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Integrated temperature and DO effect on the lab scale A^2O process: Performance, kinetics and microbial community

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ABSTRACT

In this work, it was aimed to investigate the integrated DO and temperature effect on the lab scale A^2O process treating low carbon/nitrogen (C/N) wastewater. The system performance, kinetics and microbial community dynamics during operation were studied. The chemical oxygen demand and NH_4^+-N removal usually were above 80% and 95% in both phases, respectively. The NO_3^--N and PO_4^{3-}-P removal in low DO were better than high DO. Temperature had no significant difference in nutrients removal ($p > 0.05$). The maximal ammonia oxidizing rate (AOR) and nitrite oxidizing rate (NOR) (3.62 mg N g^{-1} VSS·h^{-1} and 5.57 mg N g^{-1} VSS·h^{-1}, respectively) were observed at low DO and temperature condition. The high-throughput sequencing demonstrated low DO condition could enrich both *Nitrosomonas* and *Nitrospira*, which consistent with the AOR and NOR results. Moreover, the NO_3^--N and PO_4^{3-}-P removal and microbial community results suggested that low DO was favorable for the potential of denitrifier and denitrifying phosphorus accumulating organisms. Therefore, low DO operation even low temperature is feasible for low C/N wastewater treatment, which provide important insights for low C/N wastewater treatment.

1. Introduction

Biological nutrient removal (BNR) processes are most widely adopted in wastewater treatment plant (WWTPs) for nitrogen and phosphorus removal due to their various advantages (Huang et al., 2017). To date, many biological treatment processes have been widely applied in WWTPs for nitrogen and phosphorus removal, such as anoxic/oxic (AO), anaerobic/anoxic/oxic (A^2O), University of Cape town (UCT) and 5-stage Bardenpho process (Ge et al., 2010). These processes were severely deteriorated by reflow ratio. The high reflow ratio not only brought high nitrate but also high DO into anoxic zone, which restricted the denitrification and P-release (Patel and Nakhla, 2006; Zhao et al., 2018). Carbon source is an essential element to obtain excellent nitrogen and phosphorus removal in these traditional BNR processes. These processes reveal relatively outstanding nitrogen and phosphorus removal efficiency in treating wastewater containing enough carbon sources. However, the simultaneous nitrogen and phosphorus removal efficiency will be greatly deteriorated if the lack of carbon sources or the chemical oxygen demand (COD) (Liu et al., 2018), due to both nitrogen and phosphorus removal requires adequate carbon source but the denitrifying bacteria would preferentially use carbon sources than phosphorus accumulating organisms (PAOs) (Wang et al., 2015). So the carbon/nitrogen (C/N) ratio is crucial for evaluating the availability of carbon source in wastewater (Ge et al., 2017).

The domestic wastewater is typical low C/N wastewater in China (Huang et al., 2017; Zhao et al., 2016), in consequence, it is difficult to obtain excellent nutrient removal performance (Qian et al., 2017). For low C/N wastewater, many effective ways to improve nutrient removal performance have been well documented, such as adding additional carbon sources, chemical phosphorus removal and step feed (Ge et al., 2010; Huang et al., 2017; Kim et al., 2015). Adding additional carbon sources was one of the most effective ways for low C/N wastewater. Methanol, ethanol, acetic acid, propionic acid, and glucose have served to additional carbon sources for nitrification/denitrification (Sun et al., 2010). Coagulant, the most utilized for chemical phosphorus removal, was dosed to form precipitation to remove phosphorus (Kim et al., 2015). As for step feed, Ge et al. (2010) proposed modified step feed process to enhance nitrogen and phosphorus removal performance. Due to its excellent performance of nutrient removal, it was demonstrated to

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be an effective method for low C/N wastewater. However, adding additional carbon sources will waste organic resources and increase the operating costs. Chemical phosphorus removal not only increases the expense but also introduces new pollution. New equipment and pipelines are required for step feed, which is not suitable for existing WWTPs. Thus, modifying the operation parameters (e.g. DO or hydraulic retention time (HRT), etc.) and excavating their treatment efficiency fully are necessary.

According to our knowledge, traditional high-rate BNR systems were operated with extensive aeration to ensure high nutrient removal efficiency. As indicated in the study by Liu and Wang (2013), complete nitrification was accomplished in bench scale complete-mix reactors with 10 and 40 day solids retention times (SRT) after a long term operation (operation period > 400 days) with low DO condition (0.37 and 0.16 mg l^{-1}, respectively). Recently, Keene et al. (2017) also documented the stable and efficient high rate BNR was achieved in a pilot scale enhanced biological phosphorus removal (EBPR) plant at low DO conditions. In the 16-month period, stepwise decrease aeration was applied to reach DO about 0.3 mg l^{-1}, and nearly 90% phosphorus was removed and 70% nitrogen was denitrified. However, the low DO concentration inhibits the growth of nitrifying bacteria leading to incomplete nitrification has ever been proved by previous research (Park and Noguera, 2004). Therefore, the effect of low DO on BNR and functional bacteria should be further investigated. Actually, temperature, varies significantly with seasonal change, is also a major factor associated with nitrogen and phosphorus removal in WWTPs. The main reason is that the functional bacteria related to nutrient removal is affected by temperature. Considerable research have investigated the impact of temperature on functional bacteria, such as ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), PAOs and glycogen accumulating organisms (GAOs) (Kim et al., 2008; Liu et al., 2014; Panswad et al., 2003).

As previous studies were mainly focused on individual factor affecting the performance or microbial population, and limited studies were carried out under integrated conditions. Besides, many researches were performed under conditions of adequate nutrients. Few studies have been conducted on the treatment of low C/N wastewater. Based on the above backgrounds, the purpose of this work was to investigate the integrative effect of DO and temperature on treatment of low C/N wastewater. A^2O process, as a representative of most full-scale WWTPs, was adopted in this project for treating the low C/N wastewater. Two identical lab scale reactors were operated under different DO and temperature to investigate the performance and the dynamic of microbial community during the operation process. The ammonia oxidation rate (AOR) and the nitrite oxidation rate (NOR) were used to characterized the bioactivity of biomass and the dynamic of microbial community were revealed by high-throughput sequencing. This is the first study to investigate the integrated effects of DO and temperature on the performance and dynamic of microbial communities for treatment of low C/N wastewater, which could not only take one step forward in understanding the factors influencing the reactor performance, but also provided important insights for the treatment of low C/N wastewater.

2. Materials and methods

2.1. Seed sludge

The seed sludge was taken from a secondary settler in a WWTP (a full-scale A/O treatment plant in Tianjin, China) on Jun 30, 2017, which showed well nitrogen and phosphorus removal efficiency in long-term operation. The mixed liquor suspended solids (MLSS) of seed sludge was 5.6 g l^{-1} and volatile suspended solids (VSS) is 4.1 g l^{-1}. The sludge volume index (SVI) of seed sludge is 89 mL g^{-1}, which had a good settling property and only had limited filamentous bacteria acted as floc backbone. 5.5 L of seed sludge was inoculated in the 8.5 L A^2O reactors. The MLSS and VSS after seeding were about 3.5 g l^{-1} and 2.6 g l^{-1} in reactors, respectively.

2.2. Wastewater and synthetic wastewater

In phase I, the domestic wastewater was used as influent. The daily domestic wastewater was collected from the septic tank in the living area of Tianjin Chengjian University (Tianjin, China). The wastewater was characterized with low C/N of average 3.4, the main characteristics were: COD = 141.4 ± 30.8 mg l^{-1}, NH_4^+-N = 42.0 ± 12.0 mg l^{-1}, NO_2^--N = 0.03 ± 0.02 mg l^{-1}, NO_3^--N = 0.60 ± 0.73 mg l^{-1}, PO_4^{3-}-P = 3.56 ± 1.44 mg l^{-1}.

To reduce the impacts of wastewater quality fluctuations and ensure the data is comparable, the synthetic wastewater based on the characteristic of domestic wastewater used throughout the phase II to provide the influents of A^2O reactor. The synthetic low C/N wastewater feeding to the A^2O reactor was prepared every day. As the presence of oil and grease in the domestic wastewater, so the Tween 80 was added in synthetic wastewater. The COD (about 150 mg l^{-1}) was provided with CH_3COONa and Tween 80. The details of synthetic low C/N wastewater (per liter) were described as follows: CH_3COONa, 0.1281 g; Tween 80, 0.0249 g; $(NH_4)_2SO_4$, 0.2357 g; KH_2PO_4, 0.0219 g; $MgSO_4{\cdot}7H_2O$, 0.1 g; $CaCl_2$, 0.0555 g; $NaHCO_3$, 0.250 g; 10 mL trace element solution and 10 mL vitamin solution. The detail composition of trace element solution and vitamin solution were also described in Tables S1 and S2 (Fan et al., 2017). The purity of the above chemicals were all analytical reagent (AR), and they were purchased from Tianjin Jinke Fine Chemical Research Institute.

2.3. Reactor setup and operation

The experiment was performed in the lab scale A^2O reactor. The lab scale A^2O reactor diagram was depicted in Fig. S1. It was consisted of an anaerobic zone (1.0 L), an anoxic tank (1.5 L), two identical aeration tank (6.0 L) and a settler, all of which was made of transparent polymethyl methacrylate (PMMA). Two parallel A^2O reactors were set up under different temperature. Operation temperature of reactor 1 (R1) was controlled at low temperature with a thermostatic water jacket around the reactor. However, the reactor 2 (R2) was operated at ambient temperature under seasonal change. The approximately 17 h of HRT was achieved by feeding 12 L wastewater continuously per day. About 300 mL mixed liquor was discharged from aeration tank per day, to obtain the 30 day SRT. The MLSS of aeration zone was returned to anoxic zone (recycling ratio: 200%), and sludge from settler was also pumped back to anaerobic zone (recycling ratio: 100%).

The A^2O system was performed for a duration of 137 days, which could be divided into two phase based on the different DO concentration. In phase I (1–65 day), the DO was 2–3 mg l^{-1}, however, the DO was decreased to 0.5 mg l^{-1} in phase II (68–137 day). To keep the two reactors in the same starting state at phase II, the sludge of two reactors at the end of phase I was mixed and then added into two reactors, respectively. During the operational period, the DO, pH and temperature were monitored regularly. They were measured with a multi-parameter water quality analyzer (HQ30d, HACH, USA), which with LDO101 and PHC301 electrode (HACH, USA).

2.4. Batch tests

Batch assays were used to evaluate the activity of bacteria, which were undertaken on day 31, 45, 60, 78, 92, 107, 122, 137. The batch tests for determining the biomass AOR and NOR were modified the previous report (Liu et al., 2017), and a brief procedure was shown as follows. 300 mL activated sludge were collected and washed three times by deionized water, finally recovered to the initial 300 ml. Then the $(NH_4)_2SO_4$ and $NaNO_2$ was added to make the concentrations of NH_4^+-N and NO_2^--N were 50 mg l^{-1} and 20 mg l^{-1}, respectively. Enough carbon