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Treatment of domestic sewage and leachate using a moving bed hybrid bioreactor

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ABSTRACT

The treatment of domestic sewage using a moving bed hybrid bioreactor (MBHBR) was investigated by varying the concentrations of chemical oxygen demand (COD) (156 ± 14 – 1141 ± 78 mg/L), ammonia ($\text{NH}_3\text{-N}$) (2 ± 0.08 – 48.24 ± 0.93 mg/L), phosphorus ($\text{PO}_4\text{-P}$) (97 ± 3.2 – 281 ± 2.65 mg/L), and hydraulic retention times (HRTs). Initially, the reactor was operated at start-up mode (COD: 156 ± 14 mg/L), and gradually the COD concentrations were increased from phase-I (257 ± 24 mg/L) to phase-V (1141 ± 78 mg/L) by reducing the HRTs from 48 to 3 h. A significant improvement in COD removal, maximum up to 96%, was observed at a concentration of 858 ± 51 mg/L (phase-IV), then a sudden drop up to 81% was observed at a higher concentration of 1141 ± 78 mg/L (phase-V). Due to the increase in COD concentrations, average $\text{NH}_3\text{-N}$ removal was dropped from 85 to 32% from phase-I to Phase-V, respectively. In addition, the stability of MBHBR was studied to understand its performance in shut-down and shock loading phases. The co-treatment of sewage and leachate was also performed in MBHBR (COD: 864–881 mg/L and HRT: 6 h), which showed a COD removal of 77%–80% and $\text{NH}_3\text{-N}$ removal of 60%–94%. *Proteobacteria* was identified as a predominant species in MBHBR, which played a significant role in the co-treatment of sewage and leachate. The maximum biodegradation rate (r_{max}) and half-saturation constant (K_s) were estimated as 13.3 mg/L h and 361.8 mg/L, respectively.

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1. Introduction

With the increase in population growth and decreasing availability of fresh water in India, there is an acute need for efficient management of water resources through sewage recycling (Choudhary et al., 2016). Though sewage treatment plants are widely used in urban cities, rural populations do not have adequate facilities for sewage treatment. The sewage generated from household activities mainly comprises gray water. The sewage is characterized by moderate to high chemical oxygen demand (COD), ammonia ($\text{NH}_3\text{-N}$), and phosphorus ($\text{PO}_4\text{-P}$), which is challenging to treat by conventional methods and could cause pollution in receiving water bodies (Ogbonna et al., 2000). Various techniques such as constructed wetlands (CWs), up-flow anaerobic sludge blanket (UASB), facultative aerated lagoon (FAL), activated sludge process (ASP), and sequencing batch reactor (SBR) are widely used for the decentralized treatment of sewage in rural areas (Kalbar et al., 2012). The techniques applicable for sewage treatment in rural areas are low cost, simple to operate, and require higher land. The CWs are suitable for their operational simplicity during wastewater treatment in rural areas (Álvarez et al., 2017). ASP and SBR are less preferred due to higher capital and operating costs. However, SBR

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produces better effluent quality than ASP, which can be recycled (Kalbar et al., 2012). Currently, researchers are focusing on developing sewage treatment to be more efficient, requiring less carbon footprint than conventional treatment methods. Aerobic treatment has lower temperature sensitivity and lesser opportunities for odour generation while producing a much higher effluent quality.

The moving bed bioreactor (MBBR) is one of the novel bioreactors that consists of suspended bio-carriers moving freely due to aeration flourishing the development of biofilm (Ødegaard, 2006). The performance of MBBR is affected by several factors includes hydraulic retention time (HRT), dissolved oxygen (DO), carrier size and shape, carrier percent fill, diffusion, mixing, and aeration rate (Kawan et al., 2016). It is designed as a compact biological treatment system with a lesser footprint, accounting for 10%–20% of conventional ASP (Cimbritz et al., 2010). The MBBR is integrated with ASP, resulting in a hybrid configuration known as integrated fixed-film attached sludge system (IFAS) with both suspended and attached biomass to improve nitrification and remove BOD and phosphorus (Campo et al., 2020; Mannina et al., 2020). The moving bed hybrid bioreactor (MBHBR) is a modified version of IFAS consisting of suspended (moving bed) and attached (fixed bed) biomass with both aerobic and anaerobic environments that efficiently treat high-strength sewage. In the moving bed system, the microbes are in the form of flocs or biofilm grown over the bio-carrier tend to move freely in the mixed liquor due to the continuous aeration. In the fixed bed system, inert supporting media consisting of rock, plastic, or sponge are widely used to retain microbes, wastes, and extracellular polymers (Shah et al., 2017). The MBHBR has excellent microbial retention by enhancing the formation of thick biofilms over the bio-carrier results in good contact of the sewage with the biofilm than the conventional attached-growth systems (Ozgun et al., 2013). Due to this phenomenon, a higher mass transfer occurs, allowing the further breakdown of the complex compounds into simpler ones by sustaining higher loading rates. Gonzalez-Tineo et al. (2020) used a combination of the anaerobic–aerobic hybrid reactor showed better efficiency for removal of COD (99%) and ammonium (99.8%) at an organic loading rate (OLR) of 10 kg COD/m³ d. The porous bio-carriers like polyurethane sponges are widely used to retain more biomass because of high porosity, which is efficient for operating at shorter HRTs (Chen et al., 2019). The sponge helped to obtain a quicker start-up, while offering much higher removal rates of COD and NH₃-N (Zhang et al., 2017). This novel property of polyurethane sponge makes it an ideal bio-carrier for low-cost municipal and industrial sewage treatment.

Domestic sewage generated from human activities consists of high ammonia, nitrate, phosphorus, solid content, various chemicals, and biodegradable organic compounds (Chan et al., 2009), which serves as a better growing medium for microbial culture than industrial sewage. Trace elements enhance the metabolic activities of microorganisms by uptaking the nutrients such as nitrogen and phosphorus and increase the efficiency of sewage treatment. However, landfill leachate treatment requires a more sophisticated technique due to its physico-chemical properties, especially very high COD, BOD, and total ammoniacal nitrogen (TAN), which lasts for more than a decade if it remains untreated (Brennan et al., 2017). The characteristics of landfill leachate have a high degree of variability in terms of both quality and quantity. Thus, treatment of domestic sewage along with leachate is viable and economical to control influent characteristics because the pollutant load and flow rate significantly vary with time (Neczaj et al., 2007). Chaudhari and Basheer (2008) used a submerged aerobic fixed film (SAFF) reactor for the combined treatment of domestic sewage and landfill leachate (3%–8% v/v). The COD removal efficiency was dropped from 75% to 49% at 24 HRT with an increase in the percentage of leachate.

The main focus of this study is to evaluate the performance of the MBHBR for the treatment of domestic sewage and leachate. This project aims to design a MBHBR consisting of a moving bed integrated with a fixed bed, using polyurethane sponges as bio-carrier to enhance domestic sewage and leachate treatment. This study also reveals the optimal operating conditions of the MBHBR at various concentrations of COD, NH₃-N, PO₄-P, and HRTs.

2. Materials and methods

2.1. Seed culture and synthetic sewage

The seed culture was collected from the aeration tank of the sewage treatment plant (STP), Dadri, India. The inoculum was developed by acclimatization before adding it into the MBHBR. The synthetic sewage with the slight modification described by Chen et al. (2019) was used to acclimatize the inoculum and feed into the MBHBR. The synthetic sewage was prepared by adding following constituents in water (mg/L): Glucose (180–1260), FeCl₃·6H₂O (7.5–52.5), CaCl₂(10–70), (NH₄)₂SO₄(225–1575), KH₂PO₄(35–350), K₂HPO₄(150–1050), MgSO₄(30–210), NaHCO₃(150–1050), MnSO₄(0.1–0.7), CuSO₄(0.1–0.7). Analytical grade chemicals with 99% purity were used to prepare the synthetic sewage having a pH of 7.7 ± 0.05.

2.2. MBHBR set-up

The MBHBR consists of two sections (moving and fixed bed) made of Perspex column (Fig. 1). The total volume of the MBHBR is 8.5 L (1219 mm height × 94 mm diameter) with a working volume of 7.9 L (moving bed: 4.23 L and fixed bed: 3.67 L). Four sampling ports are there in the MBHBR for the collection of samples (Fig. 1). The moving bed section is separated from the fixed bed with perforated disks to allow sewage flow between the two sections. The sewage was fed to MBHBR at the influent sampling port by a peristaltic pump, and the effluent was collected from the top sampling port (Fig. 1). An air compressor and air sparger were used to constantly provide air at a flow rate of 3 L/min to the MBHBR.

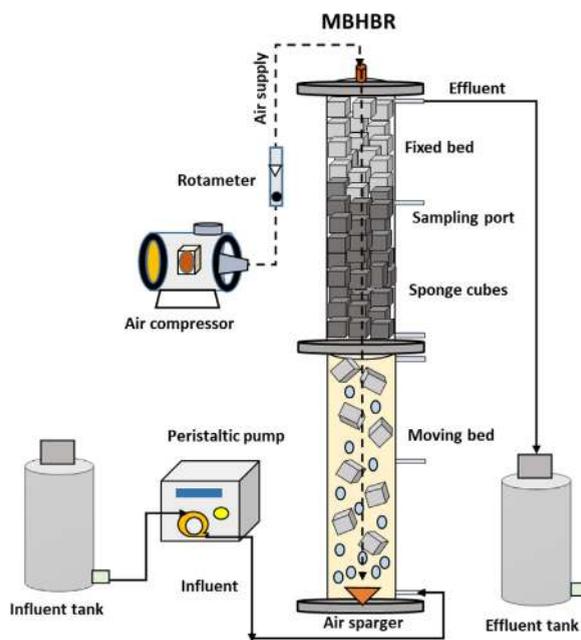


Fig. 1. Experimental set-up of MBHBR.

2.3. Bio-carrier

In moving bed and fixed bed systems of MBHBR, polyurethane sponges were used as a bio-carrier for retaining the biomass. The sponge was purchased from a local vendor and sponge cubes were prepared of uniform dimensions of $20 \times 20 \times 20$ mm with a specific surface area of nearly $0.95 \text{ m}^2/\text{g}$ and a density of 29 kg/m^3 . Sponge cubes were washed with tap water and dried in the oven at $60 \text{ }^\circ\text{C}$ for 2 h before using in MBHBR.

2.4. Start-up and operation of MBHBR

In start-up phase, the inoculum with a mixed liquor suspended solids (MLSS) concentration of $5.34 \pm 0.61 \text{ g/L}$ was fed into the MBHBR, enhancing it to grow effectively over the surface of the moving bed and fixed bed sponge medium. A longer start-up period was observed for the development of biofilm in the fixed bed system. The synthetic sewage with a COD of $156 \pm 14.1 \text{ mg/L}$ was maintained to acclimatize the microorganism for 10 days. In this phase, the MBHBR was operated in closed-loop conditions concerning microorganisms to enhance the growth by preventing its loss. The sewage was fed continuously at a constant flow rate using a peristaltic pump into the bottom of MBHBR, comprising of moving bed and passed up to the upper part of the reactor consisting of a fixed bed (Fig. 1). The influent and effluent samples were collected weekly twice from the sampling ports to determine the performance of the MBHBR. The MBHBR was operated at room temperature ($25 \pm 2 \text{ }^\circ\text{C}$). The process methodology for the operation of MBHBR is shown in supplementary Fig. S1.

In MBHBR, the COD concentration of synthetic sewage was gradually increased up to $1141 \pm 78 \text{ mg/L}$ in five different phases (I–V) by successively reducing the HRTs up to 3 h. After the successful operation up to phase-V, the effect of shut-down and shock loading on MBHBR was studied to assess its stability. During the shock loading (phases: VIII–X), the concentrations were increased further to nearly twofold ($1991 \pm 127 \text{ mg/L}$), threefold ($3256 \pm 155 \text{ mg/L}$), and fourfold ($4199 \pm 209 \text{ mg/L}$) with respect to phase-V. Moreover, the MBHBR was fed with real domestic sewage (source: STP, Dadri, India) and leachate (source: Ghazipur landfill site, Delhi, India) to evaluate its performance for its wide application.

2.5. Analytical methods

The influent and effluent samples were collected from MBHBR for determining the wastewater quality parameters. The physical and chemical parameters such as pH, MLSS, COD, $\text{NH}_3\text{-N}$, $\text{NO}_3\text{-N}$, and $\text{PO}_4\text{-P}$ were analyzed twice per week. The testing of the samples was carried out by following the standard methods recommended by the American Public Health Association (APHA, 2005).

2.6. Microbial community analysis

After the end of phase XII, a homogenized sample was collected from MBHBR and allowed to settle for 5 min to get concentrated sludge and preserved at $-20\text{ }^{\circ}\text{C}$ for the metagenomics study (Tran et al., 2020). The DNA extraction was done using commercially available kits (Qiagen, Zymo Research, ThermoFisher, USA). The microbial community were characterized via 16S rRNA sequencing. The bacterial V3–V4 region of 16 s rRNA gene was amplified using the primers V13F (5'AGAGTTTGATGTTGGCTCAG3') and V13R (5' TTACCGCGGCMGCSGGCAC3'). The polymerized chain reaction (PCR) was performed with an initial denaturation at $95\text{ }^{\circ}\text{C}$ for 15 s, annealing at $60\text{ }^{\circ}\text{C}$ for 15 s, elongation at $72\text{ }^{\circ}\text{C}$ for 2 min with a final extension at $72\text{ }^{\circ}\text{C}$ for 10 min, and hold at $4\text{ }^{\circ}\text{C}$ for 25 cycles (Ji et al., 2019). Ampure beads were used to purify the amplicons from each sample in order to remove the unused primers. Additional 8 cycles of PCR were performed with the help of Illumina barcoded adapters to prepare the sequencing libraries.

The raw data quality control (QC) was done using FASTQC and MULTIQC, followed by trimming of adapters and low-quality reads by TRIMGALORE. The trimmed reads are further taken for processing, which includes merging paired-end reads, chimeria removal, operational taxonomic unit (OUT) abundance calculation, and estimation correction. This is achieved by Uclust (QIIME) program, which enables highly accurate investigations at the genus level. Taxonomic classification was done using the Greengenes database, NCBI.

2.7. Biokinetic in MBHBR

The biokinetic constants describe the kinetic behavior of MBHBR, were determined using modified Monod model as shown in Eq. (1) (Padhi and Gokhale, 2016):

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

where, V is the total working volume (L) of MBHBR, Q is the flow rate of sewage (L/h), C_i and C_e are the influent and effluent COD concentrations (mg/L), respectively, C_{ln} is the log mean concentration $[(C_i - C_e)/\ln(C_i/C_e)]$, K_s is the half-saturation constant (mg/L), and r_{max} is the maximum biodegradation rate per unit volume (mg/L h).

3. Results and discussions

3.1. Performance of MBHBR

The MBHBR quickly adapted to the conditions, took 10 days start-up time to achieve a stable effluent concentration. After the acclimatization of biomass in the start-up phase, the biodegradation of the organic compound, $\text{NH}_3\text{-N}$, and $\text{PO}_4\text{-P}$ present in the synthetic sewage was investigated by operating the MBHBR in continuous mode. The performance of the MBHBR was investigated over a period of 155 days (phases: I–XII). The MBHBR was operated with various increasing influent COD (156 ± 14.1 – 4199 ± 209 mg/L), $\text{NH}_3\text{-N}$ (2 ± 0.08 – 48.24 ± 0.93 mg/L), and $\text{PO}_4\text{-P}$ (97 ± 3.18 – 281 ± 2.65 mg/L) by gradually decreasing the HRTs from 48 to 3 h in various phases until the pseudo-steady state was reached for each phase (Table 1). The attributing factors like pH and air flow rates were monitored throughout the study to maintain an optimum environment for the growth of microorganisms in MBHBR.

In phase-I, an influent COD concentration of 257 ± 24 mg/L was maintained in MBHBR for 16 days. Maximum removal of 86% was observed, which could be due to the higher HRT (48 h) maintained during this phase (Fig. 2). At the same time, $\text{NH}_3\text{-N}$ removal of 85% was observed at a lower COD concentration, forming a nitrate of about 16.5 mg/L in this phase (Fig. 3). However, the removal of $\text{PO}_4\text{-P}$ was less ($\sim 27\%$) at an influent phosphorus concentration of 97 ± 3.18 mg/L in phase-I (Fig. 3). Typical activated sludge process (ASP) can only remove $\text{PO}_4\text{-P}$ ranges between 20%–40% by aerobic heterotrophic bacteria, which utilizes phosphorus for their metabolic activities (Morse et al., 1998). Also, the presence of the aerobic conditions and nitrates in the reactor inhibits the environment required for the growth of the phosphate accumulating organisms (PAOs), which adversely affects the removal of $\text{PO}_4\text{-P}$ (Zheng et al., 2014).

In phase-II, the COD concentration was maintained at 403 ± 23 mg/L for 23 days at an HRT of 24 h. Significant COD removal was observed, achieving a maximum reduction of 96% on day 51 (Fig. 2). However, the average removal efficiency of the COD in this phase is nearly 87% (Table 1). This shows a continuous improvement in the performance of the MBHBR, which might be due to the enhancement of the growth of microorganisms in the moving bed and fixed bed that entraps the organic compounds and treats efficiently by giving high quality treated effluents (Xiong et al., 2018). A significant increase in $\text{NH}_3\text{-N}$ removal up to 99% was observed on 51st day of the operation because of a lower influent COD concentration of 435 mg/L. However, an average $\text{NH}_3\text{-N}$ removal of 93% was observed in this phase shows the potential of MBHBR, which released nitrate at a concentration of 91 ± 18.8 mg/L in the effluent stream. This indicates that nitrifiers are dominant at lower COD concentration plays a vital role in the nitrification by converting $\text{NH}_3\text{-N}$ to nitrate present in the sewage (Xiong et al., 2018). Fig. 3 shows that there is significant removal of $\text{NH}_3\text{-N}$ in phase-I and II. However, the $\text{NH}_3\text{-N}$ removal was dropped from phase-III onwards due to a gradual increase in COD concentration, which significantly impacted its removal. The removal of COD increases as time progresses, with an average removal of 94% was observed while maintaining nearly 632 ± 90 mg/L of influent COD concentration in phase-III. However, the removal of $\text{NH}_3\text{-N}$ drastically decreased up to

Table 1
Operating conditions of the MBHBR.

Phases	Period (d)	Wastewater (%)		Influent COD (mg/L)	HRT (h)	Removal (%)
		Synthetic	Real			
Start-up	0–10	100	0	156 ± 14	–	
I	11–27	100	0	257 ± 24	48	86
II	28–51	100	0	403 ± 23	24	87
III	52–79	100	0	632 ± 90	12	94
IV	80–103	100	0	858 ± 51	6	86
V	104–123	100	0	1141 ± 78	3	81
Shut-down						
VI	124–129	100	0	868 ± 22	6	94
VII	130–135	100	0	888 ± 12	6	90
Shock loading						
VIII	136–139	100	0	1991 ± 127	6	94
IX	140–143	100	0	3256 ± 155	6	63
X	144–147	100	0	4199 ± 209	6	54
Co-treatment of sewage and leachate (volumetric ratio: 80:20% v/v)						
XI	148–151	0	100	864 ± 90	6	77
XII	152–155	0	100	881 ± 70	6	80

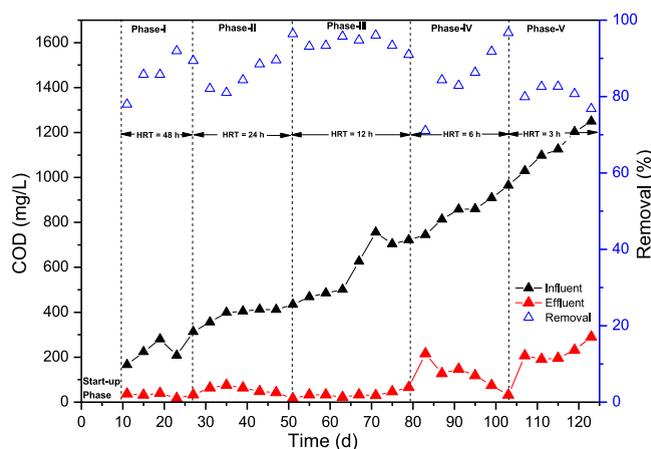


Fig. 2. Removal of COD at various phases of operation in MBHBR.

70% in phase III, subsequently forming a $\text{NO}_3\text{-N}$ of 21.6 ± 1.8 mg/L. It is possibly due to the increase in COD concentration, due to which heterotrophic bacteria are dominant over nitrifiers that inhibit the removal of $\text{NH}_3\text{-N}$ present in the sewage (Padhi and Gokhale, 2016). Thus, the removal of COD was not impacted, but the removal of $\text{NH}_3\text{-N}$ dropped significantly. Similarly, due to gradual increase in the $\text{PO}_4\text{-P}$ concentrations in phases II and III shows a significant decline in $\text{PO}_4\text{-P}$ removal up to 13%, which could be due to a rise in the concentration of organic compound, gives a detrimental effect on the activity of PAOs (Torresi et al., 2019).

Significant changes have been observed in the performance of the MBHBR due to a gradual increase in influent COD concentrations during phases IV and V. The MBHBR shows the highest COD removal of 96% on day 103 in phase-IV (Fig. 2). This shows an improvement in the overall performance of MBHBR with varying COD concentrations and HRTs over the entire period of operation. However, the average COD removal was 86% in phase-IV at an influent COD concentration of 858 ± 51 mg/L (Table 1). The COD removal was dropped subsequently in phase-V up to 81% due to the higher concentration of COD (1141 ± 78 mg/L). In phase-IV, the average $\text{NH}_3\text{-N}$ removal was about 53%, with the lowest removal of 42% on 90th day and the highest removal of 70% on 105th day at an influent $\text{NH}_3\text{-N}$ concentration of 48 ± 1 mg/L. The maximum $\text{PO}_4\text{-P}$ removal of 13% was observed on 115th day of operation while showing an overall removal of 8% in phase-IV. Phosphate removal is not as efficient as the removal of COD and $\text{NH}_3\text{-N}$, possibly due to a limited number of PAOs in the aerobic environment (Torresi et al., 2019). An increase in $\text{PO}_4\text{-P}$ removal can be achieved by integrating anaerobic with aerobic (or anoxic) reactors (Salehi et al., 2019). Polyphosphate uptake requires advanced polishing treatment, as its removal is complex and beyond the scope of this study. The phase-V lasted for about 19 days, where insignificant removal of $\text{NH}_3\text{-N}$ and $\text{PO}_4\text{-P}$ were observed due to a higher COD concentration of 1141 ± 78 mg/L, although a good reduction ($\sim 81\%$) in COD was observed at 3 h HRT in this phase. This ensures that the MBHBR is quite promising for removing a wide range of pollutants from sewage. The effluent pH of MBHBR was gradually reduced from 7.3 ± 0.3 in

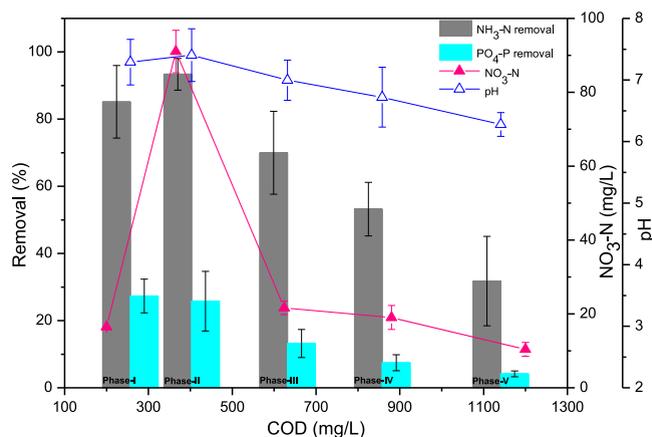


Fig. 3. Effect of COD on removal of NH₃-N and PO₄-P, NO₃-N formation, and pH.

phase-I to 6.27 ± 0.19 in phase-V due to increased CO₂ in the effluent released during the biodegradation at higher COD concentrations (Fig. 3). The NH₃-N removal was also significantly dropped in subsequent phases because of decreased pH (Chakraborty et al., 2020).

MBBRs are extensively used to treat sewage and leachate due to their simplicity in operation and robustness of the system. Organic loading, type of nutrients, bio-carrier, and HRT play a significant role in the performance of the MBBR. The performance of various MBBRs for COD removal at different operating conditions is provided in the supplementary Table S1. It has been observed that MBBR efficiently treats sewage and leachate at COD concentrations between 192 to 17600 mg/L at different operating conditions, with removal efficiencies varying in the range from 68 to 99% (supplementary Table S1). In this study, the COD removal (77%–80%) in MBHBR showed comparable results concerning other MBBRs used by previous researchers.

3.2. Stability of the MBHBR

The stability of the MBHBR was investigated during the shut-down and shock loading, and its effect on reactor performance was studied (Fig. 4). The effect of aeration and influent feed concentration on the performance of MBHBR was studied during the shut-down phases (VI and VII). Whereas the effect of increasing feed concentrations was studied during the shock loading phases (VIII–X) of MBHBR. In phase-VI, during the shut-down study, the aeration of MBHBR was stopped for 2 days by ensuring the continuous supply of the influent COD (868 ± 22 mg/L) at an HRT of 6 h (Fig. 4). When the reactor resumed its operation, no significant drop in COD removal was observed, which showed an overall COD removal efficiency of about 94%, clearly signifies the excellent performance of the reactor over such harsh environmental conditions. The higher removal efficiency obtained during this phase is due to the feast-famine strategy, which shows high potential for removing the organic micro-pollutants in MBBRs (Liang et al., 2021). Further, the influent feed to the reactor was interrupted for 2 days without stopping the aeration supply in phase-VII. After the resumption of the operation of MBHBR, a slight decrease in COD removal (90%) was observed. The results obtained during the shut-down phase ensure that the performance of MBHBR is influenced more by influent feed rather than the supply of air (Padhi and Gokhale, 2017). However, no significant removal of NH₃-N and PO₄-P was observed during the shut-down study. Hence, MBHBR is highly effective for removing organic compounds during this phase.

The performance of MBHBR was also evaluated under three different shock-loading conditions by sudden increasing the influent concentrations to understand the stability of the system (Fig. 4). In phase VIII, the influent feed concentration was nearly doubled (1991 ± 127 mg/L) with respect to phase-V, and the reactor was operated at 6 h HRT for 3 days (Table 1). A higher COD removal of 94% with insignificant NH₃-N and PO₄-P removal was observed in this phase. The robust performance of MBHBR with high organic concentrations shows a significant occurrence of biological activity within the system. The influent COD concentration was increased further up to 3256 ± 155 mg/L in phase IX. After stabilizing the system for 3 days, the COD removal was dropped up to 63%, which shows the efficiency of MBHBR even at significantly higher COD concentrations. The performance of MBHBR was also studied at very high influent COD (4199 ± 209 mg/L) in phase-X of the shock loading phase, which shows 54% COD removal, slightly lesser than the phase IX. Reduction of overall reactor performance due to the successive application of shock loading might be due to inhibition effect and limited biological activity (Mohammad et al., 2017). The results obtained from this study highlight that the MBHBR is a viable option and highly efficient for the extensive treatment of sewage.

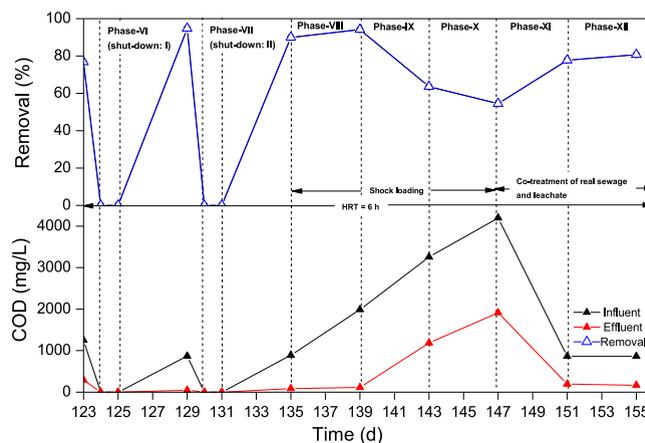


Fig. 4. Effect of shutdown, shock loading, and co-treatment of sewage and leachate on performance of MBHBR.

Table 2

Characteristics of real sewage and leachate.

Parameters	Units	Sewage	Leachate	Indian standards for treated sewage discharge ^a
pH	–	7.68 ± 0.57	8.65 ± 0.4	6.5–9.0
Electrical conductivity	mS/cm	354 ± 40	30.6 ± 3.1	–
Total solid (TS)	mg/L	1338 ± 17	21580 ± 1047	–
Total volatile solid (TVS)	mg/L	431 ± 216	3670 ± 380	–
Total suspended solid (TSS)	mg/L	936 ± 189	2061 ± 35	100
Total chemical oxygen demand (TCOD)	mg/L	292 ± 26	7870 ± 860	250
Soluble chemical oxygen demand (SCOD)	mg/L	176 ± 34	5220 ± 400	–
Ammonia (NH ₃ -N)	mg/L	2.46 ± 0.05	199 ± 20	50
Nitrate (NO ₃ -N)	mg/L	6.8 ± 0.24	24.8 ± 0.52	10
Phosphate (PO ₄ -P)	mg/L	166 ± 7.07	615.3 ± 65	5
Biochemical oxygen demand (BOD ₅)	mg/L	234.6 ± 5.1	2364 ± 120	30
Turbidity	NTU	14.2 ± 2.5	703 ± 17	–

^aChakraborty et al. (2020).

3.3. Co-treatment of real sewage and leachate

The characteristics of real sewage and leachate are summarized in Table 2. The real sewage (~80% v/v) and leachate (~20% v/v) contributing a COD in the range of 864–881 mg/L fed into the MBHBR (Table 1). The co-treatment of real sewage and leachate was investigated in MBHBR over one week (148–155 days) of operation at 6 h HRT (Fig. 4). This shows an excellent COD removal of 77%–80%, along with a significant reduction of NH₃-N (60%–94%) due to its lower concentration (9 ± 0.1 mg/L). However, the PO₄-P removal is negligible. This study indicates that a consistent removal of organic carbon and NH₃-N was observed during the co-treatment of sewage and leachate. Thus, the results ascertain that the MBHBR is a viable alternative for simultaneously treating real sewage and leachate. A more than 20% leachate could be detrimental for sewage treatment plants (Campos et al., 2019).

In recent studies, the co-treatment of sewage and leachate was studied using various bioreactors such as ASP (Ferraz et al., 2016), constructed wetland (Saeed et al., 2020), SBR (Ranjan et al., 2016), and submerged aerobic biofilter (SAB) (Ferraz et al., 2014), etc. The MBHBR showed promising results with 77%–80% removal of COD and 60%–94% removal of NH₃-N at 6 h HRT. It has been observed that various bioreactors were adopted in the past to co-treat sewage and leachate, which is limited to a low volumetric ratio (<20% v/v) of leachate, in order to increase the efficiency of the system. However, with a higher volumetric ratio (~20% v/v) of leachate in this study with increasing COD concentrations (864–881 mg/L), the MBHBR showed a better performance. Table 3 shows the performance of various bioreactors for co-treatment of sewage and leachate with comparison to MBHBR.

A limited study has investigated the feasibility of co-treatment of real sewage and leachate using the biological process in India. Ranjan et al. (2016) investigated the co-treatment of old landfill leachate (0%–40% v/v) and sewage by varying the HRT in SBR. The SBR produced an optimum result at 6 d HRT and 20% v/v landfill leachate by removing COD (60%–70%), NH₃-N (93%), and PO₄-P (80%) efficiently. Similarly, Chakraborty et al. (2020) used SBR for co-treatment landfill leachate (20% v/v) and municipal sewage in India. The SBR effectively removed COD (65%), NH₃-N (89%), and PO₄-P (64%) simultaneously at concentrations of 1732, 144, 2.2 mg/L, respectively. The MBHBR used in this study effectively removed COD (77%–80%) and NH₃-N (60%–94%) during the co-treatment of sewage and leachate, which indicates the stability and robustness of the MBHBR.

Table 3

Performance of various bioreactors for co-treatment of sewage and leachate.

Type of bioreactor	Advantages	Limitations	COD concentration (mg/L)	COD removal (%)	NH ₃ -N concentration (mg/L)	NH ₃ -N removal (%)	References
Moving bed hybrid bioreactor (MBHBR)	<ul style="list-style-type: none"> Resist high shock load at short HRT Retention high biomass 	<ul style="list-style-type: none"> High cost of bio-carrier and biomass growth inside the carrier is difficult to quantify Continuous aeration required for bio-carrier movement 	864–881	77–80	9 ± 0.1	60–94	Present study
Activated sludge process (ASP)	<ul style="list-style-type: none"> Lower operating and maintenance cost 	<ul style="list-style-type: none"> Poor refractory matter removal and inability to treat high organic loadings 	300 ± 21	60	48 ± 5.4	50	Ferraz et al. (2016)
Constructed wetland	<ul style="list-style-type: none"> Low-cost alternative technology 	<ul style="list-style-type: none"> Performance is inconsistent Large area and continuous monitoring required 	836.3 ± 79	66	149.3 ± 34	99	Saeed et al. (2020)
Sequencing batch reactors (SBR)	<ul style="list-style-type: none"> Direct co-treatment is feasible for the removal of COD and NH₃-N No biomass inhibition up to 20% landfill leachate 	<ul style="list-style-type: none"> Post-treatment may require for complete removal of COD and NO₃-N to meet discharge standards 	1000–2000	50–85	30–200	56–90	Ranjan et al. (2016)
Aerobic granular reactor (AGR)	<ul style="list-style-type: none"> Higher settling velocity with high biomass retention Resist high shock load and has a small footprint 	<ul style="list-style-type: none"> Requires longer start-up period High instability of granules and post-treatment may require to meet disposal standards 	1080 ± 106	65	340 ± 13	42	Ren et al. (2017)
Submerged aerobic biofilter (SAB)	<ul style="list-style-type: none"> Low footprint and allows high biomass retention 	<ul style="list-style-type: none"> The fixed film in biofilter causes clogging and flow channeling 	300 ± 141	50–70	37.5 ± 10	75–85	Ferraz et al. (2014)
Anaerobic–anoxic–aerobic (A2/O) bioreactor	<ul style="list-style-type: none"> Enhanced the biological nitrogen and phosphorus removal 	<ul style="list-style-type: none"> It needs a large volume of the reaction tank Optimum operating conditions need to be maintained 	118	77	34.4	95	Yu et al. (2010)
Subsurface wastewater infiltration system (SWIS)	<ul style="list-style-type: none"> Simple in construction, easier management, and low energy consumption Able to remove COD, NH₃-N, and PO₄-P completely 	<ul style="list-style-type: none"> Denitrification is limited, results in low total nitrogen (TN) removal. 	277–372	86–87	101–250	99–100%	Chen et al. (2021)

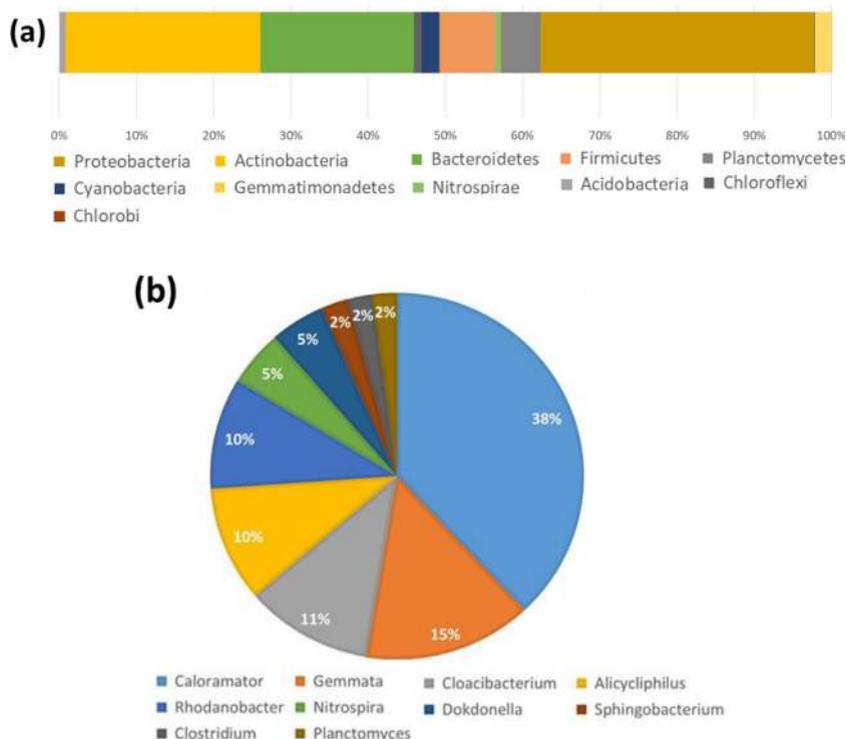


Fig. 5. Microbial community by phylum level (a) and genus level (b) in MBHBR.

3.4. Metagenomic analysis

The bacterial community was dominated by phylum *Proteobacteria* (~35%) followed by *Actinobacteria* (~25%), *Bacteroidetes* (~20%), *Firmicutes* (~9%), *Planctomycetes* (~7%), *Cyanobacteria* (~3%), *Gemmatimonadetes* (~3%), and *Nitrospirae* (~1%) etc. (Fig. 5a). *Proteobacteria* and *Actinobacteria* constituted more than 50% of the microbial community found in the MBHBR, which plays a vital role in the degradation of the organic and nitrogenous compounds (Lopez-Lopez et al., 2012). *Proteobacteria* was the most predominant in MBHBR, responsible for organics, nitrogen, and phosphorus removal. The results of this study are consistent with the research conducted by Ji et al. (2019). In addition, the presence of *Bacteroidetes* plays a significant role in the degradation of macromolecules, xenobiotics, and proteins. A previous study also reported the presence of *Bacteroidetes* in mature landfill, which plays an active role in the leachate treatment (Guo et al., 2015). The presence of *Planctomycetes* in the MBHBR results in anaerobic ammonium oxidation (Kong et al., 2017). In addition, *Nitrospira* was also detected, which is efficient in complete nitrification but needs longer generation time, preventing its proliferation in MBHBR (Xiong et al., 2018). As per the metagenomic study conducted in MBHBR, *Caloramator* (38%), *Gemmata* (15%), and *Cloacibacterium* (11%) were found to be the dominant bacteria at the genus level (Fig. 5b). The genus *Caloramator* can degrade the carbohydrate and protein present in oil refinery sludge (Wang et al., 2016). The *Gemmata* sp. can grow better on sponge media and adapted to extensive environmental conditions (Kaboré et al., 2019). Hence, the presence of a diverse microbial community shows a wider metabolic potential in MBHBR.

3.5. Determination of biokinetic constants in MBHBR

The biokinetic constants (r_{\max} and K_s) were estimated by plotting a graph between $(1/C_{in})$ and $[(V/Q)/(C_i - C_e)]$ as shown in supplementary Fig. S2. The r_{\max} and K_s were estimated as 13.3 mg/L h and 361.8 mg/L, respectively. The r_{\max} and K_s obtained in this study are higher than the values reported previously (Sonwani et al., 2021), indicating the efficiency of MBHBR.

3.6. Practical applications and future prospects of MBHBR

The MBHBR provides excellent biomass retention and requires a lesser footprint, which helps in both nitrification-denitrification for complete nitrogen removal. The MBHBR is a promising alternative that can sustain high shock and volumetric loads by generating low sludge (Xiong et al., 2018). Chen et al. (2008) successfully used MBHBR to treat landfill

leachate with high COD concentrations between 7500–17 600 mg/L. The MBHBR can be used as an upgrade for existing facilities to enhance pollutant removal. However, the high cost of biofilm carriers is the primary limiting factor for its large-scale applications.

The MBHBR has demonstrated a great potential for the co-treatment of domestic sewage and landfill leachate. However, further investigation is required to determine the performance of MBHBR at a high volumetric ratio (>20%) of leachate during the co-treatment. In addition, the fate and removal of emerging contaminants from sewage and leachate can be investigated using MBHBR. To enhance the PO₄-P removal efficiency, the integration of MBHBR with other biological techniques needs further investigation. It would be interesting to conduct an analysis utilizing different types of biofilm carriers at different filling ratios to determine the performance of MBHBR. The application of MBHBR at the field scale is still in the early stage, which can be optimized to improve its performance. Additionally, there is an urgent requirement to evaluate the techno-economic feasibility of MBHBR before its full-scale application.

4. Conclusions

The MBHBR used in this study is effective for the treatment of sewage and leachate. The MBHBR was operated by varying the concentrations of COD, NH₃-N, PO₄-P, and HRTs. The maximum COD removal of 96%, NH₃-N removal of 93%, PO₄-P removal of 27% was observed at an influent COD, NH₃-N, and PO₄-P concentrations of 858 ± 51, 33.8 ± 4.2, and 97 ± 3.2 mg/L, respectively. The co-treatment of sewage and leachate was effective in MBHBR, showing the COD and NH₃-N removal in the range of 77%–80% and 60%–94%, respectively. The results show consistent removal of COD and NH₃-N throughout the study, which indicates the stability and robustness of the MBHBR. However, with increasing COD concentrations, the removal of NH₃-N and PO₄-P was gradually decreased. It was also found that the shorter HRT (6 h) results in increasing COD removal, while longer HRT is suitable for the effective removal of NH₃-N present in sewage. *Proteobacteria* is the predominant species in MBHBR, effectively treated the sewage and leachate. The biokinetic constants, r_{\max} and K_s were obtained as 13.3 mg/L h and 361.8 mg/L, respectively. The finding of this study would help design a large-scale MBHBR for the treatment of sewage and leachate simultaneously.

CRedit authorship contribution statement

Vikalp Saxena: Data curation, Formal analysis, Investigation, Methodology, Resources, Writing – original draft. **Susant Kumar Padhi:** Conceptualization, Validation, Visualization, Supervision, Writing – review & editing. **Uday Jhunjhunwala:** Data curation, Formal analysis, Investigation, Methodology, Resources.

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.

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Appendix A. Supplementary data

Supplementary material related to this article can be found online at <https://doi.org/10.1016/j.eti.2021.101998>.

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