Digestion properties of intracellular polymers (IPS) and extracellular polymeric substances (EPS) and influences of EPS on anaerobic digestion of sludge

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Abstract: Anaerobic digestion is a method adopted to treat sludge, but its effectiveness is limited by the existence of extracellular polymerics substances (EPS). For the first time, this study separated sludge into intracellular polymers (IPS) and EPS to investigate each of their digestion properties and the influences of EPS. We found: (1) EPS anaerobic digestion was finished in three days and was much quicker than IPS; (2) the removal of organic matter in EPS was higher than IPS; and (3) biogas yield of EPS (266.6 mL/g-VS) was higher than IPS (229.2 mL/g-VS). To examine the influences of EPS on sludge anaerobic digestion, we compared the EPS-removal sample with the control sample and found that EPS could restrict biogas production during sludge anaerobic digestion. After removing EPS, cumulative biogas production and biogas yield were improved by 9.4% and 28.8%, separately. The whole biogas production process was also shortened by 7 days after removing EPS. Future studies could decide whether EPS is needed to be removed based on their purposes on anaerobic digestion outcomes (e.g. prioritize the production of biogas or the removal of organic matter).

Keywords: Sludge; IPS; EPS; anaerobic digestion

1. Introduction

Excess sludge is a by-product of wastewater and sewage treating process. Treating and disposing sludge has gradually become a burden for many countries and regions due to its high cost, huge load and high risk (Yamada et al., 2015; Yang et al., 2015a). To solve above issues, anaerobic digestion is a method adopted to treat sludge with the purposes of fuel (biogas) production, organics stabilization and mass reduction (Appels et al., 2008; Chen et al., 2008; Han et al., 2019). However, conventional sludge anaerobic digestion has some limitations, such as low biogas production, low organic matters removal and long sludge retention time (Yamamoto et al., 2006; Zhang et al., 2012).

Sludge is consisted of many small core microbial colonies and large quantities of water. Each core microbial colony can be regarded as a matrix filled with cells and extracellular polymeric substances (EPS) (Fig. 1). EPS is the exterior material outside microorganisms' cell in the sludge matrix. It has many critical roles in stabilizing sludge's characteristics, such as via stabilizing flocs, causing adhesion, forming a protective barrier to harmful substances, accumulating hydrolytic enzymes, and preventing desiccation (Li and Ganczarczyk, 1990; Appels et al., 2011). Many studies have demonstrated that EPS plays a great role in sludge flocculation, settling, and dewatering (Liu and Fang, 2003; Peng et al., 2012) but the stable and complex matrix structure formed by EPS has also been considered as one of the main reasons restricting the sludge anaerobic digestion (Carrère et al., 2010; Peng et al., 2012). Thus, disrupting this structure could be a prospective approach to improve the performance of sludge anaerobic digestion.

Many studies focused on directly disrupting this stable structure and improving the digestion efficiency by applying various pretreatment methods, such as ultrasonication and ozonation (Carrère et al., 2010; Guan et al., 2018; Kor-Bicakci et al., 2019). Although different pretreatment methods improve sludge anaerobic digestion in different ways, the enhanced digestion effectiveness is mostly correlated to the existence of EPS, such as the left quantity of EPS, layers of EPS and amount of organic matters in EPS (Carrère et al., 2010; Ebenezer et al., 2015). Significantly, several studies have report that separating EPS from the sludge matrix could enhance the pretreatment efficiency (Gayathri et al., 2015; Ebenezer et al., 2015). However, few studies have studied the role of EPS in sludge anaerobic digestion directly. Here the sludge anaerobic digestion is defined as the biological transformation of sludge organic substances into biogas under anaerobic conditions. Although it is well-known that sludge biomass contains EPS and intracellular polymeric substances (IPS), the digestion properties of sludge EPS and IPS, and the influence of EPS on sludge anaerobic digestion are little studied (Yu et al., 2013; Zhou et al 2017).

To address above gaps, this study separated the sludge into IPS and EPS, and studied the anaerobic digestion properties of sludge IPS and EPS, respectively. Additionally, performances of IPS and EPS of producing biogas were studied and discussed. As the main ingredients, protein and carbohydrate in IPS and EPS were also measured to corroborate the anaerobic digestion results. The final section synthesizes all measured peformances of IPS and EPS in anaerobic digestion and discusses the influence of EPS on sludge anaerobic digestion.

2. Materials and Methods

2.1 Sewage sludge and inoculums

Sewage sludge used in this study was collected from the secondary settling tank of a municipal wastewater treatment plant in Beijing, China. The sludge sample was stored at 4 °C to maintain its biological activity after collecting from the plant. The sludge sample was then thickened by gravity settling. The total chemical oxygen demand (TCOD) of the sludge after thickening is 26,575 mg/L, the total solids (TS) is 28,767 mg/L, the soluble chemical oxygen demand (SCOD) is 137 mg/L, the carbohydrate is 4,816 mg/L, the protein is 8,312 mg/L, and the pH is 7.15. The seed culture used in this study is obtained from the mesophilic anaerobic digester in the same wastewater treatment plant.

2.2 Separation of EPS and IPS

To investigate the effects of EPS on anaerobic digestion, sludge needs to be separated into EPS and IPS. The separation approach we adopted is the thermal treatment method suggested by Yu et al. (2013). First, 200 mL sludge sample was centrifuged for 10 min at a speed of 6,000 rpm. Second, the supernatant was filtered through a 0.45 μm membrane. Third, the residue was re-suspended to its original volume using phosphate-buffered saline (0.790% NaCl, 0.024% KH_2PO_4, 0.142% Na_2HPO_4 and 0.020% KCl) which could prevent cell lysis. Fourth, the mixed liquor was heated at 60 °C for 1 h and centrifuged for 15 min at a speed of 6,000 rpm. Fifth, the supernatant was filtered again through a 0.45 μm membrane. The filtrate gathered from first two filtration steps is EPS, and the residue is IPS. (*PS: To facilitate analysis, IPS, EPS and 200 mL of raw sludge samples were all diluted with Milli-Q water to 500 mL before anaerobic digestion experiments*.)

2.3 Anaerobic digestion experiments

Batch anaerobic digestion tests were in 1L serum bottles. EPS, IPS and raw sludge were digested separately, and three duplicates are set up for each group. Each serum bottle was filled with 500 mL of substrate and 66 mL of inoculums. The control-test group was filled with the raw sludge sample and inoculums, the blank-test group was filled with only inoculums and two duplicates are set up for blank-test and control-test groups. Every reactor was purged with N_2 gas for 10 mins to remove air. After an anaerobic condition was ensured, all reactors were transferred into a water-bath shaking incubator (Anting Sci. Instrument Inc., China) to keep a temperature of 36 ± 1℃ and an agitating speed of 100 rpm.

2.4 Analytical methods

The TS and VS were measured according to the Standard Methods (APHA, 2005), the pH was by a pH tester and the COD was by COD meter (CTL-12, Huatong Inc., China). The sludge sample was centrifuged for 10 min at a speed of 12,000 rpm within a centrifuge (TGL16, Yingtai Inc., China) and was immediately filtered through a 0.45μmmembrane to measure the SCOD. Regarding the measurement of TCOD, the sludge sample was treated with a NaOH dosage of 0.5mol/L for 24 h, and then filtered through a 0.45μm membrane. Consequently, the COD of the filtrate could be used as the TCOD (Fang et al., 2014). Proteins were measured by following the Bicinchoninic Acid (BCA) method (Yang et al., 2015b), and carbohydrates were measured by the phenol-sulfuric acid method (Yang et al., 2015b). Most anaerobic bacteria are strict anaerobic bacteria and their performance of producing gas would be limited even by exposing to oxygen in the air (Novaes, 1986). To block air inlet, the biogas production was measured by using a water feed apparatus (Tang et al., 2017). The reported values of biogas volume of IPS, EPS and raw sludge anaerobic digestion were the measured values minus that of the blank group.

3. Results and discussion

3.1 Sludge matrix separation performance

To explore the anaerobic digestion properties of EPS and IPS, and the effects of EPS on sludge anaerobic digestion, this study separated the sludge matrix into EPS and IPS at first. After separation, the total solid content (TS) of IPS and EPS were 24,621 and 7,358 mg/L, the concentration of protein were 7,078 and 1,452 mg/L, and carbohydrates were 3,888 and 388 mg/L, respectively. The EPS content is an important indicator of sludge matrix separation performance. Clearly, the TS of EPS accounted for 23% of the sludge TS (sum of EPS and IPS), which was within the normal range of 20~25% (Frølund et al., 1996). Moreover, the ratio of protein to carbohydrate in EPS is another important indicator of sludge matrix separation and was about 3.7

in this study, which was in the normal range from 0.5 to 21.2 (Liu and Fang, 2003). Above results indicate that the separated EPS and IPS were in normal conditions and could be used for next-step anaerobic digestion.

3.2 IPS and EPS anaerobic digestion properties

3.2.1 Biogas production

Biogas production of IPS and EPS anaerobic digestion was studied, and the results were showed in Fig. 2. Fig.2b shows that the process of EPS anaerobic digestion was almost ended within three days. It is much quicker than that of IPS anaerobic digestion. The final cumulative biogas production after IPS and EPS anaerobic digestion were 733 mL (Fig. 2a) and 83 mL (Fig. 2b), respectively. The biogas yield of EPS was 266.6 mL/g-VS_{added}, which was 16.3% higher than that of IPS anaerobic digestion. The higher biogas yield of EPS might be contributed by various kinds of organic matters contained in the sludge IPS and EPS (Liu and Fang, 2003). Gayathri et al. (2015) reported that the biogas yield of IPS anaerobic digestion was 435 mL/g-VS_{added} after ultrasonic pretreatment, which was higher than that of this study. The reason of this increase might be that ultrasonic pretreatment enhanced the biodegradability of intracellular materials.

Above figures also indicate that most of the produced biogas originated from intracellular materials. The biogas production ratio of IPS and EPS to sludge (the sum of IPS and EPS) was 89.8 % and 10.2%, respectively, for IPS and EPS anaerobic digestion. This result was reasonable as IPS counted for most of the sludge total solids.

3.2.2 Organic matter removal

Organic matter (VS) removal is also an important indicator of sludge anaerobic digestion. Organic matter in the EPS was more efficiently removed than that in the IPS after anaerobic digestion. The final VS removal efficiency was 33.3% and 27.8% in the EPS and IPS anaerobic digestion, respectively. Most of the previous studies reported that 30%~50% of sludge EPS organic matters could be biodegraded during anaerobic digestion (Carrère et al., 2010), which was consistent with the result in this study. However, Dai et al. (2013) reported that the final VS removal of EPS was only 24% after anaerobic digestion of dewatered sludge, which might be because part of easily degradable EPS, such as supernatant and slime, was already removed in the dewatered sludge.

3.3 Protein and carbohydrate degradation

Protein and carbohydrate are primary components of both sludge IPS and EPS, and thus their degradation is vital for both IPS and EPS digestion. Degradation properties of protein and carbohydrate in the IPS and EPS were studied, and the results were shown in Fig. 3. Final protein and carbohydrate degradation efficiencies were 25.6% and 36.5% in the IPS anaerobic digestion and were 34.4% and 40% in the EPS anaerobic digestion (Fig. 3), respectively. The higher digestion efficiencies of carbohydrate in both the IPS and EPS digestion above illustrates that carbohydrate could be easier to be degraded than protein in anaerobic digestion process. This finding is consistent with other studies, such as Yang et al. (2015b), which found that degradation efficiency of protein was lower than that of carbohydrate during sludge anaerobic digestion. The removal efficiency of protein and carbohydrate in the EPS were both higher than that in the IPS, contributing to the similar VS removal in the EPS and IPS anaerobic digestion.

Regarding the protein and carbohydrate degradation pattern, a clear difference of EPS and IPS digestion was found. Throughout the whole IPS digestion process, carbohydrate was degraded before protein because initial rapid carbohydrate hydrolysis could restrict protease formation (Fig.

3a) as reported by Yang et al. (2015b). However, during EPS anaerobic digestion, the degradation of protein and carbohydrate was almost synchronous (Fig. 3b). Yang et al. (2015b) argued that carbohydrate was degraded before protein during raw sludge anaerobic digestion.

Effects of EPS on sludge anaerobic digestion

It is widely believed that EPS will restrict the sludge anaerobic digestion performances (Appels et al., 2008; Chen et al., 2008; Yu et al., 2008; Subramanian et al, 2010; Xu et al., 2011; Han et al., 2019). However, most studies were performed by comparing sludges with different EPS concentrations or compositions. In this study, we separated EPS and IPS, which could also be regarded as EPS-removed sludge. Thus, comparing IPS and the untreated sludge sample (the control) could give us clear insight on how EPS impacted the sludge digestion.

Biogas production is one of the most important indicators of anaerobic digestion performance. The effect of EPS on biogas production was synthesized and is presented in Fig. 4. As shown in Fig. 4, the biogas production pattern was influenced not greatly by EPS. In both of EPS-removed sample and control groups, the biogas production had a quick production phase before day 21, and a slow production phase after day 21. Regarding the cumulative biogas production, it was 733 mL for the EPS-removed sample and 670 mL for the control. Final cumulative biogas production was improved by about 9.4% after removing EPS, which further confirmed that the EPS had a restrict effect on biogas production. As for the biogas yield, it was 229.2 mL/g-VS for the EPS-removed sample and 178 mL/g-VS for control, respectively. The biogas yield was improved by about 28.8% though EPS removal, suggesting that EPS could restrict the conversion of organic matters into biogas during sludge anaerobic digestion. This result was consistent with what Kavitha et al. (2014) found in their study. The higher biogas yield might be because EPS removal could relieve the restrict effect of sludge matrix, which accelerated the hydrolysis step during sludge anaerobic digestion. The biogas yield of EPS-removed sample (229.2 mL/g-VS) was significantly higher than that produced by peracetic acid oxidation pretreated sludge (180 mL/g-VS) as found by Appels et al. (2011).

Additionally, this study identified that the biogas production process could be accelerated after removing EPS. The biogas production process of EPS-removed group was almost ended on day 28 for the EPS-removed sample, which was seven days earlier than that for control (35 days) (Fig. 4). This finding also suggests that the existence of the EPS restricts the production of biogas. Namely, EPS could restrict biogas production during sludge anaerobic digestion, suggesting that EPS removal might be a feasible way to enhance biogas production.

Moreover, the effect of EPS on organic matter removal was also studied. The final VS removal efficiencies were 27.8% for EPS-removed sample and 34.1% for the control group respectively (Fig.4). This finding suggests that the presence of EPS could have a positive effect on VS removal during sludge anaerobic digestion. This finding might be attributed to the fact that the organic matters in EPS were easily degraded than that in IPS.

4. Conclusions

This study specially separated sludge into EPS and IPS to examine their anaerobic digestion performances separately. Results showed that the process of EPS anaerobic digestion was much more rapid than that of IPS anaerobic digestion. Almost 90% of produced biogas originated from IPS during sludge anaerobic digestion versus 10.2% from EPS. The final VS removal efficiency of EPS anaerobic digestion was 33.3%, which was higher than that of IPS anaerobic digestion (27.8%). In both IPS and EPS digestion, carbohydrate was more efficiently degraded than protein.

Regarding the effects of the EPS on sludge anaerobic digestion, the study explored that EPS could restrict biogas production volume and rate. EPS removal improved the cumulative biogas production and biogas yield by 9.4% and 28.8% respectively and shortened the biogas production process by 7 days. However, this study is not arguing to remove the EPS in all sludge anaerobic digestion process as the presence of EPS can help to remove organic matter. Thus, future studies could decide whether EPS is needed to be removed based on their purposes on anaerobic digestion outcomes (e.g. prioritize the production of biogas or the removal of organic matter).

Conflicts of interest

There is no conflict to declare.

Data Availability Statement

Some or all data, models, or code generated or used during the study are available from the corresponding author (Email: myponesky@163.com) by request, including methodology of sampling procedure for evaluating carbohydrates and proteins, characteristics of inoculum (TS, VS, pH), reactor's design, etc.

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List of figure captions

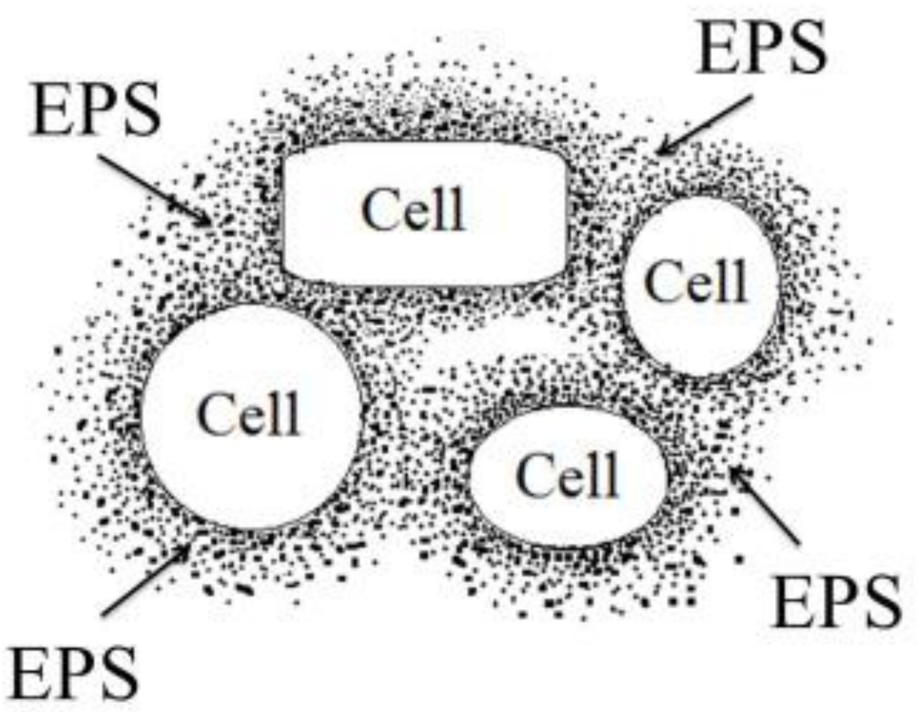


Fig.1 Structure of a sludge matrix

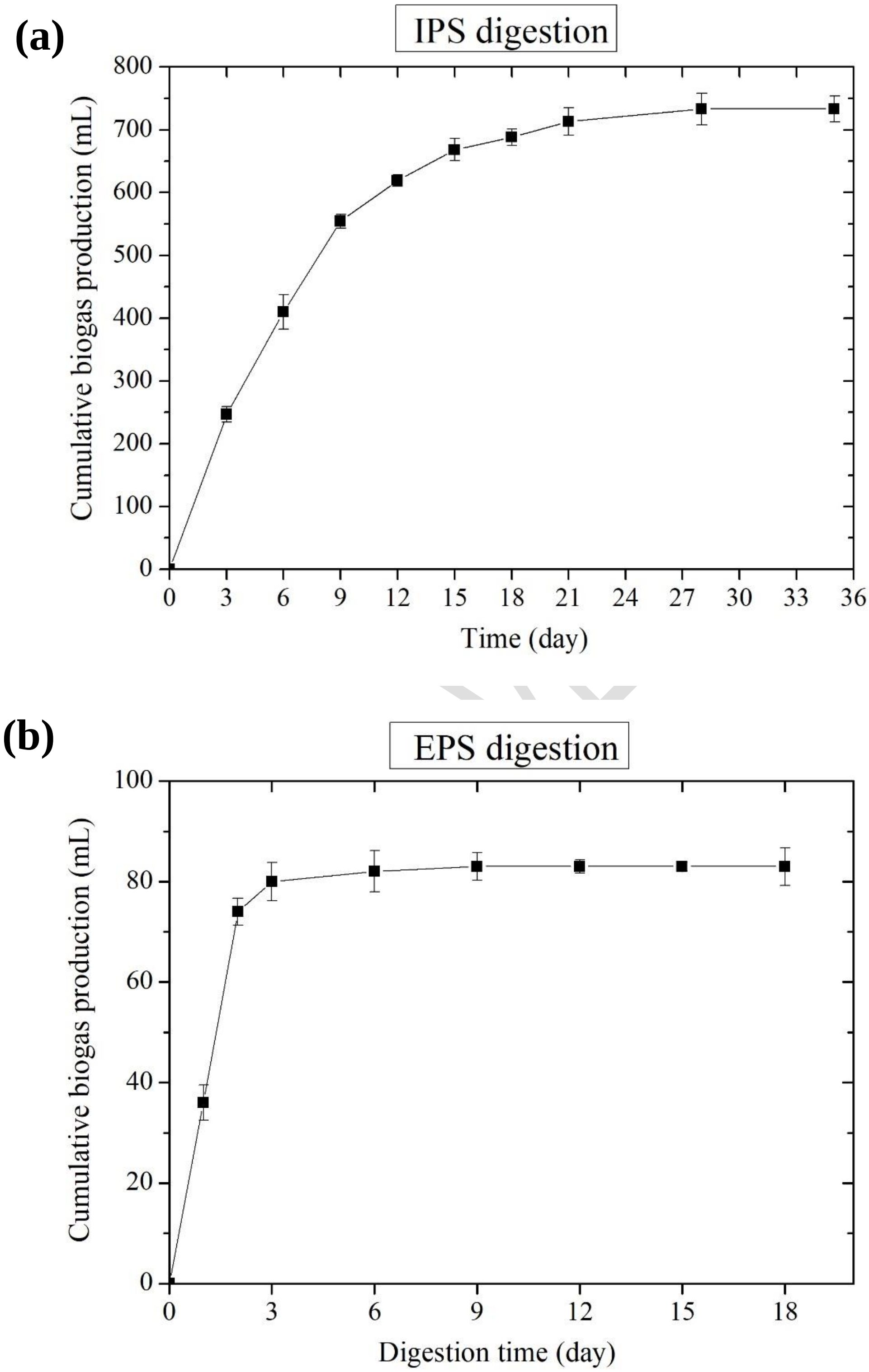
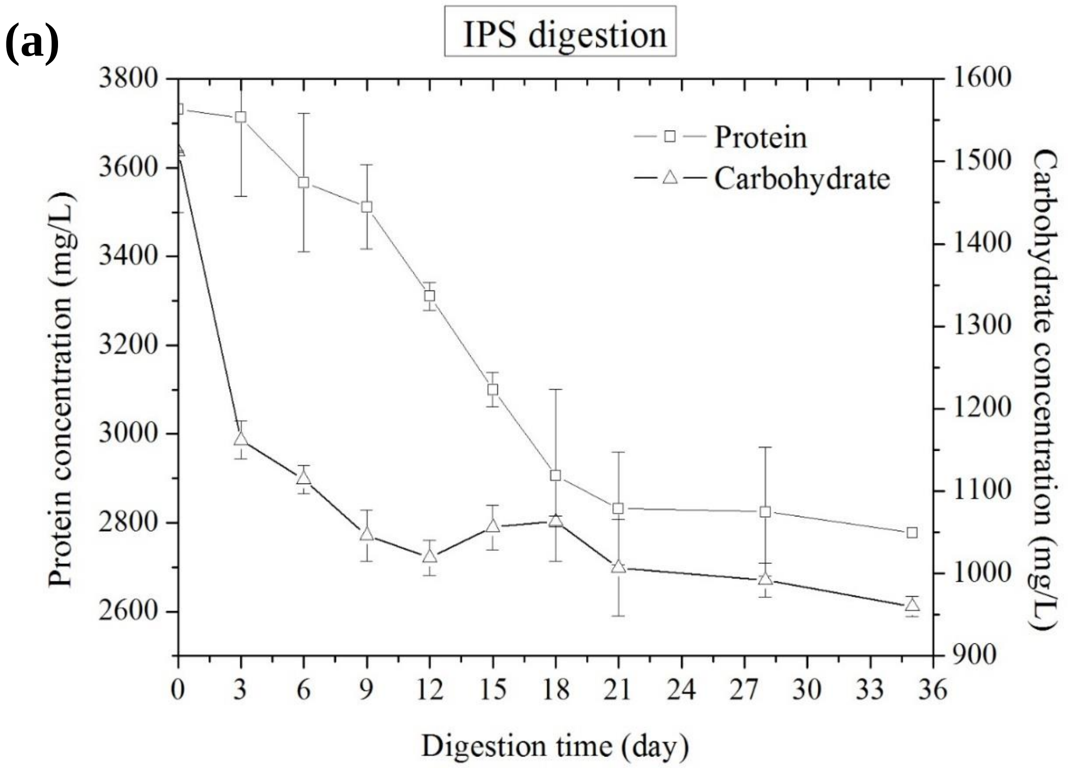


Fig. 2. Biogas production of (a) intracellular polymers (IPS) and (b) extracellular polymeric substances (EPS) anaerobic digestion.


(a)
IPS digestion
Protein
Carbohydrate
Protein concentration (mg/L)
Carbohydrate concentration (mg/L)
Digestion time (day)
3800
3600
3400
3200
3000
2800
2600
1600
1500
1400
1300
1200
1100
1000
900
0
3
6
9
12
15
18
21
24
27
30
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36

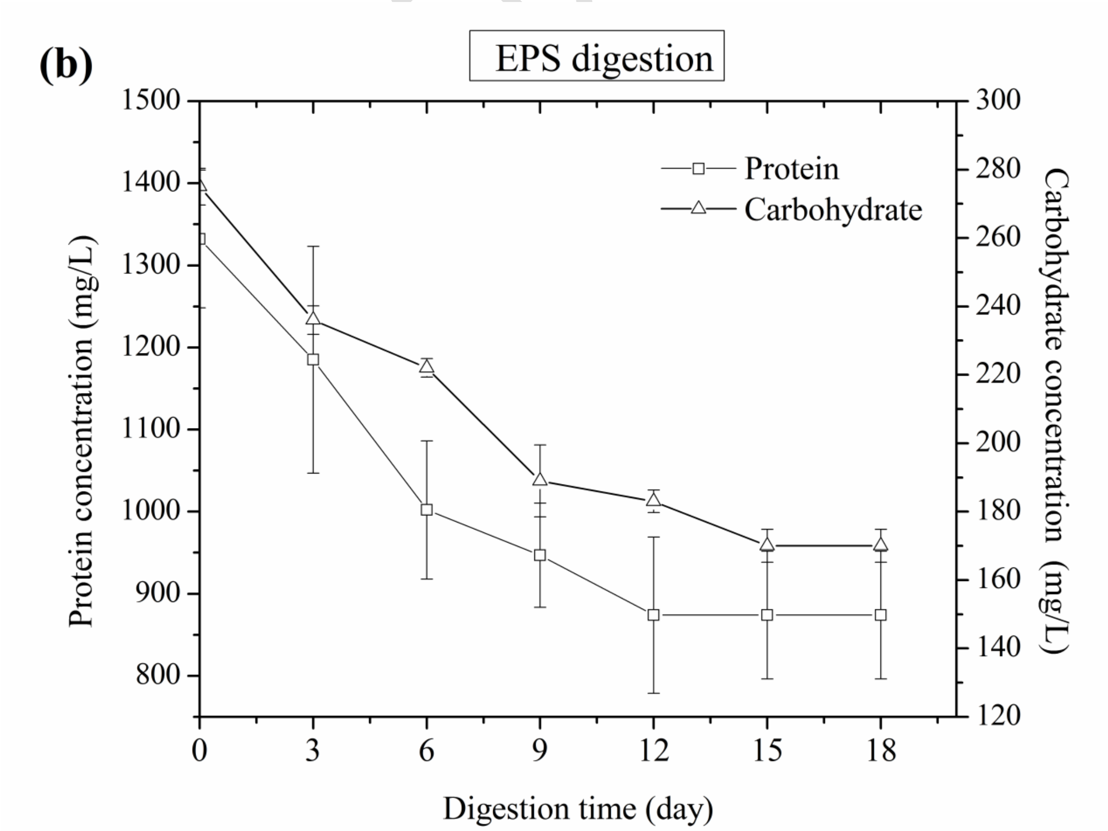
(b)
EPS digestion
Protein
Carbohydrate
Protein concentration (mg/L)
Carbohydrate concentration (mg/L)
Digestion time (day)
1500
1400
1300
1200
1100
1000
900
800
300
280
260
240
220
200
180
160
140
120
0
3
6
9
12
15
18

Fig. 3. Evolution of protein and carbohydrate concentrations during (a) intracellular polymers (IPS) and (b) extracellular polymeric substances (EPS) anaerobic digestion.

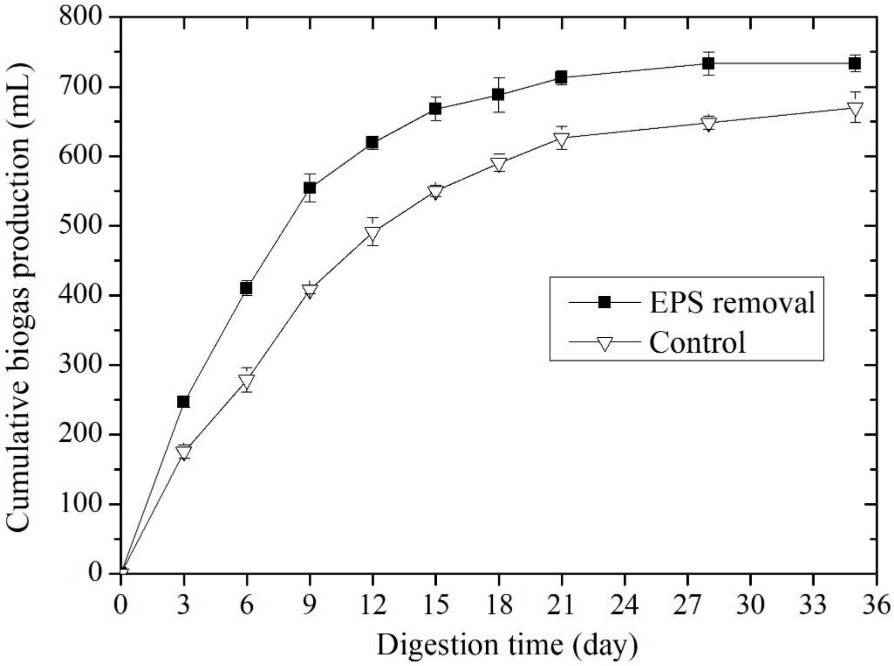

Fig. 4. Effects of extracellular polymeric substances (EPS) on biogas production during sludge anaerobic digestion.

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