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The acceleration of sludge granulation using the chlamydospores of *Phanerochaete* sp. HSD

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ABSTRACT

In the present paper, a novel method to accelerate sludge granulation is presented. Inoculation with chlamydospores of *Phanerochaete* sp. HSD accelerated sludge granulation during the treatment process of phenol wastewater, and the sludge granulation rate reached 66 ± 2% on day 7, 32 days earlier than that of the control inoculated with activated sludge only. Aerobic granule in R1 (AG_{R1}) showed an annual ring-like multilayer structure and a primary core also existed in the nuclear area of the granule. The mechanism of rapid granulation revealed that the chlamydospore could survive in phenol wastewater and form the primary matrix on which aerobic granule was developed layer by layer. In addition, AG_{R1} developed in a phenol uptake system to counteract the adverse effects of phenol inhibition. Higher tolerance toward wastewater with high phenol strength was exhibited, and the maximum specific phenol degradation rate reached 1.54 g phenol g^{-1} VSS day^{-1}.

1. Introduction

Extensive studies have been conducted on aerobic granulation in biological wastewater treatment since the 1990s. In contrast with conventional activated sludge, aerobic granule features many advantages such as better settling ability, stronger microbial structure and higher capability to withstand shock loads [1]. Aerobic granule is also believed to have the potential to respond to the challenges of pollutant removal from wastewater. The capability of the granule to treat heavy metal, phenol, and high strength organic wastewaters has been tested [1–3].

One of the bottlenecks impeding the application of aerobic granule is the need to reduce the start-up period of aerobic granulation system. Numerous reports [4–6] indicate that a long period of time is needed to culture mature aerobic granules. Full granulation in the reactor may take more than 30 days even using synthetic wastewater [7]. Sludge granulation becomes more difficult during treatment of real wastewaters, especially those with high strength or toxicity. Jiang et al. [8] reported that mature aerobic granules appeared on week 5 in the treatment process of phenol wastewater; Wang et al. [9] found that stable granules with sizes of 2–7 mm were obtained in a reactor fed with brewery wastewater after a nine-week operation. In addition, aerobic granules were formed after 75 days during the treatment process of saline wastewater [10]. A considerably longer period for granulation may be required for large-scale operations, although no granulation period has been reported under a full-scale plant setting. Several studies have focused on accelerating the granulation process with activated sludge as inoculum. However, the improvement achieved was still limited [7,11,12].

In the present work, a novel method to accelerate sludge granulation was evaluated. By inoculating chlamydospores of strain HSD to the sequencing batch reactor (SBR), aerobic granules were rapidly cultivated during the treatment process of phenol wastewater. These granules possessed higher tolerance and better biodegradation ability in relation to phenol. In addition, frozen dissection technique (FDT) was used to investigate the sludge granulation mechanism. Therefore, this study could provide a new approach for the rapid development of aerobic granular sludge for the treatment of wastewater, especially containing phenolic compounds.

2. Materials and methods

2.1. Chemicals and microorganism

All chemicals used were of spectral or analytical grade. *Phanerochaete* sp. HSD was obtained from Laboratory of Applied and Environmental Microbiology, Henan Normal University, China.

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2.2. Preparation of chlamydospores

Strain HSD was incubated on a potato dextrose agar (PDA) plate and sub-cultured for 3 days at 35 °C. Subsequently, mycelial suspension was prepared and used as the inoculum for further studies. The mycelial suspension (2 ml) was inoculated to a 250 ml flask containing 100 ml liquid medium composed of the following: 10.0 g/l glucose, 2.5 g/l diammonium tartrate, 2.0 g/l KH_2PO_4, 0.8 g/l $MgSO_4{\cdot}7H_2O$, 0.3 g/l $MnSO_4$, 0.5 g/l $CaCl_2$ and 0.7% trace element solution. The trace element solution includes: 0.5 g/l Glycine, 1.0 g/l NaCl, 0.1 g/l $FeSO_4{\cdot}7H_2O$, 0.1 g/l $CoSO_4$, 0.1 g/l $ZnSO_4{\cdot}7H_2O$, 10 mg/l $CuSO_4{\cdot}5H_2O$, 10 mg/l $AlK(SO_4)_2{\cdot}12H_2O$, 10 mg/l H_2BO_3 and 10 mg/l $Na_2MoO_2{\cdot}2H_2O$. The flask was placed in a shaking incubator with a speed of 150 rpm at 35 ± 1 °C. After three days, the cultures were centrifuged at 5000 rpm and the mycelial pellets were harvested, lyophilized, and ground in a mortar. The mortar was washed with sterile water to obtain the chlamydospore suspension of strain HSD.

2.3. Operation of reactors

Tests were carried out in an SBR system. The system, under the control of a microcomputer timer switch, was composed of two 4 l cylindrical SBRs (55 cm in height and 10 cm in diameter). Both SBRs were equipped with thermometer, pH meter, dissolved oxygen (DO) analyzer, heater, aerator, and stirrer. The sludge inoculation process was as follows. At 30 °C, one reactor (R1) was inoculated with 10 ml of chlamydospore suspension containing 0.5 g chlamydospores and 1 l activated sludge (MLSS 12 g/l) obtained from the Xinxiang Municipal Wastewater Treatment Plant, China. Another reactor (R2), as the control, was inoculated with 1 l activated sludge. During the start-up phase (day 1–2), the reactors were fed with 1 l synthetic wastewater containing the following: 2.0 g/l glucose, 0.7 g/l phenol, 2.0 g/l NH_4Cl, 2.0 g/l KH_2PO_4, 0.8 g/l $MgSO_4{\cdot}7\ H_2O$, 0.1 g/l $CaCl_2$ and 0.5 mg/l $MnSO_4$. After two days, glucose was gradually removed from the synthetic wastewater and the influent volume was increased from 1 to 2 l. The influent pH, superficial upflow air velocity, DO concentration, and rotary speed of the stirrer were controlled at the levels of 6.5, 3.0 cm/s, 4.6 mg/l, and 50 rpm, respectively. Operational phases of SBR were as follows: feeding phase (10 min); aerobic phase (4.5 h); settling phase (30 min); and discharge and idle phases (50 min).

2.4. Surface and interior structure of granule

The surface and interior of aerobic granule were observed by scanning electron microscope (SEM, AMRAY-1000B, Japan) and transmission electron microscope (TEM, JEM-1400, Japan), respectively. Furthermore, FDT was used to investigate the interior structure of the granule. Aerobic granule was placed on a metal chuck and rapidly frozen to −20 °C in a refrigerator. At this temperature, the granule became rock hard and easy to dissect. The actual structure however, was maintained. After the freezing, the aerobic granule was anatomized by a dissecting scalpel under an anatomical microscope to view the interior structure of the granule.

2.5. Analytical methods

Chemical oxygen demand (COD), phenol, mixed liquor volatile suspended solids (MLVSS), mixed liquor suspended solids (MLSS), and sludge volume index (SVI) were analyzed according to standard methods [13]. Particle size was determined using an image analysis system (Image-Pro Plus, V4.0, Media Cybernetics) with an Olympus SZX9 microscope. The chlamydospores were counted using the direct count method in a blood cell counting chamber. Extracellular polymer substances (EPS) in the granules were analyzed according to an earlier method [14]. Saccharide content was measured using the phenol/sulfuric acid method [15] and protein content was measured by the modified Lowry method [16].

The granulation rate (GR) of sludge was measured according to the method described by Wang et al. [17]. Aerobic granules were separated from flocculent sludge using a sieve (Q200-R20/3, Xinxiang, China). The weight of aerobic granules ($W1$) and total weight of sludge sample ($W2$) were obtained, and GR was calculated as

$$\mathrm{GR}(\%) = \frac{W1}{W2} \times 100 \quad (1)$$

The physical strength of granule was measured. The integrality coefficient (%), defined as the ratio of the weight of residual granules vs. the total weight of granules after 5 min of shaking at 200 rpm on a platform shaker, was measured using the method of Ghangrekar et al. [18]. A lower integrality coefficient means greater granular strength.

Specific phenol degradation rates were calculated from data of phenol degradation time course tests. Separate batch tests were conducted at different initial phenol concentrations. The kinetic analysis of phenol degradation was performed based on Haldane's equation for an inhibitory substrate [19]:

$$V = \frac{V_{\max} S}{K_s + S + S^2/K_i} \quad (2)$$

where V and $V_{\max}$ (g phenol g^{-1} VSS day^{-1}) are the specific phenol degradation rate and maximum specific phenol degradation rate, respectively; S (mg/l) is the phenol concentration; K_s (mg/l) is the half-saturation constant; and K_i (mg/l) is the inhibition constant.

3. Results and discussion

3.1. Preparation of chlamydospores

Most spores produced by microbes are resistant to high temperatures, humidity, and other unfavorable conditions. Generally, spores fully develop after a state of dormancy or hibernation. Chlamydospore is the resting spore of several kinds of fungi. A higher Mn^{2+} concentration (1.67–2.33 mmol/l) was proven to be capable of inducing the overproduction of chlamydospores of the strain HSD in our previous report [20]. Thus, the liquid medium containing 0.3 g/l of $MnSO_4$ was used to prepare the chlamydospores. After 3-day incubation, small mycelial pellets (Fig. 1a) with the diameter of 0.2–0.9 mm appeared in the flasks. These pellets were composed of a large number of chlamydospores (Fig. 1a and b). The SEM image of normal hyphae of strain HSD (Fig. 1c) shows that the morphologies of normal hypha and chlamydospore are different. Normal hypha is filamentous whereas chlamydospore is a global or elliptic reproductive cell with a thick wall. The lyophilized mycelial pellets were ground and the suspension containing a large number of chlamydospores and a few chlamydospore masses was prepared. After counting under the microscope, approximately 6×10^7 chlamydospores were found in 1 ml suspension.

3.2. Cultivation of aerobic granules

During the start-up phase, microbes in both R1 and R2 grew rapidly. The color of sludge gradually changed from brown to yellow. The MLSS curves of the two reactors (Fig. 2a) indicate a sharp decline during the start-up phase because majority of the dispersed sludge with poor settling ability were washed out with the effluent. After day 4, the MLSS concentration in both reactors started to ascend, and stabilized after day 12. The MLSS concentration in R1 was higher than in R2 throughout the test period. The effluent SS concentrations in both reactors dropped sharply during the initial 15 days, and stabilized in the remaining period. The SVI in both reac-

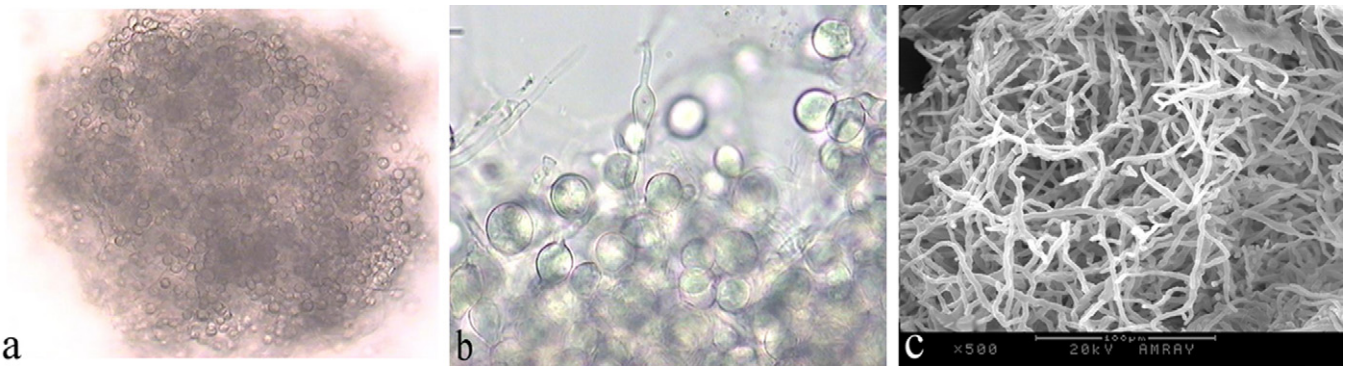

Fig. 1. Morphologies of chlamydospores and hyphae of strain HSD. (a) The overview of small mycelial pellet forming in the liquid medium containing 0.3 g/l of $MnSO_4$ (40×); (b) the micrograph of chlamydospores (100×); (c) SEM image of normal hyphae (500×).

tors (Fig. 2b) improved during the operation. After day 7, SVI in R1 (27–40 ml/g) was significantly lower than that in R2 (70–118 ml/g).

On day 3, small aerobic granules were visible in R1. A large number of aerobic granules (AG_{R1}) appeared on day 5. The sludge GR reached 66 ± 2% (Fig. 3a) on day 7. The granular size range (in %) during the initial 12 days is illustrated in Fig. 3b. By day 9, the granules were composed of 74% of sludge in R1. The COD removal in R1 reached 98% after a 6 h treatment after day 3, as the influent phenol concentration increased from 200 to 900 mg/l. Even after a 150-day operation, the same COD removal performance was maintained. Aerobic granules remained stable during this period, although a few large size granules (6.0–7.0 mm in diameter) appeared in the reactor after day 85.

In R2, several flocs with the diameter of 2.1–5.0 mm appeared on day 14. On day 30, the sludge started to show a granulation trend. Aerobic granules (AG_{R2}) with an average diameter of 1.2 mm developed in the reactor on day 39 with a GR of 37 ± 1%. As mentioned above, the appearance of granules in R1 is about 32 days earlier than that in R2. The only difference between the two reactors is that R1 was inoculated with the chlamydospores. Therefore, the rapid sludge granulation is attributed to chlamydospores.

3.3. Surface and interior structure of granule

On day 11, AG_{R1} was in spherical or spheroidal shape with compact appearance. Two types of granules (i.e. white and celadon colored) were observed in R1 (Fig. 4a). The difference in color was due to different types of microbes distributed on the granule surface. Observation of microbes on the surface through SEM (Fig. 4b) showed that AG_{R1} had an uneven appearance, with many spheroidal and filamentous microorganisms on its surface (Fig. 4c). Inside the granule, filamentous fungi were dominant (Fig. 4d). However, some spheroidal fungi (Fig. 4e) and bacteria (Fig. 4f) were observed as well.

In the present study, the interior structure of AG_{R1} was studied by FDT. A big core (C_b) was separated (Fig. 5a) after a granule was dissected. Mucilaginous liquid substances, or EPS [14], enveloped the white C_b (Fig. 5b). After removing these dopes, C_b was frozen and dissected. The AG_{R1} formed had an annual ring-like multilayer structure when viewed its cross section (Fig. 5c). In addition, a small core (C_s) appeared in the nuclear area of the granule. Because the more diminutive nucleus could not be found inside the C_s even under microscope, the C_s was believed to be the primary core. Thus, we hypothesized that aerobic granule from R1 developed layer by layer on the basis of a primary core. In addition, FDT was applied on AG_{R2}. However, AG_{R2} did not have the annual ring-like multilayer structure and also, no obvious small core was found in its interior.

3.4. Mechanism of accelerated granulation with chlamydospores

A primary core existed in the interior of AG_{R1} but not in AG_{R2}. The only difference between R1 and R2 is that R1 was inoculated with the chlamydospore. Thus, the chlamydospore probably induced the core formation. A test was conducted to validate whether the chlamydospore could induce the formation of the primary core. Two groups of SBRs (G1 and G2), each with a volume of 0.5 l, were used. Each reactor contained 250 ml wastewater with phenol concentration of 1.0 g/l, and 50 ml activated sludge. The G1 consisting of two reactors (R1 and R2) was specially inoculated with the diluted chlamydospore suspension (0.1 ml). G2 (R3 and

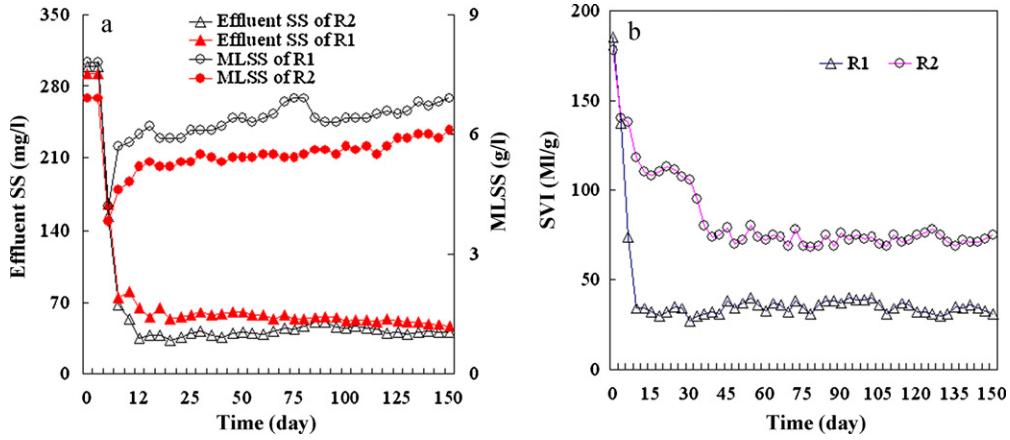

Fig. 2. Variations of MLSS, effluent SS and SVI of sludge in R1 and R2. (a) MLSS and effluent SS varied with time; (b) SVI varied with time.

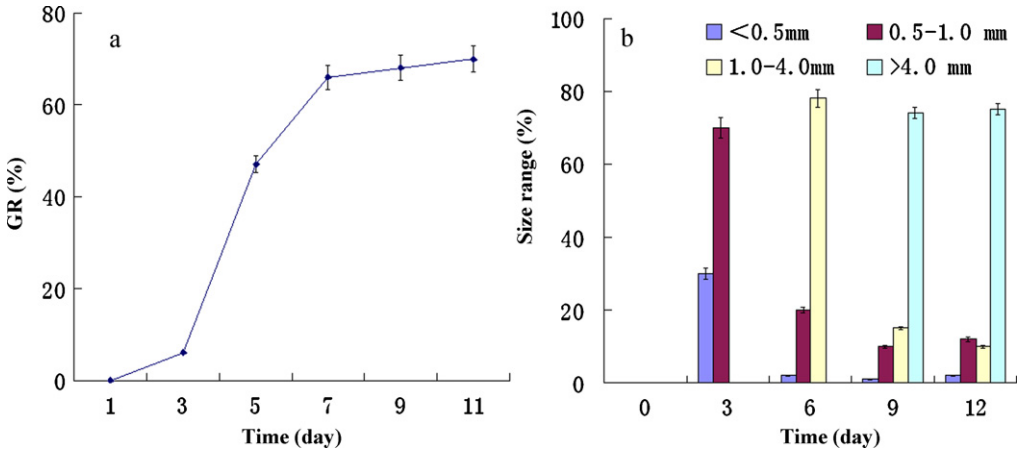

Fig. 3. GR and size range of aerobic granules in R1 during initial 12 days. (a) GR varied with time; (b) size range varied with time.

R4) was used as the control. After a 3-day incubation, almost all of the chlamydospores in G1 (Table 1) has germinated and formed miniature spherules with the size of 0.1–0.5 mm. This indicates that the chlamydospores survived in the reactor and formed cores. No spherule appeared in G2, indicating that the spherules or initial cores were not formed by inoculated activated sludge. These facts imply that it is a specific method to induce the formation of spherules using the chlamydospores. The resistance of chlamydospores to 1.0 g/l of phenol facilitated the survival of strain HSD in phenol wastewater, and the effective self-immobilization ability of chlamydospores resulted in the appearance of numerous spherules in the reactor.

Another test was conducted to investigate whether the spherule could provide the primary core for granulation. All the spherules from G1 were stained by colorizing using crystal violet, a dye which can integrate with the cell wall of microbes. These marked spherules were returned to G1 and cultivated again. Four days later, the aerobic granules were randomly picked out. The granules (Fig. 5d) are white, and thus the purple cores (the marked spherules) can be easily identified inside the granules under the microscope. This indicates that the granules are developed on the basis of the marked spherules. In summary, granulation of AG_{R1} was probably attained due to that (a) the chlamydospores survived in the phenol wastewater and miniature spherules were formed; and subsequently (b) the spherules served as primary cores for the development of aerobic granules.

Liu and Tay [21] have pointed out that the appearance of primary matrixes is a very crucial step in initiating activated sludge gran-

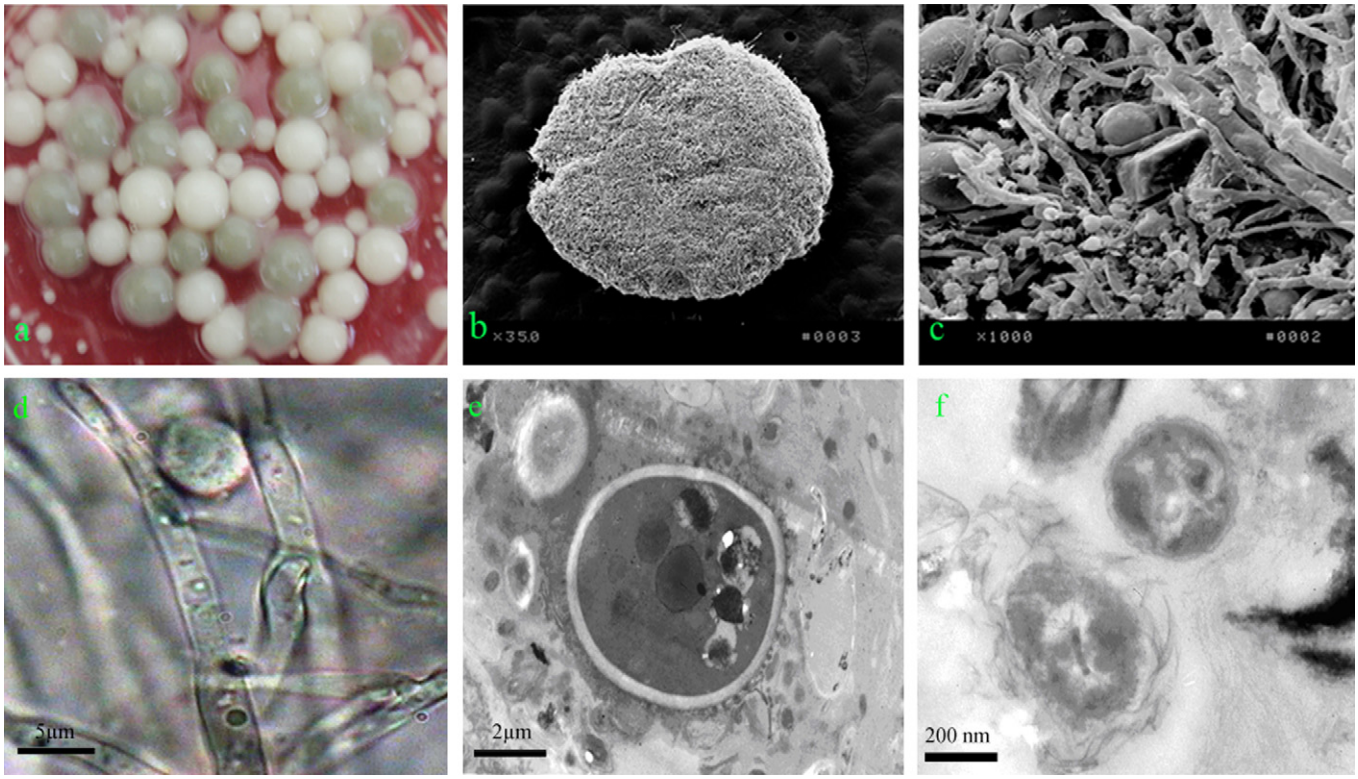

Fig. 4. Images of aerobic granules in R1 on day 14. (a) The overview of aerobic granule; (b) SEM image of aerobic granule (35×); (c) microbes on the surface of aerobic granule (1000×); (d) microbes in the interior of aerobic granule; (e) TEM image of orbicular fungus in the interior of aerobic granule; (f) TEM image of bacteria in the interior of aerobic granule.

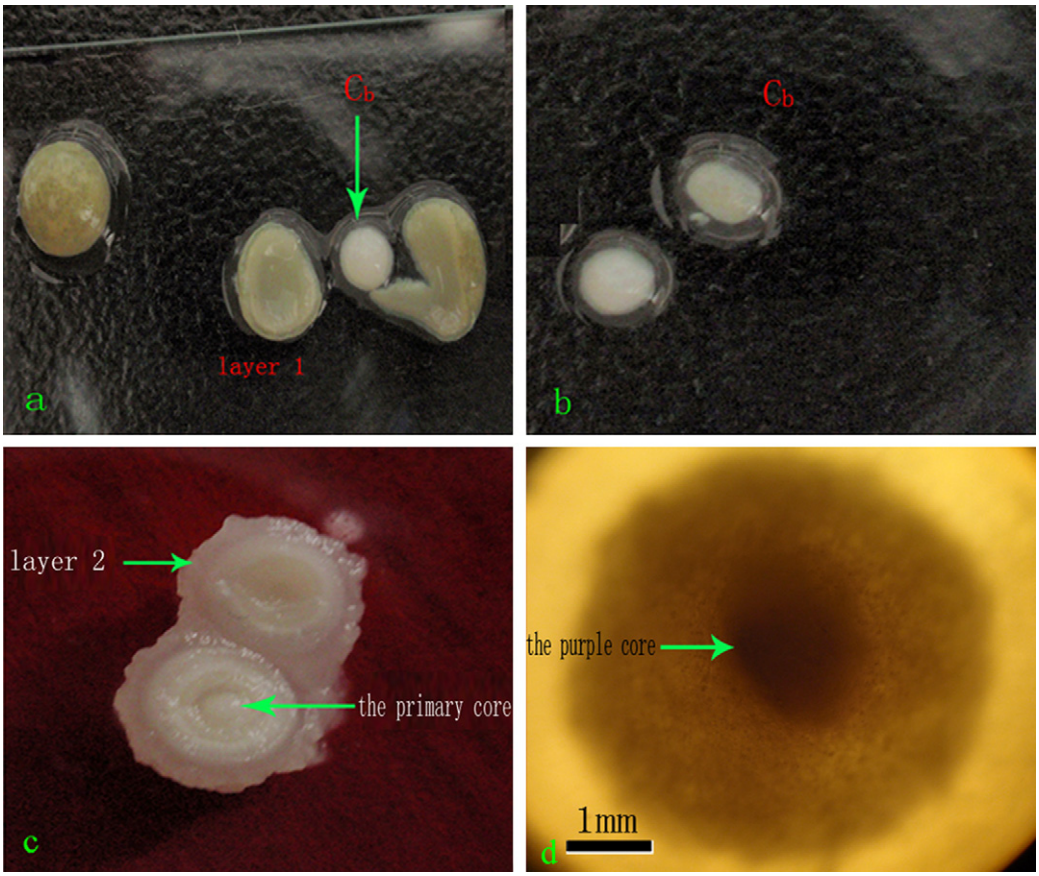

Fig. 5. The interior structure of aerobic granule (a) aerobic granule (day 27) after the dissection; (b) the overview of C_b; (c) the annual ring-like multilayer structure of C_b; (d) micrograph of aerobic granule (day 5) induced by the primary core in the reactor.

ulation. The primary core in AG_{R1} obtained in the present study presents a convincing evidence for their hypothesis which states that sludge granulation can be accelerated if the primary matrix is provided rapidly. The spherules or the primary cores appeared in R1 on day 3 in the present work. This rapid core appearance accelerated sludge granulation. In R2, the AG_{R2} did not have obvious cores. The granulation in R2 occurred at a considerably slower rate than R1, indicating that the addition of chlamydospores accelerated the granulation process.

3.5. *P/S* ratio and granule size

Both aerobic granule and C_b were enveloped by EPS (Fig. 5). The presence of EPS in granules has been studied in relation with structural integrity of aerobic granules [22,23]. Cai et al. [24] have reported that the ratio of protein to total saccharide (*P*/*S*) increases by 2.5 times during the formation of aerobic granule. In the present work, the same result was obtained. *P*/*S* ratios of AG_{R1} and AG_{R2} reached 6.49 and 4.27 respectively. These are 3.1 and 2.0 times higher compared with that of activated sludge (2.09). Statistical analysis indicates that *P*/*S* ratio is correlative with granule size in the granulation process of sludge in R1, and the correlation is significant at the 0.01 level (Fig. 6).

A test was conducted to explain why *P*/*S* ratio increased with granule size. After the dissection, aerobic granules with different sizes were divided into two parts (*P*1 and *P*2). *P*1 consists of the exterior layers (layer 1 + layer 2) and *P*2 contains the interior matrix (the remaining parts except layers 1 and 2). The *P*/*S* ratios of the

Table 1
The numbers of chlamydospores, spherules and mature aerobic granules in reactors.

Parameters	G1		G2	
	R1	R2	R3	R4
Number of chlamydospores seeded to reactor	300	110	0	0
Number of spherules appearing in reactor	299	108	0	0
Number of mature aerobic granules appearing in reactor	299	108	0	

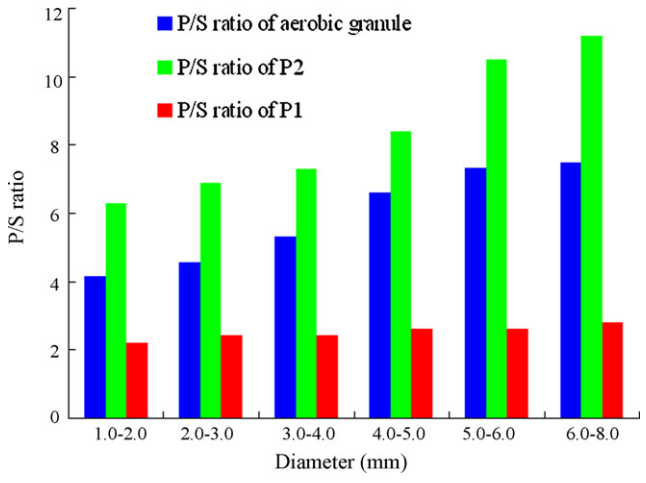

Fig. 6. *P*/*S* ratios of *P*1, *P*2 and aerobic granules (day 40) varied with granule size.

two parts were determined. For the granules with different sizes, *P*/*S* ratios of *P*1 were stable (2.2–2.8). However, the *P*/*S* ratios of *P*2 (6.3–11.2) which increased with granule size were significantly higher than those of *P*1, suggesting that proteins, rather than saccharides, were enriched in the interior matrix of the granule. The difference of microbial compositions in *P*1 and *P*2 probably influenced the *P*/*S* ratio. Fig. 4c and 4d show that filamentous fungi are the dominant microbes in *P*2, and *P*1 contains more bacteria. The bacterial *P*/*S* ratio is considerably higher than those of yeast and filamentous fungi [25]. Thus the more bacteria in *P*1 would result in the higher *P*/*S* ratio. In addition, the dominance of fungi in *P*2 would result in a lower *P*/*S* ratio. However, this is inconsistent with the determined *P*/*S* ratio data in the present work. Thus, the difference of *P*/*S* ratios of *P*1 and *P*2 cannot be explained from the aspect of microbial composition. However, saccharide consumption by microbes in the granule interior is reasonable, because microbes in a starvation state will use any potential carbon source to compensate the substrate shortage. Microbes on the granular surface can obtain substrates more easily. Therefore, the consumption of EPS such as saccharides mainly occurred inside the granule. This can explain why the *P*/*S* ratio of *P*1 was low and stable. The number of starving microbes in the granule increases with the volume accretion of *P*2, caused by the augmentation of granule size. The microbes consume an increasing amount of carbohydrates from EPS, which leads to the increasing *P*/*S* ratio of *P*2, and finally a high *P*/*S* ratio of the whole aerobic granule. This observation is interesting because researchers [24] have reported that higher *P*/*S* ratio causes changes in the surface characteristics of aerobic granules, such as the decrease of surface negative charge and the increase of hydrophobicity. The results of the present study indicate that the higher *P*/*S* ratio of interior matrix caused the increasing *P*/*S* ratio of the whole granule.

3.6. Comparison of characteristics between AG_{R1} and AG_{R2}

Sludge characteristics of AG_{R1} and AG_{R2} are compared in Table 2. The AG_{R1} has more advantages over AG_{R2} such as better settling performance, higher VSS/TSS, more compact microbial structure, and lower water content. Especially, the granulation period in R1 is much shorter than that in R2. The biodegradation performance of the granules from R1 and R2 in relation to phenol was compared using a batch experiment at different initial phenol concentrations based on the phenol removal. More rapid decrease in phenol concentration was observed in R1 (Fig. 7a) than in R2 (Fig. 7b). The maximum specific degradation rate of AG_{R1} reached 1.54 g phenol g^{-1} VSS day^{-1} at 450 mg/l of phenol. In addition, AG_{R1} remained stable at phenol concentration as high as 2200 mg/l, and phenol removal reached 83.4% after a 32 h treatment. Specific phenol degradation rates of AG_{R1} at different phenol concentrations are shown in Fig. 7c. The higher values of K_s (540 mg/l) and K_i (270 mg/l) obtained from the kinetic analysis of phenol degradation show that AG_{R1} developed a phenol uptake system to counteract the adverse effects of phenol inhibition. AG_{R2} could degrade phenol at a maximum specific rate of 0.88 g phenol g^{-1} VSS day^{-1} when the initial phenol concentration was 200 mg/l. After a 16 h treatment, 95% removal efficiency was achieved in the reactor only when phenol concentration was below 1000 mg/l. The significantly deteriorated performance of the reactor, expressed by such consequence as the breakup of the granules, was observed in R2 at the phenol concentration above 1800 mg/l.

AG_{R1} possesses the better tolerance and biodegradation ability toward phenol wastewater in contrast with AG_{R2} and the

Table 2
Comparison of sludge characteristics of AG_{R1} (day 7) and AG_{R2} (day 39).

Parameters	AG_{R1}	AG_{R2}
Culture time (day)	7	39
Mean diameter (mm)	3.7	1.2
SVI (ml/g)	32 ± 2	74 ± 5
VSS/TSS (%)	91.1 ± 0.3	89.7 ± 0.2
Density (g/cm^3)	1.0054 ± 0.1	1.0037 ± 0.1
Integrality coefficient (%)	0.0865	0.0912
Water content (%)	98.13 ± 0.1	98.44 ± 0.1
V_{max} (g phenol g VSS^{-1} d^{-1})	5.84	3.70
K_s (mg/l)	540	420
K_i (mg/l)	270	180

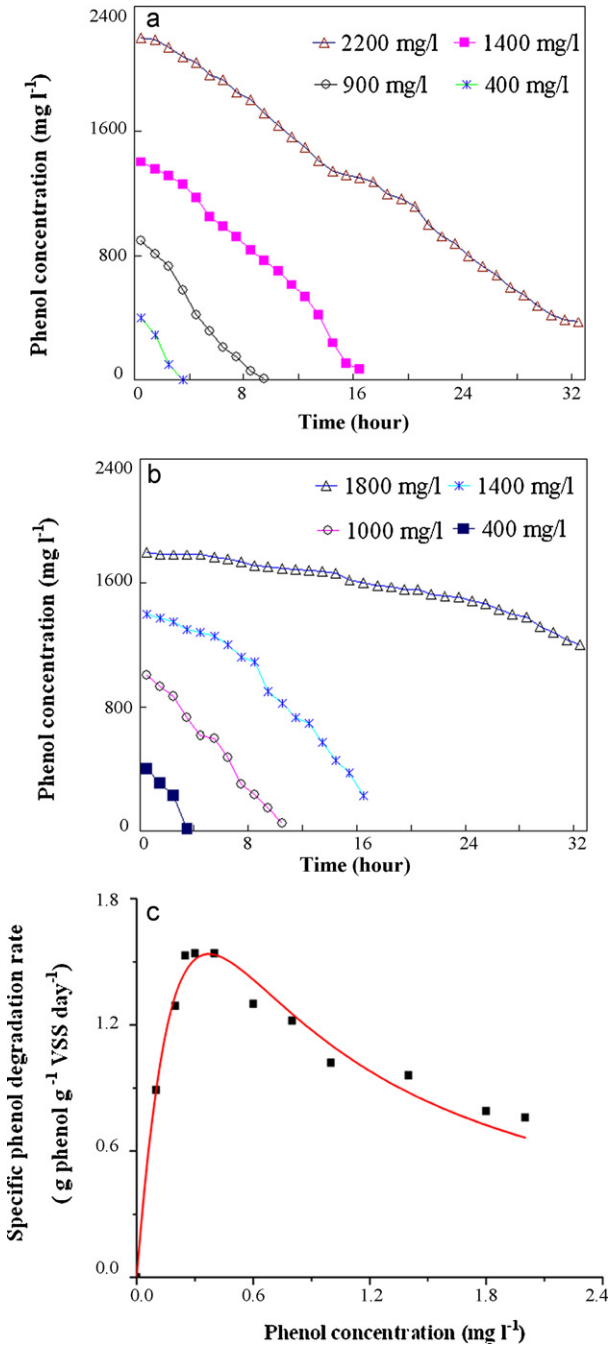

Fig. 7. Batch phenol degradation test with aerobic granules. (a) AG_{R1} at initial phenol concentrations of 400, 900, 1400 and 2200 mg/l; (b) AG_{R2} at initial phenol concentrations of 400, 1000, 1400 and 1800 mg/l; (c) specific phenol degradation rates of AG_{R1} at different phenol concentrations.

results presented in other studies [8,11,26,27]. The reason can be explained by the following aspects: (i) the particular multilayer structure gives AG_{R1} the robustness to withstand high phenol concentrations; and (ii) the addition of chlamydospores provides more fungi population for phenol degradation. On one hand, the chlamydospores improved the removal performance of the reactor by increasing the effective biomass; on the other hand, since manganese peroxidase (MnP) [28] produced by strain HSD can biodegrade phenol, its appearance in R1 resulted in higher biodegradation ability of AG_{R1}. During the 150-day operational period, MnP was detected at the enzymatic level of 250–480 U/l in R1 but not in R2, indicating certain activity of strain HSD was maintained in the granules of R1.

To the best of our knowledge, there is little information available in literature about the acceleration of sludge granulation using the method presented here. Compared with previous methods such as inoculating biogranules or microbes with self-aggregation ability to reactors [11,12,17,29,30], the proposed method showed that the chlamydospores not only survived in the reactor, induced formation of miniature spherules, and presented primary cores to accelerate sludge granulation, but also improved the treatment capability and tolerance of reactor to phenol wastewater. Moreover, since MnP secreted by strain HSD [20] can biodegrade a series of toxic substances aside from phenol, aerobic granules cultivated by the proposed method could also be used to treat other wastewaters. Thus, the acceleration of sludge granulation by the chlamydospore is effective, and can be extensively exploited to design a rapid, high-rate aerobic granulation system. Further study should be addressed on scale-up of the granulation process with chlamydospores and the development of cost-effective approaches. Moreover, synthetic wastewater containing glucose was used during the start-up phase in this study. However, the supplementation of glucose is unfeasible for the reactor in the scale-up treatment of industrial wastewaters. Therefore, mass-generation of aerobic granules with glucose-free phenol wastewater or other wastewaters should also be considered in future work.

4. Conclusions

Inoculating the chlamydospores of *Phanerochaete* sp. HSD with activated sludge to SBR is an effective method to accelerate sludge granulation. Aerobic granules were rapidly cultivated during the treatment of phenol wastewater on day 7, which was 32 days earlier than that of control. The aerobic granule cultivated by this method had an annual ring-like multilayer structure, and a primary core induced by the chlamydospore appeared in the nuclear area of the granule. Rapid granulation in R1 is proposed based on the following observations: (i) chlamydospores inoculated in SBR survived in phenol wastewater and formed miniature spherules; and (ii) the spherules served as the primary matrix for the layered growth of microbial films. The developed granules had higher *P*/*S* ratio than the granules which developed using only activated sludge as inoculum. In addition to the short culture time, AG_{R1} possesses better tolerance and biodegradation ability toward the wastewater with high phenol strength.

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References

[1] D. Gao, L. Liu, H. Liang, W.M. Wu, Aerobic granular sludge: characterization, mechanism of granulation and application to wastewater treatment, Crit. Rev. Biotechnol. 31 (2011) 137–152.
[2] Y. Liu, H. Xu, S.F. Yang, J.H. Tay, A general model for biosorption of Cd^{2+}, Cu^{2+} and Zn^{2+} by aerobic granules, J. Biotechnol. 102 (2003) 233–239.
[3] A. Carucci, S. Milia, G.D. Gioannis, M Piredda, Acetate-fed aerobic granular sludge for the degradation of 4-chlorophenol, J. Hazard. Mater. 166 (2009) 483–490.
[4] L.L. Liu, Z.P. Wang, J. Yao, X.J. Sun, W.M. Cai, Investigation on the formation and kinetics of glucose-fed aerobic granular sludge, Enzyme Microbiol. Technol. 36 (2005) 712–716.
[5] Y.Q. Liu, J.H. Tay, Cultivation of aerobic granules in a bubble column and on airlift reactor with divided draft tubes at low aeration rate, Biochem. Eng. J. 34 (2007) 1–7.
[6] Y.M. Zheng, H.Q. Yu, S. Liu, X.Z. Liu, Formation and instability of aerobic granules under high organic loading conditions, Chemosphere 63 (2006) 1791–1800.
[7] D. Gao, L. Liu, H. Liang, W.M. Wu, Comparison of four enhancement strategies for aerobic granulation in sequencing batch reactors, J. Hazard. Mater. 186 (2011) 320–327.
[8] H.L. Jiang, J.H. Tay, S.T.L. Tay, Aggregation of immobilized activated sludge cells into aerobically grown microbial granules for the aerobic biodegradation of phenol, Lett. Appl. Microbiol. 35 (2002) 439–445.
[9] S.G. Wang, X.W. Liu, W.X. Gong, B.Y. Gao, D.H. Zhang, H.Q. Yu, Aerobic granulation with brewery wastewater in a sequencing batch reactor, Bioresour. Technol. 98 (2007) 2142–2147.
[10] M. Figueroa, A. Mosquera-Corral, J.L. Campos, R. Méndez, Treatment of saline wastewater in SBR aerobic granular reactors, Water Sci. Technol. 58 (2008) 479–485.
[11] S.T.L. Tay, B.Y.P. Moy, H.L. Jiang, J.H. Tay, Rapid cultivation of stable aerobic phenol-degrading granules using acetate-fed granules as microbial seed, J. Biotechnol. 115 (2005) 387–395.
[12] V. Ivanov, X.H. Wang, S.T.L. Tay, J.H. Tay, Bioaugmentation and enhanced formation of microbial granules used in aerobic wastewater treatment, Appl. Microbiol. Biotechnol. 70 (2006) 374–381.
[13] APHA, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.
[14] S.S. Adav, D.J. Lee, K.Y. Show, J.H. Tay, Aerobic granular sludge: recent advances, Biotechnol. Adv. 26 (2008) 411–423.
[15] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356.
[16] B. Frolund, R. Palmgren, K. Keiding, P.H. Nielsen, Extraction of extracellular polymers from activated sludge using a cation exchange resin, Water Res. 30 (1996) 1749–1758.
[17] H.L. Wang, G.L. Yu, G.S. Liu, F. Pan, A new way to cultivate aerobic granules in the process of papermaking wastewater treatment, Biochem. Eng. J. 28 (2006) 99–103.
[18] M.M. Ghangrekar, S.R. Asolekar, K.R. Ranganathan, Experience with UASB reactor start up under different operating condition, Water Sci. Technol. 34 (1996) 421–428.
[19] K. Watanabe, S. Hino, N. Takahashi, Responses of activated sludge to an increase in phenol loading, J. Ferment. Bioeng. 82 (1996) 522–524.
[20] H.L. Wang, P. Li, G.S. Liu, X. Li, J.M. Yao, Rapid biodecolourization of eriochrome black T wastewater by bioaugmented aerobic granules cultivated through a specific method, Enzyme Microbiol. Technol. 47 (2010) 37–43.
[21] Y. Liu, J.H. Tay, State of the art of biogranulation technology for wastewater treatment, Biotechnol. Adv. (2004) 22533–22563.
[22] M.C. Cammarota, G.L. Sant'Anna, Metabolic blocking of exopolysaccharides synthesis: effects on microbial adhesion and biofilm accumulation, Biotechnol. Lett. 20 (1998) 1–4.
[23] Y.Q. Liu, Y. Liu, J.H. Tay, The effects of extracellular polymeric substances on the formation and stability of biogranules, Appl. Microbiol. Biotechnol. 65 (2004) 143–148.
[24] C.G. Cai, J.S. Liu, W.M. Cai, Action mechanism of extracelluar polymers on the aerobic granulation, China Environ. Sci. 24 (2004) 623–626.
[25] D.Q. Zhou, Essential Microbiology, second ed., Higher Education Press, Beijing, 2002.
[26] S.S. Adav, M.Y. Chen, D.J. Lee, N.Q. Ren, Degradation of phenol by aerobic granules and isolated yeast *Candida tropicalis*, Biotechnol. Bioeng. 96 (2007) 844–852.
[27] S.S. Adav, D.J. Lee, Single-culture aerobic granules with *Acinetobacter calcoaceticus*, Appl. Microbiol. Biotechnol. 78 (2008) 551–557.
[28] H.L. Wang, P. Li, M. Pang, Z.J. Zhai, G.L. Yu, G.S. Liu, J.M. Yao, Rapid decolorization of azo dyes by a new isolated higher manganese peroxidase producer: *Phanerochaete* sp. HSD, Biochem. Eng. J. 46 (2009) 327–333.
[29] L.L. Hu, J.L. Wang, X.H. Wen, Y. Qian, The formation and characteristics of aerobic granules in sequencing batch reactor (SBR) by seeding anaerobic granules, Process Biochem. 40 (2005) 5–11.
[30] M.V. Ganesan, V. Saravanan, Formation and hydrodynamic characteristics of aerobic granules in an activated sludge system, Environ. Technol. 28 (2007) 217–224.