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Enhancing Aerobic Granular Sludge Formation in Domestic Wastewater Through Mg^{2+} Augmentation

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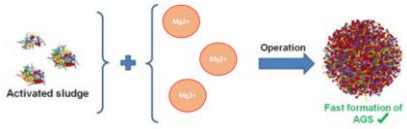
GRAPHICAL ABSTRACT



ABSTRACT

This study investigates the optimal Mg^{2+} concentration for enhancing aerobic granular sludge (AGS) formation in domestic wastewater treatment. Domestic wastewater and activated seed sludge samples were sourced from a local treatment plant, and three Mg^{2+} concentrations (130 mg/L, 160 mg/L, and 260 mg/L) were tested alongside a control sample without Mg^{2+}. Each sample was supplemented with magnesium sulfate and cultivated in four lab-scale sequential batch reactors. The reactor with 160 mg/L Mg^{2+} demonstrated the highest chemical oxygen demand (COD) removal efficiency (96.93%) and optimal granule stability due to an enhanced extracellular polymeric substance (EPS) production, specifically proteins. Higher concentrations (260 mg/L) caused EPS destabilization, negatively affecting granule formation. The 160 mg/L concentration provided the best balance for rapid AGS formation, settling, and COD removal, aligning with the literature on the role of divalent cations in sludge granulation. These findings present a promising approach for improving wastewater treatment efficiency through controlled Mg^{2+} augmentation.

Keywords: Mg^{2+} concentration, Aerobic Granular Sludge, Chemical Oxygen Demand, Extracellular Polymeric Substances, Wastewater treatment.

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1. INTRODUCTION

Wastewater treatment is essential for safeguarding the environment and public health by removing contaminants, reducing water pollution, and spreading waterborne diseases[1]. Biological treatment technology, which utilizes microorganisms like bacteria, fungi, and algae, provides a cost-effective and environmentally sustainable solution for efficiently breaking down organic matter and eliminating pollutants [2][3].

Activated sludge (AS) is a widely used biological treatment method in municipal and industrial wastewater treatment[4]. It is a suspended-growth process where a concentrated microbial culture degrades organic matter under aerobic conditions, forming biological flocs for sludge-water separation in a clarifier[5]. Activated sludge is highly efficient in treating BOD, COD, and other nutrients, producing high-quality effluent without harmful chemicals[6]. However, conventional activated sludge systems face limitations that impact their performance. The small size of activated granules results in a high surface area-to-volume ratio, which increases drag force and hampers effective settling. This decreases the settling velocity and retention time, complicating the sludge-liquid separation process and reducing effluent quality. Consequently, larger land footprints are required for additional clarifiers to separate solids [7].

Aerobic granular sludge (AGS) was introduced as a better alternative for solid-liquid separation. Under aerobic conditions, conventional activated sludge can form compact and dense AGS aggregates, enhancing settling velocity and reducing retention time[8]. AGS allows for higher biomass concentrations and simultaneous nitrification, denitrification, and phosphate removal in a single treatment tank, reducing land footprint by 50-70% and energy consumption by 30-48% compared to conventional methods[9].

Despite its advantages, AGS stability remains an issue. Mineral accumulation and increased granule sizes can lead to AGS collapse, reducing efficiency[10]. Factors such as temperature, pressure, microbial composition, C/N ratio, and wastewater type contribute to AGS instability,

necessitating constant adjustments[11]. Divalent metal ions like Fe^{2+}, Ca^{2+}, and Mg^{2+} have been shown to promote stable AGS formation, enhancing microbial aggregation and granulation[12].

While research on Mg^{2+} impact on AGS exists, it is somewhat outdated, and the results vary due to changes in wastewater characteristics and limited studies on varying Mg^{2+} concentrations. Most research has focused on synthetic wastewater, which lacks organic matter present in real wastewater. Furthermore, there were no specifications on the optimum Mg^{2+} range to improve aerobic respiration as well as the side effects of excessive Mg^{2+} addition.

This study aims to investigate the effect of different Mg^{2+} concentrations on AGS formation in domestic wastewater. The development of granules and settling abilities will be observed through analytical methods, and the extracellular polymeric substance (EPS) content of proteins will be analyzed to understand the impact of Mg^{2+} augmentation on granule stability.

2. EXPERIMENTS

2.1 Seed Sludge Sampling

Seed sludge was obtained from a local wastewater treatment plant by collecting activated sludge mixed with wastewater from the aeration tank using a plastic pail. This mixture was poured into a measuring cylinder for settling. After 30 minutes of settling, the top layer of wastewater was removed, and the thick activated sludge was transferred into polyethylene bottles using a plastic funnel. A total of 30 litres of sludge was obtained, filtered, and stored in a refrigerator at 4°C in the MJIIT lab.

2.2 Domestic Wastewater Sampling

The wastewater was sampled from the influent wastewater tank. The same plastic pail was used to collect the wastewater sample. A total of 45 litres of influent wastewater was collected into two polyethylene bottles. Both bottles were taken back to the lab and refrigerated at 4°C.

2.3 Wastewater Samples Preparation

In this experiment, three concentrations of Mg^{2+} were studied based on the range of metal ion concentrations utilized in past research on the cultivation of aerobic granular sludge. The concentrations used were 130 mg/L, 160 mg/L, and 260 mg/L of Mg^{2+}. Magnesium ion was obtained from solid magnesium sulfate ($MgSO_4$) due to its ability to readily dissolve in water, providing a reliable source of Mg^{2+} ions for the wastewater and promoting the production of extracellular polymeric substances (EPS) within granules.

The influent wastewater was mixed with $MgSO_4$ at the desired concentrations before the cultivation process. Each sample, mixed with magnesium sulfate, was kept separately and tagged with its specified concentration of 130 mg/L, 160 mg/L, and 260 mg/L. A fourth sample was prepared as a control with no addition of magnesium sulfate.

To produce wastewater solutions with different concentrations of Mg^{2+} ions, solid magnesium sulfate ($MgSO_4$) was weighed using analytical balance before being poured into the wastewater and mixed until completely dissolved. The required amount of magnesium sulfate for each concentration was calculated using Equation 1 as shown below:

$$C = \frac{n}{V} \quad (1)$$

Where C is the concentration (in moles per liter, M), n is the number of moles of solute, and V is the volume of solution in litres.

The Mg^{2+} concentrations of 130 mg/L, 160 mg/L, and 260 mg/L were selected based on existing studies and preliminary experiments. Research has shown that Mg^{2+} concentrations between 100 mg/L and 300 mg/L can significantly impact aerobic granular sludge (AGS) formation. Li et al. (2009) reported that concentrations within this range promote faster EPS production and better microbial aggregation. Preliminary trials in this study also indicated that 160 mg/L is particularly effective at maintaining granule stability without the adverse effects seen at higher concentrations like 260 mg/L

2.4 Reactor Set-Up and Operation

The cultivation of AGS was conducted using four identical sequencing batch reactors (SBR) offering flexibility in controlling the AGS formation process. Each reactor had a total volume of 3L, equipped with 2 running aeration pumps. The aeration pumps were set at 2.5 L/min for optimum microbial reactions. Reactors were set with two columns each, designated for different Mg^{2+} concentrations: control, 130 mg/L, 160 mg/L, and 260 mg/L. Wastewater samples were mixed with $MgSO_4$ beforehand to achieve the desired Mg^{2+} concentrations. The SBRs operated under a 3-hour cycle consisting of influent addition, aeration, settling, and effluent withdrawal, with a 15-minute idle period. The reactors were maintained at 18 °C in a controlled laboratory environment and operated for 20 days to observe AGS formation.

2.5 Volumetric Exchange Rate

All four reactors were seeded with a volumetric exchange rate of 50:50 sludge to wastewater ratio. The ratio is commonly used in developing aerobic granular sludge. The controlled reactor was seeded with 50% raw wastewater

and 50% activated sludge. Meanwhile, the experimental reactors were seeded with 50% activated sludge and 50% raw wastewater with the augmentation of 130 mg/L, 160 mg/L, and 260 mg/L Mg^{2+} respectively. All four reactors were aerated with the same type of air pump at the same flow rate for the SBR.

2.6 Chemical Oxygen Demand Analysis

To assess the impact of different Mg^{2+} concentrations on organic matter removal efficiency, Chemical Oxygen Demand (COD) analysis was conducted following the USEPA Reactor Digestion Method. After settling in the sequential batch reactor (SBR), effluent from the top layer was collected and divided into four labeled beakers representing control, 130 mg/L, 160 mg/L, and 260 mg/L samples.

During effluent collection, the DRB 200 Reactor was preheated to 150ºC. High-range HACH COD vials were labeled accordingly, including a blank sample prepared with distilled water. Each vial was filled with 2.00 mL of the respective sample or distilled water, with care taken to prevent burns from the exothermic reaction during vial handling.

After tightly closing the vial caps, they were gently inverted to mix the reagent. The prepared vials were then heated for two hours in the preheated DRB 200 reactor. Following heating, the reactor was switched off, and vials were left to cool for approximately 20 minutes.

Once cooled, COD values were measured using the DR3900 Laboratory VIS Spectrophotometer with the 435 COD HR program. The blank vial was inserted to zero the instrument, and then replaced with the control sample for reading. The process was repeated for experimental vials, and data were recorded accordingly.

2.7 Mixed Liquor Suspended Solids Analysis

Initially, the dry weight of an empty crucible was measured using an analytical balance, and the weights were recorded. One-litre samples of mixed liquor were obtained from all four SBRs using a measuring cylinder. Vacuum filtration was conducted using a vacuum pump connected to a vacuum flask with a filter membrane and an evaporating dish. The Whatman filter paper was placed in the filter holder and wetted with distilled water to secure the funnel. The mixed liquor samples were poured into the filter holder and filtered within 10 minutes, up to a maximum of 1 litre.

The filter and collected solids were washed with three successive 10 mL portions of distilled water to remove any dissolved solids. After filtration, the filter was dried in an oven set at 104±1°C for at least one hour. Once cooled, the filter was weighed, excluding the pan. This cycle of drying, cooling, desiccating, and weighing was repeated until a stable weight was obtained. Finally, MLSS values were calculated using Equation 2 as shown below.

$$\frac{weight\ final\ (g) - weight\ initial\ (g)}{sample\ volume\ (L)} = MLSS\ \left(\frac{g}{L}\right) \qquad (2)$$

2.8 Suspended Solids Analysis

Suspended solids were measured to calculate the sludge volume index (SVI). The suspended solids were measured using an Imhoff cone. One litre of mixed liquor samples was collected from the control, 130 mg/L, 160 mg/L, and 260 mg/L samples. The mixed liquor was filled into the Imhoff cone and left to settle for 45 minutes. The cone was moved forward and backward several times to ensure all the sediments settled down. After 45 minutes, the volume of settleable solids was recorded from the graduated scale at the top of the solid layer. All the settleable solids were recorded.

2.9 Sludge Volume Index (SVI) Analysis

From the measured MLSS and suspended solid, the sludge volume index was measured using this formula:

$$SVI\ \left(\frac{mL}{g}\right) = \frac{Settled\ sludge\ volume\ \left(\frac{mL}{L}\right)}{MLSS\ \left(\frac{g}{L}\right)}\ x\ 1000\ \left(\frac{mg}{g}\right) \qquad (3)$$

From the measured SVI value, a graph of SVI against time was plotted to study the settling characteristic of the sludge.

2.10 Granules Sieving Process

A sieving method was conducted to estimate the granule size distribution. The sludge sample was taken from the bottom of the sampling points for control, 130 mg/L, 160 mg/L and 260 mg/L reactors and each sludge sample was divided into four fractions using laboratory sieves with various diameters (0.2 mm, 0.6 mm, 1.0 mm, 1.5 mm). The sludge particles were gently submerged in water and shaken to let smaller particles pass through the openings. The procedure was repeated until all three sieves were utilized.

After sieving, the AGS collected on each sieve was weighed. The total mass of the sample was calculated by summing the weights of all size fractions. For each size fraction, the percentage of the total mass represented by that fraction was calculated by dividing the weight of the individual size fraction by the total mass and multiplying by 100. The results were then tabulated for each reactor.

2.11 Extracellular Polymeric Substance (EPS) Extraction and Analysis

To study the stability of granules cultivated, EPS extraction was carried out using the centrifugation method. First, 3g of sludge was extracted from each of the four SBR tanks and placed into centrifugation tubes. Demineralized

water was added to each tube, and the tubes were gently shaken by hand. The tubes were then centrifuged at 4000 rpm and 4°C for 20 minutes. The supernatant was collected in a glass beaker, and the pellet was discarded. The supernatant was used for protein analysis.

Protein (PN) estimation was performed using the modified Lowry method by FrØlund et al. (1995), with bovine serum albumin as the standard. This method was chosen for its clarity, effectiveness, and ability to maintain EPS integrity throughout the extraction process. The protein content of the granules represents the stability of the granules.

3. RESULTS AND DISCUSSION

COD content was successfully removed from the domestic wastewater in both control and experimental reactors, indicating positive microbial growth as the microorganisms consumed the organic material with the introduction of aeration. Table 1 lists the average effluent COD concentration and removal percentage in the four reactors after 20 days of operation.

Table 1 Average effluent COD concentration and Percentage of COD Removal in Reactors

Reactor	Average Effluent COD Concentration (mg/L)	Percentage of COD Removal (%)
Control	60.4	83.55
130 mg/L	55.3	85.71
160 mg/L	28.7	96.93
260 mg/L	30.4	96.26

After 20 days, reactors with varying Mg^{2+} concentrations showed improved organic matter removal efficiency. The reactor with 160 mg/L Mg^{2+} achieved the highest COD removal (96.93%), indicating Mg^{2+} augmentation's positive impact. Mg^{2+} facilitates coagulation and microbial aggregation, enhancing EPS secretion and COD removal efficiency. Initial rapid COD drops suggest microorganism starvation post-sampling. From day 2, stabilized COD removal patterns were observed, with Mg^{2+} augmented samples consistently showing lower effluent COD. Fig 1 and 2 illustrate the significant efficiency improvement, especially at 160 mg/L of Mg^{2+}. Mg^{2+} concentrations between 160 mg/L and 260 mg/L were identified as optimal for enhancing aerobic granular sludge formation and improving wastewater treatment efficacy.

The reactor with 160 mg/L of Mg^{2+} achieved the highest COD removal at 96.93%. This can be attributed to the fact that Mg^{2+} enhances microbial aggregation and EPS production, which improves the overall sludge structure and facilitates better substrate uptake [16]. Divalent metal ions

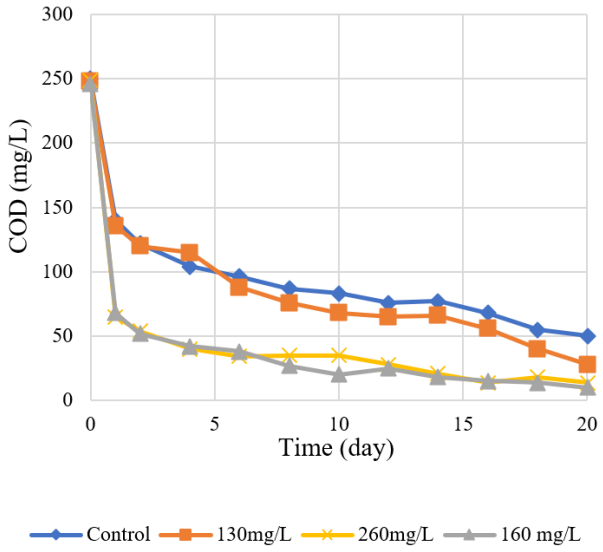

Fig. 1. Effluent COD value for (♦); control system (▲); 160 mg/L Mg^{2+} augmentation (■); 130 mg/L Mg^{2+} augmentation (×); 260 mg/L Mg^{2+} augmentation

like Mg^{2+} can interact with negatively charged EPS molecules, promoting tighter microbial floc formation, leading to faster and more efficient COD reduction [16]. The findings are consistent with Jiang et al. (2003) and X. M. Li et al. (2009), who reported that Mg^{2+} facilitates rapid granule formation and boosts microbial performance [15][16]. Higher concentrations like 260 mg/L, although effective, may result in diminishing returns due to an imbalance in EPS composition, thus making 160 mg/L the optimal concentration for enhanced COD removal.

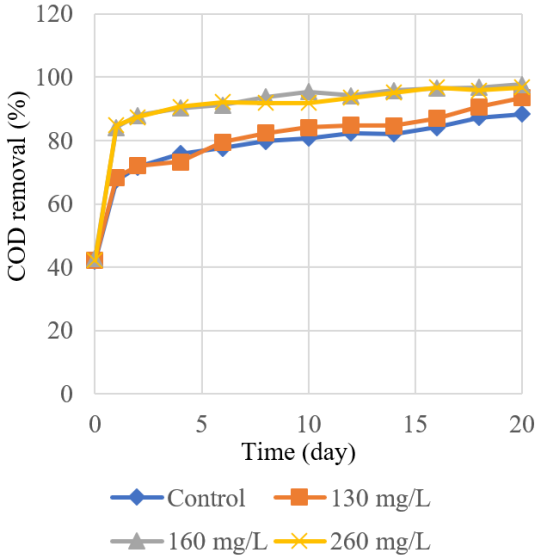

Fig. 2. COD removal percentage for (♦); control system (▲); 160 mg/L Mg^{2+} augmentation (■); 130 mg/L Mg^{2+} augmentation (×); 260 mg/L Mg^{2+} augmentation

The results of this study align with those of X. M. Li et al. (2009), who demonstrated that Mg^{2+} improves sludge settling and granule formation [16]. At concentrations around 160 mg/L, EPS production is optimal, leading to more compact and dense granules, which corresponds with the 96.93% COD removal observed here. Compared to lower concentrations like 130 mg/L, which exhibit delayed granulation, or higher concentrations like 260 mg/L, which cause EPS destabilization, 160 mg/L provides the best balance. This is further supported by Jiang et al. (2003), who also noted similar trends in metal ion augmentation for AGS formation [15].

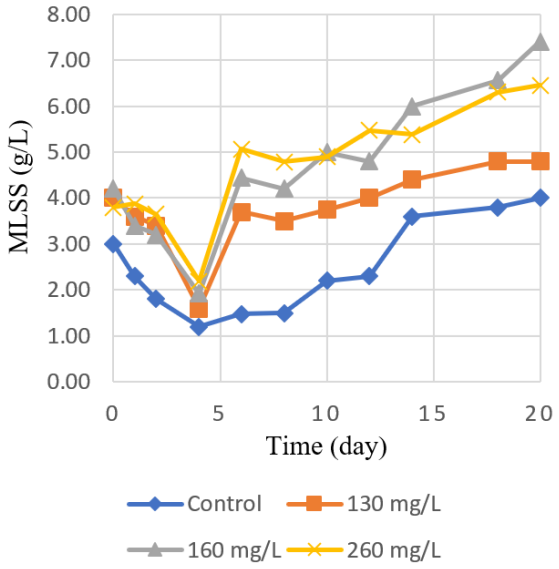

Fig. 3. MLSS value for (♦); control system (▲); 160 mg/L Mg^{2+} augmentation (■); 130 mg/L Mg^{2+} augmentation (×); 260 mg/L Mg^{2+} augmentation

Fig 3 shows the MLSS pattern throughout experiment. In the initial stages, the absence of aerobic granule formation results in low MLSS values across all samples, with biomass exhibiting a loose structure. Additionally, from day 0 to 4, low initial settling of activated sludge (AS) in the SBR leads to MLSS reduction as the AS trying to adapt to the new environment. Subsequently, sludge concentration fluctuates due to floc washout followed by granule dominance. After day 4, MLSS values increase significantly as activated sludge adapts and started to secrete EPS.

Among the three experimental samples the 160 mg/L shows the highest MLSS value near the end of cultivation compared to 130 mg/L and 260 mg/L samples. Interestingly, from day 4 until 12 the samples 260 mg/L reach the highest MLSS value compared to the others. At 260 mg/L, the Mg^{2+} concentration may be too high, leading to adverse effects on the AGS formation. High concentrations of divalent cations can disrupt the balance of extracellular polymeric substances (EPS) that are crucial for granule stability. This disruption can result in the disintegration of granules and a subsequent reduction in MLSS. Throughout the experiment, MLSS generally increases, with Mg^{2+} augmented reactors experiencing a more rapid rise compared to the control set, indicating Mg^{2+} 's positive impact on biomass concentrations and granular sludge settling characteristics in wastewater treatment.

Fig 4 illustrates the sludge volume index (SVI) for the four samples. Initially, SVI values slightly increased, peaking at 110 mL/g for the control set, indicative of disintegrated sludge structures hindering settling. However, after day 4, all samples exhibited decreased SVI values due to increased EPS secretion and granule compactness from the feast and famine cycle. Notably, the control set consistently had the highest SVI, suggesting slower settling without magnesium ions' positive charge interaction with organic matter. Furthermore, SVI showed an inverse relationship with MLSS, as increased MLSS over 20 days corresponded to decreased SVI, indicating improved settling with granule formation and reduced floc washout during drainage cycles, thereby increasing biomass content.

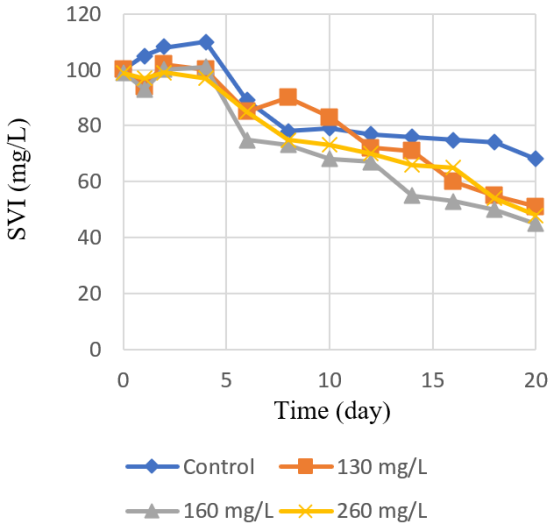

Fig. 4. SVI value for (♦); control system (▲); 160 mg/L Mg^{2+} augmentation (■); 130 mg/L Mg^{2+} augmentation (×); 260 mg/L Mg^{2+} augmentation

The slight increment in SVI observed at 130 mg/L after day 5 can be explained by incomplete granulation at this concentration. During the initial stages, flocs may still dominate over granules, causing less efficient settling. Magnesium ions at 130 mg/L may trigger EPS production but do not fully stabilize the granular structure, leading to higher SVI values compared to 160 mg/L, where granules are more compact. Previous studies have suggested that incomplete or unstable granulation can cause fluctuating SVI values.

The protein content (PN) in extracellular polymeric substance (EPS) is crucial for the sludge granules' stability as proteins have a high content of negatively charged amino acids, allowing them to form electrostatic bonds with multivalent cations like calcium and magnesium[13]. These bonds help stabilize the aggregate structure of granules. Based on the PN analysis results, all samples exhibited an increase in PN values over 20 days, attributed to the support provided by excess Mg^{2+} in polysaccharide formation within granules. Polysaccharides contribute to a robust framework essential for stable granular structure maintenance[14] . The interaction of polysaccharide functional groups, such as OH, with Mg^{2+} further enhances structural stability by forming a rigid, non-deformable polymeric gel-like matrix[14].

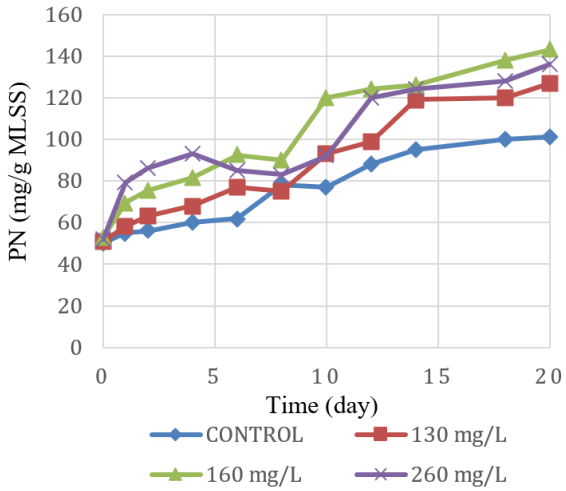

Fig. 5. PN value for (♦); control system (▲); 160 mg/L Mg^{2+} augmentation (■); 130 mg/L Mg^{2+} augmentation (×); 260 mg/L Mg^{2+} augmentation

The 160 mg/L reactor demonstrated the highest PN concentration at 143 mg/L, while the control reactor showed the lowest, slowing down the granulation process. Interestingly, despite higher Mg^{2+} concentration, the 260 mg/L reactor did not exhibit the highest PN content. Fig 5 highlights a significant drop in PN concentration on day 8 for the 260 mg/L reactor, indicating potential EPS composition alterations due to excessive Mg^{2+}. Thus, the optimal Mg^{2+} concentration for maximum PN content was observed at 160 mg/L, with concentrations above 260 mg/L potentially impacting EPS composition and reducing PN content, resulting in less compact and dense granules compared to those at 160 mg/L.

The granule size distribution analysis revealed significant insights into the effect of Mg^{2+} concentration on granule formation and maturation. Initially, the majority of granules in all reactors were below 0.2 mm, indicating early-stage development[14]. However, notable differences emerged over time. In the control reactor (Fig 6), granule growth was slow, with sizes mainly between 0.2 mm and 0.6 mm. This slow growth resulted in less dense granules, explaining the high SVI observed during the cultivation period.

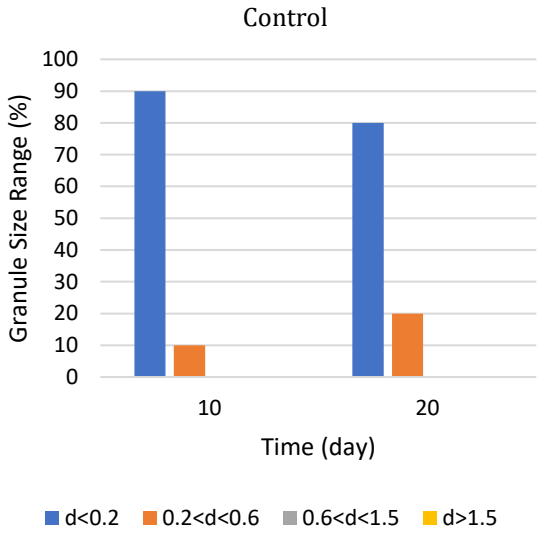

Fig. 6. Size distributions (by weight) of granules of control reactor A: d < 0.2; B: 0.2 < d < 0.6; C: 0.6 < d < 1.5; D: d > 1.5 (unit in mm)

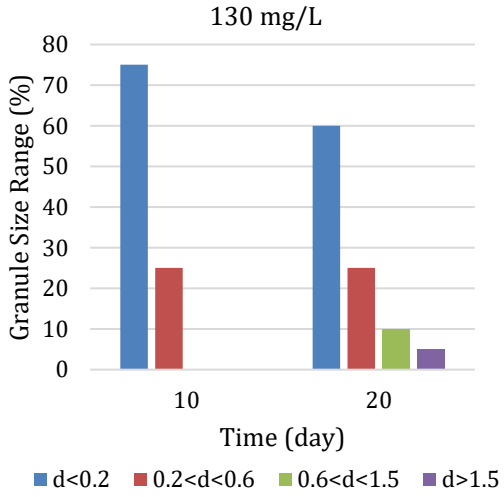

Fig. 7. Size distributions (by weight) of granules of 130 mg/L reactor A: d < 0.2; B: 0.2 < d < 0.6; C: 0.6 < d < 1.5; D: d > 1.5 (unit in mm)

Contrastingly, the 130 mg/L reactor (Fig 7) exhibited accelerated granule growth, surpassing the control reactor by achieving sizes larger than 0.6 mm within 10 days. This acceleration is attributed to magnesium ions stimulating higher EPS production, crucial for granule stabilization and maturation[15]. The 160 mg/L reactor (Fig 8) demonstrated the fastest granule growth, with mature structures above 0.6 mm within 10 days. By day 20, granules

above 1.5 mm constituted a significant percentage, indicating optimal conditions for rapid maturation. In the 260 mg/L reactor (Fig 9), granule growth was slightly slower than the 160 mg/L reactor, with a majority of granules below 0.2 mm. By day 20, however, granules above 0.6 mm increased, although growth rates were lower compared to the 160 mg/L reactor.

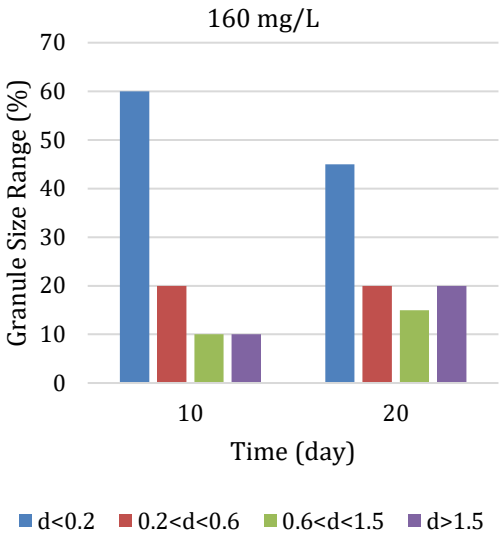

Fig. 8. Size distributions (by weight) of granules of 160mg/L reactor A: d < 0.2; B: 0.2 < d < 0.6; C: 0.6 < d < 1.5; D: d > 1.5 (unit in mm)

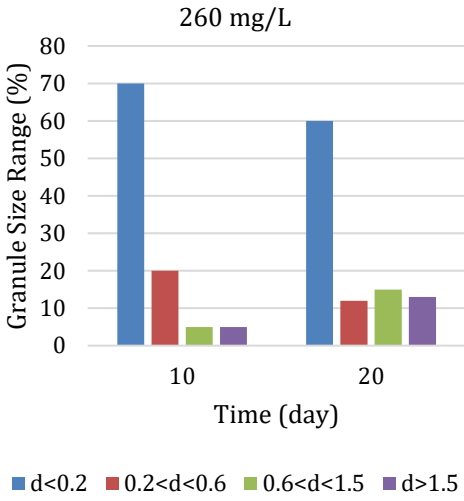

Fig. 9. Size distributions (by weight) of granules of 260 mg/L reactor A: d < 0.2; B: 0.2 < d < 0.6; C: 0.6 < d < 1.5; D: d > 1.5 (unit in mm)

Overall, Mg^{2+} presence accelerated granule formation and reduced maturation time [16]. However, concentrations exceeding 260 mg/L hindered growth, possibly due to EPS breakdown or microorganism respiration inhibition. Thus, Mg^{2+} facilitates earlier and larger aggregate formation, expediting sludge maturation within 20 days compared to the conventional 30 days.

In this study complete granulation of AGS is defined as achieving 15% of granules larger than 0.6 mm. Both the 160 mg/L and 260 mg/L reactors reached this threshold within 20 days, showing higher biomass concentration and earlier visible granules compared to the control and 130 mg/L reactors. The 130 mg/L reactor achieved 10% granules above 0.6 mm, indicating better growth than the control, which had none by day 20. These results indicate that Mg^{2+} concentrations between 160 and 260 mg/L significantly improve biomass retention and accelerate the granulation process.

4. CONCLUSION

This study demonstrates that Mg^{2+} concentration plays a critical role in enhancing aerobic granular sludge (AGS) formation and performance. The optimal Mg^{2+} concentration was found to be 160 mg/L, which yielded the highest COD removal efficiency (96.93%) and improved granule stability through enhanced extracellular polymeric substance (EPS) production, particularly proteins. The results showed that while 260 mg/L promoted early granulation, it led to EPS destabilization, indicating that excessive Mg^{2+} can negatively impact sludge stability. The 160 mg/L reactor consistently produced stable granules, reduced the sludge volume index (SVI), and accelerated granule formation from 30 to 20 days. This concentration provides a balance between rapid AGS formation and effective organic matter removal, aligning with findings from previous studies on the benefits of divalent cation augmentation in AGS systems. Future research could explore the combined effects of other metal ions like Ca^{2+} and Fe^{2+} to further enhance AGS performance. These findings offer valuable insights for optimizing wastewater treatment processes through precise Mg^{2+} dosing, contributing to more sustainable and cost-effective management practices.

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