Environmental Pollution 243 (2018) 326–335

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Dynamic membrane bioreactor (DMBR) for the treatment of landfill leachate; bioreactor's performance and metagenomic insights into microbial community evolution☆

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ARTICLE INFO

Article history:
Received 20 May 2018
Received in revised form
6 August 2018
Accepted 27 August 2018
Available online 31 August 2018

Keywords:
Dynamic membrane
Landfill leachate
Nitrogen removal
Ammonia conversion
16 rRNA
Metagenomics

ABSTRACT

The use of dynamic membranes as a low-cost alternative for conventional membrane for the treatment of landfill leachate (LFL) was investigated in this study. For this purpose a lab-scale, submerged pre-anoxic and post-aerobic bioreactor configuration was used with nylon mesh as dynamic membrane support. The study was conducted at ambient temperature and LFL was fed to the bioreactor in gradually increasing concentration mixed with tap water (from 20% to 100%). The results of this study demonstrated that lower mesh pore size of 52 μm achieved better results in terms of solid-liquid separation performance (turbidity <10 NTU) of the formed dynamic membrane layer as compared to 200 and 85 μm meshes while treating LFL. Consistently high NH_4^+-N conversion efficiency of more than 98% was achieved under all nitrogen loading conditions, showing effectiveness of the formed dynamic membrane in retaining slow growing nitrifying species. Total nitrogen removal reached more than 90% however, the denitrification activity showed a fluctuating profile and found to be inhibited by elevated concentrations of free nitrous acid and NO_2^--N at low pH values inside the anoxic bioreactor. A detailed metagenomic analysis allowed a taxonomic investigation over time and revealed the potential biochemical pathways involved in NH_4^+-N conversion. This study led to the identification of a dynamic system in which nitrite concentration is determined by the contribution of NH_4^+ oxidizers (*Nitrosomonas*), and by a competition between nitrite oxidizers (*Nitrospira* and *Nitrobacter*) and reducers (*Thauera*).

1. Introduction

Landfill leachate (LFL) is undoubtedly one of the most challenging wastewaters to treat (Hashisho and El-Fadel, 2016). The aspect of variation in its quality and quantity (Renou et al., 2008) plus the presence of refractory organics, heavy metals and emerging contaminants (Kjeldsen et al., 2002) demands a more holistic treatment approach intended to meet increasingly stringent environmental and regulatory standards (Robinson, 2017). (LFL), in fact, demands an evolutionary design of the treatment scheme that is adaptable to the dynamic nature of LFL (Alvarez-Vazquez et al., 2004).

In this regard standalone treatment options have certain limitations, for an instance, biological treatment is sensitive to shock loading and reported to have limited performance when leachate's BOD_5/COD ratio is lower than 0.1 (Ahmed and Lan, 2012). Similarly, physico-chemical treatment options, although very efficient, may have limited application due to their high cost and environmental footprints (Ahmed and Lan, 2012; Kurniawan et al., 2006). Therefore, modern LFL treatment systems adopt a hybrid treatment scheme by combining biological (activated sludge systems, anaerobic digesters or lagoons) and physico-chemical treatments (flocculation/precipitation, chemical oxidation, adsorption and reverse osmosis) that showed to have promising results (Ahmed and Lan, 2012; Hashisho and El-Fadel, 2016; Renou et al., 2008; Garbo et al., 2017).

Membrane assisted biological treatment has gained major attention as an efficient and advanced hybrid treatment option for

☆ This paper has been recommended for acceptance by Dr. Harmon Sarah Michele.

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https://doi.org/10.1016/j.envpol.2018.08.090
0269-7491/

treating challenging wastewaters, including LFL (Ahmed and Lan, 2012). The consistent biological performance of membrane bioreactors (MBRs) in treating LFL regardless of its different characteristics was attributed to the retention of high biomass concentration and associated metabolic products, favourable to degrade even slowly biodegradable organic content (Ahmed and Lan, 2012; Alvarez-Vazquez et al., 2004; Xu et al., 2017). However, fouling phenomena is the main bottleneck in widespread MBR and it becomes even more serious when dealing with wastewater containing high amount of foulants like LFL, containing excessive amount of humic and fulvic acids that have reported to aggravate membrane fouling (Sutzkover-Gutman et al., 2010; Xu et al., 2017).

A new perspective for a possible evolution of MBR systems is the advent of dynamic membrane (DM) technology that is cheaper and more sustainable, yet comparable to conventional MBR systems in many aspects (including high solids retention and efficient treatment performance etc.) (Ersahin et al., 2012; Hu et al., 2016). DM is a purpose-built fouling layer (cake layer) made up of mixture of colloidal matter and biomass flocs (Zhang et al., 2014) that is used as a mean of solid-liquid separation medium instead of conventional membranes. The main highlights of DM technology include its reproducibility, lower maintenance, high flux (J) operation at low transmembrane pressures (TMP) with modest energy consumption (Alibardi et al., 2014; Ersahin et al., 2012; Zhang et al., 2014).

The application of DM for treating wastewater, including synthetic (Alibardi et al., 2014; Ersahin et al., 2016; Saleem et al., 2016) and municipal streams (Hu et al., 2016; Liu et al., 2009; Y. Xiong et al., 2016) have been extensively studied and very few studies on high strength, complex streams such as LFL (Saleem et al., 2018; Xie et al., 2014) have been carried out. Since DM is a biological fouling layer and its performance is linked with the chemical and biological makeup of the constituent sludge plus the hydraulic regime and bioreactor configuration of the system (Ersahin et al., 2017; Xiong et al., 2016), major variations have been reported for different parameters including transmembrane pressure (TMP), effluent flux and solids removal performance etc. (Ersahin et al., 2012).

In their studies, Saleem et al. (2018) and Xie et al. (2014) have observed low COD removal, very limited total nitrogen (TN) removal performance and poor effluent quality while treating LFL using DM. The information regarding the application of DM in treating LFL is still inadequate to bring about a general consensus over its performance. Similarly, the composition of the microbiome inhabiting the membrane bioreactors used for landfill leachate treatment is a crucial component for the performance of the system. Despite the microbiome can be investigated by isolation and cultivation of the microbial species, the use of cultivation-independent techniques based on high-throughput sequencing is attracting increasing attention (Remmas et al., 2017). Among these methods, amplicon sequencing is frequently applied, and allowed for example to reveal the dominance of candidate Saccharibacteria in membrane bioreactors (Remmas et al., 2017) or the inhibition of nitrification determined by leachate's content glycerol addition (Remmas et al., 2016). The biodiversity of microorganisms involved in greenhouse gases emission rates was also investigated, and it was found to be correlated with hydraulic loading rate (Nuansawan et al., 2016). More recently, the possibility to recover the genomes of the species composing the microbial community (genome-centric metagenomics), and the prediction of their functional role, led to an unprecedented level of resolution in the study of the microbiome (Campanaro et al., 2016).

Therefore, the main objective of this study was to assess the development and performance of DM while coupling it with a pre-anoxic and post-aerobic bioreactor for treating LFL targeting biological TN removal. The behaviour of the DM along with the biological performance of the system was evaluated in three successive phases during the experimentation. Application of state-of-the-art metagenomics approaches allowed for the first time to have a comprehensive overview of the "leachate microbiome" and its functional capabilities. Furthermore, this study also provides a thorough discussion on the diversity and evolution of the microbial community structure, starting from the initial inoculum during the bioreactor operation while treating LFL. The results obtained in this study are expected to fill the knowledge gap and to provide new insights on the use of DMs for treating LFL.

2. Materials and methods

2.1. Experimental setup and DM operational control

The study was conducted in a continuously mixed pre-anoxic (0.9 L) and post-aerobic (2.5 L) bioreactor made of 9 mm thick Plexiglas (Fig. 1). DM filtration was carried out in a submerged configuration inside the aerobic bioreactor provided with a hydrostatic water head of 8 cm. DM was developed over commercially available polyamide nylon meshes (200, 85 and 52 μm) (Supplementary material, Table S1). After 24 days of continuous bioreactor operation (end of phase 1) the 200 μm mesh was initially replaced by an 85 μm mesh and immediately after 1 day with a 52 μm mesh due to biomass loss with the effluent. DM module consisted of a supporting plastic cylindrical frame (15 mm diameter and 70 mm height) weaved with a single sheet of nylon mesh. The supporting frame was provided with uniformly distributed rectangular openings (5mm X 3mm) to carry out filtration. The effective filtration area was approximately 61% of the total surface area of the cylindrical frame measuring around 19 cm^2. In phase 2 (from day 25 to day 74) the single DM module was evaluated for its performance, however, in phase 3 two identical modules were used in parallel due to the concern of excessive fouling.

Peristaltic pumps (Watson Marlow SCI 400) were used to control bioreactor's hydraulics for permeate extraction, sludge recirculation (4–6 times of the effluent flow) and for feeding the reactor with LFL (Fig. 1). The feed peristaltic pump was connected to a level controller submerged inside the anoxic bioreactor to maintain the active volume of the system.

Dissolved oxygen (DO) was provided through aeration by using fine air bubble diffusers submerged inside the aeration tank. Air diffusers were connected to an aeration pump (Maxima R, Rolf C. Hagen Inc.) through an air flowmeter (Cole Parmer 1-800-323-4340). DO concentration was maintained above 2.0 $mg\,L^{-1}$ during the entire study. Continuous mixing was facilitated by magnetic stirrers (Komet Variomag Maxi), rotating at a constant rate of 300 and 400 rpm for the anoxic tank and aerobic tanks respectively.

DM cleaning was performed manually with a brush without any chemical treatment whenever the TMP value exceeded 15 kPa (set as an arbitrary upper limit for TMP) or effluent flux reduced to less than 5 $L\,m^{-2}\,h^{-1}$ (LMH) to maintain the HRT of the system around 10 d during the incremental phase of LFL addition.

2.2. Inoculum and feed

The initial inoculum was obtained from the aerobic bioreactor of a full-scale municipal wastewater treatment plant located in Padova (Italy) applying conventional activated sludge process. The inoculum had a total suspended solids (TSS) and volatile suspended solids (VSS) concentration of 5.50 $g\,L^{-1}$ and 3.76 $g\,L^{-1}$ respectively. The bioreactor was fed with gradually increasing concentration (20%–100%) of raw LFL mixed with tap water during the entire bioreactor operation. LFL samples were collected from a non-