学校编码：10384　　分类号＿＿＿密级＿＿

学　　号：20620100153628　　UDC＿＿＿＿＿＿＿＿＿＿

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：　2　0　1　4　年　月

论文答辩日期：　2　0　1　4　年　月

学位授予日期：　2　0　1　4　年　月

答辩委员会主席：＿＿＿＿＿＿

评　　阅　　人：＿＿＿＿＿＿

2014 年 9 月

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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这个问题，要从减少细菌吸附着手。

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

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