Bioresource Technology 219 (2016) 53–63

Contents lists available at ScienceDirect

Bioresource Technology

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

Effects of hydraulic retention time and carbon to nitrogen ratio on micro-pollutant biodegradation in membrane bioreactor for leachate treatment

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HIGHLIGHTS

- Evaluate the micro-pollutant biodegradation under six HRT-C/N MBR conditions.
- C/N ratio can contribute micro-pollutant biodegradation via bacteria community.
- Phenolic compounds and PAEs were achieved in HRT 12 h.
- The degrading enzymes and their activities were systematically quantified.

ARTICLE INFO

Article history:
Received 30 April 2016
Received in revised form 20 July 2016
Accepted 22 July 2016
Available online 25 July 2016

Keywords:
Hydraulic retention time (HRT)
C/N ratio
Leachate
Membrane bioreactor (MBR)
Micro-pollutants
Enzyme activity

ABSTRACT

This research investigated the biodegradation of the micro-pollutants in leachate by the membrane bioreactor (MBR) system under six treatment conditions, comprising two C/N ratios (6, 10) and three hydraulic retention time (HRT) durations (6, 12, 24 h). The experimental results indicated that the C/N 6 environment was more advantageous to the bacterial growth. The bacterial communities residing in the sludge were those of heterotrophic bacteria (HB), heterotrophic nitrifying bacteria (HNB) and ammonia oxidizing bacteria (AOB). It was found that HB and HNB produced phenol hydroxylase (PH), esterase (EST), phthalate dioxygenase (PDO) and laccase (LAC) and also enhanced the biodegradation rate constants (k) in the system. At the same time, AOB promoted the production of HB and HNB. The findings also revealed that the 12 h HRT was the optimal condition with regard to the highest growth of the bacteria responsible for the biodegradation of phenols and phthalates. Meanwhile, the longer HRT duration (i.e. 24 h) was required to effectively bio-degrade carbamazepine (CBZ), *N*,*N*-diethyl-m-toluamide (DEET) and diclofenac (DCF).

1. Introduction

Leachate is a type of high strength wastewater with high concentrations of organic contaminants, organic and inorganic micro-pollutants, including phenols, phthalates and pharmaceuticals, which are the highly toxic, carcinogenic and estrogenic compounds. Many international organizations, including the World Health Organization (WHO) and the United States Environmental Protection Agency (EPA), have classified these micro-pollutants as the endocrine disrupting chemicals due to their harmful effects on the reproductive system, neuro-development and immune system (Huang et al., 2010). Because of the adverse impacts on human health and the environment, pesticides, phenols and phthalates are on the list of priority pollutants in leachate.

In the leachate treatment, the conventional biological treatment technologies, e.g. the activated sludge (AS), sequencing batch reactor (SBR), often encounter certain degradation and removal efficiency limitations. On the contrary, the membrane bioreactor (MBR) technology, a novel and effective system for biological wastewater treatment, possesses many advantages over the activated sludge (AS) process, including the higher biodegradation efficiency, smaller footprint and less sludge production. In

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http://dx.doi.org/10.1016/j.biortech.2016.07.094
0960-8524/

addition, the MBR system could achieve the impressive organic degradation, efficient nutrients removal as well as excellent retention of the suspended solids, thus mitigating the limitations inherent in the conventional AS system.

However, the effectiveness of the MBR technology as a barrier against the micro-pollutants, such as the endocrine disrupting chemicals (EDCs), pesticides and pharmaceuticals, remains inconclusive. In fact, the MBR performance with regard to the micro-pollutant biodegradation is subject to a number of determinants, including the solid/sludge retention time (SRT), hydraulic retention time (HRT), temperature, pH, biomass concentration and the properties of the compounds (Tadkaew et al., 2011). Previous research on the application of MBR to leachate treatment focused on the SRT and reported that the sludge retention time and the micro-pollutant degradation were positively correlated (Mozo et al., 2011).

Similarly, the micro-pollutant degradation and wastewater treatment performance could be enhanced by varying the HRT durations in the MBR system because, according to Win et al. (2016) and Zhang et al. (2016), the variations in HRT influence the bacterial communities residing in the treatment system and the system efficiency with regard to the daily treated water volume. Specifically, Prasertkulsak et al. (2016) investigated the degradation of the pharmaceutical compounds in the MBR system and reported the biodegradation efficiency over 80% under the 3 h HRT (i.e. short) condition with mixed liquor suspended solids (MLSS) of 13 g/L, a high biomass concentration relative to the short HRT duration. In addition, Zolfaghari et al. (2015) examined the removal of di (2-ethylhexyl) phthalate (DEHP) in the synthetic municipal wastewater using MBR with mixed liquor volatile suspended solids (MLVSS) of 17 g/L under two short HRT durations of 4 h and 6 h. The authors documented that the DEHP was removed by both the adsorption and the biodegradation, with the latter accounting for 74% of the overall removal efficiency.

In the MBR operation, the high biomass concentration (i.e. high MLSS) could contribute to the reduction in the biomass transfer efficiency, the increased oxygen demand and the serious membrane fouling and shortened membrane shelf life (Kaya et al., 2013). Kumar et al. (2012) and Zhu et al. (2014) conducted the biological treatment experiments under the C/N ratios of 1–7 and noted that the high microbial diversity under higher ammonia concentrations was a crucial factor that influenced the treatment efficiencies of the system. The varying ratios represent the relative amounts of carbon and nitrogen, or carbon and ammonia, available to the microorganisms in the biological treatment system, which in turn influence the bacterial community and the enzyme production of the heterotrophic bacteria and nitrogen transforming organisms. According to Fernandez-Fontaina et al. (2016), the nitrifying condition in the treatment system and the micro-pollutant removal efficiencies were positively correlated. Furthermore, the different microbial communities under different treatment conditions influenced the biological removal of the micro-pollutants (Fernandez-Fontaina et al., 2016). On the other hand, Boonnorat et al. (2016) documented that the nitrifying condition contributed little to the biodegradation of the micro-pollutants (i.e. low micro-pollutant biodegradation) and that the micro-pollutant biodegradation performance was enhanced in the presence of the heterotrophic bacterial and heterotrophic nitrifying bacterial (HNB) groups, which are the important bacteria in the micro-pollutant biodegradation.

Currently, there exists limited research on the effects of varying HRT and C/N conditions in the MBR system on the micro-pollutant biodegradation and the bacterial population dynamics. In principle, varying the HRT durations results in different biomass concentrations and efficiencies of the micro-pollutant degradation. Boonnorat et al. (2014a) applied the MBR technology to treating the municipal landfill leachate and reported that the optimal hydraulic retention time (HRT) of the MBR process was 24 h.

In the reactor operation, the bacterial enzymes play a crucial role in the micro-pollutant degradation. The use of enzymes in the treatment of leachate micro-pollutants is considerably less harmful to the environment than are the conventional chemical-based treatments. Phenol hydroxylase (PH) is an enzyme responsible for the phenol biodegradation, and esterase (EST) and phthalate dioxygenase (phthalate dioxygenase oxygenase (PDO) and phthalate dioxygenase reductase (PDR)) are the key enzymes in the phthalates biodegradation (Batie et al., 1987; Zhang et al., 2004; Peerzada et al., 2006; Rehdorf et al., 2012). Laccase (LAC) is an enzyme capable of removing the phenols, aromatic micro-pollutants and pharmaceuticals in the wastewater (Nguyen et al., 2016), and ammonia monooxygenase (AMO) is an enzyme that can degrade micro-pollutants (Fernandez-Fontaina et al., 2016). Specifically, PH, EST and PDO are the enzymes produced by the heterotrophic bacteria (HB); the heterotrophic nitrifying bacterial (HNB) group is responsible for PH, EST, PDO and AMO; and the ammonia oxidizing bacteria (AOB) produce AMO (Zhang et al., 2004; Peerzada et al., 2006; Rehdorf et al., 2012; Fernandez-Fontaina et al., 2016).

Boonnorat et al. (2016) investigated the micro-pollutant biodegradation performance in the MBR using the bacterial enzymes under various SRT and C/N conditions; and reported that the biodegradation performance was positively correlated to the length of SRT. The finding could be attributed to the higher enzyme activity, particularly under the no-sludge withdrawal condition where an abundance of nutrients promotes the enzyme activity and enhances the treatment performance.

Meanwhile, this current research aims to investigate the effects of various HRT (6, 12, 24 h) and C/N ratio (6 and 10) conditions (i.e. six HRT-C/N conditions). In this research, the MLSS was 7 g/l so as to minimize the aeration demand, membrane fouling and subsequent shortened membrane lifetime, and unnecessarily frequent system cleaning, while still achieving the efficient treatment and high micro-pollutant biodegradation. The micro-pollutants of interest were the compounds typically present in the leachate: bisphenol A (BPA), 2,6-di-*tert*-butyl-phenol (2,6-DTBP), di-butyl-phthalate (DBP), di-(ethylhexyl)-phthalate (DEHP), carbamazepine (CBZ), *N*,*N*-diethyl-m-toluamide (DEET) and diclofinac (DCF).

2. Materials and methods

2.1. Membrane bioreactor setup and operation

This research used the MBR tanks with a working volume of 12 L. Inside the tanks was a membrane module with 0.4 μm pore size for solid-liquid separation. In the experiments, the membrane permeate was intermittently withdrawn with a suction pump in a 10-min on/5-min off cycle without sludge withdrawal. In addition, the reactors were operated under room temperature with a pH of 7 (the typical leachate pH) and dissolved oxygen (DO) of 5 mg/l.

The leachate influent was obtained from the garbage trucks operating on the university (i.e. RMUTT) premises and in the surrounding communities. The characteristics of the leachate were tabulated in Table 1. The C/N ratio of the leachate influent was 10, closely resembling that of the landfill site. In this research, the nitrogen concentration in the influent was adjusted with $(NH_4)_2SO_4$ to evaluate the micro-pollutant biodegradation performance under the C/N 6 condition (Boonnorat et al., 2016).

In this research, the sludge was withdrawn from the university's (RMUTT) central wastewater treatment system and the initial MLSS was 7 g/l. Prior to the reactor operation, the sludge was cultivated in the leachate for 60 days. As previously stated, the MBR