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# Enhancement of sludge dewaterability with filamentous fungi *Talaromyces flavus* S1 by depletion of extracellular polymeric substances or mycelium entrapment

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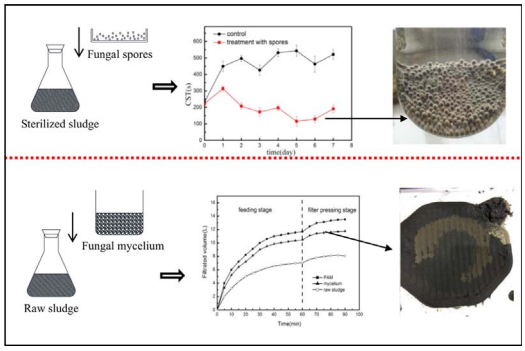
## GRAPHICAL ABSTRACT




## ARTICLE INFO

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## ABSTRACT

This study was conducted to explore the mechanism of dewaterability improvement of waste activated sludge by the filamentous fungus *Talaromyces flavus* S1. When the fungal spores were inoculated to the sterilized sludge, the sludge dewaterability was significantly improved by 48.1% and the reasons can be attributed to sludge pellet formation and degradation of extracellular polymeric substances, in particular the slime-EPS and loosely-bound EPS (LB-EPS). With the addition of fungal mycelium into the either sterilized sludge or non-sterilized sludge, the values of CST decreased by 74.0% and 43.7%, respectively, suggesting the fungal mycelium can improve the sludge dewaterability. After conditioned by the mycelium, the sludge cake by the diaphragm filter press was thicker and showed less water content than the control sludge. The results in this study demonstrated that the *Talaromyces flavus* S1 can serve as an environmentally friendly biological dewatering agent and has a promising application potential in the future.

## 1. Introduction

With the persistent increase of the waste activated sludge (WAS) generation from the wastewater treatment plants all around the world, the volume reduction of WAS has become one of the most important strategies in WAS management. WAS treatment and subsequent

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disposal account for up to approximately 60% of the operating cost of the wastewater treatment plant (Wang et al., 2017). Dewatering of WAS has attracted more and more interest because of the significant effect of volume reduction on the storage, transport, and disposal of produced WAS.

Traditionally, a large amount of organic or inorganic chemicals are used in the process of sludge dewatering as conditioners. Use of chemical polymers has many disadvantages, including high cost, negative effects on the following anaerobic digestion, corrosion, and potential ecological risks during the land application of biosolids (More et al., 2010). There is a strong need to seek biological, safe and environmentally friendly methods to achieve sustainable dewatering of WAS.

A wide variety of filamentous fungi exist in sewage sludge that plays vital roles in the wastewater treatment process. Recently, several filamentous fungal species have been found to be useful for dewatering and other treatment processes of the WAS such as settling, organic compounds degradation, etc., under controlled operating conditions (Molla et al., 2012; Mannan et al., 2005). The filamentous fungal mycelium can form a network facilitating sludge flocculation and dewatering (More et al., 2010). Filamentous fungus strengthens the floc structure and allows the formation of larger and stronger flocs that improve the sludge settling, resulting in sewage effluent with low turbidity. For example, a filamentous fungal strain *Penicillium expansum* BS30 was used to simultaneously improve the sludge settling and dewaterability (Subramanian et al., 2008). Because of the effectiveness in enhancing sludge dewaterability, filamentous fungi conditioning was regarded as a promising novel method to replace the synthetic chemicals for the conditioning of WAS (Fakhru'l-Razi et al., 2007; Molla et al., 2012).

Wang et al. reported that the degradation of extracellular polymeric substances (EPS) by filamentous fungus *Mucor* sp. GY-1 improved the sludge dewaterability (Wang et al., 2015). However, some questions still remained in the mechanism of dewaterability improvement by filamentous fungus. For example, due to the existence of various and complicated indigenous microorganisms in raw sludge, the dewaterability improvement probably cannot be directly attributed to the inoculated filamentous fungus since the fungus was inoculated into the unsterilized raw sludge. On the other hand, Jin et al. demonstrated that the physicochemical properties change could improve the dewaterability of the raw sludge without any inoculum when it was cultured for a long time (Jin et al., 2004). What's more, many previous studies were conducted at a lab scale, and only rare studies were carried out in real diaphragm filter press.

Therefore, the objectives of this study are (1) to investigate the potential of an acid-tolerant filamentous fungus *Talaromyces flavus* S1 in the conditioning of WAS for dewatering, (2) the detailed mechanism of dewaterability enhancement by filamentous fungus *T. flavus* S1 and (3) the characteristics of filamentous mycelium entrapment and its practical dewatering effect on the raw WAS in a diaphragm filter press. The results in the present study will be very helpful to shed a novel insight into the mechanism of dewaterability enhancement by the *Talaromyces flavus* S1.

## 2. Materials and methods

### 2.1. Sludge sample and filamentous fungal strain

Waste activated sludge used in this study was collected from the gravity thickener tank of the Taihu New City Wastewater Treatment Plant in Wuxi City, Jiangsu Province, China and stored at 4 °C in polypropylene bottles for subsequent use. The characteristics of the sludge were: pH was 7.43, solid content was 3.71%, organic matter content was 46.58% (VS/TS), the specific resistance to filtration (SRF) value of this sludge was $1.71 \times 10^{13}$ m/kg and the capillary suction time (CST) value was 86.9 s. Sludge was sterilized at 121 °C for 30 min for the experiment as sterilized sludge.

The filamentous fungus *T. flavus* S1 was isolated from sewage sludge sample with rose bengal medium (RBM) and potato dextrose agar medium (PDA) plates by the serial dilution technique to screen the fungal strain for the study (Murugesan et al., 2014). The fungal universal primers *Its*1 (5′-TCCGTAGGTGAACCTGCGG-3′) and *Its*4 (5′-TCCTCCGCTTATTGATATGC-3′) were used for PCR amplification in fungal internal transcribed spacer (ITS), then the PCR products were sequenced and identified by Sangon Biotech (Shanghai) Co., Ltd China. The obtained 18S rRNA sequences were compared with sequences in the GenBank database using the NCBI Blast search program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Closest cultured and uncultured relatives were retrieved from the database. A neighbor-joining tree was made based on the 18S rRNA gene sequences determined in this study and related reference sequences. Alignment and phylogenetic analysis were performed with the MEGA 4.1 (Beta) software. The procured phylogenetic tree was shown in Supplementary Fig. 1. The fungal strain S1 was saved in China General Microbiological Culture Collection Center (CGMCC) with the preservation number 13768. The isolated fungal strain was grown for 3–5 days on PDA at 30 °C. Then it was maintained at 4 °C for the preparation of fungal spore suspension liquid and inoculum to be used in sludge treatments.

### 2.2. Fungal spore inoculation experiment

The fungus on PDA was repeated washed by 10–15 mL sterilized distilled water, and then the flushing fluid was collected in triangular flask. Subsequently, the suspension was diluted three times and incubated in a gyratory shaker at 150 rpm and 28 °C for 24 h and the suspension was used for subsequent inoculation experiment. The spore concentration of the suspension was diluted to $2.0 \times 10^7$ spores/mL measured by the hemocytometer counting method (Ryoo, 1999).

The fungal spore inoculation was conducted in a series of 250 mL conical flasks each containing 100 mL of sterilized or unsterilized sludge sample. The spore suspension (fungal inoculum) of 1 mL was added into the flasks. Based on the growth cycle of *T. flavus* S1 in the pre-experiment, all flasks were incubated at 30 °C and 150 rpm for 7 days in orbital shakers. Sludge without inoculation of fungi was served as control. Sludge samples were collected every day at pre-determined intervals and analyzed for pH, SRF, CST etc.

### 2.3. Mycelium inoculation experiment

Fungal spore suspension was acquired using the methods described in Section 2.2 and the spore concentration of the suspension was diluted to $1.0 \times 10^7$ -$5.0 \times 10^7$ spores/mL. For the fungal mycelium preparation, prepared fungal spore suspension was pre-cultured in potato-dextrose broth (PDB) media at 30 °C and 130 rpm for 7 days in orbital shakers until the mycelium formation. Then the different quantity of mycelial culture (with culture solution) was inoculated in sterilized or unsterilized sludge samples according to the inoculum sizes of 30% based on the volume ratio, and the sludge without mycelium inoculation was set as control.

At the same time, the mycelial culture was separated with centrifugation (4000 rpm and 20 °C for 15 min). The sediment was regard as mycelium sediment and the suspension liquid contained fungi secretion. Subsequently, the sediment and suspension were added to sterile sludge as well as unsterilized sludge. The dewaterability of sludge samples was further analyzed, meanwhile, sludge samples without inoculation of fungi and addition of cationic polyacrylamides (PAM) were served as control to evaluate the improvement of dewaterability.

### 2.4. EPS extraction and analysis

The extraction method of EPS: slime-EPS, loosely-bound EPS(LB-EPS), tightly-bound EPS(TB-EPS) was used by the modified protocol

(Domínguez et al., 2010). The sludge samples (50 mL) were centrifuged at 4000 rpm for 15 min, and organic matter in the supernatant was the slime-EPS. Then the sludge pellets were washed with 0.05% (w/w) NaCl solution. After that, the collected sludge pellets were re-suspended by 0.05% (w/w) NaCl solution to its original volume. The mixtures were sheared by a vortex mixer for 10 min. Thereafter, the suspensions were centrifuged at 7400 rpm for 15 min and the bulk solution and solid phase were collected separately. The organic matter in the supernatant was the loosely-bound EPS (LB-EPS). The residual sludge pellets were resuspended to the original volumes (50 mL) with 0.05% (w/w) NaCl solution, followed by the horizontal oscillation at 25 °C and 150 rpm for 10 min. Afterward, the samples were extracted by heating in a water bath at 80 °C for 30 min, and then the tightly-bound EPS (TB-EPS) were harvested by centrifuging at 15,000 rpm and 20 °C for 20 min to remove remaining sludge components. Organic matter in the bulk solution was the TB-EPS. The particulates and low-molecular-weight (MW) metabolites in the slime EPS, LB-EPS, and TB-EPS solutions were removed using the 0.45 μm acetate cellulose membranes and the dialysis tubes of molecular weight cut off (MWCO) of 3500 Da (Shanghai Sangon Biotechnology, China), respectively, before chemical analysis.

### 2.5. Pilot-scale sludge dewatering experiment

The sterilized PDB liquid medium was added into the fermenter (7 L) and the fungal spore suspension was inoculated with 1:1000 (volume ratio). The mycelium was obtained after 7 days fermentation at the temperature of 20 °C and the pH of 6.63. Then the *T. flavus* S1 mycelium and PAM was added to the sludge with solid content of 3.71%, respectively, with the amount of 30% (volume ratio) and 1.5‰ (g/kg dry matter of sludge) based on the pre-experiment results. The mixture of sludge was tested on a 5 L bench-scale diaphragm filter press (XAGY0.6/250-15U) for dewatering experiment. The parameters were shown in Table 1.

### 2.6. Physical and chemical analysis method

The total solids of the sludge (TS), volatile solids (VS) and pH were determined according to Standard Methods (APHA, 1998). The carbohydrate contents in the EPS solutions were measured by the phenol–sulfuric method (Herbert et al., 1971). The contents of proteins in the EPS solutions were determined using Lowry–Folin method (Lowry et al., 1951). For dewaterability, CST was measured using a capillary suction timer (Triton Electronics Type 304 M) with 6 mL of well mixed sludge samples. SRF was determined using the Buchner funnel test (Lo et al., 2001). All experiments were conducted in triplicates and the data presented are mean and standard deviations. Statistics analysis was performed by the software JMP 8.0. ANOVA to determine the significance of difference wherever applicable.

## 3. Results and discussion

### 3.1. Effect of inoculum of fungal spores on unsterilized and sterilized sludge

The fungal spores of *T. flavus* S1 were inoculated into the raw sludge without sterilization to explore the effect of fungal growth on the dewaterability. The CST and SRF are two widely used indicators to represent the dewaterability of the sludge. The changes of CST and SRT during the growth of the fungus *T. flavus* S1 are shown in Fig. 1.

Usually, the sludge with a high value of CST cannot be dewatered easily (Neyens et al., 2003). In Fig. 1a, CST values of fungal spores inoculated sludge and the control sludge (without fungal spore) decreased gradually in the first two days. For the control sludge, it decreased from 20.9 s at initial to 13.7 s in 2 days. Meanwhile, the value of CST declined significantly from 21 s to 12.4 s in fungal spores inoculated sludge. It can be seen that the CST values of two kinds of sludge fluctuated between 12 s and 16 s, but generally maintained stable during the next 5 days, showing the similar trend and no difference with the fungal spores inoculation treatment ($P > 0.05$). For the SRF values of the fungal spores inoculated sludge and the control sludge (Fig. 1b), they also decreased significantly in 4 days and changed slightly in the next 3 days. In the fungal spores inoculated sludge, it changed from $2.76 \times 10^{12}$ m/kg at initial to $0.83 \times 10^{12}$ m/kg on day 4, while in the control sludge, it declined from $1.94 \times 10^{12}$ m/kg on day 0 to $0.97 \times 10^{12}$ m/kg on day 4.

Although the CST and SRF values of two kinds of sludge indicated that the dewaterability of sludge was improved somewhat, there is no significant difference between the fungal spores inoculated sludge and the control sludge. The improvement of the dewaterability of the two kinds of sludge under the aerobic culture might result from the declined pH values in the sludge (data not shown). This result proved that the inoculation of fungal spores of *T. flavus* S1 didn't improve the dewaterability of the sludge. In the study by Wang et al. (2015), the fungi were inoculated into the unsterilized sludge and the dewaterability improvement was observed. According to the results in Fig. 1a in the present study, the dewaterability of control sludge without fungus inoculation was also improved after 7 days aerobic culture, so the role of the filamentous fungi in the dewatering process should be investigated deeply.

To exclude the influence of the growth of indigenous microorganisms on the sludge dewaterability, the raw sludge was sterilized before the inoculation of fungal spores of *T. flavus* S1. The changes of CST and SRF are showed in Fig. 1c, d.

After sterilization, the inoculated fungal spores can grow well because of the absence of competition from the indigenous microorganisms in sludge. As shown in Fig. 1c, d, the sludge dewaterability was deteriorated greatly with the sterilization, leading to higher SRF and CST values initially. However, with the inoculation of fungal spores, the CST value decreased obviously from 222.9 s to 115.7 s on the fifth day. While in the control sludge, the CST values increased significantly from 232.7 s to 521.6 s after 7 days culture (Fig. 1c). There is a huge gap of the CST values between the two kinds of sludge, indicating the growth of the fungal spores influenced the dewaterability significantly ($P < 0.01$). In Fig. 1d, the SRF values of sludge inoculated with fungal spores decreased from an initial value of $7.58 \times 10^{12}$ m/kg to $5.1 \times 10^{12}$ m/kg after 4 days culture, and the control sludge had a much higher SRF value at $19.7 \times 10^{12}$ m/kg after 4 days culture. Although the SRF of sludge with the inoculation of spores did not decrease sharply, remarkable difference was observed between the fungal spores inoculated and control sludge ($P < 0.01$).

In the fungal spores inoculated sludge, the *T. flavus* S1 grew very well, which greatly improved the dewaterability of the sludge (More et al., 2010). As a comparison, the inoculated fungal spores cannot grow well in the unsterilized sludge, which leads the slight

**Table 1**
Relevant parameters of the diaphragm filter press.

| External size of diaphragm filter press (mm) | | | Volume of filter chamber (L) | Filter area ($m^2$) | Feeding sludge pressure (MPa) | Squeezing pressure (MPa) | Time of feeding sludge (min) | Time of squeezing (min) |
|---|---|---|---|---|---|---|---|---|
| Length 1500 | Width 500 | Height 600 | 5 | 0.6 | 0.6 | 0.8 | 30 | 40 |

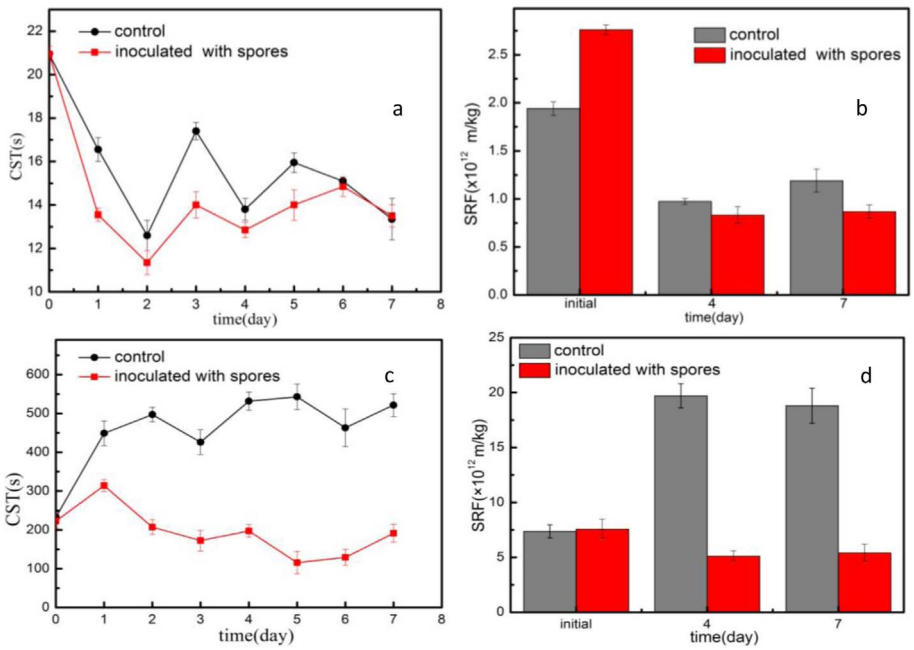


**Fig. 1.** The changes of CST (a), SRF (b) in unsterilized sludge with or without inoculation of fungal spores and CST (c), SRF (d) in sterilized sludge with or without inoculation of fungal spores.

improvement of the sludge dewaterability. This also indicated that the fungal spores of *T. flavus* S1 cannot win the competition over the indigenous microorganism in a complex microbial environment of raw sludge. Finally, it should be noted that the dewaterability deterioration of sterilized sludge during the incubation was attributed to the large amount of released EPS in sludge, which caused the very high values of CST and SRF (see the results in Section 3.2).

### 3.2. Change of the sludge EPS with inoculation of fungal spores

It is well known that the EPS in the sludge influences the sludge dewaterability significantly. Usually, the EPS can be divided into three kinds of EPS, namely, the slime EPS, loosely-bound EPS (LB-EPS) and tightly-bound EPS (TB-EPS) (Zhou et al., 2014). Furthermore, different types of EPS have various impacts on the sludge dewaterability (Huo et al., 2014).

The changes of slime EPS, LB-EPS and TB-EPS were shown in Fig. 2a, c and e, and the compositions of three kinds of EPS were shown in Fig. 2b, d and f, respectively. For the slime EPS, there was a significant difference ($P < 0.01$) between the control sludge and fungal spores inoculated sludge (Fig. 2a). The concentration of slime EPS in fungi inoculated sludge descended sharply from initial 3715 mg/L to 1297 mg/L on the day 4 and maintained stable, while in the control sludge, the initial slime EPS was 3688 mg/L and increased to 3790 mg/L. As shown in Fig. 2b, the polysaccharide content in slime EPS decreased from 3485 mg/L to 1070 mg/L (decreased by 69.3%) and from 3491 mg/L to 2740 mg/L (descended by 21.5%) in fungi inoculated sludge and control sludge during the 7 days of incubation, respectively. However, the protein content in slime EPS didn't show significant difference ($P > 0.05$) between the control sludge and fungal spores inoculated sludge.

As for the LB-EPS in fungal spore inoculated sludge, it gradually decreased from 803 mg/L to 274 mg/L on day 7. The LB-EPS in control sludge was much higher but showed a similar change trend to the spore inoculated sludge, decreasing slowly from 805 mg/L to 496 mg/L on day 7. Polysaccharide content in LB-EPS of the control sludge decreased from 716 mg/L to 417 mg/L during the incubation period, whereas in fungal spores inoculated sludge it decreased from 719 mg/L to 104 mg/L after 7 days incubation, indicating polysaccharide degradation in LB-EPS was reduced by the inoculation of *T. flavus* S1.

The concentrations of TB-EPS in two kinds of sludge changed slightly, which fluctuated at about 175 mg/L in the whole culture period (Fig. 2e). Specifically, polysaccharide content in the TB-EPS of the two kinds of sludge fluctuated in the range of 75–150 mg/L. The protein content in both of LB-EPS and TB-EPS changed little, and its content was relatively less than the polysaccharide.

Generally, it can be seen that the degradation of slime EPS and LB-EPS, especially the slime EPS, due to the growth of *T. flavus* S1 spores contributed to the dewaterability improvement of fungal spore inoculated sterilized sludge. The LB-EPS had the same trend with the slime EPS, while the disparity between the fungal spore inoculated sludge and the control sludge looked narrower than that in Fig. 2a. It might be that LB-EPS was hard to be degraded by *T. flavus* S1 compared to slime EPS during the incubation. Apparently, the TB-EPS was the most difficultly degraded EPS by fungi, because it was tightly bound with the sludge floc and the fungi cannot degrade this type of EPS easily.

It should be noted that the polysaccharide was the main content of the sludge EPS. There are some different views about the contributions from polysaccharide and protein in sludge EPS. Some previous studies found that the protein content had important impact on the sludge dewaterability (Yu et al., 2008), but the polysaccharide was also reported to be an important factor (Dai et al., 2017). In the present study, we found that the polysaccharide content had a more important impact on the dewaterability improvement of sludge. The reason was that the polysaccharides were a major component in slime EPS and LB-EPS, and it can be more easily degraded by inoculated fungi spores than the protein.

### 3.3. Effect of fungal spores inoculation on sludge particle size

The distribution of sludge particle size was analyzed on day 0, 2, 4 and 6, respectively. The change of sludge particle size during the incubation of *T. flavus* S1 spores are shown in Fig. 3.

As shown in Fig. 3a, the particle size of initial sterilize sludge was mostly distributed from 0 to 80 μm, accounting for 96.69% and the percentage content of sludge particle larger than 100 μm was 1.06%. After 2 days incubation, the particle size distribution of control sludge changed little and the distribution curves were almost overlap. However, the particle size of the fungi inoculated sludge became larger, and the percentage of particle size larger than 100 μm was 6.24% on day 2. As shown in Fig. 3b, the distribution of sludge particle size of control

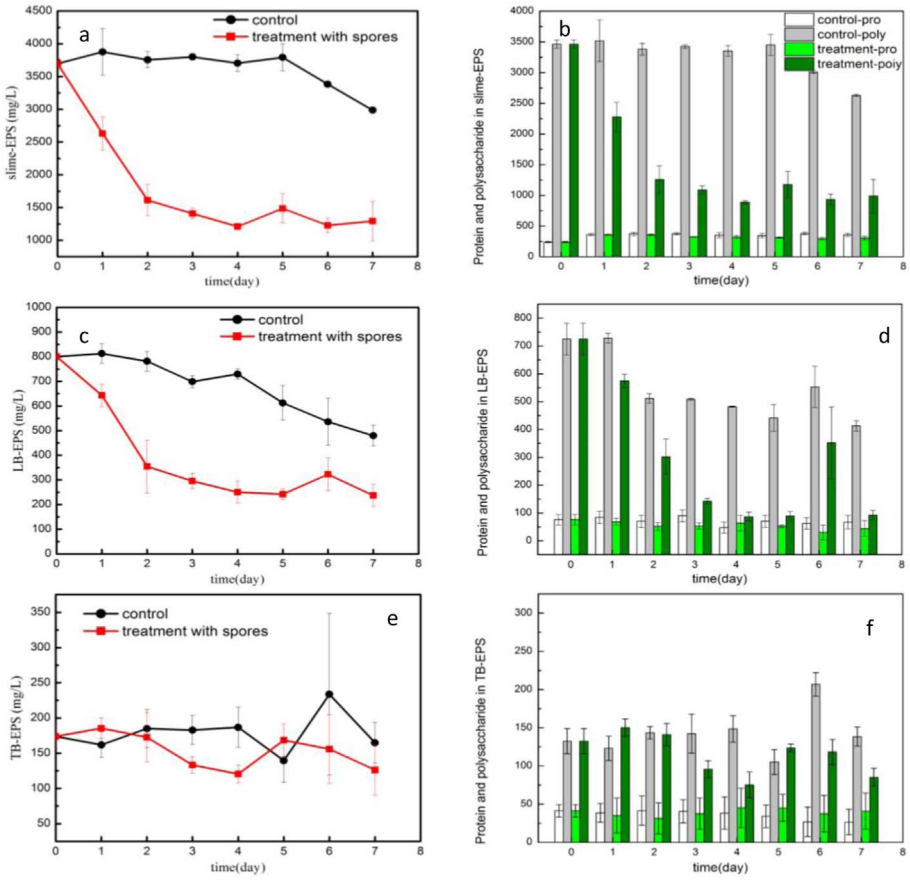


**Fig. 2.** Change of EPS in sterilized sludge, (a) slime EPS, (b) polysaccharide and protein content of slime EPS; (c) LB-EPS, (d) polysaccharide and protein content of LB-EPS; (e) TB-EPS, (f) polysaccharide and protein content of TB-EPS.

sludge on day 4 was similar to the day 0. On contrast, the percentage content of sludge particles with the size of 0–80 μm and larger than 100 μm of fungal spore inoculated sludge decreased from 96.69% to 85.12% and increased significantly to 10.76% on day 4. After 6 days incubation, the percentage of particles size larger than 100 μm increased to 13.79% (Fig. 3c) and finally the sludge flocs aggregated

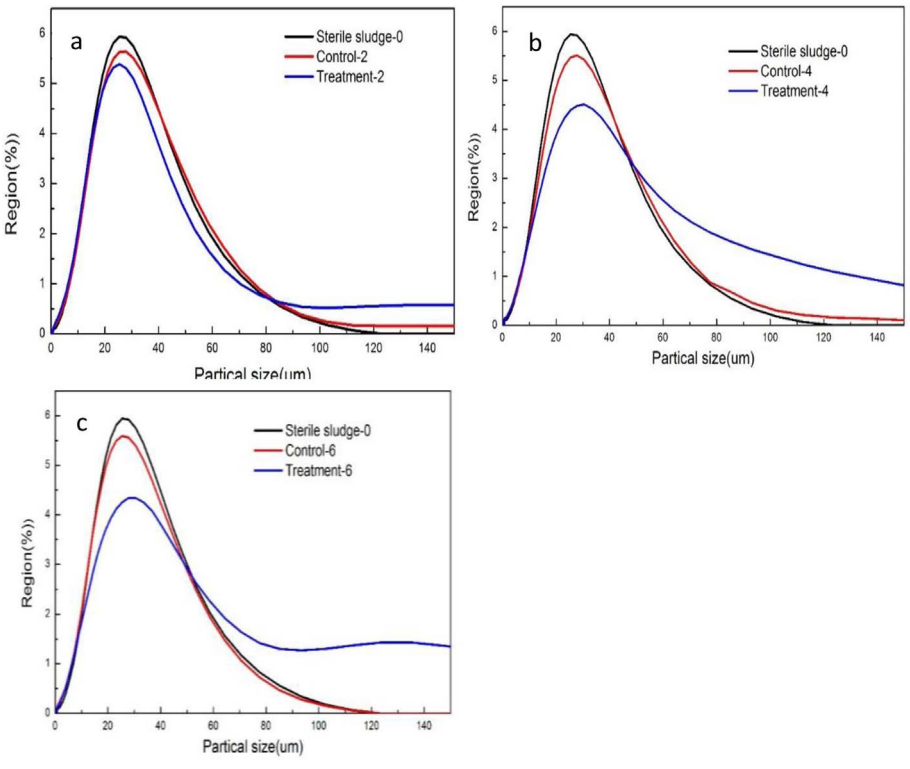


**Fig. 3.** Change of sludge particle size after inoculation of fungal spores on day 2 (a), day 4 (b) and day 6 (c). Sterile sludge-0 in figures a,b,c represents the initial sludge particle size before the spores inoculation.

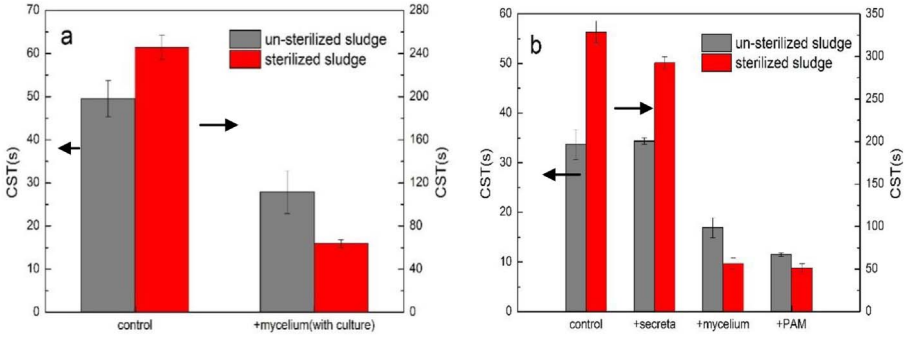


**Fig. 4.** Change of CST by fungal mycelium with culture solution (a) and separated fungal mycelium or fungal secretion as well as the PAM (b) in sterilized and un-sterilized sludge.

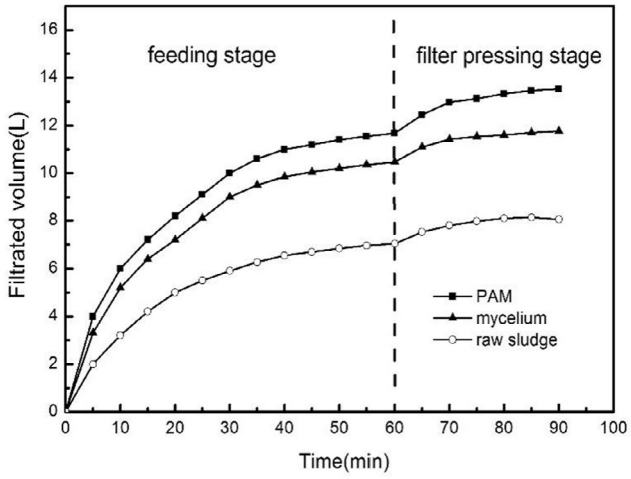


**Fig. 5.** Influence of sludge dewatering rate by *T. flavus* S1 mycelium and PAM.

together and form sludge pellets with the average diameter at about 6 mm in fungal spore inoculated sludge. This is because of the growth of the fungal spores in the sterilized sludge and the fungal mycelium entrapped the sludge particles to form sludge pellets (Murugesan et al., 2014). The sludge pellets formation in fungal spore inoculated sludge also helps improve the dewaterability of the sludge which was proved by the CST and SRF data in Fig. 1.

### 3.4. Impact of fungal mycelium on sludge dewaterability

After investigation of the dewaterability mechanism by inoculation of *T. flavus* S1 spores in sterilized sludge, to explore the impact of fungal mycelium on the dewaterability of sludge, the impact of *T. flavus* S1 mycelium was tested in sterilized and unsterilized sludge, respectively.

In Fig. 4a, CST decreased from 49.5 s at initial to 27.85 s after the addition of fungal mycelium solution (with the culture solution) in the unsterilized sludge. Meanwhile, for the sterilized sludge, it declined significantly from 245.7 s to 63.9 s after addition of fungal mycelium solution. It can be seen that the CST of unsterilized sludge and sterilized sludge decreased by 43.73% and 73.99%, respectively, indicating a significant improvement of dewaterability with the treatment of fungal *T. flavus* S1 mycelium solution. Moreover, the sterilized sludge showed a better dewaterability improvement than the unsterilized sludge.

Because the mycelium solution also contains polysaccharides substances which were secreted by the culture of fungal spores in medium, these polysaccharides substances might play the flocculation effect of the sludge particles and result in the improvement of dewaterability (Liu et al., 2016). In order to identify whether the dewaterability improvement of sludge was attributed to the flocculation effect or the entrapment effect of fungal mycelium, the mycelium and its culture solution which includes the secrets components were separated and tested their independent impact on sludge dewaterability. As a comparison, the widely used chemical flocculant PAM was also applied to show the effect of fungal mycelium on dewaterability of sludge. Fig. 4b showed the CST value change of the unsterilized and sterilized sludge by the addition of the fungal secretion and fungal mycelium itself, respectively.

The CST value of the initial sludge was 33.7 s, however, the values changed to 43.4 s and 16.9 s, respectively, after addition of the fungal secreta and mycelium in unsterilized sludge. For the sterilized sludge, CST of the initial sludge, secretion treatment and mycelium treatment sludge were 328.7 s, 292.5 s and 56.8 s, respectively. CST of secretion treatment sludge and mycelium treatment sludge decreased by 11.01% and 82.72%, suggesting that it was the fungal mycelium improved the dewaterability rather than the fungal secreta.

Additionally, the PAM effect on sludge dewaterability was tested as a comparison, the CST value dropped by 65.88% and 84.33% in unsterilized and sterilized sludge, respectively. The dewaterability improvement by the fungal mycelium is very close to the traditional chemical flocculant. This furtherly proved that the fungal mycelium was a good biological conditioning agent to improve the sludge dewaterability.

### 3.5. Evaluation of fungal mycelium dewatering effect by diaphragm filter press

Due to the absence of the practical effect of the fungal mycelium on the sludge dewatering, the *T. flavus* S1 mycelium solution was added into the raw sludge and pressed by a 5 L scale diaphragm filter press. The filtering characteristics during the sludge dewatering are shown in Fig. 5 and the sludge cake and their surface morphology pictures are shown in Supplementary Fig. 2.

The dewatering process was divided into sludge feeding stage and sludge pressing stage and the change of filtrate volume represents the dewaterability of the sludge. As shown in Fig. 5, the filtration process of three kinds of sludge was almost completed in the sludge feeding stage. The filtrate volume of the untreated raw sludge was much less than the PAM conditioning sludge and fungal mycelium conditioning sludge. The filtrate volume of PAM conditioning sludge was slightly higher than that of the fungal mycelium conditioning sludge, but they were very close, indicating that the dewatering efficiency of the chemical conditioning and fungal mycelium were similar. At the end of the whole filter process, the filtrate volumes of the untreated raw sludge, PAM conditioning sludge and fungal mycelium conditioning sludge were 8.06 L, 13.52 L and 11.76 L respectively.

The thickness of sludge cake is an indicator to evaluate the dewaterability of the sludge. With a thicker sludge cake, the sludge dewaterability is better. After treatment, the thickness of the sludge cake conditioned by fungal mycelium was higher than the untreated raw sludge. On the other hand, the water contents of the raw sludge cake and fungal mycelium conditioned sludge were different. The water contents of the untreated raw sludge cake, fungal mycelium conditioned sludge cake and PAM conditioned sludge cake were 78.91%, 68.87% and 61.36%, respectively. Finally, the surface morphology of

untreated raw sludge and fungal mycelium conditioned sludge were different. The particle size of the raw sludge was tiny and very even, however, the particle size of conditioned sludge was getting bigger and the sludge flocs aggregated together, which apparently was in favor of liquid filtration and finally the sludge dewaterability. These observations are all consistent with the CST data of the previous sections. All these results indicated a remarkable fact that the *T. flavus* S1 mycelium improved the dewaterability of the sludge.

## 4. Conclusions

The mechanism of dewaterability improvement of the WAS by the filamentous fungus *Talaromyces flavus* S1 was explored. With the inoculation of fungal spores into the sterilized sludge, the dewaterability was improved by 48.09% and pellets formation and degradation of slime-EPS and loosely-bound EPS were the two major reasons for the dewaterability improvement of sludge. The fungal mycelium improved the sludge dewaterability thanks to the entrapment of sludge particles. The practical application of fungal mycelium in diaphragm filter press demonstrated that the *Talaromyces flavus* S1 could be an environmental friendly and promising biological agent for the sludge dewatering.

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## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.08.185.

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