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The process research of anaerobic flora disintegrating excess sludge

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ABSTRACT

Large amounts of sewage sludge are produced from activated sludge processes, causing serious secondary pollution. Thus, the development of stable, safe, and low-cost methods to dispose excess sludge has gradually become a popular research topic. The anaerobic/anaerobic microorganism method shows good prospects for industrialization because of its advantages such as low cost and no secondary pollution. The current study focuses on the effect of anaerobic microorganisms on waste activated sludge during anaerobic fermentation. In this research, the changes of organic compounds, extracellular enzymes, and size distributions in the process of anaerobic flora disintegrating excess sludge were measured to clarify the disintegrating process. In various disintegrating products, COD was observed to be suitable for characterizing the degree of sludge disintegration. The disintegration efficiency of anaerobic flora was 34.86% or 3.5 times higher than that of the anaerobic environment. Lipase played an important role in breaking cell walls. There was a dynamic equilibrium in the sludge disintegration and reuse of disintegration products. The change in size distribution showed that easily degraded materials accumulated and large granular sludge was destroyed. The present study lays the foundation for future studies on the optimization of conditions for sludge disintegration.

Keywords: Excess sludge; Anaerobic flora; Disintegration; Process

1. Introduction

Activated sludge processes are used worldwide and large amounts of sewage sludge are produced from these processes. According to the incomplete statistics in 2012, sewage sludge (moisture content 97%) was yielded over 97 million t/a in China [1]. Proper treatment procedures must be established to deal with excess sludge, because this substrate contains large amounts of heavy metals and pathogens. However, most available treatment methods require high energy consumption and use chemicals which could result in secondary pollution [2,3]; these methods are not suitable for large-scale application.

The main methods currently used to dispose excess sludge are sanitary landfills and anaerobic digestion. Sanitary landfill is not sustainable because they occupy large areas of landfill space [4], and anaerobic digestion is only suitable for large wastewater treatment plants [5]. Hence, establishing a stable, safe, and low-cost method to dispose excess sludge is urgently needed.

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Cell-lysing methods, which can quickly kill bacteria in the sludge to release a matrix that other bacteria can reuse, are widely applied in sludge reduction processes [6]. The biggest advantage of cell-lysing methods is easy to realize in engineering application, which only needs to increase the related treatment device in the sludge return line. Another outstanding advantage is the decomposition of degradation products would use the dissolved oxygen in the aeration tank, which need not increase the aeration rate more [7]. Considering factors such as the cost and stability of various cell-lysing methods, anaerobic or anaerobic microbial digestion methods appear to be more favorable for sludge reduction than other methods.

Unfortunately, existing anaerobic or anaerobic microbial digestion methods, such as the oxic-settling-anaerobic (OSA) activated sludge process, present obvious disadvantages such as slow digestion speed and the need for large anaerobic zone volumes and long retention times [8]. In Xings' study [9], for example, the total volume of the anaerobic function carrier was 66 L, far larger than that of the aerobic zone volume (16 L). These disadvantages greatly affect the practical application of anaerobic microbial digestion methods.

The anaerobic environment [low oxidation–reduction potential (ORP)] and disintegrating ability of anaerobic microorganisms are the main factors influencing sludge reduction during anaerobic or anaerobic microbial digestion [10,11]. While decreasing the ORP to increase the speed of reduction is not feasible (high cost and upper limit), improving the disintegrating ability of anaerobic microbial is feasible in theory. In our previous study [12,13], an anaerobic bacteria group containing six anaerobic strains was screened; this group showed a higher sludge reduction effect than the naturally domestication sludge in smaller volumes (e.g. when the anaerobic zone is 11.8% of the aerobic zone and less than the 37.78% of the natural sludge, volume decreased by 68.74%).

As the mechanism of anaerobic microbial disintegration of sludge cells is unclear [14], optimization of this process and its industrial application are limited. Hydrolysis acidification theory is generally used to explain the disintegration process of sludge cells; however, our previous studies showed some phenomena which did not conform to this theory. For example, the amount of large molecular weight materials (molecular weight >100,000) was larger than that of low molecular weight materials (molecular weight <4,000) in the anaerobic zone effluent. The pH of anaerobic zone effluents did not decrease significantly, which shows that acidification of the quantity may be limited. On one hand, previous studies have reported that the quantity of fatty acids in the anaerobic zone effluent is low [9], which indicate that controlling the process of disintegration during hydrolysis is possible. On the other hand, in our previous work [12], high molecular weight materials in the anaerobic zone effluent did not accumulate in the aerobic zone but were reused by aerobic sludge quickly. Thus, sludge cells may be reused without the need to disintegrate into smaller molecules.

In the related degradation studies, because the microbe quantity in the sludge could not be measured exactly, the degradation degree is usually described by the nutrients in the solution which are released from the excess sludge. The related results show that protein, polysaccharide, and other carbohydrates are the main components of organic compounds in sludge [15]. Generally, the common indexes include soluble COD, protein, total N, DNA, NH_4^+-N, PO_4^{3-}-P, and volatile fatty acid. On the other hand, the first step of sludge degradation is to break the floc and zoogloea of sludge [16]. So, the size distribution of sludge is an obvious index of sludge degradation.

To clarify the principles of organic compound release and to determine the active anaerobic bacteria secreting enzymes during excess sludge disintegration, a system of excess sludge disintegration by anaerobic flora was established. In this system, changes of organic compounds, extracellular enzymes, and size distributions were determined.

2. Materials and methods

2.1. Waste-activated sludge

Waste-activated sludge (WAS) was obtained from the secondary sedimentation tank of Shihezi municipal wastewater treatment plant (Shihezi, Xinjiang, China). The characteristics and particle size distribution of raw (unfermented) WAS are shown in Table 1 and Fig. 1, respectively.

2.2. Complex microorganism

The mixed anaerobic flora included six anaerobic strains screened from acclimated sludge originating from a drainage system in Shihezi, China. The six

Table 1
Characteristics of the raw WAS

Item	Value
pH	6.7
MLSS (g/L)	10.7
MLVSS (g/L)	7.8
TCOD (mL/L)	13,600

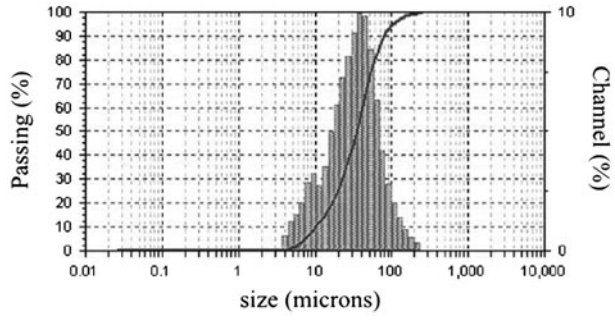

Fig. 1. Particle size distribution of raw (unfermented) WAS.

strains included *Clostridium bartlettii* (DSM16795), *Bacteroides* sp. (control 168), *Alkaliphilus metalliredigens* (QYMF), *Clostridium botulinum* (ATCC19397), *Bacillus cereus* (NVH0597-99), and *Bacillus thuringiensis* (IBL200). The strains were cultured separately for 72 h in a shake flask. Then the six strains were mixed together and cultured for 72 h in a larger shake flask and then the flora was obtained. The medium is the peptone beef medium (beef extract 0.3%, peptone 1.0%, sodium chloride 0.5%, and pH 7.4–7.6).

There are three reasons of selecting the anaerobic flora instead of anaerobic sludge in this research. First, the disintegrating ability of anaerobic flora is much higher than anaerobic sludge, which has greater advantage in future industrial applications. Second, to guarantee the accuracy of experiments, the disintegrating ability of unit mass anaerobic bacteria should be consistent. Using the flora could better meet this requirement. Finally, the optimizations of relevance would be conducted in the future experiments, which would be realized by using the flora.

2.3. Treatment system and operation

Tests to reduce sludge were carried out in shake flasks with a working volume of 500 mL. The raw (unfermented) WAS and anaerobic bacterial liquid were centrifuged (5 min, 5,000 rmp); the settling sludge and wetting bacteria were obtained. Oxygen in the flasks was removed from the headspace by nitrogen gas purging for 15 s. The shake flasks were then sealed with parafilm and placed in air bath shaker (150 rpm) at 35 ± 1°C. After 48 h of cultivation, the sludge mixture was centrifuged (5 min, 6,000 rpm) and the COD, protein content, nitrogen content of ammonium, phosphorus content of phosphate radicals, protease enzyme activity, lipase enzyme activity, and amylase enzyme activity in the supernatant were determined.

The experiments included five series of A–E, which are shown in Table 2. A (no nutrients condition), B (cultured condition), C (only nutrients), and D (sludge autolyzed) were set as control, and E was the anaerobic flora disintegrating excess sludge system. There were three parallel samples of each series and all measurements were repeated at least three times.

Actually, the concentration of various nutrients and enzymes in the disintegrating process are dynamic changes. The final values may not explain the disintegrating process. So, a continuous experiment was conducted for 96 h with a working volume of 500 mL similar to the test E described above. Samples were obtained every 12 h to determine the aforementioned parameters.

On the other hand, the structure of sludge floc had a protective effect on the disintegration. If the anaerobic flora could only use the sludge of smaller particle size, the disintegrating effect could not be very well. So, the change of sludge size distribution (the samples included excess sludge and anaerobic flora because the two sludge could not be separated) in the disintegrating process (system E) was also measured. Samples were obtained every 4 h in prophase and extended to 12 h in anaphase. There were three parallel samples and all measurements were repeated at least three times.

2.4. Analytical methods

The analyses of COD, NH_4^+ -N, and PO_4^{3-}-P were conducted in accordance with standard methods [17]. The soluble protein was determined by the BCA method using a protein determination kit (Sangon Biotech Co. Ltd).

Protease was measured by the Folin phenol reagent method [18]; here, the *K* value used was 95.24. Lipase was measured by lipase checkerboard [19] (Nanjing Jiancheng Bioengineering Institute), and amylase was measured using the national standard method [20]. Sludge size distribution was determined using a laser particle size distribution instrument (S3500 Microtrac, American).

All the chemicals were analytically pure and purchased from Sinopharm Chemical Reagent Co. Ltd.

3. Results and discussion

3.1. The nutrient concentrations measurement evaluations in sludge disintegration

The COD of five series are shown in Table 3. After culturing, no significant difference in the CODs of B (1,081) and C (1,075) was observed. It showed that

Table 2
Materials in various systems

	Wetting bacterium (g)	Ammonium chloride (g)	Glucose (g)	Settling sludge (g)	Distilled water (mL)
A	0.3	/	/	/	350
B	0.3	0.5	0.5	/	350
C	/	0.5	0.5	/	350
D	/	/	/	5	350
E	0.3	/	/	5	350

although the nutrients were easy to use, the growth rate of the anaerobic flora is slow which would be caused by the shortened fermentation time. This phenomenon meant that the changes brought by the anaerobic flora growing may not be considered.

Because only the soluble COD could be used effectively by the aerobe sludge, it was usually measured to indicate the hydrolysis degree of sludge [21]. After completely digesting, the COD of 5.0 g wet WAS (same as the WAS added to the treatment system) was 49.2 mg. So, the disintegration efficiency would be expressed by the ratio of the soluble COD.

When the nutrients were insufficient (D), the WAS released a spot of organic nutrients which was equal to 4.9 mg COD (14 mg/L * 0.35 L). Hence, 9.96% sludge was disintegrated under conditions equivalent to the OSA process, which was much lower than the related research; for example, in Chen's study [22], the sludge reduction efficiency ranged from 23 to 58% (ORPs ranging from +100 to −250 mV). There were two possible reasons: one was the higher ORP (+50 to 0 mV) which decreased the disintegrating ability and the other was the higher sludge concentration (14.29 g/L compared to 8.64 g/L), which would bring a higher resistant ability. But the higher sludge concentration could reduce the volume of anaerobic region, which was a large advantage in the industrial application.

When the nutrients are insufficient (A), autolysis occurs so that anaerobic flora can maintain self-growth. However, the autolysis degree was lower, which only released 5.95 mg COD (17 mg/L * 0.35 L). But in the subsequent discussion, this condition must be considered if the insufficient condition existed.

Table 3
Effect of complex anaerobic microorganism on COD (mg/L)

	A	B	C	D	E
COD (mg/L)	17	1,081	1,075	14	63

In E, there were two materials including the anaerobic flora and WAS. The possible reactions included the following conditions. First, if the anaerobic flora could not use the sludge and the disintegrating products of sludge, the anaerobic flora and sludge would both autolyze. So, the concentration of COD should be 31 mg/L (17 mg/L + 14 mg/L). Second, if the anaerobic flora could only use the disintegrating products of sludge, the anaerobic flora could be of normal growth and only the sludge was autolyzed. So, the concentration of COD should be 14 mg/L (from the sludge disintegration) or lower than that (some used by the anaerobic flora). In fact, the concentration of COD in E was 63 mg/L, which was obviously higher than the above conditions. This indicated that another condition occurred. The anaerobic flora could disintegrate the sludge to release the nutrients. So, the anaerobic flora was of normal growth, and the sludge was disintegrated by the anaerobic environment and flora. All of the COD was released from the sludge. If considering the utilization of anaerobic flora, the COD released from WAS would exceed 63 mg/L.

If, assuming that, the COD originated from the disintegration by the anaerobic environment in E was similar with that in D (14 mg/L), the residual COD would originate from the disintegration by the anaerobic flora which was 49 mg/L (63 mg/L − 14 mg/L). So, it was 17.15 mg COD (49 mg/L * 0.35 L) which was equivalent to 1.74 g (17.15 mg/49.2 mg * 5 g) sludge disintegrated. Then the disintegration efficiency of anaerobic flora was 34.86% or 3.5 times that of anaerobic environment. These results showed that anaerobic flora was more effective in disintegrating sludge than anaerobic environment. On the other hand, the disintegration efficiency of 44.82% (9.96% + 34.86%) had basically reached the level of OSA system (40–50%) [2]. But the higher ORP level in this research meant more simple equipment with a lower cost. On the other hand, the higher sludge concentration meant higher treatment ability in unit volume which brought a lower cost. Finally, the disintegration efficiency of anaerobic flora could be increased by condition optimization, which could also bring a lower cost.

Therefore, the anaerobic flora had a better application potential.

As other substances could be released during disintegration, the protein content, nitrogen content of ammonium, and phosphorus content of phosphate radicals were also determined (Fig. 2).

The protein content in each system was high, and two sources of the protein were identified: hydrolysis of cells and extracellular enzymes secreted by the microorganisms [23]. The sludge released more protein when it disintegrates (A and D), because the main dry weight of the sludge was protein. Compared to WAS, anaerobic flora released more protein likely because of the different compositions of these two cell types. Anaerobic flora could undergo normal growth without autolysis when nutrients are sufficient. So, the protein in system B could be composed of extracellular enzymes that the flora secreted because of the absence of protein in extra nutrients (C). The protein content in E was slightly higher than the sum of that in B and D, which was obviously lower than the predictive value. On the one hand, in the previous discussion, more sludge was disintegrated in E than that in D, which meant a large release amount of protein. On the other hand, because the sludge was more difficult to use than the nutrients in B, the anaerobic flora should secret more extracellular enzyme which also caused a higher protein concentration [24]. Then, obviously a higher protein concentration was predicted. This phenomenon may be caused by anaerobic flora using lot of protein, which was proved by a much higher protein concentration released by the anaerobic flora autolysis. On the other hand, the protein concentration was a little lower than that of the related studies in OSA system [25]. This phenomenon also indicated that the released protein was reused by the anaerobic flora. So in this system, the protein concentration was not suitable to express the disintegration efficiency.

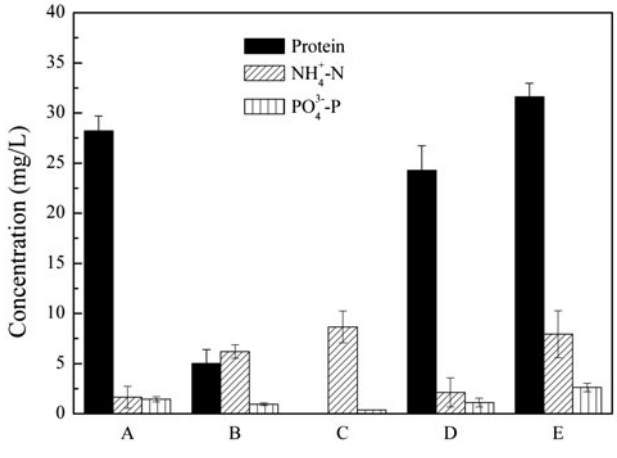

Fig. 2. Protein, nitrogen content in ammonium, and phosphorus content in phosphate radicals.

Fig. 2 shows that lower amounts of nitrogen content in ammonium were released when the nutrients were insufficient; higher amounts of nitrogen content in ammonium was released when the WAS was disintegrated by anaerobic flora. If the nitrogen content in ammonium of D was attributed to anaerobic conditions, then 5.81 mg/L of that system E subtracted system D was caused by microorganism. Its 2.8 times the release amount of anaerobic environment, and this proportion was less slightly than the proportion of COD. Its probably because of the different absorptive capacity that microorganism absorbed different nutrients.

The cell membrane is composed of phosphoric acid [26]; thus, the extent of sludge disintegration can be characterized by determining the phosphorus content in the fermentations systems. The phosphorus content of phosphate radicals released was low when nutrients were sufficient (B). When WAS was disintegrated by anaerobic flora, the amount of phosphorus content of phosphate radicals released was higher at about 1.05 times of the amount released by the anaerobic environment, which was also lower than the proportion of COD. On the other hand, the phosphorus content of phosphate radicals in E was obviously lower than that of related studies in OSA system [25], which also was caused by the reuse of anaerobic flora.

In general, because the anaerobic flora selectively absorbed nutrients, the amounts of nutrients remaining in the fermentation solution were different. From the aforesaid determined proportion, the use of COD to characterize disintegration efficiency was appropriate.

3.2. The enzyme concentrations measurement evaluations in sludge disintegration

In the process from the sludge floc to the dissolved nutrients, the extracellular enzymes played an important role. The amount of extracellular enzymes could be seen as the magnitude of degradation ability. The enzyme activities of three systems (A, B, and E) were shown in Fig. 3. In different systems, the variations in enzyme amounts secreted by anaerobic flora showed significant differences.

No significant change in amylase activity was observed among the different systems studied. Amylase hydrolyzes starch and glycogen. However, since bacterial cell walls are mainly composed of peptidoglycans and lipids; amylase plays a role in sludge

degradation only after cell disruption. Consequently, the amylase activities observed among the three systems were not very different.

Protease activities varied according to the growth conditions. Higher protease activity was observed when more nutrients were found in the fermentation system. Thus, the protease activity was related to the nutrient contents in fermentation systems.

The lipase activity was low when the nutrients in the fermentation system had a simple structure. When the nutrients were composed of bacterial cell walls (A and E), however, lipase activities increased (e.g. compared with system B) and remained higher than the activities of the other two enzymes. Lysozymes function in cell lysis, but are absent in most microorganisms. Bacterial cell walls contain massive amounts of lipid compounds; so, lipase plays an important role in breaking cell walls. Cell wall breakage improves sludge disintegration.

3.3. Changes in nutrients concentrations of sludge disintegration

In previous discussions, it was indicated that the disintegration efficiency calculation was influenced by the anaerobic flora using nutrients. So, the changes of nutrients in the disintegration process of 96 h were measured every 12 h under the conditions of E.

Variations in each nutrient during the continuous experiments are shown in Figs. 4 and 5. The trends observed for all nutrients were nearly identical. Nutrient contents first increased rapidly and reached maximum levels after 16–20 h. These levels then decreased slowly, and reached minima at about 60–72 h. Finally, nutrient contents slowly increased. The trend was obviously different to that in OSA system. Because there is only release of nutrients in OSA system, the relationship between the concentration of degradation products and time is a direct ratio, which had only one different ratio in different studies [10,25,27]. But in the systems studied, two factors affect the amount of nutrients produced: production by sludge disintegration and utilization by anaerobic flora. These two factors maintain a type of dynamic equilibrium.

At early days of fermentation, the nutrients in the systems were insufficient and anaerobic flora secreted extracellular enzymes to hydrolyze the sludge to obtain more nutrients; this activity resulted in an increase in nutrient contents. During this time, the anaerobic flora was in the early growth stage and required fewer nutrients. The required nutrients increased with the growth of anaerobic flora. As the nutrients accumulated in the system, neonatal cells no longer had any need to secrete extracellular enzymes to obtain nutrients. Thus, the rate of sludge disintegration maintained the release of nutrients, but the rate of utilization continuously increased. Consequently, the amount of nutrients in the system decreased. The increasing number of anaerobic flora caused a decrease in available nutrients in the system; thus, the flora secreted extracellular enzymes to obtain nutrients once more, resulting in an increase in the rate of sludge disintegration and in the amount of nutrients produced.

Over 96 h of disintegration, various nutrients reached stable levels after 48 h. Stable levels were achieved during the production of nutrients when sludge disintegration and nutrient utilization of anaerobic flora were balanced. Therefore, it may be inaccurate that the disintegration efficiency was calculated based on the data from 48 h of fermentation.

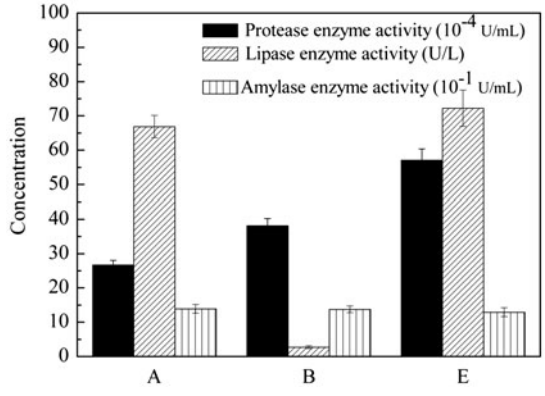

Fig. 3. Protease, lipase, and amylase enzyme activities.

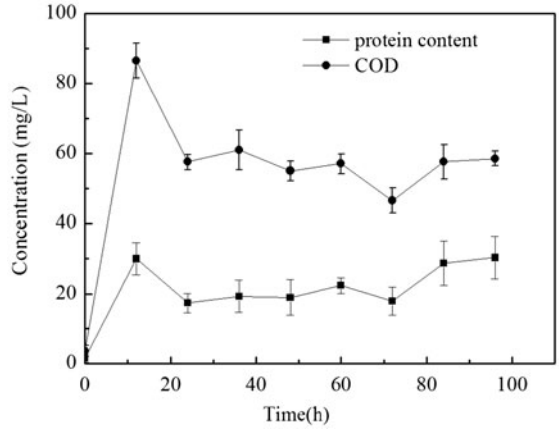

Fig. 4. Changes in COD and protein concentrations over 96 h of fermentation.

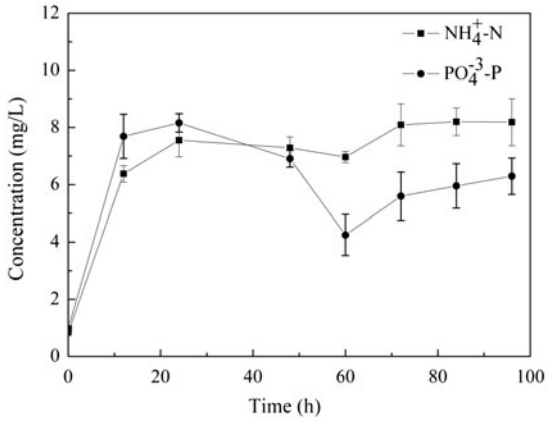

Fig. 5. Changes of nitrogen content in ammonium and phosphorus content in phosphate radicals over 96 h of fermentation.

For example, if calculation was based on COD of 12 h, the degradation product was 25.375 mg [(86.5 mg/L − 14 mg/L) * 0.35 L] and the disintegration efficiency could reach 51.58% (25.375 mg/49.2 mg), which was much higher than that of 48 h (34.86%). The total disintegration efficiency reached 61.53% (51.58% + 9.96%), which reached the higher level of that in OSA system [2,10]. On the other hand, COD was held constant from 48 to 96 h. It was impossible that the disintegration was not continuous. A scientific explanation was that the degradation products were reused by the anaerobic flora. Then three questions need to be considered further.

First, how to calculate the consumptive amount of COD which should be added in calculating the disintegration efficiency? One possible method was measuring the rate of anaerobic flora using the degradation products. Then the consumptive amount of COD could be calculated based on the rate and treatment time.

Second is how to set the disintegration time in the practical application? In OSA system, the retention time of sludge usually was set by considering two factors. One was setting an enough long time to maintain the ORP level. The other was selecting a suitable time to balance between the disintegration efficiency and treatment ability [10]. In this research, the ORP level need not be considered because it was maintained at a higher level. On the other hand, although the disintegration efficiency was increased in a long retention time too, the long retention time was not suitable because of the growth of anaerobic flora. The main purpose of disintegration was to obtain degradation products. If excessive degradation products were reused and more anaerobic sludge grew, the anaerobic sludge should be discharged as excess sludge which was not anticipant.

In respect of these problems, the third question must be considered. Whether the equilibrium state of nutrient production and utilization reveals be maintained in the practical application? In maintaining the equilibrium state, more degradation products were used by the anaerobic flora which brought some disadvantages. First, the rate of anaerobic flora using the degradation products was obviously lower than that of aerobic sludge, indicating a large treatment volume. Second, there was substrate inhibition in the treatment process. If the degradation products were ample, the anaerobic flora did not secret more extracellular enzymes, which would limit the increase of disintegration ability. If the degradation products were removed continuously and placed into an aerobic environment to be reused, anaerobic flora would maintain a starvation status and the disintegration rate could be improved effectively. However, it must be paid attention to that the residual degradation products must be able to maintain the normal growth of anaerobic flora. So the best conditions should be that, an appropriate amount of degradation products was moved from the treatment system continuously and the excess sludge was added continuously. How to realize this process is an important optimization direction in the future studies.

3.4. Changes in enzyme levels of sludge disintegration

Changes in the amylase and lipase activities in the continuous experiments were shown in Fig. 6. The lipase activity increased slowly later in the experiments, which verifies the hypothesis that the secretion of extracellular enzymes is inhibited in the presence of adequate nutrients. High, but stable levels of amylase activity further confirm that the nutrients in system were adequate. Thus, increasing lipase activities in the fermentation system is an important method to increase its disintegration efficiency.

3.5. Relationship between particle size distribution and sludge disintegration

In the anaerobic degradation process, the organic substances are released from the cracked aerobic cells and then degraded, and converted to low molecular weight materials. The size distribution of sludge during the disintegration process was shown in Table 4. The particulates of original sludge were normally distributed (Fig. 1), which was consistent with that in other research [28].

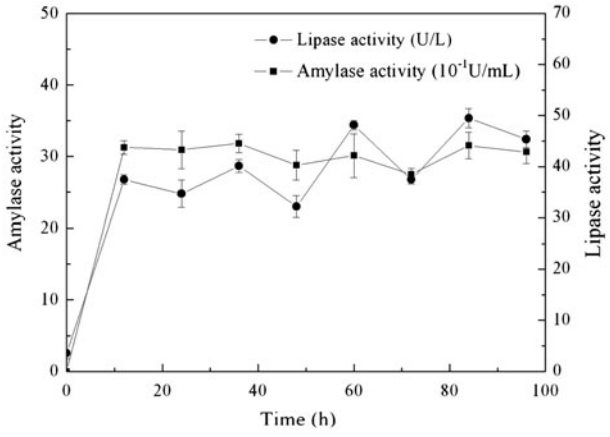

Fig. 6. Changes of amylase and lipase activities over 96 h of fermentation.

At the beginning of the disintegration process, the quantity of low molecular weight materials (0–10 μm) decreased, obviously, because anaerobic flora preferentially degraded small particles of the sludge and the release rate of low molecular weight materials was low. As disintegration effects began to increase, the quantity of low molecular weight materials increased, which meant that easily degraded materials began to accumulate. At the same time, the quantity of moderate molecular weight materials (10–50 μm) began to decrease, which meant that part of the sludge structure had been damaged [28]. However, the amount of moderate molecular weight materials remained constant instead of decreasing slowly, which may be caused by supplementation from large molecular weight materials. The quantity of large molecular weight materials (>50 μm) increased sharply early in the treatments, probably because of the addition of anaerobic flora. The size distribution of anaerobic flora was 50–100 μm (data not shown). The quantity of materials of 50–100 μm decreased as the sludge disintegrated, but the quantity of materials larger than 100 μm showed the opposite trend. These large-molecular weight materials may originate from growing anaerobic flora. The increase in larger sizes meant that the anaerobic flora grew well in the system. On the other hand, the disintegration efficiency was not well considering from the size distribution because the average particle size did not decrease, which was obviously different from that of other studies [6,29]. This phenomenon was probably caused by the selective disintegration and growth of anaerobic flora.

Because only the disintegration effect existed, the size distribution in other studies was still normally distributed with a decrease in average particle size [28,29]. But in this study, the size distribution was not normally distributed because of the effect of anaerobic flora. Considering the treatment time as 48 h in previous studies and various nutrients reached the stable levels after 48 h, the size distribution at 48 h was discussed which is shown in Fig. 7. Moderate molecular weight materials significantly decreased and low molecular weight materials increased, which indicated that more moderate molecular weight materials than low molecular weight materials were degraded in the present system. As aerobic cells cracked, large molecular weight materials were released that explained their increased amount in the system.

The change in size distribution showed that the sludge can be used without destroying the structure for a lower cost.

Table 4
Percentage of size distribution as a function of time

Time (h)	0–10 μm (%)	10–50 μm (%)	50–100 μm (%)	>100 μm (%)
0	11.6 ± 0.23	69.8 ± 0.63	13.2 ± 0.73	5.4 ± 0.18
4	6.6 ± 0.013	67.3 ± 0.26	18.8 ± 0.39	7.3 ± 0.21
8	5.4 ± 0.053	67.9 ± 0.72	19.3 ± 0.28	7.4 ± 0.62
12	6.1 ± 0.17	64.1 ± 0.36	19.7 ± 0.73	10.1 ± 0.73
24	6.6 ± 0.27	65.1 ± 1.03	19.0 ± 0.69	9.3 ± 0.28
36	6.9 ± 0.27	64.4 ± 0.92	18.8 ± 0.84	9.9 ± 0.19
48	6.9 ± 0.19	66.3 ± 0.95	17.2 ± 0.53	9.6 ± 0.63
60	7.4 ± 0.18	66.5 ± 0.99	17.1 ± 0.92	9.0 ± 0.62
72	7.6 ± 0.28	65.7 ± 0.83	16.7 ± 0.63	10.0 ± 0.27
84	8.3 ± 0.37	67.0 ± 0.82	16.2 ± 0.50	8.5 ± 0.89
96	7.9 ± 0.28	66.6 ± 0.87	16.5 ± 0.53	9.0 ± 0.73

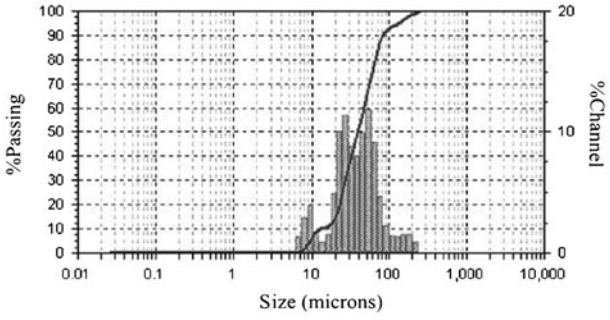

Fig. 7. Size distribution of sludge particulates after 48 h into the fermentation process.

4. Conclusion

The effect of anaerobic flora on sludge decay under anaerobic conditions was investigated in the present study. The main results are as follows:

(1) Anaerobic flora could disintegrate WAS and use the resulting material as nutrient source. The disintegration efficiency of anaerobic flora was much higher than that of anaerobic conditions, which was 3.50 times, 1.19 times, 2.80 times, and 1.05 times calculated based on the COD, protein, NH_4^+-N, and PO_4^{3-}-P, respectively. Considering the flora selectivity absorbing nutrients, it was more suitable to use COD to characterize the degree of sludge disintegration.

(2) In three extracellular enzymes, lipase activity had a large difference in the different nutrient conditions, which were higher than 65 U/L in condition of bacteria cell and lower than 5 U/L in condition of simple nutrient. So, it was indicated that lipase played an important role in breaking cell walls.

(3) According to the changes of nutrients in the continuous experiments (96 h), there was a dynamic equilibrium in the sludge disintegration and the reuse of disintegration products. This phenomenon suggested that the actual disintegration efficiency was probably much higher than that calculated from COD. If the nutrients are removed, the disintegration efficiency may be improved because the substrate was shortage.

(4) The change in size distribution showed that easily degraded materials accumulate and large granular sludge was destroyed. This finding indicated that the sludge can be used without destroying the structure for a lower cost.

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References

[1] J. Zhang, D.X. Wang, C. Hu, C.H. Feng, Status of sludge treatment and disposal of urban sewage treatment plants in Hainan province, Environ. Sci. Technol. 36 (2013) 328–331.
[2] X.M. Zhai, X. Gao, M.M. Zhang, L. Jia, J.S. Guo, Analysis of carbon balance and study on mechanism in anoxic-oxic-settling-anaerobic sludge reduction process, Environ. Sci. 33 (2012) 2444–2450.
[3] P. Romero, M.D. Coello, J.M. Quiroga, C.A. Aragón, Overview of sewage sludge minimisation: Techniques based on cell lysis-cryptic growth, Desalin. Water Treat. 51 (2013) 5918–5933.
[4] H.P. Yuan, X.F. Yan, C.F. Yang, N.W. Zhu, Enhancement of waste activated sludge dewaterability by electro-chemical pretreatment, J. Hazard. Mater. 187 (2011) 82–88.
[5] N.K. Salihoglu, G. Karaca, G. Salihoglu, Y. Tasdemir, Removal of polycyclic aromatic hydrocarbons from municipal sludge using UV light, Desalin. Water Treat. 44 (2012) 324–333.
[6] J.Y. Liu, G.F. Zhao, C. Duan, Y.F. Xu, J. Zhao, T. Deng, G.R. Qian, Effective improvement of activated sludge dewaterability conditioning with seawater and brine, Chem. Eng. J. 168 (2011) 1112–1119.
[7] C.M. Mark, V. Loosdrecht, M. Henze, Maintenance, endogeneous respiration, lysis, decay and predation, Water Sci. Technol. 39 (1999) 107–117.
[8] A.M. Corral, M.K. De Kreuk, J.J. Heijnen, Effects of oxygen concentration on N-removal in an aerobic granular sludge reactor, Water Res. 39 (2005) 2676–2686.
[9] Q. Feng, A.F. Yu, L.B. Chu, X.H. Xing, Performance study of the reduction of excess sludge and simultaneous removal of organic carbon and nitrogen by a combination of fluidized- and fixed-bed bioreactors with different structured macroporous carriers, Biochem. Eng. J. 39 (2008) 344–352.
[10] G.H. Chen, K.J. An, S. Saby, E. Brois, M. Djafer, Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process), Water Res. 37 (2003) 3855–3866.
[11] G.H. Chen, S. Saby, M. Djafer, H.K. Mo, New approaches to minimize excess sludge in activated sludge systems, Water Res. 44 (2001) 203–208.
[12] P. Cao, The flora constructing of treating tomato paste wastewater in aerobic–anaerobic coupled system and its application, Peking University, PhD Thesis, 2012.
[13] P. Cao, C. Li, S.M. Wen, Y.B. Tong, J.R. Ni, Effect of adapted constructed flora on continuous coupled aerobic–anaerobic process for treatment of tomato paste wastewater, Fresenius Environ. Bull. 20 (2011) 1649–1654.

[14] Q. Feng, A.F. Yu, L.B. Chu, H.Z. Chen, X.H. Xing, Mechanistic study of on-site sludge reduction in a baffled bioreactor consisting of three series of alternating aerobic and anaerobic compartments, Biochem. Eng. J. 67 (2012) 45–51.
[15] APHA/AWWA/WEF, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 2008.
[16] M. Barjenbruch, O. Kopplow, Enzymatic, mechanical and thermal pre-treatment of surplus sludge, Adv. Environ. Res. 7 (2003) 715–720.
[17] C. Bougrier, H. Carrère, J.P. Delgenès, Solubilisation of waste-activated sludge by ultrasonic treatment, Chem. Eng. J. 106 (2005) 163–169.
[18] C.E. McDonald, L.L. Chen, The Lowry modification of the Folin reagent for determination of proteinase activity, Anal. Biochem. 10 (1965) 175–177.
[19] R.C. Lawrence, T.F. Fryer, B. Reiter, Rapid method for the quantitative estimation of microbial lipases, Nature 213 (1967) 1265–1265.
[20] GB5521-89, Method for determination of alpha-amylase activity in cereal and cereal products colorimetric method [S]. China, 1997.
[21] E.U. Cokgor, S. Oktay, D.O. Tas, G.E. Zengin, D. Orhon, Influence of pH and temperature on soluble substrate generation with primary sludge fermentation, Bioresour. Technol. 100 (2008) 380–386.
[22] S. Saby, M. Djafer, G.H. Chen, Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process, Water Res. 37 (2003) 11–20.
[23] Y.J. Higashi, J.L. Strominger, Structure of a lipid intermediate in cell wall peptidoglycan synthesis: A derivative of a C55 isoprenoid alcohol, Proc. Nat. Acad. Sci. 57 (1967) 1878–1884.
[24] F. Wang, S. Lu, M. Ji, Components of released liquid from ultrasonic waste activated sludge disintegration, Ultrason. Sonochem. 13 (2006) 334–338.
[25] W.B. Jin, J.F. Wang, Q.L. Zhao, J.K. Lin, Performance and mechanism of excess sludge reduction in an OSA (oxic-settling-anaerobic) process, Environ. Sci. 29 (2008) 726–732.
[26] G.P.C. Salmond, Secretion of extracellular virulence factors by plant pathogenic bacteria, Annu. Rev. Phytopathol. 32 (1994) 181–200.
[27] M. Torregrossa, G. Di Bella, D. Di Trapani, Comparison between ozonation and the OSA process: Analysis of excess sludge reduction and biomass activity in two different pilot plants, Wat. Sci. Technol. 66 (2012) 185–192.
[28] T.H. Kim, S.R. Lee, Y.K. Nam, J. Yang, C. Park, M. Lee, Disintegration of excess activated sludge by hydrogen peroxide oxidation, Desalination 246 (2009) 275–284.
[29] C.X. Gong, J.G. Jiang, S.H. Yang, Effects of Fenton oxidation with ultrasonic coupling on sludge particle size and soluble substances, Chin. J. Environ. Sci. 33 (2013) 293–297.