Co-selection of Altered Nitrogen Metabolism and Multidrug Resistance in Wastewater Microbiomes under Long-Term Fluoride Stress

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ABSTRACT: Industrial fluoride (F^-) wastewater threatens the stability of biological treatment systems. However, the adaptive evolution of nitrogen-transforming microorganisms and the spread of antibiotic resistance under long-term F^- stress remain unclear. This study systematically investigated the nitrogen removal performance, microbial community structure, and resistome evolution in a sequencing batch bioreactor under prolonged (120-day) stress from 20 mg/L F^-. The results indicated that long-term F- exposure severely inhibited nitrification, decreasing the ammonia removal efficiency from 99.6% to 74.7% and the specific ammonia oxidation rate (SAOR) by 23.8%. In contrast, a significant enrichment of denitrifying bacteria (e.g., Thauera abundance increased by 274%) enhanced denitrification, boosted the specific nitrate reduction rate (SNRR) by 48.7%, and maintained a stable total inorganic nitrogen (TIN) removal efficiency (64.5%). To counteract F^- toxicity, the microbial community exhibited multi-level adaptations, including enhanced secretion of extracellular polymeric substances (EPS), upregulation of antioxidant and energy metabolism genes, and increased F- efflux capacity. Crucially, F^- stress co-selected for broad-spectrum antibiotic resistance genes (ARGs) and heavy metal resistance genes (MRGs), with total abundances increasing by 19.7% and 31.2%, respectively. Network analysis confirmed that denitrifying bacteria, which gained a competitive advantage under F^- stress, were the primary hosts of these resistance genes. This study reveals the evolutionary mechanisms of nitrogen-converting microbes in the treatment of fluoride-containing wastewater and warns of the potential risks of resistance gene proliferation induced by F^- pollution.

Keywords: Fluoride; Nitrogen transformation; Antibiotic resistance genes (ARGs); Co-selection

Contents : This study investigated the effects of long-term stress from low-concentration F^- (20 mg/L) on the nitrogen transformation function and multidrug resistance of activated sludge. We observed that F^- stress significantly inhibited nitrification (SAOR decreased by 23.8%) but maintained stable total nitrogen removal by promoting the denitrification process (SNRR increased by 48.7%). The root cause was the remodeling of the microbial community: less tolerant nitrifying bacteria were inhibited, while highly adaptive denitrifying bacteria (e.g., Thauera and Hydrogenophaga), which increased EPS secretion and enhanced F^- efflux, were significantly enriched. However, it is alarming that F^- stress significantly increased the abundance of antibiotic resistance genes (ARGs) and heavy metal resistance genes (MRGs) in the activated sludge, and the primary hosts of these resistance genes were the very denitrifying bacteria enriched by F^-. This result reveals that F^- pollution, while altering the microbial nitrogen cycling function, may also induce the co-selection risk of multidrug resistance, providing a scientific basis for a more accurate assessment of the biosafety risks of biological treatment systems for fluoride-containing wastewater.