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Wastewater ammonia removal using an integrated fixed-film activated sludge-sequencing batch biofilm reactor (IFAS-SBR): Comparison of suspended flocs and attached biofilm

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ABSTRACT

Integrated fixed-film activated sludge (IFAS) that combines the activated sludge (suspended flocs) and attached biofilm has been applied in municipal wastewater treatment to promote nitrification. This study investigated the changes in reactor performance and bacterial community structure in response to different organic loadings (500, 250, 150 mg/L COD) in an integrated fixed-film activated sludge (IFAS) reactor operated under sequencing batch mode. Changes of bacterial population in the suspended flocs and attached biofilm were compared. Biomass in the suspended flocs and in biofilm decreased as the organic loading decreased. The specific nitrification rate was highest when COD was only 150 mg/L in the feeding, which highlighted the effects of competition between heterotrophic bacteria and autotrophic bacteria over oxygen and space. Besides, lack of carbon sources limited denitrification activities. The percentages of nitrifying related genes over total genes were higher in biofilms as compared to those in activated sludge flocs, indicating biofilm was a more favorable habitat for nitrifiers. In addition, extracellular polymeric substance (EPS) in biofilm and suspended flocs changed differently in response to the organic loading changes, which might also be related to the microbial population changes.

1. Introduction

Nitrogen (N) and phosphorous (P) are essential elements for microorganism growth. High concentrations of N and P in a water body will lead to eutrophication, posing threats to aquatic life (U.S.EPA, 2007). Also, a high free ammonia concentration in water is toxic to aquatic life. High ammonium concentration will lead to a high free ammonia concentration under high pH condition. Therefore, it is important to remove N and P from wastewater before releasing it into natural water reservoirs, thus nitrification efficiency is critical to the success of secondary biological wastewater treatment.

Integrated fixed-film activated sludge (IFAS) technology was developed to improve the efficiency of biological nutrient removal (BNR) in wastewater treatment plants worldwide. In the IFAS configuration, biocarriers are integrated into conventional activated sludge reactors to provide surface area for the bacterial attachment and growth. Biocarriers can also provide a longer solids retention time (SRT), as compared to the conventional activated sludge process, to facilitate slow growing nitrifying growth and promote nitrification (Kim et al., 2011; Onnis-Hayden et al., 2007). The potential effects of IFAS reactors on nutrient removal enhancement, especially nitrogen removal, had been extensively studied (Copithron et al., 2006; Johnson et al., 2005; Johnson and McQuarrie, 2002; Onnis-Hayden et al., 2011). Two main types of IFAS media that are commercially available: fixed and mobile media. The mobile media was used for this study as it facilitate high oxygen and nutrient transfer in reactors (Ye et al., 2009). Compared with continuous flow reactors, the operation of sequencing batch reactor (SBR) is more flexible. For full-scale application of SBR, parameters (like pH, concentrations of substrates, and dissolved oxygen) could be monitored by online analyzers. Biodegradation processes can be easily controlled by adjusting the timers or programed controllers. No clarifier is needed for SBR since the sedimentation will take place in the reactor itself (U.S.EPA, 1999). In this study, A combined IFAS and SBR technology was applied. The combination could improve the nitrification efficiency of conventional SBR and reduced the capital cost of upgrading existing

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reaction tanks.

Previous researchers suggested that the operational conditions of wastewater treatment reactors, including temperature, pH, and carbon/nitrogen (C/N) ratio, would affect the composition of the bacterial community (Bassin et al., 2012; Xia et al., 2008). The C/N ratio significantly affects the growth of and competition between heterotrophic and autotrophic nitrifying bacteria. The chemical oxygen demand (COD) represents the carbon source and energy source for heterotrophic bacteria. Nitrification is composed of two steps. Ammonium is used by autotrophic ammonia oxidizing bacteria (AOB) to produce nitrite in the first step of nitrification. In the second step of nitrification, nitrite is oxidized to nitrate by nitrite oxidizing bacteria (NOB). When heterotrophic bacteria are present in a reactor, a high COD will promote the growth of heterotrophs and increase the competition between heterotrophs and autotrophic nitrifying bacteria for space and oxygen, affecting the nitrification efficiency negatively (Bassin et al., 2012; Kim et al., 2011; Onnis-Hayden et al., 2011). However, the acclimation of heterotrophic bacteria on the support media in a reactor can also facilitate the biofilm development of nitrifying bacteria. Extracellular polymeric substances (EPS, also known as “slime”) are high molecular weight substances secreted by bacteria that enhance the attachment of bacteria to the support media. Heterotrophic bacteria produce high quantities of EPS, whereas nitrifiers are slow growing bacteria that usually lack EPS (Furumai and Rittmann, 1994; Tsuneda et al., 2001). Bassin et al. (2012) found that the EPS secreted by a large amount of heterotrophic bacteria enhanced the biofilm accumulation of nitrifying bacteria during a reactor startup stage.

To promote the efficiency of biological wastewater treatment using IFAS technology, it is important to understand how the bacterial community changes in response to different operational conditions (Xia et al., 2008). Attached biofilm and suspended flocs are two major bacterial aggregates in an IFAS reactor. However, few studies have focused on bacterial community changes in both attached and suspended phases of a bioreactor and the related EPS production in response to different organic loadings. This study investigates the bacterial community compositional differences in biofilm and suspended activated sludge under different organic loadings in a bench scale IFAS reactor. The nitrifying activities of bacteria in both phases were analyzed with emphasis on comparing the impact of the organic loading on both suspended and attached phases that including (1) bacterial community dynamics—i.e., the distribution of AOB, NOB, and heterotrophic bacteria; (2) EPS production dynamics; and (3) nitrification and COD removal efficiency. The design of this experiment provides guidelines for a full-scale application of IFAS. The comparison between suspended flocs and attached biofilm enables a better understanding of the stability of these two aggregates in response to operational condition changes.

2. Method and material

2.1. Reactor operation

One reactor (800 mL) was operated in sequencing batch mode with decreasing COD concentration in influent over time from September to December 2014. Synthetic wastewater (see Appendix in supplementary material for the recipe) was used as reactor influent (Zeng et al., 2003). Ammonium-nitrogen concentration in the influent was kept at around 50 mg/L. As the COD concentration decreased and the ammonium nitrogen did not change, the C/N ratios were 10:1 in Phase 1, 5:1 in Phase 2, and 3:1 in Phase 3. Sodium acetate was used as carbon source to adjust the C/N ratio.

Each cycle of the sequencing batch reactor (SBR) was 4 h and 48 min, which included 5 min of feeding, 3 h and 50 min of reaction, 40 min of settling, and 13 min of fluid withdrawal (Fig. 1). The volume exchange ratio was about 37.5%. The hydraulic retention time was 12 h and 48 min. The reactor was filled with polyethylene carriers 50% by volume (Bioflow 9) (RVT Process Equipment GmbH Company). The carriers were cylindrical with 9 mm diameter, 7 mm height, and a specific area of 800 m^2/m^3. The carrier density was 145 kg/m^3, which is lighter than water. The low density enabled carriers to suspend and circulate in the reactor. Before Phase 1, the carriers were cultivated for one month to develop the biofilm. Air was pumped and diffused to the bottom of the reactor to provide oxygen to the reactor and drive carriers to flow throughout the reactor. The dissolved oxygen (DO) level in the reactor was maintained at 7–8 mg/L.

Reactor influent and effluent were sampled every day (or every two days when the bioreactor stabilized). The system was considered stable if the effluent characteristics and mixed liquid volatile suspended solids (MLVSS) were unchanged for one week. All samples were filtered (0.45 μm pore) before being analyzed for COD, NH_4^+-N, NO_3^--N, NO_2^--N concentrations with reagent kits (methods 8000, 10205, 10206, 10207, respectively, Hach Company, Loveland, Colorado) within 2 h of collection.

2.2. Biodegradation kinetics

To better understand nitrification activities, SBR cycle tests were conducted at the end of each phase. Mixed liquid samples were collected at the start of the reaction (0 min) and 5, 10, 15, 25, 35, 50, 65, 95, 125, 185 min, and the end of the reaction (230 min). The pH of the samples was measured at the sample collection points. Samples were centrifuged at 3000 g for 1 min, filtered (0.45 μm pore), and analyzed for COD and NH_4^+-N. The NH_4^+-N uptake rate was determined as the linear regression of NH_4^+-N concentration over time divided by the VSS in the system (Bassin et al., 2012).

2.3. Biomass in activated sludge and biofilm

To determine growth in the suspended phase, mixed liquor suspended solids (MLSS) and MLVSS were measured at the end of each C/N ratio conditions using *Standard Methods* 2540B (APHA, 2011). The biomass attached to carriers was measured using standard methods with modifications. Briefly, five carriers were gently rinsed with demineralized water to remove loosely attached biomass, then soaked with 25 mL ultrapure water in polyethylene centrifuge tubes. Carriers were sonicated for 10 min, then vortexed at maximum speed for one minute to detach biofilm from the carriers. The detached biofilm was measured using *Standard Methods* 2540B (APHA, 2011). The biofilm TSS and VSS were measured using *Standard Methods* 2540E (APHA, 2011).

2.4. Extracellular polymeric substance (EPS) analysis

EPS in the activated sludge was extracted using the formaldehyde–NaOH method with modifications (Liu and Fang, 2002). 10 mL sludge was collected in a 15 mL polyethylene centrifuge tube and 0.06 mL of 36.5% formaldehyde was added to the tube. The tube contents were mixed by inversion for several times and stored at 4 °C for one hour. After one hour, the solution was mixed with 4 mL 1 N NaOH and stored at 4 °C for three hours. The tubes were then centrifuged at 6000 g for 20 min and the supernatant was collected, filtered (0.22 μm pore), and dialysed for 24 h. To extract biofilm EPS, carriers were sonicated in ultrapure water for 10 min, then the liquid mixture was treated with the method described above. Polysaccharides in the EPS were analyzed by methods developed by DuBois et al. (1956). EPS protein content was determined using a