Performances of sequential denitrification and partial nitrification process for treatment of landfill leachate

Çöp szinti suyu arıtımı için denitrifikasyon ve kısmi nitrifikasyon proseslerinin performansı

Ahmet DUYAR¹, Vildan CIFTCIOGLU², Gokhan CIVELEKOGLU³, Keveser CIRIK⁴

¹University-Industry-Public Coll., Research-Develop.-App. Centre, Kahramanmaraş Sutcu Imam Univ, Kahramanmaraş, Turkey
ahmedduyar@ksu.edu.tr

²Graduate School of Natural and Applied Sciences, Kahramanmaraş Sutcu Imam University, Kahramanmaraş, Turkey.
vakgu88@gmail.com

³Department of Environmental Engineering, Faculty of Engineering, Akdeniz University, Antalya, Turkey.
gcivelekgolu@akdeniz.edu.tr

⁴Department of Environmental Eng., Faculty of Eng. and Architecture, Kahramanmaraş Sutçü Imam Univ., Kahramanmaraş, Turkey.
kcirk@ksu.edu.tr

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Abstract
This study aims at investigating the sequential denitrification and the partial nitrification performance of aerobic moving bed reactor (AnoxMBBR)-aerobic sequencing batch reactor (AeSBR) to remove ammonium-nitrogen from landfill leachate (LFL). For this purpose, AnoxMBBR and AeSBR were set up and operated at a cycle time of 48-h. The both reactor performances were evaluated by chemical oxygen demand (COD), dissolved organic carbon (DOC), inorganic carbon (IC), ammonium (NH₄⁺), nitrite (NO₂⁻), nitrate (NO₃⁻), total nitrogen (TN), color (Pt-Co and RES) and pH parameters. Additionally, the AeSBR performance was evaluated in terms of free ammonia (FA) and free nitrous acid (FNA) concentrations. In the sequential system, total removal efficiency of COD and ammonium was about 75% and 65%, respectively. In AnoxMBBR, also, NO₃⁻ removal efficiency was about 55%. The partial nitrification was successfully occurred in AeSBR and the nitrate accumulation at 24-h and 48-h was about 1630.16 and 1702.92 mg/L, respectively. The results of this study suggest that use of sequential denitrification/partial nitrification is an effective way to remove COD and ammonium from raw LFL. However, additional treatment methods to this sequential system can be applied as pretreatment and/or post treatment for achieving the desired water quality because effluent TN and COD values are still not meet with the discharge standards of 40 mg N/L and 600 mg COD/L.

Keywords: Landfill leachate, Partial nitrification, Moving bed biofilm reactor, Sequencing batch reactor.

1 Introduction
Landfill is the most commonly used method for the disposal of solid waste all over the world because of some advantages such as easy set-up and low cost compared to other disposal methods [1],[2]. Over 2 billion tons of solid waste worldwide is collected annually and around 95% of collected municipal solid waste is disposed in landfill site [3]-[5]. However, the formation of landfill leachate (LFL), which produces by physicochemical-biological decomposition of solid wastes and rainfall percolation through solid wastes, is the major disadvantage of this method [6]. The LFL is a highly complex wastewater as it contains a large variety of contaminants such as ammonium-nitrogen, organic matter, heavy metals and xenobiotics matter [1],[7]-[11]. High ammonium nitrogen concentrations in LFL causes serious environmental problems such as eutrophication and ammonium toxicity that inhibits photosynthesis by free ammonia (FA) under alkaline conditions (pH>8.0) [12],[13]. Therefore, various physicochemical [14],[15] and biological treatment [16] methods have been extensively investigated for removing ammonium-nitrogen from LFL in literature [1]. Compared with physicochemical methods, biological treatment methods have important advantages, such as; their cost effective, low sludge production capacity and ecofriendly nature [17],[18]. Activated sludge [16] Anomox [19], partial nitrification/denitrification [20], anoxic/oxic (A/O) process

*Corresponding author/Yazılıan Yazar
are among biological technologies which have been extensively used for LFL treatment. The anoxic-aerobic systems to remove simultaneous organic matter and nitrogen is suggested as an effective treatment method to decrease high organic loading that causes inhibition of complete/partial nitrification process and competition between autotrophic nitrifiers and heterotrophic denitrifiers [22]. In recent years, sequential denitrification and partial nitrification in A/O systems have also attracted attention of researchers to remove nitrogen from LFL [6],[23] due to its advantages of low oxygen consumption for nitrification and saving carbon source for denitrification [24]. The key of sequential denitrification/partial nitrification relies on nitrite accumulation by enrichment of ammonium oxidizing bacteria (AOB) and selectively inhibition or washout of nitrite oxidizing bacteria (NOB) [24]. Thus, many studies have investigated the effect of various parameters as pH, temperature, dissolved oxygen (DO) concentration, sludge retention time (SRT), inhibitors, FA and FNA on the AOB accumulation and NOB inhibition [25]-[30]. Anthonisen et al. [12] reported that FA concentration is inhibited both AOB and NOB, but NOB (1.0-10 mg FA/L) is more sensitive than AOB (10-150 mg/L). Also, Gabarró et al. [29] and Welander et al. [31], reported that nitrite accumulation could occur in high FA and FNA concentration. Additionally, pH has a significant role on partial nitrification (AOB enrichment) because it affects the chemical equilibrium of FA and FNA [12],[32],[33].

Thus, the main objective of this study was to investigate the sequential denitrification/partial nitrification process for simultaneous ammonium-nitrogen and organic matter removal from raw landfill leachate using sequential AnoxMBBR and AeSBR. The system performance was evaluated by COD, DOC, IC, NH₄⁺, NO₂⁻, NO₃⁻, TN, color (Pt-Co and RES) and pH parameters. Additionally, the impact of FA and FNA concentrations on partial nitrification in AeSBR was evaluated.

2 Material and method

2.1 Characteristics of raw LFL and microbial culture

The raw medium age LFL was collected once a month from leachate balancing pond influent of a sanitary landfill site in Kahramanmaras, Turkey which operated for over five years. It is well known that the BOD/COD ratio of medium (5-10 years) and old (>10 years) LFL are 0.1-0.3 and <0.1, respectively, while this ratio is >0.3 in young LFL (< 5 years) [1]. The characterization of raw medium age LFL are shown in Table 1.
a TOC-TN analyzer (Shimadzu TOC-VCPN/TNM-1, Kyoto, Japan). NH₄⁺, NO₂⁻ and NO₃⁻ ion concentrations were determined by an ion chromatography ( Dionex ICS-3000, Sunnyvale, CA, Japan) with IonPac AS19 analytical and IonPac AG19 guard columns. Eluent was prepared from 9 mM sodium carbonate and 20 mM methane sulfonic acid and was pumped at flow rate of 1 ml/min. COD measurements were carried out using COD cuvette test kits, according to HACH method described by USEPA (Hach Method No. 8000). Morphology of adhered biofilm on carrier material was determined by SEM-EDS analysis. Surface and cross-section morphologies of the carrier material were directly observed using SEM (ZEISS/EVO LS10, Thornwood, NY, USA) after coating with Au-Pd. The inorganics on carrier material was analyzed using the EDS coupled with SEM. Color analyses as Pt-Co (465 nm) and RES (436 nm, 525 nm and 620nm) units were spectrometrically carried out by HACH DR 2500 (Dusseldorf, Germany), according to the APHA Standard Methods and the standards of European Norm EN ISO 7887. The RES (m⁻¹) parameters for each wavelength were calculated using Eq. 1.

\[ RES(m^{-1}) = (A/d)xf \]  

Where A is the absorbance of the sample collected in both reactors, d is the optical path length of the cell (mm) and f is the conversion factor between mm and m, which is 1000. The free ammonia concentration in each reactor was determined according to equation described by Østergaard N, [35] (Eq. 2);

\[ \frac{[NH_3]}{[TAN]} = \left(1 + \frac{10^{pH}}{10^{(\frac{2729}{T(K)})}}\right)^{-1} \]  

Where NH₃, TAN and T (K) are free ammonia concentration (mg/L), total ammonia concentration (mg/L) and temperature as Kelvin unit, respectively.

Additionally, the concentration of FNA (HNO₂⁻N) was also calculated by following Eq. 3 in which FNA, \( S_{N(NO_2^-)} \) and T are free nitrous acid concentration (mg/L), dissolved nitrate nitrogen concentration (mg/L) and temperature as Celsius unit, respectively [12].

\[ FNA = \left( S_{N(NO_2^-)} \right) (e^{2300/(273+T)})^{1.10pH} \]  

### 3 Results and discussion

#### 3.1 The performance of anoxic moving bed reactor

The heterotrophic denitrification process has been widely used to remove nitrate-nitrogen from LFL because of its cost effective, easy operation and eco-friendly nature [36],[37]. The NO₃⁻ in this process was reduced to NO₂⁻ and further to nitrogen gas by heterotrophic denitrifying bacteria under anoxic conditions [38]. It is well known that the biological treatment of LFL is extremely difficult because it contains toxic matters. In this part of the study, the denitrification performance of AnoxMBBR was investigated. The cycle time and reactor temperature during this part were kept constant at 48 hours and 30±1 °C, respectively. The NH₄⁺, NO₂⁻, NO₃⁻, DOC, COD, IC and color profile of AnoxMBBR are shown in Figure 1. The nitrate was added to the AnoxMBBR as an electron acceptor and initial nitrate concentration was adjusted to about 450 mgNO₃⁻/L. The nitrate was rapidly decreased and reached to about 206 mg/L at first 12-h, corresponding to about 55% NO₃⁻ removal efficiency. After 12 h, the NO₃⁻ removal was quite limited and nitrate concentration observed as about 197 mg/L at end of reaction time. Li et al. [39] studied a lab-scale moving bed biofilm reactor (MBBR) for denitrification of reverse osmosis concentrate collected from wastewater reuse plant. They reported 73.2% ± 19.5% NO₃⁻ removal efficiency [39]. Besides, the nitrite was not detected during denitrification using AnoxMBBR of raw LFL Figure 2(a).

![Figure 2. The NH₄⁺, NO₂⁻, NO₃⁻ and TN. (a) COD and DOC. (b) Color as Pt-Co and Res. (c) IC and pH. (d) Profiles of AnoxMBBR used in LFL treatment.](image-url)
Additionally, ammonium concentrations increased to about 1404 mg/L and 1443 mg/L at 30-h and 36-h, respectively. The effluent ammonium concentration were reached to about 1346 mg/L at the end of cycle time of 48-h. These decreases in ammonium concentrations were mainly due to using of ammonium during microbial assimilation of anoxic microorganisms [40] while increases in ammonium concentrations during cycle time of anoxic operation were due to biological degradation of proteins and amino acids based on organic nitrogen in LFL [41]. It is known that the conventional heterotrophic denitrification is a biological process that produces inorganic carbon as a source of alkalinity and increases the pH of the reactor. Thereby, the inorganic carbon concentration in the reactor increased from 1725 mg/L±25 to 1790±30 mg/L at first 6-h Figure 2(d). Also, the inorganic carbon was estimated that adsorb by biofilm on kaldnes K1 material in Anox MBBR Figure 3(d). Additionally, the influent pH was around 6.5 throughout this operation. The effluent pH of AnoxMBBR increased gradually to 8.3 due to the IC/alkalinity production by denitrifying bacteria throughout cycle time Figure 2(d). Similar to COD removal and NO₃⁻ removal, color removal increased rapidly during the first 12 hours, thereafter color concentration increased slightly at the end of cycle time Figure 2(c). The maximum color removal efficiency as Pt-Co was obtained as 19.6% at the end of first 12-h. Furthermore, RES measurements showed similarity to the Pt-Co results, corresponding to color removal efficiency as RES436, RES525 and RES620 at the 12-h were 20.3%, 24.6%, 24%, respectively Figure 2(c).

The determination thickness of the biofilm and characterize the morphology of the biofilm attached on carrier material was determined by SEM images Figure (3). The SEM images of biomass that grew as a biofilm on surface of carrier materials were showed in Figure 3(a) and 3(b). It seems that the inner surface of the Kaldnes K1 carrier material covered by biofilm and resulted in the formation of effective and dense biofilm. Wang et al. [42] reported that they observed similar formation of the effective biofilm on carrier material. SEM images showed that the different microorganisms consisted rod-shaped and filamentous cells on carrier material Figure 3(b). The results may verify that exist of filamentous cells could act framework between carrier material and biofilm. Also, SEM images in cross-section of carrier material demonstrated that the thick of biofilm was about 2.671-4.262 μm Figure 3(c).

Additionally, matters on the carrier material were quantify by EDS. In EDS, C, N and O was due to mainly cellular components. The EDS results also showed absorbed and accumulated of inorganics such as Mg, Al, Si, Na, In, K and Ca on biofilms Figure 3(d). Similar inorganic elements, e.g. Mg, Al and Ca on the carrier material were detected by Vilchez et al. [43]. This demonstrated that inorganic elements could bridge the cells and biofilm and contributed to the formation of biofilm. Additionally, matters on the carrier material were quantify by EDS. In EDS, C, N and O was due to mainly cellular components. The EDS results also showed absorbed and accumulated of inorganics such as Mg, Al, Si, Na, In, K and Ca on biofilms Figure 3(d). Similar inorganic elements, e.g. Mg, Al and Ca on the carrier material were detected by Vilchez et al. [43]. This demonstrated that inorganic elements could bridge the cells and biofilm and contributed to the formation of biofilm.

Figure 3. SEM images (a), (b) and (c) and EDS results of attached biofilm on Kalnes K1 (d).
3.2 The performance of aerobic sequencing batch reactor

AeSBR was fed with anoxically treated wastewater to evaluate the nitrification performance with COD, DOC, IC, NH₄⁺, NO₂⁻, NO₃⁻ and color parameters. Figure 4 shows COD, DOC, IC, NH₄⁺, NO₂⁻, NO₃⁻ and color profiles at cycle time of 48-h. At the start of the operation, NH₄⁺ and COD concentrations in AeSBR were about 1238.9 mg/L and 2956.8 mg/L, respectively. However, NH₄⁺ and COD concentrations in AeSBR were about 1238.9 mg/L and 2956.8 mg/L, respectively. However, NH₄⁺ and COD concentrations decreased sharply at the first 24-h of operation, corresponding to 64.5% and 45.5% removal efficiency, respectively. Additionally, effluent NH₄⁺ and COD concentrations were approximately 444.2 mg/L and 1385.4 mg/L, respectively. Figure 4(a) and 4(b). Spagnia and Marsili-Libelli [44] reported that low COD removal (about 20–30%) was obtained in SBR due to the low biodegradability in the LFL.

The increasing pH from 7.00 to 8.07 at first 6h was resulted in increasing FA concentration from 6.5 mg/L to 43 mg/L while variation in FNA concentration was not significant. The pH of 8.07 after 6-h was gradually decreased to 6.14 at the end of 48-h and thereby, FNA concentration increased from 0.1 mg/L to 2.61 mg/L. Similarly, Vadivelu and Keller [47] reported that they inhibited biosynthesis of the nitrobacter at the FA concentration of 6 mg/L and/or FNA concentration of 0.02 mg/L. Additionally, Anthonisen et al. [12] reported that FNA concentration inhibited over 3.5 mg/L for NOB and range of 10-150 mg/L for AOB. The percentage DOC removal efficiency in AeSBR for 48-h reached to 44.5% and the variation of TN concentration was negligible due to oxidation of ammonium-nitrogen to nitrate-nitrogen Figure 4 (a) and 4(b). Similar to NH₄⁺ removal and COD removal, IC concentration decreased rapidly during first 24-h and then, the IC removal was negligible. The initial IC concentration of 1586 mg/L were decreased to 80.05 mg/L at end of first 24-h, corresponding to IC removal efficiency of 94.4% Figure 4(d). Additionally, color removal as Pt-Co and RES was negligible throughout this part Figure 4(c).

4 Conclusion

In this study, the medium age LFL was treated using sequential anoxic moving bed reactor-aerobic sequencing batch reactor. The denitrification of raw LFL in the AnoxMBBR were successfully operated at cycle time of 48-h. The increasing NH₄⁺ concentration in AnoxMBBR showed that organic nitrogen was converted to inorganic nitrogen due to its ammonification. The dense biofilm layer formation on Kaldnes K1 carrier material was detected by SEM analysis. Besides, EDS analyses illustrated that both organic and inorganic matter was contributed to the formation of the biofilm layer. The complete ammonium oxidation was not observed in the AeSBR because FA and FNA concentrations play an important role on nitrite oxidizing bacteria. However, partial nitrification was achieved in AeSBR even at high FA and FNA concentration, corresponding to 43 and 2.6 mg/L, respectively. In AeSBR, high IC removal was also observed due to consumed alkalinity in partial nitrification. At the end of the sequential system, COD and NH₄⁺ removal efficiencies were around 86% and 65%, respectively. This study showed that the sequential treatment system could offer an attractive alternative to remove ammonium and COD from high strength wastewater. However, effluent color and COD values were still not meet to the discharge standards of 260-280 Pt-Co and 500-700 mg COD/L for solid Waste Recovery and Disposal Facilities in Table 20.6 of the Water Pollution Control Regulation of Turkey [48]. Therefore, additional treatment methods should be included in this system.
5 Author contribution statements
In the scope of this study, Ahmet DUYAR contributed to the formation of the idea, literature review, obtaining and evaluating the results, visualization, writing and reviewing the manuscript. Vildan CIPTICOGLU contributed to the literature review and visualization. Golhan CIVELEKOGLU contributed to the formation of the idea, evaluating the results, and writing and reviewing the manuscript. Keverir CIRIK contributed to the formation of the idea, supplying the materials used, evaluating the results, and writing and reviewing the manuscript.

6 Ethics committee approval and conflict of interest statement
There is no need to obtain permission from the ethics committee for the article prepared.
There is no conflict of interest with any person/institution in the article prepared.

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