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Impact of saline wastewater on the behaviour of aerobic granular sludge (AGS)

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ABSTRACT

This study delves into the impact of influent salinity on the efficacy of aerobic granular sludge (AGS) in wastewater treatment. It emphasizes the influence of salinity on vital biological processes like organic elimination, denitrification, nitrification, and phosphate removal while assessing the applicability of AGS technology under varying salinity conditions. The research further examines how salinity shapes microbial diversity and structure within the AGS framework. Utilizing three separate sequential batch reactors with distinct NaCl concentrations (2000, 5000, and 10000) mg/l, the research analyzed aspects such as sludge properties, settling speed, sludge volume index, and the efficiency of COD and NH4-N removal. Findings underscored that increased salinity levels from, (2000 to 10000) mg/l adversely affected AGS operations, resulting clear drop in removal efficiencies: from 94% to 68% for COD, from 91% to 52% for NH4, from 80% to 20% for TN and caused reducing in settling speeds, granular deterioration,. Moreover, the microbial composition within the AGS was notably influenced by escalated salinity concentrations. This study offers crucial perspectives on the potential challenges of employing AGS technology for saline wastewater treatment and underscores the importance of additional investigations in this domain.

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1. INTRODUCTION

food industries, pickling, pharmaceutical, textile and other industries release a considerable amount of salts in their effluents[1]. Salt is thought to be an inhibitor of various biological processes, including organic elimination, denitrification, nitrification and phosphate removal. [1, 2], because salinity causes decreasing in oxygen transfer and in oxygen uptake, inhibition for nitrite-oxidizing bacteria (NOB), accumulating for polyphosphate- organisms (PAO) and effect on anoxic and aerobic phosphate uptake [2, 3]. As well as many of the studies show that increasing in salinity effects on particular organisms and caused to increase osmotic pressure which has negative effects on , granular formation [4]. Iraqi wastewater from municipalities is classified as saline wastewater, particularly in the southern portion of the country, where salinity levels ranged from (2000 to 10000)mg/l [5], that reduce the efficiency of the conventional activated sludge system and leads to produce effluent with high concentrations of pollutants [6]. In recent years researchers approach to investigate more effective and intensive treatment methods for wastewater that resist different severe pollution conditions like moving bed bioreactor (MMB), microbial desalination cell (MDC) and aerobic granular sludge system (AGS)[7,8,9,10]. AGS is stand out of these method that it is distinguished by its small footprint and its removal ability of organic load and nutrients which can be achieved in one basin instead of multiple basins as in conventional activated sludge system that reduce the construction and the operation cost [7, 11]. In this process the biomass is self-immobilized (without any carrier) in small and dense aggregates called granules by using feast -famine regime. This structure of biomass prevent

microorganisms from deterioration, that maintain high biomass concentration and accelerate the treatment process with high removal efficiency [11, 12], studies about salinity effect on the performance of granular sludge process are very limited especially which related to the salinity effect on the microbial morphology and diversity, these studies found that aerobic granules formation were faster and became more smoother and regular with high influent salt concentration while the porosity of these granular decreased and caused increasing in nitrate removal and decreasing in phosphate removal [13, 14]. The current study aims to find out the validity of AGS technology to treat wastewater under different concentrations of salinity and to investigate the influence of these concentrations on the microbial diversity

2. METHODS

2.1 batch rector's set-up

Three batch reactors: RA, RB, and RC (Figure 1), which were employed for AGS cultivation. Each of these reactors was designed as a graduated glass cylinder measuring of 40 cm in length and 6 cm in diameter, having a functional volume of 1 liter. Aeration was facilitated from the bottom of each reactor using an air diffuser joined to an air pump. The air flow rate for each reactor was precisely managed using a portable rotameter paired with a manual control valve [15,16].

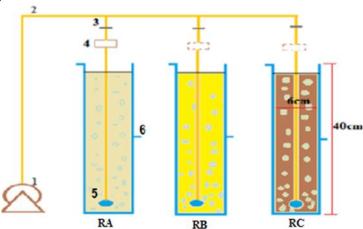


Figure 1: schematic of (1.air pump 2. distributed pipe 3. control valve 4. air flow meter 5. air diffuser 6. effluent point)

2.2 influent compositions

Three reactors, labeled as RA, RB, and RC, were set up using flocculated sludge sourced from the Al-Rustamiya wastewater treatment facility in Baghdad city in Iraq. Before use, large debris was removed from the sludge by filtering it through a 0.2 mm mesh. The reactors started with an initial MLSS (Mixed Liquor Suspended Solids) concentration of approximately 3000 mg/l. They were exposed to synthetic wastewater with COD of 1000mg/l and volumetric loading rate 1g.l/day. Each reactor receiving a distinct NaCl concentration: RA at 2000 mg/l, RB at 5000 mg/l, and RC at 10000 mg/l. The other constituents of the synthetic wastewater are detailed in Table 1. Initial MLSS values for these reactors was 3000mg/l, where the Sludge Volume Index (SVI) were (160, 156, and 125) ml/g for RA, RB, and RC respectively . Every fifth day, samples were collected from each reactor for evaluation.

Table 1: Constituents of influent synthetic wastewater

ISSN: Printed: 3078-9656, Online: 3078-9664, paper ID: 69

Influent constituents	RA	RB	RC
NaCl	2000	5000	10000
CH ₃ COONa	1282	1282	1282
NH ₄ Cl	191	191	191
K ₂ HPO ₄	27.3	27.3	27.3
NaH ₂ PO ₄ ·2H ₂ O	18.6	18.6	18.6
CaCl ₂	29.5	29.5	29.5
MgCl ₂ ·6H ₂ O	54.2	54.2	54.2
Trace element solution	0.1	0.1	0.1

2.3 Operation conditions

The reactors were run on cycles of 12-hour include 2min for feeding, 11.91 hr of aeration with superficial velocity of 3.5 cm/sec 1min for settling and 2min for discharging At the end of each cycle. the operation period was 70 days started with 30 minutes of settling for the first 15 days to limit biomass wash out, and then it was gradually reduced to 5 minutes on day 30 until reach 1 minute on day 70 of reactor operation. [14,15]. The discharge operation was carried out using a siphon after a sludge settling period through a rubber tube that draws the effluent from a location placed at the reactor's mid height.

2.4 Analysis equipment and instruments

Wastewater samples from these reactors were obtained and subsequently examined in the laboratories affiliated with the chemical engineering department at the University of Technology. Additionally, tests were carried out at the environment and water directorate within the Ministry of Science and Technology. These tests adhered to the APHA 2005 standard methodologies for water and wastewater analysis [17]. The COD levels were determined using a Lovibond spectrophotometer, while NH4-N and TN were gauged with a DR-5000 spectrophotometer from Hatch. The DO and pH measurements were taken using a CX-401 multifunction meter from Eutech Instrument. Total dissolved solid (TDS) levels were ascertained using the HANNA instrument, model HI9811.

For biomass determination weekly sludge samples from each reactor were collected to determine MLSS, settling velocity, and SVI. The MLSS was calculated using the 2540 D 'Method APHA 2005 [17]. To ensure well mixed samples, reactor liquors were mixed during aeration, and 25 ml of mixed sludge from each reactor was removed and filtered through dried and weighted filtering paper with a size of 40m. These filter sheets were then dried in an electrical oven at 105oC for 24 hours. Following drying, the samples were weighed, and the MLSS was computed as follows:

MLSS(mg/l) = (dried sludge weight(mg))/(volume of sample (ml)) *1000(1)

The time it took for individual granules to fall from a height of 40 cm in a measuring cylinder was used to calculate the settling velocity of sludge.

Settling velocity of granule = (40 cm) / (falling time min).....(2) So that:

Average settling velocity of AGS = \sum (Settling velocity of granule* number of granules %) /100(3)

Sludge volume index (SVI) was monitored at the end of aeration phase according to APHA method of 2710D[17]. This was done by determining the volume of the settled sludge in the 1 litre reactor after 30 minutes.

SVI (mV_0) = (v_0) | (MV_0) = (v_0) | (MV_0) | ($MV_$

 $SVI(ml/g) = (volume \ of \ settled \ sludge \ (ml) \)/(MLSS(g)) \dots (4)$

For microorganism's observation, the structure of the flocculated sludge and aerobic granules in the reactors was examined using both a stereo-microscope and an optical microscope equipped with a digital camera. The granules' microstructure was probed using a Scanning Electron Microscope (SEM) at the Ministry of Science and Technology's Materials Research Directorate once they achieved equilibrium. By analyzing images from the digital camera, the size variations of the aerobic granules were determined from random granule samples placed in Petri dishes.

3. RESULTS AND DISCUSSION

ISSN: Printed: 3078-9656, Online: 3078-9664, paper ID: 69

3.1 Sludge characteristics

The obtained sludge was characterized using Biomass concentration MLSS, settling velocity and sludge volume index values.

Biomass concentrations: As depicted in Figure 2, the initial 25 days of operation saw a consistent reduction in MLSS concentrations in reactor RA, primarily because of significant sludge washout. Meanwhile, the MLSS concentrations in reactors RB and RC were comparatively more stable. This could be attributed to their higher influent salt concentrations (5000 mg/l for RB and 10000 mg/l for RC) which might have acted as catalysts, accelerating granule development and minimizing sludge washout compared to RA's 2000 mg/l salt concentration. However, after 35 days, the trend changed. The decline in RA's MLSS slowed due to the formation of granules (refer to Figure 3). By the 50-day mark, a slight rise in MLSS concentration in RA was observed, peaking at 2000 mg/l by the operation's end, with granule dimensions varying from 0.5 to 2.5 mm (see Figure 4). Conversely, RB and RC experienced a drop in MLSS levels as their granules began to break down, granule sizes in RB ranged from 0.3 to 1 mm and in RC, they remained under 0.5 mm. The high salinity in RB and RC led to increased osmotic stress, making conditions unfavorable for many bacterial species to thrive. Additionally, the heightened salinity could escalate water's viscosity, subsequently reducing the transfer rates of substrate and oxygen, leading to granule degradation in RB and RC. Consequently, there was a substantial washout of biomass, which saw MLSS concentrations in RB and RC drop to 1700 mg/l and 1610 mg/l respectively by the 70-day mark. This outcome aligns with findings from [11], who determined that increment in the concentration of salt from 0 to 30 g/L led to about a 60% decrease in MLSS.

Settling velocity: Salinity also influenced settling velocity, as illustrated in Figure 5. During the initial 30 days, both RB and RC exhibited superior settling properties, registering 22 and 17.5 m/hr respectively, in contrast to RA. However, after operating for 70 days, the settling velocities of RB and RC declined, ending at 10 and 6.5 m/hr respectively. This decline can be attributed to the granules becoming more porous and eventually breaking apart. Conversely, in RA, the settling velocity consistently increased, culminating at 23.5 m/hr by the end of the observation period.

Sludge volume index SVI: The trends observed in settling velocity align with the SVI values for these reactors, as illustrated in Figure 5. Over the operational period, the SVI for RA steadily decreased, reaching its lowest at 50 ml/g by the end. In the initial 30 days, SVI improved for RB and RC, registering values of 40 ml/g for RB and 50 ml/g for RC. However, post the 35-day mark, as the settling velocity dropped, the SVI values in RB and RC rose to (75 and 90)ml/g respectively. Sludge behavior in this study has the same trend of granular sludge abstained by Winkler et al.,[13].

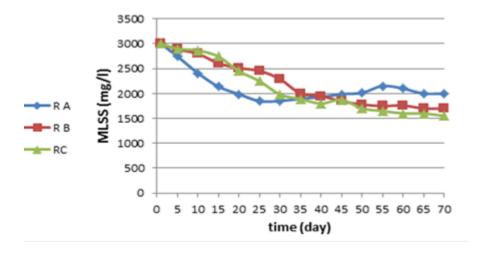


Figure 2: MLSS Concentrations in RA, RB, and RC Throughout the Operation Period..

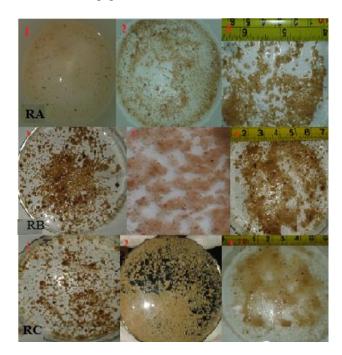


Figure 3.: Evolution of Granule Development in RA ,RB, and RC Over a 70-Day Period (1. At Day 10, 2. At Day 35, 3. At Day 70).

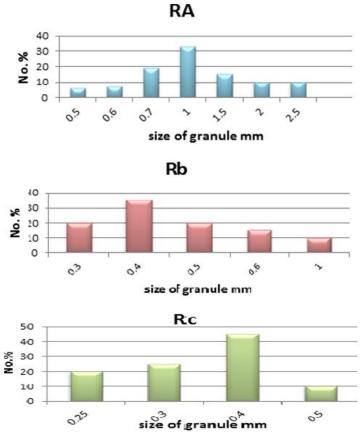


Figure 4: Size Variation of Granules in RA,RB and RC at the Conclusion of the Operational Phase.

ISSN: Printed: 3078-9656, Online: 3078-9664, paper ID: 69

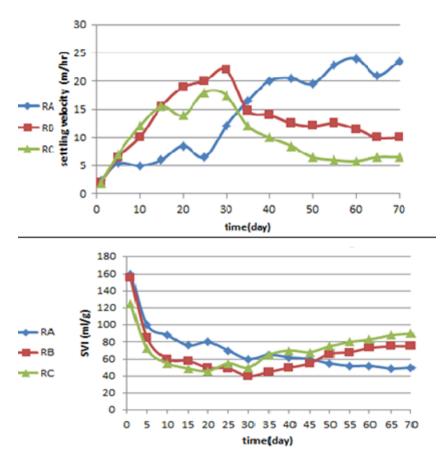


Figure 5: SVI Measurements and Settling Speeds in RA, RB, and RC Over the Course of the Operation.

3.2 Removal of COD

Figure (6) display the variations in influent and effluent COD across the reactors RA, RB, and RC. During the first 30 days, both RB and RC show cased significant COD removal efficiencies, with RB's efficiencies ranging from 68% to 90% for effluent levels of 157-49 mg/l, and RC's efficiencies ranging from 71% to 88% for effluent COD levels of 142-58.5 mg/l. In contrast, RA showed a more gradual improvement in COD removal efficiency during the same time, ranging from 55% to 82% for effluent values of 220-88 mg/l. The quicker granule formation in RB and RC, when compared to RA, was influenced by their elevated salt concentrations, promoting faster granule aggregation in these reactors.

Between days 35 and 70, the trends varied. RA saw an enhanced COD removal efficiency, peaking at 94%, attributed to granule formation. On the other hand, RC's efficiency receded to 68%, mainly due to its heightened salinity, which elevated osmotic pressures, making conditions less conducive for several bacterial species. Meanwhile, RB maintained a steady removal efficiency of 82% by the end of the 70-day operational span. The observations suggest that salt concentrations exceeding 5000 mg/l influence the efficiency of COD oxidizers, specifically heterotrophic bacteria. These observations align with the findings of Leiro in 2011, which highlighted that wastewater treatments containing salt concentrations of 5000 mg/l and 10000 mg/l led to decreases in COD removal efficiency by approximately 5% and 10.5% respectively [Leiro, 2011]. Additionally, Winkler et al. found that increased salt concentrations initially caused granules to float, which resulted in a decrease in settling velocity and biomass because of sludge washouts [13,14].

ISSN: Printed: 3078-9656, Online: 3078-9664, paper ID: 69

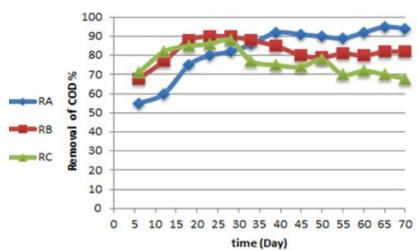


Figure 5: Efficiency Trends of COD Removal in RA, RB, and RC Throughout the Operational Duration.

3.3 NH4-N removal

The performance trends for NH4-N removal in the reactors, as illustrated in Figure 6, revealed that during the initial 30 days, all reactors, RA, RB, and RC, experienced increased NH4-N removal efficiency as the granulation process developed. By day 45, RA's efficiency in NH4-N removal had surged to a peak value of 94%, which translates to an effluent NH4-N concentration of 1.54 mg/l. In contrast, RB and RC displayed diminished NH4-N removal efficiency due to increased granule degradation, resulting in efficiencies of 70% and 58%, respectively.

In the duration between days 45 to 70, RA and RB's NH4-N removal efficiencies remained relatively stable, culminating at 91% and 75% by the end of the period, corresponding to effluent NH4-N concentrations of 2.2 mg/l and 6.2 mg/l respectively. However, RC experienced a steady decline, finishing with a removal efficiency of 52%, which corresponds to an effluent concentration of 12 mg/l.

This suggests that the lower salt concentration in RA (2000 mg/l) didn't negatively impact the biological activity of nitrobacter bacteria (responsible for ammonium oxidation). In contrast, the higher salt concentration in RC (10000 mg/l) seems to have suppressed ammonium oxidizer activity, thereby impeding the nitrification process. Additionally, as depicted in Figure (7), salt penetration into RC's granules appeared to reduce the population of denitrifies bacteria. These bacteria are pivotal for the denitrification process, which when inhibited, leads to nitrogen build-up and a decline in TN removal. By the operation's end, RA had achieved an 80% TN removal rate, whereas RB and RC were at 36% and 20%, respectively. The current results were confirmed by Leiro et al., research which also offers relevant insights. While treating fish canning wastewater containing 10g of salt, 3.2mm granules were formed in the first 75 days of operation. However, these granules deteriorated after 350 days, with declining in the TN removal rate around 26% [18,19] Moreover, Pronk et al. highlighted that elevating salinity to 20g can lead to the total inhibition of nitrate removal [14].

But they were contradictory with Qiulai, and et. al. findings which presented that removal process of nitrogen compounds is unaffected when salt concentration rise up to 20000 mg/l [12]

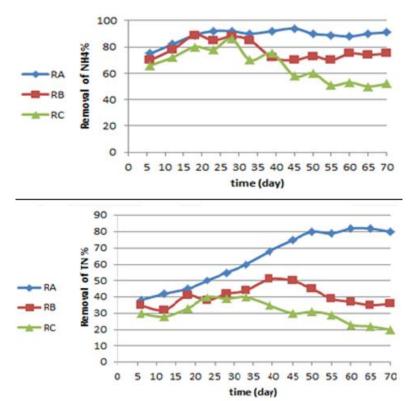


Figure 7: Ammonium Nitrogen (NH4-N) Elimination and Total Nitrogen (TN) Reduction Rates in RA, RB, and RC Throughout the Operational Phase.

3.4 Microbiology observation

Salt concentration clearly influenced AGS characteristics and the efficiency of removal, as indicated by earlier findings. A significant impact of salt on the microbial populations of AGS was evident from microscopic and SEM imagery. Figure (8) reveals that a minimal salt concentration in RA didn't severely disrupt the density of microbial communities, but it did shift the species dynamics. Rather than observing the typical protozoa and ciliates, a high presence of rotifers and some fungal filaments was noted, suggesting that these organisms might be more resilient to salinity than others[20]. An abundance of rotifers on the granular surface might contribute to a higher consumption rate of substrate and oxygen on the granular surface . which could explain the smaller granule sizes in RA, often being less than 2.5mm. The SEM image labeled 2 provides further details, indicating the presence of some extracellular polymeric substances (EPS) and bacterial clusters of cocci and rod shapes enveloping the granule's exterior. Among these, a large proportion consisted of Nitrosomonas and Nitrobacter ammonium oxidizing bacteria (AOB) as well as heterotrophic bacteria. Increasing the salt concentration in RB to 5000 mg/l led to a noticeable decline in parasitic microorganisms on the granular surface. This observation is evident in RB, which displays only a sparse presence of rotifers. Moreover, SEM imagery illustrates the abundant salt crystals that accumulate over the granular surface. In contrast, reactor RC-1 reveals that with its heightened salinity of 10000 mg/l, resulted in the complete absence of rotifers. The only visible microorganisms were fungal filaments and yeast, which are known to be halophilic, meaning they thrive in high salt environments and can resist challenging conditions [11,20]. RC -2 and RB-2 provide insights into how internal salt accumulation in granules might have led to the disappearance of denitrifying bacteria, and the consequent nitrogen buildup resulting in granular degradation. Hence, high salinity levels have been identified as factors that can restrict and modify the microbial diversity within AGS. This observation aligns with the findings of Moussa et al., who highlighted that salinity impacts the biological function of nitrifying bacteria, ultimately slowing down ammonium oxidation [4]. Additionally, it's recognized that rising salinity levels can affect biomass structure, settling attributes, solubility, and oxygen movement within the liquid phase [13,20].

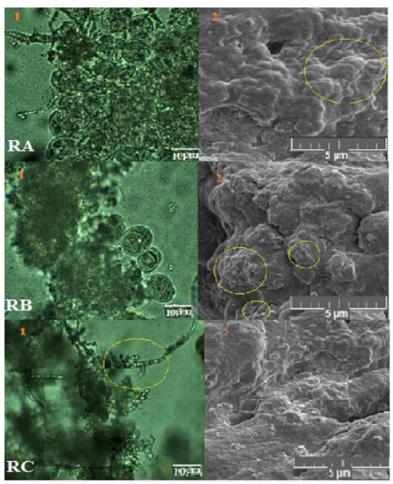


Figure 8: Granule Surface Analysis in RA, RB and RC: (1. View through Optical Microscopy 2. SEM Imaging).

4. CONCLUSION

Elevated salinity levels in wastewater influent have been observed to adversely affect the structural integrity of aerobic granular sludge. This is primarily due to the creation of a hostile environment that hinders the growth of various crucial microorganisms. As a result, there's a noticeable decline in the removal efficiency of organic materials and essential nutrients. Specifically, as salinity levels surged from 2000 mg/l to 10000 mg/l, we witnessed a drop in removal efficiencies: from 94% to 68% for COD, from 91% to 52% for NH4, and from 80% to 20% for TN. Additionally, a rise in salt concentration not only diminished the settling velocity of the granules but also escalated the SVI values, ranging from 50 to 90 ml/g. Consequently, managing and moderating salt concentrations in wastewater is of paramount importance to maintain the efficacy of treatment processes.

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BIOGRAPHIES OF AUTHORS (10 PT)

The recommended number of authors is at least 2. One of them as a corresponding author.

Please attach clear photo (3x4 cm) and vita. Example of biographies of authors:

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