International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-12, Issue-1; Jan, 2025
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.121.1

# Combined effects of ultrasound power and DO on nitrogen removal in a low-intensity ultrasound (LIU)-assisted sequencing batch biofilm reactor

Onwuagbu Chukwubuike Chiemelie, Dr. Xin Zhou*, Okeke Ikechukwu Shadrack, Paul Afreh

College of Environmental Science and Engineering, Taiyuan University of Technology, Taiyuan 030024, China.
Dr. Xin Zhou: (Corresponding Author): zhouxin@tyut.edu.cn
Okeke Ikechukwu Shadrack: ikechukwuprime@163.com
Paul Afreh: luoaifei0030@link.tyut.edu.cn

Received: 03 Dec 2024,

Receive in revised form: 08 Jan 2025,

Accepted: 15 Jan 2025,

Available online: 19 Jan 2025



***Keywords*— Batch biofilm reactor; Ultrasound; Ammonia; Nitrification; Biofilm; ANAMMOX**

***Abstract*—*Ultrasonication is a sustainable biophysical technology used in sludge treatment. Studies show it enhances microbial activity and pollutant removal from wastewater under optimal conditions. This study examines ultrasonic irradiation's effect on nitrogen removal efficiency and extracellular polymeric substances (EPS) formation in Sequencing Batch Biofilm Reactors (SBBRs). Four reactors with different ultrasonic powers (0 W, 180 W, 270 W, and 360 W) and dissolved oxygen (DO) concentrations were tested to explore the interaction between ultrasonic treatment and nitrogen transformation. Results show that moderate ultrasonic power (180 W and 270 W) significantly improves ammonia nitrogen and total nitrogen (TN) removal rates, reaching up to 99.4% and 91.7% at a 2 mg/L DO concentration. Higher power (360 W) increased EPS production, especially tightly bound EPS, enhancing biofilm stability and microbial protection, but did not improve nitrogen removal. Thus, balancing ultrasonic intensity and DO is crucial for optimal performance. Microbial analysis indicates ultrasonic treatment alters microbial diversity, promoting species and aiding nitrification and denitrification. This study shows that controlled ultrasonic irradiation can enhance SBBR efficiency by adjusting microbial activity and biofilm structure, improving wastewater treatment.*

## HIGHLIGHTS

- High-power ultrasound was utilized to aid in the nitrogen removal in an SBBR.
- AOB and ANAMMOX bacteria grow at higher ultrasonic power.
- *Ca. Kuenenia* was enriched with an increase in ultrasonic power.
- Changes in EPS highly corresponded with ultrasound irradiation.
- The mechanism of anammox enhancement in SBBR through high-power ultrasound was proposed.

## I. INTRODUCTION

Recently, Researchers have known many types of biological ways of treating wastewater, such as sequencing batch reactor activated sludge, bio-film reactor, anoxic/oxic systems, anaerobic/anoxic/oxic processes, cyclic activated sludge technology, oxidation ditch, and

adsorbendum biodegradation (Zhang et al., 2022). However, these methods also face challenges such as inadequate nitrogen removal, high power consumption, and poor environmental adaptability, which leads to noncompliant discharge effluent with stringent regulations and sustainability strategies (Qu et al., 2019). Thus, more research and instruction of these and other new procedures is needed to get over these limits and to guarantee the productive and maintainable treatment.

Sequencing batch biofilm reactors (SBBRs) can simultaneously remove nitrogenous chemicals by intrinsic nitrification and denitrification as the system operates in a fill-and-draw manner (Iaconi et al., 2002; Prendergast, 2005). Key factors that influence SBBR functionality and are widely studied for the enhancement of nitrogen removal efficiency are hydraulic retention time, aeration flow, and biofilm density (Ding et al., 2011; Wang et al., 2015; Xiang et al., 2016). Gains in treatment efficiency and cost reduction have been achieved through the application of intelligent control systems and the use of fibrous carriers for biofilm adhesion (Ding et al., 2011; Yuan, 2014). Despite SBBRs showing tolerance to different salinities (She et al., 2016), handling recalcitrant chemicals and lowering nitrous oxide emissions remain issues (Chen et al., 2021; Xiang et al., 2016). The integration of advanced oxidation techniques, such as ultrasonic irradiation, holds promise for further enhancing nitrogen removal efficiency.

Ultrasound, which consists of sound waves above 20 kHz, has been known to possess remarkable directional and penetrative capacity (Zhang et al., 2022). Ultrasonication is an eco-friendly method for sludge treatment that enhances biological activity and pollutant extraction from wastewater (Tyagi et al., 2014). It promotes gas, liquid, and oxygen transport (Chisti, 2003) while reducing mass transfer resistance through local turbulence (Rokhina et al., 2009). This process increases cell membrane permeability and accelerates metabolism and growth. When calibrated correctly, ultrasonic intensity can create beneficial ruptures in cell walls without irreversible damage (Sinisterra, 1992). Low-intensity ultrasound modifies cell membrane structures, decreasing the viscosity of phosphodiester bilayers and improving enzyme activity (Lin & Wu, 2002). It destabilizes the extracellular polymeric substance (EPS) matrix, boosting EPS concentration and protein synthesis while enhancing cell permeability. This facilitates the release of intracellular enzymes, improving treatment efficiency, although excessive intensity may lead to cell damage and reduced viability (Liu et al., 2003).

Ultrasonication generates shear stresses from cavitation bubbles, which improve enzyme-substrate interactions and accelerate enzymatic processes. When applied appropriately, low-intensity ultrasound can promote cell growth and enhance enzyme activity. Conversely, prolonged exposure risks damaging cells and potentially leading to cell death. Research indicates that ultrasonic treatment effectively reduces chemical oxygen demand (COD) and ammonium nitrogen (NH4+-N) levels in wastewater (Tian et al., 2021). For instance, ultrasonic recirculation has been successfully employed to process excess sludge, meeting established effluent standards. Hong-Cui (2012) reported that initial ultrasonic pretreatment of 8-hydroxyquinoline wastewater achieved a 40.4% reduction in CODcr.

A two-stage aerobic biochemical treatment achieved a 99.4% elimination of total Chemical Oxygen Demand (CODcr). Low-intensity ultrasound improved COD removal of low-temperature SMBR sewage treatment, being the most effective intensity 0.27 W/L after 20 min. For $NH4^{+}$-N removal, exposure to ultrasound for 15 min was ideal, demonstrating the necessity of striking the right balance between power density and power duration (Ding et al., 2011). Ultrasound enhances COD and nitrogen removal rates (Wünsch, 2004; Zhang et al., 2011) and promotes cell proliferation and enzyme activity (Biradar et al., 2010; Liu et al., 2007) when used with biological treatments. The present study dealt with the coupling technology of ultrasonic irradiation and Sequencing Batch Biofilm Reactors (SBBRs) established in previous studies to enhance nitrogen removal via biofilm destruction and microbial activity.

This research investigates Submerged Biological Biofilm Reactors (SBBRs) exhibited with ultrasonic irradiation for the preservation of the environment. Through a three-phase experimental design, it examines the effect of high-power ultrasound on biological denitrification, structures of extracellular polymers and microbial community under various carbon-to-nitrogen (C/N) ratios and ammonia concentration levels. The study aims to optimize conditions for maximum nitrogen removal and the cultivation of beneficial microbial communities.

## II. MATERIALS AND METHODS

### 2.1 SBBR running device

Figure 1 shows the experimental running device and reactor schematic. Four parallel reactors, each with a 500 ml working volume filled 50% with PU (Polyurethane) as a biofilm carrier, were tested. Reactor 1 served as the control (no ultrasound), while reactors 2, 3, and 4 received different ultrasonic powers. Aeration was supplied via an air compression pump connected to an aeration disk at the bottom, regulated by a mass flow meter for DO control.

Temperature was maintained at 30±2°C using a water bath system.

The SBBR reactors operated in cycles: influent, aeration (11.5 h), sedimentation (20 min), and discharging (5 min) (Figure 2), with a water discharge ratio of 2/3 and cycle durations of 24 or 48 hours based on phase characteristics.

Ultrasonic irradiation was applied for 10 min during aeration. After filling, reactors R1, R2, and R3 were placed in the ultrasound chamber with the rod penetrating the water to a depth of 20 mm. Parameters like power and irradiation time were adjustable post-irradiation.

*Fig.1: Experimental device*

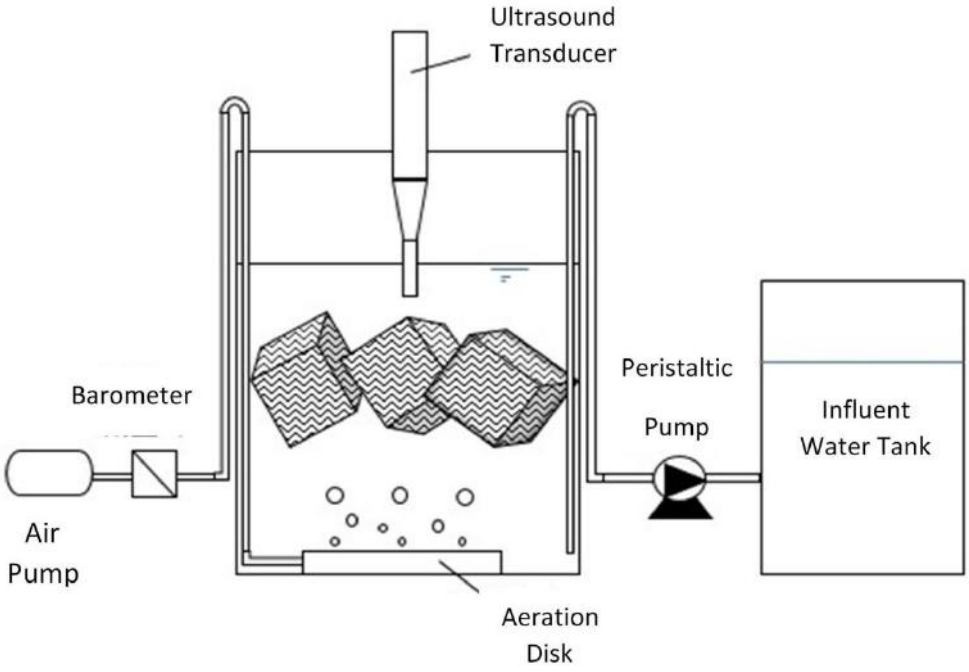



*Fig.2: SBBR scheme*

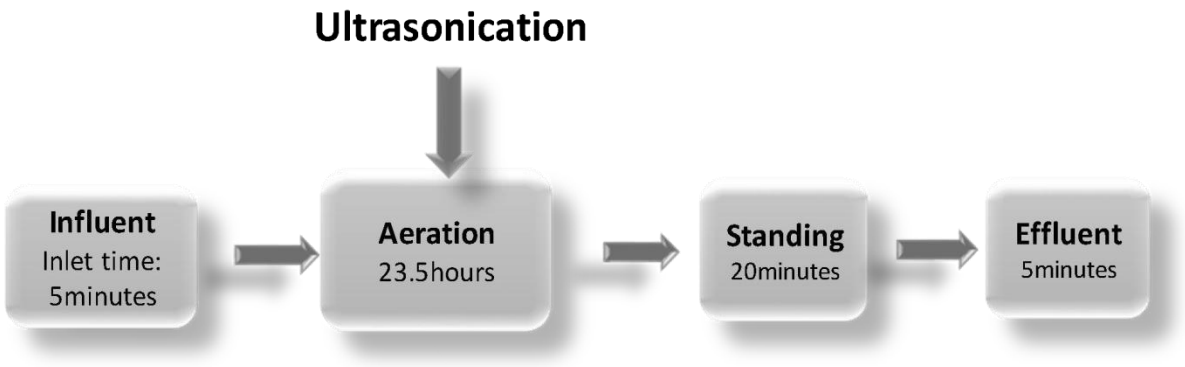



*Fig.3: Operating steps*

### 2.1.1 Running phases

Our studies, as outlined in Table 1, had three distinct running stages. The initial phase was conducted with varying influent ammonia concentrations between 200 and 400 mg/L. The second phase was conducted with varying C/N ratios, whereas the third phase was executed under conditions of elevated influent ammonia concentrations and high-power ultrasonication.

*Table 1: Details of the Running phases*

| Running Phase | Influent Ammonia (mg/L) | C/N Ratio | Ultrasound Power (W) | Irradiation time (Min) | Water Discharging Ratio | Cycle Duration |
|---|---|---|---|---|---|---|
| P1 | 200/300/400 | 3 | 0/180 | 0/10 | 3/5 | 24H |
| P2 | 300 | 2.5/1.5/2.5/3.5 | 0/180/180/180 | 0/10/10/10 | 3/5 | 24H |
| P3 | 400/600/800/1000 | 2 | 0/180/360/540 | 0/10/10/10 | 3/5 | 48H |

### 2.2 Sludge inoculation and influent water

The feed sludge for this experiment was sourced from the SBR aeration tank of a wastewater treatment plant in Shanxi Province. After 24 hours of aeration to restore its activity, the sludge was mixed with water and PU biofilm filler for domestication, with conditions continuously monitored. Three uniform biofilm fillers were used in each of the four reactors.

Municipal water, along with glucose, NH4Cl, and KH2PO4, supplied carbon, ammonia, and phosphorus while maintaining an N/P ratio of 5:1. NaHCO3 was added to keep the reactors basic, and a nutrient solution provided essential trace elements. Anhydrous glucose served as the carbon source due to its availability.

*Table 2: Details of trace elements*

| **Trace Elements** | $CoCl_26H_2O$ | $MnSO_4$ | H3BO4 | $NiCl_26H_2O$ | $ZnSO_47H_20$ | $FeCl_3$ | $CuSO_4$ |
|---|---|---|---|---|---|---|---|
| **Contents** | 0.15 | 0.12 | 0.15 | 0.19 | 0.12 | 1.5 | 0.03 |

### 2.3 Analysis and Calculation Methods

#### 2.3.1 Analysis

The effluent NH4+-N, NO2 -N, NO3--N concentrations, and COD concentrations were all investigated and monitored based on the standards techniques. The measurements of DO concentrations and pH in the reactors were performed using a DO meter (Model 550A, YSI, USA) and a Ph meter (Mettler TOLEDO FE20-Kit), respectively.

#### 2.3.2 Calculations

2.3.2.1 The Ammonium Loading Rate (ALR) of influent was calculated by:

$$\text{NH+4-N}$$

2.3.2.2 The Nitrite Accumulation Ratio (NAR) was calculated by formula (F1) (Yingyay et al., 2014)

$$\frac{[NO_2^- - N]_{eff}}{[NO_2^- - N]_{eff} + [NO_3^- - N]_{eff}} \times$$

2.3.2.3 The Free Ammonia (FA) was calculated by the formula (F2) (Ford et al., 1980):

$$\frac{17}{14} \times \frac{[NH_4^+ - N]_{inf} \times 10^{ph}}{exp[6334/(273+T)] + 10^{ph}}$$

2.3.3 EPS Analysis

During the experiment, biofilm Extracellular Polymer Analysis was performed. The biofilms on the filler are subjected to different steps, extracting three parts of extracellular polymers from biofilms: Soluble EPS, Loosely Bound EPS, and Tightly Bound EPS.

2.3.4 Microbial Analysis

Microbial biodiversity was analyzed using high-speed sequencing tests, calculating several indices: ACE, Chao, Shannon, Simpson, and Coverage. The ACE and Chao indices assess community abundance, while the Shannon and Simpson indices measure community diversity. A lower Shannon index indicates less diversity and a higher Simpson index also reflects lower diversity. The calculation formulas for these indices are F3, F4, F5, and F6.

$$S_{chao1} = S_{obs} + \frac{n_1(n_1-1)}{2(n_2+1)} \quad \text{(F3)}$$

$$S_{ACE} = \begin{cases} S_{abund} + \frac{S_{rare}}{C_{ACE}} + \frac{n_1}{C_{ACE}}\tilde{\gamma}^2_{ACE}, for\ \tilde{\gamma}_{ACE} < 0.80 \\ S_{abund} + \frac{S_{rare}}{C_{ACE}} + \frac{n_1}{C_{ACE}}\tilde{\gamma}^2_{ACE,}\ for\ \tilde{\gamma}_{ACE} \geq 0.80 \end{cases} \quad \text{(F4)}$$

$$\text{th} = \text{t t}\left[\frac{S_{rare}}{C_{ACE}}\frac{\sum_{i=1}^{abund} i\,(i-1)n_i}{N_{rare}(N_{rare}-1)}\right]$$

$$\text{th} = \text{t t}\left[\tilde{\gamma}^2_{ACE}\left\{1 + \frac{N_{rare}(1-C_{rare})\sum_{i=1}^{abund} i\,(i-1)n_i}{N_{rare}(N_{rare}-C_{ACE})}\right\},\ 0\right]$$

$$\text{Hh} \cdot \cdot \cdot = - \quad = \text{th} - \ln \cdot -$$

$$\text{th} \cdot \cdot -$$

$$\text{Dh t h} \cdot = \quad = \quad - \quad \text{(F6)}$$

## III. RESULTS AND DISCUSSION

### 3.1 Effects of ultrasonication on nitrogen removal in SBBR systems

The ultrasonic irradiation technique involves inserting a transducer rod into a submerged biofilm reactor (SBBR) solution at a depth of 20 mm, with a polyurethane biofilm filler occupying 50% of the reactor's volume. Ultrasonic power levels were set at 0 W, 180 W, 270 W, and 360 W for reactors R1, R2, R3, and R4, respectively, while testing various dissolved oxygen (DO) concentrations.

The influent consisted of ammonia nitrogen at 50 mg/L and chemical oxygen demand (COD) at 100 mg/L, with a water-changing ratio of 2/3, a cycle duration of 12 hours, and an irradiation time of 10 minutes. Initial DO was 3.5 mg/L, leading to low ammonia nitrogen and total nitrogen (TN) removal rates but high effluent nitrate concentrations. Increasing ultrasonic power decreased nitrate levels, while higher DO levels enhanced nitrification.

Lowering DO to 2.5 mg/L and then to 2 mg/L stabilized the ammonium load, regardless of power levels. In sonicated reactors, effluent ammonia nitrogen initially rose but then declined over time. The TN removal rate peaked at 270 W in reactor R1, indicating that optimizing DO content and power input is crucial for reducing ammonium loads in wastewater treatment systems.

Starting from cycle 30, the dissolved oxygen (DO) concentration was reduced to 1.5 mg/L, resulting in a steady decline in effluent nitrate concentrations across all four reactors. Initially, the total nitrogen (TN) removal rate dropped significantly but later increased, with the highest rates found in reactors R1 (0 W) and R2 (180 W). Increasing the DO to 2 mg/L led to gradual improvements in ammonia nitrogen and TN removal rates in reactors R2 and R3. By cycle 51, reactor R2 achieved a 99.4% removal rate for ammonia nitrogen and 91.7% for TN. Lowering the DO back to 1.5 mg/L caused decreases in R1 and R4, while reactors R2 and R3 showed increases, with R3 reaching a 99.1% ammonia nitrogen removal rate. Ultrasonic irradiation enhanced ammonia-oxidizing bacteria (AOB) activity and suppressed nitrite-oxidizing bacteria (NOB) activity, particularly under high concentrations and low temperatures. Optimal conditions for removal were established at 180 W of ultrasonic power, 10 minutes of irradiation, and a DO of 2 mg/L.

By cycle 6, nitrite accumulation rates in reactors R0 and R1 rose to 45% and 49%, respectively, increasing to over 73% by cycle 15 with ultrasonic treatment. In the third stage, at an ammonia concentration of 300 mg/L, the nitrite accumulation rate in the R1 sonicated reactor peaked at 94.4%. The average nitrite accumulation rate

was 39.02% in the R0 control reactor compared to 82.99% in R1, with R1 consistently outperforming R0 at various ammonia concentrations. The high nitrite accumulation and low nitrate levels resulted from incomplete nitrification and insufficient nitrite conversion. The TN removal rate increased during startup, with notable efficiencies of 93.59% in R0 and 99.07% in R1 at 200 mg/L influent ammonia. Ultrasonic irradiation also enhanced assimilable organic carbon (AOC) activity under high ammonia levels, with the effects being more significant under challenging conditions.

The nitrogen removal performance in both reactors was notable, with the sonicated reactor outperforming the control reactor. During the startup phase, the highest total nitrogen (TN) removal rates were 85.42% for the control reactor (R0) and 84.21% for the sonicated reactor (R1). After seven cycles, the TN removal rates were 75.45% for R0, 71% for R1, 64.16% for R2, and 61.75% for R3. Gradual increases in ammonia concentration benefit TN removal; however, excessive ammonia can harm microorganisms. A rise in ammonia nitrogen may enhance the effect of ultrasound on ammonia-oxidizing bacteria (AOB).

Figure 4 indicates that nitrogen transformation processes, such as nitrification and denitrification, are influenced by dissolved oxygen (DO) concentrations and power levels, highlighting the importance of optimizing these parameters for efficient nitrogen removal in wastewater treatment. Additionally, microbial populations involved in nitrogen cycling are sensitive to oxygen availability and other environmental factors, which can be adjusted to achieve lower ammonium and nitrate levels.

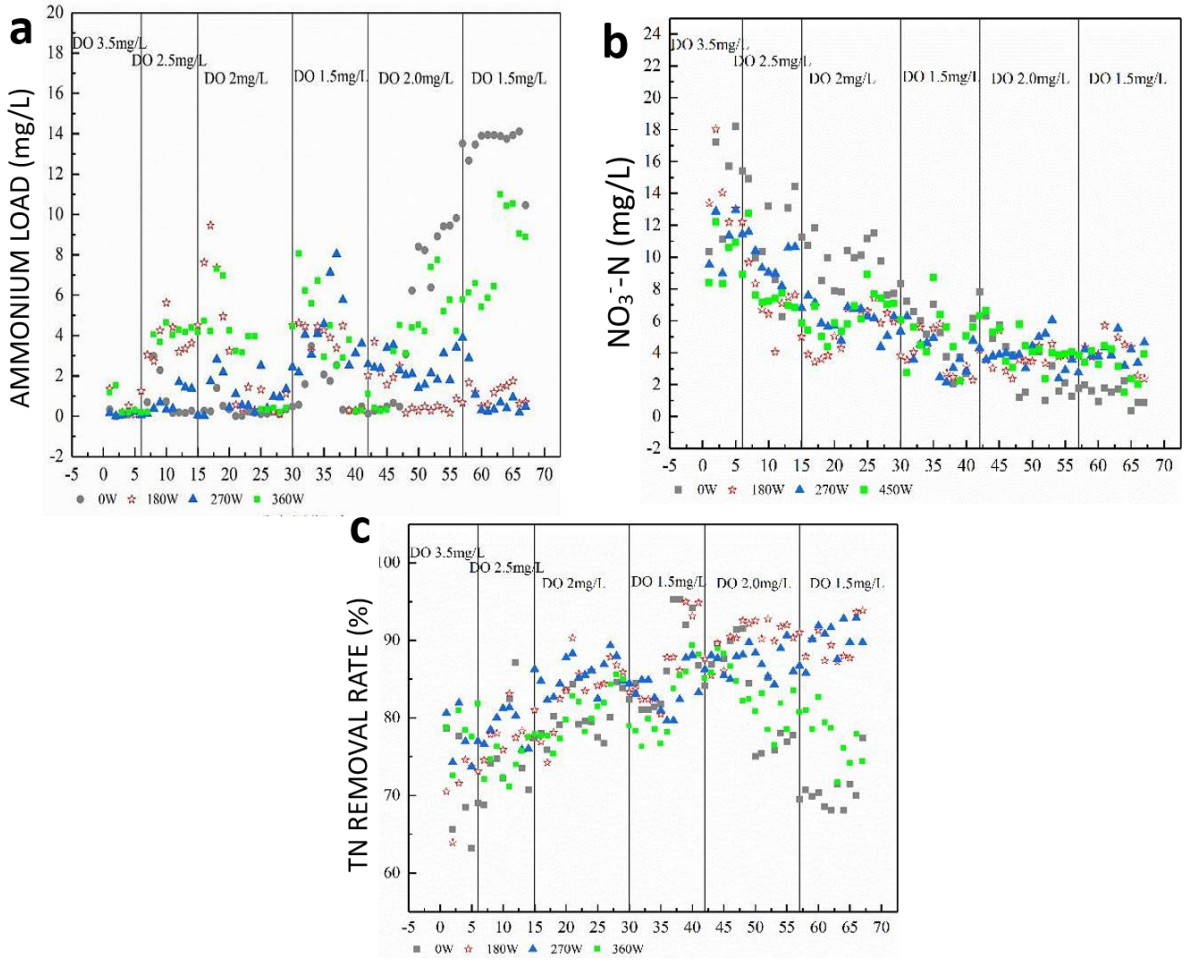



*Fig.4 (a) Effluent $NH_4^+$-N concentration; (b) Effluent $NO_3^-$-N concentrations; (c) TN removal rate.*

### 3.2. Effects of ultrasound on extracellular polymer Substances (EPSs)

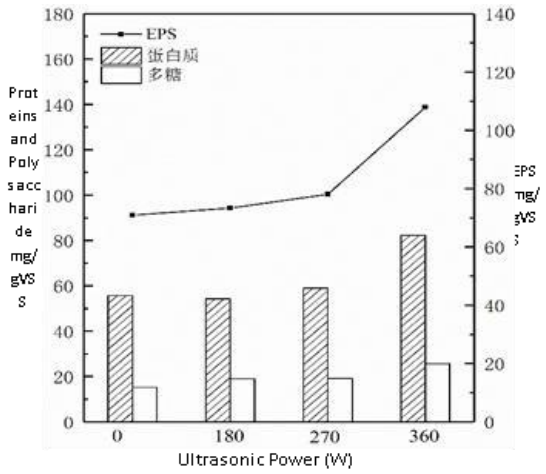
Extracellular Polymer Substance (EPS) as an organic substance secreted by microorganisms consists of carbohydrates, proteins, nucleic acids, and humic acids. Microbial survival, adhesion, and aggregation (Xiao et al., 2010; Ding et al., 2011). EPS is divided into soluble EPS (S-EPS), loose EPS (LB-EPS) and compact EPS (TB-EPS) (Sheng et al., 2010). LB-EPS and TB-EPS facilitate adhesion and aggregation, where TB-EPS possesses

potent flocculating capacities (Malamis & Andreadakis, 2009). Extracellular polymeric substance (EPS) constitutes a significant part of the biofilm formed, is involved in all aspects of biofilm biological activity, and consists of proteins and polysaccharides (Sponza, 2003) in ultrasound-enhanced (SBBR) sequencing batch biofilm reactor (SBBR) systems.

This study examined the impact of 180W ultrasonic energy on EPS during biological nitrogen removal in SBBR systems. EPS was isolated from biofilms developed in two reactors, and differences comparing control and sonicated reactor EPS contents were performed. The concentrations of S-EPS, LB-EPS, and TB-EPS in the P1 stage of the experiments were demonstrated in Table 2.

*Table 1: EPS concentration of Biofilm*

| Reactor | EPS CONCENTRATION (mg/gVSS) | | | | | |
|---|---|---|---|---|---|---|
| | S-EPS | | LB-EPS | | TB-EPS | |
| | PN | PS | PN | PS | PN | PS |
| **R1** | 14.48 | 5.22 | 21.23 | 3.65 | 19.95 | 6.34 |
| **R2** | 10.62 | 2.19 | 20.31 | 1.82 | 23.34 | 15.02 |
| **R3** | 9.61 | 2.63 | 22.96 | 4.19 | 26.38 | 12.26 |
| **R4** | 17.70 | 7.96 | 29.22 | 5.00 | 35.36 | 12.69 |




Change of EPS and its components (DO= 2.0 mg/L

Influence of influent ammonia, carbon-to-nitrogen (C/N) ratio and water changing rate on the EPS (extracellular polymeric substance) content of biofilms in 4-stage period of P1 phase (Fig. 5) This higher EPS content in sonicated reactor (R2) compared to control reactor (R1) could be associated with increased metabolite secretion which is beneficial for Anammox activity (Duan et al., 2011). Ultrasonic treatment enhances extracellular polymeric substances (EPS) production under high ammonia conditions, suggesting that the biofilm structure was potentially damaged, and microorganisms needed to produce more EPS to protect themselves (Wang et al., 2010; Gao et al., 2017).

In R1, the S-EPS content was 48.8 mg/g VSS, while in R2, it was 49.7 mg/g VSS. Protein content was 16.77 mg/g VSS for R1 and 29.87 mg/g VSS for R2, showing R2 had more protein. R2's LB-EPS was 57.3 mg/g VSS versus 36.36 mg/g VSS for R1, supplying nutrients for anaerobic ammonia-oxidizing bacteria (Gao et al., 2017). The TB-EPS content was also higher in R2 at 73.09 mg/g VSS compared to 53.55 mg/g VSS in R1. Overall, R1 had higher polysaccharide content, while R2 had superior protein content. A decrease in EPS may suggest better living conditions for anaerobic ammonia-oxidizing bacteria (Jin, 2013).

International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-12, Issue-1; Jan, 2025
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.121.1

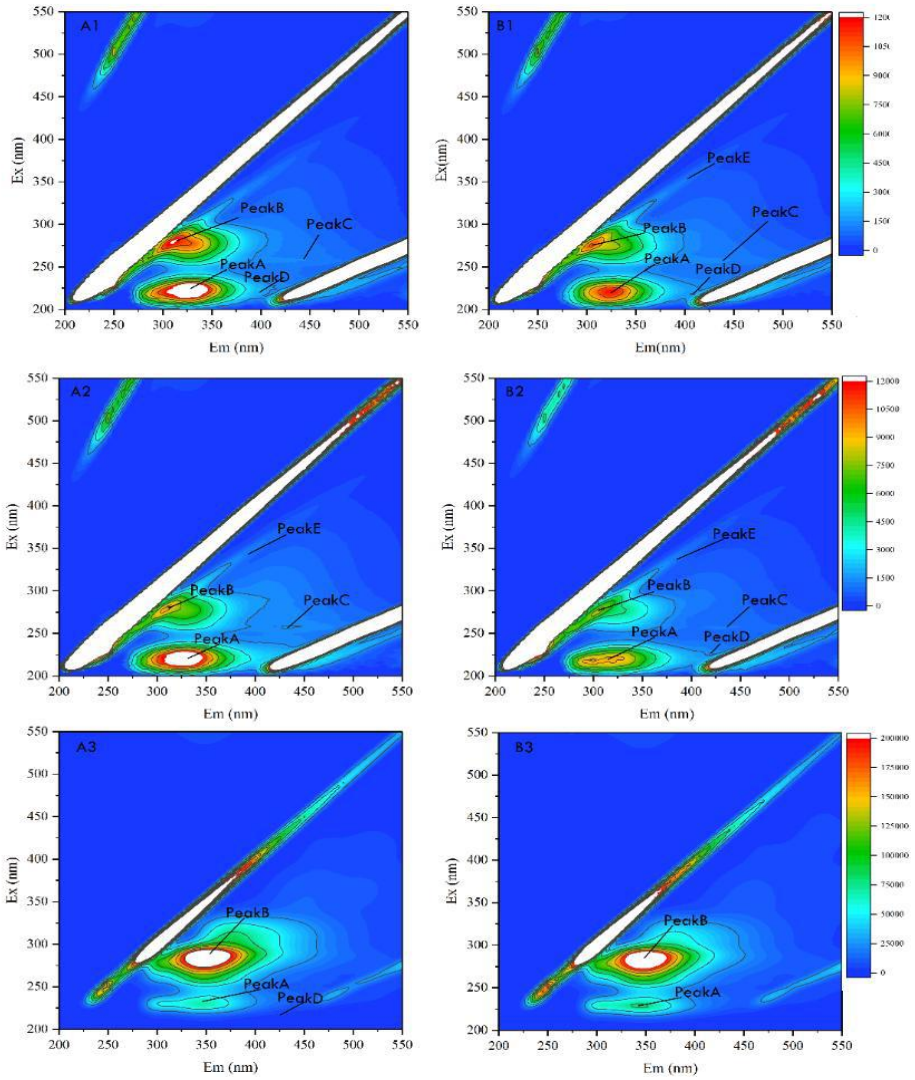



*Fig.6: 3D-EEM fluorescence spectra of EPS in R1 and R4 (A1, A2, A3. present S-EPS、LB-EPS and TB-EPS at R1/ B1, B2, B3 present S-EPS, LB-EPS and TB-EPS at R4)*

### 3.3. Ultrasonic power effects under different C/N ratios (1.5, 2.5, 3.5) on Microbial community

High-speed sequencing technology was used to analyze the microbial communities in wastewater treatment systems employing sonicated biofilm batch reactors (SBBR) with high-power ultrasound at 180W. The analysis, covering 98% of the biofilms, revealed that as the carbon-to-nitrogen (C/N) ratio increased, the Shannon index rose from 3.72 to 4.79, while the Simpson index increased from 0.03 to 0.04. The ACE and Chao1 index values for the reactors varied, with the R4 reactor having the highest Chao1 index.

At a C/N ratio of 3.5, the ACE and Chao1 indices peaked in the R3 reactor, indicating enhanced microbial richness. The microbial distribution varied significantly across reactors, mainly comprising Proteobacteria, Bacteroidetes, Planctomycetes, Acidobacteria, Candidatus Saccharibacteria, Chloroflexi, and Ignavibacteriae. Proteobacteria decreased with higher C/N ratios, particularly in the R1 control reactor compared to the sonicated R2 reactor at a C/N ratio of 2.5. In contrast,

Bacteroidetes and Candidatus Saccharibacteria increased at higher C/N ratios in the sonicated reactors, peaking at 3.5.

The study also examined the effects of varying ultrasonic powers on biofilm microbial communities. Analysis at the P1 stage showed that major genera included Ferruginibacter, Paracoccus, Simplicispira, Dokdonella, and Saccharabacteria. Proportions of Candidatus Kuenenia in reactors R1, R2, R3, and R4 were 3.08%, 1.19%, 0.35%, and 0.35%, respectively. The proportion of Ferruginibacter rose with increasing C/N ratios, while Paracoccus denitrificans, known for nitrogen loss, decreased alongside the increasing C/N ratio. In sonicated reactors, Simplicispira and Dokdonella proportions rose with higher C/N ratios, while Paracoccus denitrificans, Candidatus Kuenenia, and Nitrospira proportions declined as the C/N ratio increased.

Ammonia-oxidizing bacteria (AOB) in the four reactors were primarily Nitrosomonas, with proportions of 0.61%, 0.46%, 0.07%, and 0.26% in R1 (C/N = 2.5), R2 (C/N = 1.5), R3 (C/N = 2.5), and R4 (C/N = 3.5), respectively. The highest proportion of Nitro-Spira, a denitrifying gram-negative bacterium, was found in the R0 reactor. This indicates that the combination of the C/N ratio and high ultrasound action may inhibit AOB and nitrite-oxidizing bacteria (NOB) growth. The nitrite-removing microorganisms include AOB, NOB, denitrifying bacteria (DB), heterotrophic bacteria (HB), and anaerobic ammonia-oxidizing bacteria (AnAOB). DB utilizes nitrites and nitrates as electron acceptors to produce nitrogen.

Figure 7 shows that as the C/N ratio increased from 1.5 to 3.5, significant shifts in microbial community abundance occurred. AOB and NOB were low in number while denitrifying bacteria were abundant. ANAMMOX was also present, with Candidatus Kuenenia making up a significant portion.

**3.3.1 Functional Bacteria**

Heterotrophic nitrifying-aerobic denitrifying bacteria and facultative denitrifying bacteria coexisted in the reactors, in which most denitrifying bacteria belonged to Thermomonas, Luteimonas and Hydrogenophaga. As a Gram-negative aerobic bacterium capable of dissimilatory NO2--N reduction to N2O (Young et al., 2007), Luteimonas was involved in 5.15% of the bacterial population in reactor R4, 3.72% in control reactor, and appeared in least abundance in reactor R2. Autotrophic denitrifying hydrogen-dependent bacteria, like Hydrogenophaga, were present in all reactors, with their concentration peaking at 180W (19.50%). The control reactor predominantly composed of Azospira (Tan & Hurek, 2003), accounting for 2.13% of the total bacterial population. In addition, abundant recovery of anaerobic ammonia-oxidizing bacteria, Candidatus Kuenenia, was been identified (Wang et al., 2018).

Studies have shown that the ability of Candidatus Kuenenia to improve the competitive advantage of Anammox bacteria in sludge when subjected to ultrasound technology, leading to better ammonia removal efficiency of Anammox reactors during high nitrogen loading conditions. The proportions of Candidatus Kuenenia in reactors R1, R2, R3, and R4 were 3.08%, 1.19%, 0.35%, and 0.35%, respectively. Candidatus Kuenenia growth is inhibited at high ultrasonic power and/or at a high C/N ratio, affecting nitrogen removal. Denitrifiers were the most abundant microbial populations in the reactors (29.54%-48.5%), with higher C/N ratios resulting in a more abundant community. The control reactor R1, ANAMMOX bacteria showed the maximum activity, where almost at 2.5 level.

This ratio favors the enhanced growth of denitrifying bacteria, helping in the quick conversion of nitrate into ammonium and increasing the ammonia nitrogen amount. Besides that, an increase in Lysobacter and Ferruginibacter population at an increased ultrasonic power significantly influences other functional microbial groups. Conclusion In this respect, a higher C/N value is advantageous due to its stimulation for denitrifying bacteria.

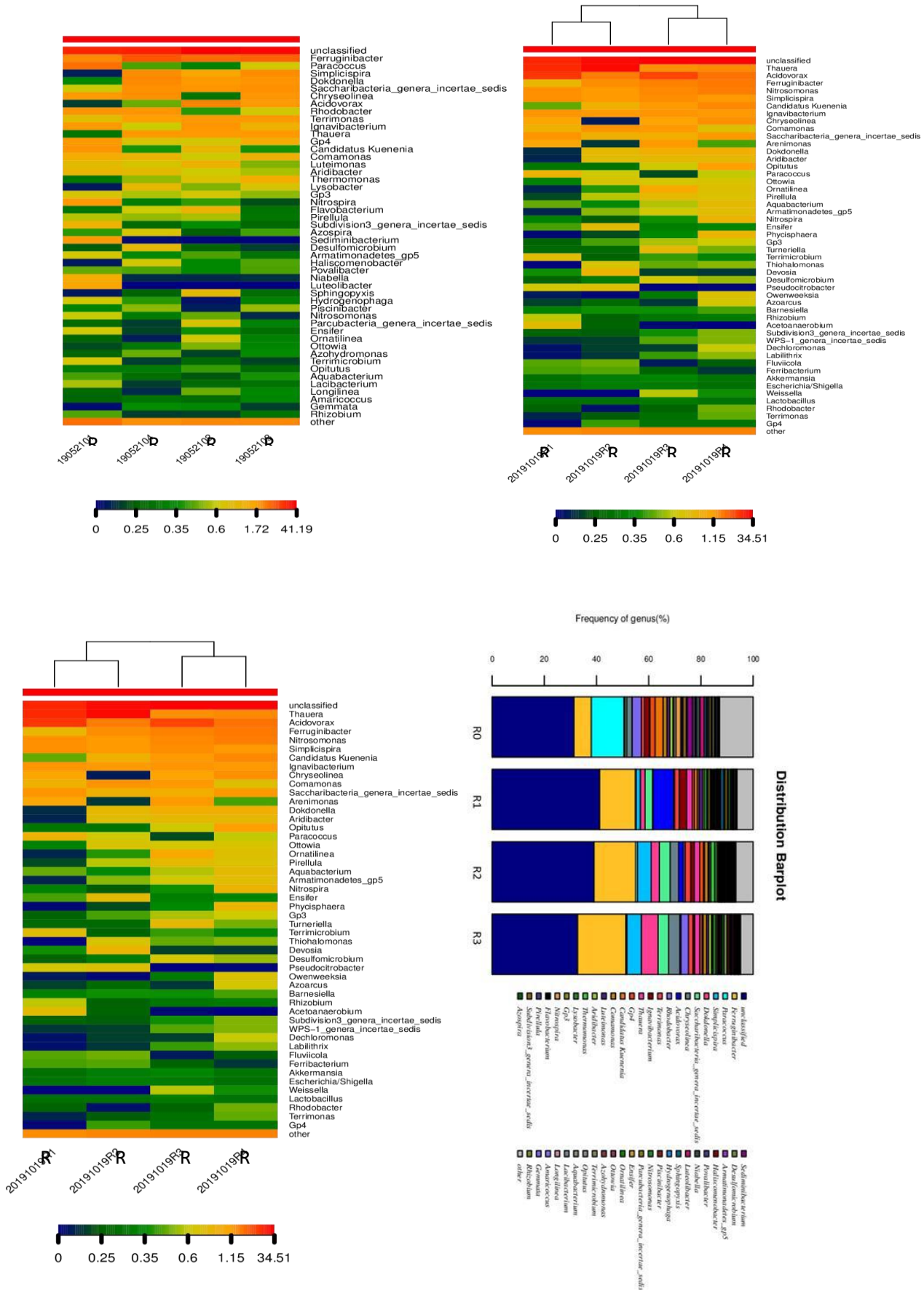



*Fig.7: Microbial Community at Genus level and Distribution Barplot*

The biodiversity of microorganisms in the Submerged Biofilm Batch Reactor (SBBR) was analyzed under various levels of ultrasound power. Significant differences in microbial composition were found across

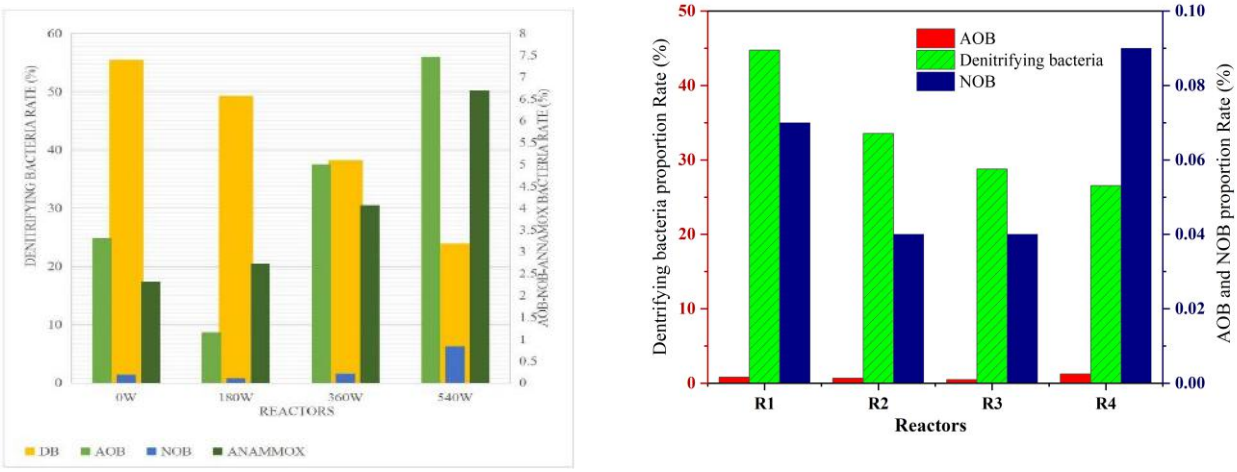
the four reactors, mainly consisting of *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Chloroflexi*, *Ignavibacteriae*, and *Verrucomicrobia.*

At the genus level, the communities were categorized into ammonia-oxidizing bacteria (AOB), nitrite-oxidizing bacteria (NOB), denitrifying bacteria (DB), heterotrophic bacteria (HB), and anaerobic ammonia-oxidizing bacteria (ANAMMOX). The AOB were primarily Nitrosomonas, with the highest proportion (7.47%) observed in the R3 reactor at 540W. *Nistrospira* dominated the NOB, also peaking in the R3 reactor. *Thauera* was the most abundant denitrifying bacteria across all reactors, and Acidovorax was also present. Increased ultrasound power led to notable changes in microbial abundance. *Paracoccus* showed the highest proportions in the control reactor, while *Candidatus Kuenenia*, a resilient ANAMMOX bacterium, exhibited greater resistance to ultrasound effects. The result demonstrates that high ultrasonic power can boost the development and multiplication of *Candidatus Kuenenia's* bacteria and raise the nitrogen removal performance of the ANAMMOX reactor under high nitrogen load working circumstances. The maximum concentration of *Acidovorax* was obtained in the control reactor. Foladori et al 2007, found that Gram-positive bacteria have a 10-15 times stronger cell wall than Gram-negative bacteria, rendering them more vulnerable to ultrasonic effects. According to Xie et al., the 2008 study observed that the species with larger cell walls may assist in the gradual enrichment of these bacteria in the reactor when ultrasound is applied.

The study analyzed relative abundances of aerobic bacteria (AOB) non-obese bacteria (NOB) and nitrite bacteria (ANAMMOX) in a sonicated reactor system. Due to the increment of the ultrasonic power, the value of AOB level also increased, maximizing in the R3 reactor at 540 W. However, NOB bacteria were normally found in low amounts, where the proportions of NOB were the highest in the R2 and R3 reactors at 360 W and 540 W, respectively. NOB can be inhibited under anaerobic conditions.

ANAMMOX bacteria, in particular, responded positively in proportion to increasing ultrasonic power, with the control reactor (R0) showing the lowest proportion and the highest proportion seen in the R3 reactor at 540 W. While the DB fraction was found to decrease as a consequence of ultrasound inhibiting DB, NOB quantities increased as ultrasonic power increased.

This resulted in promoting the growth of AOB, NOB, and ANAMMOX bacteria by 360 W and 540 W of high-power ultrasound, while inhibitory effects were observed on the growth of DB.




**A** **B**

*Fig.8: (A) Denitrifying Bacteria Rate (B) Denitrifying Bacteria Proportion Rate (%)*

## IV. CONCLUSION

The research proved that ultrasonic treatment is beneficial for nitrogen removal in SBBR systems by improving the effectiveness of nitrifying and denitrifying bacteria. Applying ultrasonic powers of 180 W and 270 W under specific dissolved oxygen conditions achieved ammonia nitrogen and total nitrogen removal rates of up to 99.4% and 91.7%, respectively. Moderate ultrasonic power improved microbial activity and the structure of the biofilm, resulting in better nitrogen removal without causing significant cellular damage.

In contrast, higher ultrasonic powers increased the production of extracellular polymeric substances (EPS), which helped protect microorganisms and maintain biofilm stability under stress; however, excessive power did not yield further improvements in nitrogen removal efficiency. The study also emphasized the interaction between dissolved oxygen concentration and ultrasonic power in shaping nitrogen transformation processes, underscoring the importance of optimal combinations of these factors for maximizing treatment efficiency. The heightened EPS production observed in ultrasonically treated reactors indicates a protective response that promotes biofilm formation and resilience.

## DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## DATA AVAILABILITY

Data will be made available on request.

## ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (NO. 21607111), Fundamental Research Program of Shanxi Province (NO. 20210302123198), the Opening Project of National Engineering Laboratory for Advanced Municipal Wastewater Treatment and Reuse Technology and State Key Laboratory of Pollution Control, Resource Reuse Foundation, Tongji University (NO. PCRRF18011) and Innovation Project for Graduate Students of Shanxi Province (NO. RC1900001671).

## REFERENCES

[1] B. Patrik Arnz, 2001. Nutrient Removal from Municipal Wastewater in Sequencing Batch Biofilm Reactors: Wasserg UTE, Munich.

[2] F. J. Erik R.Coats, 2011. Post-anoxic denitrification driven by PHA and glycogen within enhanced biological phosphorus removal: *Bioresource Technology Volume 102, Issue 2*, pp. Pages 1019-1027.

[3] Y. W. X. Y. H. X. H. T. S. S. X. T. B. W. S. Shi, 2011. Aerobic granulation for nitrogen removal via nitrite in a sequencing batch reactor and the emission of nitrous oxide: *Bioresources Technology*, vol. 102, p. 2536–2541.

[4] J. R. M. H. M. Prendergast, 2005.The efficiency of a sequencing batch reactor (SBBR) in organic carbon and phosphorus removal: *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering*, vol. 40, n° %18, pp. 1619-1626.

[5] C. Di Iaconi, A. Lopez, R. Ramadori, A. Di Pinto et R. Passino, 2004. Combined chemical and biological degradation of tannery wastewater by a periodic submerged filter (SBBR): *Water Ressources*, vol. 36, pp. 2205-2214.

[6] N. G. A. &. N. V. Majid, 2015. Ultrasonication and food technology: A review *Cogent Food & Agriculture* , vol. 1, n° %11, p. 1071022.

[7] S.-L. L. A. R. D. Vinay Kumar Tyagi, 2014.Ultrasonic Treatment of Waste Sludge: A Review on Mechanisms and Applications: *Critical Reviews in Environmental Science and Technology*, vol. 44, n° %111, pp. 1220-1288,

[8] H. Liu, 2007. Low intensity ultrasound stimulates biological activity of aerobic activated sludge: *Frontiers of Environmental Science & Engineering in China*.

[9] P. M. S. S. A.B.Pandit, 2010. Excess cell mass as an internal carbon source for biological denitrification," *Bioresource Technology*, vol. 101, no. Issue 6, pp. 1787-1791.

[10] Mahvi, A., 2009. Application of Ultrasonic Technology for Water and Wastewater Treatment: *Iranian J Publ Health*, vol. 1, p. 17.

[11] Raj B, Rajendran V, Palanichamy P, 2004. Science and technology of ultrasonics: UK.

[12] Lutz, H. T., 2016. Basics of Ultrasound: chez *Manual of diagnostic Ultrasound in Infectious Tropical Diseases*, p. 167.

[13] Zhang G, Zhang P, Gao J, Chen Y., 2008. Using acoustic cavitation to improve the bioactivity of activated sludge: *Bioresource technology*, vol. 99, n° %15, pp. 1497502.

[14] Chua SY, Adul Latif P, Ibrahim Sh., 2010. Effect of Ultrasonic Irradiation On Landfill Leachate: *Proceedings of Postgraduate Qolloquium Semester 1 2009/2010*.

[15] Thangavadivel K, Megharaj M, Mudhoo A, Naidu R., 2012. Degradation of organic pollutants using ultrasound. *Handbook on application of ultrasound: sonochemistry for sustainability. CRC Press, Taylor & Francis Group*.

[16] Sinisterra., 1992. Application of ultrasound to biotechnology an overview: *Ultrasonics*, vol. 30(3), pp. 180-5.

[17] Chisti Y., 2003. Sonobioreactors: using ultrasound for enhanced microbial productivity: *Trends in biotechnology*, p. 21(2): 89.

[18] P. V. J. Rokhina EV, 2009. Low-frequency ultrasound in biotechnology: state of the art: *Trends in biotechnology*, vol. 27(5), pp. 298-306.

[19] Y. A. W. B. C. S. A. Liu Y Y, 2003. Influence of ultrasonic stimulation on the growth and proliferation of Oryza sativa Nipponbare callus cells. *Colloids and Surfaces B: Biointerfaces*, vol. 27, p. 287– 293.

[20] Lin L D WJY. , 2002. Enhancement of shikonin production in single- and two-phase suspension cultures of Lithospermum erythrorhizon cells using low-energy ultrasound: *Biotechnology and Bioengineering*, vol. 78(1), pp. 81-8.

[21] Yu G-H, He P-J, Shao L-M, Zhu Y-S., 2008. Extracellular proteins, polysaccharides and enzymes impact on sludge aerobic digestion after ultrasonic pretreatment: *Water Research*, vol. 42(8–9), pp. 1925-34.

[22] Yang fei., 2007. Experimental study on low intensity ultrasonic enhanced biological treatment of sewage: chongqing.

[23] Yan Yixin, Liu Hong., 2006. Optimal selection of ultrasonic irradiation cycle in low intensity ultrasonic enhanced biological treatment of wastewater: *Environmental science*, vol. 27(5), p. 898.

[24] Tang Xin, Qiao Sen, Zhou Qeng., 2017. Effects of low intensity ultrasound on the activity of short-range nitrification sludge: *Journal of safety and environment,* vol. 01, pp. 267-72.

[25] Liu Y, Yoshikoshi A, Wang B, Sakanishi A., 2003. Influence of ultrasonic stimulation on the growth and proliferation of Oryza sativa Nipponbare callus cells: Colloids and Surfaces. *Bio interfaces*, vol. 27(4), pp. 287-93.

[26] Zheng M, Liu Y C, Xin J, …2015. Ultrasonic Treatment Enhanced Ammoniaoxidizing Bacterial (AOB) Activity for Nitritation Process. *Environmental Science & Technology,* vol. 50 (2), pp. 864-871.

[27] Zheng M, Liu Y C, Xu K N., 2013. Use of low frequency and density ultrasound to stimulate partial nitrification and simultaneous nitrification and denitrification. *Bioresource technology,* pp. 146: 537-542.

[28] Zhang R, Jin R, Liu G, Zhou J, Li CL., 2011. Study on nitrogen removal performance of sequencing batch reactor enhanced by low intensity ultrasound. *Bioresource technology,* vol. 102(10), pp. 5717-21.

[29] Liu H, He Y-h, Quan X-c, Yan Y-x, Kong X-h, Lia A-j., 2005. Enhancement of organic pollutant biodegradation by ultrasound irradiation in a biological activated carbon membrane reactor. *Process Biochemistry* , vol. 9, pp. 3002-7.

[30] Tan Jiangyue, Xu Zhiqiang., 2013. Study on treatment of coking wastewater by SBR method with low intensity ultrasound. *Environmental science and technology,* vol. 11, pp. 117-20 29.

[31] H. L. Xie B, 2010. Enhancement of Biological Nitrogen Removal from Wastewater by Low Intensity Ultrasound. *Water Air & Soil Pollution*, Vols. %1 sur %2211(1-4), pp. 157-163.

[32] Yan Yixin, Liu hong., 2006. Optimal selection of ratio of ultrasonic irradiation sludge in low intensity ultrasonic enhanced wastewater biological treatment. *Environmental science*, vol. 27, p. 908.

[33] L. G. S. H. Z. Q. P. Tan Jingjing, 2015. Effect of ultrasonic cytolysis on denitrification and dephosphorization system: *Municipal technology,* vol. 05, p. 122.

[34] Y. Z.-Y. I. H.-C. Li zhan-chen, 2012. Experimental study on degradation of 8-hydroxyquinoline wastewater by ultrasonic-biochemical method. *Sichuan environment*, vol. 31 (2), pp. 11-5, 2012.

[35] C. Y. J. W. Z. Z. J. Ding chi, 2014. Study on ultrasonic parameter optimization in SMBR treatment with low intensity ultrasonic. *Environmental science and management*, vol. 39 (5), pp. 88-91.

[36] C. H. Z. J. J. Y. L. Q. J. R. Yu JJ, 2013. Enhancement of ANAMMOX activity by low intensity ultrasound irradiation at ambient temperature. *Bioresource technology,* vol. 142, pp. 693-6, 2013.

[37] Yu T, Qi R, Li D, Zhang Y, Yang M., 2010. Nitrifier characteristics in submerged membrane bioreactors under different sludge retention times. *Water Research*, vol. 44, n° %19, pp. 23-30.

[38] Jin R, Liu G, Li C, Xu R, Li H, Zhang L, et al., 2013. Effects of carbon-nitrogen ratio on nitrogen removal in a sequencing batch reactor enhanced with low-intensity ultrasound. *Bioresource technology,* vol. 148:1, pp. 28-34.

[39] Tang Xin., 2015. Treatment of ammonia nitrogen wastewater by single stage autotrophic nitrogen removal process promoted by low intensity ultrasound. *Dalian university of technology, Dalian*.

[40] Xie B, Wang L, Liu H., 2008. Using low intensity ultrasound to improve the efficiency of biological phosphorus removal. *Ultrasonics sonochemistry,* , vol. 15(5), pp. 775-81.

[41] Li Na, Xie Beizhen, Liu Hong., 2010. Treatment effect of A2 / O process enhanced by low intensity ultrasound. *Proceedings of 2010 China renewable energy technology development conference. Beijing* , pp. 2781-4.

[42] Wünsch B, Heine W, Neis U., 2002.Combating bulking sludge with ultrasound.

[43] Yang shaoqiang, Xie Beizhen, Liu Hong., 2007. Low intensity ultrasonic enhanced aerobic digestion of expanded sludge. *Water and drainage*, vol. 33 (s1) , pp. 50-3.

[44] Yingyan Y, Ping L, Jinhua W, 2014. $N_2O$ emission in short-cut simultaneous nitrification and denitrification process: dynamic emission characteristics and succession of ammonia-oxidizing bacteria. *Water Science & Technology,* vol. 69, n° %112, pp. 2541-2547.

[45] Ford D L, Churchwell R L, Kachtick J W., 1980. Comprehensive Analysis of Nitrification of Chemical Processing Wastewaters. *Journal*, vol. 52, n° %1(11) , pp. 2726-2746.

[46] Hong L, Xin Y Y., 2008. Enhancement Effect of Low Intensity Ultrasound on Biological Wastewater Treatment System in Low Temperature and Design of Application on Biological Wastewater Treatment. *Environmental Science*, vol. 29(3), pp. 721725.

[47] Xiao-Meng L, Guo-Ping S, Hong-Wei L., 2010. Contribution of extracellular polymeric substances (EPS) to the sludge aggregation. *Environmental Science & Technology,* vol. 44(11), pp. 4355-4360.

[48] Ding D, Feng C, Jin Y, et al., 2011. Domestic sewage treatment in a sequencing batch biofilm reactor (SBBR) with an intelligent controlling system. *Desalination*, vol. 276(1), pp. 260-265.

[49] Sheng G P, Yu H Q, Li X Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. *Biotechnology Advances*, vol. 28(6), pp. 882-894.

[50] Malamis S, Andreadakis A., 2009. Fractionation of proteins and carbohydrates of extracellular polymeric substances in a membrane bioreactor system. *Bioresource technology,* vol. 100(13), pp. 3350-3357.

[51] Sponza D T., 2003. Investigation of extracellular polymer substances (EPS) and physicochemical properties of different activated sludge flocs under steady-state conditions. *Enzyme & Microbial Technology,* vol. 32(3), pp. 375-385.

[52] Duan X, Zhou J, Qiao S, et al., 2011. Application of low intensity ultrasound to enhance the activity of anammox microbial consortium for nitrogen removal. *ioresource technology,* vol. 102(5), pp. 4290-4293.

[53] Wang F, Ji M, Lu S., 2010. Influence of Ultrasonic Disintegration on the Dewaterability of Waste Activated Sludge. *Environmental Progress,* vol. 25(3), pp. 257-260.

[54] Gao X, Xu H, Chen W et al., 2017. Influence of extracellular polymeric substances (EPS) treated by combined ultrasound pretreatment and chemical re-flocculation on water treatment sludge settling performance. *Chemosphere,* vol. 170, pp. 196-206.

[55] Jin R C, Ma C, Yu J J., 2013. Performance of an Anammox UASB reactor at high load and low ambient temperature. *Chemical Engineering Journal*. vol. 232(10), pp. 17-25.

[56] S. Fudala-Ksiazek, A. Luczkiewicz, K. Fitobor, and K. Olanczuk-Neyman, 2014. Nitrogen removal via the nitrite pathway during wastewater co-treatment with ammonia-rich landfill leachates in a sequencing batch reactor. *Environmental Science and Pollution Research International,* vol. 21, n° %112, p. 7307–7318.

[57] Zhou Y, Oehmen A, Lim M, Vadivelu V., 2011. The role of nitrite and free nitrous acid (FNA) in wastewater treatment plants.,» *Water Ressources,* vol. 45, n° %115, pp. 4672-82.

[58] Wood PM., 1986. Nitrification as a bacterial energy source. *Special Publications of the Society for General Microbiology,* vol. 20, pp. 39-62.

[59] Li Z, Chang Q, Li S, 2017. Impact of sulfadiazine on performance and microbial community of a sequencing batch biofilm reactor treating synthetic mariculture wastewater. *Bioresource technology,* vol. 235(3), pp. 122-130.

*[60]* Yuan L, Huang Z, Ruan W, et al., 2015. ANAMMOX performance, granulation, and microbial response under COD disturbance. *Journal of Chemical Technology & Biotechnology,* vol. 90(1), pp. 139-148.

[61] Barbara Baumann, Mario Snozzi, Alexander J. B. Zehnder, Jan Roelof Van Der Meer, 1996. Dynamics of Denitrification Activity of Paracoccus denitrificans in Continuous Culture during Aerobic-Anaerobic Changes. *Journal of Bacteriology,* vol. 178, n° %115.

[62] Stouthamer A H, Boer A P N D, Oost J V D, et al., 1997. Emerging principles of inorganic nitrogen metabolism in Paracoccus denitrificans and related bacteria. *Antonie Van Leeuwenhoek,* Vols. %1 sur %271(1-2), pp. 33-41.

[63] Neef A, Zaglauer A, Meier H, et al., 1996. Population analysis in a denitrifying sand filter: conventional and in situ identification of Paracoccus spp. in methanol-fed biofilms, *Applenvironmicrobiol,* vol. 62(12), pp. 4329-4339.

[64] Koerner, H., 1993. Anaerobic expression of nitric oxide reductase from denitrifying Pseudomonas stutzeri. *Arch. Microbiol.* , vol. 159, p. 410–416.

[65] Koerner, H., and W. G. Zumft., 1989. Expression of denitrification enzymes in response to the dissolved oxygen level and respiratory substrate in continuous culture of Pseudomonas stutzeri. *Appl. Environ. Microbiol.*, vol. 55, p. 1670–1676.

[66] Mcilroy S J, Starnawska A, Starnawski P, et al., 2016. Identification of active denitrifiers in full-scale nutrient removal wastewater treatment systems. *Environmental Microbiology,* vol. 18(1), pp. 50-64.

[67] Shih-Yi S, Nian-Tsz C, Arun A B, et al., 2010. Terrimonas aquatica sp. nov., isolated from a freshwater spring. *International Journal of Systematic & Evolutionary Microbiology,* vol. 60(12), pp. 2705-2709.

[68] Gonzalez-Silva B M, Jonassen K R, Bakke I., 2016. Nitrification at different salinities: Biofilm community composition and physiological plasticity. *Water Research,* n° %195: 48, 2016.

[69] Nielsen M, Bollmann A, Sliekers O., 2010. Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. *Fems Microbiology Ecology,* vol. 51(2), pp. 247-256.

[70] Weidong W, Weiyue L, Di W, et al., 2019. Differentiation of nitrogen and microbial community in the littoral andlimnetic sediments of a large shallow eutrophic lake (Chaohu Lake, China). *Journal of Soils and Sediments,* vol. 19, p. 1005–1016.

[71] Qin Y., Han B., Cao Y. and Wang T., 2017. Impact of substrate concentration on anammox-UBF reactorsstart-up. *Bioresource Technology,* vol. 239, p. 422–429.

[72] Wang T, Zhang D, Sun Y., 2002. Using low frequency and intensity ultrasound to enhance start-up and operation performance of Anammox process inoculated with the conventional sludge. *Ultrasonics Sonochemistry,* pp. 42: 283-292.

[73] Nogaro G, Burgin A J., 2014. Influence of bioturbation on denitrification and dissimilatory nitrate reduction to ammonium (DNRA) in freshwater sediments. *Biogeochemistry,* vol. 120, n° %1(1-3), pp. 279-294.

[74] Foladori P, Laura B, Gianni A, et al., 2007. Effects of sonication on bacteria viability in wastewater treatment plants evaluated by flow cytometry—Fecal indicators, wastewater and activated sludge. *Water Research,* vol. 41(1), pp. 235-243.

[75] Mehdi Hajsardar, Seyed Mehdi Borghei, Amir Hessam Hassani, Afshin Takdastan, 2015. Optimization of wastewater partial nitrification in Sequencing Batch Biofilm Reactor (SBBR) at fixed do level. *Journal of Biodiversity and Environmental Sciences (JBES),* vol. 7, pp. 2222-304.

[76] Patrik Arnz, 2001. Biological Nutrient Removal from Municipal Wastewater in Sequencing Batch Biofilm Reactors. *Wasserg UTE, Munich*.

[77] Erik R.Coats, AlexanderMockos Frank, J.Loge, 2011. Post-anoxic denitrification driven by PHA and glycogen within enhanced biological phosphorus removal. *Bioresource Technology Volume 102, Issue 2,* pp. Pages 1019-1027.

[78] Shi, Y.J., Wang, X.H., Yu, H.B., Xie, H.J., Teng, S.X., Sun, X.F., Tian, B.H., et al., 2011. Aerobic granulation for nitrogen removal via nitrite in a sequencing batch reactor and the emission of nitrous oxide, *Bioresources Technology,* vol. 102, p. 2536–2541.

[79] Majid, I., Nayik, G. A., and Nanda, V., 2015. Ultrasonication and food technology: A review. *Cogent Food & Agriculture,* vol. 1, n° %11, p. 1071022.

[80] Vinay Kumar Tyagi, Shang-Lien Lo,Lise Appels, Raf Dewil, 2014. Ultrasonic Treatment of Waste Sludge: A Review on Mechanisms and Applications. *Critical Reviews in Environmental Science and Technology,* vol. 44, n° %111, pp. 1220-1288.

[81] Liu, Hong, 2007. Low intensity ultrasound stimulates biological activity of aerobic activated sludge. *Frontiers of Environmental Science & Engineering in China*.

[82] Pandit A.B., Prashant M.Biradar S.B.Roy S.F.D'Souza, 2010. Excess Cell Mass As An Internal Carbon Source for Biological Denitrification. *Bioresource Technology,* vol. 101, no. Issue 6, pp. 1787-1791.

[83] Xiao-Meng L, Guo-Ping S, Hong-Wei L, et al., 2010. Contribution of extracellular polymeric substances (EPS) to the sludge aggregation. *Environmental Science & Technology,* vol. (11), n° %144, pp. 4355-4360.

[84] Ding D, Feng C, Jin Y, et al., 2011. Domestic sewage treatment in a sequencing batch biofilm reactor (SBBR) with an intelligent controlling system. *Desalination,* vol. 276(1), pp. 260-265.

[85] Prendergast, J., Rodgers, M., Healy, M.G., 2005. The efficiency of a sequencing batch reactor (SBBR) in organic carbon and phosphorus removal. *Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances and Environmental Engineering,* vol. 40, n° %18, pp. 1619-1626.

[86] B. A. S. O. Nielsen M, 2010. Kinetics, diffusional limitation and microscale distribution of chemistry and organisms in a CANON reactor. *Fems Microbiology Ecology,* pp. 51(2): 247-256.

[87] Gonzalez-Silva B M, Jonassen K R, Bakke I., 2016. Nitrification at different salinities: Biofilm community composition and physiological plasticity. *Water Research,* p. 95: 48.

[88] S. I. MaintinguerI, I. K. SakamotoII; M. A. T. AdornoII; M. B. A. VarescheII, 2013. Evaluation of the microbial diversity of denitrifying bacteria in batch reactor. *Brazilian Journal of Chemical Engineering,* vol. 30, n° %13.

[89] Purkhold U., PommereningRo¨ser A, Juretschko S., Schmid M. C., Koops H. P., and Wagner M., 2000. Phylogeny of all recognized species of ammonia oxidizers based on comparative 16S rRNA and amoAsequence analysis: implications for molecular diversity surveys *Applied and Environmental Microbiology,* vol. 66, p. 5368–5382.

[90] Yan J, Hu Y Y., 2009. Partial nitrification to nitrite for treating ammonium-rich organic wastewater by immobilized biomass system. *Bioresource technology,* vol. 99(8), p. 2341-2347.

[91] Chen H, Liu S, Yang F, et al., 2009. The development of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in a single reactor for nitrogen removal 2009. *Bioresource technology,* vol. 99(4), pp. 1548-1554.