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Microbial diversity in combined UAF–UBAF system with novel sludge and coal cinder ceramic fillers for tetracycline wastewater treatment

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HIGHLIGHTS

- The main removal processes of pollutants happened at the height of 0–80 cm.
- The EPS concentration decreased with increasing reactor height in UAF and UBAF.
- The main bacteria that degraded TET were *Comamonadaceae*, *Thauera* and *Sinobacteraceae*.
- Microbial diversity became richer with gradual biodegradation of TET.

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ABSTRACT

Microbial species, community structures and functional profiles were explored to explain the strong degradation capability of the combined up-flow anaerobic filter and up-flow biological aerated filter (UAF–UBAF) system with novel sludge and coal cinder ceramsite for treating tetracycline (TET) wastewater. Concentrations of different pollutants and extracellular polymeric substance (EPS) with increasing reactor height were measured. DNA extracted from biofilm at the corresponding height was sequenced on the Illumina MiSeq platform. Results showed that the main removal process of pollutants happened at the height of 0–80 cm. The highest average removal rate was in the region of 0–40 cm. EPS decreased with rising reactor height. For the same height, EPS concentration in UAF was higher than that in UBAF. Due to the analysis of dominant population, the main bacteria that degraded TET were *Comamonadaceae*, *Thauera* and *Sinobacteraceae*. Hydrolysis mainly took place at low reactor height while acidification and acetogenesis mainly happened at high height in UAF. For UBAF, the dominant bacteria species changed from anaerobic to aerobic species. Furthermore, microbial diversity became richer with the gradual biodegradation of TET.

1. Introduction

Tetracycline (TET) is one of the most extensively used antibiotics in human activities and livestock breeding [1,2]. With the large-scale producing of TET, a great deal of pharmaceutical wastewater is generated. This kind of wastewater usually has poor biodegradability because of high TET concentration and strong bacterial toxicity. So TET cannot be completely decomposed in the sewage treatment plant and will be released into the environment. The persistent TET residues are detected in municipal wastewater, surface and ground water, which cause some potential environmental effects like the development of antibiotic-resistant bacteria [3]. Recently, most studies on the treatment of TET wastewater have been concentrated in multiple-stage treatment like hydrolysis acidification – fenton oxidation – two-stage contact oxidation processes. These complicated and hard-controlled processes suffered from long start-up time and low removal efficiencies for the strong bacteriostatic effect of TET. The growth and activity of microorganism were inhibited even when the TET concentration was low in the reactors [1]. Hence, improvements of total biomass and microbial activity in these reactors were keys to increasing the removal efficiency of TET. In order to solve this problem, the combined up-flow anaerobic filter and up-flow biological aerated filter (UAF–UBAF) system with two novel ceramics based on sludge and coal cinder were used for the treatment of TET wastewater in our study. This technology not only realized the reutilization of wastes, but also achieved advantages of short start-up time, strong tetracycline resistance and high removal efficiency of TET, COD etc. [4]. This was because that a large number of microorganisms were immobilized in this system, which contributed to the high removal rates of pollutants. However, the

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specific effects of different microbial species and the deep mechanism have not been analyzed. To solve these previous limitations, microbial secretions, species diversity, community structures and functional profiles were analyzed in this article.

To analyze microbial secretions, extracellular polymeric substance (EPS) was necessary, which had a presumed determinant role in the structure, surface charge, strength and filterability of biofilm in biological wastewater treatment processes. It could hold microbial aggregates, filamentous bacterial strains, organic and inorganic particles together to form biofilms. Therefore, extraction and analysis of EPS were vital to explain the inner reasons for the good performance of UAF–UBAF system on treating TET wastewater. EPS usually consisted of polysaccharides (PS), proteins (PN), nucleic acids, lipid, humic acid and amino acid etc. [5]. PS and PN were considered as the major components of EPS and the proportions of these components in EPS are closely related to the microbial species [6]. In this paper, two extraction methods were used to extract EPS to study the change of EPS concentration with reactor heights and determine a better EPS extraction method in combined UAF–UBAF reactors [7].

In order to analyze microbial communities in activated sludge, the molecular biological technology was used about two decades ago, which greatly improved our understanding of the microbial communities [8]. However, due to the PCR (polymerase chain reaction) bias or low throughput, the molecular methods were limited. Recently, with the development of the next-generation DNA sequencing techniques, high-throughput sequencing (HTS) has been introduced to analyze the microbial diversity [9]. With direct sequencing of the genomic DNA extracted from the environmental samples, HTS technology provided a comprehensive approach without PCR bias, which could simultaneously explore taxonomic and functional diversity of a microbial community. So far, several HTS platforms like 454 pyrosequencing and Illumina sequencing have sufficiently matured. They have been used for tackling significant biological problems [10]. In this paper, the purpose was to explore the influence of TET to the bacteria in the two reactors and the biological degradation process of TET. For this objective, four biofilm samples from UAF and UBAF at different reactor heights were tested on the newest Illumina MiSeq platform to analyze the microbial community structures and functional profiles (sequencing of bacterial 16S rDNA gene's PCR products, 16S V4 version). The microbial species and behavior in different reactors and different reactor heights were compared according to the results. The results in this study would provide theoretical guidance for the practical application of combined UAF–UBAF system in TET wastewater treatment.

2. Materials and methods

2.1. Experimental setup

The UAF and UBAF (Fig. 1) made from polymethyl methacrylate had a diameter of 200 mm with a height of 1.7 m. These two reactors were filled with novel sludge and coal cinder ceramics separately. The bulk and grain density of sludge ceramics were 435 and 790 kg m^{-3}. For coal cinder ceramics, the bulk and grain density were 896 and 1565 kg m^{-3}. Glucose, ammonium chloride and potassium diphosphate were used as carbon, nitrogen and phosphorous source respectively. USP grade tetracycline made by Sangon Biotech was added to synthetic wastewater according to the project every day. The treatment time for tetracycline wastewater was about 270 days in this system. The whole experiment could be separated to two periods. Influent COD concentration increased from 1000 mg/L to 5000 mg/L in the first stage for about 160 days. Then influent TET concentration was improved from 10 to 70 mg/L for about 110 days. Other operation conditions: HRT of UAF and UBAF were 20 and 12 h; air–water ratio of UBAF was 15:1; the temperature was low at about 16 °C. In the end, ceramics with biofilm were obtained from UAF and UBAF at the height of 35–40, 65–70 and 100–105 cm, and rapidly settled in polypropylene bottles respectively. All the samples stored in refrigerator in the temperature of 3 °C for further test.

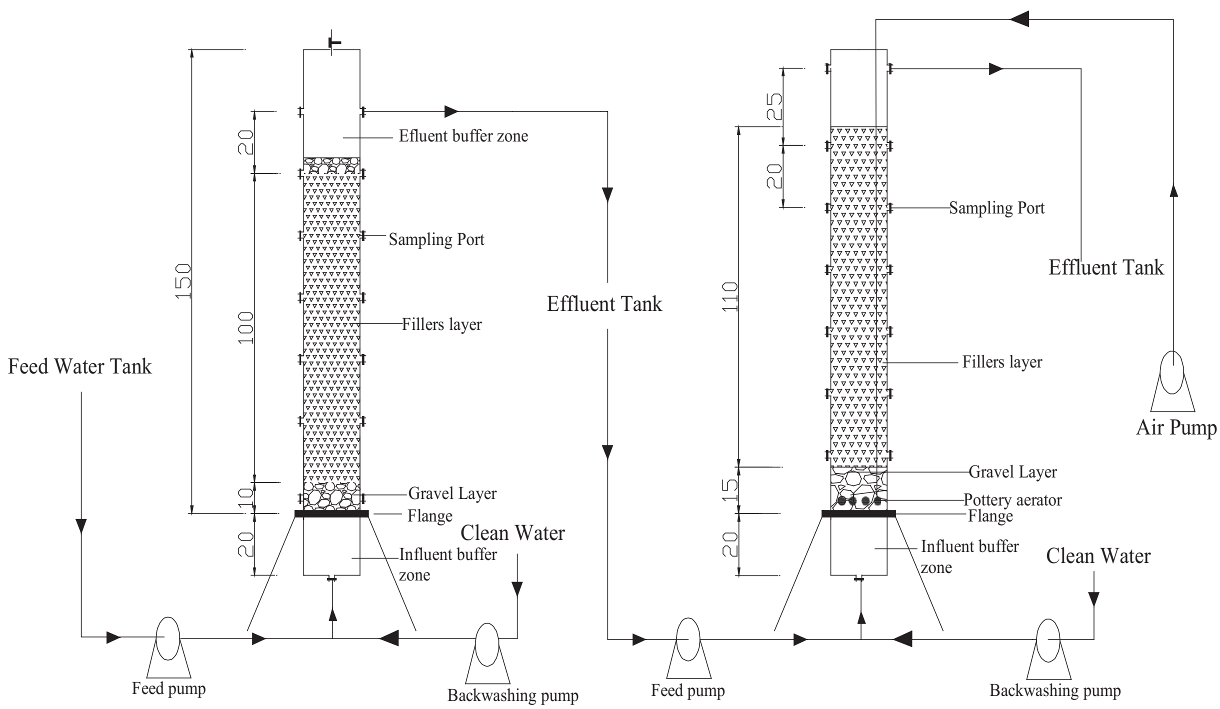

Fig. 1. Schematic diagram of the connected UAF–UBAF experimental system (dimensioning unit: cm).