学校编码：10384　　分类号_____密级_____

学　　号：22620080150142　　UDC_____

厦门大学

博 士 学 位 论 文

基于微生物细胞吸附、还原与支载的金纳米材料的制备与应用的研究

Microorganism-mediated Synthesis of Gold Nanomaterials and Their Applications

国家自然科学基金项目（21036004, 20976146）

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专 业 名 称 ：环　境　工　程

论文提交日期：2013 年 9 月

论文答辩日期：2013 年 9 月

学位授予日期：　　年　　月

答辩委员会主席：_____

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2013 年 9 月

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摘 要

金纳米材料由于其所具有的良好的生物相容性和特殊的理化性质，对其制备方法的研究和应用领域的探索一直是纳米科技的研究热点。随着纳米材料制备技术的发展，利用微生物菌体来制备或构建纳米材料的研究也逐渐引起了人们的关注。相比于传统的物理法和化学法，利用微生物还原法制备的纳米材料，具有成本低、反应条件温和、纳米颗粒稳定性好等优点；以微生物为生物模板，可以借助微生物菌体表面规整的表皮层或表皮层丰富的官能团，用于金纳米材料形貌和尺寸的调控和制备。但是，目前有关微生物法制备金纳米材料的研究中，大多采用的都是活菌，还原过程与细胞的新陈代谢过程息息相关，所制备的纳米颗粒有很大一部分是位于胞内，往往还需要通过细胞破壁、焙烧等方法将纳米颗粒从胞内分离出来，给后续的应用带来了一定的困难。而利用没有活性的微生物细胞（死菌）来制备纳米材料，所制得的纳米颗粒主要位于胞外或细胞表面，而且还原过程不依赖细胞的代谢，因此不受生长条件的限制，在反应条件的操控方面有更大的空间。目前利用死菌体来制备金纳米材料的研究还很少，尚未有利用死菌来构建金纳米材料并实现其在环境领域应用的相关报道。

本论文选取了两种微生物死菌体，通过不同的方法分别构建相应的金纳米材料。一方面，利用毕赤酵母直接还原制备了负载在菌体表面的金纳米颗粒，研究了菌体表面金纳米颗粒的形成机理，提出了一种负载型金催化剂的新制备方法，考察了 Au/菌体在 4-硝基酚（4-NP）的还原反应中的催化活性；另一方面，利用大肠杆菌为模板，抗坏血酸（AA）为还原剂，十六烷基三甲基溴化铵（CTAB）为保护剂，在三者的协同作用下制备包覆在菌体表面的金纳米线、纳米带等一维金纳米材料，提出了一种可以快速回收金的新方法。具体的研究内容如下：

首先，利用毕赤酵母死菌体细胞直接还原$[AuCl_4]^-$，获得了结合在菌体表面的金纳米颗粒。从生物吸附和生物还原的角度出发，研究了金纳米颗粒在菌体表面的形成过程机理，并考察了不同的反应条件对金纳米颗粒的形貌、粒度和分散度等的影响。结果表明：毕赤酵母对$[AuCl_4]^-$的生物吸附过程存在静电吸附、离子交换和络合作用等多种机理，而生物还原经历了 Au(III)→Au(I)→Au(0)等阶段。酵母细胞壁的甘露聚糖层为金纳米颗粒的主要形成场所，它对于金纳米颗粒

的形成、在菌体上的分散和稳定均起到了重要的作用。低的反应温度（<50 ^{o}C）、弱酸性 pH 和低浓度 $[AuCl_4]^-$（质量浓度比 $C_{[AuCl4]-}$:$C_{菌体}$<0.05）的条件下比较有利于球形纳米颗粒的形成。

其次，将负载了金纳米颗粒的菌体置于不同的溶液环境条件中，考察了外部条件对于菌体与金纳米颗粒结合强度的影响；将其作为一种新型的催化剂（Au 为活性成分，微生物细胞为载体），考察了它在 4-NP 的还原反应中的催化活性，研究了菌体种类、Au 的负载量、制备温度等条件对其催化活性的影响，并与其它载体负载的金催化剂的催化活性进行了比较。结果表明，在非强酸性溶液中，Au 与菌体之间的结合很牢固，即使置于超声场或是微波场中，也很难把纳米颗粒从菌体上剥离下来；Au/菌体对于 4-NP 的还原反应具有很好的催化活性，利用 2 g/L 的毕赤酵母菌粉与 pH 为 3、浓度为 1 mM 的 $HAuCl_4$ 溶液在 30-40 ^{o}C 反应所制得的催化剂具有最好的活性；金纳米颗粒的粒度、纳米颗粒与菌体之间的结合强度以及所使用的载体种类对于催化活性均有重要的影响。

最后，利用大肠杆菌、CTAB 和 AA 的三元协同作用制备了大量的一维金纳米材料，研究了 CTAB、AA 和菌体之间的协同作用机制，考察了大肠杆菌、CTAB、AA 和 $HAuCl_4$ 的浓度对金纳米材料的形貌和形成沉淀数量的影响。结果表明，在菌体表面形成了大量的纳米线、纳米带和纳米棒等一维金纳米材料，它们紧密地缠绕在菌体表面，使菌体细胞聚集在一起形成一些大的团聚体并且快速沉降到反应容器的底部。在制备纳米材料的同时，利用该方法还可以实现对溶液中 Au 的快速回收，回收率约为 97.56%。大肠杆菌和 $HAuCl_4$ 的浓度对于金纳米材料形貌的影响较为显著。大肠杆菌、CTAB 和 AA 的三元协同作用在 Au/大肠杆菌复合物（一维金纳米材料）的形成和快速沉降过程中起到了关键的作用。

关键词：微生物；金纳米材料；应用；催化剂

Abstract

The research on the preparation and application of gold nanomaterials has been a hot-spot in nanotechnology field due to their good biocompatibility and special physical/chemical properties. With the development of nano-materials preparation technology, utilizing the microbial cells to prepare or assemble nanomaterials has gained increasing attention. Compared with the conventional physical and chemical methods, the microorganisms-mediated synthesis method exhibits several obvious advantages such as low cost, mild reaction conditions and good stability, etc. Utilizing microbial cells as template can make full use of rich functional groups on the cell surface, which is more favorable for the morphology and size-control of the gold nanomaterial. However, the current studies on the microorganism-mediated synthesis was focus on the living microorganism. The reduction process is closely related with metabolism. The main drawback of utilizing live microorganism is that part of the as-synthesized nanoparticles are intracellular and separating nanoparticles from cell is necessary, posing difficulties in subsequent processing and applications. In contrast to live microorganism-mediated reduction, as metabolic processes are not viable in dead cells, most metal ions would be uptaken by the cell wall and prohibited from penetrating the intracellular membrane, thus leading to the formation of nanoparticles on the cell surface. Few studies using dead microorganisms to synthesize nanoparticles have been reported. There is no reports about compositing the gold nanomaterials by dead microorganism and developing their application in environmental field.

In this thesis, two different microbial cells were used to fabricate gold nanomaterials. Gold nanoparticles (AuNPs) were synthesized through the directly reduction of $HAuCl_4$ by *Pichia pastoris* (*P. pastoris*). The formation mechanism of AuNPs and the catalytic activity of Au/microorganism towards the reduction of 4-nitrophenol (4-NP) were also investigated. Then *Escherichia coli* (*E. coli*) was

employed as bio-template and ascorbic acid (AA) as a reducing agent, cetyl trimethyl ammonium bromide (CTAB) as a protective agent, repectively. One dimensional gold nanomaterials can be prepared through the cell-CTAB-AA coordination. This work also propose a new method for the rapid recovery of gold in the solution.

Firstly, AuNPs bound to the cell surface can be obtained through the directly reduction of $HAuCl_4$ by *P. pastoris*. The formation mechanism of AuNPs on the cell surface was studied from the biosorption and bioreduction perspective. The influence of reaction conditions on the morphology, size and dispersion of AuNPs were also investigated. The results revealed that several mechanisms (such as electrostatic adsorption, ion exchange and complexation. etc) were involved in the biosorption process and the bioreduction process underwent the stages like Au(III)→Au(I)→Au(0). Mannosan layer in the cell wall was supposed to be the major sites to synthesize AuNPs and played an important role in the dispersion and stability of AuNPs. Low reaction temperatures (<50 ^{o}C), weak acidic pH and low concentrations of $[AuCl_4]^-$ were more favorable for the synthesis of spherical nanoparticles.

Secondly, the binding intensity between AuNPs and microbial cell was studied by placing the Au/microorganism composite in different solution. The catalytic activity of Au/microorganism towards the reduction of 4-NP was evaluated and the effect of the parameters including microbial species, Au loading and catalyst preparation temperature on the catalytic performance were also investigated. The results indicated that the interaction between AuNPs and microbial cell is very strong in the non- heavily acidic solutions. It was difficult to strip the nanoparticles from the cells even if the microwave or untrasonic field were employed. The Au/microorganism exhibited good catalytic activity toward the reduction of 4-NP. The optimal catalyst preparation condition is 2 g/L yeast powder reacted with 1 mM of $HAuCl_4$ at pH=3 and temperature of 30-40 ^{o}C. Size of AuNPs, the binding strength between AuNPs and cell and the type of support were demonstrated to have a great impact on the catalytic activity.

Finally, the one dimensional gold nanomaterials were prepared by employing *E.*

coli as bio-template. The ternary coordination mechanism of cell-CTAB-AA were studied. The effect of *E. coli*, CTAB, AA and $HAuCl_4$ concentration on the morphology of as-synthesized gold nanomaterials and quantity of precipitation were also investigated. The results showed that a large number of one dimensional Au nanostructures such as gold nanowires or nanobelts grew around the *E. coli* cells, inducing the rapid aggregation of Au and the *E. coli* cells. Besides the preparation of Au/*E. coli* composite, this method also provided a new way to recover Au from the solution and the recovery rate was about 97.56%. The ternary coordination of cell-CTAB-AA was demonstrated to play a vital role in the formation of gold nanomaterials and rapid precipitation of Au/*E. coli* composite.

Key Words: Microorganism; Gold nanomaterials; Application; Catalysts

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