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**Research Article**

# Combined treatment of domestic wastewater with landfill leachate using aerobic moving bed bioreactor (AeMBBR)

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## ABSTRACT

In this study, the treatment of landfill leachate (LFL) and domestic wastewater using an aerobic moving bed biofilm reactor (AeMBBR) was investigated. AeMBBR was filled with 30% (v:v) biocarrier material (Kaldnes K1). The tested volumetric ratio of landfill leachate was 10%. The impact of varying hydraulic retention times (HRTs) (24–12-6 h) at a constant dissolved oxygen (DO) of 3.2 mg/L was investigated for system optimization. AeMBBR was successfully operated (HRT: 6h) for LFL and domestic wastewater treatment corresponding to 94%, and 78% ammonium nitrogen (NH4-N) and total organic carbon (TOC) removals, respectively. Additionally, *Proteobacteria* (66%) have been identified as the predominant culture in the biofilm layer, which plays a significant role in the co-treatment of domestic wastewater and LFL. Considering the results obtained; it was found that a significant amount of ammonium nitrogen was successfully removed.

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## INTRODUCTION

Landfill leachate is the result of the percolation of water through waste deposits that have been subject to aerobic and anaerobic microbial decomposition [1–4]. Landfill leachate typically contains high levels of COD, heavy metals, ammonia—nitrogen, and inorganic salts [5–7]. The characteristics of this wastewater vary depending on factors such as the composition of the solid waste, landfill operation, hydrology, and climate. High ammonium nitrogen ($NH_4^+$-N) concentrations in LFL cause serious environmental problems such as eutrophication and ammonium toxicity, which inhibit photosynthesis via free ammonia (FA) under alkaline conditions (pH>8.0) [8, 9]. Therefore, $NH_4^+$-N must be removed from the LFL.

Co-treating landfill leachate with domestic wastewater can be a cost-effective solution that promotes the degradation of organic pollutants through dilution [10]. This treatment option has the added advantage of being able to utilize existing wastewater treatment plants, eliminating the need for new investments. Various physicochemical [11, 12] and biological treatment [13] methods are available to remove $NH_4^+$-N from LFL and domestic wastewater. Biological treatment methods have important advantages compared to physicochemical methods, such as being cost-effective, having low sludge production capacity, and being environmentally friendly [14, 15]. The treatment of domestic sewage wastewater mixed with landfill leachate poses a major challenge due to the high concentration of ammonium in the leachate, which requires effective biological nitrogen removal. Activated sludge [16], anammox [17], and nitrification-denitrification [18] processes are among the biological technologies that have been extensively used for LFL and domestic wastewater treatment. The nitrification process is a good option for achieving complete $NH_4^+$-N removal [19].

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**Table 1.** Previous studies in different reactors

| Reactor type | Wastewater | Reactor volume (L) | Temperature (°C) | HRT (day) | Volumetric ratio (%) | Removal efficiency (%) | Ref |
|---|---|---|---|---|---|---|---|
| MBBR-MBR (Moving bed biofilm reactor and aerobic membrane reactor) | Leachate | 5 | 32–25 | 48 | – | COD: 74.2 $NH_4^+$-N: 99.7 | [22] |
| MBHBR (Moving bed hybrid bioreactor) | Domestic sewage and leachate | 7.9 | 25 | 6 | 4 | COD: 77–80 | [23] |
| SAFF (Submerged aerobic fixed film) | Domestic sewage and leachate | | | 24 | 8 | COD: | [24] |
| SBR (Sequencing batch reactors) | Domestic wastewater and leachate | 2 | 25 | 0.43 1–10 | 5 | COD: 73 | [25] |
| Active sludge | Leachate | 2 | 22 | – | 5–20 | COD: 16–88 | [13] |
| Active sludge | Domestic wastewater and leachate | 6.1 | – | | 5–25 | – | [26] |

HRT: Hydraulic retention times; Ref: References.

Many studies have focused on new treatment technologies for the co-treatment of LFL with domestic wastewater. The Moving Bed Biofilm Reactor (MBBR) is a rapidly developing technology for the treatment of wastewater [20]. MBBR is a biological treatment process based on conventional activated sludge and biofilter processes. The biomass present in the MBBR exists in two distinct structural forms: a suspended solid phase and a biofilm that is attached to the carrier. MBBR is more effective for nitrification than conventional activated sludge (CAS) systems due to the lower density and size of the CAS floc [21]. Table 1 shows previous studies using different reactors.

This study examines the nitrification process in an MBBR used for the simultaneous treatment of domestic wastewater and landfill leachate. The main research questions are as follows:

- What is the nitrification efficiency of the MBBR under different operating conditions?
- What factors can affect the nitrification efficiency of the MBBR?
- Which microbial communities are most prevalent in wastewater treatment?

## MATERIALS AND METHODS

### Characteristics of Domestic Wastewater and LFL

The LFL utilized in the AeMBBR studies was sourced from the Kahramanmaraş landfill in Türkiye. Domestic wastewater was collected from a treatment plant in Kahramanmaraş, Türkiye, and stored in a refrigerator at a temperature of +4°C to prevent microbial growth, following standard methods. The properties of the real wastewater used in this study are listed in Table 2. The aerobic reactor was inoculated with sludge from the activated sludge tank of the Kahramanmaraş Domestic Wastewater Treatment Plant.

**Table 2.** Properties of domestic wastewater and LFL

| Parameters | Domestic wastewater | Landfill leachate (mg/L) | Parameters | Domestic wastewater | Landfill leachate (mg/L) |
|---|---|---|---|---|---|
| COD (mg/L) | 645±100 | 7000–14000 | TSS (mg/L) | – | 1190±100 |
| TOC (mg/L) | 150±10 | 4000–11000 | Sulfate (mg/L) | – | 100±10 |
| $NH_4$-N (mg/L) | 40±10 | 1700-3600 | Copper (mg/L) | – | 2.572±0.6 |
| $NO_2$ (mg/L) | – | 330±10 | Zinc (mg/L) | – | 0.029±0.01 |
| $NO_3$-N (mg/L) | 9±2 | 2–50 | Iron (mg/L) | – | 2.575±0.5 |
| Phosphorus (mg/L) | – | 80±20 | Cadmium (mg/L) | – | 0.001 |
| Sulfide (mg/L) | – | 100±10 | Total chromium (mg/L) | – | 0.255±0.05 |
| TN (mg/L) | – | 1800±100 | Lead (mg/L) | – | 0.00025 |
| BOD (mg/L) | 327±100 | 1200±300 | Mangan (mg/L) | – | 0.1±0.05 |
| pH | 7.5-8.0 | 7.5–8.2 | Nickel (mg/L) | – | 0.625±0.1 |
| Color (Pt-Co) | 100±20 | 6380–9200 | Phenol (mg/L) | – | 20±2 |
| Color (RES) | | | Calcium ($Ca^{2+}$) | – | 150±10 |
| 436 | | 220±20 | Free Chlorine (mg/L) | – | 0.6±0.1 |
| 525 | | 90±10 | VSS (mg/L) | – | 850±20 |
| 620 | | 55±5 | | | |

**Aerobic Moving Bed Bioreactor (AeMBBR) Operation**

The schematic diagram of the AeMBBR used in this study is shown in Figure 1. The continuous flow AeMBBR consisted of a 5L tank with an effective working volume of 3L. In the AeMBBR, polyethylene Kaldnes K1 was used as a bio-carrier to retain the biomass. The Kaldnes K1 has a specific surface area of nearly 500 $m^2/m^3$ and a density of 0.95 $g/cm^3$. To account for the reactor volume and potential biofilm growth on the support material, the fill rate of the Kaldnes K1 supplement was chosen as 30%.

Landfill leachate was added to the domestic wastewater at a rate of 10% by volume. The reactor was operated continuously at a DO concentration of 3.2 mg/L. The initial concentration of mixed liquid-suspended solids (MLSS) in the AeMBBR was adjusted to approximately 4000 mg/L. Sludge was not withdrawn from the reactor which infers that theoretical SRT was infinity (HRT 24 and 12 h). However, at 6 h HRT, the sludge was maintained at an SRT of 15–20 days and with MLSS of 3500–4400 mg/L. The contents of the reactor were stirred at 250 rpm, and the operating temperature was maintained at room temperature. The DO, temperature, and pH of the reactor were measured with external probes and recorded daily. The pH of the AeMBBR was maintained at 7–7.5, with the use of $H_2SO_4$ and NaOH to correct the pH. The operating conditions of the AeMBBR are listed in Table 3.

The performance of AeMBBR was utilized under different HRTs. Initially, the HRT and dissolved oxygen (DO) concentrations in AeMBBR were maintained at 24 hours and 3.2 mg/L, respectively. Then, the HRT was reduced to 12 hours. Finally, the HRT was further reduced to 6 hours.

**Analysis**

Suspended solids in the mixed liquor (MLSS) were measured using standard methods [27]. A DO meter (YSI5000, YSI Company, USA) was used to measure dissolved oxygen (DO), and the pH value was measured with a pH meter (Thermo, Orion 4 Star, Indonesia). Each sample was centrifuged at 4000 rpm for 5 minutes (Eppendorf Centrifuge 5415R, Hamburg, Germany) and then filtered through a 0.45 µm pore size syringe filter (Sartorius AG, Göttingen, Germany) before analysis. The COD measurements were conducted using the dichromate-closed reflux Colorimetric Method as defined by Standard Methods (5220 D). The TOC-TN analyzer (Shimadzu TOC-VCPN/TNM-1, Kyoto, Japan) was used to analyze total dissolved organic carbon (TOC) and total carbon (TC). IonPac AS19-CS19 analytical and ion chromatography (Dionex ICS-3000, Sunnyvale, CA, USA) were used to determine ammonium ($NH_4^+$), nitrite ($NO_2^-$), and nitrate ($NO_3^-$)

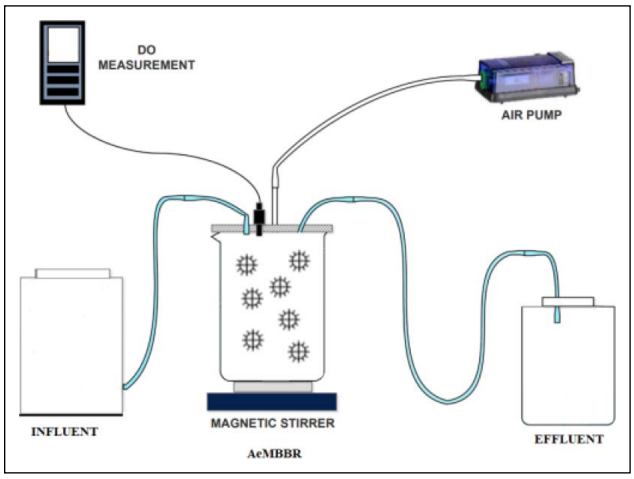


**Figure 1**. Experimental set-up of the AeMBBR.

concentrations with IonPac AG19 guard columns. The eluent was prepared by dissolving 9 mM sodium carbonate ($Na_2CO_3$) and 20 mM methane sulfonic acid in water, and then pumping the solution at a flow rate of 1 mL/min. To measure the mass of the biofilms, the three carriers were dried at 105°C for 2 hours and weighed. Subsequently, the dried carriers were combined with a 0.1 mol/L hydrochloric acid solution for 24 hours at a temperature of 80°C. Following this, the mixture was subjected to ultrasonic treatment for one hour. They were then washed several times with distilled water until all biofilm was removed from the carrier. The carrier sample was subjected to a second drying process and then weighed. The biofilm mass was calculated according to Piculell et al. [28].

**Illumina MiSeq Sequencing Analysis**

The Illumina MiSeq method was used to analyze bacteria on the contaminated Kalnes K1 surface (biofilm) and mixed liquor sample (supernatant). DNA was extracted from bacterial samples collected at 24 hours HRT in AeMBBR using the EurX Gene MATRIX Tissue & Bacterial DNA Purification Kit, according to the manufacturer's protocols. This sample was taken under the highest contamination operating conditions. The V3 and V4 domains of the bacterial 16S rRNA gene fragments were amplified using the forward primer 5'TCG TCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGN GGCWGCAG and reverse primer 5'GTCTCGTGGGTCCGGAGATGTGTATAAAGAGACAGAGGACTAC HVGGTATCTAATC, along with 12.5 µL of the reverse primer and 12.5 µL of 2X OFF Hot Start PCR mix. The temperature cycling conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 25 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, with a final step at 72°C for 5 minutes.

**Table 3.** AeMBBR operational conditions

| Reactor | Days | HRT (h) | Dissolved oxygen concentration (DO) (mg/L) | Kaldnes K1 (%) | LFL: DW (%) |
|---|---|---|---|---|---|
| AeMBBR | 0–34 | 24 | 3.2 | 30 | 10 |
| | 34–70 | 12 | | | |
| | 70–107 | 6 | | | |

HRT: Hydraulic retention times; AeMBBR: Aerobic moving bed bioreactor; LFL: Landfill leachate.

## RESULTS AND DISCUSSION

### The Performance of the Aerobic Moving Bed Bioreactor (AeMBBR)

The variation of biofilm density and MLSS concentration during the study is shown in Figure 2. The MLSS concentration was kept stable at between 3500–4500 mg/L in AeMBBR during the whole operation. Initially, during the 24 h of HRT, the density of the biofilm in Kaldnes K1 remained constant at an average of 1000±100 mg/L for

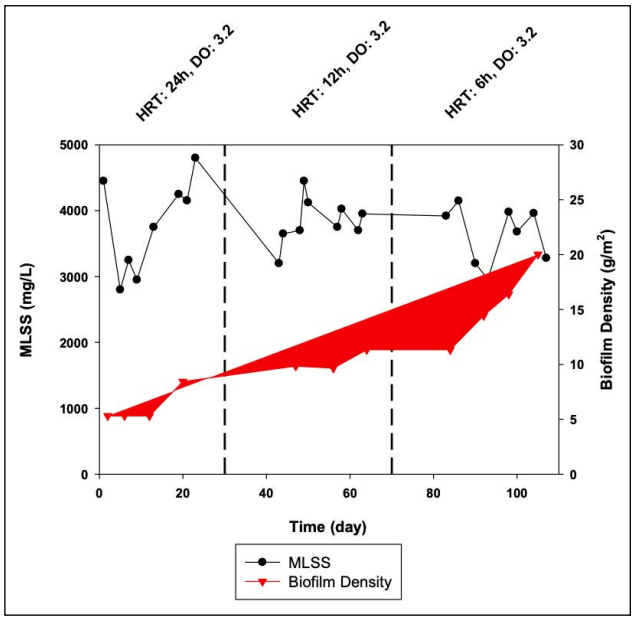


**Figure 2**. Evolution of MLSS concentration and biofilm density in the AeMBBR system.

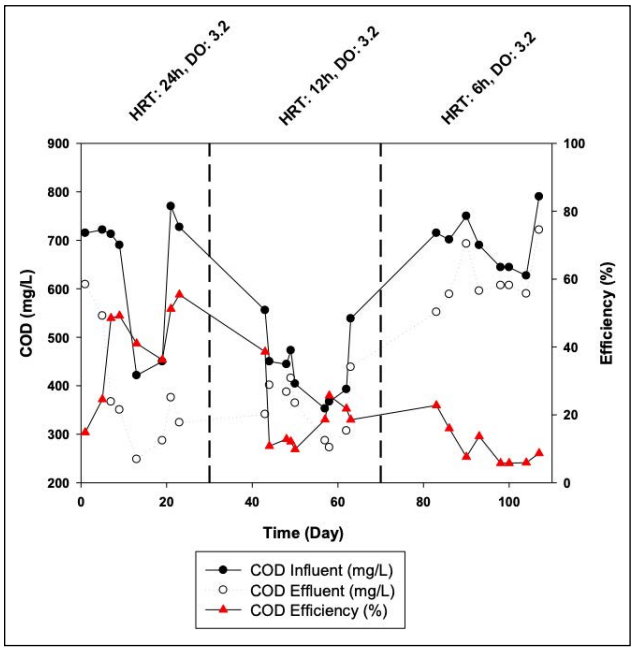


**Figure 3**. The COD removal performance of AeMBBR.

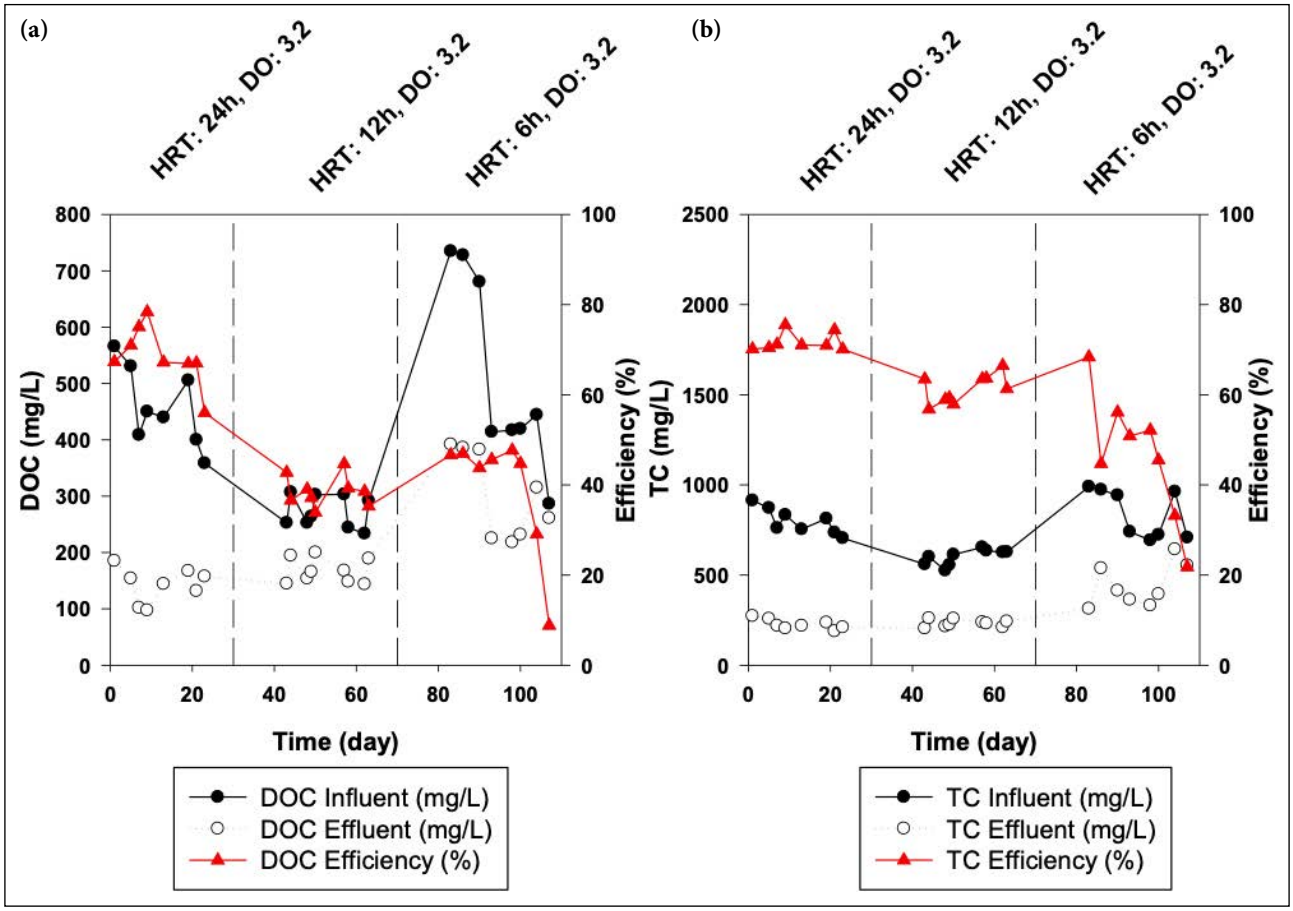


**Figure 4**. TOC **(a)** and TC **(b)** removal performance of AeMBBR.

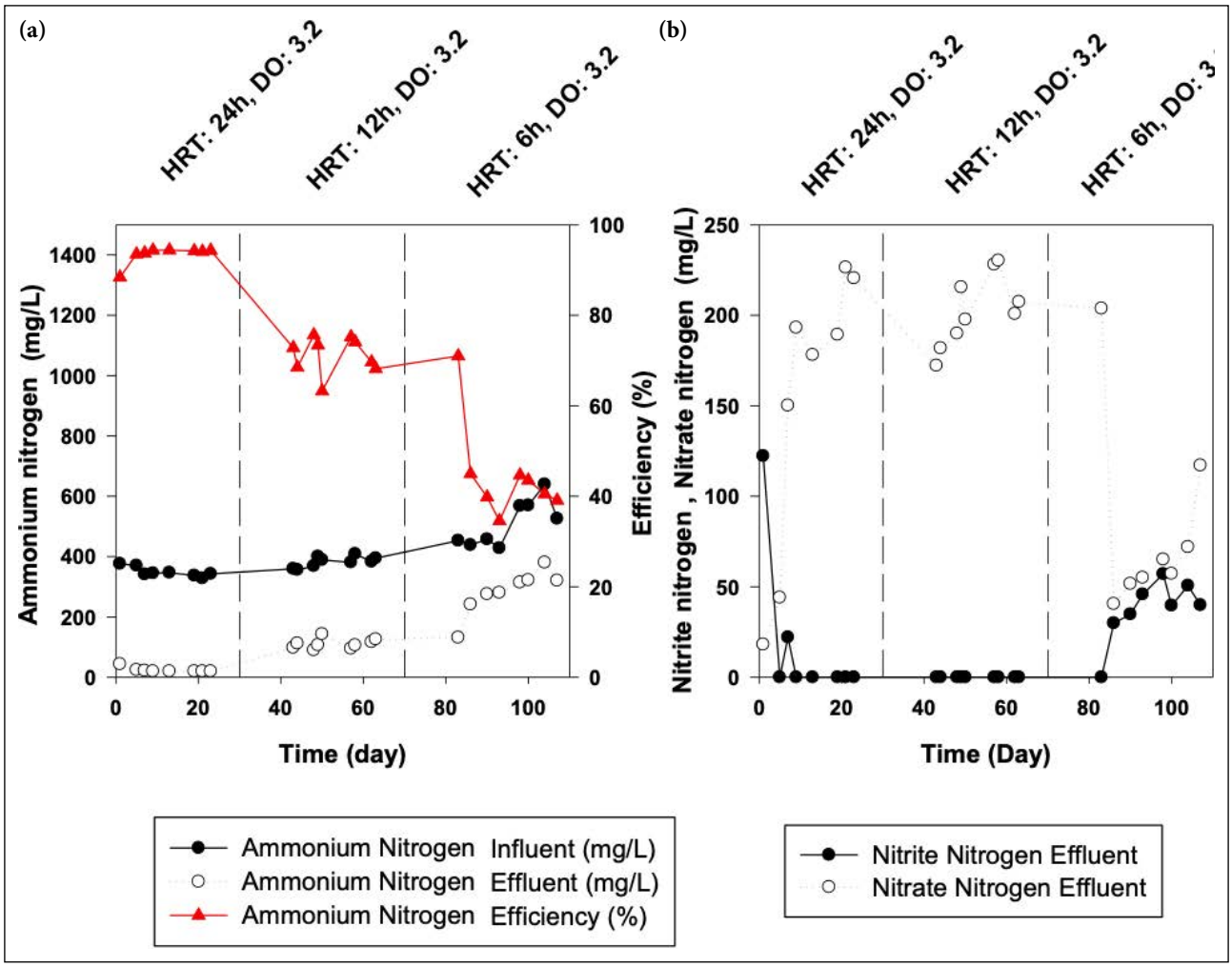


**Figure 5**. Performance of AeMBBR for NH4-N removal **(a)** and $NO_2$-N and $NO_3$-N accumulation **(b)**.

the first 12 days. After this day, the biofilm layer gradually increased. When HRT was reduced from 24 h to 12 h (days 34–70), a linear increase in biofilm density was observed. The average biofilm density at 12 h of HRT was 1542 mg/L. As seen in Figure 2, an increase in biofilm density was observed as HRT decreased. At 6 h of HRT, the average biofilm density increased to 3000 mg/L. Kozak et al. [29] observed that the biofilm-forming rate at 6 h was faster than that of 24 h and 12 h.

The effects of different HRTs on COD removal from combined LFL and domestic wastewater in the AeMBBR study are illustrated in Figure 3. The initial COD concentration varies between 350 and 800 mg/L depending on the characteristics of the landfill leachate. During the first 34 days of the study, the AeMBBR achieved a COD removal efficiency of 40%, resulting in an effluent COD concentration of 388 mg/L at 24 h. When HRT was reduced to 12 h (days 34–70), the COD removal efficiency was an average of 21%. Duyar et al. [22] reported that low HRT can negatively affect COD removal efficiency due to high loading rates and toxicity. Similarly, during days 70–107, reducing HRT from 12 h to 6 h resulted in decreased COD removal efficiency, corresponding to a 12% removal efficiency. It was difficult to compare our results with studies on COD removal efficiency in AeMBBR due to the highly challenging and variable concentrations of landfill leachate. Çeçen and Çakıroğlu [13] operated an activated sludge reactor for combined leachate and domestic wastewater treatment and achieved about 56% COD removal. Ferraz et al. [30] investigated the treatment of combined LFL and domestic wastewater using a submerged aerobic biofilter (SAB) system and achieved about 80% COD removal at 24 h HRT.

Figure 4 shows TOC and TC removal performance in the AeMBBR. Between days 0–34, average TOC and TC removal efficiency in AeMBBR were 68% and 71%, respectively, at 24 h HRT. When HRT was decreased to 12 h (days 34–70), the average TOC and TC removal efficiency was 40% and 61%, respectively. During days 70–107, the decreasing HRT from 12 h to 6 h resulted in decreasing TOC and TC removal efficiency, corresponding to 39% and 46% removal efficiency. According to Kozak et al. [29], this was probably due to the excessive biofilm production leading to mass transfer limitations.

The effect of HRT on ammonium nitrogen removal efficiency is shown in Figure 5a. During days 0 to 34, the average $NH_4^+$-N removal efficiency in the AeMBBR reactor

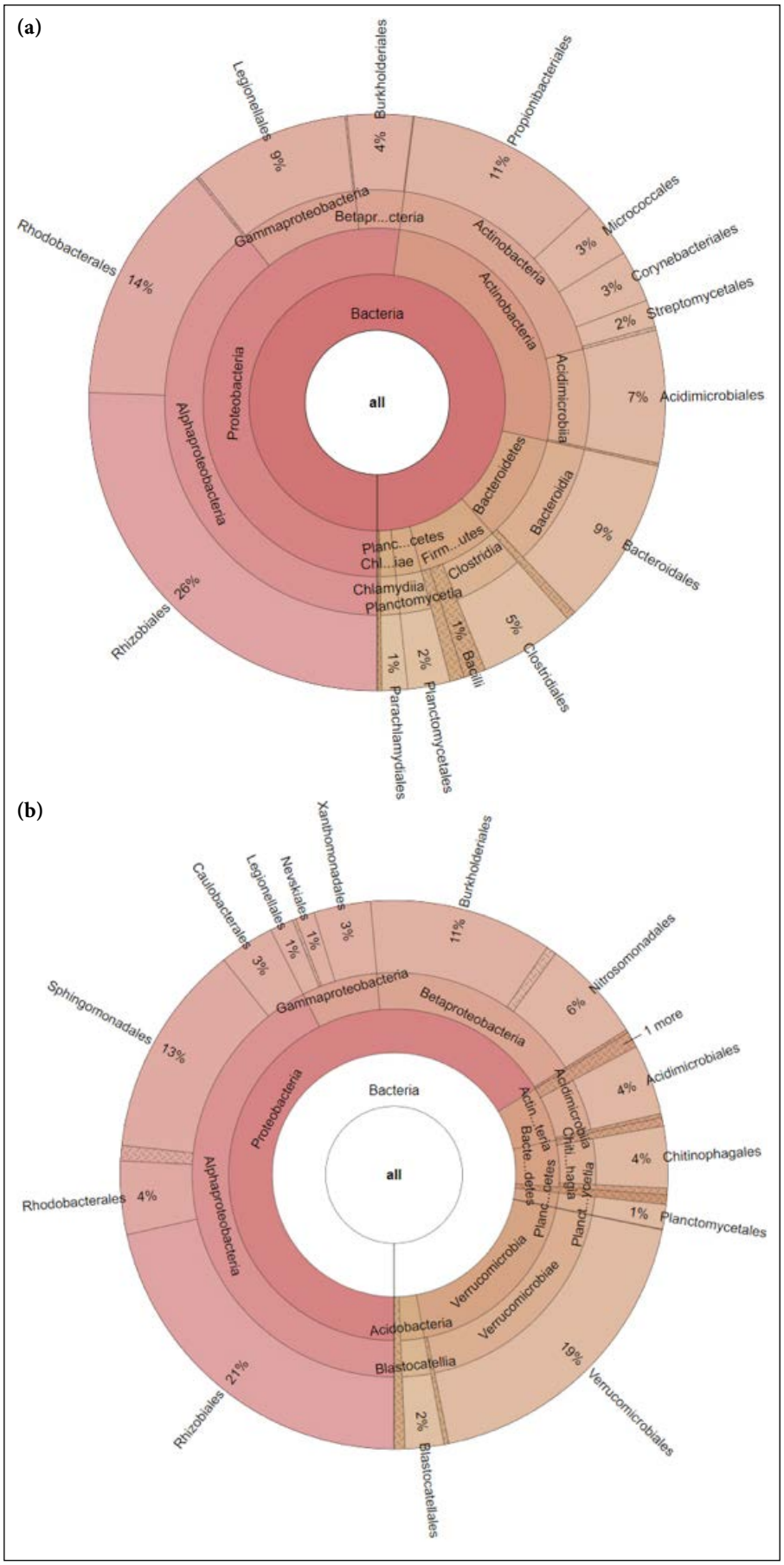


**Figure 6**. Krona chart of the microbial population in the supernatant **(a)** and biofilm **(b)** in AeMBBR with phylogenetic levels kingdom, phylum, class, order, family, and genus.

was 93%, corresponding to an effluent $NH_4^+$-N concentration of 23 mg/L at 24 h HRT. Chakraborty et al. [31], investigated the treatment of combined LFL and domestic wastewater using a sequenced batch reactor (SBR) system and achieved approximately 35% ammonium nitrogen removal at 2.5 days of HRT. $NH_4^+$-N removal efficiency decreased to 71% when HRT was reduced from 12 h to 6 h. Similarly, at 6 h HRT, reactor $NH_4^+$-N removal efficiency decreased significantly (47%). Nogueira et al. [32] reported that low HRT leads to increased loading rates, which negatively affects nitrifies as they compete with heterotrophic bacteria for substrates. Li et al. [33] reported that nitrification efficiency increased by 41% by reducing HRT from 10 h to 5 h, and the decrease in HRT increased ammonia oxidation activity.

As can be seen in Figure 5b, the accumulation of $NO_2$-N in the first 7 days was significantly high, but after this day, $NO_2$-N was not observed in the effluent and the $NO_3$-N accumulation was 152 mg/L $NO_3$-N. This was an indication that nitrification was taking place in the reactor. Similarly, no accumulation of $NO_2$-N was observed at 12 hours of HRT, and the accumulation of $NO_3$-N gradually increased, corresponding to 202 mg/L of $NO_3$-N. With the reduction of HRT to 6 hours, the $NO_2$-N accumulation decreased to 37 mg/L $NO_2$-N and the $NO_3$-N effluent concentration to 82 mg/L.

**Microbial Community Structures**

Microbial community analysis was examined using 16S rRNA gene amplicon sequencing for supernatant and biofilm samples collected from AeMBBR at 24 h HRT (Optimum condition). In AeMBBR, *Proteobacteria* predominated in the phylum group. As shown in Figures 6a and 6b, *Proteobacteria* were detected in 52% and 66% of the supernatant and biofilm, respectively. Many studies have shown that *Proteobacteria* always predominate in industrial wastewater treatment plants [34, 35]. In addition, the main bacterial group, *Proteobacteria*, includes facultative and aerobic bacteria that can remove nitrogen and organic matter [22, 36]. *Actinobacteria* (26%), *Bacteroidetes* (10%), *Firmicutes* (7%) and *Planctomycetes* (2%) are other phyla groups in the supernatant, while *Verrucomicrobia* (19%), *Actinobacteria* (5%), *Bacteroidetes* (5%), *Acidobacteria* (2%) were other groups of phyla in the biofilm. Matar et al. [37] reported that the Actinobacteria phylum is abundant in activated sludge. In addition, *Bacteroidetes* are commonly found in nitrogen removal systems [38, 39]. *Alphaproteobacteria* was dominant at the class level in the supernatant and biofilm which was 39% and 43%, respectively. *Actinobacteria* (19%), *Bacteroidia* (9%), *Gammaproteobacteria* (9%), *Acidimicrobiia* (7%), *Clostridia* (5%), *Betaproteobacteria* (4%), *Planctomycetia* (2%) were other class levels in the supernatant. Similarly, *Verrucomicrobiae* (19%), *Betaproteobacteria* (17%), *Gammaproteobacteria* (6%), *Acidimicrobiia* (4%), *Chitinophagia* (4%), and *Blastocatellia* (4%) were another class level in the biofilm.

## CONCLUSION

In this study, the nitrification process was successfully carried out in the landfill leachate and domestic wastewater treatment using AeMBBR. Our findings revealed $NH_4$-N removal around 94% in 24h HRT. Microbial community structures showed that *Proteobacteria* were the dominant culture responsible for removing N and C in AeMBBR. This study showed that the co-treatment system could provide a desirable option for removing $NH_4$-N from wastewater. However, it was also determined that an additional denitrification system should be implemented to effectively remove the nitrate that was formed.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

## CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## USE OF AI FOR WRITING ASSISTANCE

Not declared.

## ETHICS

There are no ethical issues with the publication of this manuscript.

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