#### [JOURNAL OF ENVIRONMENTAL SCIENCES XX \(2017\) XXX](http://dx.doi.org/10.1016/j.jes.2017.08.012) – XXX



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# Effects of tourmaline on nitrogen removal performance and <sup>2</sup> biofilm structures in the sequencing batch biofilm reactor

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# 10 ARTICLE INFO ABSTRACT

- 12 Article history:
- 13 Received 15 May 2017
- 14 Revised 2 August 2017
- 15 Accepted 21 August 2017
- 16 Available online xxxx
- 36 Keywords:
- 37 Tourmaline
- 38 Nitrogen removal performance
- 39 Biofilm structures
- 40 Population dynamics
- 41 Sequencing batch biofilm
- 42 reactor (SBBR)
- 43

**Structures in the sequencing batch biofilm reader of the Sequencing batch biofilm reader of the set of the set** The effects of tourmaline on nitrogen removal performance and biofilm structures were 17 comparatively investigated in two identical laboratory-scale sequencing batch biofilm reactors 18 (SBBRs) (denoted SBBR1 and SBBR2) at different nitrogen loading rates (NLRs) varying from 19  $(0.24 \pm 0.01)$  to (1.26  $\pm$  0.02) g N/(L·day). SBBR1 was operated in parallel with SBBR2, but SBBR1 20 was filled with polyurethane foam loaded tourmaline (TPU) carriers and another (SBBR2) filled 21 with polyurethane foam (PU) carriers. Results obtained from this study showed that the 22 excellent and stable performance of SBBR1 was obtained. Ammonia nitrogen removal and total 23 nitrogen removal were higher in SBBR1 than that in SBBR2 with increase of NLR. At an NLR of 24  $(0.24 \pm 0.01)$  g N/(L·day), the majority of the spherical and elliptical bacteria were surrounded by 25 the extracellular polymeric substance (EPS) and bacillus or filamentous bacteria in two SBBRs 26 biofilms. When NLR increased to (1.26  $\pm$  0.02) g N/(L·day), the clusters were more obvious in the 27 SBBR1 biofilm than that in the SBBR2 biofilm. Bacteria in SBBR1 were inclined to synthesis more 28 EPS, and the formed EPS could protect the bacteria from free ammonia (FA) under extreme 29 condition NLR (1.26  $\pm$  0.02) g N/(L·day). The results of polymerase chain reaction-denaturing 30 gradient gel electrophoresis analysis showed that the microbial community similarity in SBBR2 31 decreased more obviously than that in SBBR1 with the increase of NLR, which the microbial 32 community in SBBR1 was relatively stable. 33

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#### **Introduction**

 Biofilm process is one of biological nitrogen removal technol- ogy in wastewater treatment (Liang et al., 2010; Zou et al., [2016; Ahmad et al., 2017\)](#page--1-0). Higher sludge retention time and different oxic and anoxic zones are advantages of biofilm systems for the slowly growing bacteria. Besides, biofilms provide better protection for the functional bacteria partici- pating in the nitrogen removal [\(Zekker et al., 2017](#page--1-0)). During this process, the biological carrier has significant influences on the

growth, structure, and activity of the biofilm. Therefore, the 58 development of the new carrier has been a hot topic in the 59 field of wastewater treatment. It is a good option to use highly 60 porous three dimensional carriers, which could provide the 61 desired microenvironment for microbial growth ([Ali et al.,](#page--1-0) 62 [2015; Luo et al., 2016\)](#page--1-0). Polyurethane foam (PU) as the carriers 63 with high mechanical strength present the ideal perfor- 64 mances in this regards for the growth of biomass ([Le et al.,](#page--1-0) 65 [2016](#page--1-0)). Therefore, PU carrier has been extensively used in the 66 wastewater treatment due to its physicochemical properties 67

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 such as high porosity, appropriate pore size, low density, and 69Q5 so on ([Li et al., 2016](#page--1-0); Moawed et al., 2017). A biofilm reactor equipped with PU carriers has been proven as an efficient and inhibition-tolerant system to perform the nitrification and denitrification process [\(Tan et al., 2013](#page--1-0)).

 Tourmaline is characterized by a cyclic structure of boron silicate mineral that possesses unique physical–chemical properties, including the continuously releasing the ions 76 (Ca<sup>2+</sup>, Mg<sup>2+</sup>, etc.), producing an electrostatic field and releasing 77 rare microelements ( $Fe^{2+}$ , Mn<sup>3+</sup>, etc.) [\(Nakamura and Kubo,](#page--1-0) [1992; Emme et al., 2005](#page--1-0)). The general chemical formula of 79 tourmaline can be written as  $XY_3Z_6S_6O_{18}(BO_3)_3W_4$ , where X is 80 Na, Ca, K, or vacancies; Y is  $Mg^{2+}$ , Fe<sup>2+</sup>, Mn<sup>2+</sup>, Al, Fe<sup>3+</sup>, Mn<sup>3+</sup>, or 81 Li; Z is Al,  $Fe^{3+}$ ,  $Cr^{3+}$ , or Mg; and W is OH, F, or O (Yavuz, 1997). The spontaneous and permanent pole is one of the most important properties of tourmaline, which can produce an electric dipole, especially in small granules with diameters of several microns or less (Liu et al., 2016). Tourmaline has been paid more attention in the environmental field in recent years. Many studies have attempted to combine tourmaline and PU into a single reactor (Yang et al., 2013; Tan et al., 2017). However, most studies mentioned above have focused on 90Q6 pollutant removal efficiency (Zhang et al., 2011), fast start-up of reactor ([Yang et al., 2013\)](#page--1-0), strategies to stabilize the process ([Qiu et al., 2011](#page--1-0)), and bacteria activity (Wei et al., 2008; Wang [et al., 2016; Tan et al., 2017](#page--1-0)). However, there are few reports related to the effect of tourmaline on biofilm structures and microbial community.

 In this study, we focused on the comparative investigation of effects of tourmaline on sequencing batch biofilm reactors (SBBRs). The aims of this study were to (1) investigate the effects of tourmaline on the nitrogen removal performance, (2) investi- gate the effects of tourmaline on the biofilm structures, and (3) demonstrate population dynamics of the microbial community. The research is expected to provide useful information on enhancing the nitrogen removal process by tourmaline.

# 105 1. Materials and methods

## 106 1.1. Materials

 The tourmaline in 0.4-μm-sized powder was purchased in Chifeng region (China). Polyurethane foam loaded tourmaline (TPU) carrier was prepared using waterborne polyurethane and tourmaline powder as described in our previous study (Yang et [al., 2013](#page--1-0)). The loading of tourmaline powder was 150–175 kg/m<sup>3</sup> 112 PU.

#### 113 1.2. Reactor

 The experiments were performed in two identical laboratory- scale SBBRs (denoted SBBR1 and SBBR2) and each reactor composed of plexiglass with heights of 50 cm and internal diameters of 30 cm corresponding to an effective volume of 35 L. The filling ratio of the carriers in SBBRs was 80%. SBBR1 was operated in parallel with SBBR2, but SBBR1 filled with TPU carriers and another (SBBR2) filled with PU carriers. The influent was pumped into a network of distribution holes at the bottom of biofilm system using a peristaltic pump, and the effluent was

collected through the sampling hole at the middle of the system. 123 The operational temperature of the reactors was maintained at 124 20°C by thermostatic water jackets.

#### 1.3. Influent 126

In order to modify influent nitrogen loading rate (NLR) accurately, 127 the reactors were fed with synthetic wastewater. Ammonia 128 nitrogen was supplemented to mineral medium as required in 129 the form of NH4Cl. The concentrations used varied depending on 130 the experimental periods. The unaltered compositions of syn- 131 thetic wastewater in this study were (in  $g/L$ ): glucose 0.35, KH<sub>2</sub>PO4 132 0.01, CaCl<sub>2</sub>·2H<sub>2</sub>O 0.00565, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.3, KHCO<sub>3</sub> 1.25, FeSO<sub>4</sub> 133 0.00625, EDTA 0.00625 (Trigo et al., 2006), and 1.25 mL/L of trace 134 elements solution [\(van de Graaf et al., 1996](#page--1-0)). The pH of 135 the synthetic wastewater was adjusted between 7.5 and 7.8 by 136 1 mol/L HCl and 1 mol/L  $\text{Na}_2\text{CO}_3$  before feeding into the reactors. 137

#### 1.4. DNA extraction and PCR-DGGE analysis 138

us, 2000). In general cremental orientation of the experimental periods. The unitarity of  $\Gamma_{\rm F}$ <sup>2</sup> ( $\Gamma_{\rm F}$ <sup>3</sup> ( $\Gamma_{\rm F}$ <sup>3</sup> ( $\Gamma_{\rm F}$ <sup>3</sup>),  $\Gamma_{\rm F}$ <sup>3</sup> ( $\Gamma_{\rm F}$ <sup>3</sup>),  $\Gamma_{\rm F}$ <sup>3</sup> ( $\Gamma_{\rm F}$ <sup>3</sup>),  $\Gamma_{\rm F}$ <sup>3</sup> (Biofilms were detached from TPU and PU by 1-min sonication 139 (45 W, 50/60 Hz). Genomic deoxyribonucleic acid (DNA) from the 140 above biomass was extracted using a Bacterial Genomic DNA 141 Extraction Kit (50 T, TaKaRa, China) according to the supplier 142 instructions. In order to reveal the population dynamics in both 143 reactors, the microbial community structure of these biofilm 144 were investigated by polymerase chain reaction-denaturing 145 gradient gel electrophoresis (PCR-DGGE) analysis. The V3 regions 146 of 16S rDNA genes were amplified using universal primers F338 GC 147 (5′-CGCCCGCCGCGCGCGGCGGGCGGGGCGGGGGCACGGGGGGA- 148 CTCCTACGGGAGGCAGCAG-3′) and R518 (5′-ATTACCGCGGCTGC 149 TGG-3′) as previous report (Tan et al., 2013). A gel document 150 system equipped with a digital graphic printer (UP-897MD, Sony, 151 Japan) was used to visualize the PCR-DGGE band profile. DGGE 152 profiles were analyzed by Quantity One 4.3.0 (DGGE gel image 153 analysis software, Bio-Rad, USA) at different operational periods. 154 Bands with a relative intensity of less than 0.2% of the sum of all 155 band intensities were discarded. Similarity matrix on the DGGE 156 profiles were calculated according to Dice coefficients. 157

## 1.5. Experimental process 158

According to the influent NLR, the whole operational process of 159 both the reactors was divided into four periods, namely, period-I, 160 II, III, and IV. The reaction was performed with the progressively 161 increasing nitrogen loadings (Table 1). As the seeding sludge 162 was inoculated with activated sludge obtained from a biological 163 tank of Taiping municipal wastewater treatment plant, the 164 start-up period of SBBRs wouldn't need an adaptive period. Q7 The seeding sludge was brown with loose morphology, mean 166 size of  $(82.5 \pm 6.4)$  µm, and sludge volume index after 30 min 167 of sedimentation (SVI<sub>30</sub>) of (71.6  $\pm$  5.5) mL/g. The seeding 168 sludge had a total suspended solids (TSS) concentration of 169 (6260  $\pm$  211) mg/L and volatile suspended solids (VSS) concentra- 170 tion of  $(4650 \pm 175)$  mg/L, corresponding to a VSS/TSS ratio of 171 74.3%. For these two reactors, each operational cycle consisted of 172 10-min feeding, 180-min aerobic reaction, 40-min settling, and 173 10-min decanting. Wastewater in these reactors was intermit- 174 tently aerated by an air-blower to improve oxygen transfer to 175 wastewater, in which the dissolved oxygen was adjusted to a 176

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