Journal of Environmental Management 212 (2018) 186–197

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Performance and microbial shift during acidification of a real pharmaceutical wastewater by using an anaerobic sequencing batch reactor (AnSBR)

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ARTICLE INFO

Article history:
Received 16 October 2017
Received in revised form
17 January 2018
Accepted 21 January 2018
Available online 22 February 2018

Keywords:
Acidification
Anaerobic sequencing batch reactor
Etodolac
Pharmaceutical wastewater

ABSTRACT

In this study, a lab-scale anaerobic sequencing batch reactor (AnSBR) was used for the acidification of a pharmaceutical wastewater sourced from etodolac chemical synthesis tanks. The effects of the organic loading rate (OLR), and etodolac and sulfate concentrations on the acidification rate and microbial community in AnSBR were investigated at 35 °C with a hydraulic retention time (HRT) of 37 h, a pH of 5, and OLRs up to 5.2 kgCOD/m^3·day. The AnSBR accomplished a 60% acidification ratio and 50–60% etodolac removal at OLRs up to 2.6 kgCOD/m^3·day. However, at OLR = 3.9 kgCOD/m^3·day, acidification was not achieved due to sulfite inhibition; pre-ozonation was applied to overcome this sulfite inhibition. Although etodolac and COD removals were improved, the wastewater was not successfully acidified. Real-time polymerase chain reaction (Q-PCR) and fluorescent in situ hybridization (FISH) analyses revealed that acidification was inhibited by the dominance of sulfate reducing bacteria (SRB) over acidification bacteria in the AnSBR. However, increasing the OLR to 5.2 kgCOD/m^3·day led to toxicity stress in the SRB due to increased sulfite concentrations. Sulfate load fundamentally affected acidification process and microbial community composition. The presence of etodolac with concentration up to 56 mg/L did not have a significant effect on VFA production and the microbial community.

1. Introduction

Pharmaceutical wastewater contains various organic and inorganic components such as spent solvents, catalysts, additives, reactants and pharmaceuticals (Sreekanth et al., 2009). The composition of chemical synthesis process-based pharmaceutical wastewater varies widely and may contain: 375–64000 mgCOD/L, 200–10800 mgBOD_5/L, 8–1575 mgTN/L, 50–120 mgTP/L, and pH 1.9–9.2 (Oktem et al., 2006; Chen et al., 2008a; Gadipelly et al., 2014; Li et al., 2015a), as content may vary depending on the manufacturing process and production scale even within same factory. Hence, it is difficult to successfully treat pharmaceutical wastewater. Pharmaceutical wastewater has traditionally been treated using physico-chemical and aerobic biological processes (Lapara et al., 2001; Kulik et al., 2008); some studies have also reported the use of ozonation, advanced oxidation (Fenton, O_3/H_2O_2, O_3/UV), and adsorption processes (Arslan-Alaton and Dogruel, 2004; Kulik et al., 2008; Vergili and Gencdal, 2015, 2017). Several researchers have investigated anaerobic treatment processes such as the up-flow anaerobic sludge blanket (UASB) (Oktem et al., 2007; Sreekanth et al., 2009; Chen et al., 2011, 2014), anaerobic membrane bioreactor (AnMBR) (Kaya et al., 2017; Hu et al., 2018), UASB-anaerobic filter hybrid reactor, and anaerobic continuous stirred tank reactor (CSTR) (Oz et al., 2003), anaerobic sequenching batch reactor (AnSBR) (Shi et al., 2015) for the treatment of pharmaceutical wastewaters from chemical synthesis. Anaerobic digestion is commonly used for management of agricultural, industrial and municipal wastewater (Hanifzadeh et al., 2017). Anaerobic digestion comprehends two stage process involving the sequential action of acid forming and methane forming bacteria. In the first stage, acid forming bacteria (facultative and anaerobic bacteria) converts the complex organic

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https://doi.org/10.1016/j.jenvman.2018.01.058
0301-4797/

compounds into simpler organics (volatile fatty acids-VFAs) and also carbon dioxide and hydrogen gases. In the second stage, the organic acids and hydrogen are converted into methane and carbon dioxide by methanogens. The efficiency of anaerobic treatment depends on both the acidogenic and methanogenic phases (De Lemos Chernicharo, 2007; Shi et al., 2017). The literature contains studies on the acidification of different wastewaters such as pesticide manufacturing wastewater (Jia et al., 2006), domestic wastewater (Bai et al., 2013), petrochemical wastewater (Wu et al., 2015) and medicine wastewater (Lv et al., 2017). Generally, acidification is the rate-limiting fermentation step (De Lemos Chernicharo, 2007; Shi et al., 2017). Therefore, studies on the anaerobic treatment of pharmaceutical wastewaters have focused on the acidification rate and organic matter removal. The acid-phase can be greatly influenced by hydraulic retention time (HRT), sludge retention time (SRT), pH, temperature, reactor configuration, and wastewater characteristics (Oktem et al., 2006). Oktem et al. (2006) investigated the effects of organic loading rate (OLR), HRT, and pH on the acidification of a pharmaceutical wastewater containing bacampicillin and sultampicilline toshylate; acidification level was reported to be 44% in that study. The microbial community structure is also very important for anaerobic treatment process as well as designing the system with proper operating parameters (Shi et al., 2017) and has also been investigated by specific groups of researchers (Chelliapan et al., 2011; Qiu et al., 2013; Chen et al., 2014; Li et al., 2015a, 2015b, 2015c; Shi et al., 2015). Chelliapan et al. (2011) showed the dominance of *Methanosarcina* and *Methanosaeta* by treating pharmaceutical wastewater with an uplow anaerobic stage reactor (UASR). Chen et al. (2014) revealed that *Firmicutes*, *Bacteroidetes*, *Thermoplasmata* and *Methanobacteria* were dominated in a UASB reactor treating dilute pharmaceutical wastewater at higher OLRs. Li et al. (2015b) reported bacterial groups phlya *Proteobacteria* and phylum *Clostridia* are responsible for the degradation and desulfonation, respectively, of p-acetamidobenzene sulfonyl chloride with a UASB reactor. Shi et al. (2015) reported *Methanobacteria*, *Methanomicrobia* and *Thermoplasmata* as predominant archaeal groups in anaerobic treatment of saline pharmaceutical wastewater in a AnSBR. The microbial community structure for acetogenic/methanogenic phases was widely studied, generally relevant research on the acidogenic phase is still limited. The presence of pharmaceutical compounds in wastewater is important from two perspectives. First, pharmaceutical compounds can be toxic to microorganisms and inhibit their activity, resulting in decreased acidification and, consequently, decreased system performance (Oktem et al., 2007; Chen et al., 2008a). Second, because the biodegradation of pharmaceutical compounds in chemical synthesis wastewaters is inefficient, pharmaceutical compounds may be released into the receiving waters even after secondary anaerobic treatment (Gadipelly et al., 2014; Monsalvo et al., 2014; Kaya et al., 2017). Etodolac is a nonsteroidal anti-inflammatory drug (NSAID) manufactured and sold worldwide that works by inhibiting enzyme synthesis (Drugbank Canada, 2016). The derivatives of etodolac shown mutagenic and genotoxic activity, while etodolac itself was found to be toxic at relatively high concentrations (Passananti et al., 2015).

To our knowledge, the effect of pharmaceutical compounds on the microbial community and acidification rate in an anaerobic sequencing batch reactor (AnSBR) treating chemical synthesis wastewater from the pharmaceutical industry remains unstudied. There is a lack of specific information on the effects of pharmaceutical compounds on the acidification process and attendant microbial community. Therefore, this study has focused on a comprehensive evaluation relating to acidification capacity and shift in microbial ecology by depending on etodolac and sulfite concentrations and organic loading rate during acidification process of a real pharmaceutical-industry wastewater generated by the chemical synthesis of a nonsteroidal anti-inflammatory pharmaceutical (etodolac). In order to determine the effects of OLR, and etodolac and sulfite concentrations on the acidification rate and microbial community, an AnSBR was operated at a mesophilic temperature (±35 °C) for an HRT of 37 h, an OLR of up to 5.2 kgCOD/m^3·day and a pH of 5. In addition, analysis of extracellular polymeric substances (EPSs), soluble microbial products (SMPs), hydrophobicity and zeta potential were performed to evaluate the performance of AnSBR. Real-time polymerase chain reaction (Q-PCR) and fluorescent in situ hybridization (FISH) analyses were carried out to characterize the microbial population.

2. Material and methods

2.1. Anaerobic sequencing batch reactor (AnSBR)

The acidogenic reactor system was provided by Electrolab, United Kingdom (FerMac 320 bioreactor fermenter). The reactor was made of quartz glass and equipped with a temperature jacket and a mixer. The reactor had a total volume of 6.4 L (working volume = 4 L) with an interior diameter of 160 cm and a height of 320 cm. The system was controlled with a pH probe, a thermometer, and a level sensor. Three internal pumps were used for wastewater feed, acid-base dosage, and foam control (see Fig. 1).

2.2. Wastewater source and characteristics

Raw wastewater was obtained from the cleaning of chemical synthesis tanks used for etodolac production in a pharmaceutical factory, located in Gebze, Turkey. The AnSBR was operated with raw wastewater for the first 435 days and then with pre-ozonated wastewater between day 454 and day 653. A lab-scale venture-injection system was used for the ozonation of the wastewater at an ozone dosage of 2 g/hour for 60 min. Ozone was generated from atmospheric oxygen with a Degremont Technologies ozone generator (Model: TOGB2). A circulation pump (Arcelik, Model: MPM/1-2) was used to circulate the wastewater through the system. The circulation loop had a volume of 2 L. The temperature of the ozone reactor was controlled by a water bath at 35 °C. The main characteristics of the raw and ozonated wastewaters are presented in Table 1. NH_4Cl and KH_2PO_4 were added to the wastewater to ensure a C:N:P ratio of 300:5:1, and some trace elements (S, Na, Ca, Zn, Mg, Co, Ni, Fe, Mn and Cu) were also supplemented for biological growth.

2.3. Seed sludge and reactor operation

The AnSBR was inoculated with granular sludge from a UASB reactor treating beer-industry wastewater. The sludge featured 175 g/L TSS and 105 g/L VSS. The operating parameters of the AnSBR were selected as follows: pH = 5, T = 35 °C, and HRT = 37 h. The pH and temperature was kept constant automatically by the system during the operation. In the AnSBR, the steps were controlled by a programmable logic controller. The total operational cycle spanned 24 h and included the following steps: 30 min of feeding, 20 h of mixing, 3 h of settling, and 30 min of discharging. The agitation rate was 100 rpm during the reaction period. 2.4 L of wastewater was fed to the reactor during the cycle, yielding an exchange ratio of 60%. Samples were taken for the analysis in the last step of the cycle. The AnSBR was operated in two parts consisting of five stages total over 653 days. In the Part I, the reactor was operated with raw wastewater through day 453 in three-stages: The OLR was adjusted to 1.6 kgCOD/m^3·day at an influent concentration of 2500 mg COD/