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ORIGINAL PAPER

# Aerobic granulation and nitrogen removal with the effluent of internal circulation reactor in start-up of a pilot-scale sequencing batch reactor

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**Abstract** Aerobic granular sludge was successfully cultivated with the effluent of internal circulation (IC) reactor in a pilot-scale sequencing batch reactor (SBR) using activated sludge as seeding sludge. N removal was investigated in the start-up of aerobic granulation process. Initially, the phenomenon of partial nitrification was observed and nitrite accumulation rates ($NO_2^-$-N/$NO_x^-$-N) were between 84.6 and 99.1 %. It was potentially caused by ammonium oxidizing bacteria (AOB) in the seeding activated sludge, high external environmental temperature (~32 °C) and free ammonia (FA) concentration. After 50 days' running, the aerobic granules-based bioreactor demonstrated perfect performance in simultaneous removal of organic matter and ammonia nitrogen, and average removal efficiencies were maintained above 93 and 96 %, respectively. The maximum nitrogen removal efficiency of 83.1 % was achieved after the formation of aerobic granules. The average diameter of mature aerobic granular sludge mostly ranged from 0.5 to 1.0 mm. Furthermore, one typical cyclic test indicated that pH and DO profiles could be used as effective parameters for biological reactions occurring in the aerobic/anoxic process. The obtained results could provide further information on the cultivation of aerobic granular sludge with practical wastewater, especially with regard to nitrogen-rich industrial wastewater.

**Keywords** Aerobic granular sludge · Sequencing batch reactor (SBR) · Pilot scale · Nitrogen removal

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## Introduction

Aerobic granular sludge was extensively reported as a microbial self-immobilization process with an approximately spherical external appearance [1]. Due to its unique granule attributes, aerobic granular sludge has been successfully developed for the treatment of a wide variety of practical wastewater, including high strength wastewater containing organics, nitrogen, phosphorus and heavy metals [2–5]. To date, aerobic granule-based technology has been considered to be an environment-friendly and cost-effective method for a full-scale biological application in the future [6].

Compared to continuous microbial culture, aerobic granular sludge has been widely cultivated in sequencing batch reactors (SBRs) which are helpful to achieve self-immobilization process attributed to their cycle configurations for settling fast and frequent repetition of distinct feast and famine conditions. So far, most of the influencing factors including organic loading rates [7], hydrodynamic shear force [8], settling time [9] and operational period [10] were investigated with synthetic or practical wastewater under well-controlled laboratory-scale conditions. However, little information could be obtained from aerobic granular sludge technology under pilot-scale conditions

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with practical industrial wastewater [11]. There is still little information about scaling up experience and analysis of the aerobic granulation process. Thereby, it is desirable to explore a feasible of aerobic granulation for treatment of practical industrial wastewater under pilot-scale operational conditions.

The aim of the present study was to investigate the feasibility of cultivating aerobic granules in a pilot-scale SBR fed with the effluent of internal circulation (IC) reactor, and to provide support to future studies on the formation and application of aerobic granular sludge. To our knowledge, there are very limited studies using effluent of IC reactor as a substrate to cultivate aerobic granular sludge. Due to the effluent of IC reactor is one of the nitrogen-rich industrial wastewater resulting from the poor capability of anaerobic granular sludge in the complete removal of nutrients, N removal was studied in the start-up of aerobic granulation process. The obtained results could help to promote engineering application in relation to nitrogen-rich wastewater.

## Materials and methods

### Experimental set-up

The investigation was carried out using a pilot-scale SBR in a soy protein production company wastewater treatment plant (WWTP), as shown in Fig. 1. The pilot-scale SBR had a working volume of 1470 L (0.6 m in diameter and 5.2 m in height). The reactor was operated at a volumetric exchange ratio of 50 % with a cycle of 8 h, resulting in the hydraulic retention time (HRT) of 16 h. The aeration rates were kept between 10 and 12 $m^3$/h through an air compressor (W-0.36/8, Shanghai Feng Bao Manufacturing Co. Ltd., China). Influent wastewater was introduced at the bottom of the reactor by a water pump from a storage tank (1000 L). The reactor was operated under different DO concentrations in the range of 0.2–7.0 mg/L. A time controller was installed to achieve the reactor automatic operation.

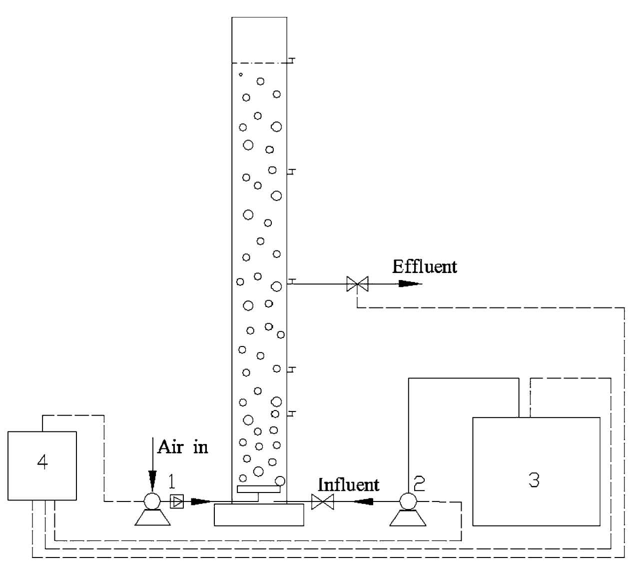


**Fig. 1** A schematic diagram of pilot-scale SBR in a soy protein production company WWTP in this study. *1* Air pump, *2* influent pump, *3* water storage tank, *4* time controller

### Wastewater composition

There were three types of water (soy protein wastewater, effluent of IC reactor and tap water) mixed for the influent of the SBR. The effluent of IC reactor was the main wastewater in this study. The composition of the effluent of IC reactor mineral medium was as follows: COD of 600–1000 mg/L, $NH_4^+$-N of 250 ± 50 mg/L, pH of 6.6–7.2, alkalinity of 2000–2200 mg/L. The raw soy protein wastewater as an external carbon source was added for complete dentrification due to the insufficient source of carbon in the effluent of IC reactor. The composition of raw soy protein wastewater was as follows: COD of 12000–16000 mg/L, TN of 330 ± 15 mg/L, pH of 4.0–4.2. The raw soy protein wastewater was added to ensure the influent COD/N ratio approximately at 100:10. Different volume of tap water was used for the dilution of influent wastewater to avoid the initial loading is too high to the SBR. As a result, the composition of the influent wastewater was adjusted as follows: COD of 800–1800 mg/L, $NH_4^+$-N of 80–160 mg/L, pH of 6.8–7.2, alkalinity of 900–1300 mg/L. The ratio of alkalinity to $NH_4^+$-N was kept above 8.0 mg/mg to satisfy the growth requirement of nitrifying bacteria.

### Operating conditions

To achieve better effluent quality not only in organic matter but also nitrogen removal, the reactor was operated by alternating anaerobic and aerobic reaction in this study. The operation of SBR was consisted of 25 min for influent filling, 75 min for anaerobic process, 300 min for aeration and 15 min for effluent removal. The settling time was stepwise decreased from 40 to 20 min for washout of free-living and floc-forming microorganisms. The temperature of the reactor was maintained at outdoor temperature (25–35 °C).

### Seeding sludge

The reactor was directly inoculated with fresh activated sludge taken from a full-scale aeration unit for the treatment of soy protein wastewater in the WWTP. Initially, 490 L of seeding sludge was added into the pilot-scale reactor, giving the mixed liquor suspended solids (MLSS) of 2.20 g/L and sludge volume index (SVI) value of 125.6 mL/g, respectively.

### Analytical methods

The effluent samples of the reactor were analyzed for the concentrations of COD, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N and TN using the respective standard methods [12]. The pH values were measured by a pH meter (PHS-3B, Shanghai Precision and Scientific Instrument Co., Ltd., China). DO concentration was determined by a DO meter (JPB-607A, Shanghai Precision and Scientific Instrument Co., Ltd., China). The granules size was measured regularly using an image analysis system (Image-pro Express 5.1, Media Cybernetics). The granule morphology of sludge was observed by an optical microscope (IX51, Olympus Co., Ltd., Japan). The micro-structure and predominant bacterial morphologies of the granules were observed using a scanning electron microscopy (Guanta FEG 250, FEI Ltd.).

## Results and discussion

### COD removal

The pilot-scale SBR was operated for 50 days in total for start-up of aerobic granulation, and the influent COD concentrations were increased from 1015.96 to 1995.96 mg/L by adding raw soy protein wastewater. Influent and effluent COD concentrations as well as COD removal efficiency of the pilot-scale SBR are shown in Fig. 2. It can be seen from Fig. 2 that the practical industrial wastewater exhibited an unstable behavior with a wide fluctuation of influent organic matter. From days 1 to 20, the effluent

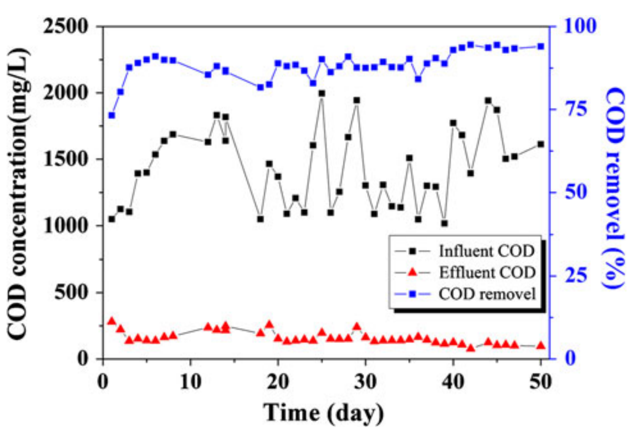


**Fig. 2** Influent and effluent COD concentrations as well as COD removal efficiency of the pilot-scale SBR

COD concentrations were stepwise decreased from 280.34 to 152.17 mg/L because the inoculated sludge gradually started to acclimate to the influent wastewater. Afterwards, the effluent COD concentrations were stable between 76.68 and 125.01 mg/L at a volumetric exchange ratio of 50 %, indicating that high activities of heterotrophic bacteria were achieved when the flocculent sludge developed into aerobic granular sludge. Kim et al. [7] showed that the optimum organic loading rate for aerobic granulation of the SBR was 2.5 COD kg/m$^3$ day. In the present case, aerobic granules were formed under an average organic loading rate of 2.2 COD kg/m$^3$ day, which was close to the above-mentioned report and therefore provided a further driving force for either aggregation or granulation. Previous studies have been well documented that aerobic granular sludge technology stabilized perfect organic matter removal efficiency for treatment of various organic wastewater. Ni et al. [13] reported that aerobic granulation of activated sludge in a pilot-scale SBR for the treatment of low-strength municipal wastewater. The average COD removal efficiency kept at 90 % during a long term bioprocess. Schwarzenbeck et al. [14] cultivated aerobic granular sludge in a SBR-treating dairy wastewater. The removal efficiency of organic matter reached to 90 % after complete granulation and separation of the biomass from the effluent during 20 weeks. Su and Yu [15] cultivated aerobic granules using soybean-processing wastewater and COD removal efficiency was above 95 % during the formation of aerobic granules. The result of this study indicated that the pilot-scale granular SBR also demonstrated a good performance in organic carbon removal using the effluent of IC anaerobic reactor.

### $NH_4^+$-N removal of the pilot SBR

Figure 3 shows the influent and effluent $NH_4^+$-N concentrations as well as $NH_4^+$-N removal efficiency of the pilot-scale SBR. There was an obvious valley of the influent $NH_4^+$-N concentration caused by the unstable effluent of IC wastewater from days 25 to 35, resulting in the sudden increase of influent COD/N ratios to higher levels. It was previously reported that heterotrophic bacteria as dominant bacteria in a nitrogen removal system under high COD/N ratios, which could create negative impacts on nitrifiers by sequestering nitrogen and consuming oxygen [16]. As a result, the $NH_4^+$-N removal efficiency decreased to 77.1 % under the influent COD/N ratio was 12.5 at day 27.

The $NH_4^+$-N removal efficiencies were improved between 94.0 and 98.3 % at the end of study. The stable $NH_4^+$-N removal capacity of the reactor indicated a good enrichment of nitrifiers, which could be contributed to the gradual formation and characterization of aerobic granular sludge. Previous research demonstrated that a good

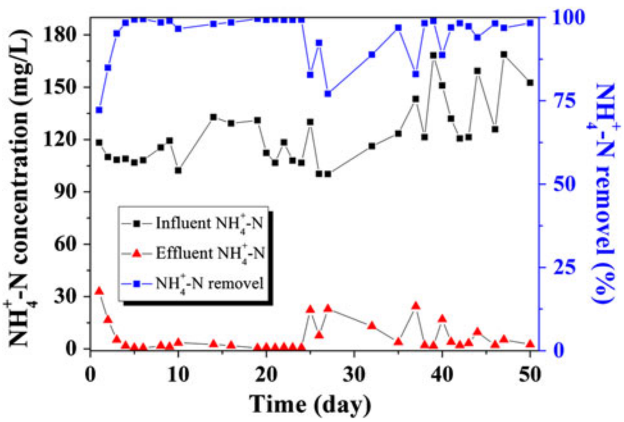


**Fig. 3** Influent and effluent $NH_4^+$-N concentrations as well as $NH_4^+$-N removal efficiency of the pilot-scale SBR

performance in nitrogen removal due to the composition of granular microbial communities, including heterotrophic, aerobic bacteria grown on the outside, ammonium-oxidizing bacteria in the middle and facultative and anaerobic bacteria in the core of the granules [17, 18].

## Effluent nitrite and nitrate of the pilot SBR

Since one aim of this work was to explore N removal process in the pilot-scale SBR, the effluent $NO_2^-$-N and $NO_3^-$-N concentrations were monitored in the aerobic granulation start-up phase (Fig. 4). Data present that the phenomenon of partial nitrification was observed in the initial of the study, as evidenced nitrite accumulation rates ($NO_2^-$-N/$NO_x^-$-N) were between 84.6 and 99.1 % in the effluents. It had been well documented that partial nitrification in Sharon process is a highly efficient and cost-effective way for removing high strength ammonia from wastewater [19].

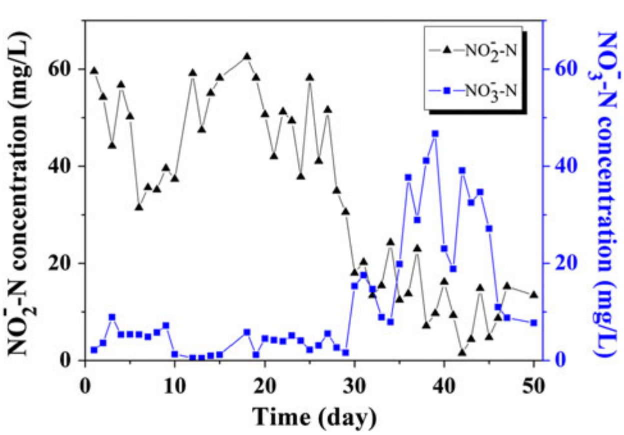


**Fig. 4** Effluent $NO_2^-$-N and $NO_3^-$-N concentrations of the pilot SBR

The result indicated that the nitrifying activity of the system was caused by ammonia-oxidizing bacteria, can be attributed to three possible mechanisms in this work. Firstly, it might have been possible that the seeding sludge contained a certain amount of ammonia-oxidizing bacteria, as evidenced by the fact of high nitrite accumulation rates in the initial stage (Fig. 4). Secondly, the temperature provided another external condition for the enrichment of ammonia-oxidizing bacteria. Previous studies showed that high temperature (30–35 °C) is an essential strategy to encourage a faster growth of ammonium oxidizing bacteria than nitrite oxidizing bacteria for microbial selection [20]. In the present case, the outdoor temperature was higher than 28 °C in summer, while the effluent of IC reactor temperature was 35–40 °C at the same time, resulting in the ultimate influent temperature was more than about 32 °C. Thirdly, the inhibitory effect of free ammonia (FA) on the metabolism of nitrobacter has been widely researched in recent years. The FA concentration in the reactor could be estimated by the expression proposed by the following equation [21]:

$$\mathrm{FA\,(mg/L)} = \frac{17}{14} \times \frac{[\mathrm{NH_4 \cdot N}] \times 10^{\mathrm{pH}}}{\exp[6334/(273+T)] + 10^{\mathrm{pH}}} \quad (1)$$

The influent FA concentration was therefore calculated approximately as 1.31–1.89 mg/L in the process of nitrite accumulation. Previous research showed that the FA inhibition threshold was 0.1–4.0 mg/L for ammonia-oxidizing bacteria [21, 22]. Therefore, it was likely the FA concentration inhibited the activity of nitrite-oxidizing bacteria even at very low levels. Nitrifying systems are affected by the influent COD/N ratios. A high growth rate for heterotrophs could create negative impacts on nitrifiers by sequestering nitrogen and consuming oxygen. The very slow growth rate of nitrite oxidizing bacteria magnified this negative impact. From days 30 to 50, the nitrogen conversion pattern was changed from partial nitrification to full nitrification. It could be confirmed that nitrate bacteria appeared and gradually became the dominant nitrifying bacteria in the system.

## Nitrogen removal of the pilot SBR

Figure 5 presents N removal efficiency of the reactor during the aerobic granulation process. In the initial stage of study, TN removal efficiencies were stepwise increased from 18.6 to 53.5 % due to the gradual acclimation of the inoculated sludge to the influent wastewater, even though the phenomenon of nitrite accumulation was existed in the reactor. After approximately 10 days' operation, $NH_4^+$-N concentrations in the effluent were undesirably increased from 1.9 to 22.4 mg/L (Fig. 3). Furthermore, there were between 41.9 and 68.3 mg/L of $NO_x^-$-N concentrations

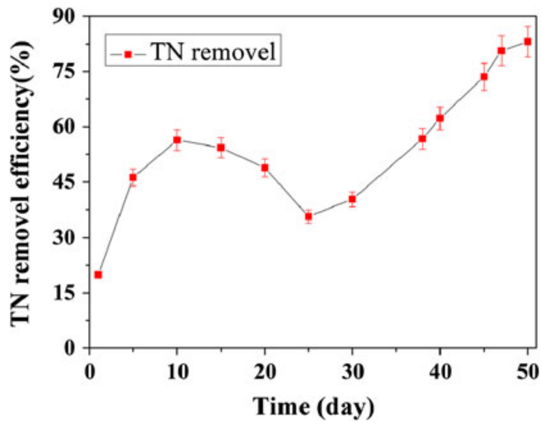


**Fig. 5** Nitrogen removal efficiency of the reactor during the aerobic granulation process

remained in the effluent which implied that the denitrification process was not accomplished completely (Fig. 4). As a result, TN removal efficiencies were not ideal in this stage and decreased to 36.3 % at day 25. The instability of reactor was similarly observed by De Kreuk et al. [23], which could be attributed to the biomass washout caused by the fluctuation of influent wastewater. With stepwise formation of granules, TN removal efficiencies were improved to between 73.5 and 83.1 %. This TN removal efficiency was comparable to reactors fed with dairy effluent [14, 24] and better than other types of substrates reported by prepared sanitary wastewater [25] and domestic wastewater [26] operated with long term bioprocess stability. Schwarzenbeck et al. [14] reported that a stable N removal efficiency was achieved after the last period of the experiment (weeks 15-20). Similar situation was also reported by Arrojo et al. [24] using an industrial wastewater coming from a dairy analysis laboratory as influent. Nitrogen removal efficiency was stable at 70 % during more than 300 days operation. The results of this study showed that the granule reactor inoculated with activated sludge was ideal for biological nitrogen removal in start-up of SBR treating the effluent of IC reactor.

## Microbial characteristics of granules

The seeding sludge was typical activated sludge with a fluffy, irregular, loose morphology with brown color (Fig. 6a). At day 15, it could be seen the initial formation of small aerobic granular sludge (Fig. 6b). At day 30, advanced microorganisms could be detected around aerobic granular sludge (Fig. 6c). Afterwards, the average diameter of mature aerobic granular sludge mostly ranged from 0.4 to 1.0 mm (Fig. 6d). Rough surface could be obtained from Fig. 6e by SEM. Figure 6f indicated that cocci were the dominant species at the surface of the aerobic granular sludge. The results revealed clearly that it is possible to cultivate aerobic granular sludge in SBR fed with the effluent of IC reactor.

## Analysis of COD and nitrogen removal in one operating cycle

A typical cyclic test was designed to further study the biological removal of COD and $NH_4^+$-N in start-up of the SBR system. The profiles of COD, $NH_4^+$-N, pH and DO values in one operating cycle on day 48 were shown in Fig. 7. It was found in Fig. 7a that COD concentration decreased immediately in the first aerated 120 min and relatively slowly within the following 180 min. The rest 180 min of the aerobic phase were the existence of starvation time, which is an important factor on aerobic granulation process for granule stability emphasized and developed by Liu et al. [27]. As for $NH_4^+$-N, it started to decline after the COD depletion due to the process of nitrification. At the end of aerobic phase, the average COD and $NH_4^+$-N removal efficiencies were 93.8 and 94.4 %, respectively. The result indicated that the aerobic granules-based bioreactor demonstrated good performance in simultaneous removal of organic carbon and nitrogen after successfully start-up of the reactor.

The parameters of pH and DO can be correlated to the operational cyclic status in reference to the nutrient removal in biotreatment [28]. Figure 7b shows the track analysis data that the corresponding profiles of pH and DO for the same cycle as Fig. 6a. At aeration process, the pH values immediately decreased from 7.7 to 7.2 for nitrification and eventually reached a valley after 3 h of aeration. This valley in the pH plot indicated that the nitrification reached the end point, when 94 % of $NH_4^+$-N was removed from the system. After complete nitrification, the pH value immediately increased due to air stripping of carbon dioxide [29]. In this study, DO concentration of the mixed liquor stepwise increased from 0.2 to 6.9 mg/L with the degradation of COD and $NH_4^+$-N in aerobic phase. It was previously reported that DO concentration also provided a better indication when it was utilized to oxidize COD and ammonia in the system [30]. It can be seen from Fig. 7b that DO concentration reached a saturated value when ammonia concentration became essentially depleted and stopped degradation. Those results from this study indicated that the variation of pH and DO values could be an effective control of biological reactions occurring in an aerobic/anoxic process.

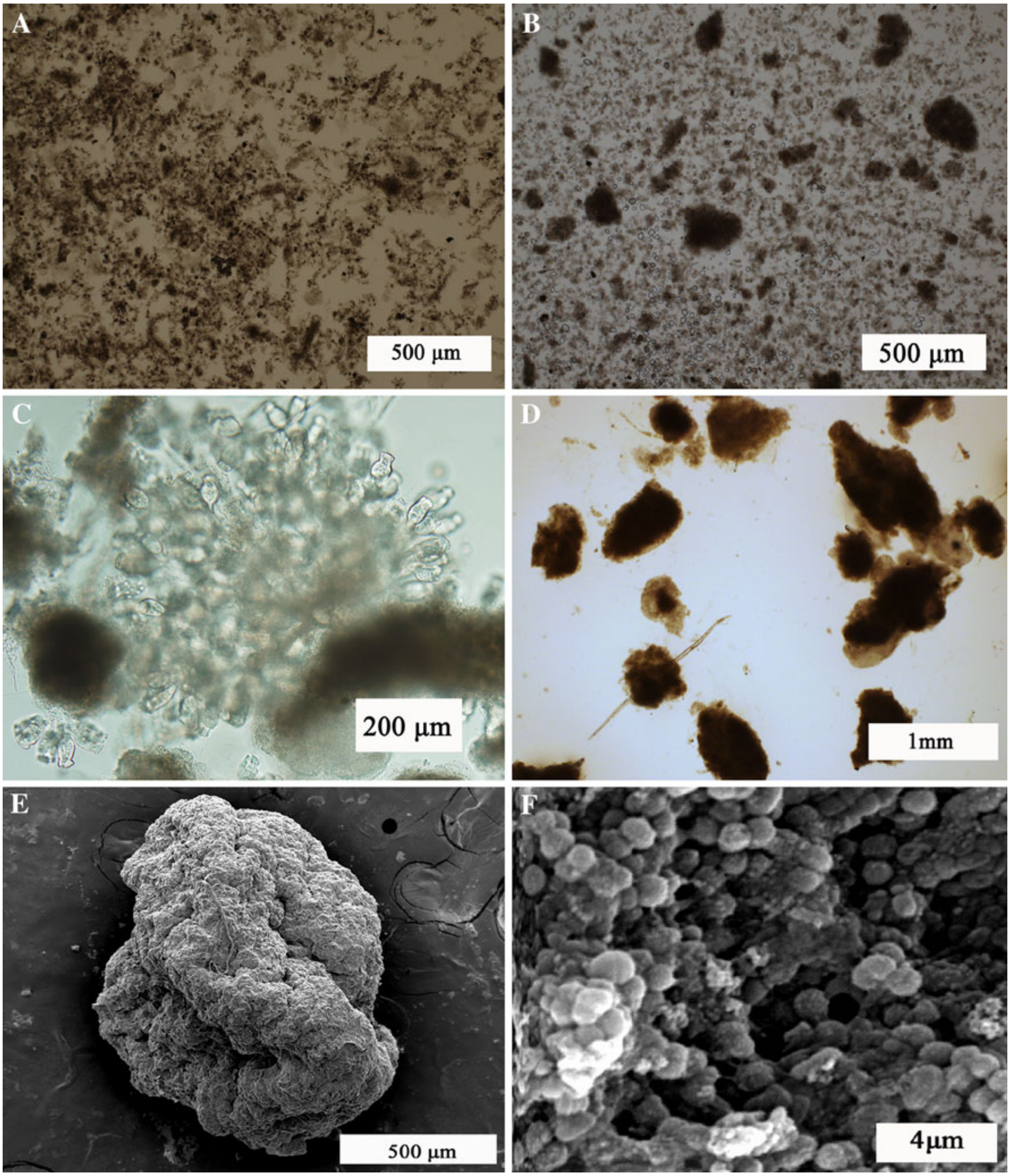


**Fig. 6** Morphology observation of aerobic granular sludge during the granulation process. **a** Seeding sludge, **b** sludge on day 15, **c** sludge on day 30, **d** sludge on day 40, **e** granule morphology of granular sludge on day 50, **f** micro-structure of the granular sludge on day 50

## Conclusions

Aerobic granular sludge was successfully cultivated with the effluent of IC reactor wastewater using activated sludge as seeding sludge. During the study, the effluent samples of COD, $NH_4^+$-N, $NO_2^-$-N, $NO_3^-$-N, and TN concentrations were analyzed. The aerobic granules-based bioreactor demonstrated good performance in simultaneous organic carbon and nitrogen removal, and average removal efficiencies were above 93 and 96 % after 50 days operation. The average diameter of mature aerobic granular sludge mostly ranged from 0.5 to 1.0 mm. One typical cyclic test implied that pH and DO profiles could be correlated to the operational cyclic status in reference to the nutrient removal in bio-treatment. This study could be helpful to the cultivation of aerobic granular sludge and facilitate its engineering in relation to nitrogen-rich wastewater.

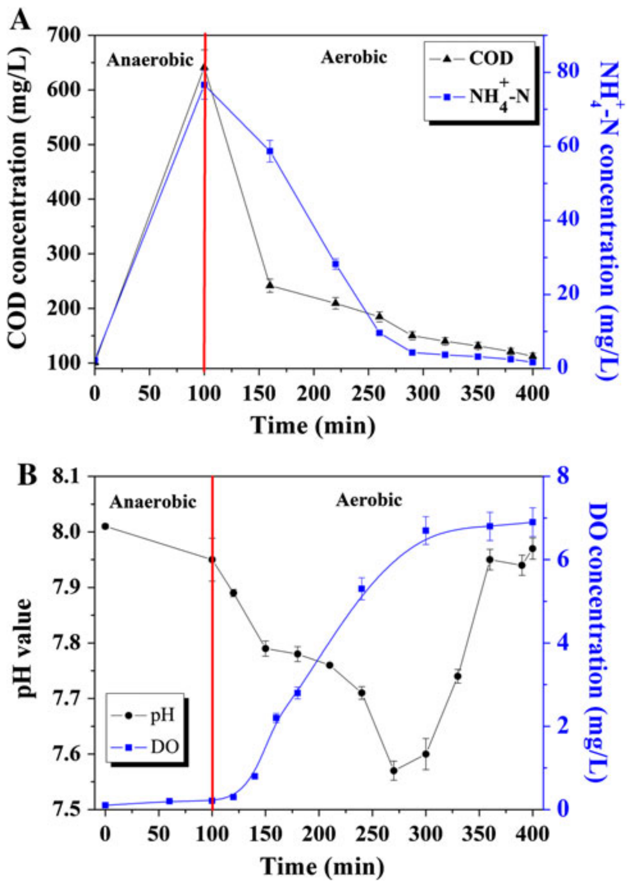


**Fig. 7** Measured profiles of COD, $NH_4^+$-N, pH and DO values in one typical cycle of the SBR in an anaerobic/aerobic phase on day 48. **a** COD and $NH_4^+$-N concentrations, **b** pH values and DO concentrations

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