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Effects of carbon sources on sludge performance and microbial community for 4-chlorophenol wastewater treatment in sequencing batch reactors

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ABSTRACT

Considering carbon sources are often supplied to satisfy the removal of high nitrogen and refractory pollutants in industrial wastewater, two sequencing batch reactors (SBRs) were used in this study to treat 1.5 ± 0.5 mg/L 4-chlorophenol (4-CP) wastewater containing ammonium nitrogen and phosphate with different carbon sources. The favorable removal efficiencies of influent COD, NH_4^+-N, PO_4^{3-}-P, and 4-CP suggested that the both SBRs were not influenced by supplying dissolved starch and sodium acetate, respectively. The phyla *Proteobacteria* and *Bacteroidetes* were dominant in both SBRs, while the dominant phylum *Candidatus Saccharibacteria* was only existed in SBR with carbon source of dissolved starch. The relative abundance of bacterial communities had significant differences at class, family, and order level in both SBRs. And the mutually dominant genus in both SBRs was only *Gemmobacter*, which was first found in 4-CP wastewater treatment. The changed extracellular polymeric substances (EPS) were related with microbial communities.

1. Introduction

Chlorophenols (CPs) wastewater, which is largely discharged from several processes such as paper pulp, wood preservation, flame retardants, pharmaceutical manufacturing and coking steelmaking, belongs to refractory industrial wastewater and is not readily degraded naturally (Gómez-Acata et al., 2018). Due to the insufficient pretreatment and unexpected leakage from CPs wastewater, CPs are extensively detected in bodies of water, soils and sediments (Zhao et al., 2017). Many researches have found that CPs in sewage and some polluted surface waters considerably vary from μg/L to mg/L, and even exceed 3 mg/L (Olaniran and Igbinosa, 2011). CPs are apt to bioaccumulation via food chains based on their hydrophobic properties, further leading to acute and chronic toxic effects on organisms (Ge et al., 2017). Thus, CPs have been already listed as priority pollutants by the United Stated Environmental Protection Agency and other countries. The discharge of CPs wastewater has caused great concerns. At present, many physicochemical methods, such as adsorption, flotation, ozonization, and wet air oxidation, have higher removal efficiency for such pollutants, while these processes are often high-cost and form some intermediate products even more toxic than parents (Monsalvo et al., 2009). In addition, the total nitrogen (TN) and total phosphorus (TP) in CPs wastewater are frequently hard to reach the discharge standards (Ma et al., 2017a). Therefore, biological processes are considered to be more eco-friendly and cost-effective for CPs wastewater treatment than physicochemical methods (Jiang et al., 2016). It has confirmed that an array of microorganisms are able to degrade CPs such as bacteria, fungi, yeasts as well as algae, and in the process of CPs biodegradation, the removal of TN and TP is also achieved (Chen et al., 2015; Wang et al., 2017).

Activated sludge process is often adopted to treat CPs wastewater, and many kinds of microorganisms are able to mineralize CPs in appropriate conditions. To implement the efficient removal of CPs in industrial wastewater, different carbon sources are often supplied to achieve cometabolic biodegradation of pollutants (Zhou et al., 2017). In addition, the organic matters in CPs wastewater are often insufficient to accomplish nitrogen removal by dentrification, and the external carbon sources are thus replenished (Plüg et al., 2015). Methanol, sodium acetate, and glucose are preferred external carbon sources in industrial and low C/N ratio wastewater treatment, and the removal of CPs as well as ammonia nitrogen is significantly influenced by different carbon sources (Majumder and Gupta, 2008; Osaka et al., 2008). Based on operational flexibility and saving operating cost, sequencing batch

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reactor (SBR) is widely used in industrial wastewater treatment (Chen et al., 2017). Moreover, in the process of CPs wastewater treatment with supplying carbon sources, the SBR has a significant effect in resisting CPs shock and reducing sludge loss (Zhao et al., 2017). To date, however, the effect of dissolved starch and sodium acetate on CPs as well as ammonia nitrogen and phosphorus removal has seldom been reported.

Activated sludge contains highly complex microbial communities, and different bacterial genera implement the pollutants removal by synergistic effect (Shu et al., 2015). Gómez-Acata et al. (2018) have found that the dominant bacterial phyla and genera for CPs biodegradation are *Proteobacteria*, *Actinobacteria*, *Firmicutes* and *Phodobacteraceae*, *Sphingobium*, *Pseudoxanthomonas,* respectively. However, the microbial diversity often changes with wastewater compositions, operation conditions of reactors, regional distributions (Zhang et al., 2012). And many bacterial groups will secrete abundant extracellular polymeric substances (EPS) during metabolism process, which are apt to cause membrane fouling once membrane technologies are adopted (Meng et al., 2017). In addition, the EPS production will increase when activated sludge is used to treat toxic and refractory wastewater (Remmas et al., 2017). Jiang et al. (2017) have reviewed that EPS is widely regarded as one of the more formidable fouling in membrane technology. In high ammonium nitrogen and refractory pollutants wastewater treatment, membrane technologies are often selected along with supplying external carbon sources (Jin et al., 2014). Thus, selecting suitable carbon source to enrich dominant bacterial genera for pollutants removal, which are able to secrete less metabolic products and alleviate membrane fouling, are meaningful. However, similar researches are seldom reported. Previous researches have found that the low sequencing depth of traditional polymerase chain reaction (PCR) approach hinders a comprehensive characterization of community structure compared with the vast genetic diversity in activated sludge systems (Zhang et al., 2012). At present, a high-throughput sequencing technique is therefore widely used to elucidate the bacterial community and compare phylogenetic affiliation for bacteria via obtaining more sequences (Shu et al., 2015). However, in the process of CPs wastewater treatment containing ammonia nitrogen and phosphate, the difference of microbial community via pyrosequencing is not studied by supplying different carbon sources.

Therefore, the aim of this study was to investigate the sludge performance and microbial community in 4-CP wastewater treatment containing ammonia nitrogen and phosphate using both SBRs, which were supplied different carbon sources of dissolved starch and sodium acetate, respectively. To our knowledge, this is the first work to present a variation in bacterial communities using high-throughput sequencing during the 4-CP treatment process by supplying different carbon sources, and ammonia nitrogen and phosphate were simultaneously removed. Meanwhile, the correlation of shifted microbial communities and EPS formation was discussed. Characterizing the bacterial communities and EPS are an attempt to provide a theoretical basis for the long-term operation strategy of high ammonium nitrogen, phosphorus, and refractory pollutants wastewater treatment, when membrane technologies are adopted and need to supply carbon sources.

2. Materials and methods

2.1. Setup and operational conditions of the bioreactor

The aerobic activated sludge was obtained from a local municipal wastewater plant in Zhengzhou, China, which was settled and discarded supernatant. Before injecting reactor, the activated sludge was washed thrice using tap water and aerated for 24 h. Two 5-L SBRs were used to investigate 4-CP wastewater treatment with different carbon sources (Fig. 1). The influent COD of both SBRs was controlled at a range of 320–420 mg/L by supplying dissolved starch and sodium acetate, respectively. One SBR supplied dissolved starch was used as reactor A,

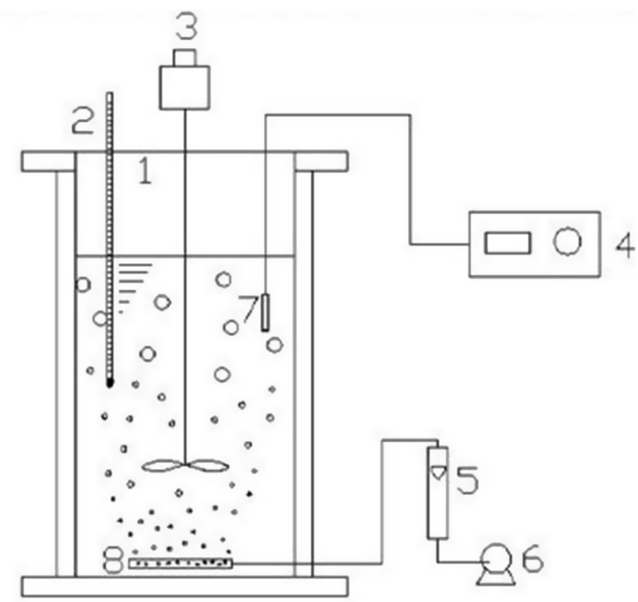

Fig. 1. Schematic diagram of SBR reactor (1-reactor; 2-thermometer; 3-stirrer; 4-DO meter; 5-gas flow meter; 6-aeration pump; 7-DO probe; 8-aerator).

while another SBR supplied sodium acetate was used as reactor B. The initial mixed liquid suspended solid (MLSS) and sludge volume index (SVI) were maintained at 2500 mg/L and 105 mL/g·MLSS. In addition to carbon source, the activated sludge was acclimated with artificial wastewater consisted of the following compositions (mg/L): NH_4Cl 100, KH_2PO_4 31.2, $MgSO_4·7H_2O$ 23.9, $CaCl_2·H_2O$ 7.6, $FeCl_3·6H_2O$ 7.0, $CuSO_4·5H_2O$ 0.047, $MnSO_4·H_2O$ 0.06, $ZnCl_2$ 0.09, $CoSO_4·7H_2O$ 0.20, and $Na_2MoO_4·2H_2O$ 0.05. The chemical reagents used were of analytical grade. The reactor pH was adjusted as 7.2 ± 0.2 through adding $NaHCO_3$ and HCl (Zhao et al., 2016).

The sequential SBR was in a 12 h cycle consisting of 0.25 h of influent filling, 11.0 h of operation period, 0.50 h of settling, and 0.25 h of effluent discharge, and 70% of volumetric exchange ratio was implemented after the SBR end. In the operation period, intermittent aeration process was adopted, i.e. 2 h aeration along with 2 h no aeration. Aeration was provided by an air pump through a porous stone diffuser to maintain the dissolved oxygen (DO) of 1.5 ± 0.5 mg/L at the initial stage. Introduced influent and discharged effluent were implemented via peristaltic pump. The operation cycle was controlled by timing switch, and the experiments were conducted at normal temperature of 19 ± 1 °C. After 3 d operation of both SBRs, each SBR was injected with 1.5 ± 0.5 mg/L influent 4-CP. Samples were taken from each SBR at influent and effluent of SBR cycle, and the concentration of NH_4^+-N, PO_4^{3-}-P, 4-CP and COD was measured and compared with both SBRs. Meanwhile, the variation of EPS production at different operation time was analyzed. Finally, at the stable operation stages of both SBRs, the diversity of microbial community was analyzed and contrasted.

2.2. Sampling and analytical methods

DO, temperature, and pH were monitored using a portable DO meter (WTW, German) and a portable pH meter (WTW, German). After the mixed liquor in both SBRs was taken and centrifuged at 4000 r/min for 5 min, the aqueous phase was used to determine COD, NH_4^+-N and PO_4^{3-}-P according to the Standard Methods of APHA, 2005, and the sludge phase was used to analyze EPS including polysaccharides and proteins. The extract methods of EPS were conducted as described by Tian et al. (2013), and the content of polysaccharides and proteins was measured using anthrone colorimetric method and Lowery Protein Quantification Kit-Rapid (Shanghai LabAide, China) according to the