial strains. The first E2 degrading bacterium *Novosphingobium tardaugens* was isolated from activated sludge (Fujii et al., 2002). Nevertheless, not all estrogen-degrading bacteria were shown to degrade recalcitrant EE2. Ke et al. (2007) isolated three strains that belonged to genera *Acinetobacter, Agromyces, and Sphingomonas*, and were able to degrade E1, E2, and E3 under both aerobic and anoxic conditions. Nonetheless, EE2 remained stable during cultivation with all three isolates. On the other hand, *Rhodococcus zopfii* and *Rhodococcus equi* isolated from activated sludge of Japanese wastewater treatment plants were shown to degrade all the four principal estrogens (E1, E2, E3 and EE2) (Yoshimoto et al., 2004). *R. zopfii*

Y50158 showing the strongest degradation activities removed completely 100 mg /L of EE2 within 24 h in this study. Another EE2-degrading bacterium, which was isolated from activated sludge of a wastewater treatment plant in China, was identified as *Sphingobacterium sp.* JCR5 (Ren et al., 2007).

In the latest study, six strains of Proteobacteria were added to the list of strains capable of EE2 transformation (Pauwels et al., 2008b). Bacterial strains isolated from compost cometabolized (an enzyme produced by microbiological metabolism that aids degradation of a contaminant) EE2 at low µg/L levels when metabolizing E1, E2, or E3. No other metabolites beside E1, E2, E3 and EE2 were detected, suggesting that total degradation and fission of aromatic rings occurred.

Generally it can be summarized that bacterial EE2 transformation is significantly affected by oxygen with higher degradation efficiency under aerobic conditions. Relatively fast bacterial degradation was recorded in agriculture soils and nitrifying activated sludge. In this case, the key role in transformation process could be attributed to AOB showing highest removal at high initial ammonia concentrations. Even higher degradation efficiencies (tens mg/L of EE2) were recorded with *Rhodococcus* and *Sphingobacterium sp.* isolated from activated sludges.

Mie`ge et al. (2009) studied that various WWTP processes were evaluated and it was found that EE2 was highly removed (70%) in the dissolved aqueous phase by a fixed biomass reactor with high sludge retention time and waste stabilisation ponds. The processes using activated sludge with nitrogen treatment were substantially more efficient than treatments. They also discovered a tendency between higher EE2 influent concentrations and removal extents.

Earlier, Johnson et al. (2005) compared 17 WWTPs in Europe based on the concentrations of nonylphenol and steroid estrogens in effluents. Treatment processes included primary chemical treatment only, submerged aerated filter, oxidation ditch, activated sludge, and trickling filter combined with activated sludge. The lowest estrogen removal was observed in the WWTP which only used primary treatment. In this study, EE2 was detected only in effluents of two WWTPs.

In a similar way, Ying et al. (2008b) described the degradation of EE2 and other estrogens in four South Australian WWTPs with different technologies. The removal rates for the estrogens were variable but consistent with the plant performance parameters such as biochemical oxygen demand, suspended solids and ammonia, and

the least efficient WWTP was that which consisted of a series of anaerobic and aerobic lagoons. The study demonstrated that E1 and EE2 were more persistent during the treatment than the other estrogens.

A poor EE2 removal was also detected in a WWTP with nitrifying activated sludge system situated in England (Kanda and Churchley, 2008). The results of a 24-h treatment experiment showed excellent removal for other estrogens and nonylphenols (97–99%), and a 98% removal of estrogenicity. Nevertheless, the removal of EE2 was only 3% which during the 7-day treatment period only increased to a mere 5.6%.

Braga et al. (2005a) reported a poor removal in an enhanced primary WWTP compared to an advanced WWTP in Australia. The difference between those two plants was primarily linked to plant performance, however, the extent to which the removal of steroid estrogens was due to bacterial metabolism rather than adsorption to the bacterial biomass remained unclear. EE2 was not detected during the study period in the influent or effluent of either WWTP. Concentrations at ng/g levels of EE2 were detected in the waste activated sludge of the two WWTPs (Braga et al., 2005b), but not in the raw sewage, suggesting that EE2 is resistant to biological treatment and is primarily removed due to sorption to sludge biomass. This was in agreement with previously reviewed data (Johnson and Sumpter, 2001). Muller et al. (2008) surveyed E1, E2, EE2 and their conjugated forms throughout an advanced WWTP. The authors quantified estrogen concentrations in water and sludge samples and investigated also the estrogenic activity by using estrogen responsive reporter cell lines. Estrogen concentrations and estrogenicity measured in the inlet and in primary treated sewage showed a weak impact of primary treatment; nonetheless, they observed a decrease of both estrogen concentration and estrogenicity during biological treatment. The removal was >90% of original concentration ranging between 200 and 500 ng/L. On the base of analysis of estrogens sorbed into the sludge, they suggested that biodegradation was the main vehicle for estrogen elimination.

Despite the application of very high initial concentrations ($2.5X10^{-5}$ to 50 mg /L), experiments using E2, and EE2, as well as activated and inactivated sludge, indicated a high adsorption affinity of the compounds to the adsorbent (Clara et al., 2004). The adsorption in this work was also found to depend on pH, where the authors observed

increasing solubility of adsorbed EE2 with elevated pH from 7 to 12. Correlation of EE2 sorption to activated sludge biomass and temperature was described (Xu et al., 2008).

The efficiency (>90%) of E1 and EE2 removal studied in municipal WWTPs under various redox conditions, demonstrated the significance of aerobic conditions for the removal of all estrogens (Joss et al., 2004). The importance of adaptation of microbial populations for the removal of estrogens (mainly E2), was shown by dramatic differences in mineralization of ^{14}C-labelled estrogenic compounds when working with biosolids from four municipal treatment plants and one industrial plant (Layton et al., 2000); in the municipal WWTP the mineralization was 70–80% during the 24 h, whereas in the industrial WWTP it reached only 4%. The mineralization of EE2 was 25–75-fold less than that of E2 and, additionally, it did not reach completion in 24 h, with only 40% mineralized to $^{14}CO2$. Changes in temperature during the process significantly affected mineralization of E2 but had no effect on the rate of EE2 metabolism (Layton et al., 2000).

Cao et al. (2008) reported a relationship between the equivalent biomass concentration and the estrogen degradation in WWTPs. Under anaerobic conditions E1 was reduced to E2, however, the extent of this reduction depended on the type of inocula (Mes et al., 2008).

No significant loss of the sum of E1 and E2, or of EE2 was observed in activated sludge during the experiment under strictly anaerobic conditions. Nevertheless, in the effluent, there was still a large fraction of E1 and E2 (adsorption to sludge accounted for a 32–35% loss from the liquid phase) but no EE2.

A work focused on potential relationship between the availability of oxygen, nitrification rate, and estrogen removal showed that EE2 was persistent under anoxic conditions in activated sludge (Dytczak et al., 2008). Under aerobic conditions, the observed level of EE2 removal was 22% within 7 h. The higher removal of estrogens was associated with higher nitrification rate, therefore, nitrifying biomass could be responsible for their removal. The fate of E1, E2, and EE2 under different redox settings was also analysed in two nitrite-accumulating sequencing batch reactors, with different sludge ages operating under aerobic conditions and anoxic/anaerobic/aerobic conditions (Pholchan et al., 2008).

The removal of EE2 under anaerobic conditions was considered to be mainly the result of sorption, nonetheless, the binding of estrogens to the sludge was apparently not as strong as the binding observed in the sludge under strictly aerobic conditions. Probably owing to nitrite toxicity and the inhibition of the monooxygenase (AMO) and the microbial population generally, EE2 removal was adversely affected when the reactor operated with sludge retention time (SRT) shorter than 5.7 days and it was significantly lower when SRT was longer than 7.5 days. In contrast, during anaerobic digestion of estrogens by sewage sludge, no influence of temperature or SRT on the process was seen (Carballa et al., 2007).

Estimations based on the measured sorption constants predict that about 50–75% of steroid estrogens will be sorbed to activated sludge during activated sludge treatment in a WWTP. Taking into consideration also the results of previous studies, the scenario of EE2 removal under aerobic conditions could be more mixed. Ternes et al. (1999) showed that E2 was oxidized and further eliminated by activated sludge from WWTP in Germany, whereas EE2 was principally persistent under selected conditions. In a similar way, Weber et al. (2005) reported persistency of EE2 in batch experiments with activated sludge from two different types of WWTP.

Further culture enrichment and isolation led to a defined mixed culture consisting of two strains, which were identified as *Achromobacter xylosoxidans* and *Ralstonia sp.* The culture used E1 and E2 as growth substrates, but not the EE2. In contrast, Forrez et al. (2009a) recorded 90% EE2 removal in an aerated nitrifying batch reactor and a complete biological EE2 removal from the synthetic effluent in a fixed bed reactor. They also showed that once established nitrifiers could maintain the EE2 degradative activity, even without further addition of ammonium (Forrez et al., 2009a,b).

It has been reported that estrogens are efficiently removed in some sewage treatment plants (STPs) which use an activated sludge process. Nevertheless, there are tremendous differences in the degree of estrogen removal among them, and in some cases, an increase in estrogenic activity or steroid estrogen concentration in the effluent and/or excess sludge has been shown, compared with that of the influent (Carballa et al., 2004)

2.14.2.3 Effects

Suspected xenoestrogens include a range of structurally diverse anthropogenic chemicals which interact with estrogen receptors and may affect the metabolism, synthesis, storage, release, transport and clearance of hormones in vertebrates (Hester and Harrison,1999). Even though the amount of estrogens detected in treated wastewater is at nanogram levels or even lower, many studies have revealed that the presence of estrogens in treated wastewater is responsible for the feminization of male fish and sexual disruption in many aquatic wildlife (JEA, 1998; IUPAC, 2003). Furthermore, these hormones caused developmental abnormalities in reptiles and invertebrates, and infertility in birds (Atienzar et al, 2001).

Endocrine-disrupting chemicals (EDCs) are at least partially responsible for disruption of reproduction and development in some wildlife populations (Tyler et al., 1998). The effects that are found include altered/abnormal blood hormone levels, reduced fertility and fecundity, masculinization of females and feminisation of males. Global efforts are now underway to develop a greater understanding of how EDCs disrupt physiological function and to develop regulatory tests for EDCs which are broadly applicable to a variety of wildlife species (Campbell et al., 1999; Kavlock et al., 1996).

Nonetheless, information on the impacts of EDCs on wildlife populations is limited to a few species in several vertebrate and invertebrate taxa. Moreover, the responses and relative sensitivities of different animal species to EDCs have not been comprehensively compared and may vary, both within and between taxa. In consequence, there are fundamental shortfalls in our knowledge of the relative significance of endocrine disruption in different wildlife taxa, and of the level of the problem of endocrine disruption in wildlife relative to other environmental stressors (Jobling et al., 2003).

In spite of the growing concern over the effects of environmental endocrine disrupters on both human and wildlife populations, little research has been conducted to determine the effects at the DNA level. This is surprising considering that estrogens are known to induce DNA damage including for instance single strand breaks, chromosomal damage, and diverse types of DNA adducts; consequently, mutations may occur. Jobling et al.(1995) investigated whether exposing larval barnacles (*Elminius modestus*) to 17β-estradiol (E2) and low concentrations of NP(4-

n-nonylphenol), both of which are thought to cause endocrine disruption, leads to detectable DNA effects using the random amplified polymorphic DNA (RAPD). DNA effects include DNA damage (e.g. DNA adducts, DNA breaks) as well as mutations (i.e. point mutations and large rearrangements) and possibly other effects at the DNA level which can be induced by chemical or physical agents that directly and/or indirectly interact with genomic DNA (Atienzar et al., 2002).

Only little is known about ecotoxicological effects of pharmaceuticals on aquatic and terrestrial organisms and wildlife, and a comprehensive review on ecotoxicological effects is lacking. Aquatic organisms are particularly important targets since they are exposed via wastewater residues over their whole life. Standard acute ecotoxicity data have been reported for a number of pharmaceuticals, nevertheless, such data alone may not be suitable for specifically addressing the question of environmental effects, and subsequently in the hazard and risk assessment (Fent, 2003). The current lack of knowledge holds in particular for chronic effects that have been only very rarely investigated.

3. MATERIALS AND METHODS

3.1 Pure culture (*Shewanella Putrefaciens)* and two chambered MFC experiments

In the first part of this study, *Shewanella Putrefaciens* and two-chambered MFC were used.

3.1.1 Two chambered MFC

The two chambered microbial fuel cells (TCMFC) were constructed using two glass bottles. Each bottle's volumes were 100 ml. Each cell compartment had three ports at the top, for electrode wire, addition and sampling of solutions, and gassing. Two compartments were separated by a Proton exchange membrane (PEM) (Nafion 117 (Dupont Co., USA)). The anode compartment was loaded with freshly prepared bacterial suspension (suspended in 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 M NaCl), vitamin, mineral solution and substrate. The cathode compartment was loaded with 50 mM Na-phosphate buffer (pH 7.0) containing 0.1 M NaCl. Nitrogen and air were continuously purged through anode and cathode compartments to maintain anoxic and aerobic conditions, respectively. (flow rate of nitrogen gas was approximately 15 ml per min). The microbial fuel cell was immersed in a water bath to maintain temperature (25°C) (Julabo FT 200 was used to supply for summer conditions and Julabo heater was used to supply for winter conditions). The air conditioner was also used to supply constant temperature.

3.1.2 Electrode Materials For Two Chambered MFC

Both anode and cathode materials of the TCMFC were platinum. Platinum wire was welded with electrodes by the manufacturer (Altınyıldız, Kapalıçarşı, Istanbul).

3.1.3 Medium for Aerobic and Anoxic Growth of *Shewanella Putrefaciens*

S. putrefacience (ATCC 8071*)* was purchased and was grown on LB (MILLER) broth. For aerobic growth, cultures of 100 ml in 250 ml flasks were shaken continuously on a cooling rotary shaker-incubator at 160 rpm at 25°C (Sartorious Certomat IS) Figure 3.1.

Figure 3.1: Sartorius Certomat IS cooling rotary shaker.

For anoxic growth, *S. putrefacience* (ATCC 8071*)* was grown on anoxically prepared 1250 ml LB (MILLER) broth in Controlled Atmospheric Chamber (Plas By Labs,USA) (Figure 3.2, Figure 3.3).

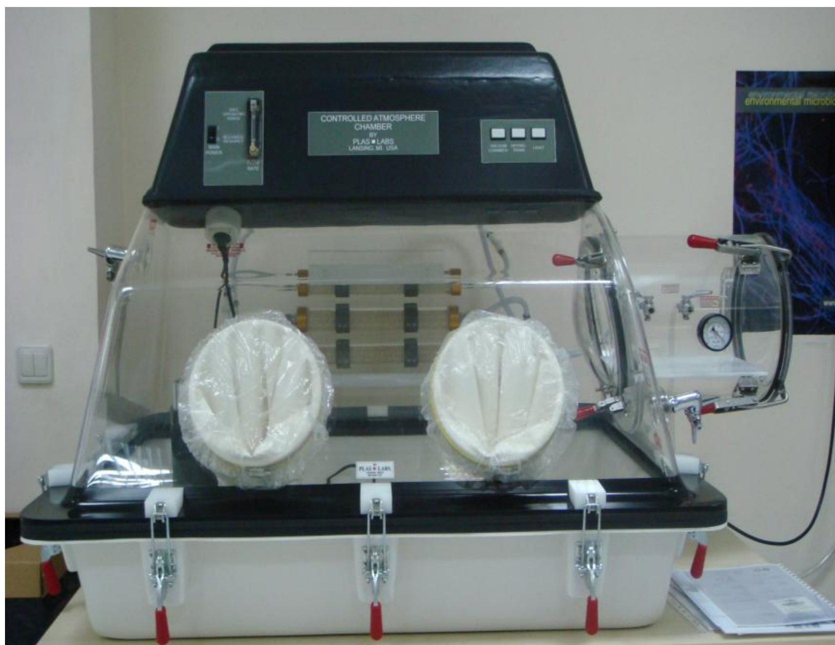


Figure 3.2: Controlled Atmosphere Chamber(PLAS LABS USA).

Figure 3.3: Anoxic growth of *S.putrefaciens* in Atmosphere Controlled Chamber.

LB (MILLER) broth in glass bottle was inoculated with 12.5 ml of an aerobically grown overnight culture and incubated without agitation. After 96 hrs of growth, the cells were harvested under anoxically conditions by a continuous centrifugation system (Hettich Rotina 420 R Centrifuge) at 4800 rpm at 4°C (Figure 3.4)

Figure 3.4: Hettich Rotina 420 R Centrifuge.

The cell pellets were washed three times with a buffer (50 mM Na-phosphate buffer (pH 7.0) containing 0.1 M NaCl). The washed cells were re-suspended in the buffer and transfer to the two chambered MFC. This system can be seen in Figure 3.5.

Figure 3.5: Two chambered MFC used.

3.2 Mixed culture in Single Chambered Microbial Fuel Cell

3.2.1 Construction of Single Chambered Flat-1-Microbial Fuel Cell (SCF-1-MFC)

Single chambered flat-1-MFC consisted of an anode and cathode placed on opposite sides in a plastic (plexiglas) cylindrical chamber 1.6 cm long by 3 cm in diameter (empty bed volume of 12 mL; anode surface area per volume of $62.5 m^2/m^3$). The anode electrodes were made of Ballart carbon paper (without wet proofing) and did not contain platinum catalyst. The carbon electrode/PEM cathode (CE-PEM) was manufactured by bonding the PEM directly onto a flexible carbon-cloth electrode containing $0.4 mg/cm^2$ of Pt catalyst (Vulcan). The PEM (Nafion 115, Dupont) was sequentially boiled in H_2O_2 (30%), deionized water, 1M H_2SO_4, and deionized water (each time for 1 h). The PEM was then hot-pressed directly onto the cathode by heating it to 100 °C at 100 bar for 4 minutes. The anode and cathode are placed on either side of a tube, with the anode sealed against a flat plate and the cathode exposed to air. When a membrane is used in this air-cathode system, it serves primarily to keep water from leaking through the cathode, although it also reduces oxygen diffusion into the anode chamber. SCF-1-MFC can be seen in Figure 3.6.

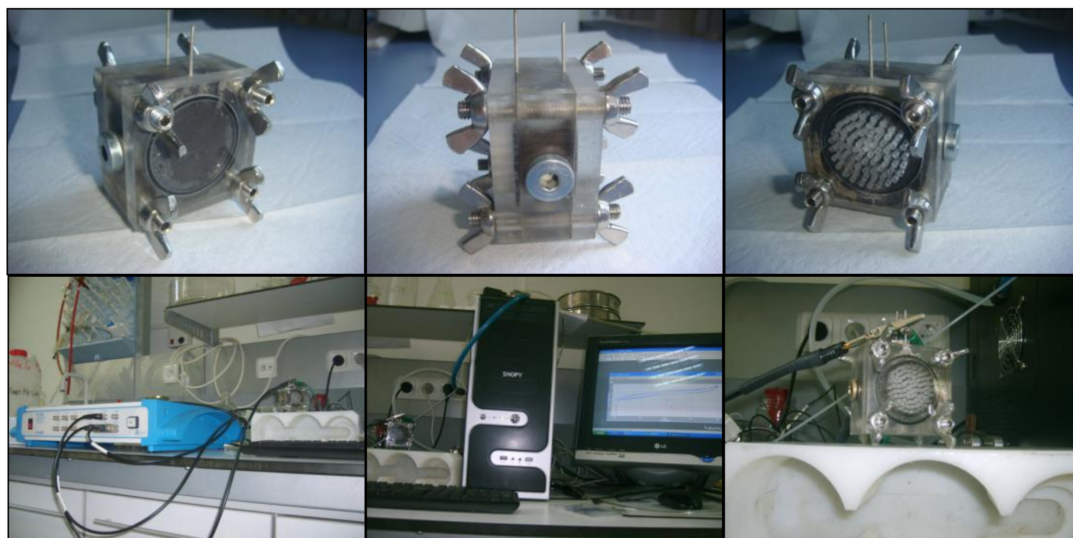


Figure 3.6: Used single chambered Flat-1-MFC in this study.

3.2.2 Construction of Single Chambered Tubular MFC (SCTMFC)

Single Chambered Tubular MFC consisted of a single cylindrical plexiglass chamber (10cm long by 3cm outer diameter; 1 cm inner diameter , empty bed volume of approximately 63 mL) containing stainless steel strap 150 cm long placed in the cylindrical plexiglass chamber as anode electrode. The carbon electrode/PEM cathode (CE-PEM) was also manufactured by bonding the PEM directly onto a

flexible carbon-cloth electrode containing 0.4 mg/cm^2 of Pt catalyst (Vulcan). The air-porous cathode consisted of a carbon/platinum catalyst/proton exchange membrane (PEM) layer was placed onto a 1 cm diameter plexiglas tube containing 2 mm diameter pores at 2 mm intervals (cathode tube) (Figure 3.7). Air flow through the tube was initially 5 mL/min but was changed to passive oxygen transfer (no forced air flow). Silver or Platinum wire was used to connect the circuit containing a 100 Ω external resistance.

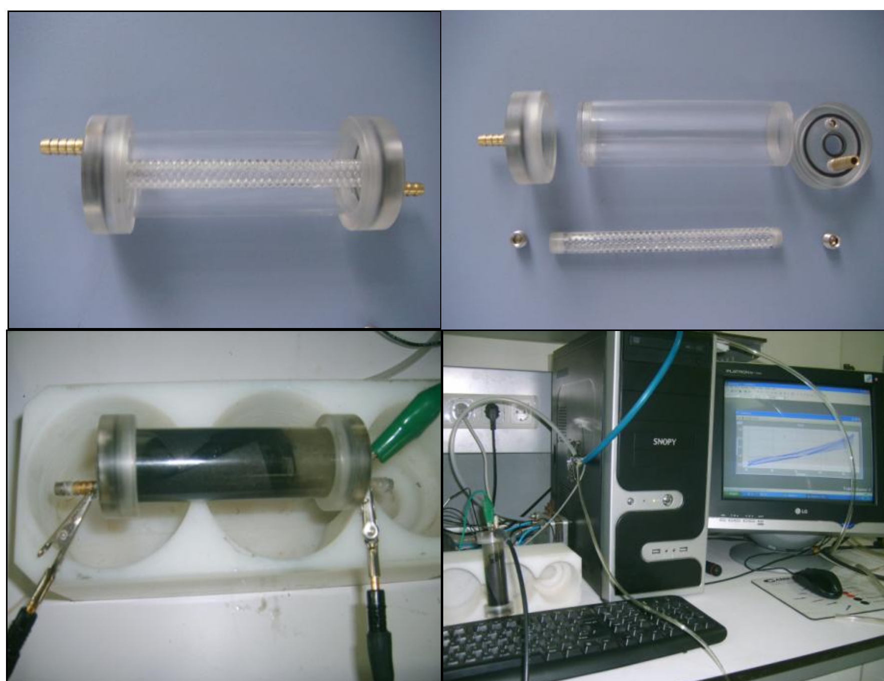


Figure 3.7: Single Chambered Tubular MFC.

3.2.3 Construction of Single Chambered Flat-2-Microbial Fuel Cell (SCF-2-MFC)

SCF-2-MFC having a 36 ml capacity anode chamber was made of plexiglass. Anode and cathode electrodes, each having 36 cm^2 surface area (6 x 6 cm), were 1,5 mm carbon cloth (CC, LLC) and cathode was coated with 1 mg/cm^2 platinum as catalyst. Electrodes were separated by a non-conductive microfiber cloth (Scotch-Brite) instead of PEM. A water impermeable layer (Tyvek, Dupont) was placed on cathode side for breathing. An Ag/AgCl reference electrode (BASI, USA) which measures +0,197 V more potential according to standard hydrogen electrode (SHE) was located in the anode chamber and connected to the electrodes for the measurement of anode and cathode potentials. Figure 3.8 and Figure 3.9 show the SCF-2-MFC and multimeter system, respectively.

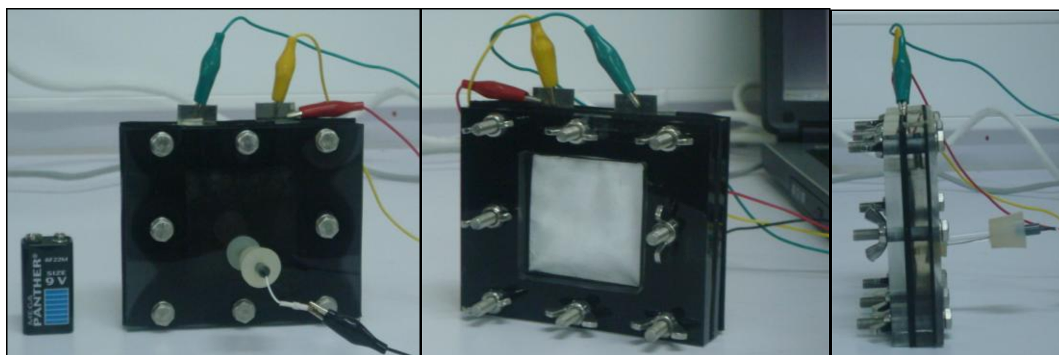


Figure 3.8: Photographs of anode side (front), cathode side (back) and side of SCF-2-MFC

Figure 3.9: Photograph of SCF-2-MFC connecting multimeter system.

3.3 Inoculum of SCF-1-MFC, SCTMFC and SCF-2-MFC.

The SCF-1-MFC was inoculated with anaerobic bacteria obtained from the anaerobic wastewater treatment plant of a brewery industry located at Merter/Istanbul and operated in batch mode for two weeks. Granular anaerobic bacteria was agitated by sonication for 30 minutes.

The anode chamber of SCF-1-MFC and SCTMFC were also inoculated with mixed culture taken from an anaerobic treatment plant (confectionery industry- Gebze, Kocaeli) and fed-batch using from 0.5 g/L to 2g/L sodium acetate (NaAc) as carbon source.

The anode chamber of SCF-2-MFC was inoculated with mixed bacterial culture from another MFC (taken from Marmara University Environmental Engineering Department) that was originally enriched from a biological nutrient removal plant (Pasaköy WWTP, Istanbul) and was fed continiously for over one year using sodium acetate as carbon source. The anode chamber unit was selected as the inoculum sampling point considering the VFA rich and oxygen free conditions that are favorable for anophilic bacteria capable of oxidizing acetate anaerobically.

3.4 Medium of SCF-1-MFC, SCTMFC and SCF-2-MFC

To feed the anode compartment of SCF-1-MFC and SCTMFC, phosphate buffered basal medium (PBBM) was used to provide minimal nutrients to support the growth of anaerobic microorganisms. The basal medium is supplemented with solutions of trace minerals and vitamins. Sodium acetate (NaAc) was used as a carbon source. Phosphate buffered basal medium (PBBM) was used to provide minimal nutrients to support the growth of anaerobic microorganisms. The basal medium is supplemented with solutions of trace minerals and vitamins. PBBM medium consist of 0.9 g/L NaCl, 0.2 g/L $MgSO_4.6H_2O$, 0.1 g/L $CaCl_2.2H_2O$, 1 g/L NH4CI, trace mineral solution 10ml/L and Resazurin solution (%2) 1ml/L. Trace mineral solution consist of 12.8 g/L nitrilotriacetic acid, 0.1g $FeSO_4.7H_2O$, 0.1 g/L $MnCl_2.4H_2O$, 0.17 g/L $CoCl_2.6H_2O$, 0.1 g/L $CaCl_2.2H_2O$, 0,. g/L $ZnCl_2$, 0.02 g/L $CuCl_2.2H_2O$, 0.01 g/L H_3BO_3, 0.01 g/L $Na_2Mo_4.2H_2O$, 1g/L NaCl, 0.017g/L Na_2SeO_3, 0.026 g/L $NiSO_4.6H_2O$ and 0.02 g/L $SnCl_2$. 10 ml Phosphate buffer and 10 ml Vitamin solution was added to autoclaved per liter PBBM medium. Vitamin solution consists of 0.002 g/L biotin, 0.002 folic acid, 0.01g/L B_6 (pyridoxine) HCl, 0.005g/L B_1 (triamine) HCl, 0.005g/L B_2 (riboflavin), 0.001g/L B_{12} crystalline (all chemicals were purchased from Merck KGaA) (Anaerobic Microbiology, 1991) All experiments were conducted in a temperature-controlled incubator set at 30 °C.

For SCF-2-MFC, the anode chambers were batch-fed with synthetic wastewater (anolyte) containing 0.74 g/L KC1, 0.58 g/L NaCl, 0.68 g/L KH_2PO_4, 0.87 g/L K_2HPO_4, 0.28 g/L NH4CI, 0.02 g/L yeast extract, 0.1 g/L $MgSO_4.7H_20$, 0.1 g/L $CaCl_2.2H_2O$ (Rozendal et al., 2006), and 1 mL of trace element solution (Rabaey et al., 2005) containing per litre: 1g $FeSO_4.7H_2O$, 70 mg $ZnCl_2$, 100 mg $MnCl_2.4H_2O$,

6 mg H_3BO_3, 130 mg $CaCl_2.6H_2O$, 2 mg $CuCl_2.2H_2O$, 24 mg $NiC1_2.6H_2O$, 36 mg $Na_2Mo_4.2H_2O$, 238 mg $CoCl_2.6H_2O$ (all chemicals were purcheased from Merck KGaA).

The SCF-2-MFC system was fed with 600 mg/L acetate for every cycle. Synthetic wastewater containing sodium acetate was stored at -20°C before using. The overall Coulombic efficiency was a function of substrate concentration and circuit resistance. Coulombic efficiency decreased from 28.3 to 13.2% when the acetate concentration increased from 80 to 800 mg/L (1000 Ω) (Liu et al, 2005).

3.5 Antibiotics and hormones

Antibiotics and hormones were studied on SCF-2-MFC. Incubator (Incucell) was used to supply constant temperature (30ºC) throughout the study.

Four different steroid estrogens (hormones), such as estrone (E1)(Sigma-Aldrich), 17-β estradiol (E2) (Sigma-Aldrich), estriol (E3) (Sigma-Aldrich) and 17-α ethinylestradiol (EE2) (Sigma-Aldrich) were used. These estrogens were prepared as 0.1, 0.5 and 1 mg/L concentartions for sequencing cycles. Estrogens were mixed with 600 mg/L acetate (containing mineral solution) and filled into the MFC.

Then, three different antibiotics which erythromycin (Sigma), sulfomethoxazole (Fluka) and tetracycline (Sigma) were used. These antibiotics were prepared as 50, 100 and 200 mg/L concentations representative to drug manufacture effluents (Lin and Tsai, 2009, Larsson et al., 2007). Antibiotics were mixed with 600 mg/L acetate (containing mineral solution) and filled into the MFC.

When MFC was filled with acetate only (or with antibiotics or with estrogen), the potential was increased to approximately 200-250 mV and continued constantly for a while, then potential decreased because substrate was comsumed (thereby one cycle was completed). When potential decreased to 5 mV, the MFC was filled fresh medium.

3.6 Electrochemical Measurement (Voltage and Current Measurements of the MFC)

The Voltage (V) across a resistor (Rext) was measured by a data acquisition module (Fluke 8846A Digit precision multimeter). Data was recorded to a personal computer in every 15 minutes. Current(I) was determined by Ohm's law (I=V/R), and power

density of the MFC was calculated as P=VI/A, where A was the surface area of anode electrode.

3.7 Calculation of current and coulombic efficiency for experiments of antibiotics and hormones.

Current (I) density was calculated for every sequential cycle as mA/cm^2*hour using integration to calculate coulombic efficiency. As it is mentioned in literature, CE equation (eq.2.16) for batch-fed is used. Coulombic efficiency (CE) is critical parameter to understand MFC performance.

3.8 Chemical Analysis

3.8.1 Sampling and COD analysis

Samples were taken from the anode chamber of the MFC and analysed. The sample was taken before the feeding (end of the every cycle). Chemical oxygen demand (COD) test kits (Merck) were used. COD kits were boiled in thermoreactor (WTW) at 148 °C for two hours. After that, the cooled samples were measured with COD spectrophotometer (WTW).

3.8.2 pH measurement

A pH meter (WTW) was used to control pH. After preparation of substrate, hormones and antibiotics solutions, pH has been adjusted to 7±0,05 with 1M and 0,1 M NaOH and 0.1 M and 1M H_2SO_4 before filling the anode compartment of the MFC.

3.9 Scanning electron micrograph (SEM) Analysis

Bacteria on the anode were examined using a scanning electron microscope (SEM) (Philips XL 305 FEG) at Gebze Institute of Science and Technology.

4. RESULTS AND DISCUSSION

4.1 Experiments with *Shewanella Putrefaciens*

4.1.1 Growth curves of *Shewanella Putrefaciens*

Shewanella putrefaciens pure culture was grown aerobically for 43 hrs. Figure 4.1 shows the Optical Dencity (OD) (660 nm) versus time graph for the bacteria. The bacteria was grown aerobically in LB (MILLER) broth. 1 ml sample was taken at one hour intervals and OD was measured. Stationary phase was observed after the 40th hour on the graph.

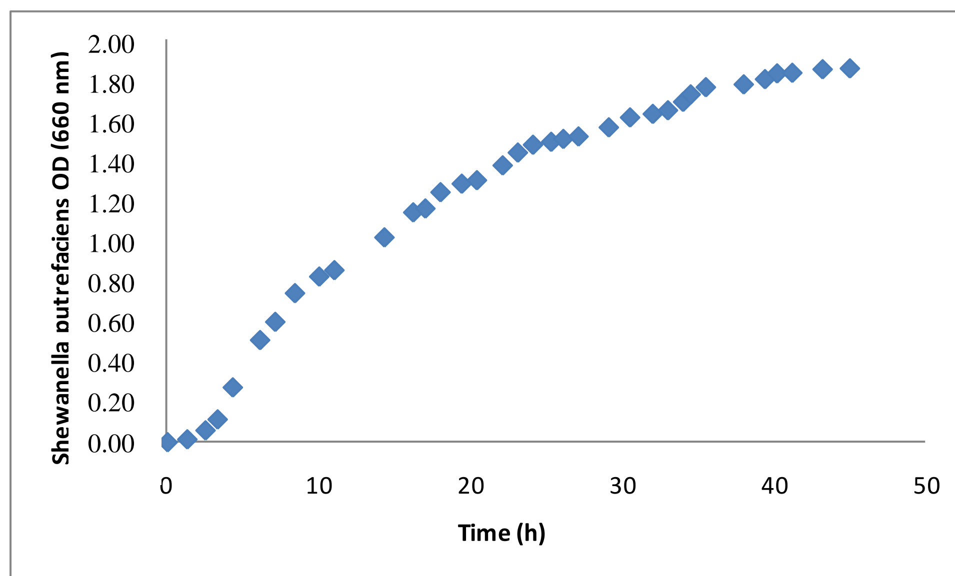


Figure 4.1: Aerobic Growth Curve of *S.putrefaciens.*

Figure 4.2 shows the photograph of *S.putrefaciens* colonies at different dilution on LB agar. Colonies have been counted and calculated using dilution factor to find the number of bacteria per mililiter.

Figure.6.3 shows the viability of *S. Putrefaciens* during the 36 hrs and demonstrated that maximum viability of the bacteria was at 19th hour. At 3 hrs time intervals, bacterial samples were diluted from $1/10^1$-$1/10^6$, and 10µL samples from dillutions were plated on LB (MILLER) agar to count bacteria.

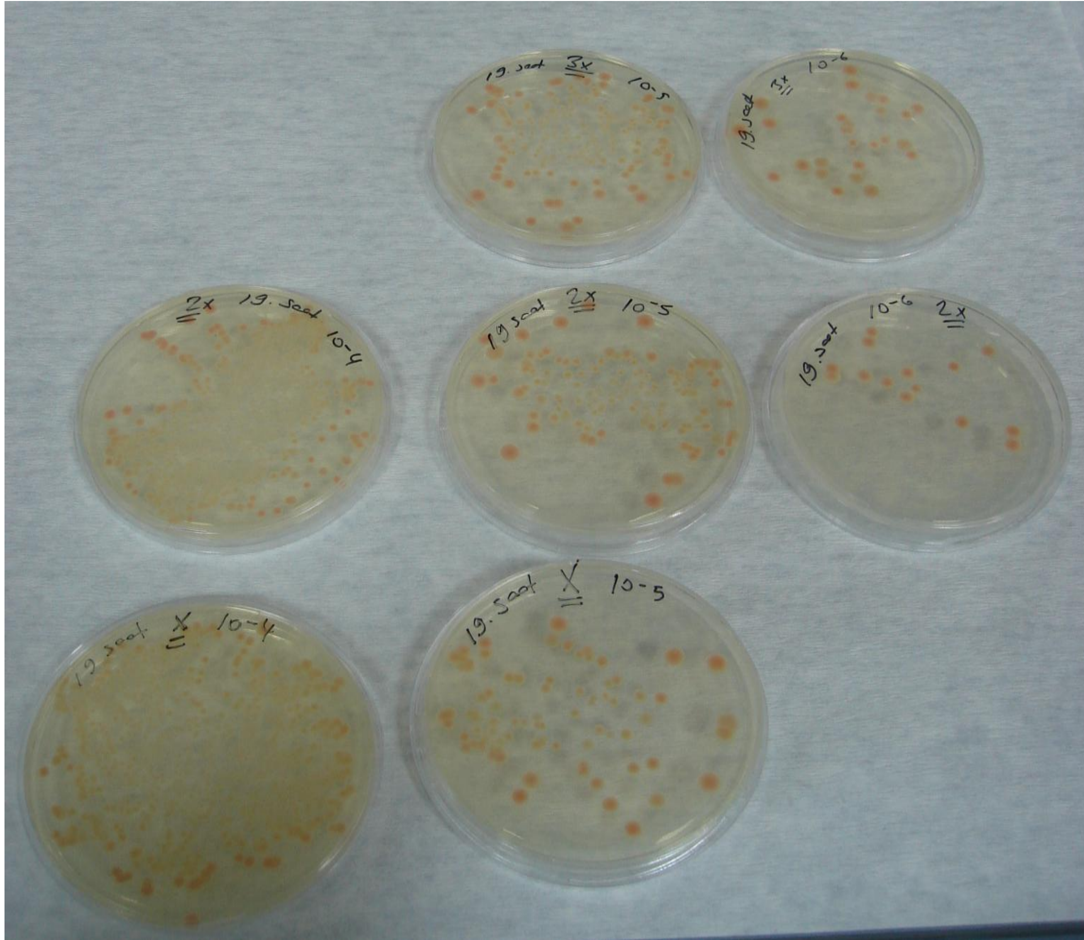


Figure 4.2: Photograph of *S.putrefaciens* colonies at different dilution on LB agar

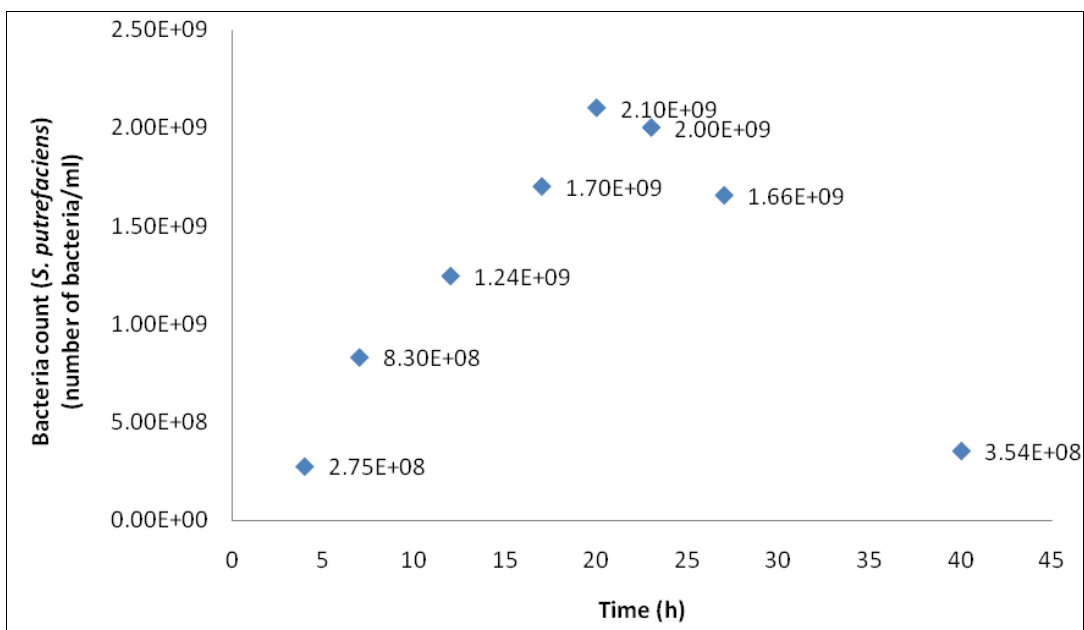


Figure 4.3: Viability Curve of *S.putrefaciens.*

In order to understand whether the strength of media has an effect of viability of *S.putrefaciens*, single (x) – double (2x) – triple (3x) strength were used (for constant pure culture inoculation) and compared (aerobically). Maximum OD was observed for 3x media. Minimum OD was observed for 2x. Figure 4.4 shows the differentiation of growth curves.

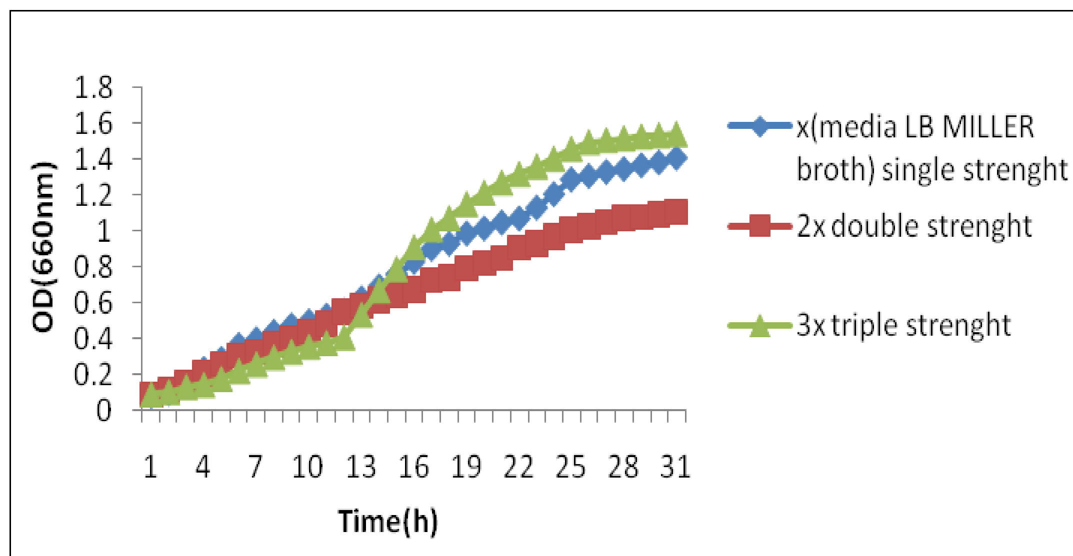


Figure 4.4: Comparison of *S.putrefaciens* Growth Curve in single-double-triple strength LB(MILLER) broth.

If the viability of these media was compared, it was observed that triple strength (3x) media was higher viability than the others. Figure 4.5. illustrates these results.

On the other hand, the experiments were performed for different initial inoculum concentrations of pure *S. Putrefaciens* culture. Single represents 10 ml, double represents 20 ml inoculum culture. Figure 4.6 illustrates that the growth curves of cultures were very close to each other.

In the comparison of single and double concentrations of culture for the viability, single concentration was optimum. Figure 4.7 shows the results.

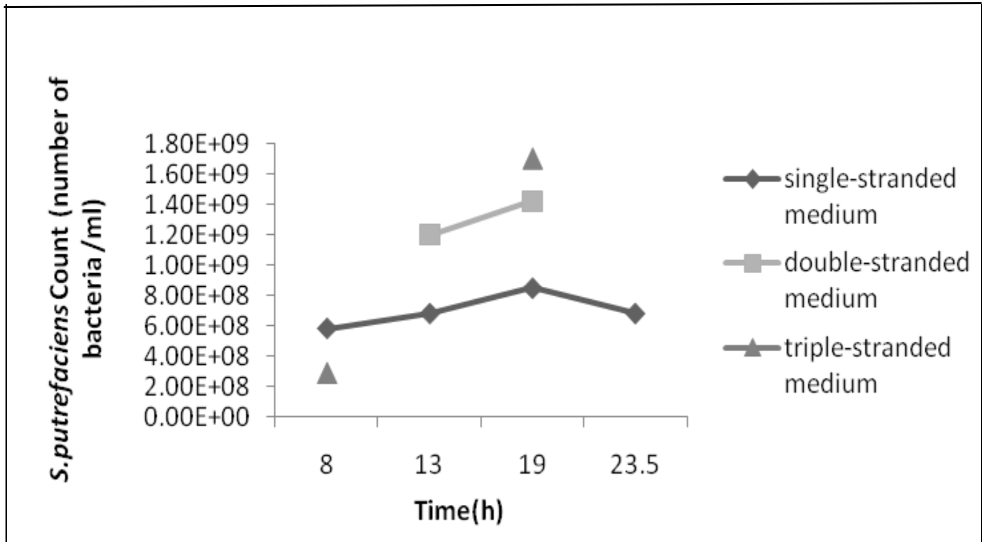


Figure 4.5: Comparison of *S.putrefacience* Viability Curve in single-double-triple strenght LB (MILLER) broth.

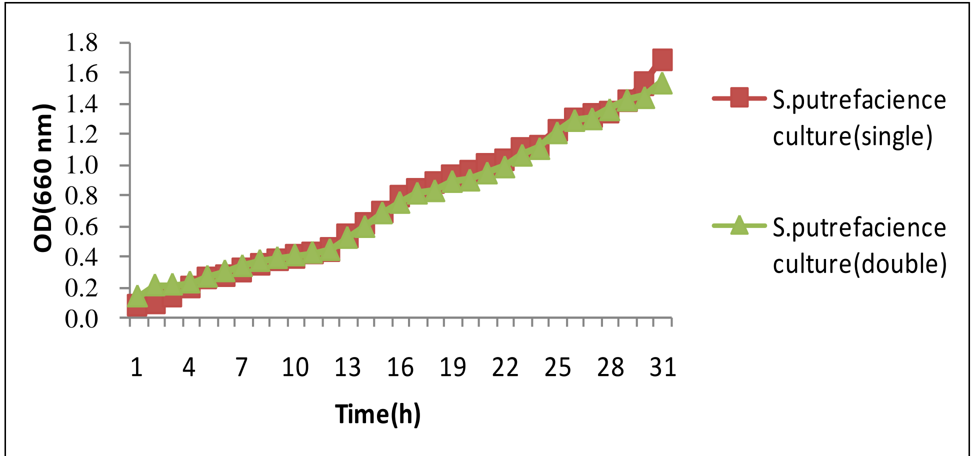


Figure 4.6: Comparison of *S.putrefaciens* growth curve in different amount of pure culture.

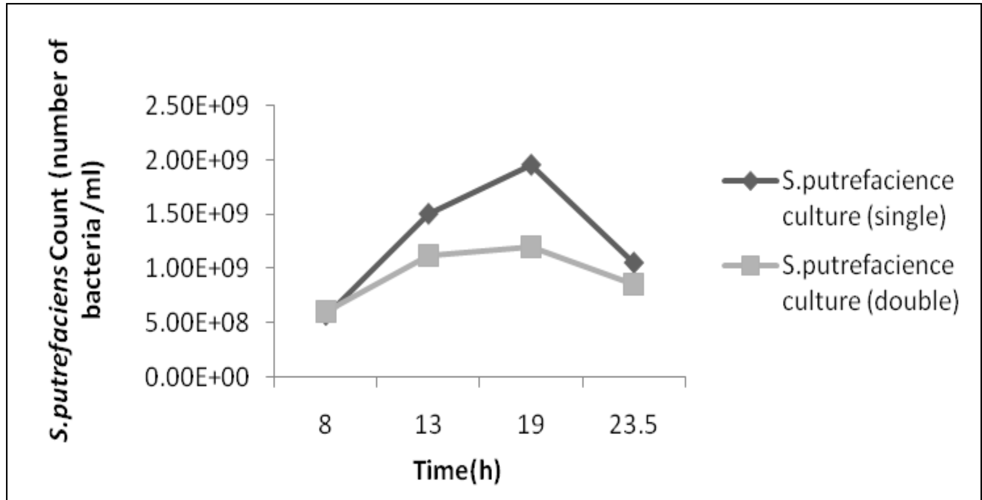


Figure 4.7: Comparison of *S.putrefacience* viability curve in single-double amount of pure culture.

4.1.2 Open circuit voltage of anoxically grown *Shewanella putrefaciens* in two chambered MFC

Before the substrate (acetate as carbon source) was added, approximately 200mV was observed from the microbial fuel cell containing suspensions of *S. Putrefaciens.* Addition of acetate (10 mM) as the fuel resulted in a rapid increase in potential (max) up to 880 mV for 1250 ml (pellet, centrifugated from 1250 ml) pure culture (Figure 4.8).

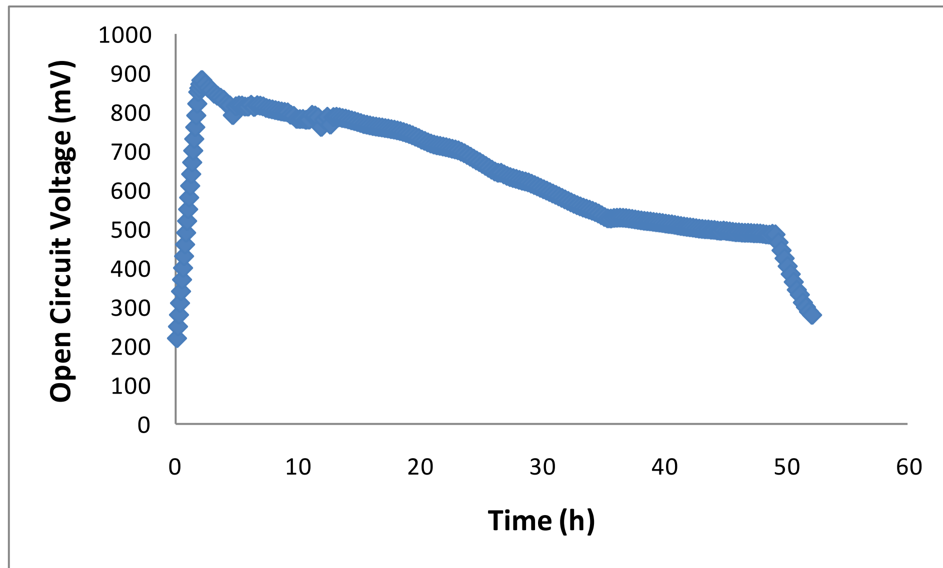


Figure 4.8: Open Circuit Voltage (OCV) of two chambered MFC containing *S.putrefaciens* (addition of 10 mM acetate and 1250 ml *S.putrefaciens*).

When the MFC was loaded with 10 mM acetate and 3750 ml pure culture , maximum potential was up to 879 mV (Figure 4.9).

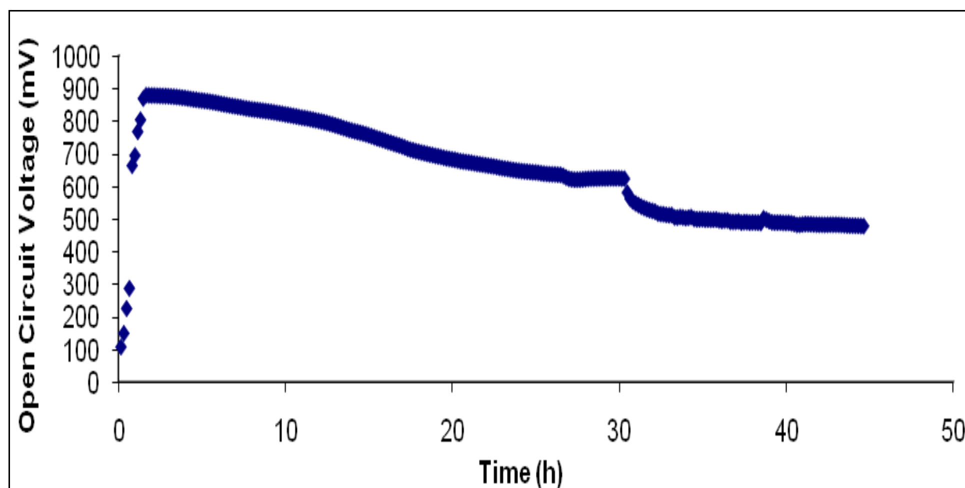


Figure 4.9: Open Circuit Voltage (OCV) from two chambered MFC containing *S.putrefaciens* (addition of 10 mM acetate-3750 ml *S.putrefaciens*).

When the results of TCMFC were compared for acetate, OCV values were not affected from amount of pure culture.

Experiments were repeated for 10 mM glucose and different volume of bacteria. Figure 4.10 shows that 2500 ml pure culture resulted in a potential of 777 mV, while figure 4.11 illustrates that 800ml pure culture resulted in a potential of 810mV, respectively. Figure 4.12 shows that 1250 ml pure culture resulted in a potential of 832mV.

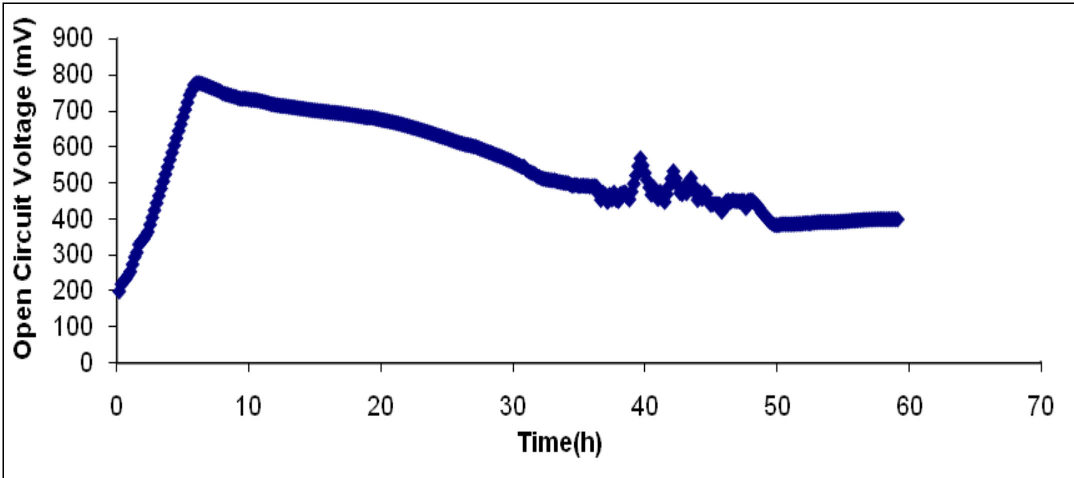


Figure 4.10: Open Circuit Voltage (OCV) development from two chambered MFC containing *S.putrefaciens* (addition of 10mM glucose-2500 ml culture).

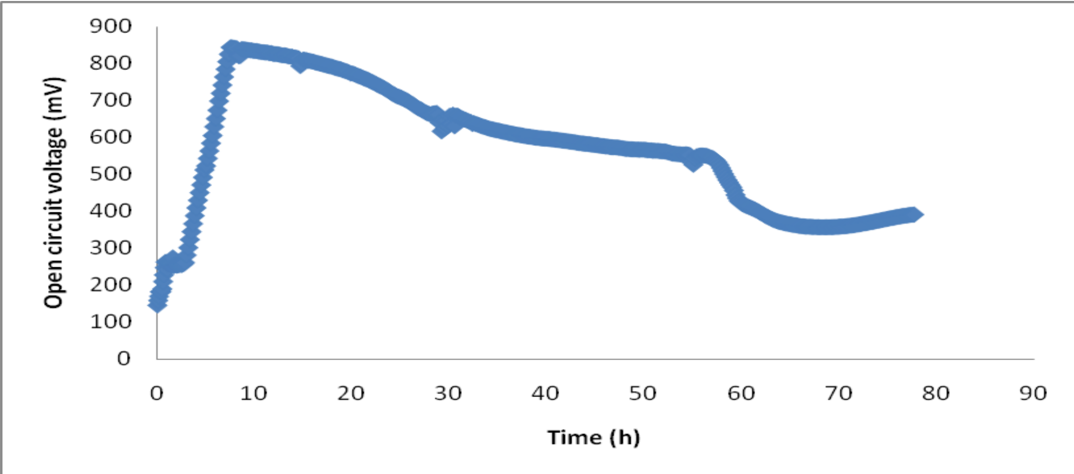


Figure 4.11: Open Circuit Potential (OCP) development from two chambered MFC containing *S.putrefaciens* (addition of 10mM glucose for 800 ml culture.

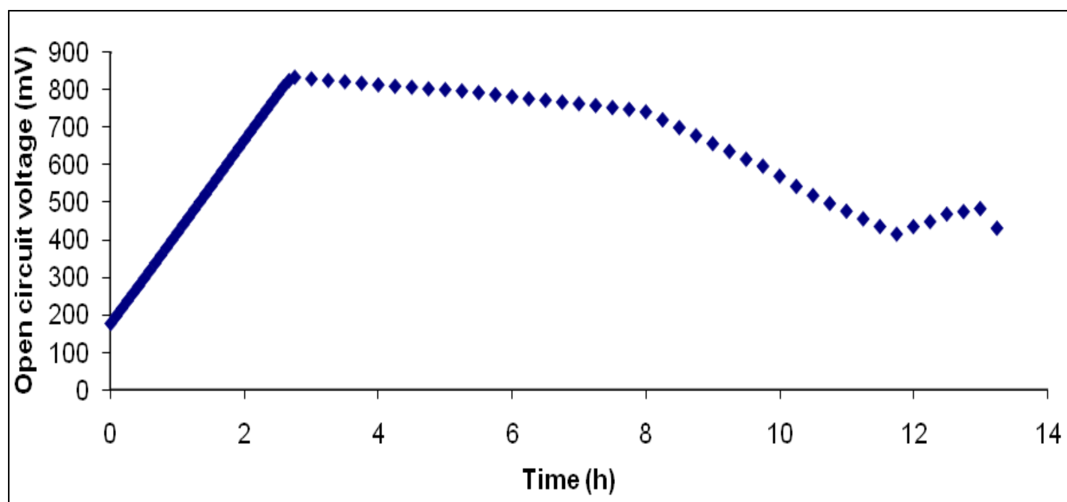


Figure 4.12: Open Circuit Voltage (OCV) from two chambered MFC containing *S.putrefaciens* (addition of 10mM glucose for 1250 ml culture).

When the results of TCMFC were compared for glucose, OCV values were close to each other , so it was not affected from amount of pure culture, like acetate.

The experiments were also conducted with ethanol and propionic acid. Addition of 10 mM ethanol as the fuel resulted in potential up to 0.670 V (Figure 4.13) while addition of 10 mM propionic acid resulted in a potential of 0.803 V (Figure 4.14).

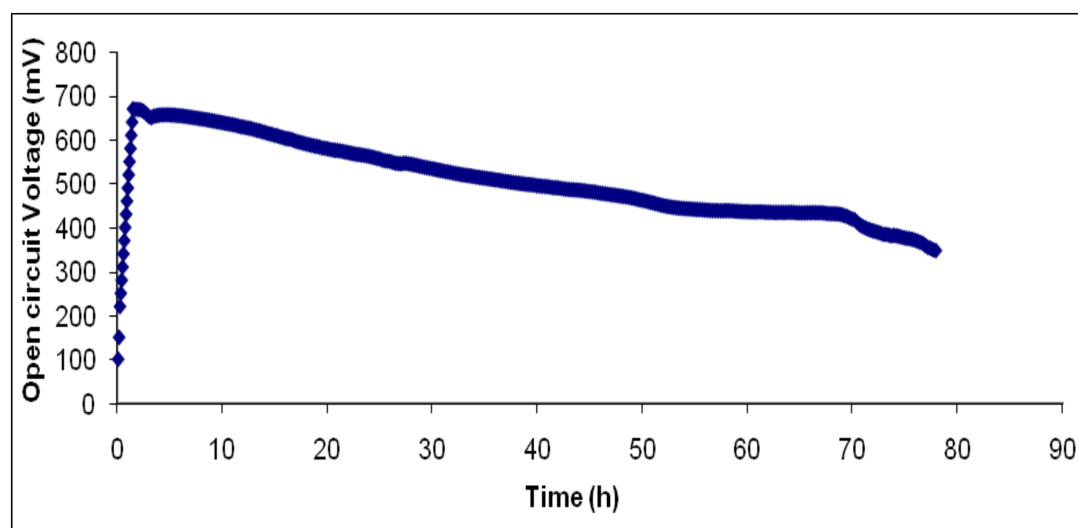


Figure 4.13: Open Circuit Voltage (OCV) from two chambered MFC containing *S.putrefaciens* (addition of 10 mM ethanol and 1250 ml pure culture)

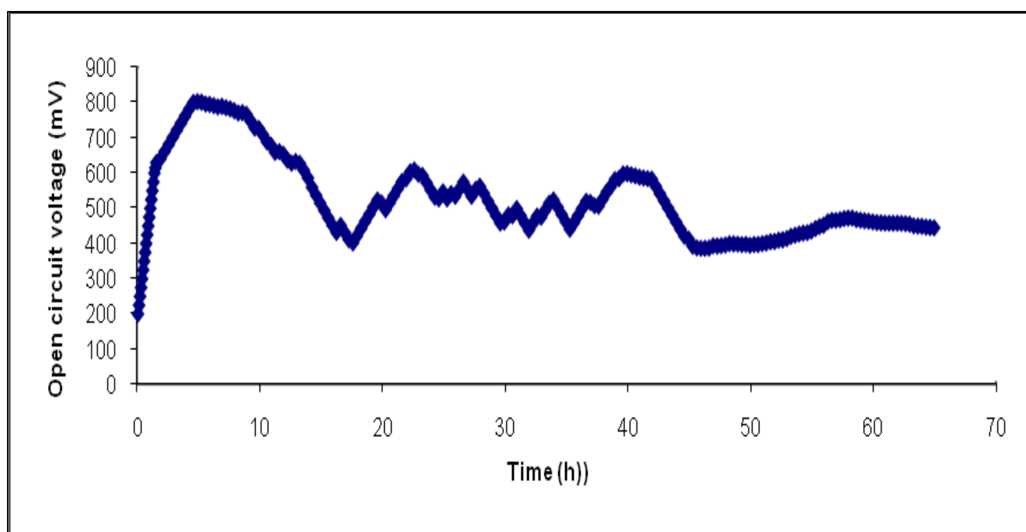


Figure 4.14: Open Circuit Potential (OCP) development from two chambered MFC containing *S.putrefaciens* (addition of 10 mM propionic acid and 1250 ml pure culture).

4.1.3 Open circuit voltage of aerobically grown *Shewanella Putrefaciens*

The anode compartment of fuel cells were loaded with freshly and aerobically prepared *S.putrefaciens* (because this culture facultative) suspension to observe potential development under open circuit conditions. The addition of glucose (10mM) as the fuel resulted in a rapid increase in potential up to 0.456 V (Figure 4.15). Comparing OCV (maximum) of aerobically growth of culture with anaerobically it was found that approximately %55 less than anaerobically.

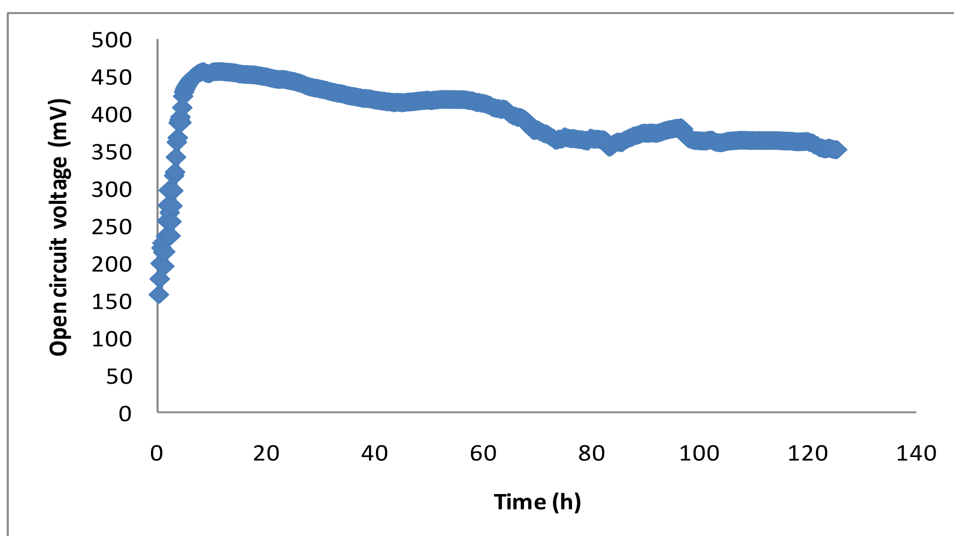


Figure 4.15: Potential development from two chambered MFC containing aerobically grown of *S.putrefaciens* (addition of 10mM glucose)

4.1.4 Power generation of two chambered MFC

When the MFC inoculated with *S.putrefaciens* and was fed with 10 mM glucose, its power density calculated as 0.8 mW/m^2. The circuit was completed with a fixed load of 5000Ω were used to determine the power generation as function of load. Current (i) was observed 4μA (average) during 20 hours. Potential(V)=iR, Power (P) was calculated as P=iV.

$$P=i^2.R=(4x10^{-6})^2.(5x10^3)=0.08\ \mu W/cm^2=0.8\ mW/m^2$$

4.1.5 Open circuit voltage from two chambered MFC (addition influent wastewater of brewery industry using *S.putrefaciens*)

The anode compartment of MFC were loaded with 1250 ml suspended *S. Putrefaciens* and influent wastewater (contains 5855mg/L COD) from brewery industry. Addition of fuel resulted in potential up to 0.630 V and this voltage continued nearly constant from 9th to 102th and dropped (Figure 4.16).

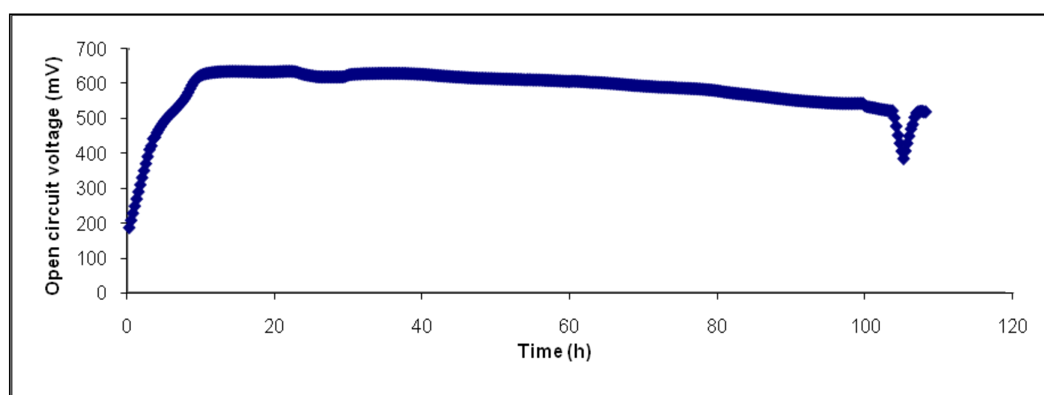


Figure 4.16: Potential development from two chambered MFC containing influent ww from brewery industry containing 1250 ml *S.putrefaciens*

4.2 Experiments with mixed culture using single chambered flat-1-MFC

4.2.1 Comparison of currents using different external resistance in single chambered flat-1- MFC

It was reported that the electrochemically active mixed cultures are enriched from activated sludge from wastewater treatment plants, sediments or the effluent of primary clarifiers (Bond and Lovley 2003; Kim et al, 2004; Lee et al., 2003; Park et al., 2001; Rabaey et al., 2004). In this study, the anode chamber of our single chambered flat -1-MFC (SCF-1-MFC) were inoculated with the sludge taken from the anaerobic unit of industrial wastewater of confectionery industry (located in Gebze, Kocaeli) before being started-up. The anaerobic unit was selected as the

inoculum sampling point considering the oxygen-less and rich VFA conditions that are favorable for bacteria capable of oxidizing acetate anaerobically. Sonicated bacteria were inoculated in anode chamber of SCF-1-MFC and the system was fed with synthetic wastewater containing of 2000 mg/L sodium acetate batchly. Different external resistances were used and shown in Figure 4.17 for 5100 ohm, Figure 4.18 for 2200 ohm, Figure 4.19 for 100 ohm, respectively.

Figure 4.17. shows that maximum current was 23 µA at 6th.hour for 5100 ohm external resistance . After the peak, this current was decreased slowly, during 42 hours. The current end value was 9.92 µA.

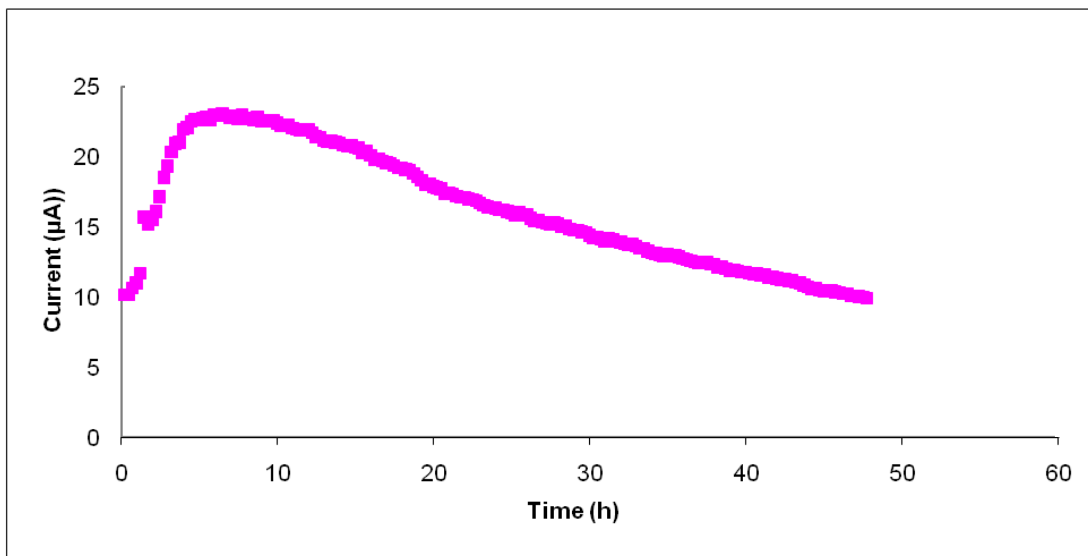


Figure 4.17: Current versus time graph for 2000 mg/L acetate using 5100 ohm external resistance at SCF-1-MFC.

Then, the external resistance was changed and current was measured. When 2200 ohm external resistance was applied, current peak value was 42.57 µA at 5th.hour for 2000 mg/L acetate. After the peak, this current was decreased slowly, during 43 hours. The current end value was 18.43µA (Fig.4.18).

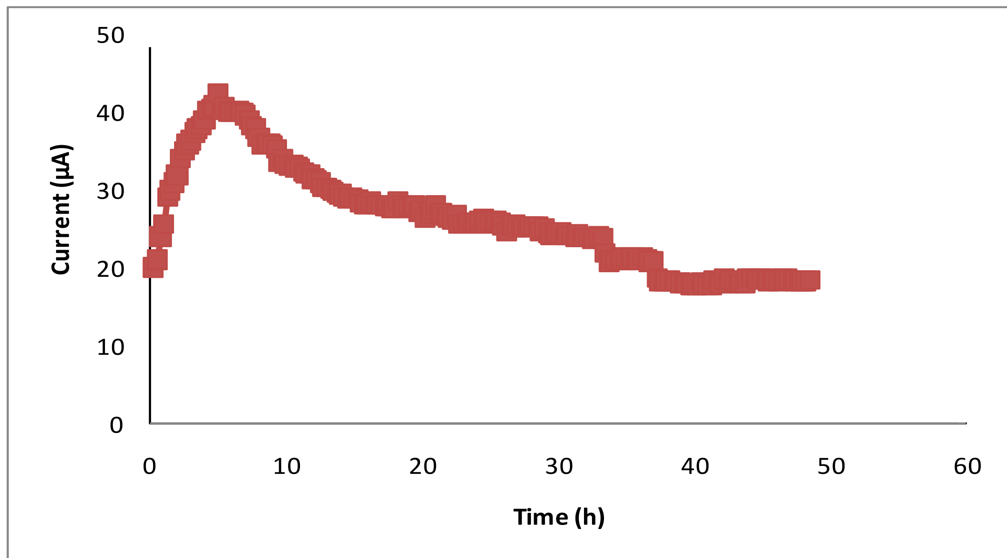


Figure 4.18: Current versus time graph for 2000 mg/L acetate using 2200 ohm external resistance at SCF-1-MFC.

When 100 ohm external resitance was applied, and 2000 mg/L aceatate was added to the system, maximum current value was 168.75 µA at 12th hour and was decreased to 72.72µA during 30 hours . When acetate was refreshed at 42th hour, the current value was reached 167.03µA. Then, this value was decreased to 11.09 µA during 92 hours. (Figure 4.19).

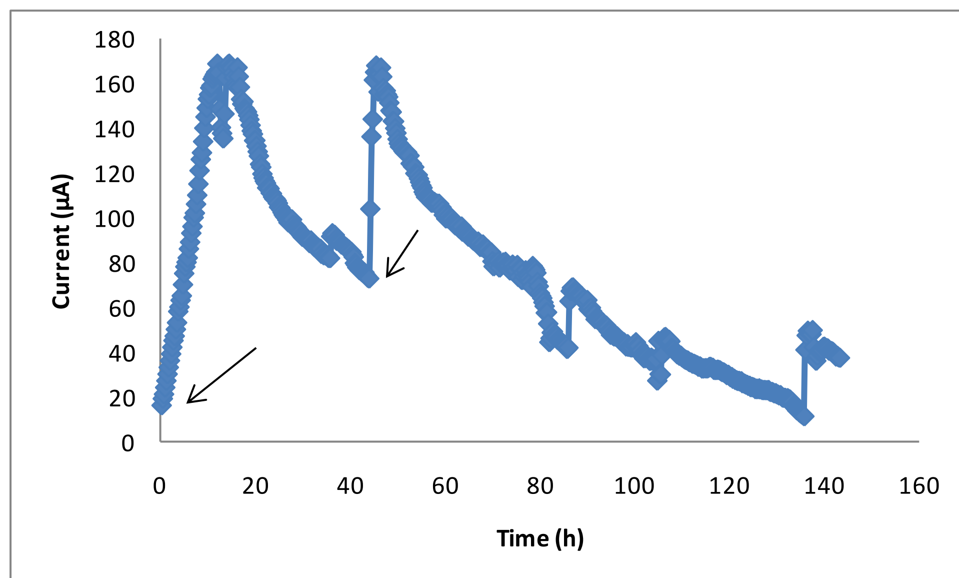


Figure 4.19: Current versus time graph for 2000 mg/L acetate using 100 ohm external resistance at SCF-1-MFC. Arrows indicate the replacement of the substrate with a fresh substrate.

It is obvious that current depends on external resistance. Current is inversely proportional with external resistance. The maximum current generations were observed 23 µA, 42 µA and 168 µA for 5100ohm, 2200 ohm and 100 ohm external resistance respectively.

Although single chambered tubular MFC was constructed, unfortunately it was not operated efficiently. Because of flow out of anolyt, it was not possible to use this system.

4.3 Scanning electron micrograph (SEM) analysis

The wide diversity of bacteria that exist in MFC reactors, driven in part by a variety of operating conditions, demonstrates the versatility of bacteria that can either transfer electrons to the electrode or can exist in the reactor as a result of symbiotic relationships with electricity-producing bacteria. Electrochemically active bacteria seem to be abundant in a variety of samples used to inoculate MFCs, including wastewaters, sludges, river and marine sediments. To control of existance of microorganisms on anode surface for SCF-1-MFC, scanning electron microscope(SEM) was used. Figure 4.20 shows SEM pictures. As it is seen, white objects should be inorganic matters, the microorganisms could not form biofilm on anode. On literature, anode was covered by microorganism by using SEM (Liu and Logan, 2004).

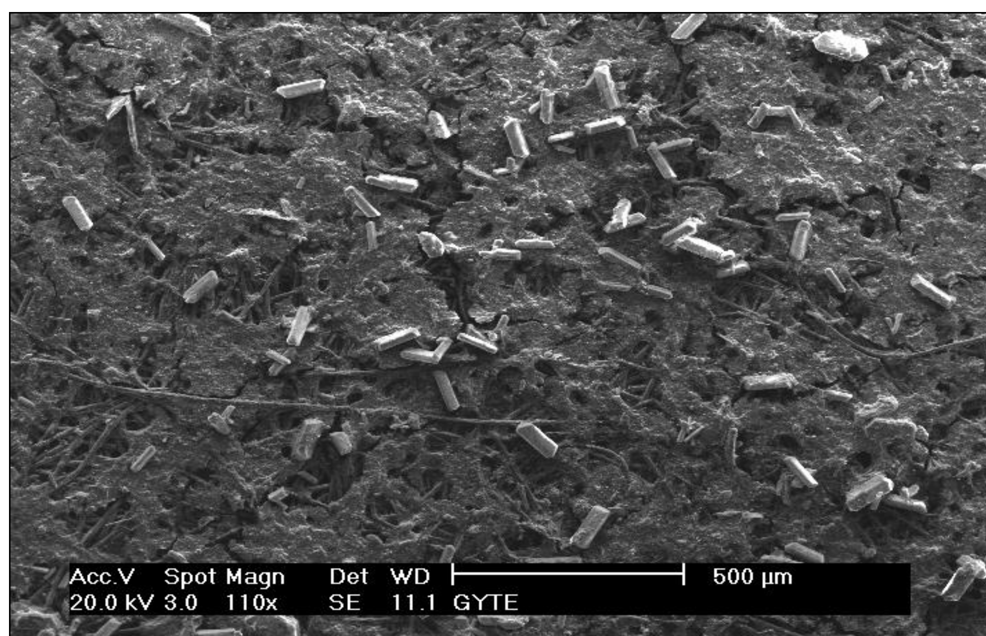


Figure 4.20: A scanning electron micrograph (SEM) for anode of SCF-1-MFC.

4.4 Voltage measurement using mixed culture microorganisms for single chambered flat-2-MFC

4.4.1 Carbon cloth cathode containing 1 mg/cm2 platinum

The anode chamber of SCF-2-MFC was inoculated with a mixed bacterial culture of another MFC (taken from Marmara University Environmental Engineering Department) that was originally enriched from a biological nutrient removal plant (Pasaköy WWTP, Istanbul) and was fed continuously for over one year using sodium acetate as carbon source, as mentioned previous chapter.

Anode material was carbon cloth (CC, LLC), cathode material was carbon cloth coated with 1 mg/cm^2 platinum as catalyst. Electrodes were separated by a non-conductive microfiber cloth (scotch-Brite) instead of PEM. A water impermeable layer (Tyvek, Dupont) was placed on cathode side for breathing. 300 Ω external resistance for during experiment.

The anode compartment was filled synthetic wastewater containing 200 mg/L acetate and inoculated with the enriched culture. It was measured and Figure 4.21 was drawn.

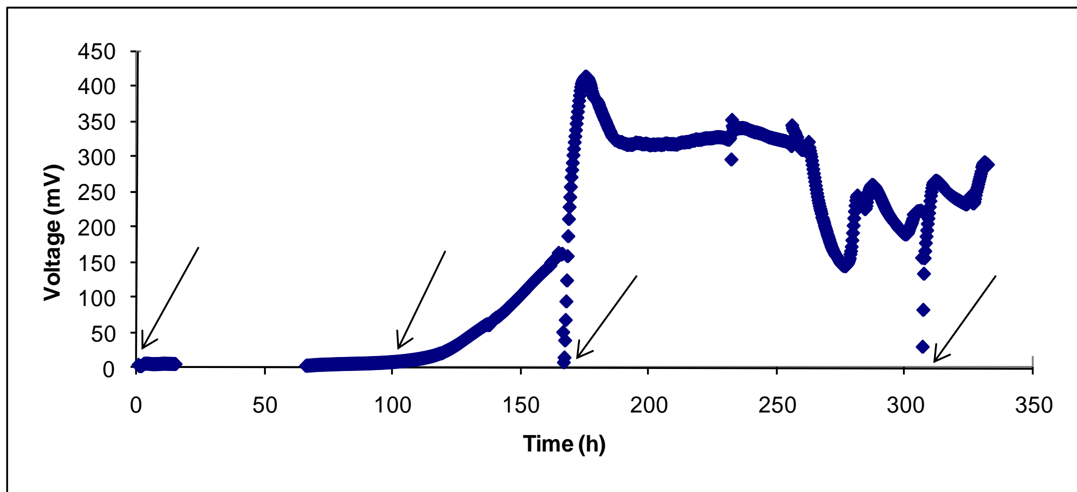


Figure 4.21: Voltage versus time graph for SCF-2-MFC using 300 ohm external resistance and batchly feeding with 200 mg/L acetate . Arrows indicate the replecament of the substrate with a fresh substrate.

Accoording to graph, acclimation time was 117 hour (after second feeding). After the feeding, voltage increased to 162 mV, then the system was loaded with acetate 3 rd time, the voltage rised rapidly to 412 mV at 175th hour . Then, the voltage dropped to 320 mV and continued nearly constant during 73 hours. After that, fluctuating values were recorded. Maximum current was calculated as 1.37 mA at 175th hour and power

density was calculated as 156.78 mW/m^2 (for 36 cm^2 anode area). COD removal was found 96±2 % .The power density value was much more than the previous studies but it was not regular, however without adding fresh media, the voltage was not dropped long time. It should be used stainless steel anode material by microorganisms. It was observed when the MFC system has been demounted, the anode material has been corroded.

4.4.2 Activated carbon cloth cathode

Some experiments was continued for SCF-2-MFC changing cathode material. Anode material was carbon cloth, but cathode material was activated carbon cloth. The reason of choosing this material is that it has much space area; therefore, more microorganisms can attach on it.

The SCF-2-MFC was fed synthetic wastewater containing 200 mg/L acetate as a carbon source. 468,5 ohm was used as external resistance. Feeding times were shown on graph by arrows (Figure 4.22). 48 hours lasts for 1st loading, 120 hours lasts for 2nd loading, 169 hours lasts for 3rd loading, 219 hours lasts for 4st loading, respectively. After the second feeding, potential was reached to 326.61 mV, current was calculated as 0,69 mA and power density was calculated as 64,39 mW/m^2.

After the third feeding, voltage was reached to 432.71 mV, current was calculated as 0,90 mA and power density was 111.01 mW/m^2. After the fourth feeding, potential was reached to 420.95 mV, current was calculated as 0.89 mA and power density was 105.06 mW/m^2. COD removal was found 95±2 % .

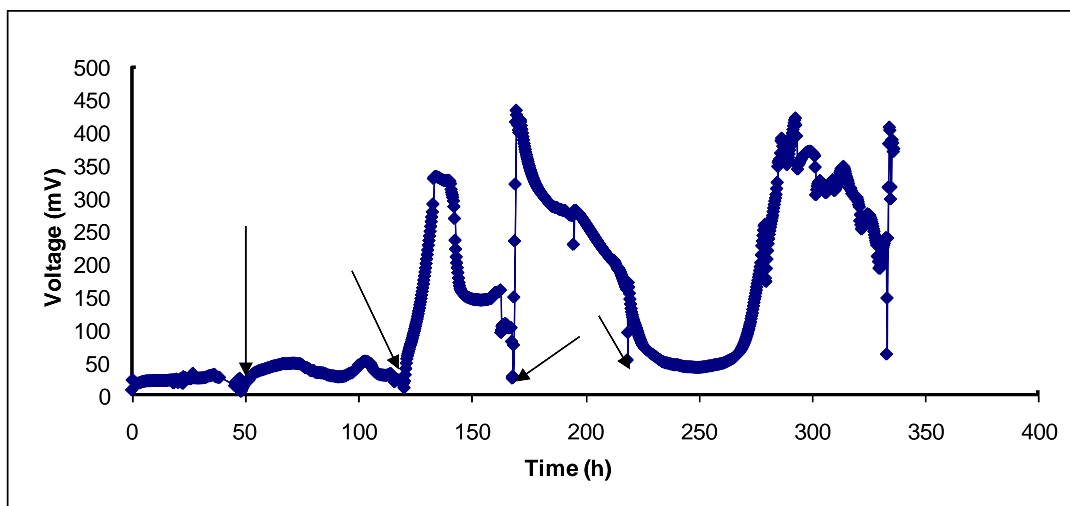


Figure 4.22: Voltage versus time graph for SCF-2-MFC for activated carbon cloth cathode.

Due to the fact that platinum is an expensive catalyst for cathode, activated carbon cloth has been used alternatively in this study because it is cheap. Maybe, it can be used alternatively at MFC studies in the future. However, it should be tried long period of time to wide uses.

4.5 Antibiotic and Hormone Experiments for SCF-2-MFC

SCF-2-MFC used as a biosensor in this part of the thesis for inhibitor matters. To compare inhibition response between asetate only feeding run and antibiotic (or hormon) plus acetate feeding run, average the currents was calculated for only asetate runs.

4.5.1 Acetate only feeding

In this study, 600 mg/L sodium acetate (NaAc) was used as carbon source. During all experimental study, 28 control cycles (runs) were measured. Average current was 0.402±0.09 $mAcm^2$/hour for all control cycles . 12 cycles of them were chosen as representatives (Figure 4.23).

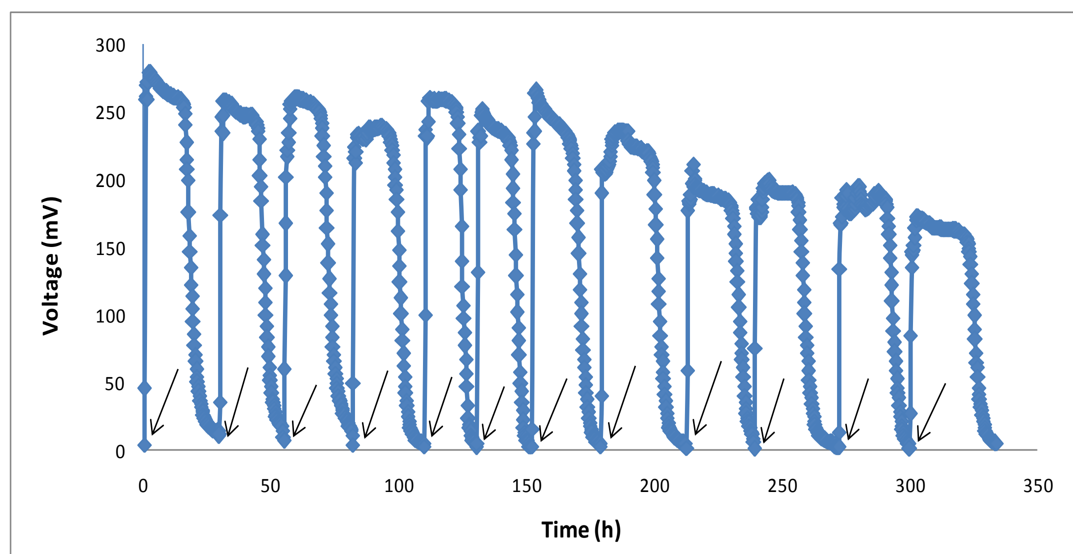


Figure 4.23: Voltage generation from NaAc only for 12 representative cycles at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

4.5.2 Antibiotics

In this study 3 different antibiotics (erythromycin, sulfamethoxazole, tetracycline) were used as inhibitor substances. During experimental study, 600 mg/L acetate was used as the carbon source and 300 Ω external resistance . All cycles were started with acetate without antibiotic. When the potential (voltage) was decreased to 5 milivolt

(mV), the MFC was filled with different concentrations of antibiotic plus acetate. Then it is loaded with acetate again. If there was fluctuating cycles, it is loaded with acetate (without antibiotic) again. In this cycle it has been confirmed that voltage field being produce by the MFC system has been recovered to its original field value. It is observed all cycles produced electricity without the addition of new bacterial inoculum. When MFC was filled with acetate without antibiotics or acetate plus antibiotics, the bacteria easily adapted to produce electricity. However, when the potential generation was evaluate during different cycles, there was discrepancy at total voltage field.

4.5.2.1 Erytromicin (ERY) Antibiotic

According to figure 4.24, 1th cycle was 600 mg/L acetate only, 2nd cycle was 50 mg/L ERY antibiotic plus 600 mg/L acetate, 3rd,4 th And 5 th cycles were acetate (600 mg/L), 6 th cycle was 100 mg/L ERY antibiotic plus 600 mg/L acetate, 7 th cycle was same concentration of acetate, 8 th cycle was 200 mg/L ERY antibiotic plus 600 mg/L acetate, 9 th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 27 hours, 2nd cycle lasts 29 hours, 3rd cycle lasts 31 hours, 4th cycle lasts 35 hours, 5th cycle lasts30 hours, 6th cycle lasts 24 hours, 7th cycle lasts 33 hours, 8th cycle lasts 23 hours, 9th cycle lasts 30 hours, totally it lasts 317 hours for ERY.

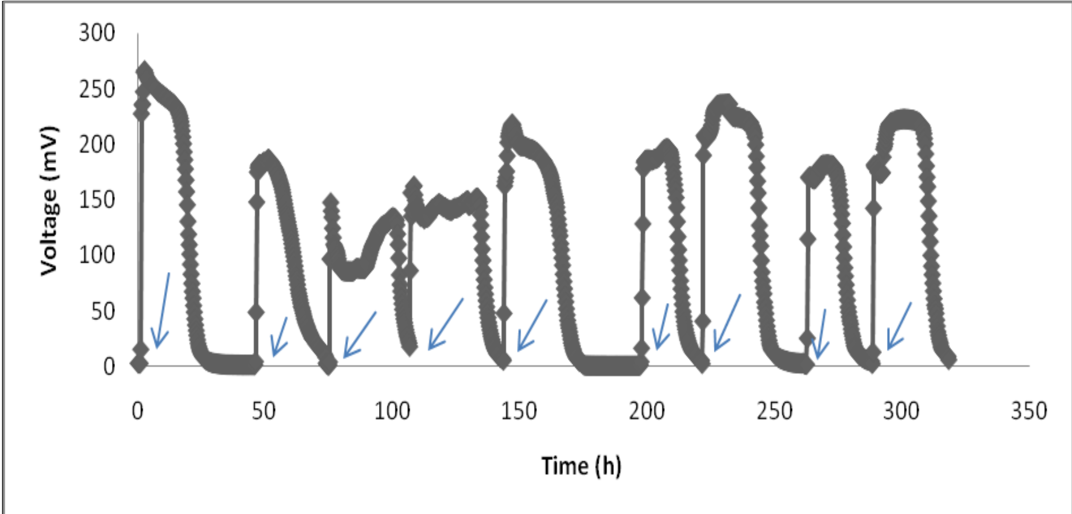


Figure 4.24: Voltage generation from acetate only and acetate with ERY antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.1. and figure 4.25, current fields from 1th to 9th cycles were 0.43, 0.27, 0.29, 0.39, 0.40, 0.26, 0.48, 0.25, 0.45 mAcm^2/hour, respectively. Current

was 0.27 mAcm2/hour, 0.26 mAcm^2/hour and 0.25 mAcm^2/hour for 50 mg/L ERY, 100 mg/L ERY and 200 mg/L ERY respectively. According to these results, there were no differences among them for current fields. But if it compare with acetate only, there were discrepancy. So, this results demonstrated that inhibition occurred. When the bacteria exposure to antibiotic firstly, could not return to original field value easily. For second and third antibiotic loading, adapted bacteria return to original value easily (only one cycle).

According to figure 4.26 for ERY, coulombic efficiency (CE) values were 0,20 for 1th cycle, 0.12 for 2nd cycle, 0.14 for 3rd cycle, 0.19 for 4th cycle, 0.19 for 5th cycle, 0.12 for 6th cycle, 0.23 for 7th cycle, 0.12 for 8th cycle and 0.21 for 9th cycle

Table 4.1: MFC performance by ERY addition.

Runs	Acetate addition mg/L	ERY addition mg/L	Effluent COD mg/L	COD efficiency %	Integral Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	15	98	61.68955	0.42831	266.1	0.2
2	600	50	56	91	38.40198	0.26662	186.45	0.12
3	600	0	25	96	41.29805	0.28648	133.94	0.14
4	600	0	21	97	57.28619	0.39752	162.36	0.19
5	600	0	15	98	58.69982	0.40752	218.22	0.19
6	600	100	124	83	37.95283	0.26345	195.96	0.12
7	600	0	16	97	68.99484	0.47905	235.69	0.23
8	600	200	270	71	35.65458	0.2475	181.56	0.12
9	600	0	16	97	64.69506	0.44918	222.39	0.21

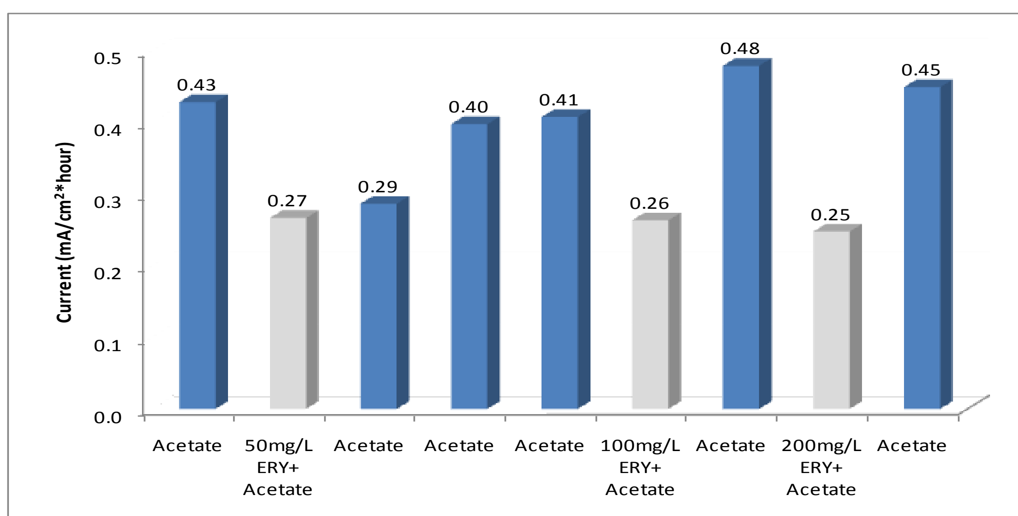


Figure 4.25: Current density per hour from acetate only and acetate with ERY antibiotic at 300 Ω external resistance.

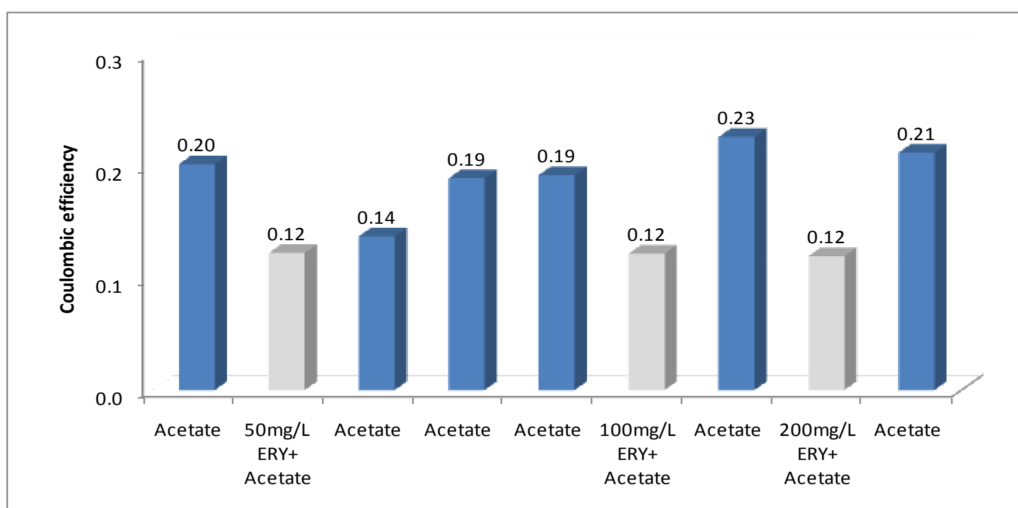


Figure 4.26: Coulombic efficiency per cycle hour from acetate only and acetate with ERY antibiotic at 300 Ω external resistance.

According to figure 4.27 for ERY, COD efficiency (%) values were 98% for 1th cycle, 92% for 2nd cycle, 96% for 3rd cycle, 97% for 4th cycle, 98% for 5th cycle, 83% for 6th cycle, 97% for 7th cycle, 68% for 8th cycle and 97% for 9th cycle.

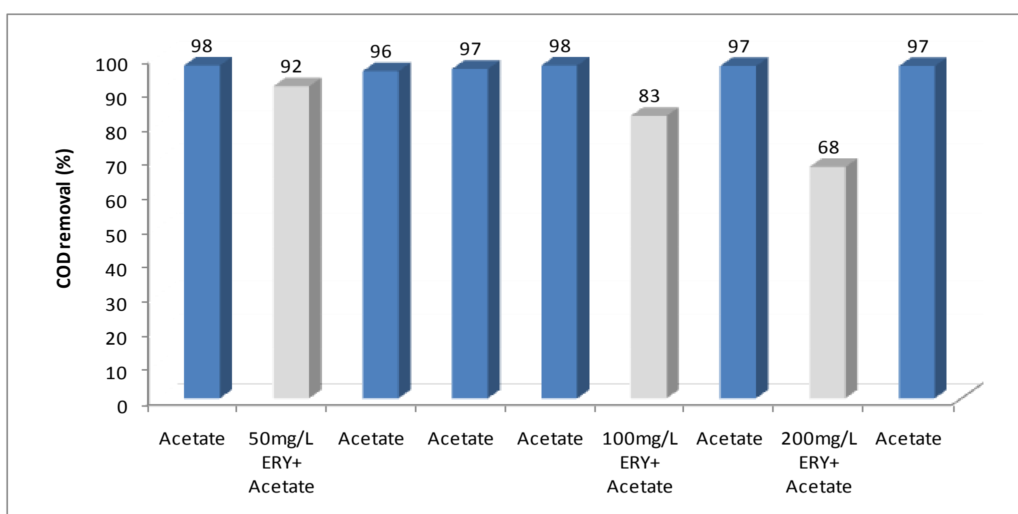


Figure 4.27: COD removal per cycle hour from acetate only and acetate with ERY antibiotic at 300 Ω external resistance.

4.5.2.2 Sulfamethoxazole (SMX) Antibiotic

According to figure 4.28, 1th cycle was 600 mg/L acetate only, 2nd cycle was 50 mg/L SMX antibiotic plus 600 mg/L acetate, 3rd cycle was 600 mg/L acetate only, 4 th cycle was 100 mg/L SMX antibiotic plus 600 mg/L NaAc, 5th cycle was same concentration of acetate, 6 th cycle was 200 mg/L SMX antibiotic plus 600 mg/L acetate and 7 th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 32 hours, 2nd cycle lasts 24 hours, 3rd cycle lasts 32 hours, 4th cycle lasts 26 hours, 5th

cycle lasts 29 hours, 6th cycle lasts 30 hours, 7th cycle lasts 27 hours, totally it lasts 268 hours for SMX.

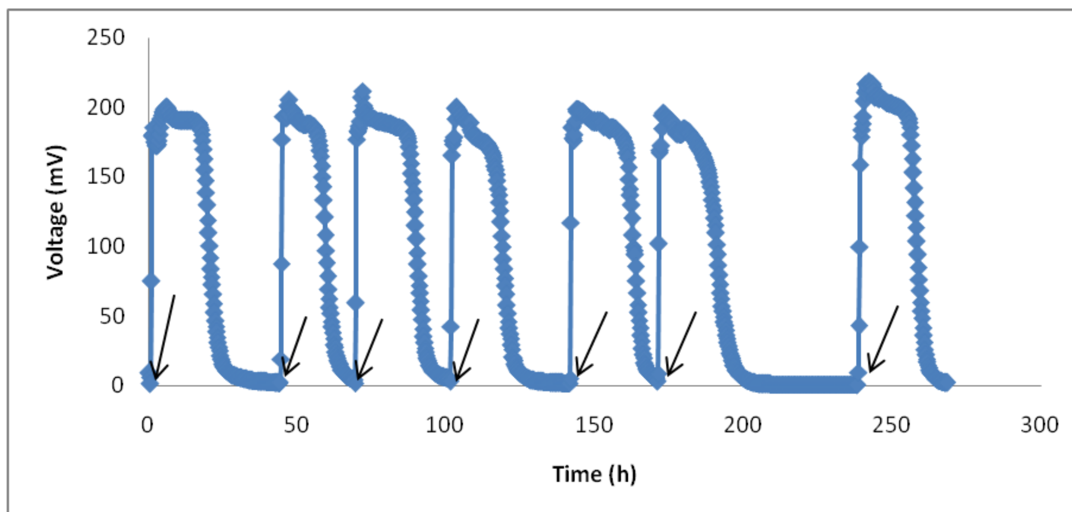


Figure 4.28: Voltage generation from acetate only and acetate with SMX antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.2 and figure 4.29, current fields from 1th to 7th cycles were 0.36, 0.28, 0.37, 0.30, 0.38, 0.34, 0.37 $mAcm^2$/hour, respectively. Currents were 0.28 $mAcm^2$/hour, 0.30 $mAcm^2$/hour and 0.34 $mAcm^2$/hour for 50 mg/L SMX, 100 mg/L SMX and 200 mg/L SMX, respectively.

Table 4.2: MFC performance by SMX addition.

Runs	Acetate addition mg/L	SMX addition mg/L	Effluent COD mg/L	COD efficiency %	İntegral Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	12	98	51.2967	0.356156	199.96	0.17
2	600	50	32	95	39.6474	0.275248	205.47	0.12
3	600	0	5	99	52.9623	0.367718	211.89	0.17
4	600	100	60	91	43.1981	0.299437	199.82	0.13
5	600	0	24	96	54.2576	0.376713	198.58	0.18
6	600	200	170	78	48.8318	0.339019	195.47	0.16
7	600	0	12	98	53.2606	0.369795	218.17	0.17

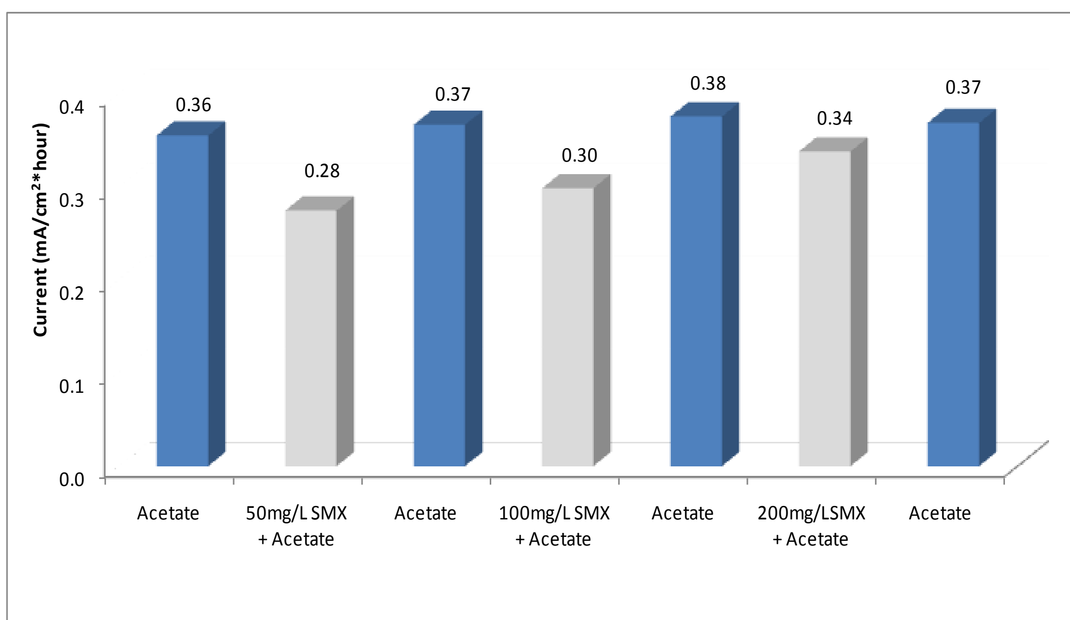


Figure 4.29: Current density per hour from acetate only and acetate with SMX antibiotic at 300 Ω external resistance.

According to figure 4.30 for SMX, coulombic efficiency (CE) values were 0,17 for 1th cycle, 0,13 for 2nd cycle, 0,17 for 3rd cycle, 0,14 for 4th cycle, 0,18 for 5th cycle, 0,16 for 6th cycle, 0,17 for 7th cycle.

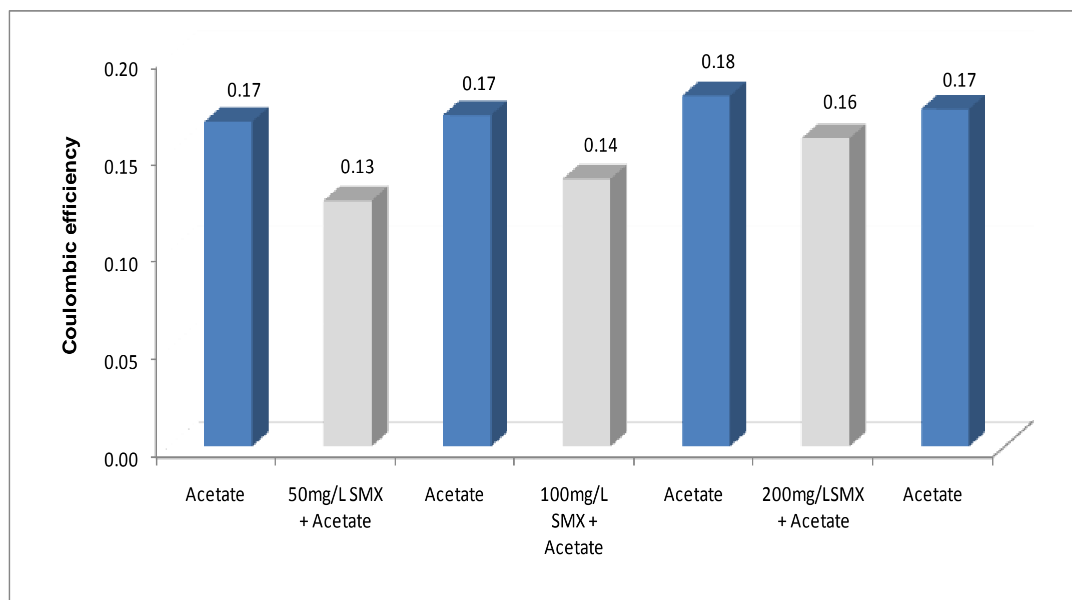


Figure 4.30: Coulombic efficiency per cycle hour from acetate only and acetate with SMX antibiotic at 300 Ω external resistance.

According to figure 4.31 for SMX, COD efficiency (%) values were 98% for 1th cycle, 95% for 2nd cycle, 99% for 3rd cycle, 91% for 4th cycle, 96% for 5th cycle, 78% for 6th cycle and 98% for 7th cycle.

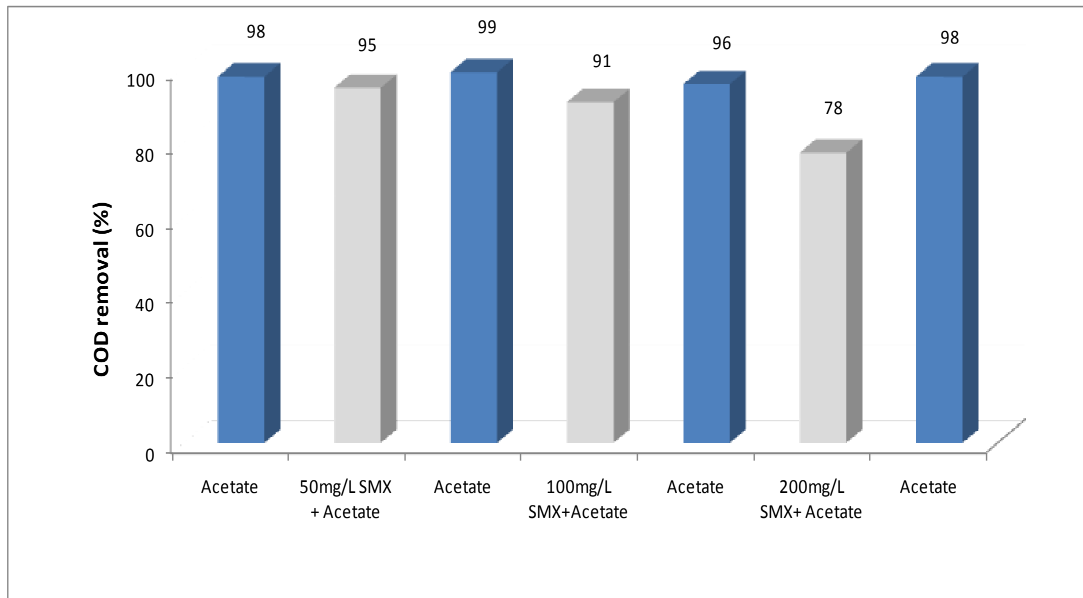


Figure 4.31: COD removal per cycle hour from acetate only and acetate with SMX antibiotic at 300 Ω external resistance.

4.5.2.3 Tetracycline (TC or TETRA) antibiotic

According to figure 4.32 for TC, 1th cycle was 600 mg/L acetate only, 2nd cycle was 50 mg/L TC antibiotic plus 600 mg/L acetate, 3rd cycle was acetate (600 mg/L) only, 4th cycle was 100 mg/L TC antibiotic plus 600 mg/L acetate, from 5 th to 8 th cycles were same concentration of acetate, 9 th cycle was 200 mg/L TC antibiotic plus 600 mg/L acetate, 10 th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 27 hours, 2nd cycle lasts 28 hours, 3rd cycle lasts 31 hours, 4th cycle lasts 30 hours, 5th cycle lasts 26 hours, 6th cycle lasts 38 hours, 7th cycle lasts 38 hours, 8th cycle lasts 34 hours, 9th cycle lasts 26 hours and 10th cycle lasts 42 hours, totally it lasts 465 hours for TC.

As it is seen table 4.3 and figure 4.33, current fields from 1th to 10th cycles were 0.36, 0.30, 0.38, 0.27, 0.25, 0.31, 0.33, 0.39, 0.26 and 0.44 mAcm^2/hour, respectively. Currents were 0.30 mAcm^2/hour, 0.27 mAcm^2/hour and 0.26 mAcm^2/hour for 50 mg/L TC, 100 mg/L TC and 200 mg/L TC, respectively.

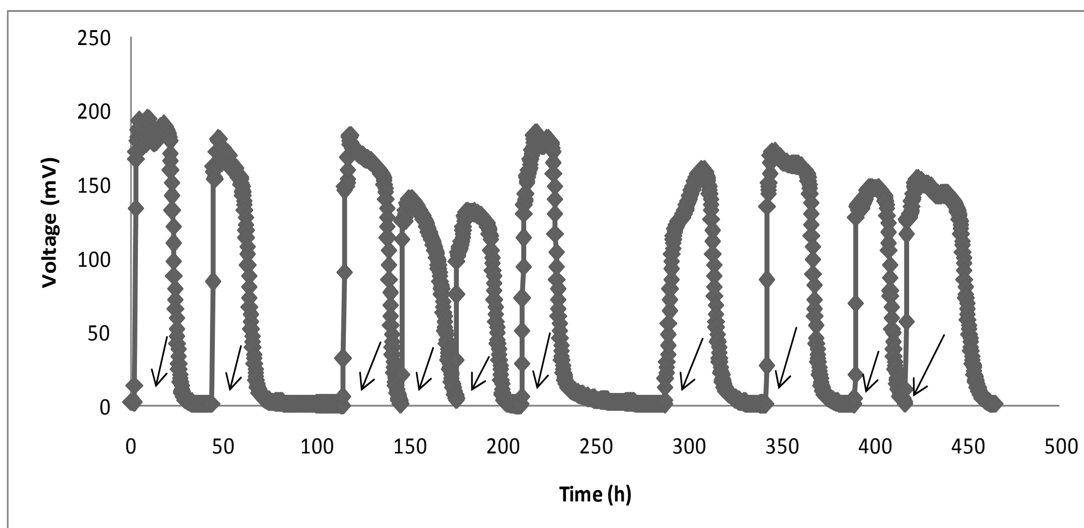


Figure 4.32: Voltage generation from acetate only and acetate with TC antibiotic at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

Table 4.3: MFC performance by TC addition.

Runs	Acetate addition mg/L	TC addition mg/L	Effluent COD mg/L	COD efficiency %	İntegraI Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	4	99	52.452273	0.364037	190.75	0.17
2	600	50	48	93	43.814774	0.304199	180.85	0.14
3	600	0	5	99	55.050569	0.382236	183.76	0.18
4	600	100	95	86	38.89252	0.270028	140.52	0.12
5	600	0	14	98	35.310034	0.245116	131.69	0.11
6	600	0	30	95	45.185865	0.313658	180.91	0.14
7	600	0	5	99	48.014575	0.333368	159,91	0.15
8	600	0	9	99	56.2322	0.39043	172,76	0.18
9	600	200	72	91	37.09454	0.25753	148	0.12
10	600	0	10	98	63.28172	0.43938	153.51	0.20

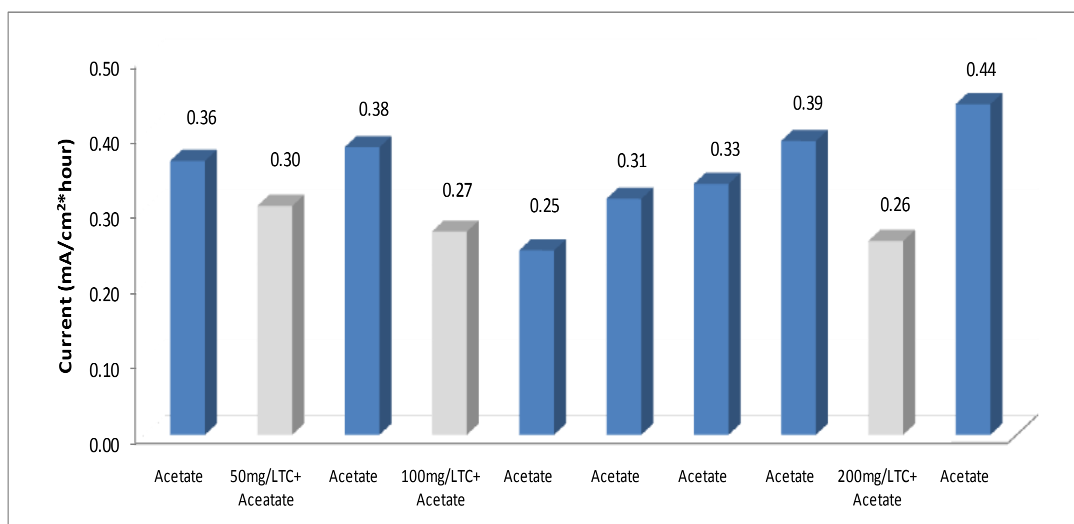


Figure 4.33: Current for per cycle hour from acetate only and acetate with TC antibiotic at 300 Ω external resistance.

According to figure 4.34 for TC, coulombic efficiency (CE) values were 0.17 for 1th cycle, 0.14 for 2nd cycle, 0.18 for 3rd cycle, 0.12 for 4th cycle, 0.11 for 5th cycle, 0.14 for 6th cycle, 0.15 for 7th cycle, 0.18 for 8th cycle, 0.12 for 9th cycle, 0.20 10th cycle.

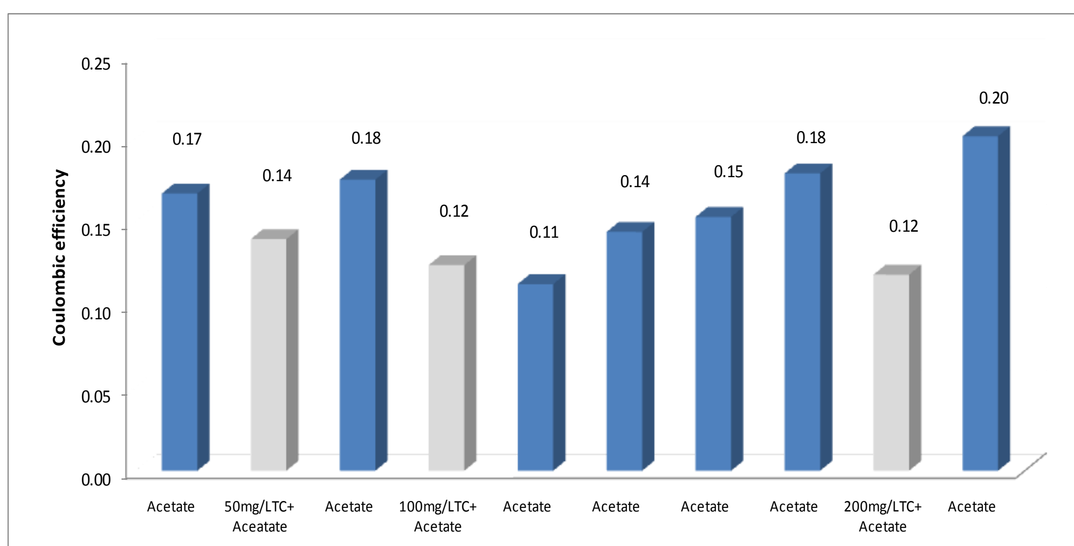


Figure 4.34: Coulombic efficiency for per cycle hour from acetate only and acetate with TC antibiotic at 300 Ω external resistance.

According to figure 4.35 for TC, COD efficiency (%) values were 99% for 1th cycle, 94% for 2nd cycle, 99% for 3rd cycle, 92% for 4th cycle, 98% for 5th cycle, 95% for 6th cycle, 99% for 7th and 8th cycles, 91% for 9th cycle and 98% for 10th cycle.

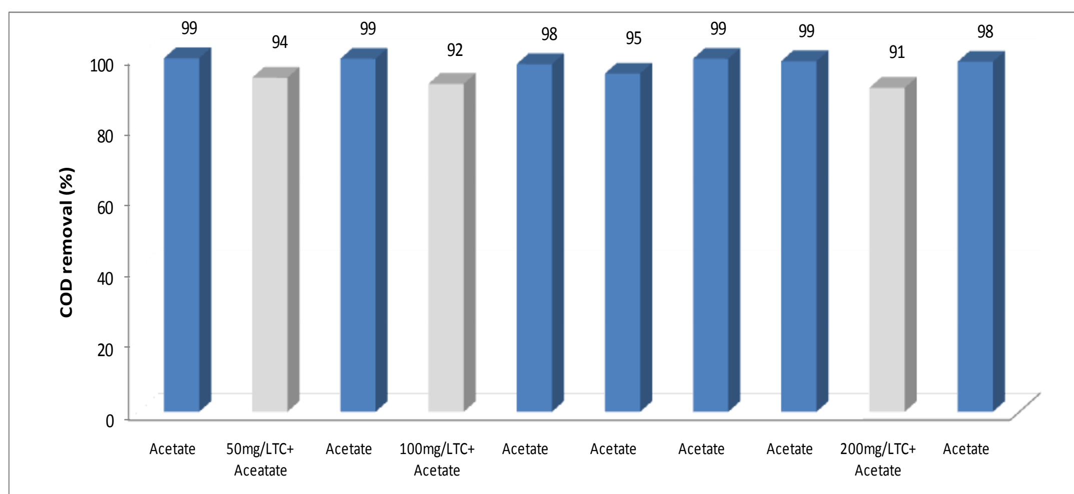


Figure 4.35: COD efficiency for per cycle hour from acetate only and acetate with TC antibiotic at 300 Ω external resistance.

When the MFC was fed with 50 mgCOD/L ERY and compared with control run, there is a significant decrease in the maximum voltage reached (30%) and the current (38%) and experiment time lasted 2 hours longer. The system's recovery to its original form took 3 runs of acetate only feeds. For 100 mgCOD/L ERY, voltage and current values obtained were 10% and 35% respectively. For 200 mgCOD/L ERY, voltage and current values obtained were 23% and 48% respectively. COD removal efficiency values decrease 7%, 15% and 29% for 50, 100 and 200 mgCOD/ml ERY respectively and no removal of antibiotics was observed. Coulombic efficiency values decreased approximately 40% for every cycle. In general, 50-200 mg/L addition of ERY caused an inhibition in the system and also the electricity production from acetate was affected negatively.

50mgCOD/L addition of SMX on acetate caused an inhibition in the system. Current and CE values decreased 23% and 29% respectively. Although the SMX concentrations increased, inhibition levels did not increase and the system reached to original form (control run) quickly. Current values decreased 18% for 100mgCOD/L SMX and 9% for 200mgCOD/L SMX, while CE values decreased 24% and 6% respectively. In the first SMX addition, the system was affected negatively; later, the system reached to original form even though increasing SMX concentrations were added. Comparing control runs, COD removal efficiency values decreased 3%, 7% and 20% for 50-100-200 mgCOD/L SMX respectively and it was understood that some part of the antibiotic, which was added, was removed.

When the MFC was fed with 50 mgCOD/L TC and compared with control run, the current decreased 17%. Then, current level reached to original value. With the addition of 100 mgCOD/L TC in the MFC, current level decreased 29%. The system's recovery to its original form took 4 runs of acetate only feeds. When the system was fed with 200 mgCOD/L TC, current decreased 34% but with the addition of acetate, it reached original value quickly. When 200 mgCOD/L TC was added the system, 66% TC removal was observed. CE value decreased 33% for 200 mgCOD/L TC.

4.5.3 Estrogens (Hormones)

In this study, natural estrogens which estrone (E1), 17β-estradiol (E2), estriol (E3) and synthetic estrogen 17α-ethinylestradiol (EE2) were used. During experimental study, 600 mg/L acetate was used as the carbon source and 300 Ω external resistance. All cycles were started with acetate without hormones. When the potential was decreased to 5 mV, the MFC was filled with different concentration hormone plus acetate. Then it is loaded with acetate again. It is observed all cycles produced electricity without the addition of new bacterial inoculum. When MFC was filled with acetate without hormone or acetate plus hormone, the bacteria easily adapted to produce electricity. During sequential cycles, there have been observed that similar voltage generation not a little discrepancy at total voltage field.

4.5.3.1 Estrone (E1) Hormone

According to figure 4.36, 1th cycle was 600 mg/L acetate only, 2nd cycle was 0,1 mg/L E1 hormone plus 600 mg/L acetate, 3rd cycle was acetate (600 mg/L), 4th cycle was 0,5 mg/L E1 hormone plus 600 mg/L acetate, 5 th cycle was acetate only, 6th cycle was 1 mg/L E1 hormone plus 600 mg/L acetate, 7 th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 30 hours, 2nd cycle lasts 24 hours, 3rd cycle lasts 27 hours, 4th cycle lasts 21 hours, 5th cycle lasts 25 hours, 6th cycle lasts 29 hours, 7th cycle lasts 23 hours, totally it lasts 183 hours for E1 hormone.

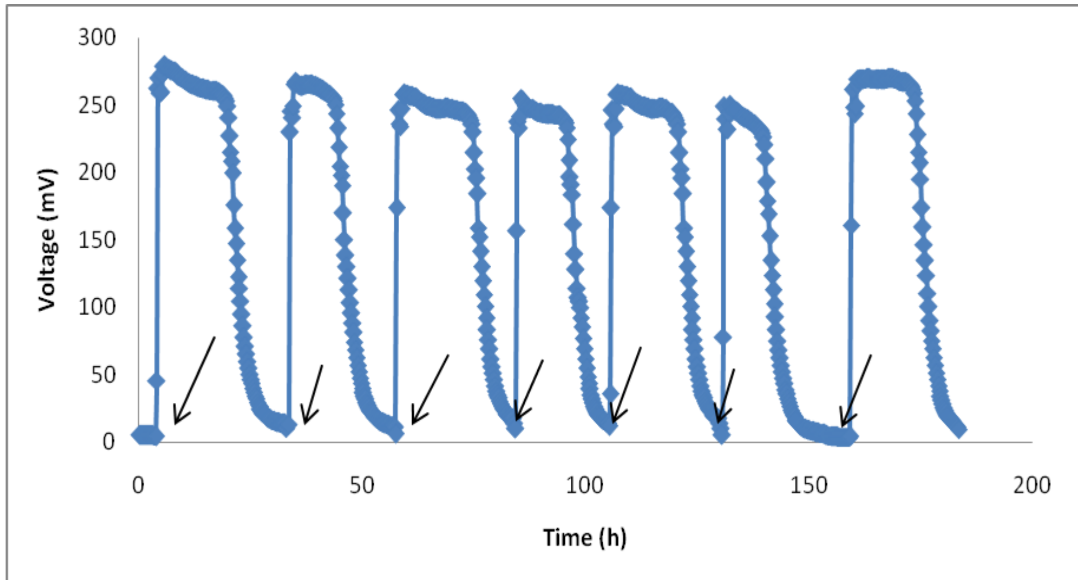


Figure 4.36: Voltage generation from acetate only and acetate with E1 hormone at 300Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.4 and figure 4.37, current fields from 1th to 7th cycles were 0.48, 0.35, 0.48, 0.35, 0.43, 0.28, 0.44 mAcm²/hour, respectively. Currents were 0,35 mAcm²/hour, 0,35 mAcm²/hour and 0,27 mAcm²/hour for 0,1 mg/L E1, 0,5 mg/L E1 and 1 mg/L E1, respectively.

Table 4.4: MFC performance by E1 addition.

Runs	Acetate addition mg/L	E1 addition mg/L	Effluent COD mg/L	COD efficiency %	Integral Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	50	92	69.8369	0.484625	279.72	0.24
2	600	0.1	100	83	50.8887	0.352917	265.81	0.19
3	600	0	52	91	69.7262	0.483742	258.49	0.24
4	600	0.5	140	77	50.1137	0.34749	255.07	0.21
5	600	0	68	89	62.3054	0.432242	257.97	0.22
6	600	1	189	69	40.2623	0.278397	250.09	0.19
7	600	0	72	88	63.9401	0.443683	270.04	0.23

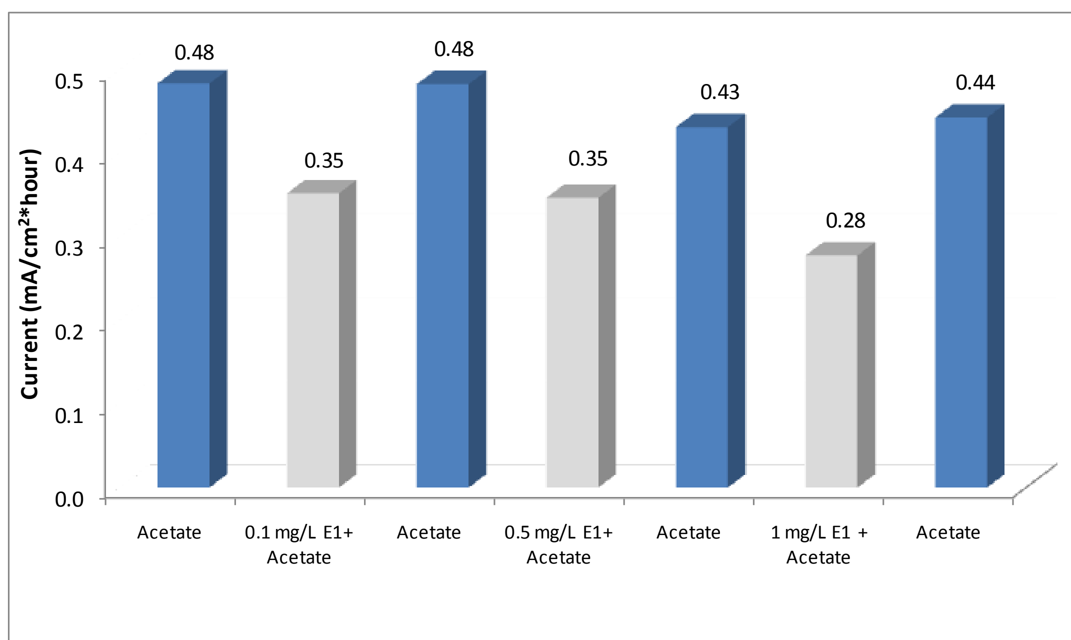


Figure 4.37: Current for per cycle hour from acetate only and acetate with E1 hormone at 300 Ω external resistance.

According to figure 4.38 for E1, coulombic efficiency (CE) values were 0.24 for 1th cycle, 0.19 for 2nd cycle, 0.24 for 3rd cycle, 0.21 for 4th cycle, 0.22 for 5th cycle, 0.19 for 6th cycle, 0.23 for 7th cycle.

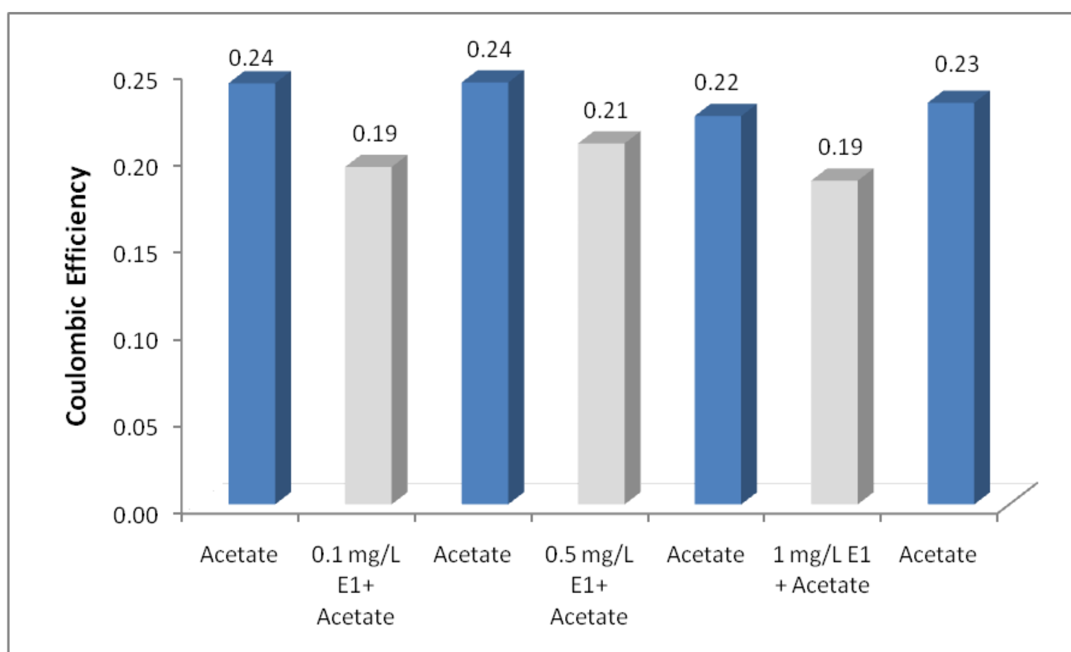


Figure 4.38: Coulombic efficiency for per cycle hour from acetate only and acetate with E1 hormone at 300 Ω external resistance.

According to figure 4.39 for E1, COD efficiency (%) values were 92% for 1th cycle, 83% for 2nd cycle, 91% for 3rd cycle, 77% for 4th cycle, 89% for 5th cycle, 69% for 6th cycle and 88% for 7th cycle.

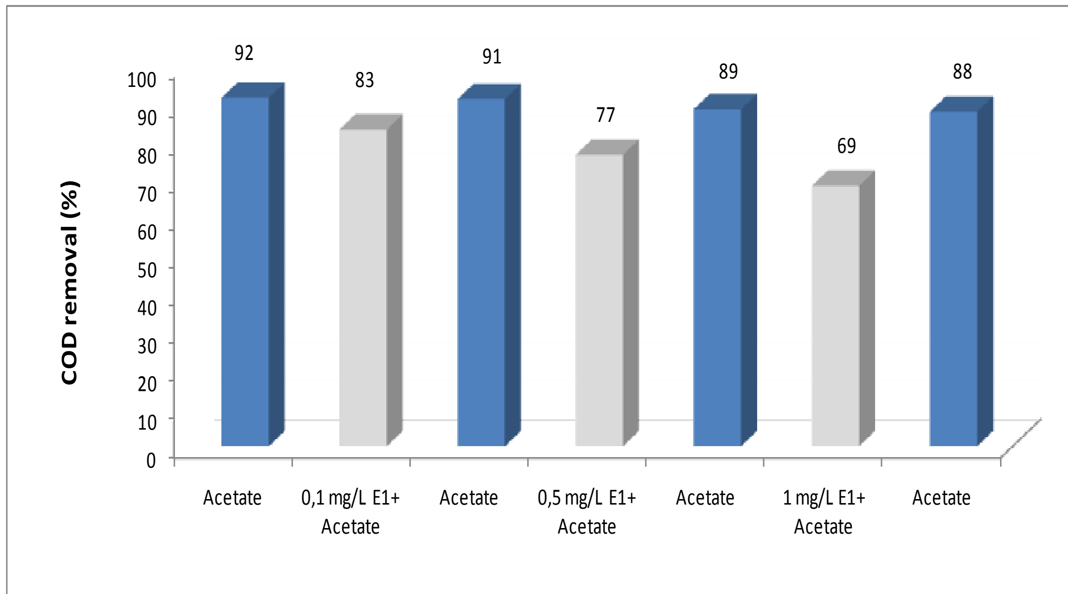


Figure 4.39: COD removal for per cycle hour from acetate only and acetate with E1 hormone at 300 Ω external resistance.

4.5.3.2 17 β-Estradiol (E2) hormone

According to figure 4.40, 1th cycle was 600 mg/L acetate only, 2nd cycle was 0,1 mg/L E2 hormone plus 600 mg/L acetate, 3rd cycle was acetate (600 mg/L), 4th cycle was 0,5 mg/L E2 hormone plus 600 mg/L acetate, 5 th cycle was acetate only, 6th cycle was 1 mg/L E2 hormone plus 600 mg/L acetate, 7 th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 25 hours, 2nd cycle lasts 22 hours, 3rd cycle lasts 24 hours, 4th cycle lasts 25 hours, 5th cycle lasts 29 hours, 6th cycle lasts 25 hours, 7th cycle lasts 27 hours, totally it lasts 200 hours for E2 hormone.

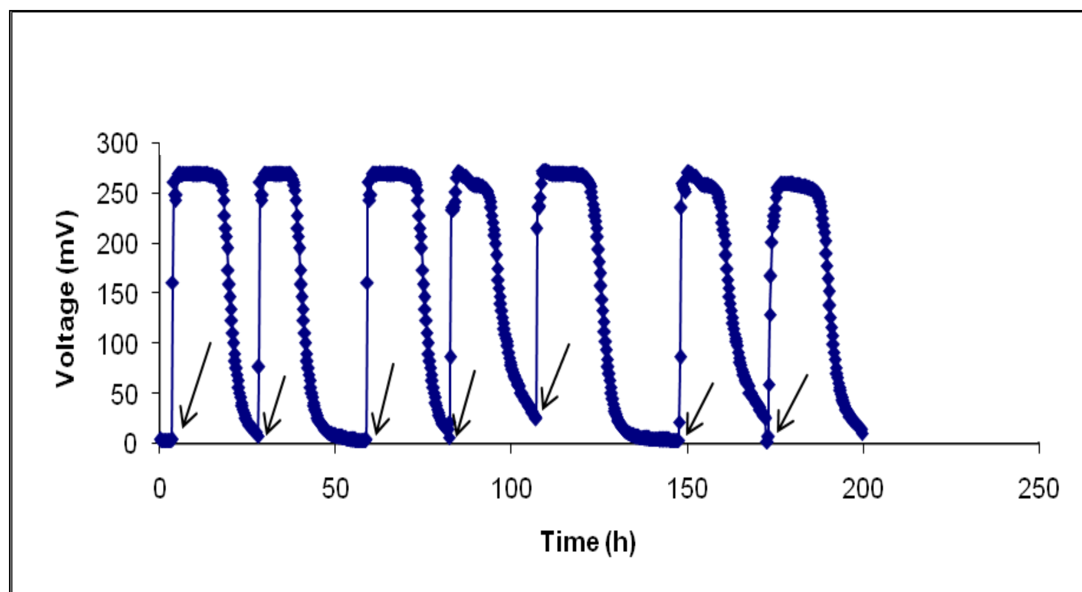


Figure 4.40: Voltage generation from acetate only and acetate with E2 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.5, .and figure 4.41 current fields from 1th to 7th cycles were 0.44, 0.33, 0.44, 0.39, 0.47, 0.39, 0.44 mAcm^2/hour, respectively. Currents were 0.33 mAcm^2/hour, 0.39 mAcm^2/hour and 0.39 mAcm^2/hour for 0.1 mg/L E2, 0.5 mg/L E2 and 1 mg/L E2, respectively.

Table 4.5: MFC performance by E2 addition.

Runs	Acetate addition mg/L	E2 addition mg/L	Effluent COD mg/L	COD efficiency %	Integral Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	72	88	63.9401	0,443683	270.04	0.23
2	600	0.1	18	97	47.1948	0.327592	270.1	0.15
3	600	0	2	100	64.7697	0.449506	271.04	0.21
4	600	0.5	30	95	56.5186	0.391759	270.67	0.19
5	600	0	2	100	69.2831	0.478295	272.4	0.22
6	600	1	36	94	56.7786	0.393598	270.55	0.19
7	600	0	2	100	64.0083	0.444112	260.22	0.20

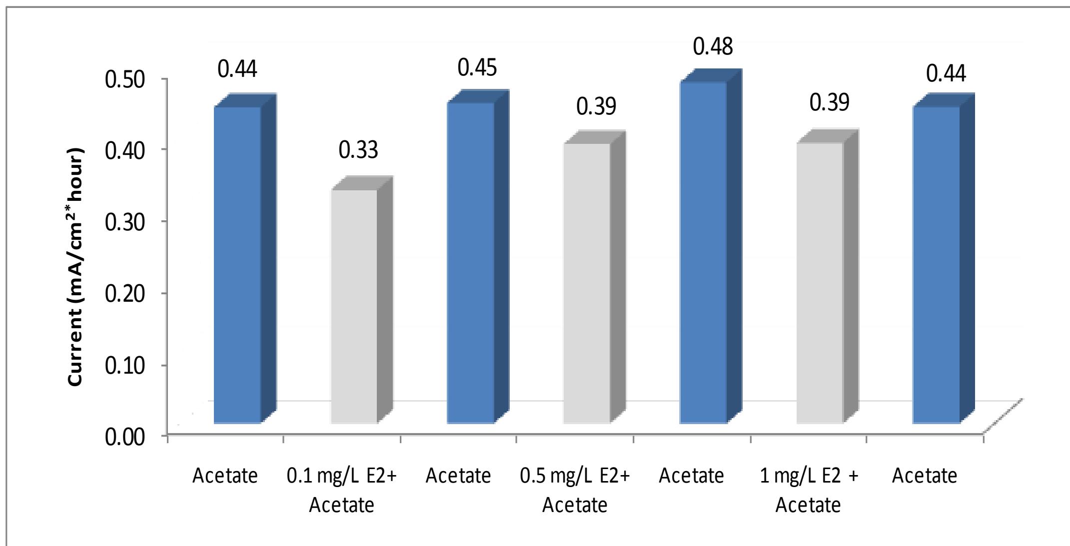


Figure 4.41: Current for per cycle hour from acetate only and acetate with E2 hormone at 300 Ω external resistance.

According to figure 4.42 for E2, coulombic efficiency (CE) values were 0.23 for 1th cycle, 0.15 for 2nd cycle, 0.21 for 3rd cycle, 0.19 for 4th cycle, 0.22 for 5th cycle, 0.19 for 6th cycle, 0.20 for 7th cycle.

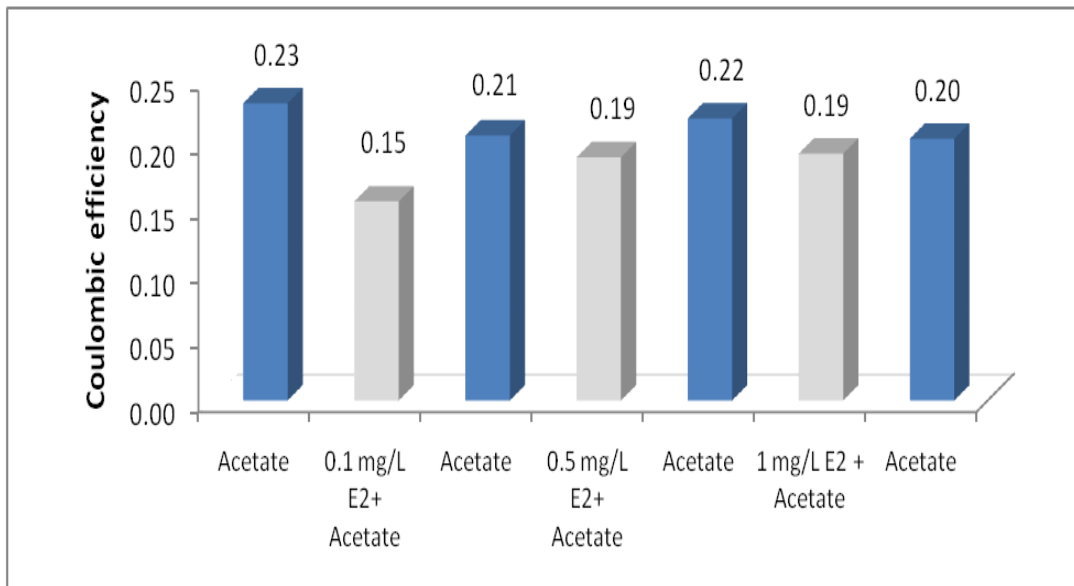


Figure 4.42: Coulombic efficiency for per cycle hour from acetate only and acetate with E2 hormone at 300 Ω external resistance.

According to figure 4.43 for E2, COD efficiency (%) values were 88% for 1th cycle, 97% for 2nd cycle, 100% for 3rd cycle, 95% for 4th cycle, 100% for 5th cycle, 94% for 6th cycle and 100% for 7th cycle.

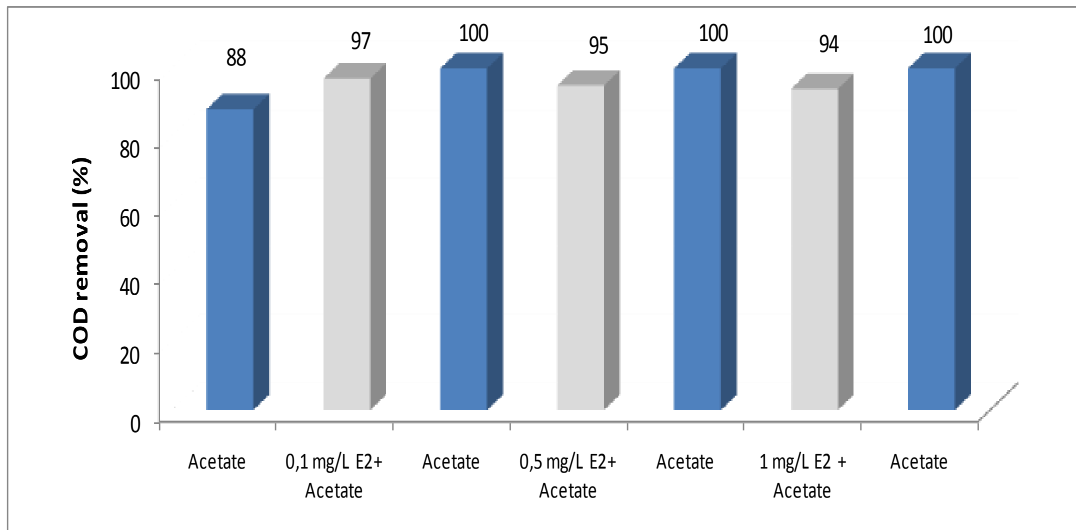


Figure 4.43: COD removal for per cycle hour from acetate only and acetate with E2 hormone at 300 Ω external resistance.

4.5.3.3 Estriol (E3) hormone

According to figure 4.44, 1th cycle was 600 mg/L acetate only, 2nd cycle was 0.1 mg/L E3 hormone plus 600 mg/L acetate, 3rd cycle was acetate (600 mg/L), 4th cycle was 0.5 mg/L E3 hormone plus 600 mg/L acetate, 5th cycle was acetate only, 6th cycle was 1 mg/L E3 hormone plus 600 mg/L acetate, 7th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 27 hours, 2nd cycle lasts 23 hours, 3rd cycle lasts 26 hours, 4th cycle lasts 24 hours, 5th cycle lasts 29 hours, 6th cycle lasts 24 hours, 7th cycle lasts 25 hours, totally it lasts 263 hours for E3 hormone.

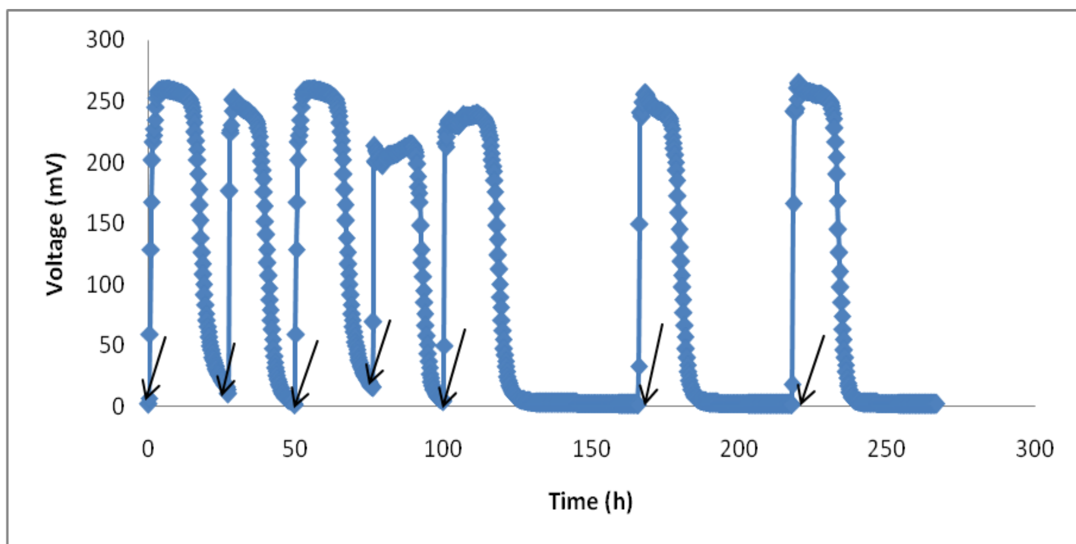


Figure 4.44: Voltage generation from acetate only and acetate with E3 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.6 and figure 4.45, current fields from 1th to 7th cycles were 0.44, 0.30, 0.45, 0.33, 0.40, 0.32, 0.38 $mAcm^2$/hour, respectively. Currents were 0.30 $mAcm^2$/hour, 0.33 $mAcm^2$/hour and 0.32 $mAcm^2$/hour for 0.1 mg/L E3, 0.5 mg/L E3 and 1 mg/L E3, respectively.

Table 4.6: MFC performance by E3 addition.

Runs	Acetate addition mg/L	E3 addition mg/L	Effluent COD mg/L	COD efficiency %	Integral Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	2	100	64.0083	0.44412	260.22	0.20
2	600	0.1	12	98	42.9826	0.29626	252.13	0.14
3	600	0	2	100	64.9358	0.45057	260.22	0.21
4	600	0.5	20	97	47.6424	0.33060	214.42	0.16
5	600	0	2	100	58.3042	0.40478	238.68	0.19
6	600	1	25	96	46.3055	0.32148	256.81	0.15
7	600	0	4	99	54.0793	0.37547	264.92	0.17

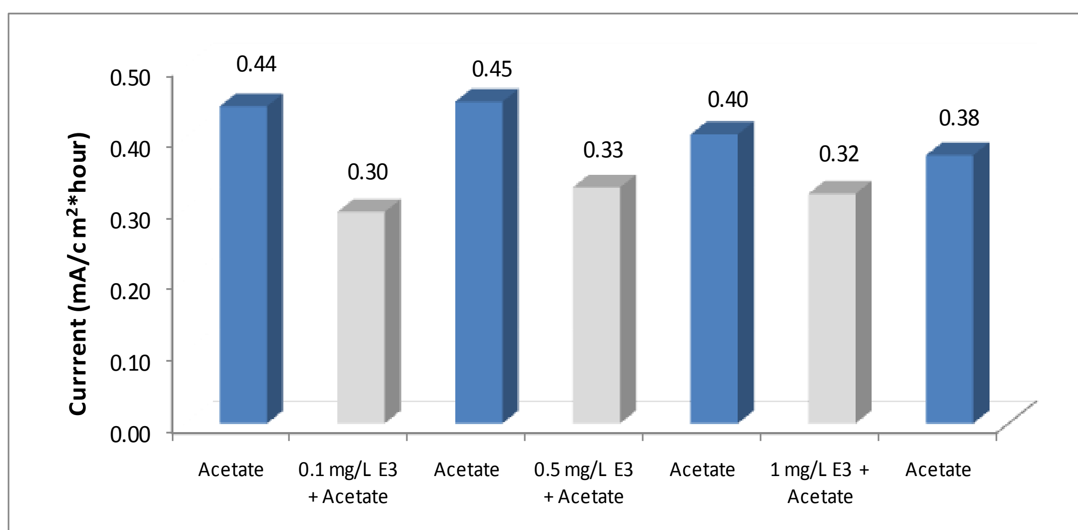


Figure 4.45: Current for per cycle hour from acetate only and acetate with E3 hormone at 300 Ω external resistance

According to figure 4.46 for E3, coulombic efficiency (CE) values were 0.20 for 1th cycle, 0.14 for 2nd cycle, 0.21 for 3rd cycle, 0.16 for 4th cycle, 0.19 for 5th cycle, 0.15 for 6th cycle, 0.17 for 7th cycle.

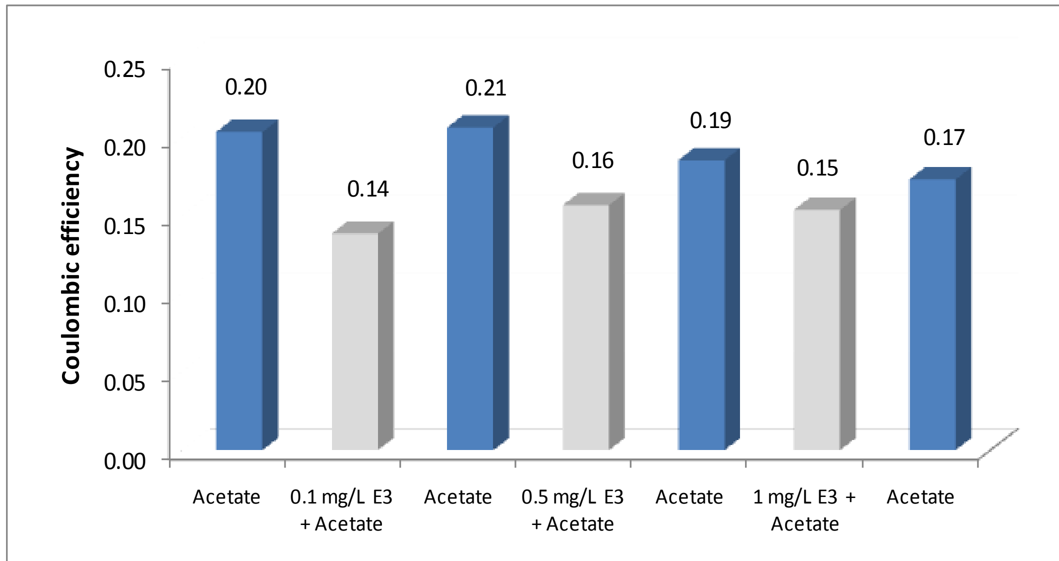


Figure 4.46: Coulombic efficiency for per cycle hour from acetate only and acetate with E3 hormone at 300 Ω external resistance.

According to figure 4.47 for E3, COD efficiency (%) values were 100% for 1th cycle, 98% for 2nd cycle, 100% for 3rd cycle, 97% for 4th cycle, 100% for 5th cycle, 96% for 6th cycle and 99% for 7th cycle.

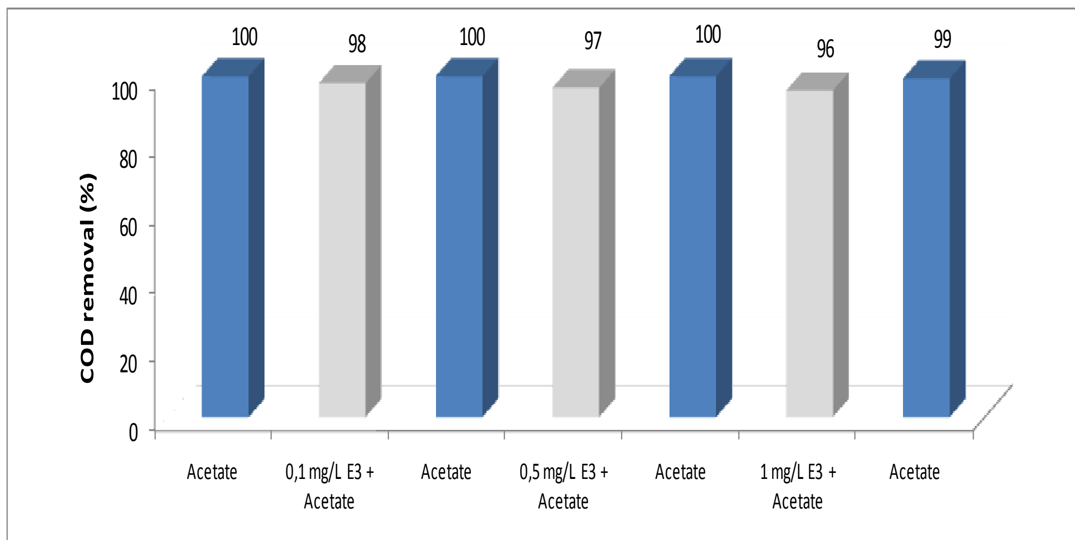


Figure 4.47: COD removal for per cycle hour from acetate only and acetate with E3 hormone at 300 Ω external resistance.

4.5.3.4 17-α Ethinylestradiol(EE2)

According to figure 4.48, 1th cycle was 600 mg/L acetate only, 2nd cycle was 0.1 mg/L EE2 hormone plus 600 mg/L acetate, 3rd cycle was acetate (600 mg/L), 4th cycle was 0.5 mg/L EE2 hormone plus 600 mg/L acetate, 5th cycle was acetate only, 6th cycle was 1 mg/L EE2 hormone plus 600 mg/L acetate, 7th cycle was acetate (600 mg/L). Approximately, 1th cycle lasts 23 hours, 2nd cycle lasts 21 hours, 3rd cycle lasts 23 hours, 4th cycle lasts 21 hours, 5th cycle lasts 23 hours, 6th cycle lasts 23 hours, 7th cycle lasts 23 hours, totally it lasts 197 hours for EE2 hormone.

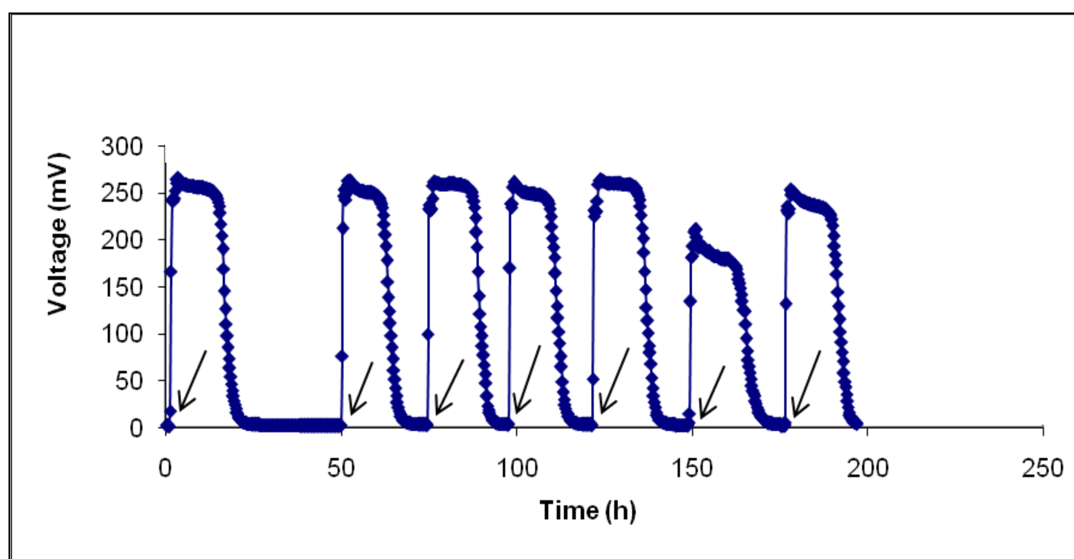


Figure 4.48: Voltage generation from acetate only and acetate with EE2 hormone at 300 Ω external resistance. Arrows indicate the replacement of consumed solution with a fresh solution.

As it is seen table 4.7 and figure 4.49, current fields from 1th to 7th cycles were 0.38, 0.32, 0.36, 0.32, 0.37, 0.28, 0.34 mAcm^2/hour, respectively. Currents were 0.32 mAcm^2/hour, 0.32 mAcm^2/hour and 0.28 mAcm^2/hour for 0.1 mg/L EE2, 0.5 mg/L EE2 and 1 mg/L EE2, respectively.

According to figure 4.50 for EE2, coulombic efficiency (CE) values were 0.17 for 1th cycle, 0.15 for 2nd cycle, 0.16 for 3rd cycle, 0.15 for 4th cycle, 0.17 for 5th cycle, 0.13 for 6th cycle, 0.16 for 7th cycle.

Table 4.7: MFC performance by EE2 addition.

Runs	Acetate addition mg/L	EE2 addition mg/L	Effluent COD mg/L	COD efficiency %	IntegraI Current (mA)	Current (mA/cm^2*hour)	Vmax (mV)	CE
1	600	0	4	99	54.0793	0.3754709	264.92	0.17
2	600	0.1	8	99	45.8060	0.318009	263.31	0.15
3	600	0	5	99	51.2563	0.35585606	259.75	0.16
4	600	0.5	9	99	45.8779	0.31849702	261.18	0.15
5	600	0	4	99	52.8175	0.36670039	260.14	0.17
6	600	1	8	99	40.4314	0.28065857	211.39	0.13
7	600	0	4	99	48.9406	0.33977702	252.62	0.16

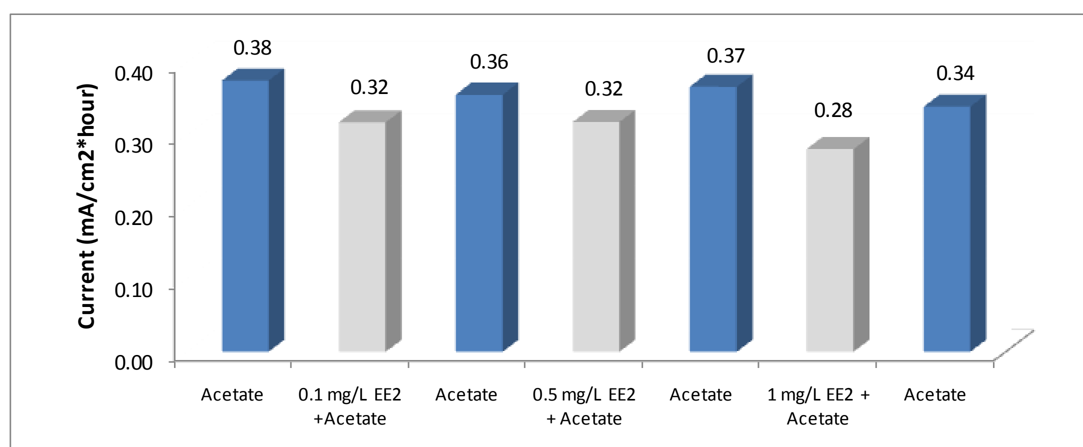


Figure 4.49: . Current for per cycle hour from acetate only and acetate with EE2 hormone at 300 Ω external resistance.

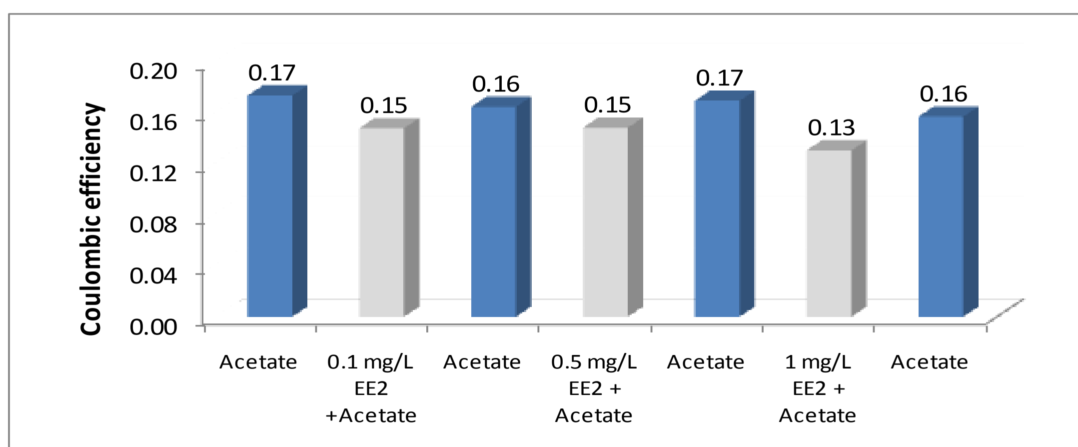


Figure 4.50: Coulombic efficiency for per cycle hour from acetate only and acetate with EE2 hormone at 300 Ω external resistance

According to figure 4.51 for EE2, COD efficiency (%) values were 99 % for all cycles.

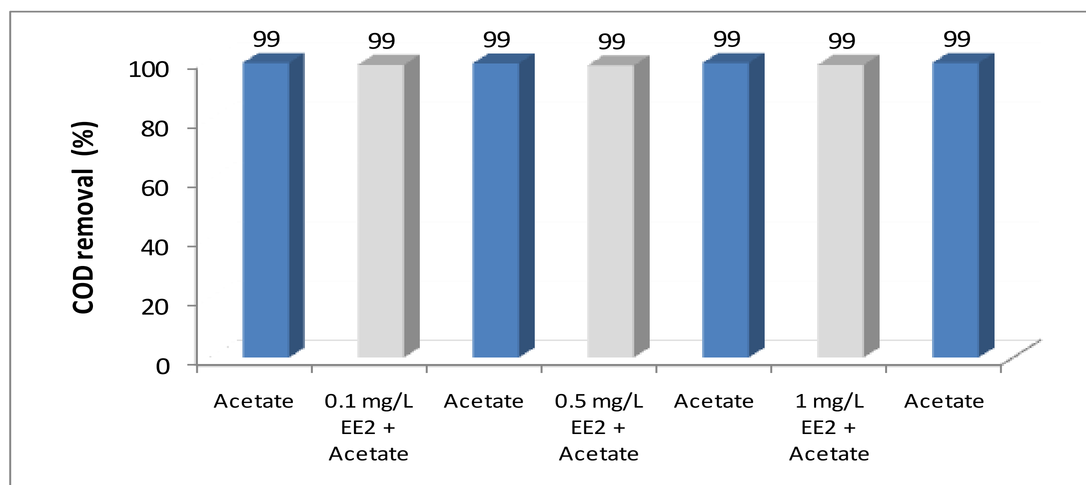


Figure 4.51. COD removal for per cycle hour from acetate only and acetate with EE2 hormone at 300 Ω external resistance.

When the MFC was fed with 0.1, 0.5 and 1 mg/L Estrone (E1) and compared with control runs, currents decreased 27%, 27% and 36% respectively. After the addition of 0,1, 0,5 ve 1 mg/L E1 to the system, it was observed that CE values decreased approximately 21% for every cycle. COD removal efficiency values decreased 83%, 77% and 66%, respectively.

When the MFC was fed with 0.1, 0.5 and 1 mg/L 17 β-Estradiol (E2) and compared with control runs, currents decreased 26%, 13% and 18% respectively. After the addition of 0.1, 0.5 ve 1 mg/L E2 to the system it was observed that CE values decreased 35%, 10% and 14% respectively. When the system was loaded with acetate only after 0.1 mg/L E2, even 0.5 and 1 mg/L E2, the system reached to original value quickly.

When the MFC was fed with 0.1, 0.5 and 1 mg/L Estriol (E3) and compared with control runs, currents decreased 33%, 27% and 21% respectively. On the other hand, CE values decreased 30, 24 and 25% respectively.

When the MFC was fed with 0.1, 0.5 and 1 mg/L 17-α Ethinylestradiol (EE2) and compared with control runs, currents decreased 15%, 11% and 23% while CE values decreased 12%, 12% and 24% respectively. When the system was loaded with 0,1 and 0,5 mg/L EE2, values of CE, Vmax and current reached to original form quickly. On the other hand, CE, Vmax and current values affected negatively with the addition of 1 mg/L EE2.

5. CONCLUSION

When it is considered in general, our thesis consists of two main subjects.

Firstly, it is purposed to generate electricity from organic compounds by using pure culture in two chambered MFC. In order to carry out this study, the experiments are achieved by creating a new laboratory substructure with the financial support taken from Turkish State Planning Organization, Istanbul Technical University and Fatih University. Long time optimization studies on this bacterium which is chosen for its genetic feature and does not require an extra chemical mediator for the gain of electrons are carried out. After the optimization, by using bacterium in the most effective way and transferring it to MFC, open circuit potentials from different organic compounds are taken. 0.8 mW/m^2 power density is attained by connecting 5000 Ω external resistance to external circuit of MFC system and using 10 Mm glucose and platinum electrodes. In his investigations, Kim et al. (2001) found that in the studies of different species of the same pure culture, the power density was 0.19mW/m^2 when woven graphite anode was used and the power density was 0.6 mW/m^2 when graphite felt anode was used. Park and Zeikus (2002) achieved 1,6 ve 1.9 mW/m^2 power density by using acetate and glucose in their studies. As it is understood that since the power density values are quite low, later on we decide to study with mixed culture bacteria in the thesis. In addition, it is decided to study with single chambered MFC in order to try different MFC configurations while making mixed culture experiments.

Different MFC system configurations are made by adapting from literature and are started to operate. Three different configurations are made as a single chambered MFC. These are named as SCF-1-MFC (single chambered flat-1-MFC), SCTMFC (single chambered tubular MFC) and SCF-2-MFC (single chambered flat-2-MFC). With SCF-1-MFC, current graphics are found and compared by changing external resistance. However, in this type of SCF-1-MFC, low currents are achieved as well. When the reason of it is analyzed, the first one is that not a good biofilm occurs on the anode surface and it is understood through the image of SEM (Scanning Electron Microscope). The other reason may be that the electrogen bacteria that will generate

electricity are not dominant on the system. Because in order to achieve electricity current efficiently, acclimation of bacteria is needed by feeding continuously for a long time.

Unfortunately, SCTMFC (Single Chambered Tubular MFC) system can not be operated since it is not possible to cover the material used as cathode on the perforated pipe and achieve the impermeability in this pipe-in-pipe system.

In the last stage of the thesis SCF-2-MFC (single chambered flat-2-MFC) is used. This MFC is operated by using microorganisms acclimated more that one year. After a short period of time system is operated, the electricity generation is started to be observed. By using 600 mg/L acetate, control runs are made. Later, inhibition studies are carried out by using different Xenobiotics (hormones and antibiotics). In other words, by using SCF-2-MFC system as biosensor, the inhibition effects of these xenobiotics on electrogen bacteria are compared with control runs which hormones and antibiotics are not used and the results are noted.

According to these results, it is proved that both antibiotics and hormones have inhibition effects on the system. 50-200 mg/L addition of ERY caused an inhibition in the system and also the electricity production from acetate was affected negatively. When the MFC was fed with 50 mgCOD/L ERY and compared with control run, there was a significant decrease in the maximum voltage reached (30%) and the current field reached (38%). For 100 mgCOD/L ERY, voltage and current values obtained were 10% and 35% anf for 200 mgCOD/L ERY, voltage and current values obtained were 23% and 48% respectively. COD removal efficiency values decreased 7%, 15% and 29% for 50, 100 and 200 mgCOD/L ERY respectively and no removal of antibiotics was observed. Coulombic efficiency values decreased approximately 40% for every cycle.

Although the SMX concentrations increased, inhibition levels did not increase and the system reached to original form (control run) quickly. Current values decreased 23% for 50 mgCOD/L SMX, 18% for 100mgCOD/L SMX and 9% for 200 mgCOD/L SMX, while CE values decreased 29%, 24% and 6% respectively. In the first SMX addition, the system was affected negatively; later, the system reached to original form even though increasing SMX concentrations were added. When compared with control runs, COD removal efficiency values decreased 3%, 7% and

20% for 50-100-200 mgCOD/L SMX respectively and it was understood that some part of the antibiotic, which was added, was removed.

With the addition of 100 mgCOD/L TC in the MFC, current level decreased 29%. The system's recovery to its original form took 4 runs of acetate only feeds. When the system was fed with 200 mgCOD/L TC, current decreased 34% but with the addition of acetate, it reached original value quickly. When 200 mgCOD/L TC was added to the system, 66% TC removal was observed. CE value decreased 33% for 200 mgCOD/L TC.

When the MFC was fed with 0.1, 0.5 and 1 mg/L Estrone (E1) and compared with control runs, currents decreased 27%, 27% and 36% respectively. CE values decreased approximately 21% for every cycle. COD removal efficiency values decreased 83%, 77% and 66%, respectively.

When the MFC was fed with 0.1, 0.5 and 1 mg/L 17 β-Estradiol (E2) and compared with control runs, currents decreased 26%, 13% and 18% respectively. After the addition of 0.1, 0.5 ve 1 mg/L E2 to the system it was observed that CE values decreased 35%, 10% and 14% respectively. When the system was loaded with acetate only after 0.1 mg/L E2, even 0.5 and 1 mg/L E2, the system reached to its original value quickly.

When the MFC was fed with 0.1, 0.5 and 1 mg/L Estriol (E3) and compared with control runs, currents decreased 33%, 27% and 21% respectively. On the other hand, CE values decreased 30, 24 and 25% respectively.

When the MFC was fed with 0.1, 0.5 and 1 mg/L 17-α Ethinylestradiol (EE2) and compared with control runs, currents decreased 15%, 11% and 23% while CE values decreased 12%, 12% and 24% respectively. When the system was loaded with 0,1 and 0,5 mg/L EE2, values of CE, Vmax and current reached to original form quickly. On the other hand, CE, Vmax and current values affected negatively with the addition of 1 mg/L EE2.

If these results were evaluated in general, it was understood that ERY and TC antibiotics had more inhibition effects on the current than SMX. When the concentrations of ERY and TC antibiotics increased, their inhibition effects on electrogen bacteria increased as well. However, a similar condition was not observed in SMX.

When hormones were evaluated in general, the inhibition in the current value did not increase although E2 and E3 concentrations were increased. On the other hand, it was observed that the inhibition in the current value increased when the concentrations in E1 and synthetic estrogen EE2 were increased.

In our experiments, the acute effects of the inhibitor materials are investigated and reported. In order to reach more clear results for future perspective, the cronic effects of the same materials have to be studied. Moreover, it is considered to find out how using more different xenobiotics affects electrogens and in this way, we can make contribution to science. The chosen antibiotics are widely used in Turkey and around the world. On the other hand, since it is observed by the researchers that widely usage of synthetic hormones in recent times has negative effects on fish, it is proved in this study that they show diversity in terms of electricity current of electrogen bacteria.

In the future, doing this kind of studies should be accelerated by drawing attention to the protection of our water sources which have the contamination potential with the uncontrolled medicine discharges.

REFERENCES

Aelterman P., Rabaey K., Pham H.T., Boon N., Verstraete W. 2006. Continious electricity generation at high voltages and currents using stacked microbial fuel cells. *Environ. Sci. Technol* ; 40:3388-94.

Aelterman, P., 2009. Microbial fuel cells for the treatment of waste streams with energy recovery. *Ph.D. Thesis*, Gent University, Belgium.

Atienzar F.A., Billinghurst Z., Depledge M.H., 2002. 4-n-Nonylphenol and 17-β estradiol may induce common DNA effects in developing barnacle larvae, *Environmental pollution*, 120,735-738.

Atienzar F.A., Cheung V.V., Jha A.N., Depledge M.H., 2001. Fitness parameter s and DNA effects are sensitive indicators of copper induced toxicity in Daphnia magna, *Toxicological Sciences*, 59,241-250.

Allen, R.M. and Bennetto, H.P., 1993. Microbial fuel cells: electricity production from carbohydrates. *Appl. Biochem. Biotechnol*. 39(2), 27-40.

Anaerobic Microbiology, 1991. *A Practical Approach* edited by P.N. Levett, Oxford University Press,.

Backhaus, T., Grimme, L.H., 1999. The toxicity of antibiotic agents to the luminescent bacterium Vibrio fischeri. *Chemosphere* 38, 3291–3301.

Backhaus, T., Grimme, L.H., 2000. Bioassays with Vibrio fischeri for the assay of delayed toxicity. *Chemosphere* 40, 821–828.

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environ. Sci. Technol.* 34 (24), 5059–5066.

Biffinger, J.C., Byrd, J.N., Dudley, B.L., Ringeisen, B.R., 2008. Oxygen exposure promotes fuel diversity for Shewanella oneidensis microbial fuel cells. *Biosens. Bioelectron*. 23, 820–826.

Braga, O., Smythe, G.A., Schafer, A.I., Feltz, A.J., 2005a. Steroid estrogens in primary and tertiary wastewater treatment plants. *Water Science and Technology* 52, 273–278.

Braga, O., Smythe, G.A., Schafer, A.I., Feitz, A.J., 2005b. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. *Environmental Science & Technology* 39, 3351–3358.

Brain, R.A., Johnson, D.J., Richards, S.M., Hanson, M.L., Sanderson, H., Lam, M.W., Young, C., Mabury, S.A., Sibley, P.K., Solomon, K.R., 2004. Microcosm evaluation of the effects of an eight pharmaceutical mixture to the aquatic macrophytes Lemna gibba and Myriophyllum sibiricum. *Aquat. Toxicol*. 70, 23–40.

Brain, R.A., Hanson, M.L., Solomon, K.R., Brooks, B.W., 2008. Aquatic plants exposed to pharmaceuticals: effects and risks. Rev. *Environ. Contam. T* 192, 67–115.

Bond, D. R.; Holmes, D. E.; Tender, L. M.; Lovley, D. R. 2002. Electrodereducing microorganisms that harvest energy from marine sediments. *Science* 2002, *295*, 483-485.

Bond DR, LovleyDR., 2003. Electricity production by Geobacter sulfurreducens attached to electrodes. *Appl Environ Microbiol*., 69:1548–55.

Boxall, A.B.A., Fogg, L.A., Kay, P., Blackwell, P.A., Pemberton, E.J., Croxford, A., 2003. Veterinary medicines in the environment. *Rev. Environ. Contam. T.* 180, 1–91.

Cajthaml T, Křesinová Z., Svobodová K., Sigler K., Rezanka T., 2009. Microbial transformation of synthetic estrogen 17α-ethinylestradiol. *Environmental Pollution* 157, 3225-3335.

Campbell, P.J., Arnold, D.J.S., Brock, T.C.M., Grandy, N.J., Heger, W., Heimbach, F., Maund, S.J., Streloke, M., 1999. *Guidance Document on Higher-Tier Aquatic Risk Assessment for Pesticides* SETAC-Europe, Brussels.

Cao, Q., Yu, Q., Connell, D.W., 2008. Degradation rate constants of steroids in sewage treatment works and receiving water. *Environmental Technology* 29,1321–1330.

Carballa, M., Omil, F., Ternes, T., Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. *Water Research* 41, 2139–2150.

Catal, T., Xu, S., Li, K., Bermek, H., Liu, H., 2008. Electricity production from polyalcohols in single-chamber microbial fuel cells. *Biosens. Bioelectron*. 24, 855–860.

Catal, T., Xu, S., Li, K., Bermek, H., Liu, H., 2008. Electricity production from polyalcohols in degradation in microbial fuel cells. *Biotechnol. Bioeng.* 100 (3), 413–422.

Chae, K.-J., Choi, M.-J., Lee, J.-W., Kim, K.-Y., Kim, I.S., 2009. Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. *Biores. Technol*. 100, 3518–3525.

Clara, M., Strenn, B., Saracevic, E., Kreuzinger, N., 2004. Adsorption of bisphenol-A, 17 beta-estradiole and 17a-ethinylestradiole to sewage sludge. *Chemosphere* 56, 843–851.

Chia M. 2002. *A miniaturized microbial fuel cell*, Technical digest of solid state sensors and actuators workshop,Hilton Head Island; 59–60.

Chang I.S., Jang J.K., Gil G.C., Kim M., Kim H.J., Cho B.W., 2004. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. *BiosensBioelectron*, 19, 607-613.

Chang IS, Moon H, Jang JK, Kim BH., 2005. Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. *Biosens Bioelectron* ;20:1856–9.

Chaudhuri SK, Lovley DR., 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat Biotechnol* 2003;21:1229–32.

Choi Y., Jung E., Kim S., Jeng S., 2003. Membran fluidity sensoring microbial fuel cell. *Bioelectrochemistry*,59,121-127

Christian, T., Schneider, R.J., Färber, H.A., Skutlarek, D., Meyer, M.T., Goldbach, H.E., 2003. Determination of antibiotic residues in manure, soil, and surface waters. *Acta Hydroch. Hydrob*. 31, 36–44.

Cheng, S.; Liu, H.; Logan, B. E., 2006. Increased Power Generation in a Continuous Flow MFC with Advective Flow Through the Porous Anode and Reduced Electrode Spacing. *Environ. Sci. Technol*. 2006, 40, 2426–2432.

Colucci, M.S., Topp, E., 2001a. Persistence of estrogenic hormones in agricultural soils: I. 17b-estradiol and estrone. *Journal of Environmental Quality* 30, 2070–2076.

Colucci, M.S., Topp, E., 2001b. Persistence of estrogenic hormones in agricultural soils: II. 17a-ethynylestradiol. *Journal of Environmental Quality* 30, 2077–2080.

Czajka, C.P., Londry, K.L., 2006. Anaerobic biotransformation of estrogens. Science of the *Total Environment* 367, 932–941.

Croal, L. R., Gralnick, J. A., Malasarn, D., Newman, D. K., 2004: The genetics of geochemistry, *Annu. Rev. Genet.,* Vol. 38, pp. 175–202.

Dantas, R.F., Contreras, S., Sans, C., Esplugas, S., 2007. Sulfamethoxazole abatement by means of ozonation. *J. Hazard Mater*. 150, 790–794.

Delong EF, Chandler P., 2002. Power from the deep. *Nat Biotechnol* ;20:788–9.

Diaz-Cruz M.S., Lopez de Alda M.J., Barcelo D., 2003. Environmental behavior and analysis of veterinary and human drugs in soils, sediments and sludge. *Trends Anal Chem*, 22:340–351.

Dokianakis, S.N., Kornaros, M.E., Lyberatos, G., 2004. On the effect of pharmaceuticals on bacterial nitrite oxidation. *Water Sci. Technol.* 50, 341–346.

Dytczak, M.A., Londry, K.L., Oleszkiewicz, J.A., 2008. Biotransformation of estrogens in nitrifying activated sludge under aerobic and alternating anoxic/aerobic conditions. *Water Environment Research* 80, 47–52.

Du Z, Li H, Gu T., 2007. A state of the art review on microbial fuel cells: A promising technology for wastewater treatment and bioenergy *Biotechnology Advances* 25, 2007 464–482.

Fent K., Weston A. A., Caminada D., 2006. Ecotoxicology of human pharmaceuticals, *Aquatic Toxicology*, 76, 122-159.

Feng, Y., Wang, X., Logan, B.E., Lee, H., 2008. Brewery wastewater treatment using air-cathode microbial fuel cells. *Appl. Microbiol. Biotechnol*. 78, 873–880.

Forrez, I., Carballa, M., Boon, N., Verstraete, W., 2009a. Biological removal of 17alphaethinylestradiol (EE2) in aerated nitrifying fixed bed reactor

during ammonium starvation. *Journal of Chemical Technology and Biotechnology* 84, 119–125.

Forrez, I., Carballa, M., Noppe, H., De Brabander, H., Boon, N., Verstraete, W., 2009b. Influence of manganese and ammonium oxidation on the removal of 17alphaethinylestradiol (EE2). *Water Research* 43, 77–86.

Fujii, K., Kikuchi, S., Satomi, M., Ushio-Sata, N., Morita, N., 2002. Degradation of 17 beta-estradiol by a gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan. *Applied and Environmental Microbiology* 68, 2057–2060.

Froehner, K., Backhaus, T., Grimme, L.H., 2000. Bioassays with Vibrio fischeri for the assessment of delayed toxicity. *Chemosphere* 40, 821–828.

Gregory KB, Bond DR, Lovley DR., 2004. Graphite electrodes as electron donors for anaerobic respiration. *EnvironMicrobiol.*, 6:596–604.

Greenman, J., Gálvez, A., Giusti, L., Ieropoulos, I., 2009. Electricity from landfill leachate using microbial fuel cells: comparison with a biological aerated filter. *Enzyme Microb. Technol*. 44, 112–119.

Giger, W., Alder, A.C., Golet, E.M., Kohler, H.P.E., McArdell, C.S., Molnar, E., Siegrist, H., Suter, M.J.F., 2003. Occurrence and fate of antibiotics as trace contaminants in wastewaters, sewage sludges, and surface waters. *Chimia*; 57, 485–491.

Gil GC, Chang IS, Kim BH, Kim M, Jang JY, Park HS, 2003. Operational parameters affecting the performance of a mediatorless microbial fuel cell. *Biosens Bioelectron*;18:327–34.

González, O., Sans, C., Esplugas, S., 2007. Sulfamethoxazole abatement by photo-Fenton toxicity, inhibition and biodegradability assessment of intermediates. *J. Hazard. Mater*. 146, 456–459.

Gorby, Y. A., Yanina, S., McLean, J. S., Rosso, K. M., Moyles, D., 2006. Electrically conductive bacterial nanowires produced by Shewanella oneidensis strain MR-1 and other microorganisms, *Proc. Natl. Acad. Sci. USA*, Vol. 103, pp. 11358–63.

Gu, C., Karthikeyan, K.G., 2008. Sorption of the antibiotic tetracycline to humic mineral complexes. *J. Environ. Qual*. 37, 704–711.

Hau, H. H. and Gralnick, J. A., 2007. Ecology and Biotechnology of the Genus Shewanella, *Annu. Rev. Microbiol*., Vol. 61, pp. 237–58, 2007.

Habermann W., Pommer .E.H., 1991. Biological fuel cells with sulphide storage capacity. *Appl Microbiol Biotechnol*;35:128–33.

Halling-Sørensen, B., 2000. Algal toxicity of antibacterial agents used in intensive farming. *Chemosphere*, 40, 731–739.

He, Z.; Minteer, S. D.; Angenent, L. T. 2005. Electricity Generation from Artificial Wastewater Using an Upflow Microbial Fuel Cell. *Environ. Sci. Technol.,* 39, 5262–5267.

Heilmann, J., Logan, B.E., 2006. Production of electricity from proteins using a microbial fuel cell. *Water Environ. Res*. 78, 531–537.

Hester R.E., and Harrison R.M., 1999. *Endocrine distrupting chemicals, Issius in Environmental Science and Technology*. Royal Society Chemistry.

Holmes DE, Bond DR, O'Neil RA, Reimers CE, Tender LR, Lovley DR. 2004. Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microbial Ecol* 48:178–90.

Holzman D.C., 2005. Microbe power. *Environ Health Persp*, 113:A754–7.

Huang, L.P., Logan, B.E., 2008. Electricity generation and treatment of paper recycling wastewater using a microbial fuel cell. *Appl. Microbiol. Biotechnol.* 80, 349–355.

Ieropoulos I, Greenman J, Melhuish C. 2003. Imitation metabolism: energy autonomy in biologically inspired robots. Proceedings of the 2nd international symposium on imitation of animals and artifacts; 191–194.

Ieropoulos IA, Greenman J, Melhuish C, Hart J.,2005. Comparative study of three types of microbial fuel cell. *Enzyme Microb Tech* 2005;37:238–45.

Jang, J.K., Pham, T.H., Chang, I.S., Kang, K.H., Moon, H., Cho, K.S., Kim, B.H., 2004: Construction and operation of a novel mediator and membrane-less microbial fuel cell, *Biochem*. 39, 1007–1012.

Jobling S, Casey D, Rogers-Gray T, Oehlmann J, Schulte-Oehlmann U, Pawlowski S, Baunbeck T, Turner AP, Tyler CR., 2003. Comparative responses of molluscs and fish to environmental estrogens and an estrogenic effluent. *Aquat Toxicol*. , 65, 205-220.

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. *Environ. Sci. Technol*. 32 (17), 2498–2506.

Jobling S., Reynolds T., White R., Parker M.G., Sumpter J.P., 1995. A variety of environmentally persistent chemicals, including some phthalate plasticizers, are weakly estrogenic., *Environmental Health and Perspectives*, 103, 582-587.

Johnson, A.C., Sumpter, J.P., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. *Environmental Science & Technology* 35, 4697–4703.

Johnson, A.C., Aerni, H.R., Gerritsen, A., Gibert, M., Giger,W., Hylland, K., Jurgens, M., Nakari, T., Pickering, A., Suter, M.J.F., Svenson, A., Wettstein, F.E., 2005. Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices.*Water Research* 39, 47–58.

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H., 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. *Environmental Science & Technology*,38, 3047–3055.

Larsson, D.G.J., de Pedro, C., Paxeus N., 2007. Effluent from drug manufactures contains extremely high levels of pharmaceceuticals. *Journal of Hazardous Material*. 148, 751-755.

Lin, A.Y.C., Tsai, Y.T., 2009. Occurence of pharmaceuticals in Taiwans's surface waters: impact of waste streams from hospitals and pharmaceutical production facilities. *Science of the total Environment*. 407, 3793-3802.

Jurgens, M.D., Holthaus, K.I.E., Johnson, A.C., Smith, J.J.L., Hetheridge, M., Williams, R.J., 2002. The potential for estradiol and ethinylestradiol degradation in English rivers. *Environmental Toxicology and Chemistry* 21, 480–488.

Kanda, R., Churchley, J., 2008. Removal of endocrine disrupting compounds during conventional wastewater treatment. *Environmental Technology* 29, 315–323.

Kang KH, Jang JK, PhamTH,Moon H, Chang IS,Kim BH. 2003. Amicrobial fuel cell with improved cathode reaction as a low biochemical oxygen demand sensor. *Biotechnol Lett* 2003;25:1357–61.

Kavlock, R.J., Daston, G.P., DeRosa, C., Fenner-Crisp, P., Gray, L.E., Kaattari, S., Lucier, G., Luster, M., Mac, M.J., Maczka, C., Miller, R., Moore, J., Roplland, R., Scott, G., Sheenen, D.M., Sinks, T., Tilson, H.A., 1996. Research needs for the risk assessment of health and environmental effects of endocrine disrupters: a report of the US EPA-sponsored workshop. *Environmental Health Perspectives* 104-4, 715–741.

Ke, J.X., Zhuang, W.Q., Gin, K.Y.H., Reinhard, M., Hoon, L.T., Tay, J.H., 2007. Characterization of estrogen-degrading bacteria isolated from an artificial sandy aquifer with ultrafiltered secondary effluent as the medium. *Applied Microbiology and Biotechnology* 75, 1163–1171.

Kidd K. A., Blanchfield P.J., Mills K.H., Palace V.P., Evans R.E., Lazorchak J.M., Flick R. W., 2007. Collapse of a Fish Population after Exposure to a Synthetic Estrogen, Proceedings of the National Academy of Sciences of the United States of America 104, 8897–8901.

Kim, B.-H., Ikeda, T., Park, H.-S., Kim, H.-J., Hyun, M.-S., Kano, K., Takagi, K. and Tatsumi, H. 1999a. Electrochemical activity of an Fe(II1)-reducing bacterium, Shewanella putrefaciens IR- I , in the presence of alternative electron acceptors. *Biotechnol. Techniques* 13(7), 475-478.

Kim, B.H., Kim, H.-J., Hyun, M.-S. and Park, D.-H., 1999b. Direct electrode reaction of Fe(lI1)-reducing bacterium, Shewanella putrefaciens. J. *Microbiol. Biotechnol*. 9(2), 127- 13 1.

Kim, B.H., Park, D.H., Shin, P.K., Chang, I.S. and Kim, H.J. 1999c. *Mediator-less biofuel cell*. U.S. Patent 5976719.

Kim, H.-J., Hyun, M.-S., Chang, I.S. and Kim, B.-H. 1999d A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, Shewanella putrefaciens. *J. Microbiol.Biotechnol*. 9(3), 365-367.

Kim HJ, Park H.S., Hyun M.S., Chang I.S., Kim M., Kim B.H., 2002., A mediatorless microbial fuel cell using a metal reducing bacterium, Shewanella putrefaciens. *Enzyme Microb Tech* ;30:145–52.

Kim BH, Chang IS, Gil GC, Park HS, Kim HJ. 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. *Biotechnol Lett* ;25:541–5.

Kim BH, Park HS, Kim HJ, Kim GT, Chang IS, Lee J, 2004. Enrichment of microbial community generating electricity using a fuel-cell type electrochemical cell. *Appl Microbiol Biotechnol* ;63:672–81.

Kim J.R, Min B, Logan B.E. 2005. Evaluation of procedures to acclimate a microbial fuel cell for electricity production. *Appl Microbiol Biotechnol*, 68:23–30.

Kim, S., Eichhorn, P., Jensen, J.N., Weber, A.S., Aga, D.S., 2005. Removal of antibiotics in wastewater: effect of hydraulic and solid retention timeson the fate of tetracycline in the activated sludge process. *Environ. Sci. Technol.* 39, 5816–5823.

Kim, S.C., Carlson, K., 2007. Temporal and spatial trends in the occurrence of human and veterinary antibiotics in aqueous and river sediment matrices. *Environ. Sci. Technol*. 41, 50–57.

Kolpin, D.W., Furlong, E.T., Meyer, M.T., Thurman, E.M., Zaugg, S.D., Barber, L.B., Buxton, H.T., 2002. Pharmaceuticals, hormones and other organic wastewater contaminants in U.S. streams 1999–2000: a national reconnaissance. *Environ. Sci. Technol*. 36 (6), 1202–1211.

Kumlanghan K. 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. *Biosensors and Bioelectronics* 22 2939–2944.

Kümmerer, K, 2009. Antibiotics in the aquatic environment – A review – Part I, *Chemosphere*, 75-417-434.

Kümmerer, K., 2004. Resistance in the environment. *J. Antimicrob. Chemoth*. 54, 311–320.

Kümmerer, K., Henninger, A., 2003. Promoting resistance by the emission of antibiotics from hospitals and households into effluents. *Clin. Microbiol. Infec.*9, 1203–1214.

Kümmerer, K., Al-Ahmad, A., Mersch-Sundermann, V., 2000. Biodegradability of some antibiotics, elimination of the genotoxicity and affection of wastewater bacteria in a simple test. *Chemosphere* 40, 701–710.

Layton, A.C., Gregory, B.W., Seward, J.R., Schultz, T.W., Sayler, G.S., 2000. Mineralization of steroidal hormones by biosolids in wastewater treatment systems in Tennessee USA. *Environmental Science & Technology* 34, 3925–3931.

Li, K., Yediler, A., Yang, M., Schulte-Hostede, S., Wong, M.H., 2008. Ozonation of oxytetracycline and toxicological assessment of its oxidation by-products. *Chemosphere* 72, 473–478.

Liu, H.; Logan, B. E., 2004. Electricity Generation Using an Air-Cathode Single Chamber Microbial Fuel Cell in the Presence and Absence of a Proton Exchange Membrane. *Environ. Sci. Technol*., 38, 4040–4046.

Liu, H.; Ramnarayanan, R.; Logan, B. E., 2004. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. *Environ. Sci. Technol.*, 38, 2281-2285.

Liu, H., Cheng, S., Logan, B.E. 2005. Power generation in fedbatch microbial fuel cells as function of ionic strength, temperature and reactor configuration. *Environ. Sci. Technol*.; 39 (14), 5488–5493.

Liu Z.D., Lian J., Du Z.W., Li H.R., 2006. Construction of sugar-based microbial fuel cells by dissimilatory metal reduction bacteria. *Chin J Biotech* ;21:131–7.

Liu, Z., Liu, J., Zhang, S., Su, Z., 2009. Study of operational performance and electrical response on mediator-less microbial fuel cells fed with carbon- and protein-rich substrates. *Biochem. Eng*. J. 45, 185–191.

Logan, B. E. 2004. Extracting hydrogen and electricity from renewable resources. *Environ. Sci. Technol*., 38, 160a-167a.

Logan, B.E. 2005. Materials and configuration for scalable microbial fuel cells. Provisional patent application, PST 2091 8, PSU 2006-3 173, Penn State University.

Logan B.E., Murano C., Scott K., Gray N.D., Head I.M., 2005. Electricity generation from cysteine in a microbial fuel cell. *Water Research* ,39: 942–952

Logan B.E., Hamelers B., Rozendal R., Schroder U., Keller J, Freguia S, 2006. Microbial fuel cells: methodology and technology. *Environ Sci Technol*;40, 5181–92.

Logan, B. E., Regan, J. M., 2006a. Electricity-producing bacterial communities in microbial fuel cells, *Trends. Microbiol.,* Vol. 14, pp. 512–18,

Logan B.E., Regan J.M., 2006b. Microbial Challenges and Fuel Cell Applications, *Environmental Science & Technology*, 5172-5180

Logan, B.E., Cheng, S., Watson, V., Estadt, G., 2007. Graphite fiber brush anodes for increased power production in air-cathode microbial fuel cells. *Environ. Sci. Technol*. 41, 3341–3346.

Logan B.E., 2008., *Microbial Fuel Cells*, Wiley Interscience, A. John Wiley and Sons, Inc, Publication

Lovley D.R. 1993. Dissimilatory metal reduction. *Annu Rev Microbial* ;47:263–90.

Lovley D.R., Coates J.D., Blunt-Harris E.L., Phillips E.J.P., Woodward J.C., 1996. Humic substances as electron acceptors for microbial respiration. *Nature* ;382:445–8.

Lovley D.R., Holmes D.E., Nevin K.P, 2004. Dissimilatory Fe(III) and Mn(IV) reduction. *Adv Microb Physiol*;49:219–86.

Lovley, D.R., 2006. Bug juice: harvesting electricity with microorganisms. *Nature Rev. Microbiol*., 4,497-508.

Lundgren M.S., Novak, P.J., 2009. Quantification of Phytoestrogens in Industrial Waste Streams, Environmental *Toxicology and Chemistry* , 28,2318-2323

Luo, H., Liu, G., Jin, S., 2009. Phenol degradation in microbial fuel cells. *Chem. Eng. J.* 147, 259–264.

Madigan, M.T. and Martinko, J.M. 2006. Brock *Biology of Microorganisms*, Pearson Education Inc., Upper Saddle River, NJ.

Melhunish C., Ieropoulos I., Greenman, J., Horsfield I., 2006. Energitically autonomous robots:food for thougt. 21-187-198 .

Mes, T.Z.D.D., Kujawa-Roeleveld, K., Zeeman, G., Lettinga, G., 2008. Anaerobic biodegradation of estrogens – hard to digest. *Water Science and Technology* 57, 1177–1182.

Mie` ge, C., Choubert, J.M., Ribeiro, L., Euse` be, M., Coquery, M., 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants –conception of a database and first results. *Environmental Pollution* 157, 1721–1726.

Min B., Logan B.E., 2004. Continuous electricity generation from domesticwastewater and organic substrates in a flat plate microbial fuel cell. *Environ Sci Technol* 2004;38:5809–14.

Min B, Cheng S, Logan B.E., 2005a. Electricity generation using membrane and salt bridge microbial fuel cells. *Water Res*;39:1675–86.

Min B, Kim J.R., Oh S.E., Regan J.M., Logan B.E, 2005b. Electricity generation from swine wastewater using microbial fuel cells. *Water Res*;39:4961–8.

Moon H., Chang I.S.,Kang K.H., Jang J.K.,Kim B.H., 2004. Improving the dynamic response of a mediator-less microbial fuel cell as a biochemical oxygen demand (BOD) sensor. *Biotechnol Lett* ,26:1717–21.

Moon H, Chang I.S., Kim B.H., 2006. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. *Bioresource Technol* ;97:621–7.

Mölstad, S., Lundborg, C.S., Karlsson, A.K., Cars, O., 2002. Antibiotic prescription rates vary markedly between 13 European countries. Scand. *J. Infect. Dis.* 34,366–371.

Muller, M., Rabenoelina, F., Balaguer, P., Patureau, D., Lemenach, K., Budzinsky, H., Barcelo, D., De Alda, M.L., Kuster, M., Delgenes, J.P., Hernandez-Raquet, G., 2008. Chemical and biological analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plants. *Environmental Toxicology and Chemistry* 27, 1649–1658.

Myers, C., R. and Myers, J., M., 2002. MtrB Is Required for Proper Incorporation of the Cytochromes OmcA and OmcB into the Outer Membrane of *Shewanella putrefaciens* MR-1, *Applied and Environmental Microbiology,* 68, 5585–5594

Nealson, K. H., Scott, J., ed. Dworkin, M., 2005. *Ecophysiology of the genus Shewanella, In The Prokaryotes,*. New York: Springer-Verlag,.

Niessen J, Schroder U, Scholz F., 2004. Exploiting complex carbohydrates for microbial electricity generation — a bacterial fuel cell operating on starch. *Electrochem Commun*;6:955–8.

Oh, S.-E., Min, B., Logan, B.E. 2004. Cathode performance as a factor in electricity generation in microbial fuel cells. *Environ. Sci. Technol*. 2004: 38 (18), 4900–4904.

Oh S.E, Logan B.E. 2005a. Hydrogen and electricity production from a food processing wastewater using fermentation and microbial fuel cell technologies. *Water Res*;39:4673–82.

Oh, S.-E., Logan, B.E., 2005b. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Appl. Microbiol. Biotechnol.* submitted for publication, doi:10.1007/500253-005-0066-y.

Oh, S. and Logan, B.E. 2006. Proton exchange membrane and electrode surface areas as factors that affect power generation in microbial fuel cells. *Appl. Microbiol. Biotechnol.* 70(2), 162-169.

Pant D., Bogaert G.V., Diels L., Vanbroekhoven K., 2009. A rewiev of the substrates used in microbial fuel cells(MFCs) for sustainable energy production, *Biosource technology*, 101, 1533-1543

Park, S., Choi, K., 2008. Hazard assessment of commonly used agricultural antibiotics on aquatic ecosystems. *Ecotoxicology* , 17(6), 526-538.

Park, D. H.; Zeikus, J. G., 1999. Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. J. *Bacteriol.*, 181, 2403-2410.

Park D.H., Zeikus J.G., 2006. Electricity generation in microbial fuel cells using neutral red as an electronophore. *Appl Environ Microb* 2000;66:1292–7.

Park D.H., Zeikus J.G., 2002. Impact of electrode composition on electricity generation in a single-compartment fuel cells using Shewanella putrefaciens. *Appl Microbiol Biotechnol*;59: 58–61.

Park D.H., Zeikus J.G., 2003. Improved fuel cell and electrode designs for producing electricity from microbial degradation. *Biotechnol Bioeng*, 81,348-355.

Patil, S.A., Surakasi, V.P., Koul, S., Ijmulwar, S., Vivek, A., Shouche, Y.S., Kapadnis, B.P., 2009. Electricity generation using chocolate industry wastewater and its treatment in activated sludge based microbial fuel cell and analysis of developed microbial community in the anode chamber. *Biores. Technol*. 100, 5132–5139.

Pauwels, B., Wille, K., Noppe, H., De Brabander, H., de Wiele, T.V., Verstraete, W., Boon, N., 2008. 17α-ethinylestradiol cometabolism by bacteria degrading estrone, 17β-estradiol and estriol. *Biodegradation* 19, 683–693.

Pham C.A., Jung S.J., Phung N.T., Lee J., Chang I.S., Kim B.H., 2003. A novel electrochemically active and Fe(III)-reducing bacterium phylogenetically related to Aeromonas hydrophila, isolated from a microbial fuel cell. FEMS *Microbiol Lett* ;223:129–34.

Pham T.H., Jang J.K., Chang I.S., Kim B.H., 2004. Improvement of cathode reaction of a mediatorless microbial fuel cell. *J Microbiol Biotechnol* ;14:324–9.

Pham, T.H., Rabaey, K., Aelterman, P., Clauwaert, P., Schamphelaire, L.D., Boon, N., Verstraete, W., 2006. Microbial fuel cells in relation to conventional anaerobic digestion technology. *Eng. Life Sci*. 6 (3), 285–292.

Pholchan, P., Jones, M., Donnelly, T., Sallis, P.J., 2008. Fate of estrogens during the biological treatment of synthetic wastewater in a nitrite-accumulating sequencing batch reactor. *Environmental Science & Technology* 42, 6141–6147.

Rabaey K, Lissens G, Siciliano S, Verstraete W., 2003. A microbial fuel cell capable of converting glucose to electricity at high rate and efficiency. *Biotechnol Lett* 2003;25:1531–5

Rabaey K., Boon N., Siciliano S.D., Verhaege M., Verstraete W. 2004. Biofuel cells select for microbial consortia that self-mediate electron transfer. *Appl Environ Microb*;70:5373–82

Rabaey K and Verstraete W, 2005. Microbial fuel cells: novel biotechnology for energy generation, *Trends in Biotechnology*,; 23 No:6: 291-298

Rabaey K., Van De Sompel K., Maignien L., Boon N., Aelterman P., Clauwaert P., 2006. Microbial fuel cells for sulfide removal. *Environ Sci Technol* ;40:5218–24.

Ren, H.Y., Ji, S.L., Ahmad, N.U.D., Dao, W., Cui, C.W., 2007. Degradation characteristics and metabolic pathway of 17a-ethynylestradiol by Sphingobacterium sp.JCR5. *Chemosphere* 66, 340–346.

Rittmann, B.E. and McCarty, P.L., 2001. *Environmental Biotechnology: Principles and Applications*, McGraw-Hill, Boston.

Robinson, A.A., Belden, J.B., Lydy, M.J., 2005. Toxicity of fluoroquinolone antibiotics to aquatic organisms. *Environ. Toxicol. Chem*. 24, 423–430.

Rodrigo, M.A., Cañizares, P., Lobato, J., Paz, R., Sáez, C., Linares, J.J., 2007. Production of electricity from the treatment of urban waste water using a microbial fuel cell. *J. Power Sources* 169, 198–204.

Rosembaum, M., Schroder U.,Scholz F., 2006. Investigation of the electrocatalytic oxidation of formate and ethanol at platinum black under microbial fuel cell conditions, *J Solid State Electrochem*, 10,872-878.

Rozendal R. A., Hamellers H.V.M., Buisman j.N., 2006. Effects of Membrane Cation Transport on pH and Microbial Fuel Cell Performance, *Environmental Science and Technology*, 40, 5206-5211.

Sarmah, A.K., Northcott, G.L., 2008. Laboratory degradation studies of four endocrine disruptors in two environmental media. *Environmental Toxicology and Chemistry* 27, 819–827.

Samuelsen, O.B., 1989. Degradation of oxytetracycline in seawater at two different temperatures and light intensities, and the persistence of oxytetracycline in the sediment from a fish farm. *Aquaculture* 83, 7–16.

Schroder U, Nieben J, Scholz F. 2003. A generation of microbial fuel cells with current outputs boosted by more than on e order of magnitude. *Angew Chem Int Ed* ;42:2880–3.

Scott, K., Murano, C., 2007. A study of a microbial fuel cell battery using manure sludge waste. *J. Chem. Technol. Biotechnol*. 82, 809–817.

Shi, J., Fujisawa, S., Nakai, S., Hosomi, M., 2004. Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium Nitrosomonas europaea. *Water Research* 38, 2323–2330.

Shantaram A., Beyenal H., Veluchamy R.R.A., Lewandowski Z., 2005. Wireless sensors powered by microbial fuel cells. *Envron Sci. Technol*, 39,5037-5042.

Soto, A.M., Justicia, H., Wray, J.W., Sonnenschein, C., 1991. P-nonylphenol: an estrogenic xenobiotic released from modified polystyrene. *Environmental Health Perspectives* 92, 167–173.

Stein N. E., Keesman K.J., Hamelers H. V.M, Straten G, 2010. Kinetic models for detection of toxicity in a microbial fuel cell based biosensor , *Biosensors and bioelectronics*, 26-7, 3115-3120

Sukul, P., Lamshöft, M., Zühlke, S., Spiteller, M., 2008. Photolysis of (14) Csulfadiazine manure. *Chemosphere* 71, 717–725.

Sun, M., Sheng, G.-P., Mu, Z.-X., Liu, X.-W., Chen, Y.-Z., Wang, H.-L., Yu, H.-Q., 2009. Manipulating the hydrogen production from acetate in a microbial electrolysis cell-microbial fuel cell-coupled system. *J. Power Sources* 191, 338–343.

Tender LM, Reimers CE, Stecher HA, Holmes DE, Bond DR, Lowy DA, 2002. Harnessing microbially generated power on the seafloor. Nat Biotechnol ;20:821–5.

Tender, L.M., Gray, S.M., Groveman, E., Lowy, D.A., Kauffman, P., Melhado, J., Tyce, R.C., Flynn, D., Petrecca, R., Dobarro, J., 2008. The first demonstration of a microbial fuel cell as a viable power supply: powering a meteorological buoy. *J. Power Sources* 179, 571–575.

Ter Heijne, A., Hamelers, H. V. M., De Wilde, V., Rozendal, R. A. & Buisman, C. J. N. 2006. A bipolar membrane combined with ferric iron reduction as an efficient cathode system in microbial fuel cells. *Environ. Sci. Technol.* 40-17, 5200–5205.

Termes T., Jos A, Siegrist,H., 2004. Scrutinizing pharmaceutical and personal care products in wastewater treatment. *Environ.Sci.Technol*, 393-399.

Ternes, T.A., Kreckel, P., Mueller, J., 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants - II. Aerobic batch experiments with activated sludge. *Science of the Total Environment* 225, 91–99.

Tiedje, J., 2002. Shewanella—the environmentally versatile genome, **Nat. Biotechnol**., Vol. 20, pp. 1118–23, 2002.

Thomulka, K.W., McGee, D.J., 1993. Detection of biohazardous materials in water by measuring bioluminescence reduction with the marine organism Vibrio harveyi. *J. Environ. Sci. Heal.* A 28, 2153–2166.

Turiel, E., Bordin, G., Rodríguez, A.R., 2005. Study of the evolution and degradation products of ciprofloxacin and oxolinic acid in river water samples by HPLC–UV/MS/MS–MS. *J. Environ. Monitor.* 7, 189–195.

Tyler, C.R., Routledge, E.J., 1998. Oestrogenic effects in fish in English rivers with evidence of their causation. *Pure and Applied Chemistry* 70, 1795–1804.

Urase, T., KikutaT., 2005. Seperate estimation of adsorbsion and degredation of pharmaceutical substances and estrogens in the activated sludge process. *Water Res*., 39-7, 1289-1300.

Url–1<*http://www.wiley.com/college/pratt/0471393878/student/activities/bacterial_drug_resistance/antibiotic_targets_web.gif*> accessed at 26.01.2011

Url–2<http://www.inchem.org/documents/jecfa/jecmono/v43jec03.gif>, accessed at 03.02.2011.

Vader, J.S., van Ginkel, C.G., Sperling, F.M.G.M., de Jong, J., de Boer, W., de Graaf, J.S., van der Most, M., Stokman, P.G.W., 2000. Degradation of ethinyl estradiol by nitrifying activated sludge. *Chemosphere* 41, 1239–1243.

Vajda A.M., Barber L.B, Gray J.L, Lopez E.M, Woodling J.D, Norris D.O., 2008. Reproductive Disruption in Fish Downstream from an Estrogenic Wastewater Effluent, *Environmental Science and Technology* 42, 3407–3414.

Vargas M., Kashefi K,. Blunt-Harris E.L., Lovley D.R., 1998. Microbiological evidence for Fe(III) reduction on early earth. *Nature* ;395:65–70.

Venkata Mohan, S., Mohanakrishna, G., Reddy, B.P., Saravanan, R., Sarma, P.N., 2008. Bioelectricity generation from chemical wastewater treatment in mediatorless (anode) microbial fuel cell (MFC) using selectively enriched hydrogen producing mixed culture under acidophilic microenvironment, *Biochem. Eng*. J. 39, 121–130.

Venkata Mohan, S., Srikanth, S., Veer Raghuvulu, S., Mohanakrishna, G., Kumar, A.K., Sarma, P.N., 2009. Evaluation of the potential of various aquatic eco-systems in harnessing bioelectricity through benthic fuel cell: effect of electrode assembly and water characteristics. *Biores. Technol*. 100, 2240–2246.

Venkateswaran, K., Moser D., Dollhopf M., Lies D., Saffarini D., 1999. Polyphasic taxonomy of the genus *Shewanella* and description of Shewanella oneidensis sp. nov. *Int. J. Syst. Bacteril.,* Vol. 49, pp. 705–24.

Wang, X., Feng, Y., Ren, N., Wang, H., Lee, H., Li, N., Zhao, Q., 2009. Accelerated start-up of two-chambered microbial fuel cells: effect of positive poised potential. *Electrochem. Acta* 54, 1109–1114.

Weber, K. A., Achenbach, L. A., Coates, J. D., 2006. Microorganisms pumping iron: anaerobic microbial iron oxidation and reduction", *Nat. Rev. Microbiol.,* Vol. 4, pp. 752–64.

Weber, S., Leuschner, P., Kampfer, P., Dott, W., Hollender, J., 2005. Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture. *Applied Microbiology and Biotechnology* 67, 106–112.

Wiatrowski, H. A., Ward, P. M., Barkay, T., 2006. Novel reduction of mercury (II) by mercury sensitive dissimilatory metal reducing bacteria, *Environ. Sci. Technol.,* Vol. 40, pp. 6690– 96.

Wilkinson S., 2000. Gastrobots –benefits and challenges of microbial fuel cells in food powered robot applications, *Journal of Autonomous Robots* 9,99-111.

Wise, R., 2002. Antimicrobial resistance: priorities for action. *J. Antimicrob.Chemoth.* 49, 585–586.

Xing, D.F., Zuo, Y., Cheng, S., Regan, J.M., Logan, B.E., 2008. Electricity generation by Rhodopseudomonas palustris DX-1. *Environ. Sci. Technol*. 42, 4146–4151.

Xu, W.H., Zhang, G., Zou, S.C., Li, X.D., Liu, Y.C., 2007. Determination of selected antibiotics in the Victoria Harbour and the Pearl River, South China using highperformance liquid chromatography-electrospray ionization tandem mass spectrometry. *Environ. Pollut*. 145, 672–679.

Xu, K., Harper Jr., W.F., Zhao, D., 2008. 17α-Ethinylestradiol sorption to activated sludge biomass: thermodynamic properties and reaction mechanism. *Water Research* 42, 3146–3152.

Yamashita, N., Yasojima, M., Nakada, N., Miyajima, K., Komori, K., Suzuki, Y., Tanaka, H., 2006. Effects of antibacterial agents, levofloxacin and clarithromycin, on aquatic organisms. *Water Sci. Technol*. 53, 65–72.

Ye, Z., Weinberg, H.S., Meyer, M.T., 2007. Trace analysis of trimethoprim and sulfonamide, macrolide, quinolone, and tetracycline antibiotics in chlorinated drinking water using liquid chromatography electrospray tandem mass spectrometry. *Anal. Chem.* 79, 1135–1144

Ying, G.G., Kookana, R.S., Dillon, P., 2003. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. *Water Research* 37, 3785–3791.

Ying, G.G., Kookana, R.S., Kumar, A., 2008a. Fate of estrogens and xenoestrogens in four sewage treatment plants with different technologies. *Environmental Toxicology and Chemistry* 27, 87–94.

Ying, G.G., Toze, S., Hanna, J., Yu, X.Y., Dillon, P.J., Kookana, R.S., 2008b. Decay of endocrine-disrupting chemicals in aerobic and anoxic groundwater. *Water Research* 42, 1133–1141.

Yoshimoto, T.,Nagai, F., Fujimoto, J.,Watanabe, K., Mizukoshi, H., Makino, T.,Kimura, K., Saino, H., Sawada, H., Omura, H., 2004. Degradation of estrogens by Rhodococcus zopfii and Rhodococcus equi isolates from activated sludge in wastewater treatment plants. *Applied and Environmental Microbiology* 70, 5283–5289.

You S., Zhao Q., Zhang J., Jiang J., Zhao S., 2006. A microbial fuel cell using permanganate as the cathodic electron acceptor. *J Power Sources* 162:1409–1415

Zuo, Y., Maness, P.-C., Logan, B.E., 2006. Electricity production from steam-exploded corn stover biomass. *Energy Fuels* 20 (4), 1716–1721.

CURRICULUM VITAE

Candidate's full name: Sevil AKTAN

Place and date of birth: Merzifon, 1969

Permanent Address: Fatih Üniversitesi, Çevre Mühendisliği Bölümü, 34500, Hadımköy, Istanbul-Türkiye

Universities and Colleges attended:

2004-2011 : PhD. Institute of Science and Technology, Environmental Biotechnology Program, Istanbul Technical University, Istanbul, Turkey.

2001-2004 : M.Sc. Institute of Sciences and Engineering, Department of Environmental Engineering, Fatih University, Istanbul, Turkey.

1988-1991 : B.Sc. Department of Environmental Engineering, Istanbul Technical University, Istanbul, Turkey.

Professional Experience:

2007- :Lecturer, Fatih University, Department of Environmental Engineering, Hadımköy, Istanbul, Turkey.

2001-2007 : Research Assistant, Fatih University, Department of Environmental Engineering, Hadımköy, Istanbul, Turkey.

2000-2001 : Specialist, Fatih University, Department of Environmental Engineering, Hadımköy, Istanbul, Turkey.

1994-1999 : Environmental Engineer, OYAK Construction Incorporated Company, Ayazaga, Istanbul, Turkey.

PUBLICATIONS

Aktan S., Salih B., (2005), Fluorescent in situ hybridization (FISH) for the detection of bacterial community in activated sludge from textile factories, Environmental Technology*,* 27, 63-69

Aktan S., Çokgör E.U., Çalli B., Çavdar P., Gücin F.,(2011) Effects of antibiotics on electricity generation in microbial fuel cells, The 8th International IWA Symposium on Waste Management Problems in Agro Industries, Ilıca, Çeşme, İzmir, Turkey, Vol 1, 407-414.

Çavdar P., Yilmaz E., **Aktan S.**, Çalli B., (2010), Bio-electricity production from wastewater using membrane-less air cathode microbial fuel cell, 5th International Ege Symposium and Exhibition (IEESE-5), Pamukkale, Denizli, Turkey.

Aktan S, Ceylan C.U., Yazıcıoğlu M.B.I., Bozkurt A., Arslan N, Gücin F., Çokgör E.U. (2008), Electricity generation by a mediator-less microbial fuel cell using S*hewanella putrefaciens*, 4th International Conference on The Ecological Protection of The Planet Earth-Environment, Maritime, Trabzon, Turkey**.**

Aktan S., Salih B. (2004), Tekstil Endüstrisi Aktif Çamurundaki Microorganizmaların FISH Metoduyla Belirlenmesi, 9. Endüstriyel Kirlenme Kontrolü Sempozyumu EKK İTÜ İnşaat Fakültesi Çevre Müh. Böl., 1,265-273

Aktan S., Şeker S, Başak B., İleri R. (2001), Tekstil Atıksularındaki Renk Sorununun İleri Aktif Çamur (İLAÇ) Metodu ile Giderilebilirliği, Ulusal Çevre ve Sanayi Sempozyumu ve Sergisi, Ulusal Çevre ve Sanayi Sempozyum ve Sergisi Mersin Bildiri Kitabı, 1 , 77-85, Mersin-Türkiye.

PROJECTS

Aktan S., Bir mikrobiyal yakıt hücresinde hidrojen bakterileri *Shewenella putrefaciens* tarafından organik atıklardan elektrik üretimi, DPT Project, Researcher, 2007-2010.