

学校编码: 10384

分类号_____密级_____

学 号: 20620100153628

UDC_____

厦 门 大 学

博 士 学 位 论 文

阴离子聚合物修饰不锈钢表面抗菌性能研究

The Study of Antibacterial Properties of Anionic Polymer

Modified Stainless Steel Surfaces

钟 丽 娟

指导教师姓名: 陈晓东 教 授

吴雪娥 助理教授

专业名称: 工业催化

论文提交日期: 2014 年 月

论文答辩日期: 2014 年 月

学位授予日期: 2014 年 月

答辩委员会主席: _____

评 阅 人: _____

2014 年 9 月

厦门大学学位论文著作权使用声明

厦门大学学位论文著作权使用声明

本人同意厦门大学根据《中华人民共和国学位条例暂行实施办法》等规定保留和使用此学位论文，并向主管部门或其指定机构送交学位论文（包括纸质版和电子版），允许学位论文进入厦门大学图书馆及其数据库被查阅、借阅。本人同意厦门大学将学位论文加入全国博士、硕士学位论文共建单位数据库进行检索，将学位论文的标题和摘要汇编出版，采用影印、缩印或者其它方式合理复制学位论文。

本学位论文属于：

1. 经厦门大学保密委员会审查核定的保密学位论文，
于 年 月 日解密，解密后适用上述授权。

2. 不保密，适用上述授权。

（请在以上相应括号内打“√”或填上相应内容。保密学位论文应是已经厦门大学保密委员会审定过的学位论文，未经厦门大学保密委员会审定的学位论文均为公开学位论文。此声明栏不填写的，默认为公开学位论文，均适用上述授权。）

声明人（签名）：

年 月 日

厦门大学学位论文原创性声明

本人呈交的学位论文是本人在导师指导下，独立完成的研究成果。本人在论文写作中参考其他个人或集体已经发表的研究成果，均在文中以适当方式明确标明，并符合法律规范和《厦门大学研究生学术活动规范（试行）》。

另外，该学位论文为()课题(组)的研究成果，获得()课题(组)经费或实验室的资助，在()实验室完成。(请在以上括号内填写课题或课题组负责人或实验室名称，未有此项声明内容的，可以不作特别声明。)

声明人(签名)：

年 月 日

摘要

细菌生物膜广泛存在于含水和潮湿的各种表面，生物膜中细菌的代谢活动可导致动植物及人类疾病发生，严重威胁人类健康，腐蚀管道和金属表面，降低传热能力，造成能源浪费。不锈钢是人类目前应用最广的金属材料之一，由于不锈钢表面细菌生物膜的生长，带来了严重的危害，而且吸附的生物膜很难清除，因此，要解决生物膜污染的问题，要从减少细菌吸着手。

Nafion 性质稳定，能够耐受高温，而且是一种生物相容型材料，由于其结构中含有磺酸基，在溶液中解离后可使修饰的表面带负电荷，由于大部分细菌表面呈负电性，因此用 Nafion 修饰后的不锈钢表面与细菌表面由于静电排斥力的作用，抑制细菌在不锈钢表面的吸附。利用沾取法在不锈钢表面镀上一层 Nafion 膜，利用静态水接触角测量仪，粗糙度测量仪，白光干涉仪，3D 激光扫描显微镜等分析手段进行表征，研究了 Nafion 修饰前后不锈钢表面性质的变化。实验结果表明：经 Nafion 修饰后的不锈钢表面粗糙度变化很小，修饰后的表面疏水性增强。分别以革兰氏阴性菌大肠杆菌和革兰氏阳性菌枯草芽孢杆菌为研究对象，考察了它们在修饰前后不锈钢表面的吸附状况，利用扫描电镜，显微镜，平板计数法等方法进行定性和定量分析。并研究了流体流速和 Nafion 修饰浓度对细菌生物膜形成的影响。实验结果表明：经过 Nafion 修饰的不锈钢片表面具有良好的抗菌效果，并且随着流速增大，剪切力增大，不锈钢表面上大肠杆菌的吸附减少。1%(wt)与 1.5%(wt)Nafion 修饰后的不锈钢抗菌性能较好。

对苯乙烯基磺酸钠分子结构中也带有磺酸基团，我们考察了对苯乙烯基磺酸钠溶液对大肠杆菌生长的毒害作用，发现对苯乙烯基磺酸钠溶液对大肠杆菌生长有抑制作用。同时以不锈钢为基底，利用 Click 反应，将对苯乙烯基磺酸钠接枝到不锈钢表面，利用衰减全反射-傅里叶红外(ATR-FTIR)、静态水接触角仪、3D 激光扫描显微镜等分析手段对不锈钢表面进行表征，发现修饰后不锈钢表面疏水性减弱。研究了修饰前后不锈钢表面大肠杆菌生物膜形成状况，考察其对大肠杆菌吸附的影响。发现接枝后的不锈钢表面对大肠杆菌生物膜形成具有良好的抑制效果。同时，实验表明修饰后的不锈钢可以重复利用，且重复抵抗细菌生物

膜的效果良好。

细菌生物膜的监测多采用离线监测，不能体现出真实的情况，影响了对细菌生物膜研究的进展。因此我们利用电化学仪器，在线记录了湖水中不锈钢表面随着细菌生物膜生长的电压变化，并且与 DAPI 荧光染色结果对比，发现电压随生物膜变化的趋势与 DAPI 染色结果一致，在此基础上初步建立了一种新型、简便的在线监测生物膜生长情况的方法。

关键词：Nafion；对苯乙烯基磺酸钠；点击反应；生物膜；抗菌性能

Abstract

Bacteria attached to the surfaces are more resistant to disinfectants than free-living cells, thus biofilm has been a serious problem in many areas, such as food, environmental and biomedical, etc., and has posed many problems, like food spoilage, public health concerns, as well as energy and instrumental wastage. Stainless steel is one of the most commonly used materials in food industry and daily life, However, the biofilms on stainless steel surfaces have brought serious problems, and the biofilms on surfaces are difficult to thoroughly remove. Exploiting effective strategies to control biofilm formation and development on stainless steel is therefore an urgent need.

Nafion membrane is superselective, thermal stable and biocompatible which has been widely used for water electrolyzes biosensors, as well as fuel cells. Nafion has also been used to improve antifouling properties. The sulfonic acid groups at the side chains of Nafion would dissociate the hydrogen ion in solution, and the remaining polymers are negatively charged, thus making the coating surfaces to reduce bacterial adhesion. Stainless steel discs were coating with Nafion by dipping, and then the surface properties were characterized by Contact Angle Measurement, roughness tester, Phase Shift MicroXAM-3D and 3D-LSM. The anti-biofilm ability of modified surfaces was tested using Gram negative bacteria *E.coli* and Gram positive bacteria *B.subtilis* as representative bacteria. The results showed that significant reduction in adherent bacteria was observed on the Nafion coated stainless steel discs for both *E.coli* and *B.subtilis*.

Sodium p-Styrene Sulfonate also has sulfonic acid groups, the toxic effects of Sodium p-Styrene Sulfonate solution on *E.coli* growth were tested, and the results showed that Sodium p-Styrene Sulfonate solution would inhibit the growth of *E.coli*. And then Sodium p-Styrene Sulfonate was grafted on stainless steel surfaces by Click-Reaction, the properties of the grafted surfaces were characterized by

ATR-FTIR, Contact Angle Measurement, and 3D-LSM. The anti-biofilm ability of grafted surfaces was studied in this work, and *E.coli* was used as representative bacteria. It has been shown that the grafted stainless steel surfaces would inhibit *E.coli* cells adhere on the surface. And the reusing grafted stainless steel surfaces retained anti-biofilm ability.

The formation of biofilm is a complex process, and it is the result of synthesized factors of mass, momentum and energy exchange. Lacking of high-quality quantitative parameters inhibits the researches on biofilm development and control. Thus developing effective and simple on-line monitoring methods to provide more data for biofilm research is important. Keithley which can record voltage data was employed in this work, and the voltage data recorded during biofilm formation was compared with the fluorescence intensity of DAPI colored biofilm. The results showed that the voltage and the fluorescence intensity increasing during biofilm formation, which meant that the changing of voltage of stainless steel during biofilm formation represented the number of bacteria adhering on the surface.

Key words: Nafion; Sodium p-Styrene Sulfonate; Click-Reaction; Biofilm; Antibiofilm

目录

摘要.....	i
Abstract.....	iii
目录.....	i
Contents	vi
第一章 前言.....	1
1.1 生物膜简介.....	1
1.1.1 细菌生物膜的定义及结构.....	1
1.1.2 生物膜的危害.....	1
1.1.3 细菌生物膜的形成过程及吸附理论.....	3
1.1.4 细菌生物膜形成影响因素.....	6
1.1.5 细菌生物膜形成过程模型.....	8
1.1.6 细菌生物膜检测方法.....	14
1.1.7 抗菌材料.....	16
1.1.8 抗菌不锈钢的研制情况.....	20
1.2 细菌.....	22
1.2.1 细菌细胞结构和分类.....	22
1.2.2 革兰氏染色的机制.....	23
1.2.3 细菌细胞结构与细菌吸附的关系.....	25
1.3 Nafion 简介	26
1.4 巯基-烯点击化学反应	27
1.5 本课题研究工作.....	30
1.5.1 课题的研究背景和意义.....	30
1.5.2 主要研究内容.....	31
第二章 Nafion 修饰不锈钢表面对大肠杆菌生物膜形成的影响	33
2.1 引言.....	33

2.2 材料与设备.....	34
2.2.1 菌株.....	34
2.2.2 主要试剂与材料.....	34
2.2.3 主要仪器与设备.....	35
2.2.4 培养基与溶液.....	36
2.3 实验步骤.....	36
2.3.1 大肠杆菌菌液制备.....	36
2.3.2 不同浓度 Nafion 的制备	36
2.3.3 不锈钢片的预处理.....	37
2.3.4 Nafion 对大肠杆菌毒性的测试	37
2.3.5 不锈钢片接触角及粗糙度的测量.....	37
2.3.6 不锈钢片的修饰.....	37
2.3.7 生物膜的形成.....	38
2.4 细菌生物膜的鉴定	39
2.4.1 平板计数法.....	39
2.4.2 显微镜观察.....	39
2.4.3 白光干涉仪.....	39
2.4.4 SEM	39
2.5 实验结果.....	40
2.5.1 Nafion 溶液对大肠杆菌的毒性	40
2.5.2 不锈钢表面修饰 Nafion 前后表面形态的变化	40
2.5.3 不锈钢表面 Nafion 修饰前后接触角测量	41
2.5.4 Nafion 修饰前后不锈钢表面粗糙度测量	42
2.5.5 Nafion 修饰不锈钢对大肠杆菌的抑制作用	43
2.5.6 不同浓度 Nafion 对大肠杆菌生物膜形成的影响	47
2.5.7 不同剪切力对不锈钢片生物膜形成的影响.....	48
2.5.8 1% Nafion 修饰后不锈钢片表面抑菌能力重复使用效率	49
2.6 小结.....	50

第三章 Nafion 修饰后不锈钢表面对枯草芽孢杆菌生物膜形成的影响	52
3.1 引言.....	52
3.2 材料与设备.....	52
3.2.1 菌株.....	52
3.2.2 主要试剂与材料.....	53
3.2.3 主要仪器与设备.....	53
3.2.4 培养基培养基与溶液.....	54
3.3 实验步骤与方法.....	55
3.3.1 枯草芽孢杆菌菌液制备.....	55
3.3.2 不同浓度 Nafion 的制备	55
3.3.3 Nafion 对枯草芽孢杆菌毒性的测试	55
3.3.4 不锈钢片的预处理.....	56
3.3.5 不锈钢片接触角测量.....	56
3.3.6 不锈钢片的修饰.....	56
3.3.7 生物膜的形成.....	56
3.4 细菌生物膜的鉴定.....	56
3.4.1 显微镜观察.....	56
3.4.2 SEM	57
3.5 实验结果与讨论.....	57
3.5.1 Nafion 溶液对枯草芽孢杆菌的毒性	57
3.5.2 不锈钢表面修饰前后接触角变化.....	58
3.5.3 修饰前后不锈钢表面粗糙度变化.....	58
3.5.4 Nafion 修饰不锈钢对枯草芽孢杆菌的抑制作用	59
3.5.5 不同浓度 Nafion 修饰对枯草芽孢杆菌生物膜形成的影响	61
3.5.6 1% Nafion 修饰后不锈钢片表面重复使用抑菌效率	62
3.6 小结.....	63
第四章 对乙烯基苯磺酸钠修饰不锈钢表面的构建.....	65
4.1 引言.....	65

4.2 反应机理.....	65
4.3 实验材料和仪器.....	66
4.3.1 主要试剂与材料.....	66
4.3.2 主要仪器与设备.....	67
4.3.3 常用溶液.....	68
4.4 实验步骤.....	68
4.4.1 不锈钢预处理.....	68
4.4.2 不锈钢表面接入对乙烯基苯磺酸钠.....	68
4.5 材料的鉴定与性能表征方法.....	69
4.5.1 材料的鉴定.....	69
4.5.2 表面形貌表征.....	69
4.5.3 表面润湿性测试.....	69
4.6 结果与讨论.....	70
4.6.1 不锈钢修饰前后接触角变化.....	70
4.6.2 不锈钢表面微观形貌.....	71
4.6.3 材料的鉴定与分析.....	73
4.7 小结.....	76
第五章 对苯乙烯基磺酸钠修饰后不锈钢抗菌性能研究.....	77
5.1 引言.....	77
5.2 材料与设备.....	77
5.2.1 菌株.....	77
5.2.2 主要试剂与仪器设备.....	77
5.2.3 主要仪器与设备.....	78
5.2.4 培养基与溶液.....	78
5.3 实验方法.....	79
5.3.1 对乙烯基苯磺酸钠对大肠杆菌的毒性.....	79
5.3.2 平板计数法测试细菌生长情况.....	79
5.4 结果与分析.....	80

5.4.1 不同浓度对苯乙基磺酸钠对大肠杆菌生长的影响.....	80
5.4.2 对苯乙基磺酸钠修饰前后不锈钢表面大肠杆菌生长情况.....	81
5.4.2 修饰后不锈钢重复利用时的抗菌效果.....	83
5.4.3 样品碱洗后对大肠杆菌生物膜形成的影响.....	84
5.5 小结.....	85
第六章 在线监测不锈钢表面生物膜生长情况的新方法.....	87
6.1 引言.....	87
6.2 材料与设备.....	88
6.2.1 菌株.....	88
6.2.2 主要试剂与材料.....	88
6.2.3 主要仪器与设备.....	89
6.2.4 培养基与溶液.....	90
6.3 实验步骤.....	91
6.3.1 不锈钢片预处理.....	91
6.3.2 细菌生物膜形成过程不锈钢表面电压监测装置.....	91
6.3.3 细菌生物膜检测.....	92
6.4 实验结果.....	92
6.4.1 电化学监测湖水中不锈钢表面电压变化.....	92
6.4.2 湖水中细菌在不锈钢表面的生长情况.....	93
6.5 小结.....	95
第七章 结论与展望.....	96
7.1 主要结论.....	96
7.2 研究展望.....	97
参考文献.....	98
博士期间发表的文章.....	123
致谢.....	124

Contents

Abstract(Chinese)	i
Abstract(English)	iii
Contents	i
Chapter 1 Review	1
1.1 Introduction of biofilm.....	1
1.1.1 Definition and structure of biofilm	1
1.1.2 Detriments of biofilm.....	2
1.1.3 Theory of bacterial adhesion.....	2
1.1.4 Influence factors for biofilm formation	6
1.1.5 Biofilm model systems	8
1.1.6 Monitoring methods for biofilm	13
1.1.7 Antibacterial materials	16
1.1.8 Current researches on anti-bacterial stainless steel.....	20
1.2 Bacteria	22
1.2.1 Structure and classification of bacteria	22
1.2.2 Mechanism of gram staining.....	23
1.2.3 Relationship between bacterial structure and adhesion	25
1.3 Introduction of Nafion	26
1.4 Introduction of Click-Reaction	27
1.5 Work of this study	30
1.5.1 Background and significance.....	30
1.5.2 The main contents	30
Chapter 2 The influences of Nafion coated stainless steel on <i>E.coli</i> biofilm	32
2.1 Introduction.....	32
2.2 Materials and equipments	33

Contents

2.2.1 Strains	33
2.2.2 Reagents and materials	33
2.2.3 Equipments and primary instruments	33
2.2.4 Medium and solution	34
2.3 Methods.....	35
2.3.1 Preparation of <i>E.coli</i> suspension.....	35
2.3.2 Preparation of Nafion solution for different concentrations	35
2.3.3 Pre-treatment of stainless steel discs.....	35
2.3.4 The toxicity of Nafion to <i>E.coli</i>	36
2.3.5 Surface morphology of stainless steel.....	36
2.3.6 Modified of stainless steel by Nafion	36
2.3.7 Biofilm formation	36
2.4 Identification of biofilm.....	37
2.4.1 Plate counting method.....	37
2.4.2 Observed by microscope.....	38
2.4.3 Phase shift MicroXAM-3D.....	38
2.4.4 SEM	38
2.5 Results.....	38
2.5.1 The toxicity of Nafion to <i>E.coli</i>	38
2.5.2 Surface morphology of stainless steel discs before and after modification	39
2.5.3 Wettability of stainless steel discs before and after modification.....	40
2.5.4 Roughness of stainless steel discs before and after modification.....	41
2.5.5 CFU of stainless steel discs before and after modification.....	41
2.5.6 Biofilm observed by microscope	43
2.5.7 Biofilm observed by Phase shift MicroXAM-3D.....	44
2.5.8 Biofilm observed by SEM	45
2.5.9 The influences of different Nafion concentrations on <i>E.coli</i>	46

2.5.10 The influences of different flow velocity on <i>E.coli</i>	47
2.5.11 The anti-biofilm ability of reused Nafion coated stainless steel	48
2.6 Summary	49
Chapter 3 The influences of Nafion coated stainless steel on <i>B.subtilis</i> biofilm.....	51
3.1 Introduction.....	51
3.2 Materials and equipments	51
3.2.1 Strains	51
3.2.2 Reagents and materials	51
3.2.3 Equipments and primary instruments	52
3.2.4 Medium and solution	53
3.3 Methods.....	53
3.3.1 Preparation of <i>B.subtilis</i> suspension	53
3.3.2 Preparation of Nafion solution for different concentrations	54
3.3.3 The toxicity of Nafion to <i>B.subtilis</i>	54
3.3.4 Pre-treatment of stainless steel discs.....	54
3.3.5 Surface morphology of stainless steel.....	54
3.3.6 Modified of stainless steel by Nafion	55
3.3.7 Biofilm formation	55
3.4 Identification of biofilm.....	55
3.4.1 Observed by microscope.....	55
3.4.2 SEM	55
3.5 Results.....	55
3.5.1 The toxicity of Nafion to <i>B.subtilis</i>	55
3.5.2 Wettability of stainless steel discs before and after modification	56
3.5.3 Roughness of stainless steel discs before and after modification	57
3.5.4 Biofilm observed by microscope	58
3.5.5 Biofilm observed by SEM	58
3.5.6 The influences of different Nafion concentrations on <i>B.subtilis</i>	59

3.5.7 The anti-biofilm ability of reused Nafion coated stainless steel.....	60
3.6 Summary	61
Chapter 4 Preparation and characterization of sodium p-styrenesulfonate modified stainless steel.....	62
4.1 Introduction.....	62
4.2 Reaction mechanism	62
4.3 Materials and equipments	63
4.3.1 Strains	63
4.3.2 Reagents and materials	63
4.3.3 Equipments and primary instruments	64
4.3.4 Medium.....	65
4.3.5 Solution.....	65
4.4 Methods.....	65
4.4.1 Preparation of <i>E.coli</i> suspension.....	65
4.4.2 Pre-treatment of stainless steel discs.....	65
4.4.2 Stainless steel modified by sodium p-styrenesulfonate	65
4.5 Identification and characterization of materials.....	66
4.5.1 Identification of materials	66
4.5.2 Characterization of surface morphology.....	66
4.5.3 Measurement of surface wettability.....	67
4.6 Results.....	67
4.6.1 Wettability of stainless steel discs before and after modification.....	67
4.6.2 Surface microtopography of sodium p-styrenesulfonate modified stainless steel.....	68
4.6.3 Identification and analysis of materials	72
4.7 Summary.....	75
Chapter 5 Sodium p-styrenesulfonate modified stainless steel for anti-biofilm application.....	76

Degree papers are in the "[Xiamen University Electronic Theses and Dissertations Database](#)". Full texts are available in the following ways:

1. If your library is a CALIS member libraries, please log on <http://etd.calis.edu.cn/> and submit requests online, or consult the interlibrary loan department in your library.
2. For users of non-CALIS member libraries, please mail to etd@xmu.edu.cn for delivery details.

厦门大学博硕士论文摘要库