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Characterization of sulfate-reducing bacteria anaerobic granular sludge and granulometric analysis with grey relation

Jing Guo and Yong Kang†

School of Chemical Engineering and Technology, Tianjin University, Tianjin 300350, China

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Abstract−We constructed a bench-scale up-flow anaerobic sludge reactor to systematically investigate the physicochemical characteristics of sulfate-reducing bacteria (SRB) anaerobic granular sludge and evaluate the granular size by a grey relational analysis. Results indicated that the granulation proportion was improved from 17.9% to 68.7% with the sulfate reduction efficiency larger than 90% under gradually shortened hydraulic retention time (HRT) and increased organic loading. Larger SRB granule sludge showed a higher specific gravity and settling velocity. The seed sludge was negatively charged, and the surface charge decreased with the incremental granular diameter. The maximal hydrophobicity and granulation proportion were 69.9% and 42.4%, respectively, for the granular diameter ranging from 1.5 to 2.5 mm. Extracellular polymeric substance (EPS) of the sludge exhibited the highest ratio of protein to polysaccharide (PN/PS) for the granular diameter in the range of 0.5 to 1.5 mm. Based on the grey relational analysis of the SRB anaerobic sludge granulation, the correlation degree of the inherent influencing factors was PN/PS>surface charge> hydrophobicity. The theoretical evaluation would be conducive to granulation control during the potential application.

Keywords: Sulfate-reducing Bacteria (SRB), Granular Sludge, Grey Relational Analysis, Up-flow Anaerobic Sludge Reactor

INTRODUCTION

Industrial wastewaters from diversified sources, such as pharmaceutical, chemical units and paper production, contain high concentrations of sulfate and organic substance [1]. The discharge of sulfate-containing wastewater continues to substantially increase in the industrialized world [2], aggravating environmental pollution and disrupting the natural sulfur cycle [3,4]. Anaerobic microbiological techniques have been widely used for the treatment of sulfate-containing wastewater for the advantages of high load rate, low energy consumption and operation cost [5,6]. Characterized by the compact structure of floc sludge resulting from the aggregation of microorganisms under suitable conditions, the sulfate-reducing bacteria (SRB) granular sludge presents a large mass transfer area, high biomass concentration and excellent settling ability in comparison with traditional floc sludge [6,7]. Especially, SRB can simultaneously remove the sulfate and organics [8,9]. Hence, it is more feasible to better develop and operate the SRB granular sludge for the sulfate wastewater treatment [10].

The up-flow anaerobic sludge reactor (UASB) for sulfate reduction, which is a single tank anaerobic digester used in wastewater treatment system, plays a crucial role in the granular operation process [11,12]. Granular sludge with stable performance is one of the most important indicators of the successful operation of a UASB reactor [10,13,14], and the granulation process would be affected by temperature [15], pH and alkalinity [16], composition and concentration of the organics [17], hydrophobicity and the surface charge of sludge [18]. Recently, investigations revealed that it is essential to correlate the EPSs with the granulation process [19-21]. Seviour et al. [22] demonstrated that the presence of polysaccharide promoted the formation of aerobic granular sludge. Li et al. [10] found that the protein increased significantly during granulation, but the polysaccharide showed little change. Moreover, the surface charge actually decreased with SRB granulation. Hao et al. [2] discovered that the hydrophobicity of the granular sludge was increased 1.7-times relative to that of inoculated sludge. It has been reported that the components of the EPS could change the surface characteristics of the bacteria and the physical characteristics of granular sludge [23]. Generally, bacteria are negatively charged in wastewater, resulting in electrostatic repulsion. The production of EPS, however, can change the surface charge of the bacteria, reducing repulsion and instead leading to bacterial agglomeration. Obviously, the surface charge, hydrophobicity, and the PN/PS in the EPS constituents can change during the granulation process of SRB anaerobic sludge, indicating the necessity to comprehensively compare and analyze the effect degree on the sludge granulation for the process regulation.

Grey relational analysis (GRA), developed by Deng [24], is an effective statistical and impacting measurement method in grey system theory that analyzes uncertain relations between a main factor and all other factors, allowing quantitative and qualitative relationships using relatively little data or with great variability in factors. The grey relational grade generated from a series of correlation function calculations was used to describe the relational grade and reflect the impact order of each chosen operational factor on granulation indicators, thereby distinguishing the key impact factors and providing the optimal scope [25]. This approach has been widely applied to various fields, such as engineering [26] and biology [27]. Xu et al. [28] reported the use of the response surface methodology

†To whom correspondence should be addressed.
E-mail: ykang@tju.edu.cn
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(RSM) coupled with GRA to evaluate the effects of experimental conditions on soluble microbial product (SMP). However, to the best of our knowledge, no study has used the GRA approach to study SRB granulation. Because of the difficulty in continuous measurement of dynamic granular growth, it is hard to characterize the granulation process. Schmid and Ahring reported that criteria for determining if granular sludge has developed in a UASB reactor were given based on the densities and diameters of the granular sludge [29]. The objective of this work was to identify the different size-fractions of the granular sludge and then the major physico-chemical characteristics of the SRB granular with the different size-fractions in the UASB reactor examined. This paper was designed to record the changes of sludge surface charge, hydrophobicity and PN/PS with different size-fractions of the granular sludge. As a simple and useful approach, GRA has been used to better understand the maximal relational factor of the SRB granular diameter evaluated with these three factors, sludge surface charge, hydrophobicity and PN/PS. It is hoped that the information generated from GRA could be useful to optimize the cultivation of SRB granules in UASB reactor. The laboratory scale was conducted using synthetic medium to facilitate the SRB granulation regulation during the potential application and enhance the SRB granular performance in the removal of sulfate for large scale commercial process.

MATERIALS AND METHODS

1. Seed Sludge and Synthetic Wastewater

The seed sludge containing SRB was initially collected from a wastewater treatment plant at Sinopec Tianjin branch (Tianjin, China). The ratio of volatile suspended substances to total suspended substances (VSS/TSS) was 0.698. After being sieved through a 30-mesh screen to remove large particles and impurities, 30% (V/V) inoculum sludge was added to medium to promote the formation and acclimation of SRB anaerobic sludge. When the sulfate reduction removal remained above 90% at 36 °C for two days, the process was repeated three times. The medium was modified according to our previous research [30], mainly including sodium lactate, sodium sulfate anhydrous, yeast extract, ammonium chloride, potassium phosphate dibasic and a small amount of trace elements. The synthetic wastewater used in this study, prepared in our lab, contained lactate serving as the organic carbon source and sulfate as energy source for SRB growth. The wastewater pH was adjusted to 6.0 using hydrochloric acid (0.1 M) and sodium hydroxide (0.1 M). All chemicals used were analytical grade and used as received without further purification.

2. Reactor and Operation

A lab-scale UASB reactor made of plexiglass was constructed as shown in Fig. 1. The reactor had an internal diameter of 70 mm and a height of 1,100 mm, with an effective liquid volume of 3.9 L. A three-phase separator on the top of the reactor was used to separate the biogas, effluent and sludge. The sludge and effluent were recirculated back to the reactor, and the biogas produced in the reactor was transferred to a gas adsorption tank. A thermostatic water bath was used to maintain the reactor temperature at 36 °C. The hydraulic retention time (HRT) was adjusted by changing the influent flow rate, and the recirculation was controlled using a variable-

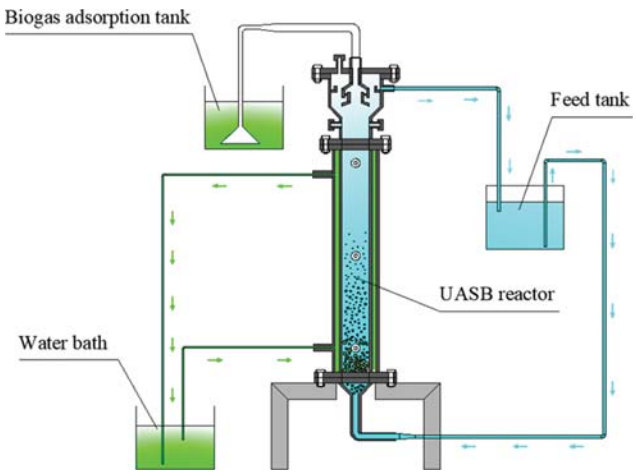

Fig. 1. Schematic diagram of the experimental setup.

Table 1. List of the operation parameters in experiments

Operating parameters	Preliminary stage	Medium stage	Later stage
HRT (h)	36	24	24
Organic loading rate (kg COD m^{-3} d^{-1})	2.33	5-2.8	5
C/S	3.5	3.3-1.8	3.3
Up-flow velocity (m h^{-1})	0.213	1.04	2.08
Operating time (d)	0-26	27-47	48-58

speed peristaltic pump. The whole experiment was operated for more than 50 days, stabilizing the biological sulfate reduction and the development of granular sludge. Experimental modifications were conducted in three stages by varying the COD loading, up-flow velocity and HRT, as shown in Table 1. Taking no account of a small aliquot of granular sludge withdrawn for sampling, the overall sludge retention time was estimated to be about 36 d.

3. Chemical and Physical Analysis

3-1. Routine Analysis

The liquid and sludge in the reactor were sampled regularly and measured immediately. The liquid samples were filtered through a 0.45 μm polyethersulfone membrane before analysis. Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to standard methods [31]. The sulfate was analyzed using a C200 multi-parameter ion specific meter (Hanna Instruments, Italy) [32]. Before the granular sludge sampling, all the sludge in the reactor was well sparged with nitrogen gas. At the end of every experimental stage, a small aliquot (20 ml) of granular sludge sample (liquid and sludge) was withdrawn using transfer pipette from the top, middle and bottom sampling point of the reactor, respectively. Then the samples from different sampling points were well mixed for further analysis. And, there are two parallel samples. The granular sludge taken from the reactor was separated using 5-mesh, 7-mesh, 12-mesh or 30-mesh screens to determine the proportion of granular sludge with a certain diameter by the weighing method. Granular sludge with a diameter greater than 0.5 mm was selected to calculate the granulation proportion; the remaining sludge was considered as flocculent sludge. The data of the granulation proportion was for the total sludge.

3-2. Microscopic Observation

The morphology characteristics of SRB granules with different diameters were observed by the Hitachi S-4800 scanning electron microscopy (SEM) (Hitachi, Japan). The granule samples for SEM were fixed with 2.5% glutaraldehyde at 4 °C for 24 h. Then the samples were washed in 0.1 M phosphate buffer at pH 7.2 for 10 min for three times and dehydrated with a gradient series of ethanol (10 minutes at 50%, 70%, 80%, and 90%, followed by three washes of 100% for 15 min). After being dried at about 37 °C for two days, the samples were coated with gold and observed under the microscope.

3-3. EPS Analysis

EPS analysis of SRB granule with different diameters was performed in triplicate. The thermal extraction procedure was conducted according to Morgan et al. [23]. The sulfuric acid-anthrone and the modified Lowry methods were adopted to determine the polysaccharide and protein contents [33].

3-4. Surface Characteristic

The surface charge of SRB granular was measured using the colloid titration technique [23], which was expressed as milliequivalents per gram of mixed liquor suspended solids with positive or negative colloidal charge: meq g^{-1} TSS. The relative hydrophobicity of the sludge was measured as adherence to hydrocarbons, as detailed by [34]. The relative hydrophobicity could be expressed as the ratio of TSS_2 concentration in the organic phase after emulsification to the concentration of TSS_1 in the aqueous phase before emulsification:

$$\text{The relative hydrophobicity (\%)} = \frac{TSS_2}{TSS_1} \times 100 \quad (1)$$

4. Grey Relational Analysis

Grey relational analysis (GRA) [24,28] was chosen to evaluate the importance of three major factors, including the surface charge, hydrophobicity and PN/PS, on the SRB granule diameter, which was conducted through data pre-processing, calculation of the grey relational absolute deviation sequences, grey relational coefficient calculation and grey relational grade calculation. The values of normalized data can indicate the influence of SRB granular sludge diameter, i.e., a larger value (with the maximum equal to 1) means favoring granulation. The grey relational coefficients offer information about the relationship between the optimal and actual normalized results. A high value of grey relational coefficient indicates that the experimental result is close to the optimal value for the single evaluation response [35]. First, the independent variables were chosen as the original data sequence:

$$x_i(j)=\{x_i(j)|j=1, 2, \cdots, k; i=1, 2, \cdots, m\} \quad (2)$$

which were then transferred into a comparable sequence for normalization before GRA analysis:

$$x_i^*(j)=\{x_i^*(j)|j=1, 2, \cdots, k; i=1, 2, \cdots, m\} \quad (3)$$

where k is the total number of factors to be considered, and m is the total number of observation data. In this study, k=3, and m=5.

Second, the absolute deviation sequences values Δ_{ij} can be calculated by Eq. (4):

$$\Delta_{ij}=|x_0(j)-x_i^*(j)| \quad (4)$$

where $x_i^*(j)$ denotes the standardization processing results of the original data and $x_0(j)$ is the reference sequence.

Following that, the correlation coefficient δ_{ij} between the reference sequence $x_0(j)$ and comparative sequence $x_i^*(j)$ was calculated using Eq. (5):

$$\delta_{ij} = \frac{\Delta(\min)+\rho\Delta(\max)}{\Delta_{ij}+\rho\Delta(\max)} \quad (5)$$

where Δ(min) is the element of minimum value in the Δ_{ij} and Δ(max) is defined as the element of maximum value in the Δ_{ij}. ρ is known as the distinguishing coefficient ranging from 0 to 1. In this study, 0.5 is considered.

Finally, the grey relational grade γ was obtained by calculating the average values of all the grey relational coefficients:

$$\gamma = \frac{1}{n}\Sigma_{j=1}^{n}\delta_{ij}(j) \quad (6)$$

RESULTS AND DISCUSSION

1. Performance of UASB

The reactor was inoculated with anaerobic sludge and synthetic wastewater for intermittent operation. It primarily consisted of the preliminary stage, medium stage, and later stage. The entire experiment was conducted for nearly 60 days. The removal performance is shown in Fig. 2. During the preliminary stage with HRT of 36 h (the first 26 days), the SRB activity and predominance was gradually improved in the anaerobic system under influent concentrations of sulfate and COD of 1,000 mg L^{-1} and ~3,500 mg L^{-1}, respectively. The experimental data of first 20 days are not provided; this period was intended to improve the activity of SRB and establish the predominance of SRB in the anaerobic system. The sulfate removal efficiency was more than 90% after the inoculation and acclimation. For the following 20 days (medium stage), the sulfate concentration increased to 1,500 mg L^{-1} with the HRT of 24 h and a higher up-flow rate of 1.04 m h^{-1}. The removal efficiency of sulfate fluctuated slightly and finally reached 90% at the end of the medium stage. In addition, the C/S ratio was reduced

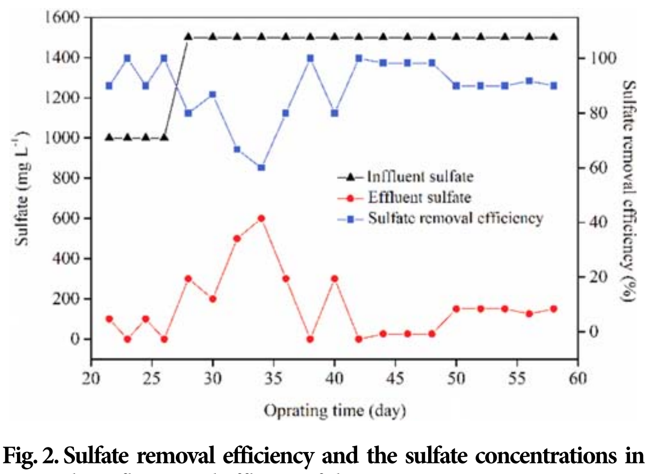

Fig. 2. Sulfate removal efficiency and the sulfate concentrations in the influent and effluent of the UASB system.

to 1.8 (sulfate concentration unchanged) by decreasing COD at the later of the medium stages. The sulfate removal efficiency was only 60%, indicating decreased activity of SRB with declining C/S ratio. The result is similar to that obtained by Bai et al. [36], who reported that the C/S ratios were decreased from 1.9 to 1.22; sulfate reduction decreased from 88% to 44% in their system. Then the organic concentration was increased to improve the sulfate removal with up-flow rate of 2.08 m h^{-1} at the later stage (48-58 days), of which the sulfate concentration and organic loading during the later stage were both similar to those exhibited during the medium stage. However, the sulfate removal efficiency was 90%, lower than at the medium stage (98.3%) for the decrease in the influent pH from 6.5 to 5.5. During this stage, the sludge granular gradually matured with the granulation percentage increased to 68.7%. Although the violent mixing of ordinary anaerobic sludge caused by the methane production, the SRB anaerobic sludge in this reactor was completely dependent on the up-flow rate [2]. So, by the process of granulation that gradual shrinkage of HRT and increasing the up-flow rate and the organic loading could facilitate granulation SRB anaerobic sludge [37].

The granulation percentage for granular sludge with particle size larger than 0.5 mm increased from 17.9% at the preliminary stage to 68.7% at the end of the experiment (Table 2), indicating the improvement of the SRB sludge granulation with increasing organic load rate. The VSS/TSS ratio meant the amount of biomass in total sludge measured as suspended solids, indicating the greater VSS/TSS ratio, the larger percentage of biomass in the reactor. The variation of VSS/TSS first increased and then decreased for the granular sludge (diameter ≥0.5 mm). When the organic load rate was increased, the VSS/TSS was improved correspondingly from preliminary stage to medium stage. While the organic load rate was further increased, the VSS/TSS ratio value at the later stage (0.693) was slightly smaller than the medium stage granular sludge (0.754). This is because with the granulation process, granular diameter would limit mass transfer, resulting in a lower biomass of larger granular sludge compared to smaller granular sludge. Additionally, the calcified wastewater would lead to increased ash in sludge and further promote sludge calcification or hardening [38]. With regard to flocculent sludge (diameter <0.5 mm), the VSS/TSS ratio decreased from 0.724 at preliminary experiment to 0.675 at end the experiment. This phenomenon was caused by lower resistance to the higher up-flow rate for flocculent sludge and then washing biomass away. Hence, it is essential to determine the VSS/TSS ratio during the reactor operation.

According to the report of optimum pH value for the SRB metabolism (5-8) [39], the initial pH value in this study was about 6.5. In Fig. 3, the effluent pH increased to about 8 upon completion of the experiment, guaranteeing dissolution of the H_2S generated by the system, which can effectively control H_2S escape [37]. At later stage, a decrease in the influent pH from 6.5 to 5.5 could result in a slight decrease for sulfate removal. This indicated that sulfate reduction occurred at low pH condition, though at a low rate. Remarkably, the decrease of the initial influent pH did not change the effluent pH obviously, which showed the reactor performance was determined by the interaction of multiple microorganisms in the reaction system.

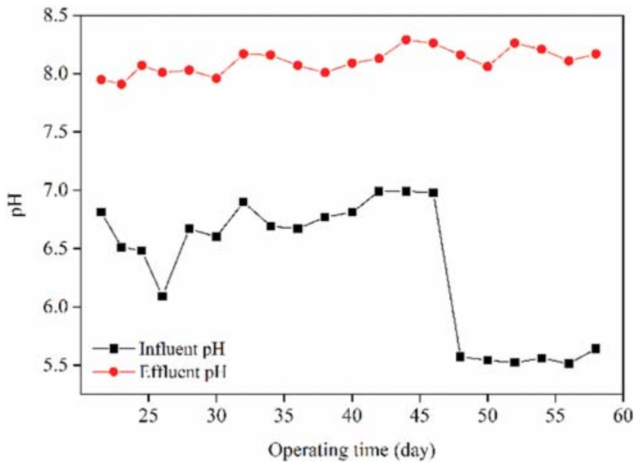

Fig. 3. Influent and effluent pH values of the UASB system.

Table 2. The VSS/TSS ratio and granulation percentage of the SRB granular at three stages

Stage	VSS/TSS		Granulation percentage (%)
	Flocculent sludge	Granular sludge	
Preliminary	0.724	0.709	17.9%
Medium	0.712	0.754	36.0%
Later	0.675	0.693	68.7%

2. Physic-chemical Characteristics of SRB Granular Sludge

The samples were collected from later stage and divided into different groups based on granular sludge diameter for physico-chemical characteristics, which are shown in Table 3 and Figs. 4-6. SRB granular sludge had remarkable settling ability; as measured by the specific gravity and settling velocity in Table 3, the specific gravity ranged from 1.018 to 1.059, and the settling velocity of the anaerobic granular sludge was 43.3-80.2 m h^{-1}. Until now the relationship between the specific gravity and granular sludge diameter was not clear. However, the specific gravity of the SRB granular sludge improved with the increased granular diameter in this study, which was inconsistent with the reported results by Beeftink et al. [40]. Generally, high specific gravity was accompanied with high density and compact structure, which implied that limitations in mass transfer would commonly occur for the larger size granules because of lower granule porosity [2]. Fortunately, higher granule density of the SRB granular sludge in this study could be against

Table 3. Physical properties of granular sludge with different granular diameters

Range of granular diameter (mm)	Specific gravity	Settling ability (m h^{-1})	Percentage (%)
0.5-1.5	1.018	43.3	27.8
1.5-2.5	1.024	64.5	42.4
2.5-3.5	1.037	71.1	12.3
3.5-5	1.059	80.2	17.5

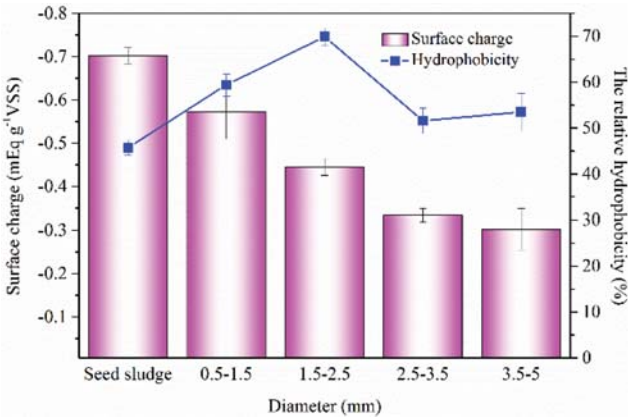

Fig. 4. The surface charge and hydrophobicity of SRB granular with different diameters.

the higher up-flow rate (2.08 m h^{-1}) effectively, which would reduce the restrictions of mass transfer and be conducive to the maintenance of internal microbial activity. Furthermore, the settling velocity of the SRB granular sludge was positively correlated with the granular diameter, which was higher than other SRB granules (18-65 m h^{-1}) [2]. The excellent settling ability kept the sludge retention, the liquid-solid separation and effluent turbidity. Overall, the granular sludge with greater specific gravity and settling velocity were important features to achieve satisfactory reactor performance.

Up to now, the hydrophobicity, surface charge, and PN/PS of the EPS constituents affecting the properties of the sludge and helping the sludge aggregation, were three important internal factors in the granulation process. The hydrophobicity, surface charge and EPS content of SRB granular on different diameters were determined and shown in Fig. 4-5. For a better interpretation and assessment of the significance of the hydrophobicity, surface charge and EPS content of SRB granular on different diameters, the results obtained were analyzed statistically in terms of analysis of variance (ANOVA), which is presented in Table 4. In these ANOVA data, a high F value and a low probability P value of the regression model illustrated whether the level means were significantly different from each other or not. These values suggested that the models were quite efficient in distinguishing the results accurately. Then the relative hydrophobicity of granular sludge of different granular diameters was evaluated by individually analyzing the TSS content in the aqueous phase and organic phase (normal hexane) by Eq. (1). From Fig. 4, the relative hydrophobicity positively increased with the diameter of granular sludge, the maximum of which was 69.9% for diameter ranging from 1.5 to 2.5 mm with the highest content of granular sludge (42.4%). This indicated that the relative hydrophobicity would significantly impact the particle size and microbial affinity through improving the bacterial adherence, decreasing the surface free energy, and accelerating the self-polymerization of granular sludge. Therefore, the relative hydrophobicity was greater when the diameters were within the 1.5-2.5 mm range; the percentage of the granular sludge was the highest (42.4%) within the same range. For the granular sludge 2.5-3.5 mm in diameter and larger than 3.5 mm, the relative hydrophobicity decreased slightly, but was still higher than that of the initially inoculated sludge.

For the surface charge (Fig. 4), it was evident that the SRB granular sludge carried negatively charged. With the increase of granule diameter, the surface charge was decreasing step by step, which facilitated the mutual-approach of sludge particles to form the granular structure. Then the granulation of flocculent sludge was visible. The surface charge decreased more significantly from −0.702 meq g^{-1} VSS for seed sludge to –0.334 meq g^{-1} VSS for 2.5-3.5 mm granular sludge, and further maintained about –0.302 meq g^{-1} VSS for 3.5-5 mm granular sludge. As with 3.5-5 mm granular sludge, almost no changes for negative charges, it should be hard for them to further aggregate. However, the higher production of EPS within this granular diameter range could bridge the sludge particles aggregation. Our findings also agree with previous research that EPS bonding could be independent of electrical neutralization.

Subsequently, we identified the granulation process with changes of EPS components and content of SRB granular sludge [41] with different diameters sampled at the end of the experiment. From Fig. 5, PN was an important component of EPS and its content was about six-times that of PS. The PN content increased from 90 mg g^{-1} VSS in the seed sludge to 160 mg g^{-1} VSS in the SRB granular sludge with particle diameter greater than 3.5 mm, while the variation amplitude of the PS content changed little during the granulation process, revealing the dominant effect of PN (mostly the hydrophobic amino acid) over PS (mostly the hydrophilic groups)

Table 4. Analysis of variance (ANOVA) of the surface charge, hydrophobicity and EPS content of SRB granular with different diameters

Source	DF[a]	Seq SS[b]	Adj MS[c]	F[d]	P[e]
Surface charge	4	0.224	0.056	39.12	0.001
Hydrophobicity	4	673.824	168.456	22.76	0.002
Protein	4	6142.456	1531.114	228.08	0.000
Polysaccharide	4	113.346	28.336	43.51	0.000

[a]Degree of freedom
[b]Sum of squares
[c]Mean sum of squares
[d]F value
[e]Probability (The level was 0.05.)

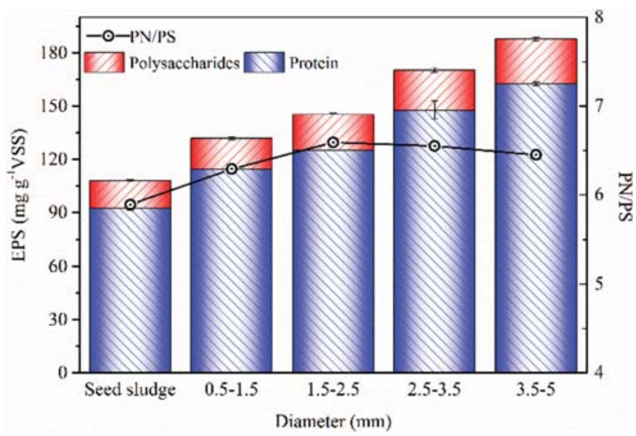

Fig. 5. Variation of EPS content of SRB granular with different diameters.

on the sludge granulation in this UASB system and the hydrophobicity of the granulated SRB sludge. Our finding is also consistent with the early study that the EPS was mostly composed of protein [10] and the high content protein was responsible for the larger granules. In a higher up-flow rate surroundings, larger granules were more susceptible to flow impact. To withstand rupture, bacteria secreted more EPS to protect themselves, which could make microorganisms connect to each other tightly, resist the higher up-flow rate and maintain granules intact; as a result, SRB granules would have a better performance. In addition, the calculated PN/PS ratio was in similar changing tendency with the sludge hydrophobicity, for the greatest PN/PS ratio with the highest percentage (42.4%) of the 1.5-2.5 mm granular sludge. The smaller changing tendency of PN/PS ratio further showed PS and PN content of the EPS maintained a certain balance in the granulation process.

3. Morphology of the Granules

The hydraulic force would significantly impact the granulation by washing away the sludge with loose structure and poor settling property and compacting the flocculent sludge to sludge granular with excellent settling property. The SRB granular sludge presented a complete structure, clear boundary and a compact and smooth surface with elliptic or spherical particles, as shown in Fig. 6(a)-6(b). It can be observed from the SEM that the granular sludge had an irregular porous surface, with a complicated channel-like structure (Fig. 6(c)) that can play a key role in nutrient substance transport and the output of metabolites. Diversified morphological (spherical, rod-shaped etc.) characteristics of bacteria were detectable in the granular sludge (Fig. 6(d)), indicating the stable structure and function of the micro-ecosystem inside the granular sludge. Additionally, the EDS of the granular sludge indicated that the content of sulfur element was the fourth highest, behind only carbon, oxygen, and nitrogen, reflecting the occurrence of sulfate reduction reactions.

4. Grey Relational Analysis

In this section, the interrelation of three major factors, including the surface charge, the relatively hydrophobicity and the PN/PS ratio, with the granulation process of SRB sludge through the granular diameter is specified. Wang et al. [41] demonstrated a positive relationship between total EPS content and surface charge as well as a positive correlation between hydrophobicity and PN/PS. Nevertheless, lower correlation coefficient between PN/PS and surface charge or hydrophobicity in this study revealed different varieties of granular sludge and culture conditions, which would lead to the diversity of granular sludge during their growth.

The GRA method was used to evaluate the influential degrees of surface charge, hydrophobicity and PN/PS on the diameter of granular sludge in the batch reactor. The grey relational grades γ of the diameter of granular sludge were determined as: PN/PS (0.8537)

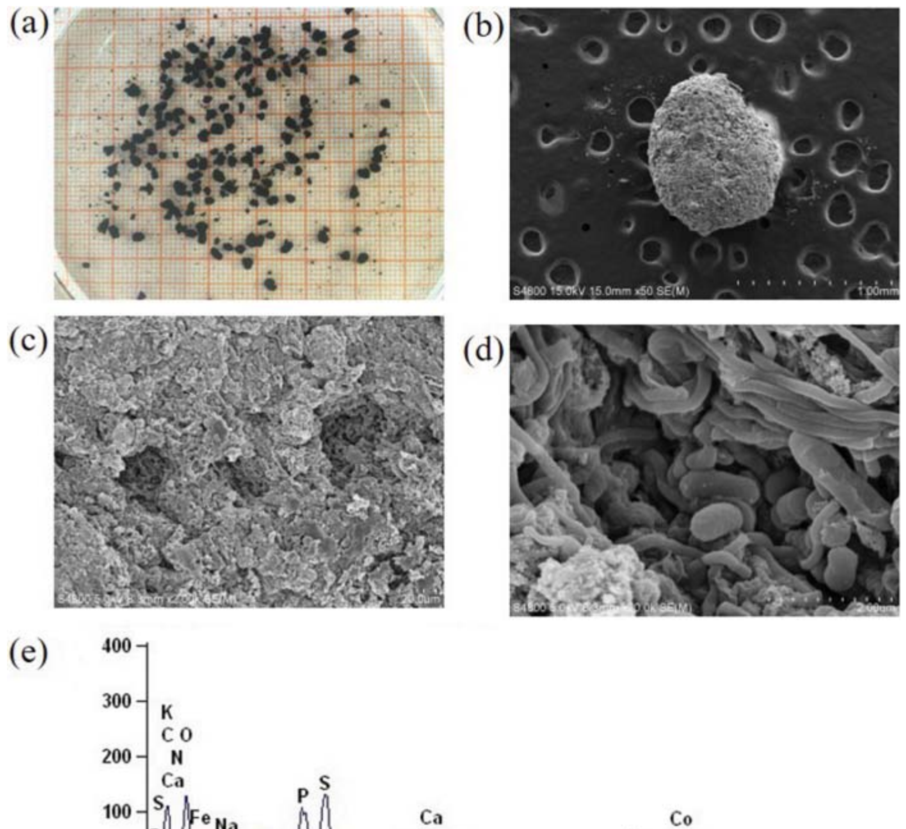

Fig. 6. Morphology of the granular sludge: photograph (size of each square: 1×1 mm) (a), SEM (b)-(d) and EDS images (e).

>surface charge (0.6952)>hydrophobicity (0.5381). According to the values of γ, PN/PS had the most noticeable effect on the diameter of SRB granular sludge, followed by surface charge. Hydrophobicity exhibited the lowest effect on the diameter of SRB granular sludge. With the reduction of the negative surface charge of the SRB granular sludge, the sludge floc would be able to be close to each other and form a stable granular sludge structure. With the increased hydrophobicity, mutual affinity between cells and adhesion performance between bacteria also increased to promote the formation of a stable granular sludge structure. The amino acid of the PN in the EPS was hydrophobic and positive charge, and the PS contained hydrophilic, negatively charged carboxyl groups, Thus, the PN/PS might affect the surface charge and hydrophobicity of the granular sludge [41], and then these three factors may jointly determine the diameter of granular sludge. The theoretical evaluation would be conducive to the granulation control during the potential application.

CONCLUSIONS

The SRB granules developed stably within the UASB rector, presenting a clear and regular outline with an irregular porous structure. The SRB granules showed greater specific gravity (1.018-1.058 g cm^{-3}) and settling ability (43.3-80.2 m h^{-1}). The hydrophobicity of the granular sludge was 69.9% when the granular diameter was in the range of 1.5-2.5 mm. The surface charge was negative and decreased with the decrease of the granular sludge diameter. PN content in the EPS increased with the particle size, while the amount of PS remained nearly constant. The correlation between the internal factors of SRB sludge granulation was PN/PS>surface charge> hydrophobicity. The PN/PS may affect the surface charge and the hydrophobic properties of granular sludge, which can affect the formation of granular sludge together.

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