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The Impact of Berberine Pharmaceutical Wastewater on Aerobic Granules Formation: Change of Granules' Size

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Abstract: As important parameters in the characterization of aerobic granulation, the shape and average diameter were related to substrates. The previous studies disclosed that the morphology change in aerobic granules was the result of growth and the relatively strong hydrodynamic shear force. No further exploration of the size distribution of the aerobic granules has been conducted. To better understand the impact of toxic compounds on aerobic granules' growth during their formation, the properties of aerobic granules were traced over 81 days in 3 sequencing batch reactors fed with acetate and berberine wastewater, especially the particle size and size distribution. The results showed that the aerobic granules were cultivated by the simulated acetate wastewater (R1), simulated berberine wastewater (R2), and effluent from an anaerobic baffled reactor (ABR) reactor which was fed with industrial berberine wastewater (R3). The reactors exhibited different COD removal efficiencies, and the MLSS and MLVSS values affected by the different substrates which were in an order of R1 > R2 > R3. However, the SVI and SOUR, which were affected by several factors, showed more complicated results. The aerobic granules had the lowest microbial activity (SOUR), while the aerobic granules in R3 had the lowest settling ability among the three kinds of granules. For the three reactors with different influent compositions, the aerobic granulation process displayed a three-stage process separately. Compared with the granules fed with berberine wastewater, the granules fed with acetate in a stable operation period showed more independence from other periods.The size distribution was affected by substrates. The aerobic granules with a range of 0.3–1.0 µm occupied 77.0%, 67.0%, and 35.7% of the volume for R1, R2, and R3, respectively. The biomass less than 0.3 µm occupied 59.1% volume in R3. The components of the substrate had a great influence on the growth of aerobic granules, not only on the diameter but also on the size distribution.

Keywords: aerobic granules; particle size; distribution; berberine; wastewater treatment

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1. Introduction

Aerobic granulation, a new form of cell immobilization for exploitation in biological wastewater treatment, is a promising technology in future wastewater treatment [1–3]. Starting in the late 1990s, extensive studies have been carried out on these technologies, especially the aerobic granulation process [2,4–6]. The characteristics of sequencing batch reactor (SBR), such as the operation cycle and its periods, settling time, the liquid volume exchange ratio, pH and influent COD concentration of substrate, the composition of influent, and the organic loading rate (OLR) have been studied since they are related to the mechanism behind granule formation [7–11]. Several hypotheses to explain the aerobic granule formation mechanism were suggested based on these studies [12–14]. For instance, Liu and Tay [15] suggested three major steps for granule formation. Physical movement is an initial step for microbial self-aggregation. In the second step, the attractive forces helped the microbial cells to stabilize to be microbial aggregations. Then, mature granules are formed under the mixed forces from extracellular polymeric substances (EPS) synthesis, growth of immobilized cells, and hydrodynamic forces. Barr et al. [2] have suggested that aerobic granules formed from a single colony of microorganisms or many independent colonies of microorganisms. Wu et al. [16] thought that a high organic loading rate and a high selection pressure are two crucial factors for aerobic granules formation in a continuous flow reactor. However, these hypotheses have not been certified by convincing experimental evidence. Further study needs to be carried out to disclose what happened in the aerobic granulation process.

In our study, the simulated acetate wastewater, simulated berberine wastewater, and industrial berberine wastewater were used as substrates for aerobic granules formation. Berberine ($C_{20}H_{18}NO_4$, BBR in short), produced by extraction from herbal plants or synthesized by chemical means, is a traditional Chinese medicine with a broad antibacterial spectrum [17–19]. In addition to being an anti-inflammatory, berberine was applied to be medicine for its antitumor, anti-oxidation, anti-Alzheimer's disease, and anti-hyperglycemic activities [20,21], which led to the extensive production and use of berberine. Inevitably, a large quantity of BBR-containing wastewaters was released into the environment. Since the discharge of BBR poses a risk to the ecosystem, it is necessary to treat it before it enters the environment [22,23]. In this study, the change in aerobic granules was observed in the granulation process. The characteristics of aerobic granules, such as suspended solids (SS), volatile suspended solids (VSS), sludge volume index (SVI), specific oxygen utilization rates (SOUR), and COD removal efficiency, were tested. Furthermore, the analysis of results tested by Malvern Mastersizer 2000 gave a new version of the aerobic granulation process.

2. Materials and Methods

2.1. Inoculum

The inoculum of R1 and R2 were collected from the municipal wastewater plant. The inoculum of R3 was collected from a pharmaceutical wastewater treatment plant. The characteristics of the inoculum were listed in Table S1.

2.2. Experiment Design and Set-Up

Experiments were performed in 3 column-type sequence batch reactors (SBR) with a working volume of 2.8 L and a diameter of 6 cm (Figure S1). Reactors were operated sequentially in 4-h cycles (4 min of influent filling, 225 min of aeration, 5 min of settling, and 5 min of effluent withdrawal) with a hydraulic retention time (HRT) of 8 h. The effluent was discharged with a volumetric exchange ratio of 50%. The aeration was supplied through a dispenser at the reactor bottom at an airflow rate of 4.0 L min^{-1}. The operation parameters are shown in Table S2.

2.3. Medium

R1 was fed with acetate synthetic wastewater. R2 was fed with synthetic wastewater with the components of acetate and berberine, and the effluent from an anaerobic baffled reactor (ABR), which was fed with industrial berberine wastewater, was pumped into R3 as an influent. The industrial berberine wastewater was collected from the separation process during berberine production with a COD concentration of 4166 $\pm$ 102 mg/L and berberine of 900 $\pm$ 100 mg/L. The components of the media feed into the three reactors were shown in Table S3. All of the reactors were cultivated with an organic loading rate of 3 kg COD $m^{-3}d^{-1}$.

2.4. Analytical Methods

SS, VSS, and SVI were measured periodically according to the standard methods [24]. SOUR was measured followed the previous studies [25,26]. Granule morphology was observed by Leica microscope (Leica DM5000 B, Wetzlar, Germany). The sizes were measured by a laser particle size analysis system (Malvern Mastersizer 2000, Malvern, UK) and analyzed by an image analysis system (Image-Pro Plus 5.0). A microscopic study of aerobic granular samples was conducted with a scanning electron microscope (SEM) (SU-70, Hitachi, Tokyo, Japan), as described previously [27]. The relationships between the samples collected at different times in R1, R2, and R3 were analyzed by systematic clustering using SPSS 26 statistical software. All samples were tested in triplicate.

3. Results

3.1. Biological Treatment Performance during Aerobic Granulation in R1, R2, and R3

The aerobic granules were cultivated in R1, R2, and R3 fed with the substrate at the COD average concentrations of 2080.9 ± 64.0 mg/L, 2067.0 ± 76.6 mg/L, 1784.3 ± 76.3 mg/L, respectively. The operation and characteristics are shown in Figures 1 and 2, respectively. Although the components were different, the COD removal efficiency of the three reactors were kept higher than 90% from day 4 to 81. In the steady period, the average COD removal efficiencies of R1, R2, and R3 were 94.63 ± 4.69%, 94.45 ± 3.77%, and 91.63 ± 3.74%, respectively, which indicated the steady operation of the three reactors. It seems that the COD removal efficiency of R3 was lower than R1 and R2.

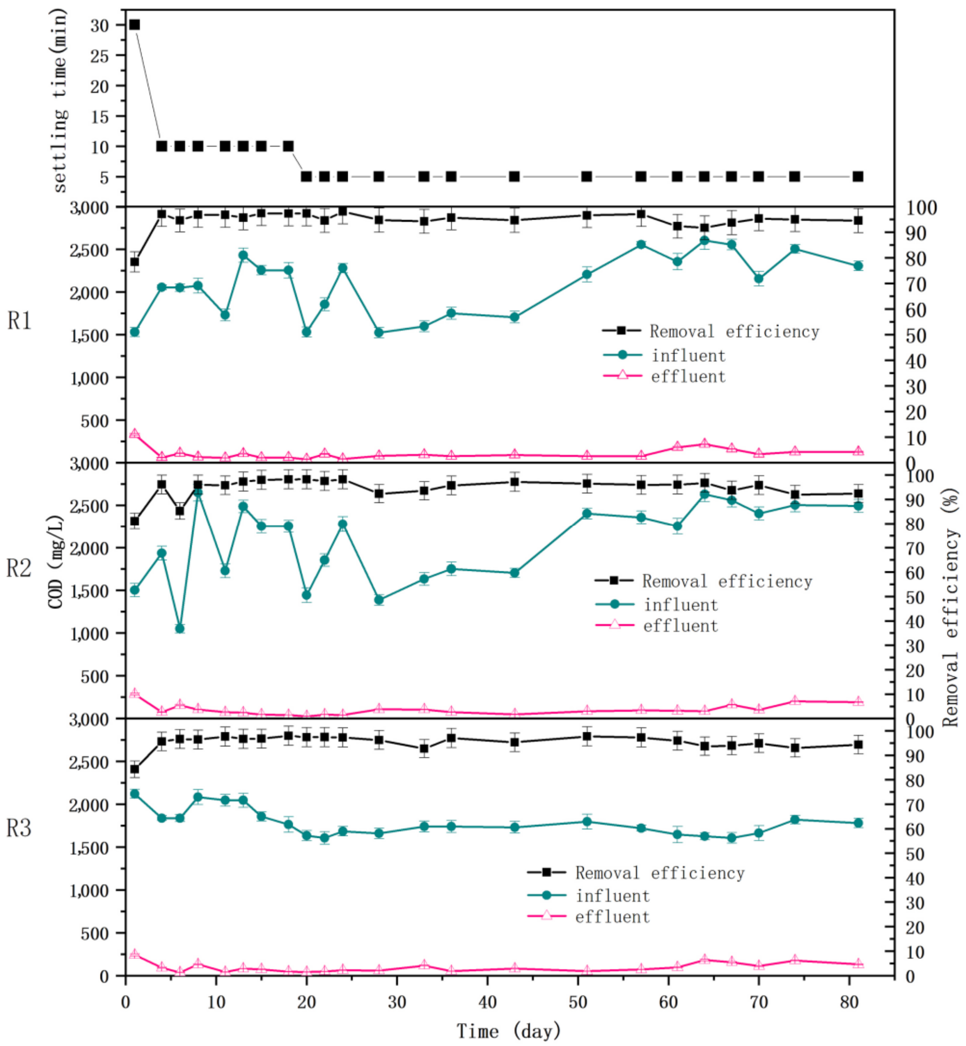

Figure 1. The pollution degradation during aerobic granules' formation processes in R1, R2, and R3.

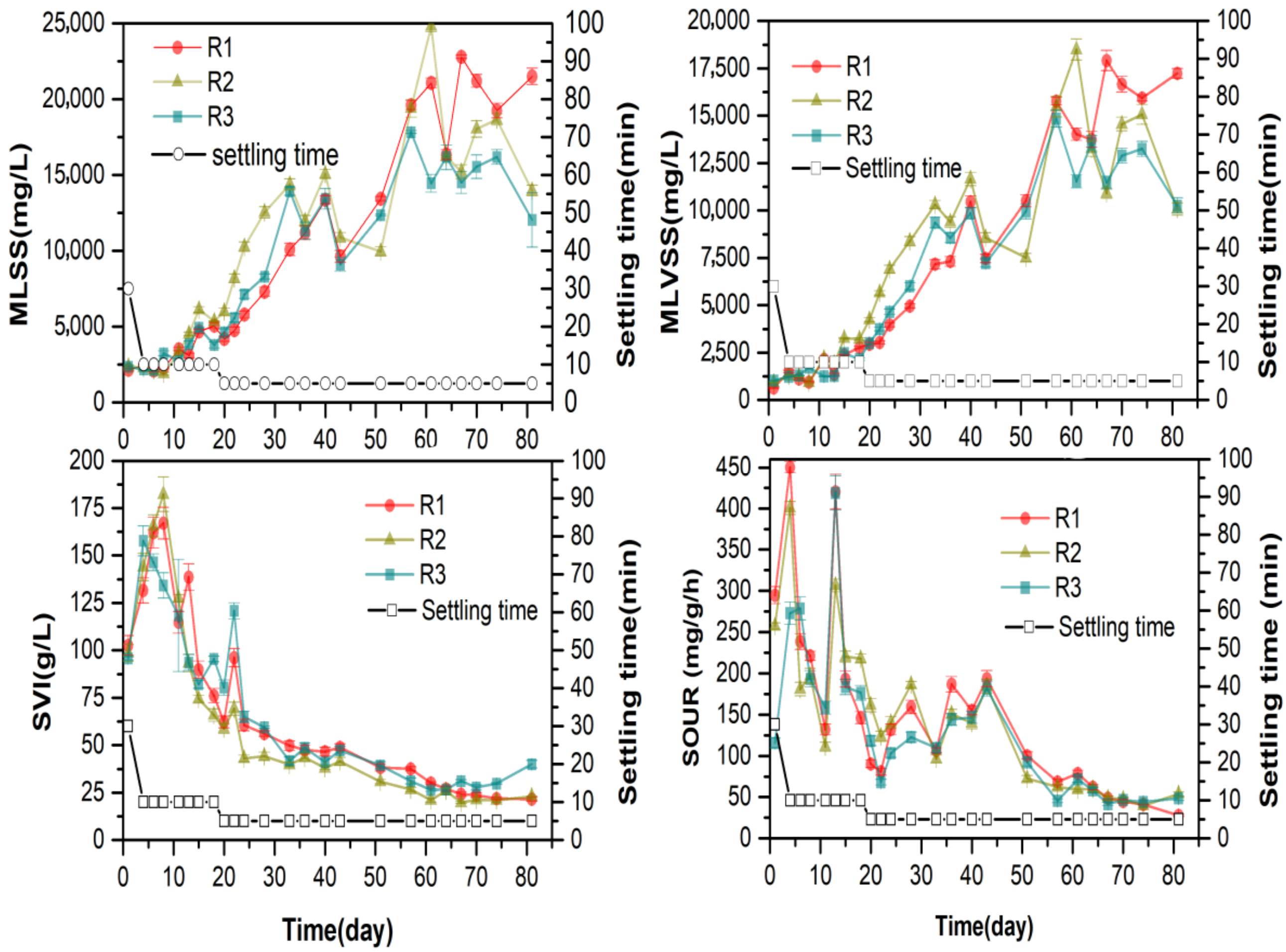

Figure 2. The variations in MLSS, MLVSS, settleability (SVI), and microbial activity (SOUR) during aerobic granules' formation process in R1, R2, and R3.

The variations in MLSS, MLVSS, settleability (SVI), and microbial activity (SOUR) during aerobic granules' formation process in R1, R2, and R3 are shown in Figure 2. The mixed liquor suspended solids (MLSS) in R1, R2, and R3 started from 2147.5 ± 85.9 mg/L, 2362.5 ± 82.7 mg/L, and 2350 ± 117.5 mg/L. The amount of biomass increased with the operation. At the steady state, the MLSS of R1, R2, and R3 was maintained at 20,355 ± 367 mg/L, 17,813 ± 362 mg/L, and 14,810 ± 863 mg/L, respectively. Thus, the amount of biomass in R1 was higher than that of R2 and R3. The SVI decreased with operation time. At a steady state, the average SVI of R1, R2, and R3 was 24.64 ± 0.99 mL/g, 21.92 ± 0.86 mL/g, and 30.20 ± 1.09 mL/g, respectively, which indicated the aerobic granules in R1 and R2 possessed better settling ability than that of R3. The mixed liquor volatile suspended solids (MLVSS) of R1, R2, and R3 have similar trends to that of MLSS.

High SOUR indicates the high microbial activity of microorganisms. On the first day, the SOURs of R1, R2, and R3 were 294.45 ± 10.31 mg/g VSS.h, 257.1 ± 3.86 mg/g VSS.h, and 115.5 ± 5.78 mg/g VSS.h, respectively. The seed sludge of R3 from the pharmaceutical wastewater treatment plant showed a low activity with low SOUR. After granulation, SOURs decreased to be 27.67 ± 1.38 mg/g VSS.h, 53.62 ± 2.68 mg/g VSS.h, and 48.22 ± 2.41 mg/g VSS.h for R1, R2, and R3, respectively. This is mainly due to a balance between the compact of the biomass and substrate inhibition. For R1, the microbial activity decreased because of the compact structure formation during granulation. For R2 and R3, the microbial activity was decided by the total effects of substrate inhibition and compact structure formation.

3.2. Effects of Berberine Wastewater on the Morphology of Aerobic Granules

The biomass grew gradually when the reactors were fed with a substrate. The change in the granules' diameters is shown in Figure 3. D[4,3] means the volume average particle size, which refers to the average diameter of the granules in previous studies [28,29]. d(0.5) refers to medial granularity and means the diameter corresponding to 50% of the cumulative particle size distribution (0 to 100%) [30,31].

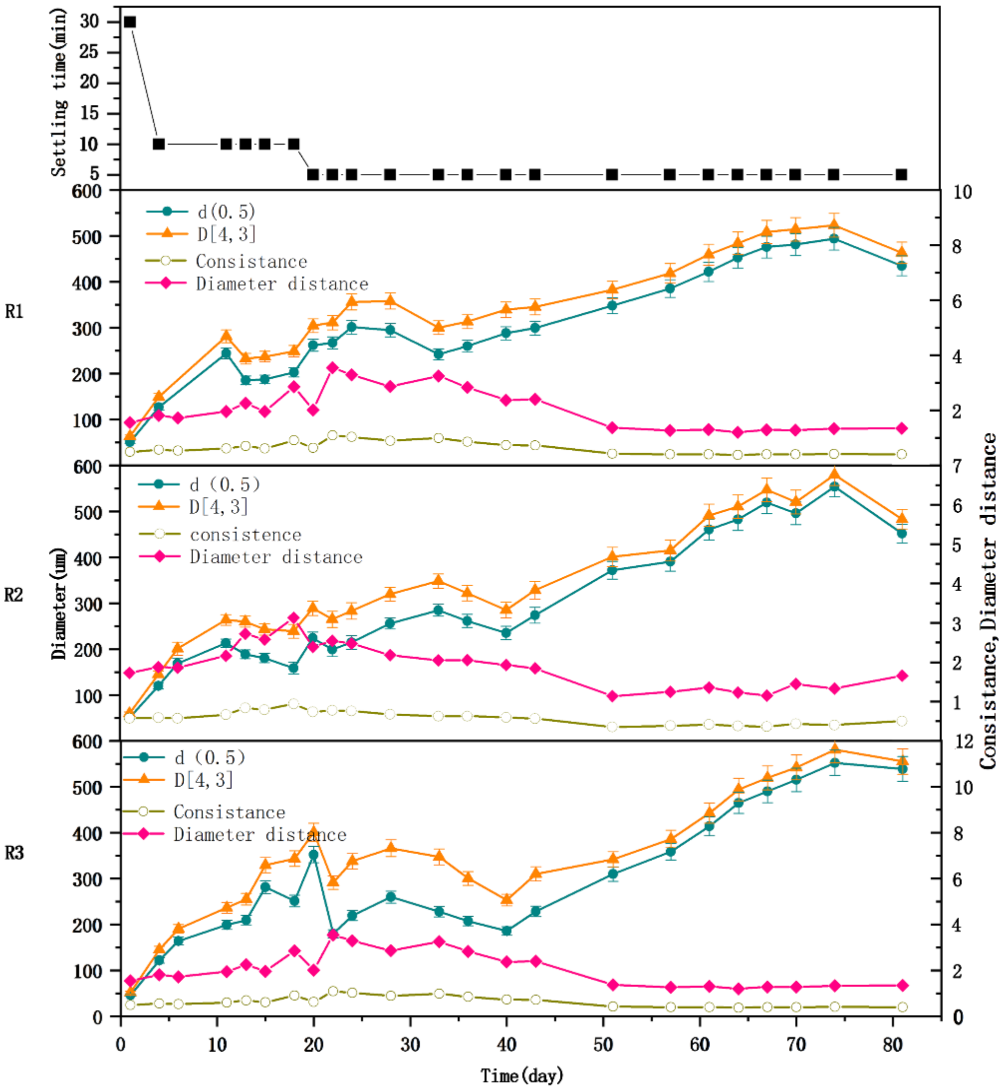

Figure 3. The change in diameter of aerobic granules in the formation process in R1, R2, and R3.

In this study, the biomass sizes in R1, R2, and R3 started from 62.84 ± 3.12 µm, 60.12 ± 3.14 µm, and 51.64 ± 2.58 µm, respectively. They were increased with the operation time. On day 51, the size of the biomass in R1, R2, and R3 increased to 382.56 ± 19.12 µm, 400.96 ± 20.95 µm, and 341.61 ± 17.08 µm, respectively. At the steady state, the average sizes of aerobic granules in R1, R2, and R3 were 469.42 ± 46.43 µm, 493.48 ± 61.08 µm, and 482.59 ± 85.05 µm, respectively. The d(0.5) of R1, R2, and R3 have similar trends to that of D[4,3].

The diameter distance indicates the measurement width distribution. The smaller the value, the narrower the distribution. The average diameter distance of R1 was 1.75 ± 0.34. For R2, the average diameter distance was 1.93 ± 0.56. The average diameter distance of R3 was 2.08 ± 0.75. Thus, granules in R1 had the smallest difference from each other when the reactor reached a steady state.

Consistency indicates the proximity between D[4,3] and d(0.5). The smaller the consistence value, the closer the D[4,3] and d(0.5), and the better the granules similar to a ball. At the steady state, the average consistency of R1 was 0.54 ± 0.11. For R2, the average consistency was 0.59 ± 0.17. The average consistency of R3 was 0.64 ± 0.23. The shape of granules in R1 was more similar to a ball.

Clustering is a technology to find an internal structure between data. Data in the same cluster are the same as each other. The further analysis of the size characteristics of aerobic granules in R1, R2, and R3 using systematic clustering (Figure 4, Tables S3–S5), the results showed that the aerobic granulation process could be divided into three stages. The samples from day 57 to day 74 in the 3 reactors were in the same cluster, which was called the stable operation period. For R1, the group including the samples from day 13 to 36 was close to the group from day 1 to day 20 and day 40 and day 43, while the group including the samples from day 51 to 74 was relatively independent of the whole operation. For R2, the group including the samples from day 51 to 74 was close to that from day 1 to 11 and day 33 to 43, then both of them were close to that from day 13 to 24. For R3, the group including the samples from day 51 to 74 was close to that from day 4 to 20 and day 40 to 43, then both of them were close to the group from 18 to 36. Interestingly, the granules fed with acetate in a stable operation period showed more independence from other periods, compared with the granules fed with berberine wastewater.

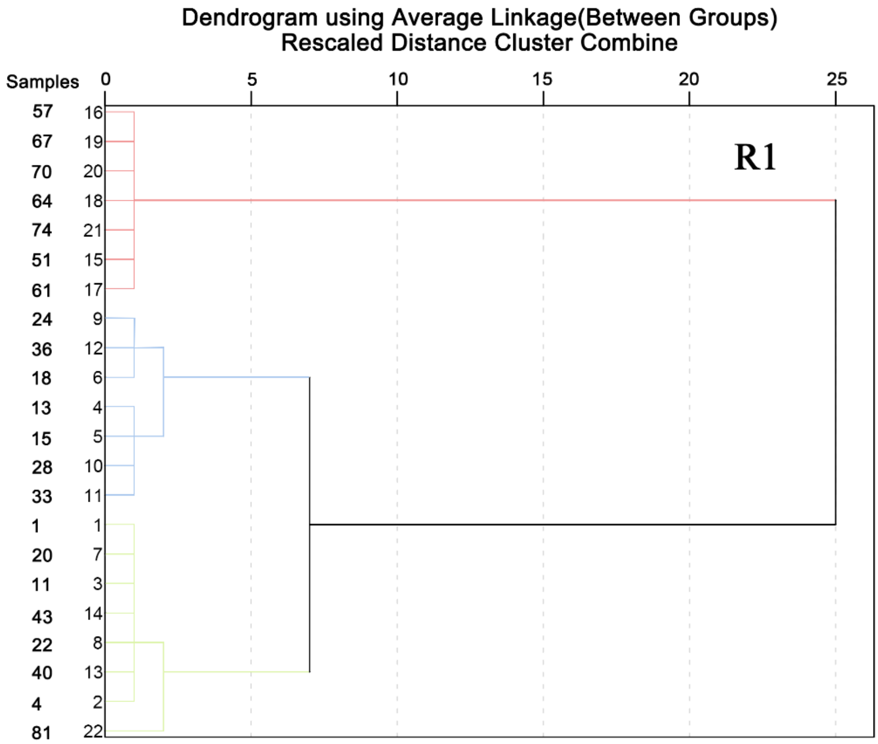

Figure 4. *Cont*.

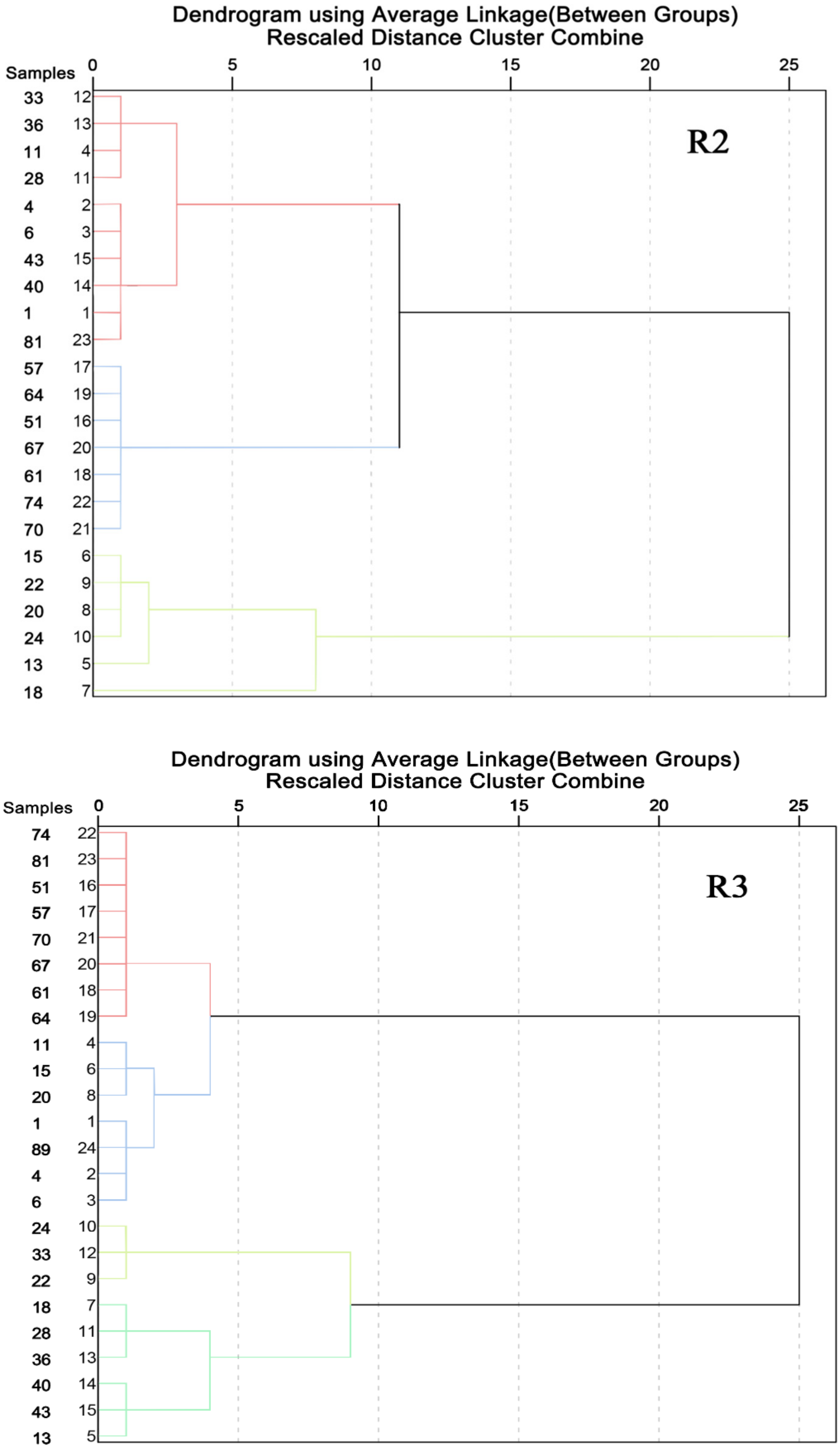

Figure 4. The clustering results of aerobic granules formation process (1) R1, (2) R2, (3) R3.

3.3. The Size Distribution of R1, R2, and R3

The aerobic granules grew under the pressure of decreased settling time and the substrate components. Figure 5 shows the size distribution of biomass in R1, R2, and R3. On day 1, the settling time was 30 min., all of the biomass in R1 and R2 was smaller than 0.3 mm, and 0.54% of the biomass was higher than 0.3 mm. From day 4 to 18, the settling time was 10 min. under the selection pressure, and R1, R2, and R3 showed almost the same size distribution. Then, the biomass grew in the sequence of R1, R2, and R3. The largest biomass size in R1 is 1.2–1.5 mm, while the largest biomass size in R2 is 1.0–1.2 mm, and the largest biomass size in R3 is 0.8–1.0 mm. On day 20, the decreased settling time (5 min) almost had no impact on the size distribution except for the biomass lower than 0.3 mm was washed out of the reactor. On day 51, the start of stable operation, the biomass with the size of 0.3–0.8 mm accounted for more than 50% volume. On day 81, granules with a size of 1.2–1.5 mm appeared in all three reactors. For R1, the biomass less than 0.3 mm occupied around 14.8% of volume, while the biomass less than 0.3 mm occupied 59.1% of volume in R3. Maybe the non-easy degradable substrate, industrial berberine wastewater, could not provide enough substance and energy for the microbial organisms to grow largely [32].

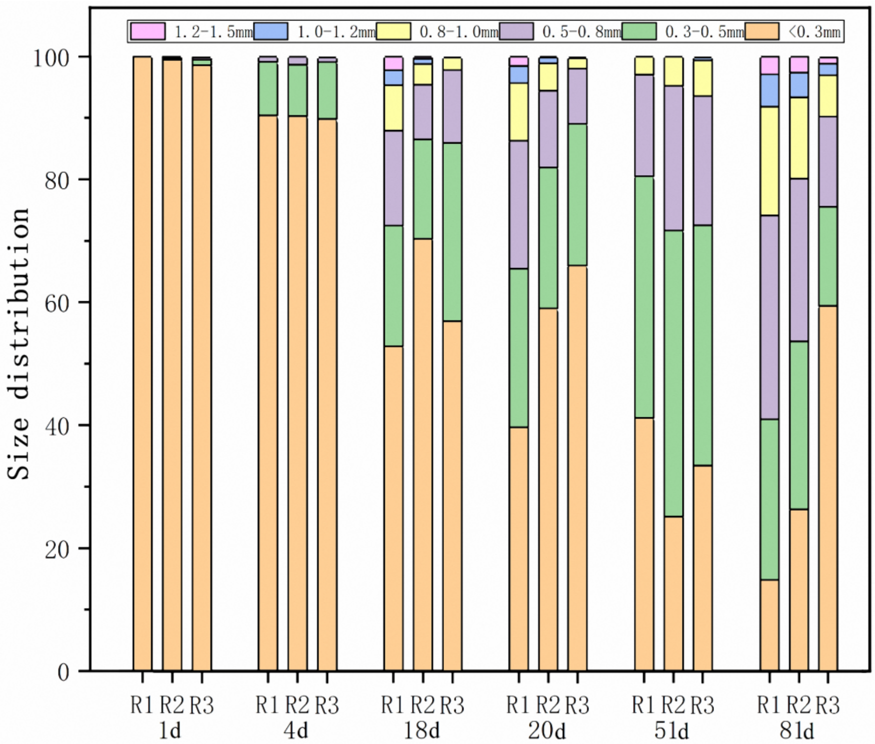

Figure 5. The granularity distribution in the formation process of aerobic granules in R1, R2, and R3.

3.4. The Change in the Shape Observation of Granules

The shape change in the aerobic granule was shown in Figure 6. The biomass in R1 grew quickly compared with the other two reactors. After 7 days of operation of the R1 reactor, aerobic granular sludge began to appear, but the reactor was still dominated by flocculent sludge. When the reactor was run for 21 days, flocculent sludge has been replaced by granular sludge. Maintaining the operating conditions of the reactors, it was observed that the particle size of granular sludge in the R1 reactor was gradually increasing, and the average particle size of the aerobic granular sludge was 600 µm after 80 days of operation, with a yellow, dense structure and smooth appearance. The biomass in R2 showed a heterogeneous appearance during the growth process. Finally, the color of aerobic granules completely changed to yellow. The aerobic granules in R2 had different sizes, and the average particle size is about 700 µm with good settling ability. A certain number of metazoans (rotifers, bell worms, etc.) on the surface of mature aerobic granular sludge were observed. The inoculated sludge of R3 was from a pharmaceutical wastewater treatment plant, with a loose structure and irregular shape. Aerobic granules appeared on day 15 with an average size of 200 µm. On day 40, the aerobic granules dominated in R3. In the steady state, the aerobic granules with different sizes coexisted in R3.

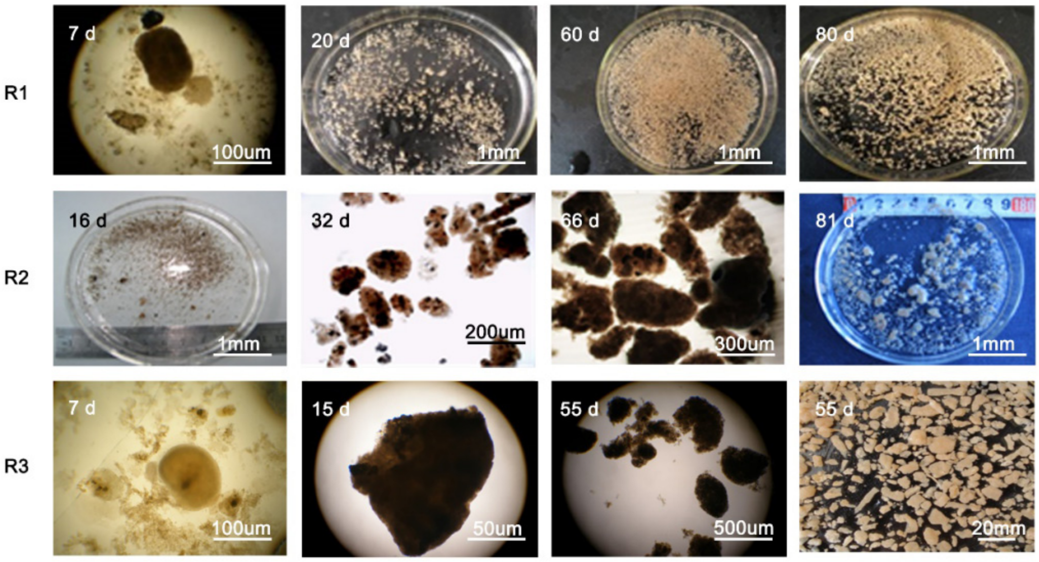

Figure 6. The image analysis of aerobic granules in the formation process in R1, R2, and R3.

The SEM observations of the matured aerobic granules in R1, R2, and R3 are shown in Figure 7. The granular sludge in R1 had a clear ellipsoid outer surface, and there is a certain amount of void structure for oxygen and organic matter transmission. The particle surface is mainly occupied by Cocci and Brevibacterium. On the surface of granular sludge in R2, the EPS was observed, which makes Cocci and Brevibacterium on the surface granular sludge closely bonded together. Compared with granular sludge in R1, the structure in R2 was more compact. This dense flora arrangement structure gave sludge good settling capability, biological activity, and impact resistance. The granular sludge in R3 was mainly composed of filamentous bacteria, which formed a loose granular sludge skeleton.

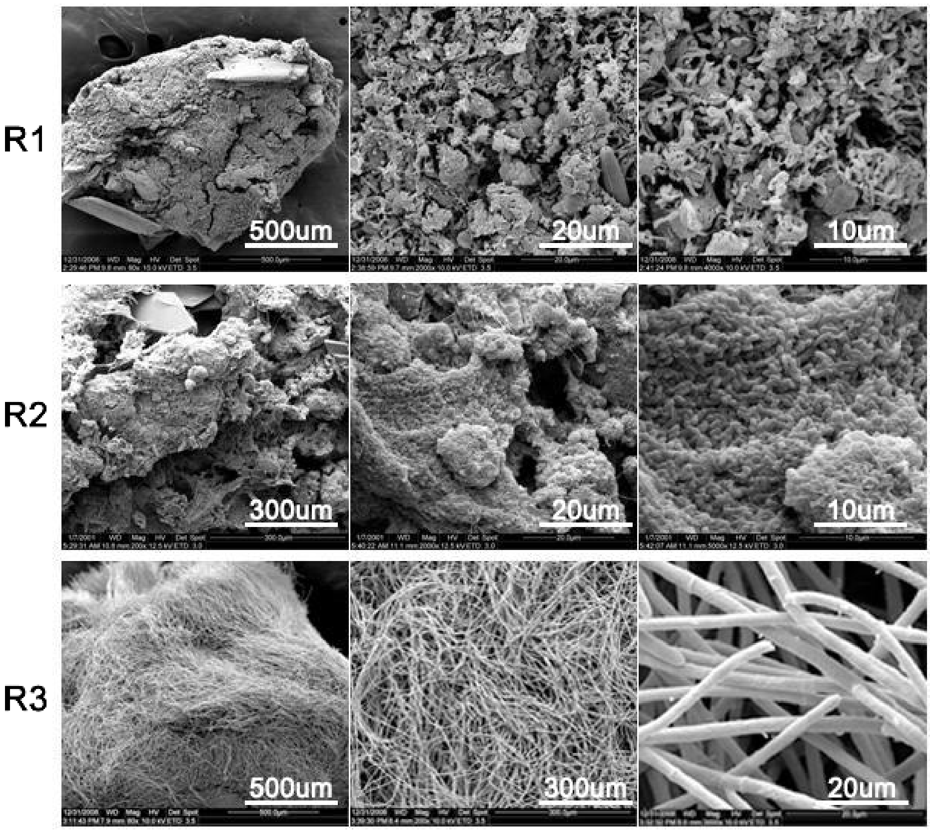

Figure 7. The SEM of matured aerobic granules in R1, R2, and R3.

4. Discussion

Aerobic granules have been successfully cultivated with a variety of substrates, including the easy degrading substrates, such as glucose [33] and acetate [34]; recalcitrant substrates, such as trichloroethylene [35]; textile wastewater [36]; toxic substrates, such as phenol [37]; and berberine [23]. In addition, aerobic granules could be developed on industrial wastewater, such as pharmaceutical wastewater [38] and brewery wastewater [39]. The type of substrates that provided the carbon source and nutrient affected the properties of aerobic granules. The granule size, microstructure and species diversity are related to the components of substrates [15]. Generally, the studies disclosed the shape of the granules, the average diameter of aerobic granules, which were the results of growth, and the relatively strong hydrodynamic shear force in aerobic reactors. No further exploration on the size distribution of the aerobic granules has been conducted.

Berberine is a kind of quaternary ammonium salt with anti-inflammatory properties which is related to the quaternary ammonium on the number C benzene ring [40]. The berberine with high concentration exerted toxicity effects on microbial organisms because the cytoplasmic membrane and deactivating enzymes of microbial cells are damaged.

Thus, in our studies, the aerobic granules R2 and R3 were cultivated with the mixture of BBR and acetate, and the effluent from ABR was fed with industrial berberine wastewater, which indicated more complicated components. For R1, acetate provided favorable food to the microbial organisms, the aerobic granules grew in a good state. For R2, the microbial organisms need to exhaust more energy to deal with the toxic food, but they obtained the compensation from acetate. For R3, the microbial organisms need to exhaust more energy to deal with the toxic food without compensation. That was why the COD removal efficiency of MLSS and MLVSS showed the order of R1 > R2 > R3.

The growth of the aerobic granules showed complicated phenomena. On one hand, the granules in R1 grew up more easily; on the other hand, it was easier to obtain a compact structure (Figure 7). For R3, the toxic food made them grow smaller and fluffy, which led to the loose structure of granules. So, the size of R1 < R2, R1 < R3, and R3 < R2. If we make a detailed observation of the 3 kinds of granules, the size of lower than 0.3 μm granules occupied 59.1% of total granules, although the average size of R1 was lower than that of R3. Combined with the diameter distance and consistency, the aerobic granules fed with an easily degradable substrate showed more even morphology and closer to a ball. SOUR and SVI also showed complicated results affected by several factors.

5. Conclusions

In this study, the aerobic granules were cultivated by the simulated acetate wastewater (R1), simulated berberine wastewater (R2), and effluent from the ABR reactor which was fed with industrial berberine wastewater (R3). The reactors exhibited different COD removal efficiencies, and MLSS and MLVSS values were affected by the different substrates which were in an order of R1 > R2 > R3. However, the SVI and SOUR, which were affected by several factors, showed more complicated results. The aerobic granules had the lowest microbial activity (SOUR), while the aerobic granules in R3 had the lowest settling ability (SVI) among the three kinds of granules. For the three reactors with different influent compositions, the aerobic granulation process displayed a three-stage process separately.

Compared with the granules fed with berberine wastewater, the granules fed with acetate in a stable operation period showed more independence from other periods. The size distribution analysis showed that the components of substrate impacted aerobic granules' size distribution. The aerobic granules with a range of 0.3–1.0 μm occupied 77.0%, 67.0%, and 35.7% volume for R1, R2, and R3, respectively. The biomass less than 0.3 μm occupied 59.1% volume in R3. The substrate had a great influence on the growth of aerobic granules. In conclusion, as a medicine with a toxic effect on microorganisms, berberine showed a great effect on the formation of aerobic granules. The further analysis of particle size data provided new insight into granule size dynamics during the aerobic granulation process.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr10040792/s1, Figure S1. The image of the aerobic granular system. A: SBR reactor, B: air pump, C: timer, D: influent pump, E: effluent pump, F: influent tank; Table S1 Physical and chemical properties of aerobic sludge as inoculum; Table S2: The composition of influent wastewater; Table S3: The characteristics of aerobic granules' diameter in R1; Table S4: The characteristics of aerobic granules' diameter in R2; Table S5: The characteristics of aerobic granules' diameter in R3.

Author Contributions: Conceptualization, P.Z. and M.C.; methodology, Y.L. and F.L.; investigation, Y.W. and F.L.; data curation, Y.W.; writing—original draft preparation, P.Z. and J.L.; writing—review and editing, M.C. and Y.Z.; project administration, Y.Z.; funding acquisition, M.C. and P.Z.; All authors have read and agreed to the published version of the manuscript.

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References

1. Beun, J.J.; van Loosdrecht, M.C.M.; Heijnen, J.J. Aerobic granulation in a sequencing batch airlift reactor. *Water Res.* **2002**, *36*, 702–712. [CrossRef]
2. Barr, J.J.; Cook, A.E.; Bond, P.L. Granule formation mechanisms within an aerobic wastewater system for phosphorus removal. *Appl. Environ. Microb.* **2010**, *76*, 7588–7597. [CrossRef] [PubMed]
3. Tay, S.T.L.; Zhuang, W.Q.; Tay, J.H. Start-up, microbial community analysis and formation of aerobic granules in a tert-butyl alcohol degrading sequencing batch reactor. *Environ. Sci. Technol.* **2005**, *39*, 5774–5780. [CrossRef] [PubMed]
4. Abdullah, N.; Ujang, Z.; Yahya, A. Aerobic granular sludge formation for high strength agro-based wastewater treatment. *Bioresour. Technol.* **2011**, *102*, 6778–6781. [CrossRef]
5. Beun, J.J.; Hendrik, S.A. Aerobic granulation in a sequencing batch reactor. *Water Res.* **1999**, *33*, 2283–2290. [CrossRef]
6. Sarma, S.J.; Tay, J.H. Aerobic granulation for future wastewater treatment technology: Challenges ahead. *Environ. Sci. Water Res. Technol.* **2018**, *4*, 9–15. [CrossRef]
7. Zhang, Y.; Tay, J.H. Toxic and inhibitory effects of trichloroethylene aerobic co-metabolism on phenol-grown aerobic granules. *J. Hazard Mater.* **2015**, *286*, 204–210. [CrossRef]
8. Adav, S.S.; Lee, D.J.; Ren, N.Q. Degradation of phenol by Acinetobacter strain isolated from aerobic granules. *Water Res.* **2007**, *41*, 2903–2910. [CrossRef]
9. Basheer, F.; Farooqi, H.J. Biodegradation of p-cresol by aerobic granules in sequencing batch reactor. *J. Environ. Sci.* **2012**, *24*, 2012–2018. [CrossRef]
10. Moghaddam, S.S.; Moghaddam, M.R.A. Aerobic granular sludge for dye biodegradation in a sequencing batch reactor with anaerobic/aerobic cycles. *Clean-Soil Air Water* **2016**, *44*, 438–443. [CrossRef]
11. Arrojo, B.; Mosquera-Corral, A.; Garrido, J.; Mendez, M.R. Aerobic granulation with industrial wastewater in sequencing batch reactors. *Water Res.* **2004**, *38*, 3389–3399. [CrossRef] [PubMed]
12. Awang, N.A.; Shaaban, M.G. Effect of reactor height/diameter ratio and organic loading rate on formation of aerobic granular sludge in sewage treatment. *Int. Biodeter. Biodegr.* **2016**, *112*, 1–11. [CrossRef]
13. Hu, L.; Wang, J.; Wen, X.; Yi, Q. Characteristic of the strain of simultaneous denitrification bacteria aerobic granule sludge in SBAR. *Process Biochem.* **2005**, *40*, 5–11.
14. Tsuneda, S.; Ejiri, Y.; Naganom, T.; Hirata, A. Formation mechanism of nitrifying granules observed in an aerobic upflow fluidized bed (AUFB) reactor. *Water Sci. Technol.* **2004**, *49*, 27–34. [CrossRef]
15. Liu, Y.; Tay, J.H. State of the art of biogranulation technology for wastewater treatment. *Biotechnol. Adv.* **2005**, *22*, 533–563. [CrossRef]
16. Wu, K.; Wu, P.; Xu, Y.; Li, Y.; Shen, Y. Formation mechanism of aerobic granular sludge and removal efficiencies in integrated ABR-CSTR reactor. *Huanjing Kexue* **2015**, *36*, 2947–2953.
17. Berberine. Available online: https://www.sciencedirect.com/topics/neuroscience/berberine (accessed on 19 February 2022).
18. Cui, X.; Zeng, P.; Qiu, G.L.; Liu, Y.; Song, Y.H.; Xie, X.; Han, L. Pilot-scale treatment of pharmaceutical berberine wastewater by Fenton oxidation. *Environ. Earth. Sci.* **2015**, *73*, 4967–4977. [CrossRef]

19. Qin, W.W.; Song, Y.H.; Dai, Y.; Qiu, G.L.; Ren, M.; Zeng, P. Treatment of berberine hydrochloride pharmaceutical wastewater by O3/UV/H2O2 advanced oxidation process. *Environ. Earth Sci.* **2015**, *73*, 4939–4946. [CrossRef]
20. Li, Y.; Yin, Y.M.; Wang, X.Y.; Wu, H.; Ge, X.Z. Evaluation of berberine as a natural fungicide: Biodegradation and antimicrobial mechanism. *J. Asian Nat. Prod. Res.* **2017**, *20*, 148–162. [CrossRef]
21. Othman, M.S.; Safwat, G.; Aboulkhair, M.; Abdel Moneim, A.E. The potential effect of berberine in mercury-induced hepatorenal toxicity in albino rats. *Food Chem. Toxicol.* **2014**, *69*, 175–181. [CrossRef]
22. Qiu, G.L. Hybrid UASB-MBR Process for Berberine Pharmaceutical Wastewater Treatment and the Process Mechanisms. Ph.D. Thesis, Beijing Normal University, Beijing, China, 2011.
23. Qiu, G.; Song, Y.H.; Zeng, P.; Duan, L.; Xiao, S.H. Characterization of bacterial communities in hybrid upflow anaerobic sludge blanket (UASB)-membrane bioreactor (MBR) process for berberine antibiotic wastewater treatment. *Bioresour. Technol.* **2013**, *142*, 52–62. [CrossRef] [PubMed]
24. *Standard Methods for the Examination of Water and Wastewater*, 21st ed.; American Public Health Association: Washington, DC, USA, 2005.
25. Zeng, P.; Moy, B.; Song, Y.H.; Tay, J.W. Biodegradation of dimethyl phthalate by *Sphingomonas* sp. isolated from phthalic-acid-degrading aerobic granules. *Appl. Microbiol. Biotechnol.* **2008**, *80*, 899–905. [CrossRef] [PubMed]
26. Keerio, H.A.; Bae, W. Experimental investigation of substrate shock and environmental ammonium concentration on the stability of ammonia-oxidizing Bacteria (AOB). *Water* **2020**, *12*, 223. [CrossRef]
27. Liu, S.; Zhang, Y.; Zeng, P.; Wang, H.; Song, Y.; Li, J. Isolation and characterization of a bacterial strain capable of efficient berberine degradation. *Int. J. Environ. Res. Public Health* **2019**, *16*, 646. [CrossRef]
28. Tay, S.T.L.; Moy, B.Y.P.; Jiang, H.L.; Tay, J.H. Rapid cultivation of stable aerobic phenol-degrading granules using acetate-fed granules as microbial seed. *J. Biotechnol.* **2005**, *115*, 387–395. [CrossRef]
29. Verawaty, M.; Tait, S.; Pijuan, M.; Yuan, Z.G.; Bond, P.L. Breakage and growth towards a stable aerobic granule size during the treatment of wastewater. *Water Res.* **2013**, *47*, 5338–5349. [CrossRef]
30. Shi, R.Q.; Pan, A.D. The application of Mastersizer 2000 in the environmental evolution. *J. Heze Univ.* **2005**, *27*, 56–61.
31. .Shu, X.; Wu, Y.C.; Tao, Q.X.; Cheng, J.G.; Xia, Y.H. An analysis on report of Mastersizer 2000 laser particle size analyzer. *Exp. Technol. Manag.* **2011**, *28*, 37–41.
32. Pronk, M.; Abbas, B.; Al-zuhairy, S.H.K.; Kraan, R.; Kleerebezem, R.; van Loosdrecht, M.C.M. Effect and behaviour of different substrates in relation to the formation of aerobic granular sludge. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 5257–5268. [CrossRef]
33. Tay, J.H.; Pan, S.; Tay, S.T.L.; Ivanov, V.; Liu, Y. The effect of organic loading rate on the aerobic granulation: The development of shear force theory. *Water Sci. Technol.* **2003**, *47*, 235–240. [CrossRef]
34. Moy, B.Y.P.; Tay, J.H.; Toh, S.K.; Liu, Y.; Tay, S.T.L. High organic loading influences the physical characteristics of aerobic sludge granules. *Lett. Appl. Microbiol.* **2002**, *34*, 407–412. [CrossRef] [PubMed]
35. Zhang, Y.; Tay, J.H. Co-metabolic degradation activities of trichloroethylene by phenol-grown aerobic granules. *J. Biotechnol.* **2012**, *162*, 274–282. [CrossRef] [PubMed]
36. Franca, R.D.G.; Ortigueira, J.; Pinheiro, H.M.; Lourenco, N.D. Effect of SBR feeding strategy and feed composition on the stability of aerobic granular sludge in the treatment of a simulated textile wastewater. *Water Sci. Technol.* **2017**, *76*, 1188–1195. [CrossRef] [PubMed]
37. Jiang, H.L.; Tay, J.H.; Tay, S.T.L. Changes in structure, activity and metabolism of aerobic granules as a microbial response to high phenol loading. *Appl. Microbiol. Biotechnol.* **2004**, *63*, 602–608. [CrossRef]
38. Cai, P.; Chen, Y.; Jiang, S.Y.; Wu, Z.; Xie, J.; Jiang, S. Treating high concentration pharmaceutical wastewater with aerobic granular sludge involves inoculating activated sludge in batch reactor, adding zeolite powder and pumping in pharmaceutical wastewater into batch reactor. Patent Application No. CN 105858873 B, 12 April 2016.
39. Wang, S.G.; Liu, X.W.; Gong, W.X.; Gao, B.Y.; Zhang, D.H.; Yu, H.Q. Aerobic granulation with brewery wastewater in a sequencing batch reactor. *Bioresour. Technol.* **2007**, *98*, 2142–2147. [CrossRef]
40. Zheng, H. Experimental and Theoretical Studies of Protoberberine Alkaloids on the Mechanism of Multiple Activities. Ph.D. Thesis, Tianjin Medical University, Tianjin, China, 2004.