Simultaneous removal of carbon, nitrogen, and phosphorus from landfill leachate using an aerobic granular reactor

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Abstract

In this study, aerobic granular reactor (AGR) was used to treat landfill leachate by changing the concentrations of chemical oxygen demand (COD) (668 ± 110 – 1149 ± 93 mg/L), ammonia ($\text{NH}_3\text{-N}$) (30 ± 3.3 – 48 ± 1.3 mg/L), and phosphorus ($\text{PO}_4\text{-P}$) (147 ± 18 – 221 ± 17 mg/L). The average COD removal was gradually reduced from 81 to 75%, increasing COD concentrations from 668 ± 110 to 1149 ± 93 mg/L. In phase I, the maximum removal of COD (94%) and $\text{NH}_3\text{-N}$ (85%) were observed at influent concentrations of 668 ± 110 mg/L and 30 ± 3.3 mg/L, respectively. Significant removal of $\text{PO}_4\text{-P}$ was observed, resulting in a maximum of up to 87%, further reducing up to 34% due to an increase in influent $\text{PO}_4\text{-P}$ concentration. The SVI_{30} reduced from 77 mL/g to 24.15 mL/g towards the end of phase III indicates the formation of granular biomass. The stability of AGR was also investigated in extreme conditions like shut-down and shock-loading phases. The treatment of real leachate diluted with wastewater ($\sim 20:80\%$ v/v) using AGR showed a significant COD, $\text{NH}_3\text{-N}$, and $\text{PO}_4\text{-P}$ removal of 62–65%, 61–93%, and 56–64%, respectively. *Proteobacteria* and *Planctomycetes* were identified as the predominant (80%) bacterial community in aerobic granules responsible for removing COD, $\text{NH}_3\text{-N}$, and $\text{PO}_4\text{-P}$ from leachate using AGR. The maximum biodegradation rate ($r_{\max}$) and half-saturation constant (K_s) in AGR were determined as 123.5 mg/L h and 309 mg/L, respectively.

Introduction

Leachates are the black-colored liquid mixture that emerged during landfill stabilization comprising biodegradable and non-biodegradable toxic compounds, whose characteristics vary geographically (Damiano et al. 2014). Excessive moisture content in the landfill flows through the porous media results in leachate generation (Bejgarn et al. 2015). Landfill leachate majorly consisting of biodegradable organic matter, humic, and fulvic acids (Shehzad et al. 2015). It also contains heavy metals, nitrogenous matter, and chlorinated organics that contaminate water bodies, groundwater, soil and adversely impact human beings (Mukherjee et al. 2015). The physico-chemical characterization of leachate is challenging to evaluate the organic content concerning its refractibility, biodegradability, composition, and origin (Baettker et al. 2020). The presence of hazardous and recalcitrant compounds in addition to micropollutants alters the physico-chemical and biological properties of leachate to a greater extent (Besha et al. 2017). Significant increase in anthropogenic activities such as applying cosmetics and personal care products, pharmaceuticals waste, harmful pesticides, and synthetic organic compounds increases leachate toxicity posing a more significant threat to nearby water bodies (Goswami et al. 2018). Thus, leachate being a potential threat to the environment, its characterization is crucial to evaluate the pollution level of the landfill site to develop an appropriate treatment process. The selection of the most suitable landfill leachate treatment process depends on leachate composition and the concentrations present in it (Mandal et al. 2017). Physico-chemical and biological methods are two general treatment techniques adopted to treat leachate (Haslina et al. 2021). Physico-chemical treatment processes efficiently remove certain pollutants from leachate but are costly and require high energy to run these processes (Kurniawan et al. 2006). Physico-chemical methods are used for pre-treatment before the biological treatment of leachate to efficiently remove the recalcitrant compounds (Aftab et al. 2019). However, huge chemical expenditure, installation, processing costs, and energy consumption restrain the full-scale landfill leachate treatment using physico-chemical methods (Luo et al. 2019).

The biological method is the best alternative evolved over the period to overcome the limitations of physico-chemical processes. The leachate is treated either aerobically or anaerobically by using microbial inoculum to degrade the organic and nitrogen content (Miao et al. 2019). The biological treatment method is the most reliable, simple, cost-effective, and widely applicable for treating emerging contaminants present in leachate (Bove et al. 2015). Various aerobic biological reactors such as activated sludge process (ASP), aerated lagoons, sequencing batch reactors (SBR), trickling filters, moving bed biofilm reactor (MBBR), rotating biological contactor (RBC), and membrane bioreactor (MBR) successfully used for the treatment of landfill leachate (Luo et al. 2019). The critical factor for the successful application of any biological process is determining the optimum conditions for enhancing the biomass growth to increase the removal of COD, $\text{NH}_3\text{-N}$, and other micropollutants found in the leachate (Anna et al. 2019). While using biological methods for treating landfill leachate, the primary concern is recalcitrant organic compounds, which are hard to treat using conventional biological methods (Zhang et al. 2019).

Aerobic granular reactor (AGR) is one of the most promising and innovative biological techniques, shown good results for treating leachate and wastewater (Wei et al. 2012; Sarma et al. 2017). The AGR is a type of SBR preferred for developing granular sludge containing dense microbial biomass with excellent settleability, high retention, smaller footprint, and less sludge generation, which can sustain high organic loading (Adav et al. 2008; Sarma et al. 2017). The AGR is widely used on a laboratory scale, although very few studies have been reported its application for pilot-scale treatment (Beun & Heijnen, 2000). Various factors such as cycle time, starvation period, hydrodynamic shear, and organic loading rate directly influence the formation and stability of the granules (Di Bellaa & Torregrossa, 2014). To the best of our knowledge, very limited studies reported treating landfill leachate using an AGR.

The main objective of this study was to evaluate the performance of AGR for the treatment of landfill leachate at various concentrations of COD, $\text{NH}_3\text{-N}$, and $\text{PO}_4\text{-P}$ at 12 h hydraulic retention times (HRT). The AGR was also operated under different shut-down and shock loading phases to evaluate its stability. A metagenomics study was performed to identify the predominant microbial community in aerobic granules, which plays a vital role in landfill leachate treatment.

Materials And Methodology

Seed sludge and leachate collection

The seed sludge and wastewater used in this study were obtained from a wastewater treatment plant (WWTP), Dadri, India. The leachate used in this study was collected from the Ghazipur landfill site situated in East Delhi, India, one of the most disruptive landfill sites in this region, deteriorating the quality of land and groundwater (Banerjee 2021). The characterization of leachate used in this study is shown in Table 1.

Synthetic leachate

The synthetic leachate was prepared by adding the following chemicals in water (mg/L): sodium acetate (500-2000), sodium propionate (250-500), humic acid (250-500), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (14-40), CaCl_2 (60-260), $(\text{NH}_4)_2\text{Cl}$ (60-600), KH_2PO_4 (50-200), K_2HPO_4 (100-400), MgSO_4 (100-200), NaHCO_3 (100-200), MnSO_4 (10-50), CuSO_4 (20-80), NaCl (30-120), Phenol (50-400). Sodium acetate, sodium propionate, humic acid, and phenol were used as sole carbon sources in synthetic leachate, whereas ammonium chloride was used as a source of nitrogen. KH_2PO_4 and K_2HPO_4 were used as a source of phosphorus, and NaHCO_3 was used as a buffering agent. All the chemicals used to prepare the synthetic leachate were of analytical grade with 99% purity with a pH of 7.5 ± 0.2 .

Experimental set-up

The study was performed in a laboratory-scale AGR made of a Perspex column with a working volume of 4 L. The height (H) and internal diameter (D) of AGR were 200 cm and 5.6 cm, respectively, with a H/D ratio of ~ 36 (Fig. 1). The influent leachate was introduced at the bottom of the AGR with the help of a peristaltic pump, and continuous aeration was provided using an air compressor with a sparger at a rate of 3 L/min. The AGR was operated at room temperature (27 ± 2 °C).

Operating methodology

During the start-up phase, seed sludge (2 L) with a mixed liquor suspended solids (MLSS) concentration of 1420 ± 272 mg/L and an equal proportion of synthetic leachate (2 L) was added to the AGR. The reactor was operated at 12 h HRT for acclimatization during this phase. Poly aluminium chloride (PAC) of 50 mL at a concentration of 20 g/L was fed to the AGR only during the start-up phase to enhance the formation of granulation from sludge (Liu et al. 2016). The synthetic leachate with a COD of 244 ± 21 mg/L was supplied to the microorganisms during this phase.

The AGR was operated in sequential batch mode with a volumetric exchange ratio of 50%. In each cycle, 50% of reactor volume was decanted after settling, and the same volume of fresh feed was added to the AGR. A settling time of 15 min was maintained in each cycle during the start-up phase to prevent biomass loss. However, the settling time was gradually reduced

up to 5 min after forming the stabilized granular biomass. The cycle time of 12 h (operated twice per day) with 30 min of feeding, 673 min aeration, 5 min settling, 2 min effluent withdrawal, and 10 min idle time were maintained. The samples were collected from the sampling port provided at the middle of the AGR by following a 50% volumetric exchange ratio. The AGR was continuously operated for about four months by gradually increasing the feed concentrations (COD, NH₃-N, and PO₄-P), and the performance was investigated during shock loading, shut-down, and treatment of real leachate.

Analytical methods

The influent and effluent samples were collected twice per week to analyze the concentrations of COD, NH₃-N, NO₃-N, PO₄-P, and MLSS as per the standard methods (APHA 2005). Sludge volume index (SVI₃₀) was determined in mL/g by collecting 1 L of sample in a measuring cylinder from AGR in aerating phase and after 30 min of settling, the settled sludge volume was measured (APHA 2005). The ratio of settled sludge volume (mL) and the concentration of MLSS provided the SVI₃₀ (Tomar and Chakraborty 2018).

Microbial community analysis

Homogenized aerobic granular sludge of 1 L was collected from the AGR after the end of phase X and allowed to settle for 5 min to get the concentrated granular sludge. The collected sample was preserved at -20 °C for the metagenomics study following the standard operating process (Tran et al. 2020). A commercially available kit (Qiagen, Zymo Research, ThermoFisher, USA) was used for DNA extraction, and 16S rRNA sequencing was used to characterize the microbial community. The primers V13F (5'AGAGTTTGTATGGTCTCAG3') and V13R (5' TTACCGCGGCMGCSGGCAC3') were amplified with the bacterial V3-V4 region of the 16s rRNA gene. The polymerized chain reaction (PCR) with initial denaturation (95 °C for 15 seconds), followed by annealing (60 °C for 15 seconds), elongation (72 °C for 2 minutes), extension (72 °C for 10 minutes), and then holding (4 °C for 25 cycles) was carried out (Ji et al. 2019). Amplicons were purified using ampure beads for removing the unused primers for each sample. Illumina barcoded adapters were utilized to perform an additional 8 cycles of PCR to prepare the sequencing libraries.

FASTQC and MULTIQC, subsequently trimming of adapters and low-quality reads by TRIMGALORE was adopted for raw data quality control (QC). Further, the trimmed reads are processed by merging the paired-end reads, removing the chimeria, followed by operational taxonomic unit (OUT) estimation using Uclust (QIIME) program, which gives accurate information at the genus level. Afterward, the Greengenes database of NCBI was used for taxonomic classification.

Results And Discussion

Start-up phase

During the start-up phase, the microbial inoculum was acclimatized in AGR for 10 days. It was observed that the MLSS concentration was increased from 1420±272 mg/L (0th day) to 2228±272.94 mg/L (10th day) during this phase, which signifies the successful acclimatization of the seed sludge. The MLSS concentrations show fluctuating behavior at the initial stage, which gradually increased over the end of operations with the increase in influent concentrations. The aerobic granules are developed during the sequencing batch operation of AGR, but the stability of granules becomes challenging during the continuous operation (Kent et al. 2018). In the start-up phase, the SVI₃₀ values were very unstable mainly because of the development of filamentous microorganisms, but progressively a steady increase in biomass concentration was observed. The results obtained in this study were consistent with the similar research by Vashi et al. (2019). The average MLVSS/MLSS ratio of 0.45 was observed throughout the operation of AGR.

COD removal

The AGR was operated at three different phases (I-III) by gradually increasing the COD concentrations from 668±110 to 1149±93 mg/L by maintaining an HRT of 12 h. A longer HRT of 12 h was beneficial for developing aerobic granulation and

accumulation of flocs by removing the suspended and filamentous microorganisms present in the AGR (Wang et al. 2021). A significant average COD removal of 81%, 71%, and 75% was observed in phase I, II, and III, respectively (Table 2). A maximum COD removal of 95% was observed on the 31st day of operation at an influent COD concentration of 668 ± 110 mg/L in phase-I (Fig. 2). Initially, a gradual increase in VSS concentration and SVI₃₀ value was observed in phase I of AGR, indicating initiation of granule formation (Fig. 3) (Tomar and Chakraborty 2018). An increase in heterotrophic microorganisms in the AGR directly correlated with the increase in the removal of the organic compounds observed in phase-I, which is similar to a recent study by He et al. (2018). The SVI profile was increased from 45 to 77 mL/gm in this phase with an average VSS of 2182 ± 102 mg/L.

In phase II, the average COD removal was 71%, which was lesser than phase-I, primarily due to increased COD concentration (908 ± 65 mg/L). The removal of COD was slightly unstable (60%) at the beginning of phase II owing to the rise in COD concentration, which gradually recovered (84%) towards the end of the operation (Fig. 2). The compact aerobic granules were observed during phase-II of the operation, signified by the rapid decrease in SVI up to 36 mL/g (Fig. 3). Nevertheless, a gradual increase in VSS was observed from 2.3 g/L to 2.8 g/L, showing the adaptability of microorganisms at higher concentrations of COD (908 ± 65 mg/L) in the influent stream.

In phase III, a slight improvement in the COD removal was observed while maintaining the influent concentration of 1149 ± 93 mg/L, which resulted in an average COD removal of 75% (Table 2). In this phase, a maximum COD removal of 85% was observed even if at a higher COD concentration than phase-II, indicating better adaptability of microbes in AGR (Fig. 2). A significant increase in VSS (5 g/L) and decrease in SVI (24 mL/g) in this phase prove the effectiveness of AGR under toxic environmental conditions (Fig. 3).

NH₃-N removal

The AGR was operated by maintaining the NH₃-N concentrations of 30 ± 3.3 , 37 ± 3 , 48 ± 1.3 mg/L in phase I, II, and III, respectively (Table 2). During phase I, a gradual increment in NH₃-N removal from 21 to 85% was observed at an influent concentration of 30 ± 3.3 mg/L (Fig. 4). Concurrently, the NO₃-N formation increased from 2.4 to 10.9 mg/L with an average concentration of 6.7 ± 2.6 mg/L in this phase. A sudden drop in NH₃-N removal (39%) was observed on the 34th day of operation in phase II due to an increase in influent NH₃-N (37 ± 3 mg/L) and COD (908 ± 65 mg/L) concentrations (Fig. 4). Subsequently, the NH₃-N removal was improved up to 68% at the end of phase II. At the same time, NO₃-N formation was also reduced from 6.7 ± 2.6 mg/L in phase I to 5.8 ± 1.4 mg/L in phase II. This indicates higher COD and NH₃-N concentrations inhibit the removal of NH₃-N by lowering the formation of NO₃-N. This might be due to heterotrophic microbes are dominant over nitrifiers at high organic concentrations (Padhi and Gokhale 2017). Nitrifying bacteria present in the aerobic granules become predominant only after the biodegradation of the organics, due to which the removal of NH₃-N was relatively less (49%) as compared to COD (71%) in phase II (Wei et al. 2012). The free NH₃-N concentration in phase II was higher than the threshold limit of nitrifying bacteria, reducing the microbial activity (Yang et al. 2004).

In phase III, the gradual improvement in NH₃-N removal from 40% on the 58th day to 61% at the end shows the competence of AGR for successful removal of NH₃-N, albeit at high COD and NH₃-N concentrations (Fig. 4). The average NH₃-N removal was 47% at an influent concentration of 48 ± 1.3 mg/L, forming a NO₃-N concentration of 4.2 ± 1.2 mg/L in this phase. Di Bellaa & Torregrossa (2014) reported denitrifying activity in AGR, which could decrease NO₃-N concentration in the effluent. The anoxic zone in the core of aerobic granules due to the inability of oxygen diffusion promotes simultaneous nitrification and denitrification in AGR (Layer et al. 2020).

PO₄-P removal

The PO₄-P concentrations of 147 ± 18 , 204 ± 12 , and 221 ± 17 mg/L was maintained in phase I, II, and III, respectively in AGR (Table 2). During phase I, a gradual increase in PO₄-P removal from 44% on 10th day to 83% on 31st day was observed with an average removal efficiency of 66% (Fig. 5). In phase II, a decline in PO₄-P removal (31%) was observed on the 34th day due to

an increase in inlet PO₄-P concentration (204±12 mg/L). The PO₄-P removal was subsequently recovered and reached a maximum of up to 47% on the 55th day of operation (Fig. 5). The average PO₄-P removal was dropped from 66% in phase I to 40% in phase II due to a sudden increase in PO₄-P concentration, which inhibits the microbial activity of phosphate accumulating organisms (PAOs) (Huang et al. 2015). With a further rise in PO₄-P concentration (221±17 mg/L) in phase III, a similar trend was observed with an overall removal efficiency of 34%. The increased toxicity of leachate in the subsequent phases of AGR inhibits the removal of PO₄-P. However, the significant reduction in PO₄-P from leachate confirmed the existence of PAOs in the aerobic granule, which typically consists of anaerobic/anoxic environment to release phosphorus followed by its uptake by aerobic microbes (Wei et al. 2012; Ren et al. 2017). The low availability of biodegradable organic matter in the old leachate could be another important factor that significantly impacts the PO₄-P removal in AGR (Ren et al. 2017).

Stability of the AGR

The AGR was investigated for its stability during extreme environments such as shut-down and shock-loading. During the 1st shut-down phase (IV), the air supply was stopped for 2 days, ensuring the feeding at 12 h HRT by maintaining an average COD concentration of 976±105 mg/L. When the AGR resumed its operation, a COD removal efficiency of 89% was observed (Table 2). However, in the 2nd shut-down phase (V), the feeding was stopped for 2 days with a continuous air supply to the AGR. After the start of AGR operation, it resulted in relatively higher removal efficiency of 93% at a COD concentration of 888±45 mg/L. This signifies the performance of AGR is affected more by the supply of air (Liu & Tay 2006). Moreover, the AGR shows significant removal of NH₃-N, i.e., 49 and 66% in phase IV and V, respectively (Table 2). But the PO₄-P removal in the shut-down phase of AGR was minimal due to the interrupted process and stringent environmental conditions (de Kreuk et al. 2005).

The shock-loading was performed in AGR at three different phases (VI-VIII) by suddenly increasing the influent COD concentrations to understand its stability. In phase-VI, the COD concentration was nearly doubled (1679±163 mg/L) and operated for 3 days showing an excellent COD removal (84%) with negligible removal of NH₃-N (31%) and PO₄-P (18%) (Table 2). Subsequently, a sudden drop in COD removal in phase VII of shock-loading up to 61% was observed at a higher COD concentration (2886±104 mg/L). The COD removal was further decreased up to 58% in phase VIII at a COD concentration of 3773±302 mg/L. At very high COD concentrations, the biological activity of microbes in aerobic granule was inhibited, due to which a gradual decrease in COD removal was observed (de Kreuk et al. 2005). The result obtained from this study shows the feasibility of AGR to operate efficiently under such harsh environmental conditions.

Treatment of real leachate

After successfully treating synthetic leachate at various increased influent concentrations, the performance of AGR was evaluated using real leachate in two different phases (IX and X). The COD concentration of real leachate collected from a landfill was very high (7588±262 mg/L) (Table 1), which may reduce the efficiency of AGR. Therefore, the leachate was diluted with wastewater (~20:80% v/v) with a COD concentration ranging 848-906 mg/L was fed to the AGR (Table 2). It was observed that the granules got destabilized when the AGR was provided with real leachate due to the toxic pollutants present in it, which was also reported by Di Bellaa & Torregrossa (2014). The treatment of real leachate diluted with wastewater was investigated in AGR over one week of operation at 12 h HRT. The significant COD removal of 62-65% was observed at influent COD concentrations between 848-906 mg/L (Table 2). Concomitantly, the NH₃-N removal of 61-93% was observed at lower NH₃-N concentrations ranging from 8-13 mg/L, generating 15 mg/L of NO₃-N in the effluent. The PO₄-P removal (56-64%) was also remarkable at 75-77 mg/L of influent concentrations. This study reveals the efficiency of AGR for simultaneous removal of COD, NH₃-N, and PO₄-P from real leachate.

The performance of various bioreactors studied over the period of time for the landfill leachate treatment, such as activated sludge process (ASP) (Ren et al. 2017), constructed wetland (Bulc 2006), aerated lagoons (Mehmood et al. 2009), SBR (Xu et al. 2020), biological filter (Stephenson et al. 2004), moving-bed biofilm reactor (MBBR) (Welander et al. 1998), membrane bioreactor (MBR) (Zolfaghari et al. 2016), membrane sequencing batch reactor (MSBR) (Tsilogeorgis et al. 2008), and RBC (Castillo et al. 2007) (Table 3). The landfill leachate was efficiently treated using various biological reactors with a wide range

of COD (~700-9000 mg/L) and NH₃-N (8-2000 mg/L) with a limited study conducted on PO₄-P removal. However, the present study showed the successful treatment of landfill leachate in AGR while removing COD, NH₃-N, and PO₄-P simultaneously with a maximum removal efficiency of 65, 93, and 64%, respectively (Table 3).

Metagenomic analysis

The metagenomics study of aerobic granular sludge confirms the presence of mixed microbial consortia dominated by phylum *Proteobacteria* (~50%) along with *Planctomycetes* (~30%), *Acidobacteria* (~10%), *Bacteroidetes* (~6%), *Verrucomicrobia* (~3%), *Nitrospirae* (~2%), *Actinobacteria* (~2%), *Cyanobacteria* (~1%), and *Firmicutes* (~0.5%), etc. (Fig. 6a). *Proteobacteria* and *Planctomycetes* constitute more than 80% of aerobic granules, play an important role in the biodegradation of COD, NH₃-N, and PO₄-P (Ding et al. 2019; Wang et al. 2021). The result was consistent with the previous study affirming *Proteobacteria* as the predominant phyla in the aerobic granular sludge (Li et al. 2020). The presence of *Acidobacteria* indicates the acidic microenvironment and helps to gain structural stability of aerobic granules due to its firmed structure (Li et al. 2020). The *Verrucomicrobia* and *Firmicutes*, although present in smaller quantities in granular biomass, but play a vital role in the biodegradation of organic matter (Zhao et al. 2020; Li et al. 2020). Similarly, *Bacteroidetes* and *Proteobacteria* help to remove nitrogen by biological nitrification and denitrification in AGR (Fang et al. 2018). At the genus level, aerobic granule was dominated by *Plantomyces* (35%), *Nitrospira* (18%), *Nannocystis* (14%), *Gemmata* (7%), *Alicyclophilus* (7%), *Hyphomonas* (5%), *Georgenia* (4%), *Prostheco bacter* (4%), *Bifidobacterium* (3%) and *Thauera* (3%) (Fig. 6b). The results obtained from this study ascertain that the diverse microbial community in aerobic granule plays a key role in the efficient biodegradation of COD, NH₃-N, and PO₄-P in AGR.

Biokinetic coefficients

The biokinetic coefficients that describe the kinetic behavior of AGR are determined from the steady-state outlet concentrations for various inlet concentrations of COD using a modified Monod equation as shown in Eq. (1) (Padhi and Gokhale, 2016). The assumptions are oxygen limitation is not present in AGR and at a steady-state, the growth rate of microorganism in equilibrium with its decay rate. Hence, the biokinetic coefficients were considered to be constant over the period of time.

$$\frac{V/Q}{C_i - C_o} = \frac{K_s}{r_{max}} \frac{1}{C_{in}} + \frac{1}{r_{max}} \quad (1)$$

where, V is the working volume (L) of AGR, Q is the flow rate of leachate (L/h), C_i is the inlet, and C_o is the outlet COD concentrations (mg/L), C_{in} is the log mean concentration [(C_i - C_o)/ln(C_i/C_o)], r_{max} is the maximum biodegradation rate (mg/L h), and K_s is the half saturation constant (mg/L). The biokinetic coefficients r_{max} and K_s were estimated from a plot between (1/C_{in}) and [(V/Q)/(C_i - C_o)]. The r_{max} and K_s values were determined as 123.5 mg/L h and 309 mg/L, respectively.

Conclusions

The present study demonstrates the feasibility of AGR for simultaneously removing COD, NH₃-N, and PO₄-P from landfill leachate. The AGR showed a maximum removal of 94% COD, 85% NH₃-N, and 83% PO₄-P at influent concentrations of 668 ± 110 mg/L, 30 ± 3.3 mg/L, and 147 ± 18 mg/L, respectively. A higher COD concentration (above 3773 mg/L) showed a detrimental effect on removing NH₃-N and PO₄-P from leachate. The shut-down study revealed the effect of aeration has a significant impact on the performance of AGR. The treatment of real leachate diluted with wastewater showed a promising result obtaining a substantial reduction of COD (65%), NH₃-N (93%), and PO₄-P (64%). The metagenomics study revealed *Proteobacteria* and *Planctomycetes* are the predominant species in aerobic granules of AGR. The r_{max} and K_s were estimated as 123.5 mg/L h and 309 mg/L, respectively. The finding of this study would help to design a large-scale AGR for the simultaneous treatment of COD, NH₃-N, and PO₄-P from leachate.

Declarations

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Authors' contributions

All the authors contributed to the conceptualization of this study. Data curation, formal analysis, investigation, methodology, and writing original draft was contributed by Vikalp Saxena; Ritik Bhatt helped in material preparation, data curation, and performing experiments in the laboratory. Writing, reviewing, editing, and supervision were performed by Susant Kumar Padhi. Lopa Pattanaik contributed to the validation, visualization, and editing of the final draft of the manuscript.

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Data availability

All the data generated and analyzed during this study are included in the manuscript.

Declaration

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

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Tables

Table 1 Characteristics of leachate

Parameters	Units	Leachate
pH	-	8.44 ± 0.5
Conductivity	(mS/cm)	28.9 ± 2.1
Total solid (TS)	mg/L	20540 ± 1039
Total volatile solid (TVS)	mg/L	3398 ± 270
Total chemical oxygen demand (TCOD)	mg/L	7588±262
Soluble chemical oxygen demand (SCOD)	mg/L	5654±152
Ammonia (NH ₃ -N)	mg/L	189±12
Nitrate (NO ₃ -N)	mg/L	25.7±1.2
Phosphate (PO ₄ -P)	mg/L	569±45
Biochemical oxygen demand (BOD ₅)	mg/L	2425±56

Table 2 Operating conditions and performance of AGR

Phases	Period (d)	Leachate (%)		Influent COD (mg/L)	COD Removal (%)	NH ₃ -N concentration (mg/L)	NH ₃ -N Removal (%)	PO ₄ -P concentration (mg/L)	PO ₄ -P Removal (%)
		Synthetic	Real						
Start-up	0-10	100	0	244±21					
I	11-31	100	0	668±110	81	30±3.3	50	147±18	66
II	32-55	100	0	908±65	71	37±3	49	204±12	40
III	56-76	100	0	1149±93	75	48±1.3	47	221±17	34
Shut-down									
IV	77-82	100	0	976±105	89	49±2.8	49	232±21	16
V	83-88	100	0	888±45	93	48±5	66	216±14	21
Shock loading									
VI	89-92	100	0	1679±163	84	47±3	31	224±16	18
VII	93-96	100	0	2886±104	61	48±2.5	12	196±20	12
VIII	97-100	100	0	3773±302	58	50±4	8	227±13	6
Treatment of real leachate (volumetric ratio: 20%)									
IX	101-104	0	100	848±81	62	13±2	61	75±5	56
X	105-108	0	100	906±101	65	8±0.5	93	77±8	64

Table 3 Performance of various bioreactors for treatment of leachate

Type of bioreactor	COD concentration (mg/L)	COD removal (%)	NH ₃ -N concentration (mg/L)	NH ₃ -N removal (%)	PO ₄ -P concentration (mg/L)	PO ₄ -P removal (%)	References
AGR	848-906	62-65	8-13	61-93 • 75-77	• 56-64	•	Present study
Activated sludge process (ASP)	811±75	50-80	130-450	60-80 • -	• -	•	Ren et al. (2017)
Constructed wetland	626 ± 164	50	496±100	51	2.33±1.5	53	Bulc (2006)
Aerated lagoons	1740	75	1241	80			Mehmood et al. (2009)
Sequencing batch reactors (SBR)	2000-8000	40-60	800-2000	70-90	-	-	Xu et al. (2020)
Biological filter	719	18	581	33	-	-	Stephenson et al. (2004)
Moving-bed biofilm reactor (MBBR)	800-2000	20	400-800	90	-	-	Welander et al. (1998)
MBR	1500±239	63.4±12.2	288±112	98.2±1.7	4.3±1.5	52.5±32.6	Zolfaghari et al. (2016)
Membrane sequencing batch reactor (MSBR)	2456	40-60	238	100	8.2	35-45	Tsilogeorgis et al. (2008)
Rotating biological contactor (RBC)	2500-9000	10-60	-	-	-	-	Castillo et al. (2007)

Figures

Figure 1

Schematic of aerobic granular reactor (AGR)

Figure 2

Removal of COD at various phases of operation in AGR

Figure 3

Characteristics of aerobic granules in terms of SVI and VSS

Figure 4

Removal of $\text{NH}_3\text{-N}$ along with the formation of $\text{NO}_3\text{-N}$ in AGR

Figure 5

Removal of $\text{PO}_4\text{-P}$ at various phases in AGR

Figure 6

Microbial community at (a) phylum level and (b) genus level in AGR

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