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厦门大学

博 士 学 位 论 文

黑曲霉菌负载金、银纳米颗粒的制备及其应用研究

Preparation and application of gold and silver nanoparticles immobilized on *Aspergillus niger*

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摘 要

近年来受日益增长的“绿色化学”概念和环境保护意识的驱动，利用微生物还原法制备金属纳米颗粒已成为一种新颖且环境友好的纳米材料制备方法。目前有关微生物还原法的研究大多采用的是活菌体，还原过程所得的纳米颗粒主要形成于胞内，影响到纳米颗粒后续利用，而利用微生物干/死菌体还原所得的金属纳米颗粒则在胞外或菌体表面上形成，有利于获得菌体负载的金属纳米颗粒。该负载型金属纳米颗粒具有易于分离回收的特性，可望作为一种新型材料在多相催化等领域得以应用。本论文以工业上广泛应用的黑曲霉菌为生物吸附、还原材料，系统研究了其对贵金属离子(金、银)的生物吸附、生物还原特性，进而制备出黑曲霉菌体负载的金、银纳米材料，分别考察菌体负载金纳米材料对染料还原的催化性能以及菌体负载银纳米材料的抗菌性能，并借助于 TEM、EDS、XRD、XPS、FTIR 和 AAS 等技术对所得纳米材料进行表征，对其结构与应用性能进行关联。主要研究内容和结果归纳如下：

首先，以黑曲霉干菌体作为生物吸附剂，对其吸附 Au(III)离子的影响条件、吸附热力学、吸附动力学进行了系统的研究，并对吸附机理进行了较为深入的分析。结果表明，黑曲霉菌对 Au(III)离子的吸附性能对溶液 pH 值具有一定的依赖性，其最佳 pH 值为 2.0～3.0；20 ℃下吸附过程分为 2 个阶段进行，分别对应于 Au(III)离子还原前和还原后的吸附，而 30 ℃、40 ℃和 60 ℃下吸附过程均无明显分段现象；当 Au(III)离子初始浓度小于 233.32 $mg\cdot L^{-1}$ 时，吸附量几乎不受温度的影响，而当初始浓度大于 367.94 $mg\cdot L^{-1}$ 时，升温明显促进了吸附量的提高。黑曲霉菌对 Au(III)离子的吸附等温线可用 Langmuir 方程很好地模拟，20 ℃、30 ℃、40 ℃和 50 ℃时 Au(III)离子饱和吸附量分别为 185.19 $mg\cdot g^{-1}$、202.02 $mg\cdot g^{-1}$、235.85 $mg\cdot g^{-1}$ 和 277.78 $mg\cdot g^{-1}$。热力学函数 Gibbs 自由能变、吸附焓变和吸附熵变的计算结果表明，黑曲霉菌对 Au(III)离子的吸附过程是一自发的吸热和熵增过程。其吸附动力学可用准二级速率方程描述，吸附活化能为 55.71 $kJ\cdot mol^{-1}$。黑曲霉菌吸附 Au(III)离子的主要作用机制是静电引力、络合作用和氧化还原作用，菌体表面的酯基、羧基、羟基和氨基等均是键合 Au(III)离子的主要官能团，其

中以羟基的贡献最大，而氨基的贡献相对较小。

其次，利用黑曲霉干菌体对 Au(III)离子的吸附还原特性制备了 AuNPs/黑曲霉菌催化剂，考察了 $HAuCl_4$ 溶液初始 pH 值、反应温度、$HAuCl_4$ 浓度、反应时间和菌体表面基团预处理等催化剂制备条件，以及染料种类、$NaBH_4$ 用量、催化剂浓度等催化反应条件对染料催化还原性能的影响，并对所得 AuNPs/黑曲霉菌催化剂进行了表征。结果表明，AuNPs/黑曲霉菌对 6 种染料均具有高的催化还原性能，其表面同时存在 Au^0、Au^+和 Au^{3+}金物种，其中氧化态 $Au^{\delta+}$在催化剂中的含量和组成(Au^+/Au^{3+})对催化剂活性影响显著。对于 MB 催化还原反应，催化剂较优制备条件为：2.0 $g\cdot L^{-1}$ 的黑曲霉菌粉与 pH 值为 5、浓度为 23.64～94.56 $mg\cdot L^{-1}$ 的 $HAuCl_4$ 溶液在 30 ℃下反应 48～72 h。在上述条件下制得的催化剂，当 MB 与 $NaBH_4$ 物质的量的比为 1:100、催化剂浓度为 55.6 $mg\cdot L^{-1}$ 时，50～70 s 可将 2×10^{-4} $mol\cdot L^{-1}$MB 完全还原。随着 $NaBH_4$ 用量的增加，MB 还原反应速率迅速增大；随着催化剂浓度的增大，MB 还原反应速率先逐渐增大后几乎不变。AuNPs/黑曲霉菌催化剂对 MB 的催化还原性能在重复使用 2 次后明显降低，对于 GTL 则重复使用 9 次后活性未明显降低。

最后，利用黑曲霉干菌体吸附还原银氨离子制备了 AgNPs/黑曲霉菌复合材料，以 *E. coli* 为测试菌株评价该复合材料的抗菌性能，通过银释出实验考察该复合材料的稳定性，研究了反应温度与 pH 值对还原过程和所得复合材料的抗菌性能及稳定性的影响，并对所得 AgNPs/黑曲霉菌进行了表征。结果表明利用黑曲霉干菌体还原制得的 AgNPs/黑曲霉菌复合材料，AgNPs 以粒度为 6.9～8.2 nm 的近球形颗粒形式均匀分布在黑曲霉菌体表面，银负载量为 3.7%～5.0%。反应温度从 30 ℃提高至 60 ℃有利于提高菌体银负载量，所得 AgNPs 粒径增大；反应 pH 值从 9.5 提高至 11.5 有利于提高反应速率，而菌体银负载量和 AgNPs 的粒径则差异不大。该复合材料对 *E. coli* 具有较好的抗菌性能，其 MIC 为 217～434 $mg\cdot L^{-1}$(以菌粉总质量表示)；500 $mg\cdot L^{-1}$ 的复合材料(银负载量为 3.8%)在 10 min 内能完全杀死 10^6 $cfu\cdot mL^{-1}$ *E. coli*，且重复使用 5 次后，其抗菌性能未有明显下降；该复合材料的抗菌性能主要与 AgNPs 粒度大小有关，AgNPs 的粒径越小其抗菌性能越强。银释出实验结果表明，AgNPs/黑曲霉菌复合材料中 AgNPs 与菌体结合牢固，单位质量复合材料释放出 Ag^+的量为 1.7～6.8 $mg\cdot g^{-1}$(占总质量分数

的 3.4%～18.4%)，提高反应温度和反应 pH 值后 Ag^{+}的释出均减少。

关键词：黑曲霉菌；金属纳米颗粒；制备；抗菌性能；催化性能

Abstract

Currently, increasing “green chemistry” concept and environmental awareness necessitate the development of environmental benign nanoparticles synthesis. As a result, the bioreduction method based on microorganisms has emerged as novel and environmental benign approach to synthesize metal nanomaterials in recent years. However, current studies on the miroorganism-mediated synthesis were focus on the living microorganism and most of the as-synthesized nanoparticles were intracellular, resulting in the difficulties in subsequent processing and applications. In contrast, the nanoparticles synthesized by inactive miroorganism were extracellular or located on the cell surface. These metal nanoparticles immobilized on cell surface can be easily recovered and applied as a novel material for heterogeneous catalytic reactions etc. This study aims at the characteristic of biosorption and bioreduction of Au(III) and Ag(I) by *Aspergillus niger*(*A. niger*), which has been widely used in industry. Gold nanoparticles (AuNPs) and silver nanoparticles (AgNPs) supported on *A. niger* were fabricated via reduction of Au(III) ($[AuCl_4]^-$) and Ag(I) ($[Ag(NH_3)_2]^+$) by dry biomass of *A. niger*. Catalytic activity of AuNPs/*A. niger* and antimicrobial activity of AgNPs/*A. niger* composite were evaluated respectively. A variety of techniques, such as TEM, EDS, XRD, XPS, FTIR and AAS were employed to characterize the composites and study the relationship between the performance and structure of the composites. The main contents and some successful results have been listed as following.

Firstly, the biosorption potential of *A. niger* for Au(III) was investigated. The biosorption mechanism, as well as the parameters influencing the biosorption of Au(III) and thermodynamics and kinetics was systematically studied. The results indicated that Au(III) biosorption was pH-dependent and the favorable pH value was about 2.0–3.0. Au(III) biosorption involved two stages corresponding to periods before and after Au(III) reduction at 20 ºC. The biosorption capacity was hardly affected by temperature at lower initial Au(III) concentrations(<233.32 $mg \cdot L^{-1}$), while

a significant increase was observed with the increase of temperature at higher Au(III) concentrations(>367.94 $mg \cdot L^{-1}$). Au(III) biosorption was found to fit the Langmuir model well and the maximum Au(III) uptake capacity was 185.19 $mg \cdot g^{-1}$, 202.02 $mg \cdot g^{-1}$, 235.85 $mg \cdot g^{-1}$ and 277.78 $mg \cdot g^{-1}$ at 20 ºC, 30 ºC, 40 ºC and 50 ºC, respectively. Thermodynamic parameters such as Gibbs free energy, enthalpy and entropy changes were calculated, revealing the biosorption process was spontaneous and endothermic and entropy increased. The biosorption process was found to conform to the pseudo-second-order rate kinetics and the obtained activation energy was 55.71 $kJ \cdot mol^{-1}$. The biosorption of Au(III) by *A. niger* mainly depended on electrostatic forces, complexation and redox. FTIR characterization illustrated that the functional groups such as ester, amino, carboxyl and hydroxyl might be responsible for the interaction between Au(III) and *A. niger* biomass and the hydroxyl groups were found to play the key role.

Secondly, AuNPs/*A. niger* catalyst was synthesized by dry biomass of *A. niger*. The catalytic activity of AuNPs/*A. niger* towards the reduction of various dyes in the presence of $NaBH_4$ was evaluated. The effect of catalyst preparation parameters including pH value of $HAuCl_4$, catalyst preparation temperature, concentration of $HAuCl_4$, catalyst preparation time and pretreatment of the function groups of biomass, and the effect of catalytic reaction conditions including dye species, dosage of $NaBH_4$ and concentration of catalyst on the catalytic performance were investigated, respectively. The results indicated that AuNPs/*A. niger* was an efficient catalyst for catalytic reduction of six organic dyes. The characterization results of catalysts indicated that gold species on the catalysts existed in the form of Au^0, Au^+ and Au^{3+}. The catalytic performance of AuNPs/*A. niger* was closely related to the content and composition (Au^+/Au^{3+}) of $Au^{\delta+}$. For MB reduction, the optimal catalyst preparation condition was 2.0 $g \cdot L^{-1}$ *A. niger* powder reacted with 23.64–94.56 $mg \cdot L^{-1}$ $HAuCl_4$ at pH=3 and 30 ^oC for 48–72 h. When the concentration of $NaBH_4$ was 100 times to that of MB and the concentration of catalyst was 55.6 $mg \cdot L^{-1}$, MB with an initial concentration of 2×10^{-4} $mol \cdot L^{-1}$ could be completely reduced within 50–70 s. The reaction rate of MB reduction process can be enhanced with the increase dosage of

$NaBH_4$. With the increase of catalyst concentration, the reaction rate increase gradually and then remain almost the same. Furthermore, for the reduction of CR, the AuNPs/*A. niger* showed excellent reusability for 9 successive cycles with no obvious decrease in catalytic effects. However, for the reduction of MB, the catalytic rate of the catalyst decreased significantly after 2 times' reuse.

Finally, AgNPs/*A. niger* composites were fabricated via the reduction of $[Ag(NH_3)_2]^+$ by dry biomass of *A. niger*. The antimicrobial property of AgNPs/*A. niger* composites was evaluated and the representative *E. coli* was used as the tested strain. The immobilization efficiencies of silver ions and AgNPs in the composites were confirmed by silver leaching experiments. The effects of temperature and pH value on the bioreduction process and the antimicrobial activity and the stability of the composites were studied. AgNPs/*A. niger* composites were also systematically characterized. The results showed that AgNPs with 6.9–8.2 nm were well dispersed on the cell surface and the Ag loadings were 3.7%–5.0%. Although increasing the reaction temperature from 30 ^oC to 60 ^oC resulted in the increase of the silver loading, the size of the as-synthesized AgNPs became larger at the same time. The increase of pH value from 9.5 to 11.5 accelerated the reaction and shortened the reaction time (from 144 h to 24 h), whereas no significant effects were observed on the Ag loading and the size of AgNPs. The AgNPs/*A. niger* composites exhibited excellent antimicrobial activity against *E. coli* and the minimal inhibitory concentration (MIC) was 217–434 $mg \cdot L^{-1}$ (based on the weight of AgNPs/ *A. niger*). The AgNPs/*A. niger* composite showed fast and long-lasting bactericidal activity toward *E. coli*. The composite with the concentration of 500 $mg \cdot L^{-1}$ (3.8% Ag loading) can fully kill 10^6 $cfu \cdot mL^{-1}$ *E. coli* after 10 min of incubation and bactericidal activity can be kept even after five rounds' recycle. The antimicrobial activity of the composite was found to be mainly depended on the size of AgNPs and smaller size exhibited better antimicrobial activity. The release of AgNPs and Ag^+ from the AgNPs/*A. niger* composites was also investigated. The results suggested that the as-prepared AgNPs were supported firmly on *A. niger* and the release of Ag^+ was 1.7–6.8 mg per gram of AgNPs/*A. niger*. Furthermore, increasing the reaction temperature or the pH value led to the decrease

of Ag^{+} release.

Key Words: *Aspergillus niger*; Metal nanoparticles; Synthesis; Antibacterial activity; Catalytic activity