

Table of Contents

Table of Contents

Table of Contents	i
Abstract	v
1. Introduction	1
1.1 Surface functionalization	1
Chemical methods	1
Physical methods	2
Biological methods	3
1.2 Peptides for enzyme immobilization.....	4
1.3 Thin-film coatings.....	6
Chemical deposition techniques	7
Physical deposition techniques.....	7
Biological deposition techniques.....	8
1.4 Failures in thin-film coatings	8
1.5 Corrosion protection of metals	12
1.6 Methods used for surface characterization	16
X-ray Photoelectron Spectroscopy	16
Field emission scanning electron microscopy.....	17
Ellipsometry	19
Contact angle	20
Hardness	21
Adhesion.....	21
2. Objectives	23
3. Experimental section.....	25
3.1 Materials	25
3.1.1 Consumables.....	25
3.1.2 Chemicals	25
3.1.3 Commercial Kits.....	26
3.1.4 Machines and equipment.....	26
3.2 Methods	28
3.2.1 DNA extraction, quantification, and sequencing	28
3.2.2 Oligonucleotides for polymerase chain reaction	28
3.2.3 Polymerase chain reaction (PCR).....	28
3.2.4 Agarose gel electrophoresis	29
3.2.5 Cloning.....	29

Table of Contents

3.2.6 Transformation.....	30
Bacterial laccases.....	30
Fungal enzymes.....	30
3.2.7 Pre-culture.....	31
Bacterial laccases.....	31
Fungal enzymes.....	31
3.2.8 Cryo-culture.....	31
3.2.9 Shake flask production of proteins.....	31
Bacterial Laccases.....	31
Fungal laccase.....	32
UPO.....	33
3.2.10 15 L fermentation of CueO-MacHis.....	33
3.2.11 Cell lysis.....	34
3.2.12 Concentration and buffer exchange.....	34
Amicon Ultra-15 centrifugation tube.....	34
Vivaflow® Cross Flow Cassettes.....	35
3.2.13 Protein purification.....	36
3.2.14 Quantification of protein concentration.....	36
3.2.15 SDS-PAGE.....	36
3.2.16 Western Blot.....	37
3.2.17 Quantification of enzyme activity.....	37
Laccase.....	37
UPO.....	38
3.2.18 Quartz Crystal Microbalance with Dissipation monitoring.....	38
3.2.19 Cleaning of material surface.....	39
3.2.20 Enzyme immobilization to material surface.....	39
3.2.21 Surface-confined enzymatic polymerization and film formation.....	39
3.2.22 Doping PPy films with zinc nanoparticles.....	40
3.2.23 X-ray photoelectron spectroscopy.....	40
3.2.24 Ellipsometry.....	40
3.2.25 Field Emission Scanning Electron Microscopy.....	40
3.2.26 Visualization of MacHis binding to Zn nanoparticles.....	41
3.2.27 Sheet resistance.....	42
3.2.28 Salt spray test & climate storage.....	42
3.2.29 Acid treatment.....	42

Table of Contents

3.2.30 Pencil hardness test	42
3.2.31 Pull-off test	43
4 Results and Discussion	44
4.1 Production of enzyme-AP fusion proteins	44
4.1.1 Generation of enzyme-AP fusion constructs	44
4.1.2 Enzyme and enzyme-AP production and purification	46
4.1.3 Enzyme and enzyme-AP activity determination	49
4.2 Establishing an enzymatic method for surface-confined thin-film coatings	49
4.2.1 Surface functionalization with enzyme and enzyme-AP	49
4.2.2 Activity of soluble and immobilized CueO and CueO-MacHis	52
4.2.3 Enzymatic polymerization of pyrrole and film formation	54
4.3 Chemical characterization of PPy-coatings	55
4.4 Morphological examination of bare and PPy-coated SST	59
4.5 Hydrophobicity of bare, enzyme functionalized, and PPy-coated SST	60
4.6 Film thickness of PPy-coatings	61
4.7 Scale-up of the enzymatic coating method	63
4.8 Enzymatic coating as a broad coating method	64
4.8.1 Chemical composition of bare and PPy-coated synthetic polymers	66
4.8.2 Morphological examination of bare and PPy-coated synthetic polymers	75
4.8.3 Conductivity measurements of bare and PPy-coated synthetic polymers	77
4.9 Stability of PPy-coatings	78
4.9.1 Acid treatment	78
4.9.2 Doping of PPy-coatings with Zn nanoparticles to boost protection	81
4.9.3 Salt spray test and climate storage	84
4.9.4 Long-term stability	86
4.9.5 Hardness	87
4.9.6 Pull-off adhesion strength	88
5 Lessons learned from using immobilized enzymes for the formation of thin-film coatings	91
6 Conclusion	94
References	95
Appendix	113
1 List of Abbreviations	113
2 List of Figures	115
3 List of Tables	121
4 List of DNA and amino acid sequences	122

Table of Contents

5 List of oligonucleotides	135
Acknowledgements	136
Declaration	137
Curriculum vitae.....	138
Publications	139