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Effects of stirring strategies on the sludge granulation in anaerobic CSTR reactor

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ABSTRACT

The anaerobic sludge granulation was studied in two laboratory-scale continuous-flow stirred tank reactors (CSTRs), which treating synthetic glucose wastewaters under a mesophilic constant temperature of 35 ± 1°C. Different stirring strategies were employed in the two CSTRs. Magnetic stirring at a rotation speed of 140 rpm was used in CSTR1 and axial continuous agitation at a speed of 90 rpm in CSTR2. It was observed that highly activated granular sludge formed in CSTR2, but flocculent sludge still remained in CSTR1 after about 45 days' cultivation. Liquid circulation analysis of the two stirring modes displayed that the overall circulation in CSTR2 was more uniform. Furthermore, calculation results proved that the hydraulic shear force produced in CSTR2 was more suitable for granules formation than it in CSTR1, and granules did form in the CSTR2. Therefore, it could be concluded that in the laboratory-scale CSTR, stirring strategy played a key role in sludge granulation. On the other hand, scanning electron microscope images of granular sludge displayed that filamentous bacterial dominated in the outer layer and bacilli surrounded by lots of extracellular polymeric substances in the inner layer. Both of these are beneficial to promote the granules formation and maintenance.

Keywords: Anaerobic CSTR; Sludge granulation; Stirring strategy; Hydraulic shear force

1. Introduction

Anaerobic wastewater treatment played an important role in the environmental engineering field, especially for the treatment of high-strength organic wastewater. There are various types of micro-organisms, including fermentative bacterial, acetogens, and methanogenes, participating in the anaerobic process, which resulting in the complexity and biodiversity of the anaerobic systems.

It is generally considered that the development of granular sludge is the key factor for the successful operation of an anaerobic reactor [1]. The main reasons include the following: (1) Granular sludge is critical for the effective separation of suspended biomass from the effluent of the wastewater treatment system, which permits the application of high hydraulic loads to anaerobic reactors without having to be

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concerned about wash-out of biologically active sludge particles, and thus make a high concentration of active anaerobic biomass remained in the reactor [2,3]; (2) sludge granulation minimizes the distances between the microbial and maximizes interspecies transfer of acetate and hydrogen between syntrophic fatty acid degraders and methanogenes [4], resulting in an effective anaerobic digestion; (3) granular sludge is better able to cope with high and fluctuating loading rates, which are discovered in upflow anaerobic sludge blanket (UASB) processes for the treatment of high-strength wastewater [5]; and (4) rapid settling velocities of granules could help to reduce the size of anaerobic reactors and decrease the construction costs.

Granular sludge was firstly observed in the UASB reactors. Owing to the numerous advantages of the granule, UASB reactors became a popular high-rate anaerobic treatment system throughout the world [6,7]. Although there are many benefits of the UASB reactor, it also suffers from a number of shortcomings. One major drawback of the UASB reactor is that it would be blocked easily due to its complex construction. Therefore, the UASB reactor is not particularly suitable for treating wastewaters with high suspended solids (SS), which restricted the utilization of UASB in treating some special wastewaters. Moreover, short circuit was similarly faced by the UASB reactors. Other problems of the UASB also existed in treating domestic wastewaters, including long start-up time, poor gas production, susceptibility to shock loading, etc. [8,9]. Therefore, in order to offset the shortage of UASB reactor in treating some specific wastewaters, exploring other types of anaerobic reactors in which granular sludge can form becomes particularly important.

Prior to the UASB reactors development, continuous stirred tank reactor (CSTR) was the most common reactor type used in the anaerobic digestion of wastewaters, especially in the treatment of high SS organic wastewater and excess sludge [10]. Benefiting from its simple configuration, CSTR possessed a better mixing characteristic and was not easy to be blocked. Additionally, it was also convenient to debug and operation. Therefore, rapid cultivation of granular sludge in CSTR reactors attracts large researcher's attention nowadays.

Sludge granulation are generally influenced by numerous extrinsic factors including temperature, pH, organic loading rate, substrate characteristics, nutrients and some heavy metals, the hydraulic shear force, microbial ecology of seed sludge, etc. [11]. In this work, all other parameters in the two CSTRs were the same except for the shear force generated from the stirrer. In order to investigate the influence of shear on anaerobic sludge granulation in CSTR reactors, two stirring strategies with different mode and intensity were employed, thereby exploring an appropriate condition that granular sludge can be formed in a relatively short period of time. Additionally, the roles of filamentous bacteria and extracellular polymeric substances (EPS) in the enhancement of the sludge granulation were also discussed.

2. Materials and methods

2.1. Cultivation of anaerobic granules

Both the seed sludge of the two CSTRs in this study were taken from the previous magnetic stirring reactor, which had worked with anaerobic flocculent sludge for about 524 days for other research projects prior to this experiment, sludge in the reactor was always maintained in flocculent and seemed unable to form granules, all operation conditions of the previous reactor was identical to the CSTR1 in this study.

Anaerobic sludge was cultivated at 35 ± 1℃ in two similar laboratory-scale CSTRs paralleled, which had a 1.5 L of headspace and a 4.5 L of liquid volume with 30 cm in height and 18 cm in diameter. Except for liquid mixing in the two CSTRs was different, that is, the liquid mixing was achieved by intermittent magnetic stirring (140 rpm) in CSTR1 and continuous impeller agitation (90 rpm) in CSTR2, all other operating conditions of the two reactors maintained identically, such as the feeding rate, mixing time, settling time, and withdraw rate. The ORP, temperature, and pH were monitored online by the probes (Mettler-Toledo, Switzerland) which connected to a data acquisition system.

The reactors were fed with a synthetic wastewater consisting of glucose as the sole carbon source. Other chemicals, such as nutrients, trace metals, and buffering compounds, were served as a supplement. The composition of synthetic wastewater and trace element used in this study are shown in Tables 1 and 2, respectively.

Table 1
Synthetic wastewater components

Components	Concentration (mg L^{-1})
COD (glucose)	2000
NH_4^+–N (NH_4Cl)	33.33
P (KH_2PO_4)	6.67
Trace element	0.11 (mL L^{-1})

Table 2
Trace element solution components

Components	Concentration ($g\,L^{-1}$)	Components	Concentration ($g\,L^{-1}$)
$FeSO_4{\cdot}7H_2O$	8	H_3BO_3	0.1
$MnCl_2{\cdot}4H_2O$	0.5	EDTA	0.05
$CoCl_2{\cdot}6H_2O$	0.88	$NiCl_2{\cdot}6H_2O$	0.036
$CuCl_2{\cdot}2H_2O$	0.035	$(NH4)_6Mo_7O_{24}{\cdot}4H_2O$	0.64
$ZnSO_4{\cdot}7H_2O$	0.1	$MgSO_4{\cdot}7H_2O$	5

2.2. Observation of anaerobic granules

Microscopic examination of sludge from the reactor was performed using an OLYMPUS-BX51 (Japan). Scanning electron microscope (SEM) was also used to observe the detailed morphology of granular sludge in CSTR2.

Granules that sampled from the CSTR2 for the analysis of SEM were fixed in 2.5% glutaraldehyde after 6 h of fixation at 4℃, and then, the granules were rinsed in phosphate buffer solution and subsequently dehydrated through a graded ethanol series (30, 50, 70, 80, 90 and two times 95 and 100%, 15 min per step). After that, the washed samples were stored in a mixture of isoamyl acetate and ethanol (1:1) and 100% isoamyl acetate in succession for 15 min. Finally, the granules were critical point dried (CPD) with carbon dioxide (CPD, Quorum/Emitech K850). The samples were sputter coated with gold and analyzed with scanning electron microscope (JSM-6510LV, Japan) at room temperature [12].

2.3. Measurement of hydrogen partial pressure (p_{H2})

Biogas composition in CSTRs was determined by gas chromatograph (Agilent 6890 N GC) equipped with a TDX-01 packed column (2 m × 0.3 mm) and a thermal conductivity detector (GC–TCD). The inert gas argon was selected as the carrier gas at a flow rate of 49.9 mL min^{-1}. Headspace gas in CSTRs was sampled by a 500-μL gas-tight syringe followed by direct injection on to the column through a septum. The percentage concentration of hydrogen was obtained and the hydrogen partial pressure can be calculated.

2.4. Other components

Particle size distribution of sludge was measured with a Malvern mastersizer laser beam diffraction granulometer over a 0–2000 μm size range. Moreover, all other analyses were carried out according to the Standard Methods for the Examination of Water and Wastewater [13].

3. Results and discussion

3.1. Analysis of mixing flow field and hydraulic shear force

In order to investigate the influence of shear force on sludge granulation under the two types of stirring strategies, the streamline maps of magnetic stirring and axial-flow stirring were, respectively, depicted (Fig. 1(A) and (B)), according to the computational fluid dynamics analyses results [14]. Research considered that the hydraulic shear force played a crucial influence on sludge granulation, so hydraulic shear forces were also calculated in this work under the two stirring conditions [15,16].

Hydraulic shear force was defined by the average velocity gradient, which was abbreviated to G. For turbulent flow ($N_{Re} > 10{,}000$), the power imparted by an impeller in a baffled tank was given by the following equations:

$$G = \left(\frac{P}{\mu V}\right)^{\frac{1}{2}} \tag{1}$$

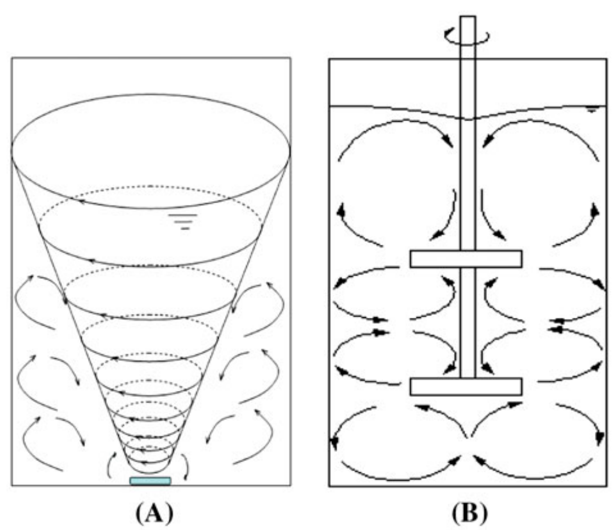

Fig. 1. Streamline maps in: (A) CSTR1; (B) CSTR2. (Compared with magnetic stirring (A), axial-flow mixing (B) could produce good convective mass transfer and it is good at the main circulation).

$$P = K_T n^3 D_i^5 \rho \quad (2)$$

$$N_{\mathrm{Re}} = \frac{D_i^2 n \rho}{\mu} \quad (3)$$

where P is the power imparted to the water per unit volume of the basin ($\mathrm{N\,m\,s^{-1}}$); μ is the absolute viscosity of the liquid, ($0.7225 \times 10^{-3}\,\mathrm{N\,s\,m^{-1}}$); V is the tank volume ($0.0045\,\mathrm{m^3}$); K_T is the impeller constant for turbulent flow (1.00) [17–19]; n is the rotation speed (140 and 90 rpm, respectively, in the CSTR1 and CSTR2); D_i is the impeller diameter (0.06 and 0.045 m, respectively), ρ is the density of the liquid ($1.032 \times 10^3\,\mathrm{kg\,m^{-3}}$); $\rho = \gamma/g_c$, γ is the specific weight of the liquid ($\mathrm{N\,m^{-3}}$), g_c is the acceleration due to gravity ($9.906\,\mathrm{m\,s^{-2}}$).

In the anaerobic system without gas circulation, the hydraulic shear force mainly depends on the stirring mode and intensity. Experimental results from Tay et al. which researched on the artificial wastewater digestion, showed that compact granules could be produced under strong hydraulic conditions. Conversely, it was difficult or impossible for sludge granulation under weak conditions [20]. Wu et al. (2009) [21] suggested the critical point of the shear force on sludge nucleation was around $8.28\,\mathrm{s^{-1}}$, which was obtained through a series of comparative experiments. The analysis results deduced that the high shear force would enhance the production of extracellular proteins, but the over-produced proteins might slow down the nucleation of sludge and made the aggregates become loose and weak. In this work, the average velocity gradient (G) of these two CSTRs was $29.17\,\mathrm{s^{-1}}$ in CSTR1 (140 rpm) and $6.42\,\mathrm{s^{-1}}$ in CSTR2 (90 rpm), respectively. Combined with streamline distributions in the two reactors, calculation of the shear forces showed that compared with magnetic stirring, axial-flow mixing could produce a milder shear force and a good convective mass transfer. Moreover, the liquid circulation in the axial-flow mixing reactor was also excellent. The results were exactly in accordance with the characteristics of axial-flow mixer reported by Hu, that is, the shear rate distributed uniformly around the blade, the mild shear production, the lower energy consumption and the uniform velocity distribution [14], all of which are beneficial for the sludge gather and granulation.

3.2. General observations

In order to evaluate sludge granulation process, the particle size distributions of sludge in the two CSTRs were determined in different time intervals (Fig. 2). The results indicated that the flocculent sludge was predominant in both of the two reactors at the beginning of the experiment. After about 45 days' cultivation, there were no significant changes in the particle size in CSTR1, which means that flocculent sludge still remained and no granules have been observed. Inversely, the dispersed flocs disappeared on the 45th day and granular sludge became predominately in CSTR2. The average particle sizes of anaerobic granules of the CSTR2 in a different period of 0, 20, 45, and 60 days were 151, 460, 646 and 742 μm, respectively. Therefore, it can be concluded that compared to magnetic stirring, axial-flow mixing had a positive effect on the sludge granulation.

Fig. 3 presented that the sludge settleability was significantly improved with granulation. In CSTR1, the sludge SVI was approximately remained in $105.5\,\mathrm{mL\,g^{-1}}$ due to the remained flocculent sludge. But in CSTR2, the sludge SVI decreased from 102.2 to $22.78\,\mathrm{mL\,g^{-1}}$. It was obviously that the granular sludge in CSTR2 exhibited a high settling velocity, which could result in a good liquid separation and high biomass retention.

3.3. p_{H_2} and the maximum specific methanogenic activities

Due to the sludge morphology in CSTR1 invariably remained in flocs, no significant reduction

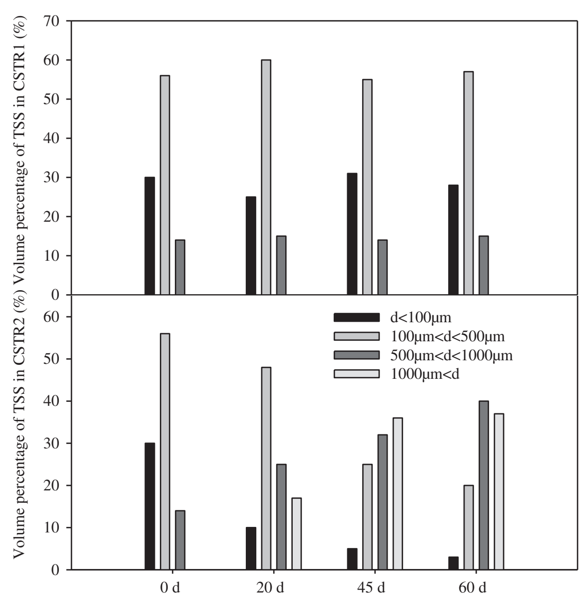

Fig. 2. Evolution of particle size distribution of anaerobic sludge. (Flocculent sludge was the major component in both of the two CSTRs at the beginning of the experiment. After approximately 45 days' cultivation, the sludge in the CSTR2 was almost granulated).

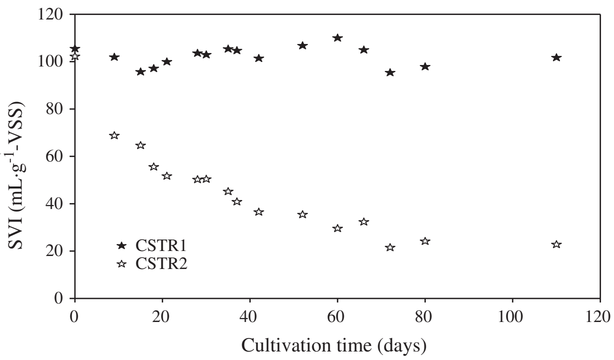

Fig. 3. Changes of sludge settleability (SVI) in the two CSTRs. (Sludge settleability was significantly improved with the sludge granulation of the CSTR2. The sludge SVI in CSTR1 was approximately remained in 105.5 mL g^{-1} and the sludge SVI in CSTR2 was decreased from 102.2 to 22.78 mL g^{-1}).

in the hydrogen partial pressure in the reactor was observed. The hydrogen partial pressure in CSTR1 was in the range of 28.1–29.3 Pa, and the maximum specific methanogenic activities (SMAs) of the sludge also maintained in an unchanged level correspondingly (Fig. 4(A)).

Fig. 4(B) shows that hydrogen partial pressure in the headspace of CSTR2 reduced from 27.3 to 1.6 Pa during the sludge granulation, and the degradation rate of propionate and butyrate were consequently improved.

According to the theory of hydrogen regulation in anaerobic digestion, due to the oxidation of propionate and butyrate are thermodynamically unfavorable, it is only possible if the intermediate products (especially for hydrogen) are utilized efficiently by the methanogenes, that is, a relative low hydrogen partial pressure is necessary (the hydrogen partial pressure should be lower than 10 Pa) [4,22]. The results showed that hydrogen partial pressure decreased to 9.34×10^{-5} atm on the 25th day with the sludge granulation, which provided a suitable thermodynamic condition for the degradation of the two volatile fatty acids, and the specific methanogenic activities of the bacteria were thereby improved.

The results also confirmed the views that the distance between bacterial was minimized as the result of the high bacterial densities in granule, which contributed to optimize the interspecies mass transfer of acetate and hydrogen between syntrophic acetogens and methanogenes [23], thus improved the degradation rate of fatty acids.

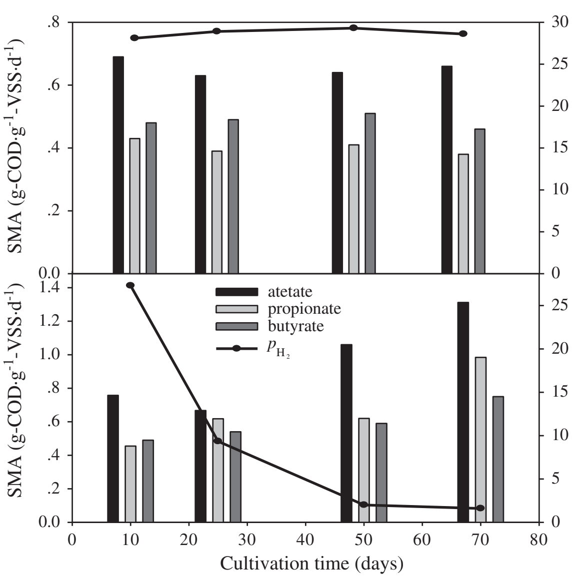

Fig. 4. Changes of p_{H2} and the bacterial activities of the anaerobic sludge in: (A) CSTR1; (B) CSTR2.

3.4. Relationships between the sludge SVI, p_{H_2} and the particle size in CSTR2

Fig. 5 shows that with the evolution of the sludge average particle size from 151 to 742 μm in 60 days' cultivation, the sludge SVI declined from 102.2 to 22.95 mL g^{-1} significantly, and the hydrogen partial pressure in the CSTR2 also decreased from 35.5 to 1.6 Pa obviously. The decrease in sludge SVI indicated that the sludge settleability improved significantly with the evolution of the granule size, which benefited from the compact structure of granule that avoided washing out from the reactor and then the small particle developed to mature granule. In addition, the granulation of sludge narrowed the distance between micro-organisms and thereby decreased the mass transfer resistance, which made the produced H_2 in the previous acetogenesis stage utilized by the hydrogenotrophic methanogenes instantly. When the H2 partial pressure decreased below 10 Pa, the

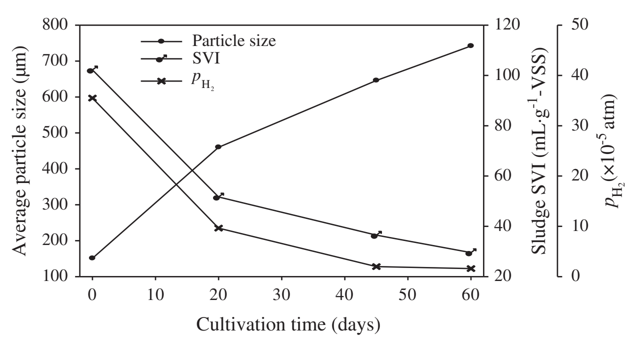

Fig. 5. The relationships between the sludge SVI, p_{H2} and the average particle size in CSTR2.

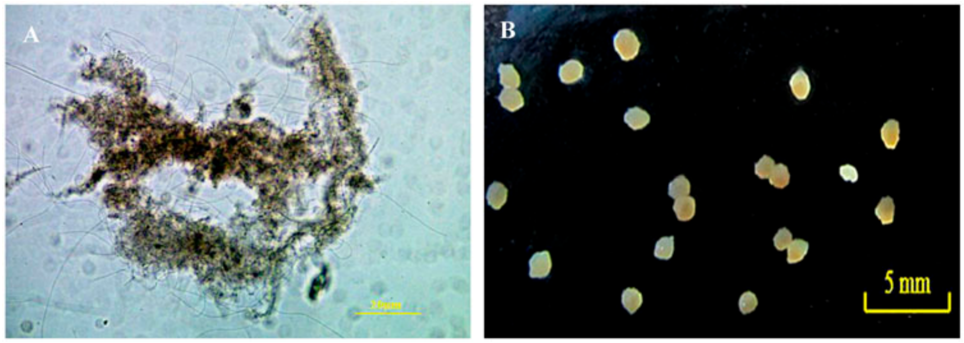

Fig. 6. The sludge morphology after 45 days cultivation: (A) CSTR1, flocculent sludge; (B) CSTR2, granular sludge. (After approximately 45 days cultivation, the sludge formed in these two CSTRs was different significantly. The granular sludge formed in CSTR2 possessed a relatively regular shape and clear outline).

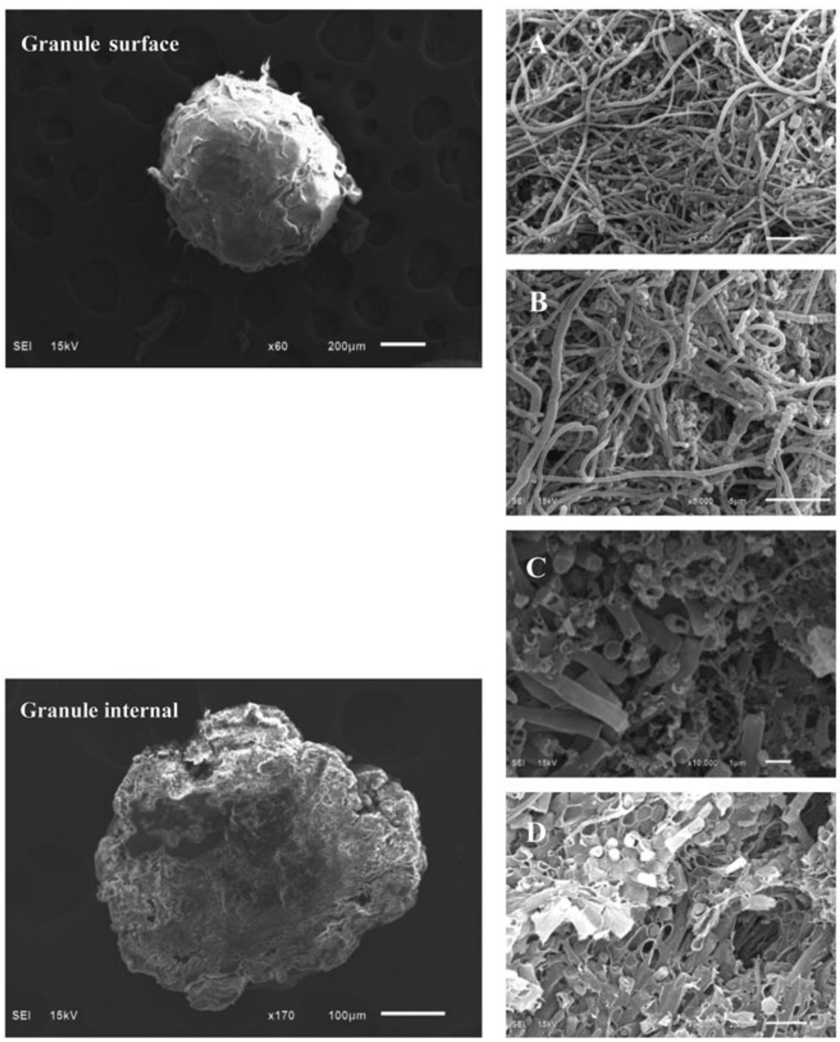

Fig. 7. SEM image of the granular sludge from CSTR2. ((A) and (B): aggregates of filamentous bacterial cells on the surface of the granular sludge; (C) and (D): arrangement of bacterial cells inner the cross-section of the granule surrounded by extracelluar polymeric substances (EPS)).

degradation of the propionate and butyrate proceeded thermodynamic favorably.

3.5. Observation of the sludge morphology

After approximately 45 days' cultivation, the sludge morphologies in these two CSTRs were different significantly. Sludge in CSTR1 was totally flocculent, but in CSTR2, it was almost granulated (Fig. 6).

The SEM image of granules obtained from the CSTR2 was presented in Fig. 7, which showed that the anaerobic granules possessed a relatively regular shape and clear outline. Filamentous micro-organisms predominated in the surface of the granule (Fig. 7(A) and (B)), and the bacillus bacteria distributed in the inner part (Fig. 7(C) and (D)). Therefore, it could be deduced that filamentous micro-organisms play a significant role in sludge granulation, which waved each other to wrap bacillus bacteria together and made the granules more and more compact.

Production of EPS was also expected to be beneficial to stimulate the sludge granulation, because that EPS could bind each other and thus made aggregates stronger [24]. SEM images displayed that lots of EPS were produced and distributed within the inner part of the granular sludge, and a large number of bacteria cells were surrounded by EPS (Fig. 7(C) and (D)). A further magnification of this structure showed that bacteria individuals were inter-woven by a network of fibrous strands like a cobweb (Fig. 7(C)), and some others were verwoben by a fibrous nest analogous to a honeycomb (Fig. 7(D)), which appear to hold the pallets intact.

4. Conclusions

The effects of stirring strategies on the sludge granulation in anaerobic CSTR were investigated in this study. According to the analysis of the streamline distribution, it could be observed that velocity distribution was more uniform under the operation condition in CSTR2. Moreover, calculation results of the shear force in the two CSTRs indicated that a milder shear force was produced in CSTR2 than CSTR1, which resulting in the sludge granulation after approximately 45 days cultivation.

Filamentous bacteria in the surface and EPS in the inner part of granules were also conducive to sludge granulation. Filamentous bacteria in the surface of granules entangled each other and wrapped bacillus bacteria together, thus made the granules compact and could resist a higher shear force. Moreover, individual cells within the granules were surrounded by lots of EPS, which formed a network of fibrous strands analogous to a cobweb or a honeycomb and made the bacteria cells interwoven by it.

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