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Simultaneous nitrogen and phosphorus removal using denitrifying phosphate-accumulating organisms in a sequencing batch reactor

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Abstract

In this study, an anaerobic/aerobic/anoxic process (referred to as an AOA process) using a sequencing batch reactor (SBR) was proposed for simultaneous phosphorus and nitrogen removal from wastewater. The AOA process was stably operated over more than one year when a certain amount of carbon substrate (40 mg-C/L in a reactor) was supplemented to inhibit aerobic phosphate uptake. The average nitrogen and phosphorus removal efficiencies were 83% and 92%, respectively. It was demonstrated that phosphate-accumulating organisms (PAOs) capable of utilizing nitrite as an electron acceptor, the so-called denitrifying phosphate-accumulating organisms (DNPAOs), could exist in the AOA process. Moreover, the ratio of anoxic phosphate uptake rate (PUR) to aerobic PUR (anoxic/aerobic PUR ratio), which indicates the fraction of DNPAOs in total PAOs, was experimentally evaluated. The results indicate that the AOA process has a much larger anoxic/aerobic PUR ratio than the conventional A_2O (anaerobic/anoxic/aerobic) and AO (anaerobic/aerobic) processes. In conclusion, the AOA process allows DNPAOs to take an active part in simultaneous nitrogen and phosphorus removal in an SBR when a suitable amount of carbon substrate is supplied at the start of aerobic conditions.

Keywords: Enhanced biological phosphorus removal (EBPR); Anaerobic/aerobic/anoxic (AOA) process; Denitrifying phosphate-accumulating organisms (DNPAOs); Sequencing batch reactor (SBR)

1. Introduction

A sequencing batch reactor (SBR), the so-called fill and draw reactor, separates operating conditions timely in a single reactor. Different from continuous-flow-activated sludge systems, various biological reactions are switchable in the same reactor. In the SBR, clarifiers and flow equalization tanks are unnecessary, and thus, costs of facilities and operation management are much lower than those of continuous flow activated sludge systems. Moreover, the SBR has benefits in that it is easy to change operating conditions, such as cycle times and flow rates [1]. Therefore, the SBR is regarded as effective, especially for small wastewater treatment plants. However, the SBR has not been applied to the simultaneous nitrogen and phosphorus removal process, such as conventional A_2O (anaerobic/anoxic/aerobic) process, which uses phosphate-accumulating organisms (PAOs), nitrifier and denitrifier, because the circulation of liquid that contains nitrate and nitrite is necessary in this process. If denitrification can be attained without any carbon substrate under anoxic condition, the circulation of liquid from aerobic to anoxic phases is unnecessary and thus nitrogen and phosphorus can be removed in a single SBR. To realize this process, we propose to use denitrifying phosphate-accumulating organisms (DNPAOs) in the SBR.

DNPAOs have metabolic characteristics similar to those of PAOs, based on the metabolic transformations responsible for enhanced biological phosphorus removal (EBPR) [2–6]. In a similar manner as PAOs, DNPAOs take up external carbon substrates and store as polyhydroxyalkanoates (PHAs) in the cell under anaerobic conditions. However, they can utilize nitrite or nitrate instead of oxygen as an electron acceptor to remove phosphorus without any extracellar carbon substrates under anoxic conditions. To use these special characteristics of DNPAOs to advantage in simultaneous nitrogen

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and phosphorus removal in the SBR, we propose an anaerobic/aerobic/anoxic (AOA) process. In this process, anoxic phosphate uptake and denitrification can be simultaneously performed by DNPAOs under anoxic conditions without any carbon substrate, because nitrification during aerobic conditions provides an electron acceptor for anoxic phosphate uptake.

Most DNPAOs are able to utilize oxygen as well as nitrate [7]. Thus, even if DNPAOs are dominant in the AOA process, aeration for nitrification allows phosphate uptake using O_2, which results in limited phosphate uptake using nitrate under subsequent anoxic conditions. In consideration of the metabolic characteristics of DNPAOs [8], the external supply of a certain amount of carbon substrate at the start of aerobic conditions is effective in inhibiting aerobic phosphate uptake. Therefore, it is important to evaluate the amount of supplemented carbon substrate at the start of aerobic conditions and to understand the optimal conditions for stable operation in the AOA process.

This study precisely evaluates nitrogen and phosphorus removal in the AOA process using an SBR and the fraction of DNPAOs in the sludge, both of which depend on the amount of carbon substrate added at the start of aerobic conditions.

2. Materials and methods

2.1. Sequencing batch reactor operation

The anaerobic/aerobic/anoxic (AOA) process was operated using an SBR with a 2 L working volume. The SBR was operated at a cycle of 8 h, consisting of a 15 min filling phase, a 90 min anaerobic phase, a 90 min aerobic phase, a 195 min anoxic phase, a 65 min settling phase and a 25 min withdrawing phase, as shown in Fig. 1. In consideration of the metabolic characteristics of DNPAOs [8], a certain amount of carbon substrate (sodium acetate, 20–40 mg-C/L) was supplemented at the start of aerobic conditions to temporally inhibit aerobic phosphorus uptake. Both influent and effluent volumes were 1 L and thus 16 h of hydraulic retention time (HRT) was maintained. At the end of anoxic conditions, excess sludge was withdrawn and sludge retention time (SRT) was maintained at 15–25 days. The activated sludge obtained from an aerobic basin of a local municipal wastewater treatment plant where nutrients were efficiently removed was used as the inoculating sludge for the SBR operation.

Synthetic wastewater of the following composition was used as the feeding solution: 384.4 mg of CH_3COONa (300 mg/L as COD basis), 49.4 mg of KH_2PO_4 (11 mg/L as $PO_4{}^{3-}$–P basis), 140 mg $(NH_4)_2SO_4$ (30 mg/L as $NH_4{}^{-}$–N basis), 14 mg of $CaCl_2{\cdot}2H_2O$, 90 mg of $MgSO_4{\cdot}7H_2O$ and 0.3 mL of nutrient solution per liter. The nutrient solution consisted of the following compounds per liter: 1.5 g of $FeCl_3{\cdot}6H_2O$, 0.15 g of H_3BO_3, 0.03 g of $CuSO_4{\cdot}5H_2O$, 0.18 g of KI, 0.12g of $MnCl_2{\cdot}4H_2O$, 0.06 g of $Na_2MoO_4{\cdot}2H_2O$, 0.12 g of $ZnSO_4{\cdot}7H_2O$, 0.15 g of $CoCl_2{\cdot}6H_2O$, and 10 g of EDTA [9].

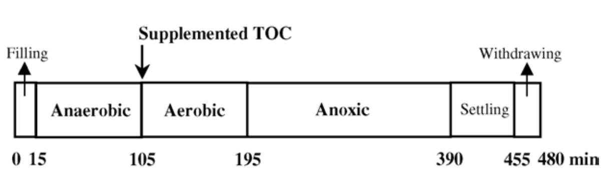

Fig. 1. SBR operating cycle in the AOA process.

2.2. Determination of phosphate uptake rate of the sludge

The activated sludge taken at the end of anaerobic conditions was divided into two batch reactors. One was exposed to aerobic conditions and the other was exposed to anoxic conditions (use of nitrogen gas and addition of 15 mg-N/L $NaNO_3$ in the reactor). Phosphate uptake rate (PUR) was estimated from the slope of the line describing the linear decrease in phosphate concentration. The ratio of anoxic PUR to aerobic PUR (anoxic/aerobic PUR ratio) was used as an index reflecting the fraction of DNPAOs [10,11].

2.3. Analytical methods

The analyses of phosphorus and mixed liquor suspended solid (MLSS) were performed according to standard methods [12]. Nitrate and nitrite concentrations were determined using a high-performance liquid chromatograph (HPLC) equipped with an anionic column (IC-Anion-PW, Tosoh, Japan) and an ultraviolet detector (UV-8011, Tosoh, Japan). Total organic carbon (TOC) was measured using an automatic TOC analyzer (TOC-5000, Shimadzu, Japan). Ammonia was measured with an ion chromatograph (DX-120, Dionex, USA) equipped with an anion column (CS10, Dionex, USA).

3. Results and discussion

3.1. Phosphorus and nitrogen removal in AOA process

The AOA process was operated for about one year. Fig. 2 shows the time course of phosphorus, nitrogen and MLSS concentrations. The effect of the amount of carbon substrate supplied at the start of aerobic conditions on phosphorus and nitrogen removal efficiency was examined.

When 20–30 mg-C/L TOC was supplied at the start of aerobic conditions, aerobic phosphate uptake was not inhibited. When the supplied TOC was increased to 45 mg-C/L on day 32, aerobic phosphate uptake was sufficiently inhibited, but nitrification was also inhibited. Consequently, there was not an electron acceptor for anoxic phosphate uptake. Therefore, phosphate was released again, resulting in the decrease in phosphorus removal efficiency.

On day 34, the supplied TOC was decreased to 40 mg-C/L. As a result, aerobic phosphate uptake was inhibited and nitri-