

## Article

# A Bioreactor Designed for Restricting Oversize of Aerobic Granular Sludge

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**Abstract:** Aerobic granular sludge (AGS) with oversized diameter commonly affects its stability and pollutant removal. In order to effectively restrict the particle size of AGS, a sequencing batch reactor (SBR) with a spiny aeration device was put forward. A conventional SBR (R1) and an SBR (R2) with the spiny aeration device treating tannery wastewater were compared in the laboratory. The result indicates that the size of the granular sludge from R2 was smaller than that from R1 with sludge granulation. The spines and air bubbles could effectively restrict the particle size of AGS by collision and abrasion. Nevertheless, there was no significant change in mixed liquor suspended solids (MLSS) and the sludge volume index (SVI) in either bioreactors. The removal (%) of chemical oxygen demand (COD) and ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N) in these two bioreactors did not differ from each other greatly. The analysis of biological composition displays that the proportion of *Proteobacteria* decreased slightly in R2. The X-ray fluorescence (XRF) analysis revealed less accumulation of Fe and Ca in smaller granules. Furthermore, a pilot-scale SBR with a spiny aeration device was successfully utilized to restrict the diameter of granules at about 300 μm.

**Keywords:** aerobic granular sludge; oversize; diameter; bioreactor; wastewater



**Citation:** Feng, H.; Yang, H.; Sheng, J.; Pan, Z.; Li, J. A Bioreactor Designed for Restricting Oversize of Aerobic Granular Sludge. *Processes* **2021**, *9*, 374. <https://doi.org/10.3390/pr9020374>

Academic Editors: Francesca Raganati, Alessandra Procentese and Bippro R. Dhar  
Received: 27 November 2020  
Accepted: 12 February 2021  
Published: 18 February 2021

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## 1. Introduction

Aerobic granular sludge (AGS) is a biological aggregate which is generally defined as more than 90% of sludge with particle size greater than 200 μm [1,2]. It was first cultured by Mishima et al. [3] in an aerobic upflow sludge blanket (AUSB) with pure oxygen aeration in 1911. AGS has excellent properties such as compact microbial structure, good settling performance, and tolerance to high organic loading [1,4]. Therefore, AGS has been evaluated for treatment of various industrial wastewaters such as textile [5], rubber [6], brewery [7], and petroleum [8] wastewater. Moreover, AGS has been used in municipal sewage, and it shows better performance than traditional activated sludge. The AGS process is considered as a promising and alternative technology for wastewater treatment. However, most of the reported AGS is still at the lab or pilot-scale [9,10], with only a few reported cases at full-scale [11,12].

The stability of AGS is one of the main reasons limiting its wide practical application [13]. Besides, the particle size of AGS is one of the major factors which exert influence on its stability. The selective pressure is necessary for the formation of AGS, generally in the way of removing the flocculent sludge with poor settling performance and retaining the sludge with good settling performance by regulating the settling time. The sludge with larger particle size shows better settling performance. The average particle size of AGS in the reactor increases gradually under selective pressure. Granular sludge cultivated by Jungles et al. [14] was about 3500 μm in diameter. Farooqi and Farrukh [15] cultivated granular sludge of an average 2000–4000 μm in diameter in SBR with different selective pressures. The results showed that with the particle size increasing, the resistance of mass

which transfers from the surface to the interior would increase so that the anaerobic zone would expand, and the lack of nutrients in the interior would further lead to the disintegration of the sludge particles, and finally to the failure of operation [16]. Toh et al. [17] separated the AGS with different particle sizes in SBR to study its properties. They drew the conclusion that the most economical and effective particle size range is 1000–3000  $\mu\text{m}$ . The effective restriction of the particle size of granular sludge is of great significance to the engineering application of it.

A great number of studies have been conducted on the physicochemical properties and treatment effects of AGS with different particle sizes [18,19]. However, methods about restricting particle size have rarely been reported. A method for restricting the particle size of AGS is sieving. Long et al. [20] manually sieved to obtain the 2000–3000  $\mu\text{m}$  granular sludge and returned it to the reactor. However, this is not easy to do in engineering applications. When the AGS was oversized, the efficiency of mass transfer would decrease and the stability would become worse. Therefore, there is a necessity to find an effective and convenient method to restrict particle size of AGS. A bioreactor which can restrict the particle size of AGS was proposed in this experiment. The spiny aeration device was adopted in this bioreactor to restrict the particle size of AGS to tackle the instability caused by its oversized diameter. The spiny aeration device could effectively restrict the particle size of AGS without additional equipment.

## 2. Materials and Methods

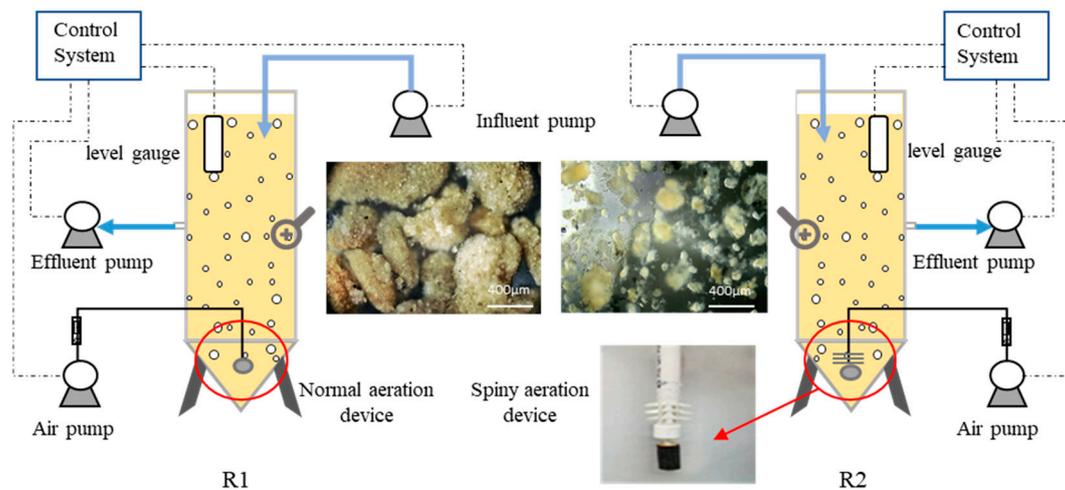
### 2.1. Inoculated Sludge and Wastewater

The inoculated sludge was obtained from the aerobic tank of a tannery wastewater treatment plant in Haining, Zhejiang Province. The mixed liquor suspended solids (MLSS) of the inoculated sludge were about 8300 mg/L and the sludge volume index (SVI) with 30 min settling time (SVI<sub>30</sub>) was about 75 mL/g. The influent water was taken from pretreated tannery wastewater. The main parameters of the wastewater were as follows: pH 6–8, chemical oxygen demand (COD) 550–800 mg/L, ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N) 105–160 mg/L, suspended solids (SS) 60–120 mg/L, and total phosphorous (TP) 1–4 mg/L.

### 2.2. Experimental Set-Up and Operation

#### 2.2.1. Lab-Scale Sequencing Batch Reactors

Aerobic granular sludge treating tannery wastewater was obtained in two lab-scale sequencing batch reactors (SBRs). The conventional aeration device was used in R1 and the spiny aeration device made of plastic material was used in R2 (Figure 1). The SBRs each had a diameter of 20 cm and a working volume of 11 L with a volumetric exchange ratio of 7/11. One period was 12 h and including the following procedures, influent (5 min), aeration (11 h), settling (5 min), drainage (20 min), and standing (30 min). The lab-scale reactors were operated for a total period of 70 days. The aeration volume was controlled at about 0.7 m<sup>3</sup>/h. The laboratory temperature was 15–30 °C during the whole period. It is worth noting that bioreactors for wastewater treatment would usually be exposed to temperature differences in a real-life scenario (Table S1), so the temperature in the lab was not constant artificially. But R1 and R2 had the same temperature. The SBRs were cleaned weekly to prevent biofilm formation on the wall.



**Figure 1.** Schematic diagram of the sequencing batch reactors (SBRs).

### 2.2.2. Pilot-Scale Sequencing Batch Reactors

A spiny aeration device was installed at the bottom of an AGS pilot-scale SBR system [21] (Figure 2). The system could dispose 120 m<sup>3</sup>/d wastewater from a town. The pilot-scale system was composed of two parallel columns (diameter of 2 m, height of 6 m, and H/D of 2.5) with 31.4 m<sup>3</sup> working volume and 50% volumetric exchange ratio. One period included fill (40 min), aeration (120 min), settling (60 min), and discharge (20 min). The system was operated continuously for more than 400 days. The agnail aeration device and air pipe layout were shown in Figure 2.



**Figure 2.** System of the pilot-scale reactor.

### 2.3. Analytical Methods

The SVI<sub>30</sub> and MLSS were measured periodically. Liquid samples in the feed and effluent were filtered periodically using 0.45- $\mu$ m filters. COD<sub>Cr</sub> was measured by fast digestion-spectrophotometric method and NH<sub>4</sub><sup>+</sup>-N was measured by Nesslerization. All these methods were in accordance with standard methods [22]. Samples were taken from the reactor at predetermined time intervals for analysis. The pH was monitored using a pH meter (PHS-3D, Shanghai). The morphology of sludge was observed by an Olympus CX31 microscope and a digital camera (Canon EOS 30D). The size of granules was analyzed by an image analysis system (Image-Pro Plus 6.0, Media Cybernetics, Rockville, MD, USA). The system reported the average length of diameters measured at 5° intervals around the

centroid of each object and then output the statistics. The X-ray fluorescence (XRF) analysis was performed using ARL ADVANT'X IntelliPower TM 4200 (Thermo Fisher, Waltham, MA, USA).

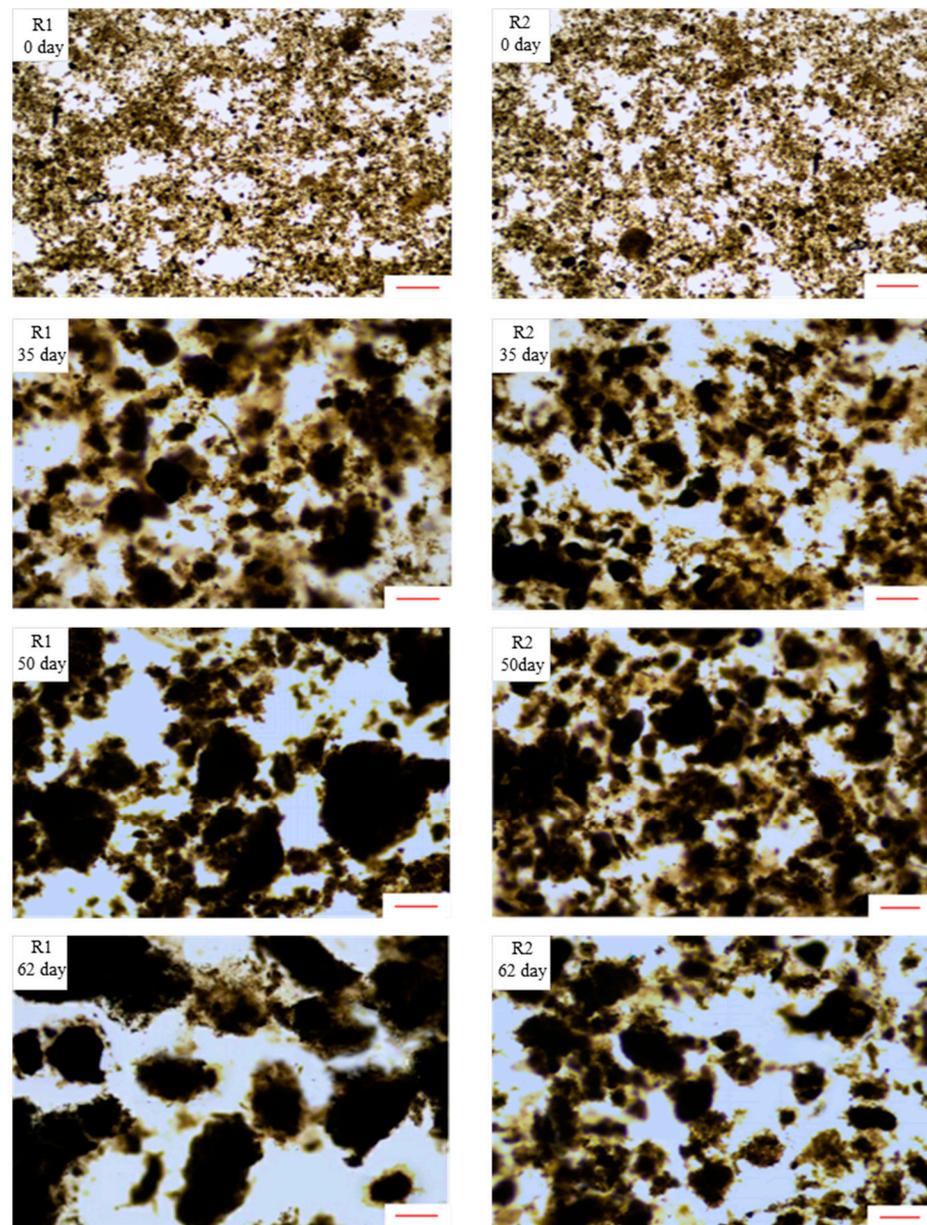
Three sludge samples acquired from the inoculation sludge and sludge from two bioreactors were freeze-dried for nucleotide extraction. Microbial DNA was extracted from AGS samples using the E.Z.N.A.<sup>®</sup> Mag-Bind Soil DNA Kit (Omega Bio-tek, Norcross, GA, USA) in accordance with the manufacturer's protocols. The final DNA concentration and purification were determined by NanoDrop 2000 UV-vis spectrophotometer (Thermo Scientific, Wilmington, DE, USA), and DNA quality was checked by 1% agarose gel electrophoresis. The V3–V4 hypervariable regions of the bacteria 16S rRNA gene were amplified with primers 341F and 805R by thermocycle PCR system (GeneAmp 9700, ABI, Waltham, MA, USA). The PCR reactions were conducted using the following program: 3 min of denaturation at 94 °C; 20 cycles of 30 s at 94 °C, 20 s at 45 °C and 30 s at 65 °C; 20 cycles of 20 s at 94 °C, 20 s at 55 °C and 30 s at 72 °C; and a final extension at 72 °C for 5 min. The PCR product was recovered using an AxyPrep DNA Gel Extraction Kit (AXYGEN, Corning, NY, USA) and quantified with a QuantiFluor TM -ST system. The high-throughput sequencing of PCR products was performed using the Illumina MiSeq platform of Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). The operational taxonomic units (OTUs) were clustered with a distance limit of 0.03, using Usearch. Microbial alpha diversity indices such as Shannon, Simpson, Chao, and the abundance-based coverage estimator (ACE) were analyzed using the MOTHUR program. The RDP Classifier with a confidence threshold of 70% was used to classify effective sequences into different taxonomy units, while the community composition was analyzed at different taxonomy levels.

### 3. Results

#### 3.1. Comparison of Sludge Characteristics

##### 3.1.1. Sludge Particle Size

The variation of sludge particle size is shown in Figure 3. The same flocculent sludge was inoculated into the R1 and R2 bioreactors, respectively, and the MLSS in both reactors after inoculation was around 1750 mg/L. After 15 days' operation, a few small particles began to appear in both reactors, but the sludge was still mainly flocs. The particle size and the number of granules in R1 were both slightly larger than those in R2. After 23 days of operation, aerobic sludge in R1 began to granulate, while AGS appeared in R2 six days later than in R1. On the thirty-fifth day, the granular sludge in both R1 and R2 had grown in size, but there was still plenty of flocculent sludge. After 62 days, the sludge in R1 was basically granular with a clear profile. The granular sludge in R2 was also clear but it still contained some flocculent sludge with a prominently smaller particle size than that in R1. The average particle size of the granular sludge was 285 µm in R1 and 190 µm in R2. The particle size distribution (PSD) was mainly within the range of 0–0.5 mm in SBR1 and SBR2. On day 62, and in SBR1, the PSD < 0.2 mm was 10.7%, while those at 0.2–0.5 mm reached 89.3%, respectively. In SBR2, the PSD < 0.2 mm was 36.2% and 0.2–0.4 mm was 63.8%.

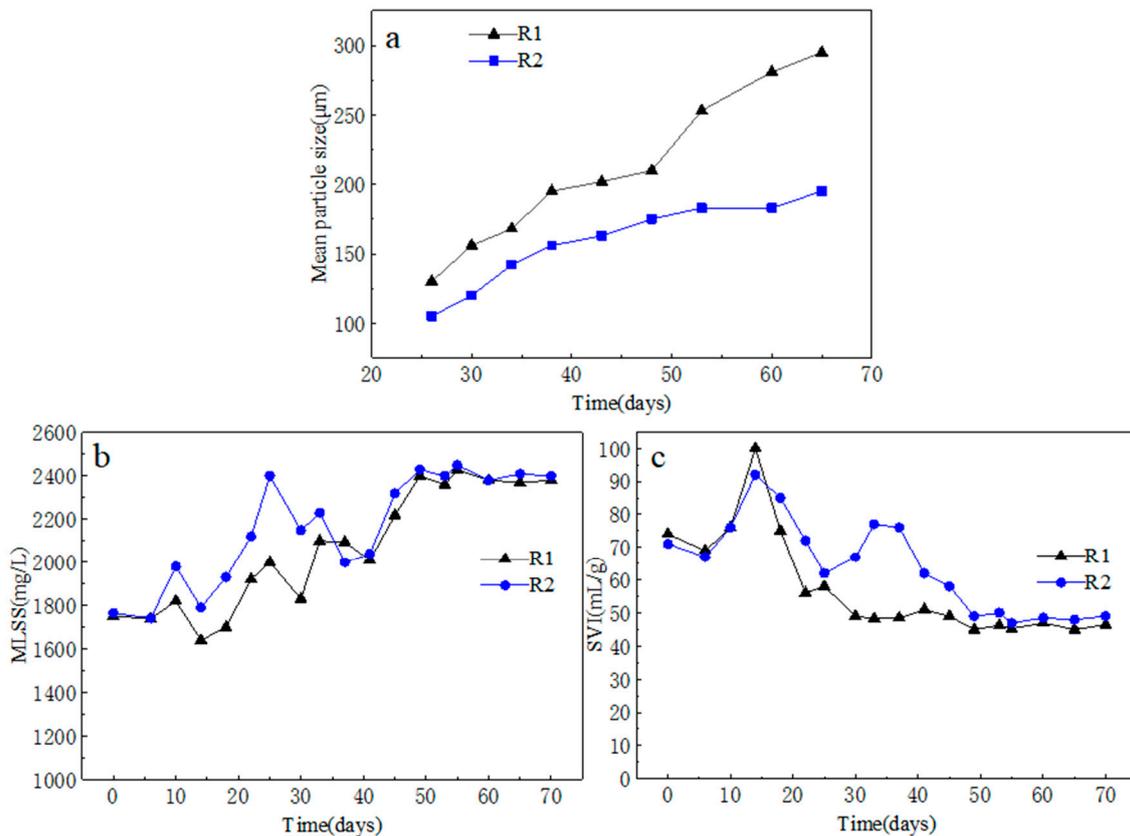


**Figure 3.** Variations in the morphology of sludge (scale bar: 200  $\mu\text{m}$ ).

### 3.1.2. Sludge Sedimentation

The initial MLSS was 1750 and SVI was 73 mL/g for both SBR systems in the first phase. On the whole, the MLSS in both reactors indicated an increasing trend while SVI indicated a decreasing trend, with both the MLSS and SVI in R1 being lower than those in R2 (Figure 4). In the first 4 days, due to the short settling time of only 5 min, sludge with poor settling performance was discharged from the bioreactor. In consequence, the MLSS in R1 and R2 decreased from 1750 to 1733 and 1730 mg/L, respectively. In the following 4 days, MLSS increased to 1819 and 1967 mg/L in R1 and R2, respectively. On the eighth day, the MLSS decreased both in R1 and R2, and the SVI increased on account of the deterioration of the sludge settling performance. On the twenty-fifth day, the MLSS in R1 and R2 manifested an increasing trend and augmented to 2003 and 2396 mg/L, respectively. After 25 days, the MLSS in R1 sustained growth in a continuously stable state at 2400 mg/L. The MLSS in R2 decreased from 2396 to 2105 mg/L from day 25 to 45. After 45 days, the MLSS in R2 began to increase, and on the sixty-fifth day, it maintained stable at about 2450 mg/L. The SVI in R1 and R2 increased to 100 and 92 mL/g, respectively, in the first 13

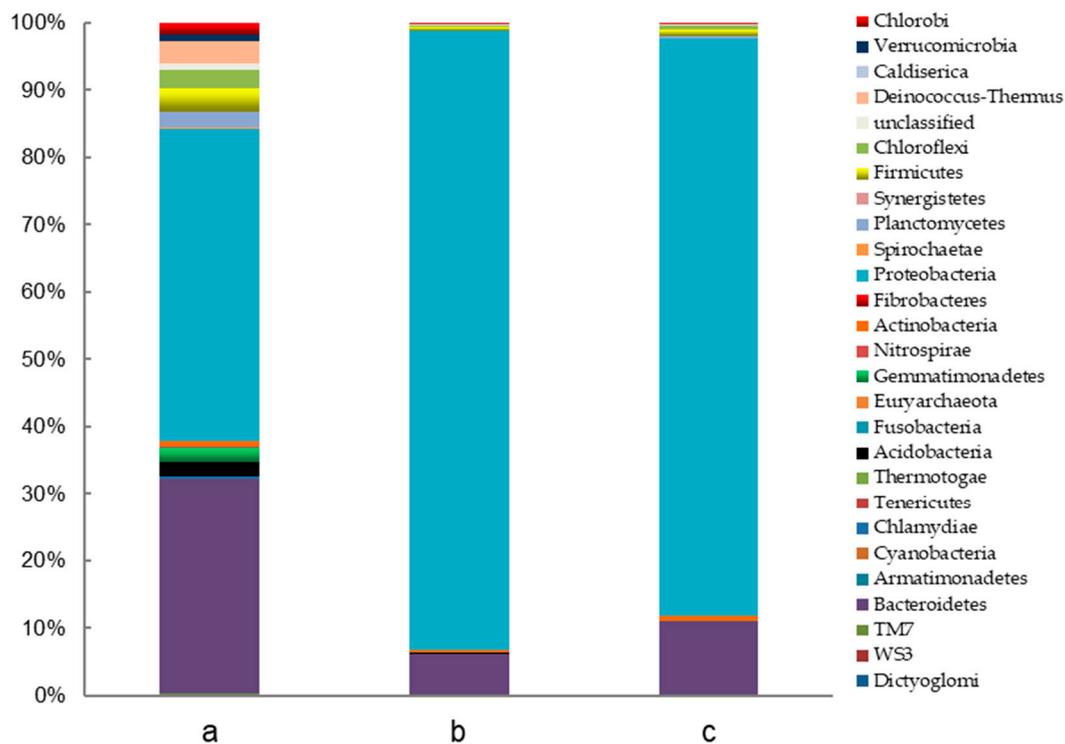
days of operation. After 13 days, the SVI in R1 continued to decrease and maintained stable at about 45 mL/g after 65 days of operation, while the SVI in R2 increased to 80 mL/g after 35 days of operation. After 35 days, the SVI in R2 began to decrease and was basically stable at 50 mL/g on the sixty-fifth day. The SVI of R2 decreased more slowly than that of R1, and eventually the SVI of the granular sludge in R2 was slightly higher than that in R1.



**Figure 4.** Variations in the mean particle size, mixed liquor suspended solids (MLSS), and sludge volume index (SVI) of sludge. ((a) mean particle size; (b) MLSS, (c) and SVI).

### 3.1.3. Microbial Community Analysis

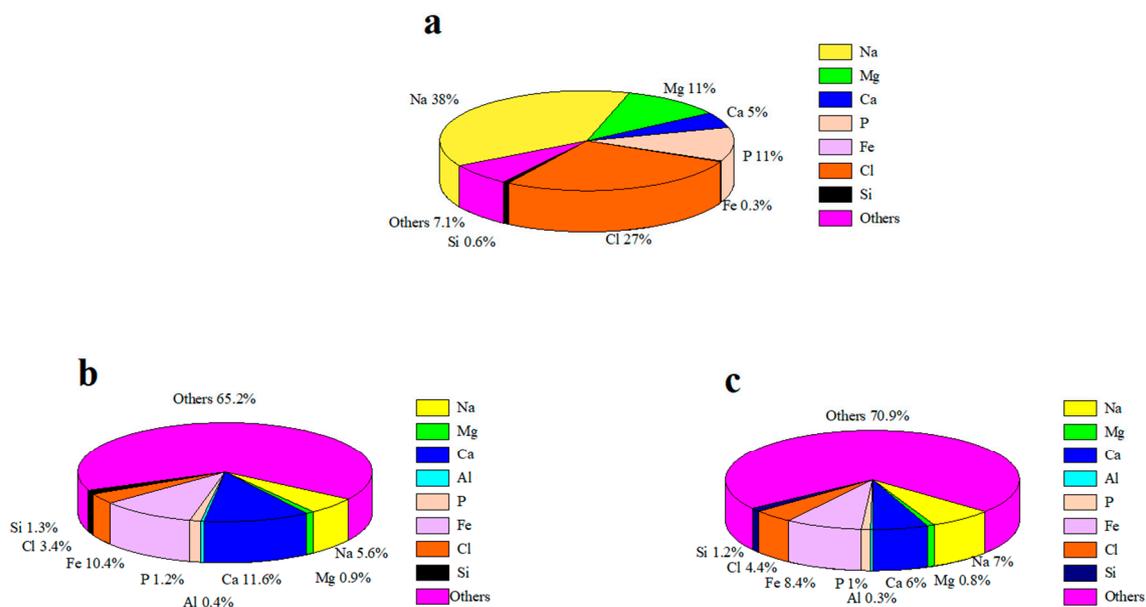
The microbial community was analyzed through high-throughput sequencing. As shown in Figure 5, the results indicated that two bacterial phyla, *Proteobacteria* and *Bacteroidetes*, were the most abundant in the inoculated sludge, accounting for 46.45% and 31.74% of the total sequence in the inoculated sludge respectively, followed by *Firmicutes*, *Deinococcus-Thermus*, *Chloroflexi*, *Planctomycetes*, *Acidobacteria*, and *Gemmatimonadetes*, accounting for 3.55%, 3.4%, 2.62%, 2.33%, 2.23%, and 2.01% in the inoculated sludge, respectively. The microbial community of R1 sludge was mainly constituted by *Proteobacteria* and *Bacteroidetes*, accounting for 91.98% and 6.27% of the total sequence, respectively. R2 was similar to R1 with *Proteobacteria* and *Bacteroidetes*, accounting for 85.86% and 10.95% of the total sequences, respectively. Compared with inoculated sludge, the amount of *Proteobacteria* in R1 and R2 rolled up, *Bacteroidetes* decreased, and other bacteria phyla also decreased.



**Figure 5.** Distribution of microorganisms at phyla level ((a) inoculated sludge; (b) sludge of R1; (c) sludge of R2).

### 3.1.4. The X-ray Fluorescence Analysis of Sludge

The XRF was adopted to analyze the element composition of feed water and AGS. It showed that raw water contains large amounts of Na, Cl, Ca, Mg, and P. The AGS accumulates elements such as Fe, Ca, Mg, and P. As shown in Figure 6a, influent samples were measured for the mass fraction of each element in the water and the mass fraction of the water, and the proportion of each element in the total solute mass was obtained by conversion. It can be seen from the figure that the proportion of the element Na in the feed water is 38%, 27% for Cl, followed by 11%, 11%, 5%, 0.6%, and 0.3% for P, Mg, Ca, Si, and Fe, respectively, and 7.1% for the other elements. The element distribution of the AGS in R1 was shown in Figure 6b. Ca and Fe account for a larger proportion, 11.6% and 10.4%, respectively, followed by Na, Cl, Si, P, Mg, Al, and other elements as 5.6%, 3.4%, 1.3%, 1.2%, 0.9%, 0.4%, and 65.2%. The element distribution of AGS in R2 was shown in Figure 6c. The elements with a larger proportion were Fe, Na, and Ca at 8.4%, 7%, and 6%, followed by Cl, Si, P, Mg, Al, and other elements at 4.4%, 1.2%, 1%, 0.8%, 0.3%, and 70.9%, respectively. Comparing the elements in feed water and AGS, it could be seen that Fe and Ca were heavily enriched in the granular sludge. The content of elements Fe and Ca in R1 was greater than that in R2, but the content of elements Na and Cl was less than that in R2. The proportions of other elements were similar in these two reactors.

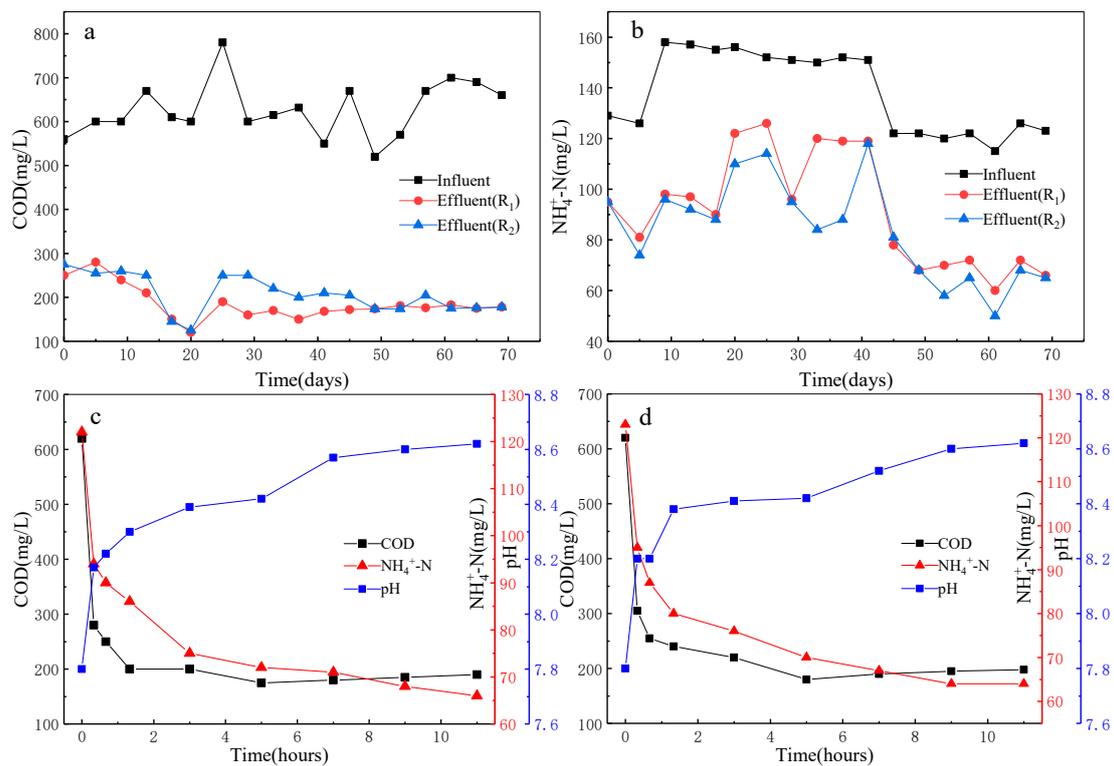


**Figure 6.** Elemental composition ((a) influent; (b) sludge of R1; (c) sludge of R2).

### 3.2. Pollutant Removal Performance

The removal effects of COD and  $\text{NH}_4^+\text{-N}$  were shown in Figure 7a,b. The concentration of COD in the influent of R1 and R2 had maintained at 500–800 mg/L, while that in effluent had been kept below 300 mg/L since the outset of operation, and it stabilized at less than 200 mg/L and the removal (%) reached 79% after 50 days of operation. The concentration of  $\text{NH}_4^+\text{-N}$  in the feed of R1 and R2 was 110–160 mg/L. After 49 days, this figure in the effluent water was basically less than 70 mg/L. The concentration of COD and  $\text{NH}_4^+\text{-N}$  in the effluent met the discharge standard [23] of water pollutants for the leather- and fur-making industry in China. In general, the COD and  $\text{NH}_4^+\text{-N}$  removal effect of R1 and R2 did not differ from each other significantly; namely, spiny aeration did not contribute to the efficiency of the pollutant removal.

After aerobic sludge granulation, the contamination removal effect of R1 and R2 in one cycle was analyzed, as shown in Figure 7c,d. The situation of R2 was similar to R1 in that the concentration of COD and  $\text{NH}_4^+\text{-N}$  declined rapidly in both reactors within a short period of time, with a slightly smaller decrease of COD and a slightly larger decrease in  $\text{NH}_4^+\text{-N}$  at the first 5 h in R2 than in R1. The pH of both R1 and R2 was constantly rising, with a pH of 7.83 in the influent and 8.63 in the effluent.



**Figure 7.** Performance of the reactors ((a) chemical oxygen demand (COD); (b) ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N), (c) COD, NH<sub>4</sub><sup>+</sup>-N, and pH of R1; (d) COD, NH<sub>4</sub><sup>+</sup>-N, and pH of R2).

### 3.3. An Attempt at the Pilot-Scale System

After 7 days, small aerobic granules could be observed. On the twentieth day, loose sludge began to conglomerate into lumps. On the fiftieth day, there was mainly AGS with a compact structure and irregular outline in the reactor. As the reactor continued to operate, the average particle size of granular sludge remained at 300  $\mu\text{m}$  and the SVI was 43 mL/g in the end. The pilot-scale reaction was operated continuously for more than 400 days and maintained high removal efficiencies for pollutants. The removal of COD was maintained at about 88% and almost all the NH<sub>4</sub><sup>+</sup>-N was removed. It could be seen that the particle size of AGS in SBR can be effectively restricted by using the spiny aeration tray, which indicated that the spiny aeration device plays an important role in restricting the particle size in the pilot-scale SBR.

## 4. Discussion

Through the analysis of sludge characteristics, the spiny aeration device effectively controlled the particle size of aerobic sludge by collision and abrasion. In the process of aeration, the granular sludge would collide with the agnail on the aeration device, and then the sharp agnail would abrade large particles, causing them to be broken (Figure 2). The granular sludge with a larger particle size was more likely to be in the lower half of the reactor so that it was more likely to come into contact with the agnail. At the same time, most of the small particles could smoothly pass through the space between agnails with small probability of being punctured.

Due to physical fragmentation, the sedimentation of the sludge showed no significant change in both bioreactors. The removal (%) of COD and NH<sub>4</sub><sup>+</sup>-N in these two bioreactors did not differ from each other greatly.

In this experiment, the *Proteobacteria* was abundant and prior in the AGS of tannery wastewater treatment. This result was similar to the research of Zhao et al. [24] and Huang et al. [25]. It is reported that *Proteobacteria* is common in AGS and can produce excessive extracellular polymers to promote flocculation of flocculent sludge and formation of AGS.

In addition, *Proteobacteria* have the ability to degrade COD and nitrogen. The number of *Proteobacteria* in AGS was much more than that in inoculated sludge, which indicated that *Proteobacteria* played an important role in the granulation of AGS. The analysis of biological composition displayed that the proportion of *Proteobacteria* decreased slightly in the R2. Most members of *Proteobacteria* are facultatively or obligately anaerobic. The larger anaerobic zone is formed in the bigger particle size of the sludge [26]. This indicated that the particle size of sludge in the R2 was effectively restricted.

This experiment result of the X-ray fluorescence analysis indicates that the larger particle size of the granular sludge was enriched with more Fe and Ca, which was consistent with the study of Liu et al. [21]. The ash in AGS contained more calcium salts (mainly calcium carbonate), and the content of calcium carbonate precipitate increased with the increase of the particle size of the granular sludge within a certain size range [27]. Other research and literature suggested that a large accumulation of calcium would undermine the biological activity [28].

Therefore, it could be seen that the particle size of AGS in SBR was effectively restricted by using the spiny aeration device, which indicated that the spiny aeration device played an important role in restricting the particle size in the SBR. Long et al. [20] enhanced the stable operation of AGS in pilot-scale SBR by manually sieving to obtain the 2000–3000  $\mu\text{m}$  granular sludge and returning it to the reactor to increase their proportion. Sieving manually can completely retain the structure of the AGS, but the high cost and low efficiency of this method make it unsuitable for engineering applications. Compared with the existing method of particle size control, using the spiny aeration device might damage the particle structure in some way, but its operability is suitable for engineering application. In addition, using the spiny aeration device did not exert influence on the removal (%) of COD and  $\text{NH}_4^+\text{-N}$  in the system.

## 5. Conclusions

A bioreactor with the spiny aeration device could effectively restrict the particle size of the AGS. The oversized AGS was more likely to collide and abrade with the spines and air bubbles. However, the spiny aeration device made no difference in the pollutant removal and sludge characteristics. By adding the spiny aeration device in the pilot-scale SBR system, the average particle size of AGS could be effectively restricted at about 300  $\mu\text{m}$ .

**Supplementary Materials:** The following are available online at <https://www.mdpi.com/2227-9717/9/2/374/s1>, Table S1: Basic climatic conditions of Hangzhou.

**Author Contributions:** Conceptualization, H.F., H.Y., and J.L.; methodology, H.F. and H.Y.; software, H.F. and H.Y.; validation, H.F., Z.P., and J.S.; data curation, H.F. and H.Y.; writing—original draft preparation, H.F.; writing—review and editing, H.F., Z.P., and J.S.; funding acquisition, J.L. supervision, J.L.; project administration, J.L. All authors have read and agreed to the published version of the manuscript.

**Funding:** The research was funded by the Major Science and Technology Program for Water Pollution Control and Treatment (2017ZX07201003), the Zhejiang Key Research and Development Program (no. 2018C03006).

**Institutional Review Board Statement:** “Not applicable” for studies not involving humans or animals.

**Informed Consent Statement:** “Not applicable” for studies not involving humans.

**Data Availability Statement:** Data sharing not applicable.

**Acknowledgments:** This work was supported by the Major Science and Technology Program for Water Pollution Control and Treatment (2017ZX07201003), the Zhejiang Key Research and Development Program (no. 2018C03006).

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

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