## Decoupling mechanisms of high-efficiency nitrogen removal and low sludge production in an encapsulated biofiller system

The concurrent goals of achieving high-efficiency nitrogen removal and minimizing excess sludge production represent a significant challenge in urban wastewater treatment, directly impacting the sustainability and circularity of urban water cycles. This study investigates the underlying mechanisms of an encapsulated biofiller Anaerobic-Anoxic-Oxic-Anoxic (EB-AAOA) system that successfully decouples these two traditionally linked processes. We reveal a multi-layered synergistic mechanism, rooted in both process-level engineering and microbial-level metabolic shifts, that enables a paradigm shift from a proliferation-driven to a maintenance-driven treatment model.

The foundation of this decoupling lies in a strategic carbon flow redirection. The system's front-end was engineered as an "endogenous carbon factory," where encapsulated hydrolytic-acidifying bacteria efficiently converted influent complex organics into volatile fatty acids (VFAs). These VFAs were subsequently consumed in a low-yield anoxic process, effectively intercepting the carbon source before it could fuel the growth of aerobic heterotrophic bacteria (AHB)—the major source of sludge in conventional systems. This design created a severe carbon-starvation environment in the oxic zone, which was found to be the critical prerequisite for both sludge suppression and the establishment of an energy-efficient partial nitrification pathway. Within this engineered oligotrophic and high-SRT environment, the microbial

community demonstrated a profound metabolic adaptation toward self-consumption. The encapsulation technology provided critical niche stabilization, creating a mature ecosystem where internal biomass recycling became the dominant metabolic strategy. This "self-consumption loop" was characterized by three key processes: endogenous respiration, cell lysis followed by cryptic growth, and predation by higher trophic-level organisms. This active, in-situ biomass reduction mechanism explains the net decrease of the system's existing biomass inventory.

The EB-AAOA system maintained >99% nitrogen removal while achieving an exceptionally low observed sludge yield (Y<sub>obs</sub>) of 0.052 g SS/g COD. This represents a reduction of over 85% compared to conventional activated sludge (CAS) processes, demonstrating a fundamental breakthrough in biomass control. Multi-omics analyses provided a complete chain of evidence, validating the genetic blueprint for the engineered low-yield pathways and the functional reality of the self-consumption response.

In conclusion, by synergistically combining process-level carbon management with the promotion of a microbial self-consumption economy, this system offers a scientifically validated pathway to transform WWTPs. It moves beyond incremental improvements, presenting a robust strategy to fundamentally address the sludge burden, thereby contributing significantly to the goals of developing more sustainable and resource-efficient cities.