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Microbial diversity and community distribution in different functional zones of continuous aerobic–anaerobic coupled process for sludge in situ reduction

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HIGHLIGHTS

- The revelation of sludge reduction mechanism was based on molecular biology.
- The relationship between microbial communities and function of sludge were linked.
- The bacteria responsible for COD & TN removal were identified.

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ABSTRACT

A continuous aerobic–anaerobic coupled (CAAC) process was operated for sludge in situ reduction during wastewater treatment. The average removal efficiencies of chemical oxygen demand (COD), NH_4^+–N, and total nitrogen (TN) reached 98.6%, 99.4%, and 72.8%, respectively. The sludge growth yield coefficient reduced to 20.2% of that achieved by the conventional activated sludge process to be about 0.1 g VSS/g COD. The sludge reduction was discussed involving with the lysis and anaerobic digestion of sludge according to the increase in levels of COD and NH_4^+–N, presence of fermentative bacteria (*Clostridium* and *Stenotrophomonas*), and morphologic characteristic of disrupted bacteria in anoxic and anaerobic zones. Furthermore, two dominant populations such as *Zoogloea* and *Sphaerotilus* in moving bed biofilm reactor (MBBR) carries biofilm, which account for 75.44% of the identified genera, play an important role in achieving the high COD removal efficiency. The existence of *Nitrosospira* and *Nitrospira* in MBBR and aerobic zones combining with denitrifying bacteria in each zone of the process enhanced the removal of nitrogen from the system.

1. Introduction

The conventional activated sludge treatment process is the most widely used for treating municipal and industrial wastewater in the world [1]. Although it is an efficient and reliable process for organic matter and nitrogen removal, but its major drawback is excessive sludge production. Currently, excess sludge (i.e. waste activated sludge) disposal is raising challenge for wastewater treatment plants due to its economic and environmental aspects, which account about half even up to 60% of the total operating costs [1,2].

Solids reduction by physico-chemical methods results in accumulation of chemicals. This may pollute the environment and also require further treatment to remove the hazardous chemicals. Wastewater sludge reduction up to 100% by biological and environment friendly method has been successfully tested at different scales [3]. Furthermore, the solution of sludge should be in situ rather than subsequently treated during wastewater treatment, since only in-place reduction of excess sludge was natural process without any external and extraneous intervention [3]. Approaches to achieve the goal of sludge in situ reduction (minimum sludge generation at place of its production) are classified as: (a) biological lysis-cryptic growth [3,4], (b) biological uncoupling of metabolism [5], (c) maintenance metabolism [6], and (d) predation [7].

In recent years, some modified bioreactors were designed and investigated for excess sludge production. The anaerobic side-stream reactor (ASSR) process has shown significant decrease in

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sludge production [8]. The Oxic-settling-anoxic (OSA) process was also effective for in situ excess sludge production by treating settled sludge (return sludge) under anoxic condition [9]. Feng et al. [10] achieved in situ reduction of excess sludge at 63.7% through a baffled reactor with repeated coupling of aerobic and anaerobic conditions during wastewater treatment, in which the presence of *Clostridia* was considered to contribute to sludge reduction.

In our previous studies, a continuous aerobic–anaerobic coupled (CAAC) process was designed for sludge in situ reduction and synchronous organics and nitrogen removal [11]. The efficiency and stability of this process is entirely dependent upon the concerted and syntrophic activity of microorganisms in it. However, the microbial diversity and community distribution in different functional zones of this CAAC process has not yet been clarified. Biodiversity and distribution of microbial communities are regarded as important indicators for functionality in wastewater treatment system [12]. Hence, the information on microbial communities of this process was worthy. However, the methodology used for microbial community structure was commonly based on sequencing 16S rDNA and *mcrA* clone libraries, and it has been difficult to cover the entire complexity of microbial composition [13]. Compared to other molecular techniques, pyrosequencing does not require subcloning or handling of individual clones, and it is superior and reliable to reveal the whole composition of microbial communities [14]. The pyrosequencing technology has been used to explore microbes in human gut, soil, oceans and bioreactors [15–17].

The objective of this study was to continue to run the lab-scale CAAC process and investigate its performance to simultaneously remove organic carbon and nitrogen and reduce the excess sludge during the treatment. SEM and the pyrosequencing methods were used to observe cell morphology and analyze the diversity and distribution of the microbial communities in each zone of the CAAC process. Then, the relationship between the microbial community structure and function of sludge in each zone of the present process was discussed in this study.

2. Materials and methods

2.1. Experimental set-up

Experiments were carried out in a lab-scale CAAC process (as shown in Fig. 1), which consists of a moving bed biofilm reactor (MBBR) connecting with an aerobic–anaerobic coupled (AAC) process. This process consisted of four zones: MBBR (Zone 1), anoxic zone (Zone 2), anaerobic zone (Zone 3), and aerobic zone (Zone 4). The CAAC process was made of Plexiglas with a total liquid volume of 18.6 L (MBBR: 4.1 L, other three zones: 14.5 L). Suspended carriers, with cross inside and longitudinal fins outside, were placed in the MBBR with a packing ratio of 30% (vol/vol). Zones 2–4 were divided into three regions by two internal baffles. Zones 3 and 4 were filled with industrial slag as fixed carrier with packing ratios of 100% and 80%, respectively. The industrial slag had a diameter of 3–5 cm, and a porous structure with porosity of 50–60%. Aeration devices were fixed on the bottom of MBBR and Zone 4. Wastewater was first pumped into the MBBR from a storage tank for organic carbon and nitrogen simultaneous removal and then flowed into the anoxic and anaerobic zones for in situ sludge reduction and total nitrogen (TN) removal, the existence of aerobic zone was in order to guarantee the effluent quality.

2.2. Characteristics of wastewater

Synthetic wastewater simulating the effluent from the anaerobic reactor for soybean wastewater pretreatment was used as an experimental influent. The characteristics and detailed chemical composition of the influent wastewater were described as follows: pH, 7.5–8.5; COD, 728.1–648.3 mg/L; TN, 42.2–65.1 mg/L; NH_4^+–N, 41.5–70.0 mg/L; total phosphorus (TP), 42.4–58.1 mg/L.

2.3. Physico-chemical analysis

Samples from different zones along the flow direction in CAAC process were obtained through centrifugation at 8000×g for 10 min. The supernatants were used to analyze COD, NO_2^-–N, NO_3^-–N, NH_4^+–N, and PO_4^{3-}–P, and the biomass samples were collected for volatile suspended solids (VSS) measurement according to Chinese SEPA Standard Methods [18]. The DO was measured using a DO electrode connected to a DO meter (JPBJ-608, Shanghai Precision & Scientific Instrument Co., China). The pH was measured using a pH meter (HI8424, Hanna Co., Italy). TN was the sum of NH_4^+–N, NO_2^-–N and NO_3^-–N rather than an independent test.

2.4. SEM sample preparation and image acquisition

SEM was carried out when the CAAC process was operated for 104 days at HRT 1.3 days. One biomass sample was obtained from suspension sludge in anoxic zone, and the other three samples were obtained from sludge attached to carriers in the MBBR, anaerobic zone, and aerobic zone, respectively. The samples were fixed

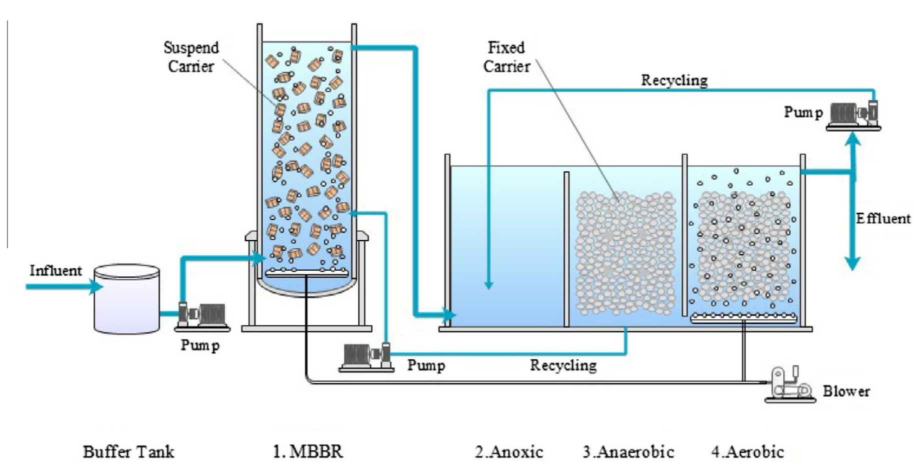

Fig. 1. Schematic of the experimental setup of continues aerobic–anaerobic coupled (CAAC) process. (1) Moving bed bio-film reactor; (2) anoxic zone; (3) anaerobic zone and (4) aerobic zone.