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研究报告

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荧光定量PCR检测生物强化SBR系统中苯胺降解菌的数量变化

Monitoring of the number changes of aniline-degrading bacterium in bioaugmented system by quantitative Real-time PCR

关键词：荧光定量PCR *Pseudomonas otitidis* strain JY9 C12O 苯胺降解率 活性污泥

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摘要：生物强化SBR系统中苯胺降解菌*Pseudomonas otitidis* strain JY9在降解苯胺废水过程中发挥重要作用.本研究根据该菌株的邻苯二酚1，2双加氧酶（C12O）的基因序列，设计特异引物，扩增特异片段并克隆到PGM-T载体中，以此重组质粒为参照，系统活性污泥中提取的总DNA为模板，应用实时荧光定量PCR方法定量检测该菌在苯胺废水生物强化SBR系统中的丰度变化.同时应用高效液相色谱法检测系统中苯胺的残留.结果表明，苯胺初始浓度在200~500 mg·L^{-1}范围内，苯胺降解率均达到96%以上.并且SBR反应系统活性污泥中每单位（mg）MLVSS的C12O基因拷贝数随苯胺浓度的升高而显著上升，最高拷贝数达到2.7×10^{9} 个，表明该菌的数量随苯胺浓度的升高而显著上升.结果显示，在苯胺去除过程中*Pseudomonas otitidis* strain JY9的投加能够稳定活性污泥中的MLVSS，当含有高浓度苯胺废水系统运行稳定时，该降解菌逐渐成为活性污泥当中的优势菌.

Abstract：Aniline-degrading bacterium, *Pseudomonas otitidis* JY9, plays an important role in bioaugmentating aniline degradation in sequential batch reactor (SBR). According to the sequence of Catechol 1,2-dioxygenase(C12O) gene from *Pseudomonas otitidis* strain JY9, a pair of specific primers for amplifying a fragment of this gene was designed and the amplification products were cloned to PGM-T vector. Using the recombinant plasmid as a reference and the total DNA abstracted from SBR activated sludge as a template, quantitative real-time PCR was performed to detect the changes of the abundance of this strain and aniline residue was detected by High Performance Liquid Chromatography (HPLC). The results showed that the aniline degradation rate reached over 96% at aniline concentration of 200-500 mg·L^{-1}. Moreover, the copies of C12O increased obviously with the increasing of the aniline concentration, and the highest copy number of gene C12O reached 2.7×10^{9}/mg MLVSS,indicating that *Pseudomonas otitidis* strain JY9 and the abundance of C12O increased rapidly. During the degradation of aniline, MLVSS concentration in activated sludge was stabilized by adding the strain JY9. When the SBR reactor steadily operated at high concentration aniline wastewater, the strain JY9 gradually become the dominant population in the activated sludge.

Key words：quantitative Real-time PCR *Pseudomonas otitidis* strain JY9 C12O aniline degradation rate activated sludge

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