



## Continuous reactor with anaerobic micro-organism to disintegrate excess sludge

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### ABSTRACT

Secondary pollution caused by excess sludge generated by sewage treatment continues to elicit an increasing amount of concern. To reduce the discharge of excess sludge in the process of sewage treatment, a continuous reactor with anaerobic micro-organism was designed and utilized to disintegrate excess sludge. After disintegrating for 360 h in this reactor, the volume of which was 4 L, the disintegration efficiency of excess sludge reached 50%. When the hydraulic retention time was 3 d, the concentrations of COD,  $\text{NH}_4^+\text{-N}$ , TP, protein, and polysaccharide increased from  $72 \text{ mg L}^{-1}$ ,  $0.04 \text{ mg L}^{-1}$ ,  $0.005 \text{ mg L}^{-1}$ ,  $42.94 \text{ mg L}^{-1}$  and  $22.69 \mu\text{g L}^{-1}$  (0 h) to  $3103 \text{ mg L}^{-1}$ ,  $3.11 \text{ mg L}^{-1}$ ,  $3.88 \text{ mg L}^{-1}$ ,  $37.32 \text{ mg L}^{-1}$ , and  $83.78 \mu\text{g L}^{-1}$  (360 h), respectively. When the hydraulic retention time was 4 d, the concentrations of COD,  $\text{NH}_4^+\text{-N}$ , TP, protein, and polysaccharide were all higher than that when the hydraulic retention time was 3 d. All these indexes rose with the anaerobic time, and rose with the increase in hydraulic retention time. Results showed that the continuous reactor with anaerobic micro-organism has a good effect on excess sludge disintegration.

*Keywords:* Excess sludge reduction; Anaerobic; Disintegration; Continuous; Reactor; Anaerobic micro-organism

### 1. Introduction

Wastewater processing has exhibited a trend of rapid growth because of the increase in the number of facilities of city sewage and industrial wastewater treatment [1]. At the end of 2013, 5,364 urban sewage factories were operated in China; daily sewage treatment capacity reached 170 million tons, the treatment ratio of urban sewage reached 94.02%, and 6.25 million tons of dry sludge was produced only in 2013 [2].

The sludge (moisture content 80%) output is expected to reach 40 million tons in 2015, as reported [3]. Excess sludge as a byproduct of sewage treatment putrefies easily and produces a bad stench. It also contains plenty of pathogenic bacteria and parasite eggs which easily lead to the spread of infectious diseases [4]. Cost of treatment and disposal accounts for 40–60% of the sewage treatment [5]. How to reduce sludge output economically and environmentally under efficient treatment has thus become a popular research issue.

Existing treatments, such as composting, incineration, and sanitary landfills, cannot realize economic and efficient sludge disposal. Source reduction method,

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including uncoupling, feeding, and strengthening recessive growth, is the ideal mean to solve the problem of excess sludge [6]. Uncoupling method involves the uncoupling between catabolism and anabolism, namely, in the intake of the substrate at the same time, the inhibition of cell proliferation results in sludge reduction. Uncoupling is simple to implement; however, although its effect is significant, it is unstable and harmful for the environment [7]. Feeding method involves establishing a variety of microbial components of the complex ecosystem by enhancing the flow of biological energy to realize sludge reduction. Although feeding method is economic and environmental friendly, it is difficult to control [8]. Strengthening the recessive growth of micro-organisms through cell lysis to achieve the release of intracellular organic matter complicates material quality transpiration from the solid phase to the liquid phase [9].

The oxic-settling-anaerobic (OSA) system is more stable and cheaper compared with those methods before. But the disintegration efficiency of excess sludge in OSA system relies on the oxidation–reduction potential (ORP) level [10]. For example, in Saby's study, the OSA system was operated with different ORP levels (+100 to –250 mV) in its anoxic tank. When the ORP level decreased from +100 to –250 mV, the sludge reduction efficiency was increased from 23 to 58% [11]. Hence, many researches are studied at –250 mV, including Jin's [12]. However, this ORP level means a strictly anaerobic condition, which is difficult to implement in engineering. Moreover, the sludge reduction efficiency can hardly be increased because the ORP level cannot be lower than –250 mV. But some anaerobic micro-organism can strengthen the disintegration process, and can break through the ceiling that ORP level brings.

Our previous research presented that anaerobic micro-organism was much more effective than anaerobic condition [13]. And a better disintegration effect of the sludge was discovered only at the ORP level ranging from 0 to +50 mV. Cheng's study got a good effect on sludge reduction in shake flask, whereas the treatment was small and batch processing. It needs more researches for increasing treatment capacity and disintegration efficiency before industrial application.

Hence, the 4 L continuous anaerobic reactor was designed to solve the problems mentioned above. Nothing but a few anaerobic bacteria were added in. Except that, there are no any additional conditions like controlling ORP level or pH. It is very convenient to operate. The disintegration effect of the sludge was discussed to provide a theoretical reference for late pilot and sewage treatment plants.

## 2. Materials and methods

### 2.1. Design and operation of the reactor

The reactor is made of glass, and the guide plate is made of plastic, which is to change the internal structure and flow velocity distribution in order to form the best position to reduce sludge sedimentation. As shown in Fig. 1, the reactor volume is 4 L (21 cm (L) × 11 cm (W) × 18 cm (H)), which is approximately 12 times that of the shake flask (the effective volume is 350 mL) [13]. The mixed zone is used for mixing the sludge, in which the effective volume is 1.5 L. And the settling zone is used for settling the solids down as much as possible in order to reduce the number of solids in the effluent, in which the effective volume is 1 L. In the circumfluence zone, the effective volume is 1.5 L, the sludge from the settling zone is mixed again here and then degraded.

### 2.2. Test materials

- (1) Waste activated sludge (WAS) was obtained from the secondary sedimentation tank of Shehezi Municipal Wastewater Treatment Plant (Shihezi, Xinjiang, China) and the sludge was cultivated in the laboratory for two weeks. Glucose, urea, and potassium dihydrogen phosphate were added at a ratio of 100:5:1 every 7 d. When the sludge flocs were stable, it was placed in the reactor. The characteristics of cultivated WAS are shown in Table 1.
- (2) The six single bacteria strains screened previously by our group included *Clostridium bartlettii* (DSM16795), *Bacteroides* sp. (controll 168), *Alkaliphilus metalliredigens* (QYMF), *Clostridium botulinum* (ATCC19397), *Bacillus cereus* (NVH0597–99), and *Bacillus thuringiensis* (IBL200). The strains were preserved in Xinjiang Production and Construction Corp.'s chemical laboratory.

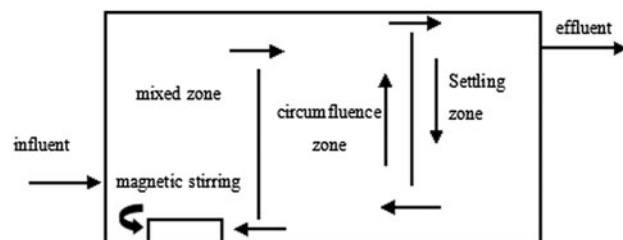


Fig. 1. Schematic of the reactor.

Table 1  
The characteristics of cultivated WAS

Item	Value
pH	6.7
MLSS ( $\text{g L}^{-1}$ )	5.48
TCOD ( $\text{mL L}^{-1}$ )	20,000
SV (%)	18
SVI ( $\text{mL g}^{-1}$ )	32.85
$\text{NH}_4^+\text{-N}$ ( $\text{mg L}^{-1}$ )	19.5
TP ( $\text{mg L}^{-1}$ )	5.24
Protein ( $\text{mg L}^{-1}$ )	194

### 2.3. Test plan

A test using continuous treatment was implemented by adding 4 L of distilled water and 3 g of anaerobic bacteria into the reactor. The sludge was pumped into the mixing zone by a peristaltic pump and disintegrated by the anaerobic bacteria. The nutrients were then released. Meanwhile, some of the nutrients were used by the anaerobic bacteria, but most of which transferred to the liquid phase.

To study the disintegration effect of the reactor, the flow of the peristaltic pump was set to  $0.9 \text{ mL min}^{-1}$ , and the hydraulic retention time (HRT) was 3 d. And to study the effect of different HRTs, the flow of the peristaltic pump was set to  $0.7 \text{ mL min}^{-1}$ , and the HRT was 4 d.

Samples were obtained every 12 h and centrifuged for 10 min at  $10,000 \text{ r min}^{-1}$  to determine the concentrations of COD, TP,  $\text{NH}_4^+\text{-N}$ , polysaccharide, and protein.

Each test was conducted for 360 h. Each sample was determined in triplicate.

### 2.4. Analytical methods

MLSS and MLVSS were determined according to standard methods [14]. Analyses of COD,  $\text{NH}_4^+\text{-N}$ , and  $\text{PO}_4^{3-}\text{-P}$  were conducted in accordance with standard methods [15]. Soluble protein was determined through BCA determination of protein concentration; the protein kit was from Solarbio Biotech Co., Ltd. Polysaccharide was measured through phenol–sulfuric acid method [16]. Determination of sludge diameter involved the use of a laser particle size distribution instrument (Microtrac S3500).

## 3. Results and discussion

### 3.1. Effect of sludge disintegration

If the sludge cells were destroyed, the organic compounds could be used again by the aerobic

wastewater treatment. Then the sludge yield could be decreased. So the changes in solids (sludge) in the system were measured when HRT was 3 d.

At 0 h, only 3 g of anaerobic bacteria and 4 L of distilled water existed. After reacting for 360 h, the effluent contained a small number of solids, as shown in Fig. 2a. The nature was obviously different between effluent and influent (Fig. 2b). The effluent was turbid but had no obvious precipitation, while the influent which was muddy and brown had obvious precipitation. A certain number of solids accumulated in the reactor, but its accumulated amount was significantly smaller than that of added sludge. So the reactor has reached the goal of design.

As shown in Table 2, the mass of the solids in the effluent was only  $0.3 \text{ g L}^{-1}$ , which is 5.5% of that in the influent. And it cannot precipitate normally (SVI cannot be tested). This result showed that the sludge floc structure was destroyed.

The particle size distribution of the influent sludge is shown in Fig. 3. The particle size was between 10 and  $400 \mu\text{m}$ , and most of it was between 50 and  $70 \mu\text{m}$ , which typical value is about  $70 \mu\text{m}$  [17]. The particle size distribution of effluent sludge when HRT is 3 d is shown in Fig. 4. The particle size was between 0.4 and  $80 \mu\text{m}$ , and most of it was between 4 and  $12 \mu\text{m}$ . The particle size distribution of the effluent sludge decreased significantly. This result suggested that the structure of some sludge floc was destroyed, and the size of the particles decreased. The particles with a small particle size ( $<1 \mu\text{m}$ ) increased while the diameter of the sludge cells was between 0.5 and  $5 \mu\text{m}$  [17], which indicated that the sludge cells were disintegrated.



Fig. 2a. Effluent quality.



Fig. 2b. Influent quality.

Table 2  
The characteristic of influent and effluent

	SV (%)	MLSS (g L <sup>-1</sup> )	SVI (mL g <sup>-1</sup> )
Influent	18%	5.48	32.85
Effluent	/	0.30	/

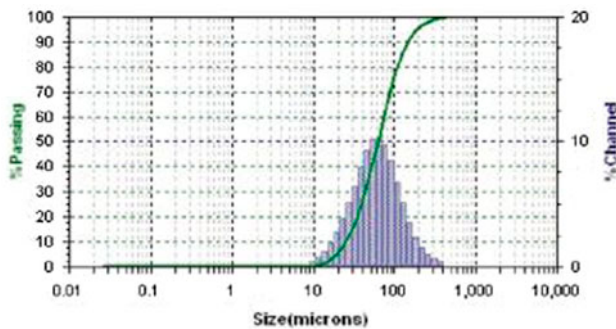


Fig. 3. Particle size distribution of influent sludge.

The MLSS of the influent was 5.48 g L<sup>-1</sup>. Hence, for the entire treatment period, the adding sludge was 106 g (5.48 g L<sup>-1</sup> × 0.9 mL min<sup>-1</sup> × 360 h × 60 min/1,000). The MLSS of the effluent was 0.30 g L<sup>-1</sup> when HRT was 3 d, and the sludge that was discharged was approximately 6 g (0.30 g L<sup>-1</sup> × 0.9 mL min<sup>-1</sup> × 360 h × 60 min/1,000). About 50 g of sludge including 3 g of anaerobic bacteria remained in the reactor after 360 h. This meant that nearly 53 g of sludge (106 g – (50 – 3) g – 6 g) was disintegrated. Hence, the average daily reduction was about 0.88 g L<sup>-1</sup>. The sludge total disintegration efficiency was about 50%, which was better than Cheng's study [13].

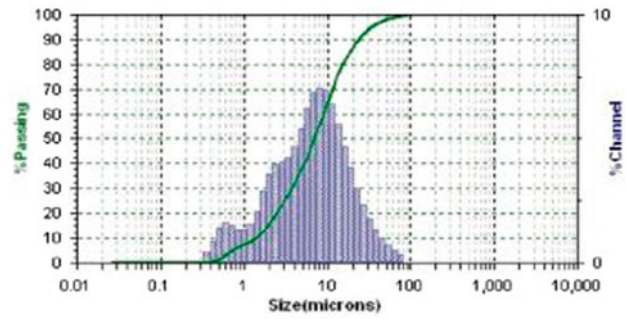


Fig. 4. Particle size distribution of effluent sludge.

In Jin's study [12], the total effective volume of the OSA system was 20.5 L, and its daily sludge reduction was about 0.64 g L<sup>-1</sup> (73% of the current reactor). In Saby's study [11], the total effective volume of the OSA system was 18.4 L. When ORP was -250 mV, the sludge production decreased about 0.13 g L<sup>-1</sup> (15% of the current reactor). However, -250 mV requires a strict anaerobic environment, which is not easy to achieve because high energy consumption is involved. While this reactor's ORP was between 0 and +50 mV, it was not strictly anaerobic and does not require additional conditions. The sludge disintegration efficiency would be better if operated at -250 mV. Considering the normal growth of anaerobic bacteria in the reactor should be more than 3 g, given that the reduction effect of this reactor is very significant.

### 3.2. Sludge disintegrate mechanism

Disintegrating the sludge is expected to transfer organic matter from the solid phase to the liquid phase, which could be used again in aerobic treatment. So the whole sludge production could be decreased. Thus, SCOD, NH<sub>4</sub><sup>+</sup>-N and TP in the liquid phase were determined to study the sludge disintegrate mechanism.

The change in effluent SCOD concentration, as shown in Fig. 5, indicated that these concentrations increased with the time of anaerobic digestion. However, the concentration of influent SCOD was unchanged, indicating that the sludge degradation ability continued to increase. Obviously, the effluent SCOD concentration of the OSA system did not present this trend under the same condition. With the extension of anaerobic time, the average daily reduction in this reactor should be raised even more. This revealed that the anaerobic bacteria could strengthen the disintegration process in sludge degradation.

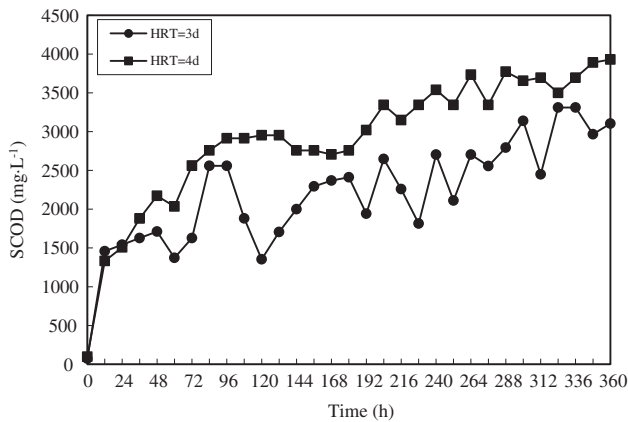


Fig. 5. Change in SCOD concentrations at different HRTs.

Cheng's study showed that under the same conditions, 5 g of sludge after disintegrating 48 h in 500 mL (the effective volume is 350 mL) shake flask, and 1.75 g of it was disintegrated by 0.3 g of anaerobic bacteria. So the average daily reduction was about  $2.5 \text{ g L}^{-1}$ , and the disintegration efficiency was 35% [13]. Although the average daily reduction is greater than this reactor, the disintegration efficiency is less than it. That is because its reaction time is only 48 h, the amount of treatment in the early stage is much higher than that in the later stage, as shown in Fig. 5.

Compared with the shake flask, this reactor's processing ability was enhanced because the extension of processing time caused the anaerobic bacteria to accumulate. In view of the microbial growth curve, the growth trend of SCOD concentration was slow at first and then fast. On the contrary, the SCOD concentration growth trend was fast at first and then slow (in Fig. 5). The likely cause was the removal of nutrients.

When HRT was 4 d, the effluent SCOD concentrations for 0–24 h were 98, 1,332, and  $1,507 \text{ mg L}^{-1}$ ; the increment rates were 1,259.00 and 13.29%. The effluent SCOD concentrations for 180–204 h were 2,757, 3,019, and  $3,343 \text{ mg L}^{-1}$ ; the increment rates were 9.50 and 10.73%. The effluent SCOD concentrations for 336–360 h were 3,695, 3,890, and  $3,929 \text{ mg L}^{-1}$ ; the increment rates were 5.27 and 1.00%. Owing to the lack of nutrients in the early stage and removing nutrients continuously, the anaerobic bacteria only obtained nutrients for their own growth by disintegration sludge. Hence, the disintegration was fast in the early stage. As anaerobic time was extended, the nutrients accumulated. The nutrients' removal rate was less than the nutrients' production rate, so the disintegration was slower in the later stage. This condition may also mean that the additional anaerobic

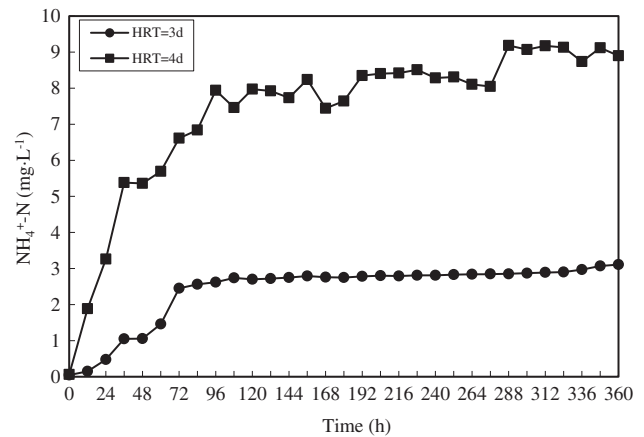


Fig. 6. Change in nitrogen concentrations at different HRTs.

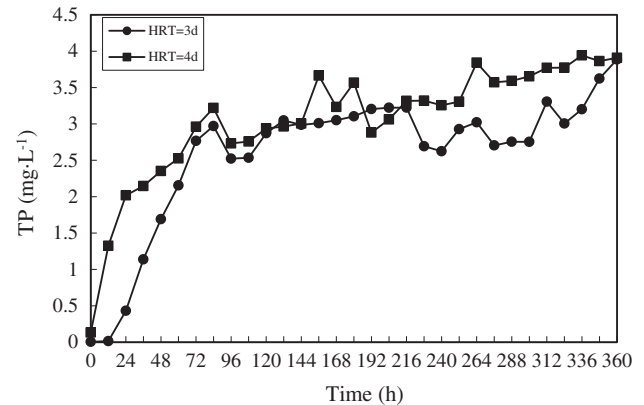


Fig. 7. Change in phosphorus concentrations at different HRTs.

bacteria in the reactor adapted to the aquatic environment and can disintegrate sludge stably.

The changes in effluent  $\text{NH}_4^+\text{-N}$  and TP concentrations are shown in Figs. 6 and 7. The microbial life structure was disintegrated by anaerobic bacteria, and the organic matter of sludge was decomposed, resulting in a large number of organic and inorganic substances dissolved into the liquid phase. In this regard,  $\text{NH}_4^+\text{-N}$ , TP, and SCOD are consistent in terms of dissolution properties. Therefore, their change trends of concentration are similar. With the extension of anaerobic time,  $\text{NH}_4^+\text{-N}$ , and TP concentrations increased; they increased sharply in the early stage of the reaction and then increased slowly in the later stage. Also, when HRT was 4 d, the concentration was higher than that in HRT of 3 d.

Inorganic nitrogen compounds were mainly derived from the release of inorganic nitrogen in the

sludge and inorganic nitrogen compounds in the cell. The anaerobic digestion of sludge mainly included hydrolysis acidification and production of acetic acid and methane. A large amount of organic acid was produced during hydrolysis acidification; this involved a process of pH decrease, and the pH of the effluent was 6.5. Therefore, nitrogen sources in the process of disintegration cannot escape in the form of  $\text{NH}_3$  [18]. Meanwhile, the possibility of nitrogen transformation from organic state to  $\text{NH}_4^+$ -N in the process of reduction is very small. Hence, the decrease in  $\text{NH}_4^+$ -N concentration was likely due to the decrease in anaerobic time.

The TP concentration of the influent was  $5.24 \text{ mg L}^{-1}$ . Hence, for the entire treatment period, the TP added to the system was  $102 \text{ mg}$  ( $5.24 \text{ mg L}^{-1} \times 0.9 \text{ mL min}^{-1} \times 360 \text{ h} \times 60 \text{ min}/1,000$ ). The average TP concentration of the effluent was  $2.60 \text{ mg L}^{-1}$  when HRT was 3 d, and the TP that was released was approximately  $51 \text{ mg}$  ( $2.60 \text{ mg L}^{-1} \times 0.9 \text{ mL min}^{-1} \times 360 \text{ h} \times 60 \text{ min}/1,000$ ). Besides, there was  $16 \text{ mg}$  ( $3.88 \text{ mg L}^{-1} \times 4 \text{ L}$ ) of TP remained in the reactor. And the concentration of TP in the discharged sludge was  $0.6 \text{ mg g}^{-1}$ , because the nature of the discharged and remained sludge was almost the same, the TP in the sludge was  $32 \text{ mg}$  ( $0.6 \text{ mg g}^{-1} \times (47 + 6) \text{ g}$ ). Considering the calculation is not very accurate, the mass of TP is equivalent ( $102 \text{ mg} - 51 \text{ mg} - 16 \text{ mg} - 32 \text{ mg} = 3 \text{ mg}$ ). The decline in TP concentration in the sludge implied that the cell was broken, and most phospholipids in the cell membrane were released into the liquid phase. This confirmed that the sludge was disintegrated well in this reactor.

However, the difference in TP concentrations at different HRTs was less than that in  $\text{NH}_4^+$ -N possibly because of the selective absorption of nutrients by anaerobic micro-organisms. The use of  $\text{NH}_4^+$ -N was higher than the use of TP. And most phosphorus-containing substances in the sludge are organic form, and mainly existing in the micro-organisms [19]. So we can guess TP nearly all from the release of micro-organisms in WAS during the reduction, and is not affected by the disintegration of floc.

Similar to SCOD and  $\text{NH}_4^+$ -N, a large number of nutrients were obtained from sludge by the disintegration of anaerobic bacteria in the early stage. The number of nutrients required for its growth in that stage was small, so TP concentration accumulated in the early stage. A wide increment rate was obtained. And the growth of micro-organisms required plenty of nutrients in the middle and later stages. Therefore, although the nutrients accumulated, the increment rates were lower than those in the previous stage.

### 3.3. Relationship between extracellular polymer and disintegration of sludge cells

The sludge in the experiment was not treated with any pretreatment, namely, the sludge cell maintained a whole floc structure before entering the reactor. Therefore, the sludge in the system needed to experience a complete floc structure, a small floc structure, an individual cell, and a breaking cell.

Protein and polysaccharide are the main components of the extracellular polymers (Extracellular Polymer Substances, EPS) in the sludge. While protein originated mainly from extracellular enzymes, which were secreted by the micro-organisms, the hydrolysis materials of cell and floc disintegrated in the sludge supernatant. Therefore, the concentrations of protein and polysaccharide can be defined as the degree of cell lysis [20].

The changes in the effluent concentrations of protein and polysaccharide are shown in Figs. 8 and 9.

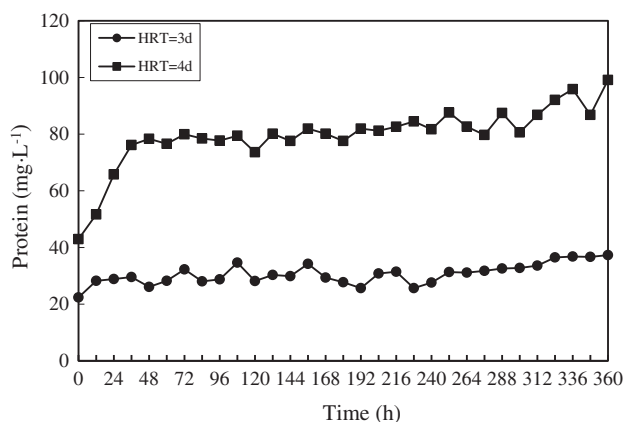


Fig. 8. Change in protein concentrations at different HRTs.

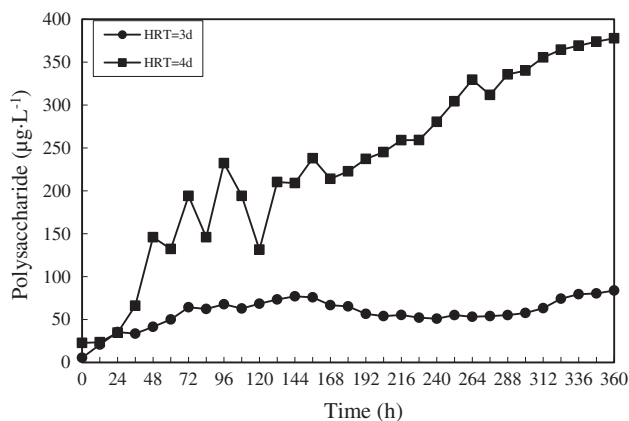


Fig. 9. Change in polysaccharide concentrations at different HRTs.

Their trends are different from those of nutrients. When HRT was 4 d, the concentration was significantly higher than that when HRT was 3 d.

This result may be attributed to the fact that sludge disintegration was mainly concentrated in the former stages when HRT was 3 d. At this point, extracellular polymer was mainly disintegrated, so the concentrations of protein and polysaccharide were low. When HRT was 4 d, most of the sludge cells were disintegrated aside from the extracellular polymer. Fig. 4 also showed the effluent particle size distribution. Therefore, protein and polysaccharide concentrations were very high. This condition meant that more disintegrated floc existed in the reactor. How to strengthen this ability is a future research direction in this field. In addition, the disintegrated products could be reused after entering the aerobic sludge treatment system and forming a complete circle. In this cycle, some of the carbon is mineralized into CO<sub>2</sub> in the aerobic phase, which can realize organic processing.

#### 4. Conclusion

- (1) A continuous reactor with anaerobic micro-organism was designed. The reactor can effectively disintegrate excess sludge. During the 360 h of experimental operation, 53 g of the sludge was disintegrated. The disintegration efficiency was 50%, and the average daily reduction was about 0.88 g L<sup>-1</sup>.
- (2) The concentrations of SCOD, NH<sub>4</sub><sup>+</sup>-N, and TP, which can reflect the effect of disintegration, tended to rise with the anaerobic time and increase in HRT. And their trends were fast at first and then slow. When HRT was 3 d, the concentrations of COD, NH<sub>4</sub><sup>+</sup>-N, and TP increased from 72, 0.04, and 0.005 mg L<sup>-1</sup> to 3,103, 3.11, and 3.88 mg L<sup>-1</sup>, respectively. When HRT was 4 d, the concentrations of COD, NH<sub>4</sub><sup>+</sup>-N, and TP increased from 98, 0.06, and 0.133 mg L<sup>-1</sup> to 3,310, 8.90, and 3.90 mg L<sup>-1</sup>, respectively. And the mass of TP is equivalent.
- (3) The concentrations of extracellular polymer also tended to rise with the anaerobic time and increase in HRT. When HRT was 3 d, the concentrations of protein and polysaccharide increased from 22.37 mg L<sup>-1</sup> and 5.37 μg L<sup>-1</sup> to 37.32 mg L<sup>-1</sup> and 83.78 μg L<sup>-1</sup>, respectively. When HRT was 4 d, the concentrations of protein and polysaccharide increased from 42.94 mg L<sup>-1</sup> and 22.69 μg L<sup>-1</sup> to 99.12 mg L<sup>-1</sup>

and 377.83 μg L<sup>-1</sup>, respectively. The concentrations of protein and polysaccharide can be used to weigh the degree of cell lysis.

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#### Glossary of Terms

MLSS	—	mixed liquor suspended solids
MLVSS	—	mixed liquor volatile suspended solids
COD	—	chemical oxygen demand
TCOD	—	total chemical oxygen demand
WAS	—	waste activated sludge
OSA	—	oxic-settling-anaerobic activated sludge process
ORP	—	oxidation-reduction potential
HRT	—	hydraulic retention time
EPS	—	extracellular polymer substances

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