

Article

Optimization of Wastewater Phosphorus Removal in Winter Temperatures Using an Anaerobic–Critical Aerobic Strategy in a Pilot-Scale Sequencing Batch Reactor

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Received: 8 December 2019; Accepted: 27 December 2019; Published: 29 December 2019



Abstract: Biological phosphorus removal using an anaerobic–aerobic sequencing batch reactor (SBR) in a low temperature can be difficult to remove, and aeration always accounts for nearly half of the total electricity costs at many wastewater treatment plants. In this study, a pilot-scale anaerobic–critical aerobic SBR (A–CA SBR) was developed for synthetic domestic wastewater. More importantly, the phase, whose concentration of diffused oxygen was controlled at 1.0–1.5 mg/L, was defined as a critical aerobic phase, which reduced expenses during the operation. To be specific, half of the ammonia was removed within 10 days and no NO_3^- -N was accumulated during the process. From the SEM and metagenome analysis, *Rhodocyclus*, *Zooglea*, *Dechloromonas*, and *Simplicispira* had the ability to remove phosphorus and NO_3^- -N simultaneously, which proved the existence of a potential double-layer sludge structure under an A–CA operational condition. All of the results disclose that the pilot-scale A–CA SBR is a reliable manipulation strategy for phosphorus removal under low temperatures, which can hopefully apply to practical wastewater remediation.

Keywords: pilot-scale anaerobic–critical aerobic SBR; winter temperature; phosphorus removal; metagenome analysis

1. Introduction

Discharging phosphorus into the river and sea without treatment will deteriorate the aquatic environment, and causes eutrophication, which aggravates the situation for the algae competing for oxygen and nutrition with aquatic organisms [1–3]. In order to avoid this situation happening, various wastewater treatment plants are used, like enhanced biological phosphorus removal (EBPR), which can effectively remove phosphorus and nitrogen [4]. Among EBPRs, a sequencing batch reactor (SBR) has been widely used because of its effective removal efficiency, good adaptation for influent water fluctuations, and space-saving characteristic [5]. There are some illustrated examples for different operation modes of SBRs. Using an anaerobic–aerobic SBR, Li et al. [2] realized a phosphorus removal efficiency of over 80% under three levels of temperature (5, 15, and 20 °C), and Panswad et al. [6] realized a soluble phosphorus of the effluent as low as 0.4 mg/L. for long anoxic–short aerobic SBR, and 4.3 mg/L of soluble phosphorus was left in high-strength wastewater of 4500 mg/L Chemical Oxygen Demand (COD) and 35 mg/L PO_4^{3-} -P under an ambient temperature [7]. Most of the operation modes mentioned above needed to supply enough oxygen for a good performance. However, supplying a long period of air could account for approximately 50% of the total energy consumption in typical wastewater treatment [8,9], which is not in accordance with the current mainstream concept of building an energy-saving society, and why a lower concentration of oxygen should be considered more in processes.

Among all of the influenced parameters, temperature is a vital factor in the phosphorus uptake process. Li et al. reported that 20 °C or possibly lower was suitable for P removal of microorganisms [2], and Liu and Li showed that temperatures below the optimum range had more significant effects on growth rate than those above the optimum range, because the activities of the microorganisms sharply decreased with the decreasing temperature. So, it was more difficult to realize phosphorus removal at a low temperature [10]. Additionally, Liu et al. [11] investigated the influence of temperature on denitrifying phosphorus removal (DPR), and found that more than 80% of phosphorus removal from DPR was obtained in the range of 20–30 °C, and the removal efficiencies went down when the temperature was below 10 °C. However, in the north of China, temperature changes drastically when the season varies. Especially in winter, wastewater is at low temperatures (11–13 °C) for a long period, which results in a negative impact on the performance of phosphorus removal [12]. Therefore, it is crucial to find a solution to treat wastewater in low temperatures properly.

It has been noted that most studies have been focused on lab-scale SBR [3,7,13,14], and pilot-scale SBR are reported on less. In this study, the phase whose concentration of diffused oxygen was controlled at 1.0–1.5 mg/L was defined as a critical aerobic phase. A pilot-scale anaerobic–critical aerobic (A–CA) SBR, which had a good adaption, high efficiency, and strong practicality [15], was designed to remove phosphorus in wastewater under cold temperatures. During the operation process, a sludge structure of the inner anoxic zone and outer-critical aerobic layer was found, and metagenome analysis proved the microorganism's variation. In addition, the optimization of the length of the critical aerobic phase was investigated in order further to obtain a minimum oxygen supply and to reduce the total running costs. Under this scenario, the operation strategy of A–CA SBR could effectively remove the total phosphorus, and partly remove the ammonia in wastewater in impoverished conditions, which implied a possibility for future application. More importantly, it is reasonable to make attempts for applying this operation mode of pilot-scale A–CA SBR into a larger scale wastewater treatment plant in the future.

2. Materials and Methods

2.1. Wastewater and Seed Sludge

The synthetic domestic wastewater with a COD of 200–350 mg/L, $\text{NH}_4^+\text{-N}$ of 15–25 mg/L, and $\text{PO}_4^{3-}\text{-P}$ of 8–15 mg/L, which stimulated the fluctuations of real wastewater in the whole experiment, contained 8 g of glucose; 2.5 g of NH_4Cl ; 0.6 g of KH_2PO_4 ; 0.4 g of MgSO_4 ; 0.4 g of CaCl_2 ; and 2.5 mL of a trace element solution, which contained (1 L) of 10 g ethylenediaminetetraacetic acid (EDTA), 0.9 g FeCl_3 , 0.15 g H_3BO_3 , 0.18 g KI , 0.03 g $\text{CuSO}_4\cdot 5\text{H}_2\text{O}$, 0.06 g $\text{MnCl}_2\cdot 4\text{H}_2\text{O}$, 0.12 g $\text{ZnSO}_4\cdot 7\text{H}_2\text{O}$, 0.15 g $\text{CoCl}_2\cdot 7\text{H}_2\text{O}$, and 0.06 g $\text{Na}_2\text{MoO}_4\cdot 2\text{H}_2\text{O}$.

The seed mixed liquor suspended solids (MLSS) was collected from the aerobic phase of a full-scale sewage treatment plant (A/A/O process) in the Nanjiao sewage treatment plant (Changchun, China). The concentration of MLSS for operation was almost 2000 mg/L.

2.2. Reactor Operation

A sequencing batch reactor (SBR), with an effective working volume of 20 L, height of 400 mm, length of 300 mm, and width of 240 mm, was configured in the present study (Figure 1), and the whole operation procedure was controlled by a computer (Version 6.2, Monitor and Control Generated System, Jiangsu, China). The SBR was operated under anaerobic–aerobic conditions for 41 days so as to acclimate the phosphorus accumulating organisms (PAOs), and operated under anaerobic–critical aerobic conditions for another 73 days to optimize the whole procedure, and between two stages, there were three days for adjustment. The temperature was controlled at 11–13 °C all the time. During stage I (41 days), each cycle consisted of the following five phases: filling (0.25 h), anaerobic (3 h), aerobic (4 h), settling (0.5 h), and withdrawing (0.25 h). Magnetic stirrers (4RK30RGN-C, MAILI, Taiwan, China, CO.) were operated during the anaerobic phase for a better mixing, and kept the concentration of dissolved oxygen under 0.5 mg/L at a rate of 100 r/min. For the aerobic phase, the air was maintained by diffusers (ACO-003, SenSen, Jiangsu,

China) in the bottom of SBR at a concentration of dissolved oxygen (DO) of 2.0 mg/L approximately, to guarantee the aerobic organism living properly. For another 73 days, each cycle consisted of the following five phases: filling (0.25 h), anaerobic (2 h), critical aerobic phase (length is always changing by the results), settling (0.5 h), and withdrawing (0.25 h). During the experiments, the duration of the critical aerobic phase changed from 2 h to 1 h, and eventually to 0.5 h, due to the treatment results, and the concentration of DO was always controlled at 1.0–1.5 mg/L.

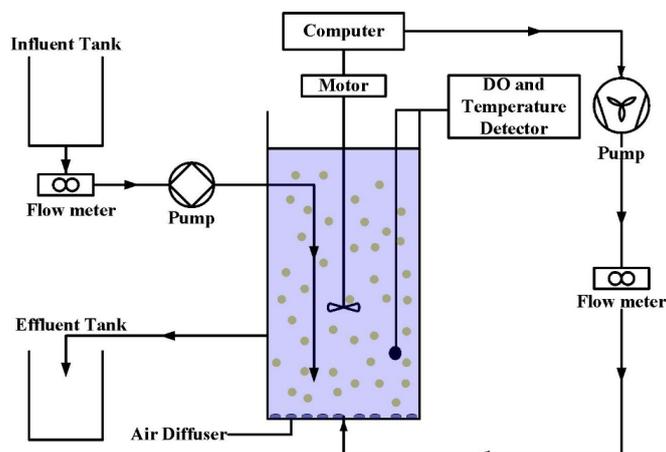


Figure 1. Configuration of a sequencing batch reactor (SBR) reactor. DO—dissolved oxygen.

The specific operation mode is shown in Table 1. Moreover, the pH was uncontrolled, except that the initial pH was adjusted to 7.0–7.5 at the beginning of each cycle. Sludge was not discharged during the whole manipulation of SBR. However, when necessary, manual sludge disposal was performed for the subsequent treatment, such as sludge dewatering. The relevant components were periodically measured to monitor the reactor performance during the whole operation.

Table 1. The operating mode of the pilot SBR at low temperature of synthetic wastewater. PAO—phosphorus accumulating organisms.

Stage	Operating Term (d)	Operation Mode	Remarks
I	1–43	Anaerobic (3 h)–aerobic (5 h)	Accumulation of PAOs.
II	45–64	Anaerobic (2 h)–critical aerobic (2 h)	Change the aerobic condition to critical aerobic condition; set the length of critical aerobic condition as 2 h.
III	65–94	Anaerobic (2 h)–critical aerobic (1 h)	Reduce the critical aerobic phase's time to 1 h.
IV	95–117	Anaerobic (2 h)–critical aerobic (0.5 h)	Reduce the critical aerobic phase's time to 0.5 h.

2.3. Physicochemical Analysis

The concentrations of COD, $\text{NH}_4^+\text{-N}$, $\text{PO}_4^{3-}\text{-P}$, $\text{NO}_3^-\text{-N}$, and $\text{NO}_2^-\text{-N}$ were analyzed according to the Standard Methods for the Examination of Water and Wastewater from the American Public Health Association before the removal of treated wastewater and after every different phase [16]. The mixed liquor suspended solids (MLSS) and settling velocity (SV) were also determined periodically using a gravimetric method. The pH was measured using a pH analyzer (PB-10, Sartorius, Gottingen, Germany), and the dissolved oxygen (DO) was monitored using a DO quick-analysis apparatus (SC200, Hitachi, Washington, DC, America). The temperature was detected by the sensor installed in the SBR reactor.

2.4. Morphological Observation

All of the selective sludge samples were observed by the scanning electron microscope (SEM) for the morphological observation of sludge so as to analyze the sludge structure. The specific procedure was the same as for Liu et al. [17].

2.5. Microbial Community Analysis

The metagenomics method manufactured by Sangon Biological Technology Company (Sangon Biological Engineering Co., Shanghai, China) was utilized to unravel the microbial community contained in the sludge, using the polymerase chain reaction followed by high-throughput sequencing (Hiseq 2500, Illumina, CA, USA). The specific procedure was the same as for Xiong et al. [18].

Sludge samples from different phases were taken at day 38 to represent the acclimation results of the PAOs in the stage I, while days 86 and 112 were selected to represent the condition of the sludge in this reactor, especially the microbial community change in the critical aerobic circumstance.

3. Results

3.1. Performances of SBR

The experiment had two different conditions according to the configuration changes, and the concentrations of COD, $\text{NH}_4^+ \text{-N}$, $\text{PO}_4^{3-} \text{-P}$, and $\text{NO}_3^- \text{-N}$, and the typical profiles for stage II, III, and IV are depicted in Figures 2 and 3, respectively.

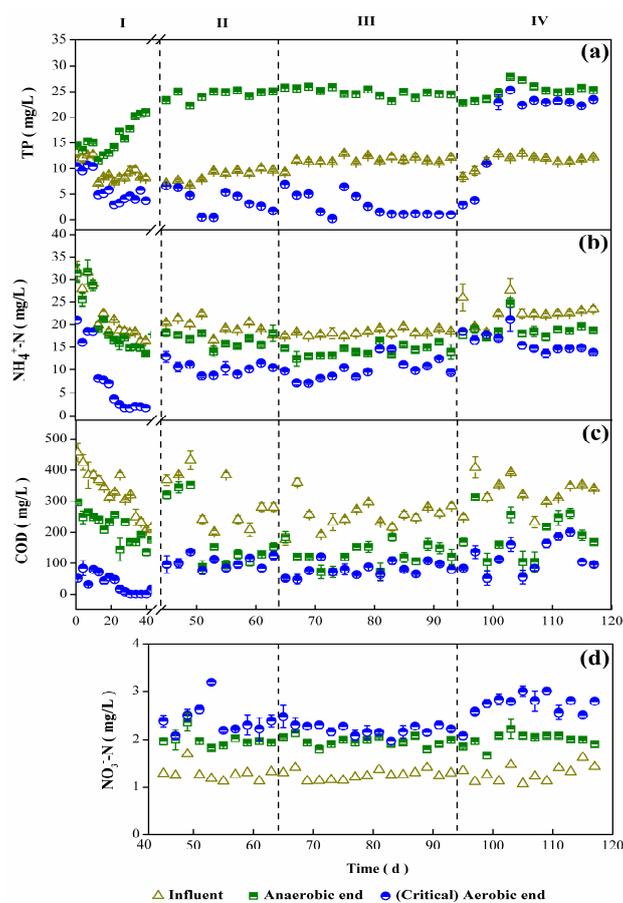


Figure 2. The overall performance of anaerobic–critical aerobic (A–CA) SBR: (a) total phosphorus (TP) removal performance during the four stages; (b) $\text{NH}_4^+ \text{-N}$ removal performance during the four stages; (c) COD removal performance during the four stages; (d) $\text{NO}_3^- \text{-N}$ removal performance during the four stages. Error bars indicate the standard deviations of the measurements.

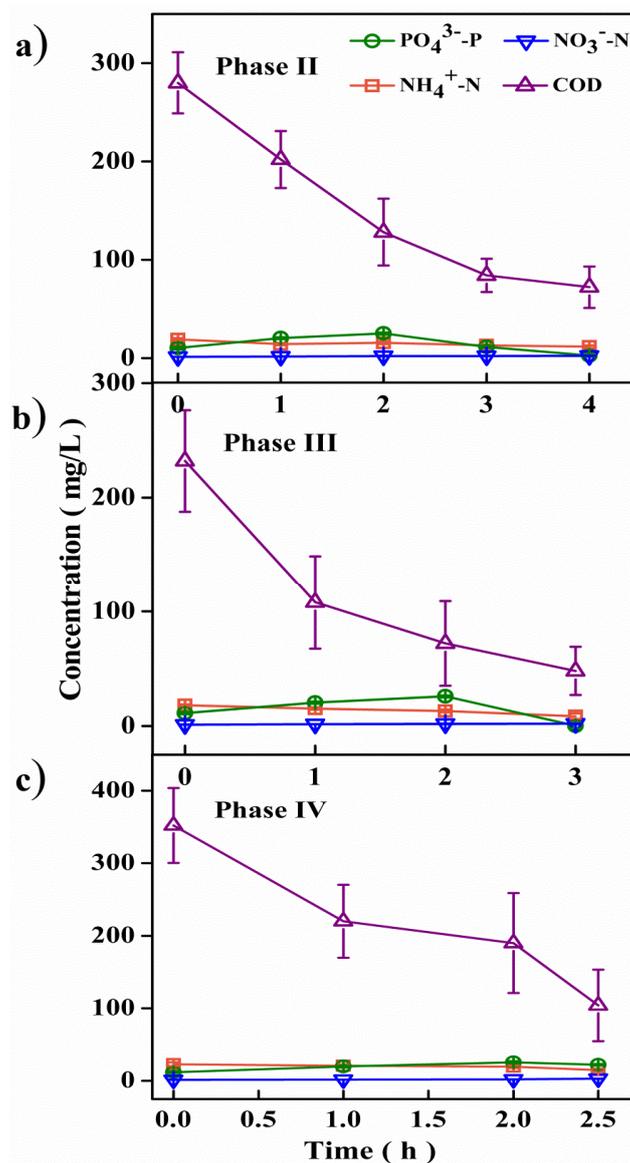


Figure 3. The concentration of various nutrients in typical cycle profiles for (a) phase II, (b) phase III, and (c) phase IV. Error bars indicate the standard deviations of the measurements.

3.1.1. Phosphorus Removals

As shown in Figure 2, the phosphorus release efficiency increased all the time, up until almost 200%, and the phosphorus removal efficiency was maintained at 85%, where the $\text{PO}_4^{3-}\text{-P}$ concentration in the effluent was below 4 mg/L at the end of stage I. These results indicated the acclimation of the PAOs was successful [19–21].

For stage II, the aerobic phase was altered into the critical aerobic phase, whose DO concentrations was 1.0–1.5 mg/L [22,23] so as to reduce energy consumption and, depending on the effluent, the length of the critical aerobic phase changed from 2 h to 1 h to 0.5 h. Then, a surprising phenomenon occurred, that the $\text{PO}_4^{3-}\text{-P}$ concentration of the effluent could be as low as below the detective line within 10 days, regardless of how many times we changed the length of the critical aerobic phase (the regular change pattern of phosphorus is in Figure 2a in blue dots). During this period, almost 100% of $\text{PO}_4^{3-}\text{-P}$ was removed. The PAOs in this system could be responsible, because PAOs are more suitable for a lower temperature (<20 °C) than GAOs, which deteriorated the phosphorus removal and competed with the nutrients with PAOs. However, 0.5 h was not suitable for this experiment (seen in stage IV in Figure 2), as the special phenomenon did not meet our expectations, while only less than 5% $\text{PO}_4^{3-}\text{-P}$

was eliminated. The typical cycle profile for the various stages exhibited in Figure 3 could validate that as well. It can be known that phase II and III achieved a good performance for the COD and NH_4^+-N , and removed almost all of the total $\text{PO}_4^{3-}-\text{P}$. However, when the length of the critical aerobic was cut to 0.5 h (phase IV in Figure 2), a worse result was elucidated, that the residual concentrations of COD, $\text{PO}_4^{3-}-\text{P}$, and NH_4^+-N were higher than before, proving that 0.5 h was not a sufficient time-length for PAOs to remove phosphorus. From a previous report, PAOs require a lag period for synthesizing enzymes for phosphorus uptake, especially when switched to a different oxygen condition (changed to critical aerobic phase in this study). Thus, under this condition, 1 h was more suitable for the PAOs to develop enzymes for phosphorus removal, rather than 0.5 h [24].

3.1.2. Nitrogen Removals

From Figures 2 and 3, the NH_4^+-N removal efficiencies in stage I for the anaerobic phase and aerobic phase were 20% and 50%, respectively, which were contributed to the demands of normal microorganism metabolism. Simultaneously, it was also revealed that stable nitrifying bacteria existed in the reactor, without obvious denitrifying bacterial. Thus, the concentrations of NO_3^--N and NO_2^--N were not measured during this stage.

Stage II to IV witnessed a relatively poor nitrification with a lower removal efficiency of NH_4^+-N than stage I. Additionally, the NO_3^--N in effluent was accumulated because of the effects of denitrification. The partly NH_4^+-N deceleration was insensitive to the length of the critical aerobic phase and the products, and NO_3^--N had a slightly higher concentration (stage II and III: <2.5 mg/L, stage IV: <3 mg/L) than the influent (1 mg/L). Furthermore, because of the low concentration (around detection limits), there was no observation of NO_2^--N during these three stages. Poor nitrification would be caused by a low operating DO concentration, because of the inhibition of the growth rate of the nitrifiers [25]. Additionally, the highest dissimilatory nitrate-to-ammonia process (DNRA) genes in glucose supported the denitrification system [26], which would dissimilate NO_3^--N to generate ammonia, was favorable for a high ammonia concentration in the effluent [27–29], and the dominated genus in the glucose denitrification supported system were *Thauera*, *Dechloromonas*, and *Zoogloea* [26], which was in accordance with the metagenome results (seen in discussions of chapter 3.2). However, the previous literature from Fan et al. [30] indicated that long SRTs reactors could improve the nitrification efficiencies even in a low DO concentration. Coincidentally, this A–CA SBR system did not generate much sludge during operation, which meant that the system had long SRTs. As a whole, a certain amount of NH_4^+-N was changed into NO_3^--N , because of the nitrification, causing an increasing concentration of NO_3^--N (Figure 2). Simultaneously, compared with stage II, part of NO_3^--N was clearly removed, which could be denoted in stage III. Generally speaking, denitrifying phosphate accumulating organisms (DPAOs) could realize the synchronous removal of NO_3^--N and $\text{PO}_4^{3-}-\text{P}$ [31] by using NO_3^--N for electron donation instead of O_2 for excess $\text{PO}_4^{3-}-\text{P}$ absorption in an anoxic environment [32–34]. Therefore, from this perspective, the existence of DPAOs in the system was demonstrated. In addition, the dominant genus of *Dechloromonas* and *Zoogloea* in the subsequent analysis also validated it properly (seen in two critical aerobic phases of Figure 5).

Conventionally, NH_4^+-N is achieved by the nitrification of ammonia to nitrate or nitrite under aerobic conditions, and NO_3^--N and NO_2^--N were removed by denitrification under anoxic conditions [35]. In our results, the DO concentration of 1.0–1.5 mg/L in our critical aerobic phase did not exert much of an impact on the NO_3^--N removal. It could be noted that the activated sludge flocs were larger, irregular, and porous under low DO conditions, which could contain an anoxic area inside the flocs, as seen in Fan et al. [30]. Inspired by this result, a hypothesis formed that the sludge might consist of two different conditions in order to meet the various oxygen demands of denitrification and phosphorus denitrifying removal, associating with various nutrients' capture in the same process. An SEM and metagenome analysis were utilized to prove this, which can be seen in Figures 4 and 5. The relevant discussions are shown in Chapter 3.2.

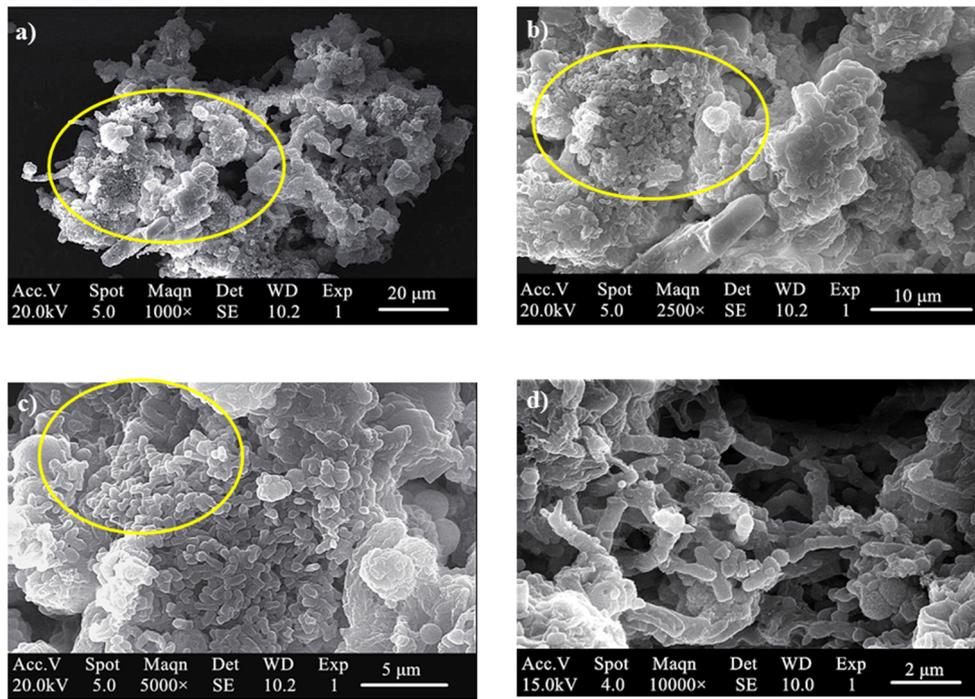


Figure 4. The SEM observations of different size of sludge: (a) 20 μm; (b) 10 μm; (c) 5 μm; (d) 2 μm.

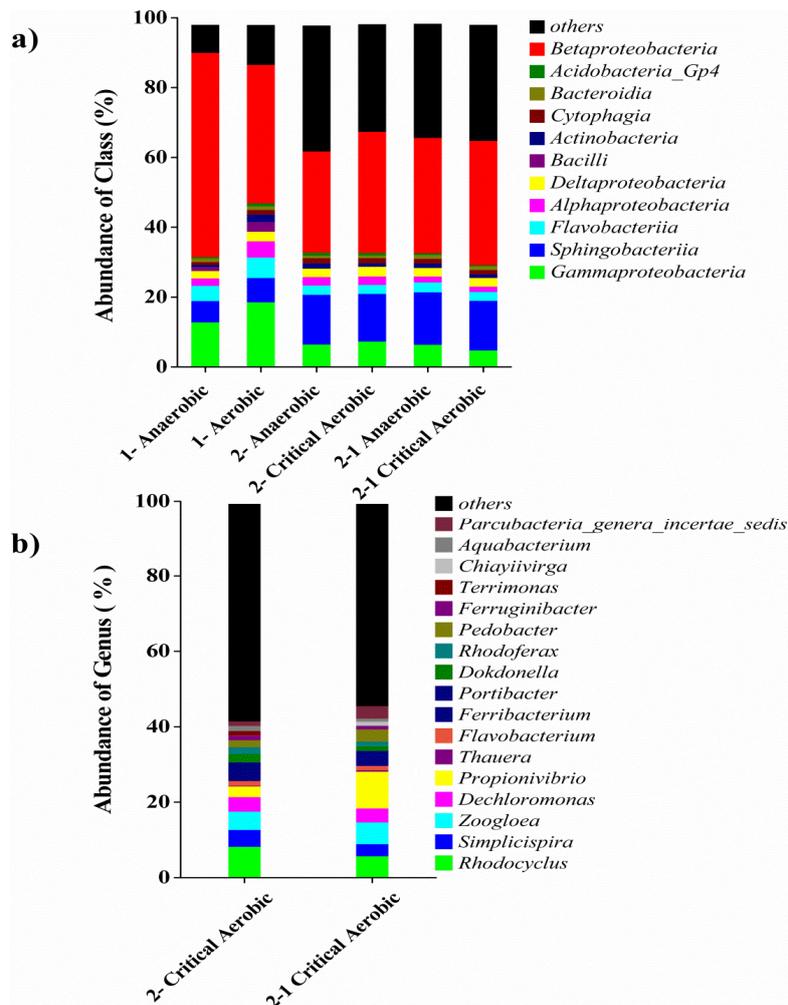


Figure 5. The results of the metagenomics analysis from the (a) class and (b) genus level.

3.1.3. COD Removals

For the COD, the influent concentration was approximately 200–350 mg/L, while it was 250–440 mg/L for the synthesized solution. The wide range of COD concentrations were used to simulate the fluctuations of the influent wastewater in the real situation. During the whole experiment, the removal efficiencies of the anaerobic phase and critical aerobic phase were moderately impacted after the configuration changes. Of the COD, 54% and 61% were accounted for by the aerobic phase and critical aerobic phase, respectively.

3.2. Sludge Structure and Microbial Community Composition

Figure 4 shows the observations from the SEM. From Figure 4a, it was obvious that the different morphology of the bacteria embedded together and cohered tightly. Figure 4b denoted that the co-existence of the rod-shaped and small coccus-shaped microorganisms. Figure 4c,d showed that filamentous bacteria existed in the entire process, and created an inner space to transfer the NO_3^- -N and NO_2^- -N for DAPOs, utilized with the help of the rod-shaped bacteria. Additionally, it was easy to distinguish them, and it was found that this was typical bacteria for the different functioning bacteria. For the phosphorus removal bacteria, they were short and rod-shaped, which could be seen directly from the SEM observations, which was similar to Wang et al.'s experiment [36]. It was observed that the abundances of *Betaproteobacteria* and *Alphaproteobacteria* were the highest in the bacteria community (the same as our results), and their SEM observations had large amounts of rod-shaped bacteria.

Nevertheless, the SEM observation results could not fully explain the double-layer structure of sludge. Moreover, the metagenome analysis was an effective technology to unravel the predominant species in various levels and their abundance in the microbial community at these stages [37,38]. The relative abundance of the microbial diversity analyzed is presented in Figure 5. The figure shows the data from a class and genus level based on the relative abundance of the microbial population. Only the top 12 taxonomic categories at a class level and representative of 17 at a genus level are summarized here. The sequences with a low similarity or no similarity, or where the V3–V4 region did not have any alignment hits against the taxonomic database and the others could not find out had been categorized as “others”.

At a genus level, it was obvious that *Rhodocyclus*, *Zoogloea*, *Dechloromonas*, *Thauera*, *Propionivibrio*, and *Simplicispira* took up a great proportion in three different sludge samples. After setting a critical aerobic phase in the process, the abundance of *Rhodocyclus*, which was the major and typical PAO in our common wastewater treatment plant [39,40], increased from less than 1% to 8.19%, and decreased to 5.69% in 1 h and 0.5 h length for critical aerobic phase, respectively. The reason was estimated to be nutrition competence. *Dechloromonas* (3.74% and 2.48%) and *Simplicispira* (4.47% and 3.21%) were the most common DPAOs for the biological wastewater treatment, which could use NO_3^- -N as the electron acceptor in an anoxic environment for oxidized organic matter [41–43], and their abundances both decreased after 0.5 h of the critical aerobic phase. *Zoogloea* was an easily adapted bacteria because they could adjust themselves either in an aerobic environment or anoxic environment for nitrifying and denitrifying [36,44]. Although the unique phenomenon had not occurred after 0.5 h, the abundance of *Zoogloea* was mildly enhanced (from 4.87% to 5.77%), which guaranteed the DNRA for NO_3^- -N removal [26]. However, the concentration of NO_3^- -N in the effluent was higher than previously, which demonstrated the denitrifying phosphorus removal was the main contributor for NO_3^- -N removal. *Thauera* was a facultative anaerobic denitrifying bacteria that could compete with the electron acceptor of the PAOs [45–48], and its abundance was always lower than 0.5%. *Propionivibrio* (2.79% and 9.75%) was one genus of the GAOs in the daily wastewater treatment plants that impeded the EBPRs efficiency in high abundance and competed for nutrition with the PAOs [49]. The decrease of its abundance evidenced the former research, that GAOs were not dominant at a low temperature compared with PAOs [6,50–52]. All of these changes elucidated the impact on the deterioration of the removal of both PO_4^{3-} -P and NO_3^- -N. On the basis of the literature, the critical aerobic phase of this experiment

needed a low concentration of O₂, in order to ensure the bacterial regularly metabolism [53], which was in accordance with our results.

4. Conclusions

In this study, we contrived a novel operation strategy of a pilot-scale anaerobic–critical aerobic SBR (A–CA SBR) for capturing the phosphorus of wastewater in cold regions. Accurately, almost the total PO₄³⁻-P was captured and no NO₃⁻-N was accumulated during the process, which were contributed to the proper critical aerobic phase length of 1 h. Moreover, from the SEM results and the metagenome analysis, *Rhodocyclus*, *Dechloromonas*, *Zoogloea*, and *Simplicispira*, which belonged to the phosphorus removal bacteria or denitrifying bacteria, co-existed in the different spaces of sludge. In essence, it was helpful for acquiring the aforementioned dominating bacteria in the subsequent artificial sludge regulation. Furthermore, the results signified that A–CA SBR was a feasible, high-efficient, and easy-handled operation strategy for domestic wastewater treatment in low-temperature conditions. Thus, this experiment not only solves the practical domestic wastewater treatment problems in winter temperatures, but also carves out an appropriate operating strategy for SBR with lower expenses. It could strongly believe that this operation mode of a pilot-scale SBR has a promising future for wastewater treatment in cold temperatures.

Author Contributions: Conceptualization and methodology, M.L. and D.Z.; investigation, M.L., B.Y., S.C., and T.M.; supervision, M.L. and D.Z.; review, D.Z.; funding acquisition, D.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Jilin Scientific and Technological Development Program, 20140204038SF.

Conflicts of Interest: The authors declare no conflict of interest.

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