Effect of inoculum source on organic matter and nutrient removals in AGS systems under saline stress

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Highlights:

- Alternating salinity pulse required shorter granulation times.
- Reactor with submerged aerated biofilter inoculum favored the removal of phosphorus and organic matter removal.
- Reactor with activated sludge inoculum favored denitrification.

Keywords: Aerobic granules; Biological treatment; Saline pulses.

INTRODUCTION

Aerobic Granular Sludge (AGS) is a recent technology that has been increasingly used to treat the most diverse types of effluents, especially domestic sewage. Due to dissolved oxygen (DO) stratification within the granule, different zones are created with oxygen gradients that are fundamental for the simultaneous removal of nitrogenous fractions, organic matter, and phosphorus (Nancharaiah & Reddy, 2018; Rollemberg *et al.*, 2018). Therefore, the larger the granule, the greater the probability of achieving better removal efficiencies.

Thus, operational strategies must be optimized to improve the removal of contaminants, increase denitrification efficiency, and favor biomass development, especially in shorter time intervals. Some optimizations reported in the literature that favor granulation use saline pulses, although studies and applications are still incipient. According to Wang *et al.* (2023), the presence of salinity selects the microorganisms that act in sedimentation due to the different microbiome sensitivities to saline stress, which increases the buoyancy forces in the system and generates greater resistance to precipitation.

The inoculum is another factor that influences the granulation process. As is known, the quality of the inoculum is fundamental to defining the microbial groups of aerobic granules and thus delineating the metabolic mechanisms that will influence the efficiency of pollutant removal. In Brazil and worldwide, activated sludge (AS) systems are the main sources of inoculum for granulation systems. To date, as far as we are concerned, no studies have been found that assess the impacts of using Submerged Aerated Biofilters (SAB) biomass, technology typically used in Brazil for decentralized wastewater treatment, as inoculum in AGS systems compared to AS.

In SAB biomass, denitrification is carried out by heterotrophic denitrifying microorganisms, which may be advantageous when using high sources of organic carbon and low oxygen concentrations (Loh et al., 2021). Furthermore, EPS production capacity depends on the microbial groups that withstand the granulation process and can influence both treatment performance and biomass stability in the long term. Gao et al. (2011) found that inoculum from AS systems had greater microbial diversity, favoring the formation of more stable and resilient granules.

In this sense, the present work sought to evaluate the effect of inoculum source on organic matter and nutrient removals in AGS systems under saline stress.

METHODOLOGY

Two identical sequential batch AGS reactors were operated under the same conditions for 53 days, differing only from the inoculum source: R1, biomass from a submerged aerated biofilter and collected from the secondary clarifier; R2, biomass from a carousel-type activated sludge system. Both reactors operated under saline stress, alternating cycles with saline feed (5 gNaCl/L) and feed without salinity. The sedimentation time was reduced by characterizing Phase I (15 min) and Phase II (5 min). Each cycle lasted 6 hours and consisted of anaerobic feeding (20 min), anaerobic reaction (100 min), aerobic reaction (214-224 min), anoxic reaction (10 min), sedimentation (15-5 min), and discharge (1 min). The engineering aspects, analytical methods, and synthetic effluent composition are described in Frutuoso et al. (2024).

RESULTS AND CONCLUSIONS

The reactors started operation with 2.5 gVSS/L, with the biomass having an VSS/TSS ratio of approximately 82%. The sedimentation time was gradually reduced over two phases to select the biomass better and form more resistant granules. Granulation is achieved when 80% of the granules have a diameter greater than 200 µm. It took 21 days in the reactor with submerged aerated biofilter inoculum (R1) and 15 days in the reactor with activated sludge system biomass (R2). These granulation times with alternating salinity pulse were shorter than in other studies in which salinity was continuous throughout each cycle, requiring approximately 30 days to be considered granular (Tang *et al.*, 2023).

Although R1 required a longer time for granulation, it presented a greater amount of biomass at the end of the experiment, reaching 6.2 gVSS/L, while in R2 the biomass concentration was only 4.7 gVSS/L. Furthermore, approximately 90% of the granules in R1 had a diameter above 1000 µm, unlike R2, whose percentage reached only 75%. This difference in the particle size profile was fundamental for some processes to benefit from R1 (Table 1), such as phosphorus removal, which was significantly greater than in R2, both in Phases I ($p = 0.03$) and II ($p = 0.04$). In the investigations by He *et al.* (2020), phosphorus removal process was inhibited at salinity levels of 20 g/L.

Organic matter removal was also favored in R1. However, it was only during Phase II when granulation was achieved and the granules were larger ($p = 0.044$). The greatest organic matter removal during the granulation process was in R2 ($p = 0.02$), reaching values of approximately 99%.

Regarding nitrogen, the denitrification rate was statistically higher in R2 in Phases I ($p = 0.03$) and II ($p = 0.02$). This difference reflects the greater nitrite accumulation in R1 during Phase II ($p < 0.001$) and the greater ammonia removal by R2 during the granulation process. As in R2, organic matter was removed lower than in R1; this remaining organic matter was likely used to support denitrification, resulting in a higher denitrification capacity than in R1.

Therefore, while the reactor with submerged aerated biofilter inoculum favored the removal of phosphorus and organic matter, the activated sludge inoculum was better in terms of nitrogen removal and NOx accumulation.

Table 1 – COD, nitrogen, and phosphorous removals in AGS systems with reactor with submerged aerated biofilter inoculum (R1) and reactor with activated sludge system biomass (R2), with sedimentation time of 15 minutes (Phase I) and 5 minutes (Phase 2). Legend: COD, Chemical Oxygen Demand; TP, Total Phosphorus.

Parameters	Phase I		Phase II	
	R1	R2	R1	R2
COD_{inf} (mg/L)	972±90	924±95	1024±86	1054±142
COD_{eff} (mg/L)	112±80	3±1	38±26	54±50
COD removal (%)	89±8	99±1	96±2	95±5
NH_4^+-N_{inf} (mg/L)	57±8	56±2	54±3	54±3
NH_4^+-N_{eff} (mg/L)	11±2	1±1	1±1	3±2
NO_2^--N_{eff} (mg/L)	5±5	7±1	11±1	7±1
NO_3^--N_{eff} (mg/L)	15±4	11±1	8±3	8±1
NH_4^+-N removal (%)	80±4	99±1	98±1	95±4
Denitrification (%)	46±10	66±2	55±14	70±3
PO_4^{3-}-P_{inf} (mg/L)	13±1	14±1	14±1	14±1
PO_4^{3-}-P_{eff} (mg/L)	7±2	13±1	11±1	12±1
TP removal (%)	43±19	7±1	24±6	18±7

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