Enhanced Antibiotic Degradation and Resistance Risk Mitigation in Microalgal-Bacterial Granular Sludge by Zero-Valent Iron-Activated Carbon: Metagenomic and Molecular Docking Insights

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ABSTRACT

In recent years, the widespread use of antibiotics has led to their frequent detection in wastewater, while also inducing the emergence of antibiotic resistance genes (ARGs). Both are recognized as emerging contaminants and have become critical issues affecting environmental and public health. Algal-bacterial granular sludge (MBGS) technology has demonstrated high potential for efficient antibiotic removal. In this study, sulfamethoxazole (SMX), a typical sulfonamide antibiotic, was chosen as the target contaminant. Two identical sequencing batch reactors (1.4 L) were established: an experimental reactor amended with 2 g/L zero-valent iron-activated carbon (ZVI-AC) (R2) and a control reactor (R1). Both were operated for 80 cycles with synthetic municipal wastewater containing 10 μg/L SMX. The degradation mechanism of SMX was analyzed by metagenomic sequencing and molecular docking, aiming to explore the effect of ZVI-AC-enhanced MBGS system on the degradation efficiency of antibiotics and the risk of ARGs transmission. The results showed that compared with the control group, the addition of ZVI-AC promoted the chlorophyll transformation and accumulation of chlorophyll *b* (3.89 mg/g vs. 2.26 mg/g) in MBGS, enhanced biomass (4.06 g/L vs. 3.67 g/L) and conductivity (875.52 μs/cm vs. 830.17 μs/cm), thereby improving SMX degradation. The degradation rate constant increased from 0.0334 h^{-1} to 0.0565 h^{-1} (an increase of 69.2%). The effluent quality met the Grade A standard of municipal wastewater. Metagenomic analysis revealed that the addition of ZVI-AC promoted the enrichment of cytochrome P450 family genes involved in drug metabolism and reduced the generation of the harmful compound TP163 (among 9 metabolites). Molecular docking further indicated that the CYP102 enzyme (a P450 family member) exhibited enhanced binding affinity with SMX (binding energy: −8.6 kcal/mol), facilitating more efficient degradation. Specifically, the enrichment of CYP450 genes and enhanced electron transport activity drove hydroxylation and S–N bond cleavage, promoting the generation of smaller molecular metabolites. Furthermore, with the addition of ZVI-AC, the biological toxicity (inhibition rate of *Escherichia coli*) of system effluent was significantly reduced by 83.96 ± 10.42%. The abundance of *g_Leptolyngbya* (a potential host of ARGs in MBGS) markedly decreased. The abundances of ARGs (*sul1*, *sul3*) and class I integron (*intI1*) were reduced by 47.3%, 31.4%, and 63.3%, respectively, and 12 fewer ARG subtypes were detected, which greatly reduced the risk of horizontal transfer of ARGs. These findings indicate that the addition of ZVI-AC can facilitate the establishment of an MBGS system capable of pollutant removal, toxicity reduction, and resistance inhibition, thereby providing an engineerable technological paradigm for the efficient removal and risk control of antibiotic emerging pollutants in wastewater treatment.

Keywords: Algal-bacterial symbiosis; Emerging contaminants; Sulfamethoxazole; Antibiotic resistance genes