Removal of tryptophan in drinking water using biological activated carbon filter
Shuai Wang, Tao Lin and Wei Chen

ABSTRACT
Tryptophan (Trp), an important nitrogenous organic compound commonly present in water sources and posing a serious threat to human health, was selected as the main object in the removal trial by utilizing a laboratory-scale biological activated carbon (BAC) column. The laboratory-scale BAC column was divided in a three-part composition: upper layer (UL) (0–20 cm), middle layer (ML) (20–40 cm) and bottom layer (BL) (40–60 cm). The removal efficiencies of Trp in the three layers were 45.4%, 86.4% and 43.2%, respectively, while the adsorption of granular activated carbon (GAC) for Trp did not show a similar tendency (the average adsorption yields were 10.98 ± 1.17 mg/g, 7.45 ± 0.80 mg/g and 3.32 ± 0.39 mg/g, respectively), which indicated that the biodegradation of microorganisms attached to the GAC played an important role. Furthermore, a high-throughput quantitative polymerase chain reaction (HT-qPCR) was utilized to determine the relative abundance of the first eight bacterial genera in the three BAC layers and results revealed the relative abundance of Aquincola, Pseudomonas and Ensifer were highest in the ML.

Key words | biological activated carbon filter, nitrogenous disinfection by-products, tryptophan

INTRODUCTION
The Yangtze River is an important water source for many provinces in China. Recently, many studies have demonstrated that the concentrations of dissolved organic nitrogen (DON) in the raw water of the Yangtze River ranges from 0.07 to 0.45 mg/L, accounting for 3–24% of the total dissolved nitrogen (Bond et al. 2012; Lin et al. 2016). The DON may be transformed into nitrogenous disinfection by-products (N-DBPs) when using chlorine or chloramines as a disinfectant, such as haloacetonitriles (HANs), haloacetamides (HAcAs) and halonitromethanes (HNMs) (Chu et al. 2012; Tan et al. 2017), which have been widely detected at low μg/L levels in drinking water (Richardson & Ternes 2014). But due to their significantly higher cytotoxicity and genotoxicity than regulated DBPs, such as trihalomethanes (THMs) and haloacetic acids (HAAs), these N-DBPs pose a serious threat to human health (Shin et al. 2013; Chu et al. 2016). The components of DON in source water are complex, including amino acids (AAs), proteinaceous compounds and nitro-compounds (Chu et al. 2010; Postigo & Richardson 2014). AAs, the potential precursors of regulated carbonaceous disinfection by-products (C-DBPs) and N-DBPs, have typical concentrations ranging within 50–1,000 μg/L in rivers and lakes, accounting for 15–35% of DON and 3–8% of dissolved organic carbon (Shang et al. 2014; Yan et al. 2014). Among them, tryptophan (Trp), tyrosine (Tyr), aspartic acid (Asp) and histidine (His) are frequently identified at a high level and show greater formation potentials (FPs) for THMs, HAAs and HANs (Chuang et al. 2012). Due to low molecular weight and low hydrophobicity, these AAs have poor removal efficiencies in conventional drinking water treatment plants (DWTPs) including coagulation, sedimentation and filtration (Chu et al. 2011a), which results in the formation of C-DBPs and N-DBPs during the subsequent chlorine or chloramine disinfection process.

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Many DWTPs (for example, Longtan drinking water treatment plant, Nanjing, China) have coupled ozonation with a biological activated carbon (BAC) filter in order to prevent the formation of DBPs prior to chlorine or chloramine disinfection. Previous research has shown that the reactivity of Asp or His decreased significantly after ozonation, but that of Trp was less influenced. Therefore, the goal of this study was to acquire knowledge on the removal efficiency of Trp associated with the BAC process and to assess the FPs of C-DBPs and N-DBPs. Meanwhile, the change of bacterial population in different BAC layers was revealed to find the dominant bacteria in the process of biodegradation for Trp using a high-throughput quantitative polymerase chain reaction (HT-qPCR). This study was aimed at the Yangtze River water, which has rarely been studied in previous research. Furthermore, much literature reporting on the Yangtze River has just aimed at the FPs of conventional C-DBPs rather than the higher cytotoxicity and genotoxicity of N-DBPs.

MATERIALS AND METHODS

Materials

Granular activated carbon (GAC), whose effective size, specific surface area, uniformity coefficient, iodine value, methylene blue value, and density of filled GAC were 0.65 mm, 950 m² g⁻¹, ≤2.1, 950 mg g⁻¹, 194 mg g⁻¹ and 400 g L⁻¹, respectively, was obtained from the actual BAC filter in a DWTP located in Nanjing. The Trp solution, purchased from NanJing WanQing Chemical Glassware Instrument Co., Ltd (Nanjing, China), had an initial concentration of 10 mg L⁻¹ after being dissolved in ultrapure water. Standards for four THMs, two HAAs and one HNM (trichloronitromethane, TCNM) were purchased from Sigma-Aldrich (St Louis, USA). The standard for one HAcAm, dichloroacetamide (DCAcAm), was purchased from Alfa Aesar (Karlsruhe, Germany). The standard for one HAN (dichloroacetonitrile, DCAN) was obtained from Supelco (Bellefonte, USA). Ultrapure water was produced with a Millipore Milli-Q Gradient water purification system (GWA-UN, MA, USA). All other materials and reagents were also purchased from NanJing WanQing Chemical Glassware Instrument Co., Ltd.

Laboratory-scale experiment

A laboratory-scale experiment was performed to simulate the BAC filtration process. The Trp solution with a concentration of 10 mg L⁻¹ was introduced into a BAC filter using a longer pump (YZ1515X, BaoDing, China). The filtration rate of the BAC filter (a column of 1.5 m in height and 0.1 m in diameter) was kept at a rate of 12 m h⁻¹. The contact time of the BAC column was 15 min. Besides the Trp solution (10 mg L⁻¹), the influent also included glucose (3 mg L⁻¹) and microelements (MnCl₂·4H₂O, 0.4 mg L⁻¹; ZnSO₄·7H₂O, 0.5 mg L⁻¹; CuSO₄·5H₂O; KH₂PO₄, 0.2 mg L⁻¹; Fe₂(SO₄)₃, 0.3 mg L⁻¹) for cultivating microorganisms attached to the GAC.

Sampling and analytical methods

During the experiment, the activated carbon particles extracted from nine sampling points (shown in Figure 1) were respectively introduced into nine conical flasks (250 mL) after being dried at 105 °C for 12 h in the oven, which could eliminate the effect of biodegradation on the removal of Trp. The 100 mL Trp solution (10 mg L⁻¹) was added into each conical flask, and then the flask was put in the digital water bath oscillator (SHY-2, ChangZhou, China) at 25 °C and 100 times per minute for 12 h. Subsequently, the water samples were filtered through a 0.22 µm membrane to determine the adsorption of the GAC for Trp by using high-performance liquid chromatography (HPLC) and fluorescence detection (Agilent A1100, USA). An Agilent ZORBAX XDB-C18 (3.0 mm × 250 mm × 5 µm) was used as the analytical column with a mobile phase consisting of A and B solutions (ratio: 95%/5%). The A solution consisted of 0.05 mol L⁻¹ NaAc and 1.5% THF (v%), and the B solution was made with 0.01 mol L⁻¹ NaAc/acetonitrile/methanol being 46/44/10 (v/v/v). Also, the flow rate was 1.0 mL/ min, and the excitation and emission wavelengths of the fluorescence detector were 254 nm and 338 nm, respectively. The limit of quantification for the HPLC for Trp is 1 mg/L.
THMs were detected using a gas chromatograph (GC, Agilent 7890B, USA) with HP-5 (30×0.25 mm × 0.1 μm) as the analytical column and split injection (ratio: 1:5). The temperature of sample inlets and oven was controlled at 200 °C and 40 °C (kept for 11 min), respectively. Two HAAs were also analyzed by the GC above, and the details were described in the previous research (Chu et al. 2012). After a liquid-liquid extraction, HAcAm (DCAcAm) was analyzed using GC with splitless injection. The temperature of sample inlets was kept at 235 °C, and the initial temperature of the oven was kept at 80 °C for 5 min, heating up to 150 °C (kept for 1 min) at the rate of 40 °C/min. The formation potentials of disinfection by-products (DBPsFP) were tested according to the approach developed by Krasner et al. (2007), which was described in detail by Chu et al. (2011a). Two volatile DBPs (DCAN and TCNM) were measured using purge and trap (OI-Eclipse 4660, USA) coupled with gas chromatograph/mass spectrometry (GC/MS), based on the USEPA method 524.2 (USEPA 1992). The limits of quantification of the GC for THMs, HAAs, DCAcAm, DCAN and TCNM are 2 μg/L, 1.5 μg/L, 1 μg/L, 1 μg/L and 2 μg/L, respectively.

The changes of dominant microbial population in different BAC layers were analyzed using the HT-qPCR. Samples collected (shown in Figure 1) and stored at 4 °C were transported to Sangon Biotech Co., Ltd (Shanghai, China) for detection.

**RESULTS AND DISCUSSION**

**The removal of Trp**

In this study, the removal of Trp was studied by investigating the transformation of nitrogen in purification with a BAC filter. As shown in Figure 2, the concentration of ammonia nitrogen (NH₄⁺) firstly increased with the biodegradation of influent Trp, reaching the maximum value in the carbon middle layer (ML), and then gradually decreased in the bottom of the BAC filter. Due to the nitrification, the variations of nitrate nitrogen (NO₃⁻) and nitrite nitrogen (NO₂⁻) were consistent with that of NH₄⁺. The average removal rate of Trp in the upper layer (UL), ML and bottom layer (BL) of the BAC filter was 45.4%, 86.4% and 43.2%, respectively. The influent nitrogen component only involved Trp; Trp was, therefore, partly absorbed by activated carbon and was biodegraded into NH₄⁺, NO₂⁻ and NO₃⁻ by the attached microorganisms. The higher concentrations of Trp and dissolved oxygen (DO) promoted the amination reaction in the UL and ML of the BAC filter, where the ammonifier could translate the Trp into NH₄⁺.
In order to further understand the transformation of the AA-like DON in the removal of Trp by the BAC process, the detection of effluent AAs was performed and the results are shown in Figure 3. As can be seen in Figure 3, the peaks at 2 min and 24.5 min retention time were always existent and did not change in size significantly. Therefore, the two peaks could come from derivatization reagents. The peak at 15.4 min retention time responded to Trp, whose fluorescence intensity (FI) decreased with the deepening of the carbon layer, having consistency with the results above. There were three new peaks, at retention times 17.5 min, 20 min and 21.5 min, that occurred in the effluent of the UL (Figure 3(b)), and those were considered to be tyrosine (Tyr), valine (Val) and phenylalanine (Phe), respectively (Azilawati et al. 2015). This indicated that new AA could be generated in the metabolism of microorganisms attached to the GAC when they biodegraded Trp. As shown in Figure 3(d), the three peaks’ intensity tended to decrease, even disappearing, which demonstrated that the three AAs formed in the UL and ML were degraded gradually.

**Removal of DBP precursors**

The AAs are usually considered as the main precursors of disinfection by-products. The variations of DBP precursors were explored by investigating the related DBPsFPs. The removal efficiencies of the DBPsFPs of the effluent, being sampled at different height outlets in the carbon layers, were calculated according to Equation (1), which also reflected the removal of DBP precursors. The results are shown in Figure 4. The removal efficiencies of most DBPsFPs except DCAN had a similar tendency, those of the effluent from the UL and BL being lower than that of the ML, while the lowest removal efficiency of DCAN precursors was in the effluent of the ML. This result was probably due to the removal efficiency of Trp in the ML being higher than in the other BAC layers, and Trp had a higher FP than most DBPs (Chuang et al. 2012). However, there were newly formed AAs associated with the biodegradation of Trp (as shown in Figure 3), those having higher concentration in the effluent of the ML. These AAs (Tyr, Val and Phe) had higher FPs of DCAN than Try (Chuang et al. 2012; Goslan et al. 2017), which resulted in the poor removal of DCAN precursors in the effluent of the ML compared with the other carbon layers. The results of Figure 3 also indicated that the newly produced AAs could be further purified by microorganisms attached to the BL carbon, where there was less newly produced AA due to the lower influent concentration of Trp. Therefore, the removal of Trp had an important influence on the variations of whole DBP precursors. The purification of AA using the BAC filter included adsorption and biodegradation (Fan et al. 2012).
Figure 3  | Chromatograms for AAs in the effluent of different BAC layers: (a)–(d) represent the effluent of 0 cm, 20 cm, 40 cm and 60 cm heights of BAC layers, respectively.

Figure 4  | Removal of FPs of chloroform (CF), dichloroacetic acid (DCA), trichloroacetic acid (TCA), dichloroacetonitrile (DCAN), dichloroacetamide (DCAcAm) and trichloronitromethane (TCNM) in different BAC layers (‘UL’, ‘ML’ and ‘BL’ represent the upper, middle and bottom activated carbon layers, respectively).
and it was therefore essential to further investigate which effect, adsorption or biodegradation, accounted for the key role in the process of Trp removal:

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\text{Removal efficiencies} = \frac{C_A - C_B}{C_A} \times 100 \tag{1}
\]

\(C_A\) – DBP formation potentials in the influent of the \(C_B\) carbon layer.

\(C_B\) – DBP formation potentials in the effluent of the 20 cm, 40 cm and 60 cm height carbon layers, respectively.

**Adsorption of GAC for Trp**

The Langmuir or Freundlich isothermal adsorption models have been commonly used for describing the adsorption characteristics of new GAC, and the fitting equations for the Freundlich and Langmuir models and analysis results using the two models are shown in Table 1 and Figure 5, respectively. As can be seen in Figure 5, there was a significantly positive correlation between the Langmuir isotherm adsorption model and the adsorption of Trp with GAC, compared with that of the Freundlich model, which had more deviation between the adsorption curve and the experimental data with the growth of the liquid phase concentration.

The utilized GAC particles associated with the BAC filter were used to further investigate the adsorption of Trp by analysis with the Langmuir isotherm adsorption model. Prior to the adsorption trial, the GAC particles were sterilized at 105°C for 12 h to eliminate the influence of biodegradation of the attached microorganisms on the removal of Trp. The results are shown in Table 2. It can be seen that the adsorption capacity for Trp decreased gradually with the deepening of the carbon layers, and the average adsorption capacity in the UL (10.98 mg/g) was higher than the other two layers (7.45 mg/g and 3.32 mg/g, respectively). This result was mainly attributed to the higher influent concentration, the driving force and the adsorption efficiency of the activated carbon in the UL of the carbon filter (Chu et al. 2012). The concentration of Trp declined with downward flow in the filter, which resulted in the removal percentages due to adsorption of GAC in the ML (17.2%) and BL (9.2%) having lower yields than the UL (20.2%). However, the total removal efficiency of Trp, associated with the combination of biodegradation and adsorption, was higher in the ML (86.4%) of the BAC filter than the UL (45.4%) and BL (43.2%). Therefore, the biodegradation of microorganisms attached to the GAC played a main role in the removal of Trp.

**Dominant bacterial population**

In the study, the laboratory-scale BAC column used the down-flow influent mode, which enabled the BAC
column to enrich more attached biomass, and *Alphaproteobacteria* and *Betaproteobacteria* could become dominant groups in the BAC effluent due to the operation mode of the filter. Meanwhile, the variations of bacterial population located in different BAC layers were investigated in terms of the level of genera by utilizing HT-qPCR. The results are shown in Figure 6. Figure 6 shows the relative abundance of the first eight bacterial genera (normalized to the corresponding 16SrRNA gene copy numbers) in the different BAC layers, and the eight bacterial genera accounted for 32.4% of the total bacterial community. The relative abundance of *Aquincola*, *Pseudomonas* and *Ensifer* in the ML was higher than that of the other two carbon layers. Previous research has demonstrated that *Aquincola* can degrade complex organic compounds, for example methyl tertiary butyl ether (MTBE), as carbon and energy sources (Ma et al. 2012). Caldera et al. (2016) found that *Pseudomonas* could transform DON into Phe, which was consistent with the phenomenon shown in Figure 3(b). Also, it was proven that *Ensifer* was able to attack effectively ether and amide bonds (Ge et al. 2014). The three bacterial genera attacked specific functional groups of Trp and degraded them, resulting in a satisfactory removal efficiency of Trp in the ML of the BAC filter.

**CONCLUSIONS**

In summary, the study revealed the impact of BAC removal of Trp on different carbon layers. The influent Trp was partly absorbed by activated carbon and biodegraded into inorganic nitrogen by the attached microorganisms, especially the three bacterial genera, *Aquincola*, *Pseudomonas* and *Ensifer*, which could biodegrade complex organic compounds as carbon and energy sources and attack actively the specific functional groups of Trp. Also, HT-qPCR analysis demonstrated that the relative abundances of *Aquincola*, *Pseudomonas* and *Ensifer* were higher significantly in the ML than the other two carbon layers and had an important influence on the transformation of Trp. Meanwhile, new AA could be generated in the metabolism of microorganisms attached to the GAC when they biodegraded Trp. Compared with the other two layers, the removal efficiencies of Trp and DBPs were both significantly higher in the ML, but the adsorption experiment did not show this tendency (the average adsorption yields were 10.98 ± 1.17 mg/g, 7.45 ± 0.80 mg/g and 3.32 ± 0.39 mg/g, respectively), which demonstrated that the removal of Trp had an important influence on the variations of whole DBP precursors and the biodegradation of BAC played an important role in the process of removal of Trp.

**Figure 6** | Relative abundance of the first eight bacterial genera in the different BAC layers (‘UL’, ‘ML’ and ‘BL’ represent the upper, middle and bottom carbon layers, respectively).
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REFERENCES


