

# Influence of Di-n-butyl phthalate (DnBP) on the Characteristics of Anaerobic Granular Sludge in an IC Reactor

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The characteristics of anaerobic granular sludge in the internal circulation (IC) anaerobic reactor and influence of particles by Di-n-butyl phthalate (DnBP) at normal temperature were conducted. The results show that, with HRT decreasing, the percentage of granular particles smaller than 0.3 millimeters and in the range of 2.0 and 3.0 millimeters had decreased while those between 0.9 and 2.0 mm increased significantly. For the extracellular polymeric substances (EPS), the protein content increased more, and the polysaccharide content had no significant changes; SS and VSS appeared to decline after an initial ascent; Specific methanogenic activity (SMA) of granular sludge firstly reduced significantly, and then gradually stabilized. At toxicity and recovery experiment, SMA and content of coenzyme both reduced sharply with the DnBP concentration increasing. When the concentration of DnBP was higher than 1.2 g L<sup>-1</sup>, the SMA and content of coenzyme F420 virtually reached zero. Regardless of changes in the activity of the sludge, the tendency of SMA was consistent with coenzyme F420. Therefore, DnBP had an inhibiting effect on the anaerobic granular sludge.

## Key words:

internal circulation anaerobic reactors, anaerobic granular sludge, granular diameter, settling velocity, specific methanogenic activity, content of coenzyme F420

## Introduction

The internal circulation (IC) anaerobic reactor is a successful development based on the UASB reactor of the third generation of high-rate anaerobic reactor. The UASB reactor is considered desirable in high-strength organic wastewater treatment because of its high biomass concentration and rich microbial diversity<sup>1–3</sup>. Compared with the UASB reactor, the IC anaerobic reactor, which has a smaller site area, higher-diameter ratio, higher organic load,

more stable outlet water quality and stronger capacity of impact-resistance load<sup>4</sup>, is suitable for a wide range of organic wastewater treatment<sup>5–7</sup>. The IC anaerobic reactor is well capable of removing COD removal and performs excellently at high loading rates at medium-high temperature, but it consumes high amounts of energy at medium-high temperature. Although the IC anaerobic reactor has lower processing ability when operated at ambient temperature, it can save energy and reduce costs. Therefore, it is important to research IC anaerobic reactor treatment at ambient temperature.

In comparison to other anaerobic technologies, such as anaerobic filter, anaerobic sequencing batch reactor, anaerobic expanded bed and fluidized bed reactors, the inner loop anaerobic reactor is highly dependent on its granulation process with a particular organic wastewater. Anaerobic granular sludge is the

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core component of the IC reactor. The stability and high performance of the IC reactor depends on the formation of anaerobic granular sludge with good settlement and high methanogenic activity. Anaerobic granular sludge reactor can prevent loss of sludge with the water, and maintain the population of highly active and well-settling anaerobic sludge in the reactor, so that the reactor can effectively remove the organic matter at higher organic and hydraulic load. Although ample research has been conducted<sup>8–11</sup> regarding the characteristics of anaerobic granular sludge in UASB reactors, EGSB reactors, and other high-rate anaerobic reactors, there is relatively limited research on granular sludge in IC reactors<sup>12</sup>, particularly, the granulation of anaerobic sludge under the conditions of continuous changes in water temperature and low organic concentrations.

In recent years, the paper recycling industry has developed rapidly due to the lack of raw materials. Compared to using fiber, waste paper recycling can reduce the pollution load, but the effluent still has a high amount of COD and BOD, therefore it is important to treat the paper-recycling wastewater. Di-n-butyl phthalate (DnBP) is a typical pollutant widely distributed in the regenerated papermaking wastewater, which is toxic and difficult to biodegrade. It is thus necessary to probe the effects of DnBP on microbial activity in the wastewater treatment.

DnBP, belonging to the family of phthalic acid esters, is the most frequently identified PAE in diverse environmental samples including groundwater, river water, drinking water, open ocean water, soil humates, lake sediments and marine sediments, and is one of the most widely used plasticizers in China. Also, DnBP is a rather stable compound in the natural environment and its toxic properties are even more important considering its high bioaccumulation rate in different organisms<sup>13</sup>.

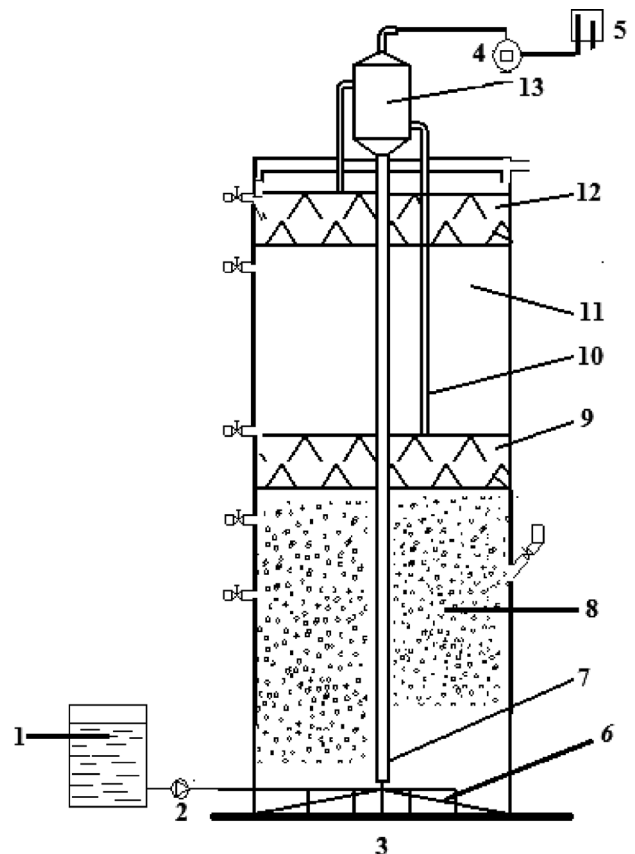
In the past few years, considerable attention has been paid to the analysis, environmental fate, general toxicity and biological degradability of these compounds in the natural environment. Numerous studies have demonstrated the biodegradation of several PAEs in wastewater<sup>14,15</sup>, in activated sludge<sup>16</sup>, in acclimated sludge<sup>17</sup>, and under anaerobic conditions<sup>18</sup>. To understand how DnBP can be degraded effectively within the WWTP facility, many studies focus on the removal mechanism and fate of DnBP in the WWTP.

In this paper, the characteristics of anaerobic granular sludge in an IC anaerobic reactor treating regenerated papermaking wastewater at ambient temperature were studied. Moreover, the influence of DnBP on the characteristics of anaerobic granular sludge in the IC reactor was exposed. In addition, the parameters of the granular sludge in different stages of treatment were investigated systematically.

## Materials and methods

### Reactor system

In this study, the IC reactor as shown in Fig. 1, was researched and developed by South China University of Technology. The reactor was cylindrical and made of Plexiglas. The reactor had a diameter of 200 mm, height of 1272 mm, and average working volume of 25.12 L.



1. Store tank; 2. Pump; 3. IC reactor; 4. Flowmeter; 5. Collecting gas bottle; 6. Inlet water distributor; 7. Recirculation tube; 8. Reaction zone of sludge bed; 9. 1<sup>#</sup> three-phase separator; 10. Riser pipe; 11. Precipitation zone; 12. 2<sup>#</sup> three-phase separator; 13. Gas storage unit

Fig. 1 – Flow chart of IC reactor

OCC papermaking wastewater from the regenerated papermaking system was used in the study. At the same time, it was necessary to add ferrous, cobalt, manganese, nickel, and other trace elements. According to the operating conditions,  $\text{NaHCO}_3$  was added to maintain pH between 6.8 and 7.2. The sludge was from Liede sewage treatment plant in Guangzhou city, China. The sludge concentration was  $71.7 \text{ kgSS m}^{-3}$ .

### Operating conditions and analytical methods

During reactor operation, COD and pH of the influent and effluent, and gas production were measured daily; MLSS, MLVSS, granular diameter dis-

tribution, setting velocity, and specific methanogenic activity were measured weekly. HRT would be changed when the removal rate of COD reached 80 % and gas production was stable.

The following parameters were analyzed according to Standard Methods<sup>19</sup>: Chemical organic demand (COD), mixed liquor suspended solid (MLSS), and mixed liquor volatile suspended solid (MLVSS). The pH was valued by glass electrode method; the distribution of the granular diameter distribution was measured according to Sieving-analysis Method<sup>20</sup>; the setting velocity was measured according to gravitational method<sup>21</sup>; specific methanogenic activity was measured according to volume method; ultraviolet spectrophotometry was used to determine the activity of coenzyme F420<sup>22</sup>; sludge EPS was extracted under conditions of dilute sulphuric acid; the carbohydrate content in EPS was measured by the anthrone method<sup>23</sup> using glucose as the standard; the protein content in EPS was measured by the modified Lowry method<sup>24</sup> using bovine serum albumin as the respective standards.

## Results and discussion

### Main physicochemical properties of the anaerobic sludge

#### Size of granules

During the operational process of IC reactor treating OCC papermaking wastewater, the characteristics of granular sludge changed greatly. Moreover, filamentous bacteria and *Methanobacterium* were the main microorganisms of the anaerobic granular sludge. The sizes of the granules at different stages are shown in Fig. 2, which shows that, with changing hydraulic retention time (HRT), the diameter of granular sludge changed greatly. Thus, with HRT decreasing, the percentage of granular particles smaller than 0.3 millimeters and those between 2.0

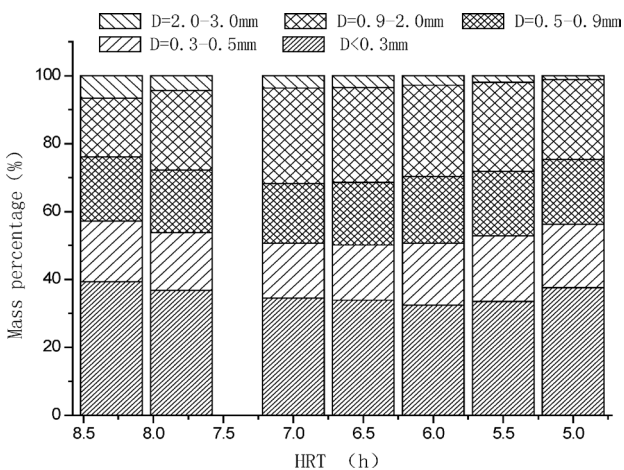


Fig. 2 – Distribution of granular sludge with HRT

and 3.0 millimeters decreased, while those between 0.9 and 2.0 mm increased significantly.

The ratio of granules having a diameter of 0.9–2.0 mm was 17.3 %, while the ratio increased to 28.2 % after treating the regenerated papermaking wastewater. In addition, there were almost no granules of diameter larger than 2 millimeters in the IC reactor because of the variation of the influent water quality and HRT. However, at HRT of 5.5 h, the average particle size became smaller. In this case, the proportion of particles larger than 0.9 mm decreased visibly. However, the percentage of granules with diameter smaller than 0.3 mm increased by 5 %, which may be due to disassociation of active sludge. The results showed that, with increased volume loading, the gas production had increased, and the shearing force of methane was enlarged by more gas disturbance, which led to the disintegration of large particles. As a result, the partial decomposition of particles larger than 0.9 mm in diameter led to the increase in 0.5–0.9-mm particles within the sludge.

#### Settling properties of granular sludge

The granular sludge is able to stay in the reactor because of its very good sedimentation performance, which is the main reason it maintained high biomass in the high-rate anaerobic reactor. As one might expect, a poor settling sludge would have a large proportion of biomass exiting from the system at low up-flow velocities, while sludge with good settling properties would remain within the test system at much higher velocities. At different reactor hydraulic retention time, the settling velocity of granular sludge with different diameters was measured, as shown in Fig. 3. The results indicate that the settling velocity of granular sludge in clean water was proportional to its diameter. More specifically, the settling velocities of granular sludge with diameters of 0.3–0.471 mm, 0.471–0.9 mm,

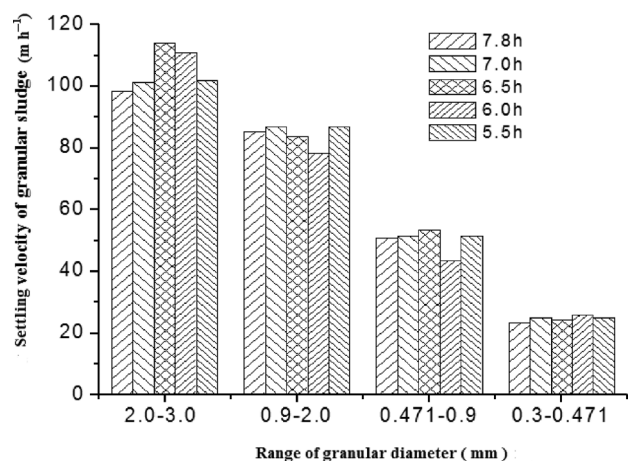


Fig. 3 – Settling velocity of granular sludge with different sizes



0.9–2.0 mm, and 2.0–3.0 mm were in the range of 23.3 m h<sup>-1</sup> to 24.89 m h<sup>-1</sup>, 43.49–51.56 m h<sup>-1</sup>, 78.19–86.94 m h<sup>-1</sup> and 98.31–116.02 m h<sup>-1</sup>, respectively. As a result, at different HRTs, the settling velocity of particles within the same size range was basically the same; with the HRT increasing, the settling velocity fluctuated slightly. Thus, the smaller the particle size, the smaller the difference in settling velocity. The reason being that, with the volume load increasing and HRT decreasing, the large-diameter internal granular sludge caused a large drop in particle density because of cell autolysis and the formation of cavity density caused by malnutrition. Thus, the larger the size of granular sludge, the less different the settling velocity.

#### Extracellular polymeric substances (EPS)

Microbial cells can produce extracellular polymeric substances (EPS), which can lead to the formation of granular sludge due to agglomeration of bacteria. EPS could provide an extensive surface area to bind microorganisms and have a significant influence on activated granular sludge. Furthermore, the sludge in the surroundings of an exopolysaccharide coating can provide sites for attraction of some organics and inorganics. Therefore, the content and composition of EPS in granular sludge had a very close relation with granule formation and stability. For years, carbohydrate was considered the main constituent of EPS, and recent studies of mixed cultures in wastewater treatment systems found that protein was also an important constituent in EPS, which is possibly due to the large quantities of exoenzymes entrapped in the EPS. In this work, polysaccharide and protein was used to characterize the content of EPS. The EPS content and composition of granules at different stages in the operation are shown in Fig. 4. As can be seen from Fig. 4,

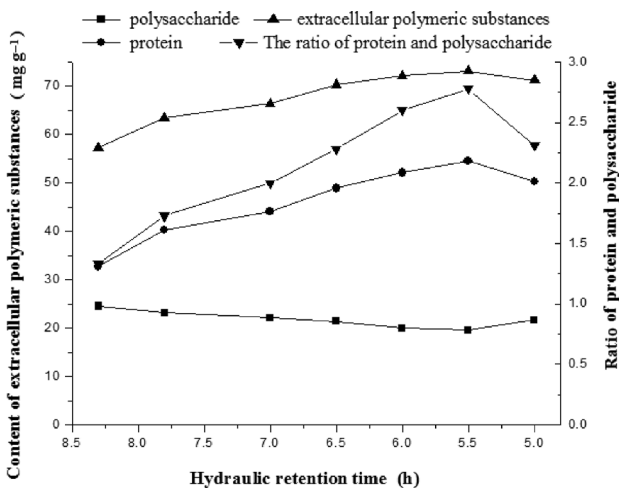


Fig. 4 – Content of extracellular polymeric substances with HRT

with the reactor hydraulic retention time increasing, the content of EPS showed the same trend with the HRT. Specifically, the protein content increased, but there were no significant changes in polysaccharide content. The ratio of protein and polysaccharide was between 1.33 and 2.31, and at different hydraulic retention times it was different as well. At HRT of 8.3 h, the ratio of proteins and polysaccharides was smallest, reaching 1.33. Compared to HRT at 5.5 h, the ratio reached the highest point (2.31); but when the HRT was reduced to 5 h, there was a sharp decline in the ratio of protein and polysaccharide. In this case, there was a slight change in the average particle size of the granular sludge, which showed that the larger the ratio of protein and polysaccharide, the larger the average particle size of granular sludge and the more stable the granular sludge.

#### Evaluation of sludge activities

##### Change of sludge concentration

According to studies, the effect of removing organisms depends on the characteristics of the granular sludge in the IC reactor. Therefore, mixed liquor suspended solids (SS) and mixed liquor volatile suspended solids (VSS) are important indicators reflecting the properties of the sludge. In addition, the average sludge concentration is mainly related to water quality, turbulent, bacterial growth rate, and other factors, so it can play a positive role in researching the sludge concentration under different operating conditions. Generally speaking, the volatile solids in the sludge are a living microbial biomass, and VSS/SS is always used to express the activity of the sludge. During the trial process, with HRT decreasing, the SS and VSS appeared to decline after an initial ascent. At HRT of 5.5 h, the SS and VSS reached maximum at 75.2 g L<sup>-1</sup>, and 55.42 g L<sup>-1</sup>, respectively. Since VSS cannot truly reflect the activity of anaerobic microorganisms, other parameters need to be determined in order to express the activity of microorganisms accurately.

##### Test of specific methanogenic activity

The specific methanogenic activity was measured in four fundamental steps, as follows: 1) Take out 25 mL of granular sludge from the reactor that has been running under normal operating conditions; 2) Vaccinate the sample into the triangular bottle; 3) Add 100 mL of water to the sludge; 4) Record the volume of methane produced. This measurement was conducted at room temperature (25 °C ~ 35 °C). The methanobacterium had the highest methanogenic activity. As shown in Fig. 5, the specific methanogenic activity changed with the operation time. Specifically, compared with the granular sludge, the methanogenic activities of the

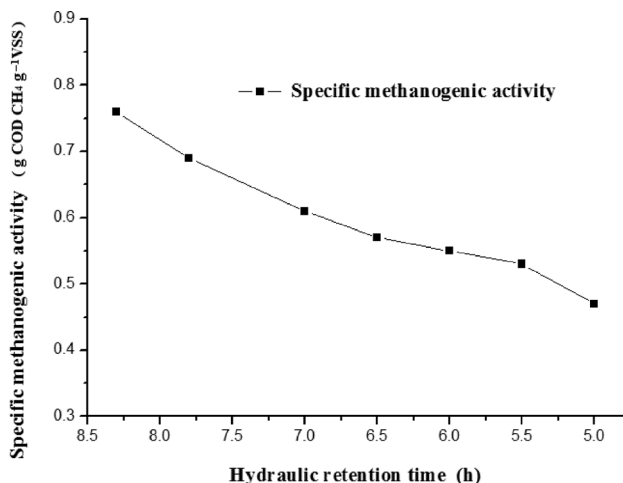


Fig. 5 – Specific methanogenic activity of granular sludge with run time

granular sludge decreased by various degrees at HRT of 8.3 h. With HRT decreasing, the specific methanogenic activity (SMA) also decreased. At HRT of 8.3 h, the sludge was domesticated for 16 days by increasing the proportion of regenerated papermaking wastewater to 10 %, 30 %, 50 %, 80 %, 100 %. The results show that, although the anaerobic granular sludge adapted to the wastewater, the SMA still declined, because the regenerated papermaking wastewater had a more complex composition. Moreover, the anaerobic sludge could not quickly adapt to the sudden change in hydraulic retention time, the sludge methanogenic activity reduced greatly, and with HRT decreasing, the activity stabilized gradually, but when HRT was reduced to 5 hours, the sludge activity dropped sharply.

#### Biological characteristics of granular sludge under DnBP

DnBP is an organic benzene derivative pollutant, it is toxic and difficult to biodegrade. According to studies, DnBP is a typical pollutant widely distributed in regenerated papermaking wastewater. In view of its toxicity, it may play an important role in the micro-study of the poisoning effect. Here, the methanogenic activity and the content of coenzyme F420 were used for the representation. Apart from adding different concentrations of DnBP, when determining the poisoning effect of DnBP on the sludge, the other steps are basically the same as for determining the methanogenic activity of granular sludge in the reactor. As shown in Fig. 6, with the concentration of DnBP increasing, the SMA and content of coenzyme both reduced sharply. When the concentration of DnBP was higher than 1.2 g L<sup>-1</sup>, the SMA and content of coenzyme F420 virtually reached zero, with no significant recovery in the

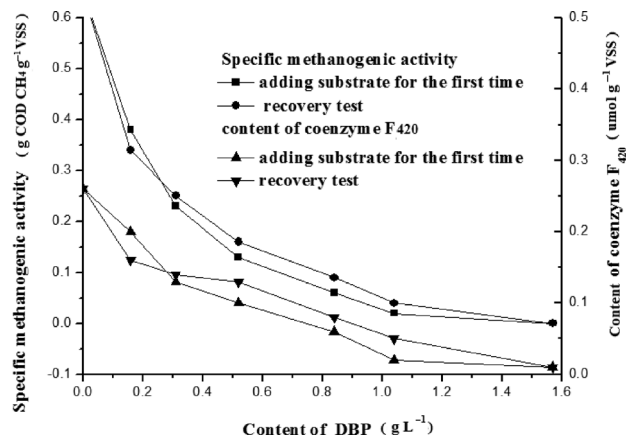


Fig. 6 – SMA and coenzyme F420 of granular sludge under different DnBP concentrations

subsequent recovery experiment. While the concentration of DnBP was less than 0.3 g L<sup>-1</sup>, the SMA decreased with decreased DnBP concentration, with slight recovery in the recovery tests. Therefore, regardless of changes in sludge activity, the tendency of SMA was consistent with coenzyme F420.

#### Conclusion

In this paper, the characteristics of anaerobic granular sludge in an IC anaerobic reactor treating regenerated papermaking wastewater at room temperature were studied. With HRT changing, the diameter of granular sludge changed greatly. With HRT decreasing, the percent of granular particles smaller than 0.3 mm and between 2.0 and 3.0 mm decreased, while those between 0.9 and 2.0 mm increased significantly. With HRT increasing, the content of EPS increased. Moreover, coenzyme F420 had a significant positive relationship with methane yields and specific methanogenic activity; with the concentration of DnBP increasing, SMA and content of coenzyme both reduced sharply.

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## Nomenclature

SS	– Suspended solids
IC	– Internal circulation
UASB	– Up flow anaerobic sludge blanket
EGSB	– Expanded granular sludge bed
COD	– Chemical oxygen demand
BOD	– Biochemical oxygen demand
DnBP	– Di-n-butyl phthalate
PAE	– Phthalic acid ester
OCC	– Old corrugated cartons
MLSS	– Mixed liquor suspended solid
MLVSS	– Mixed liquor volatile suspended solid
VSS	– Volatile suspended solids
HRT	– Hydraulic retention time

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