Comparative study of the removal of sulfate by UASB in light and dark environment

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Abstract

At present, the application of sewage treatment technologies is restricted by high sulfate concentrations. In the present work, the sulfate removal was biologically treated using an upflow anaerobic sludge blanket (UASB) in the absence/presence of light. First, the start-up of UASB for the sulfate removal was studied in terms of COD degradation, sulfate removal, and effluent pH. Second, the impacts of different operation parameters (i.e., COD/SO_4^{2-} ratio, temperature and illumination time) on the UASB performance were explored. Third, the properties of sludge derived from the UASB at different time were analyzed. Results show that after 28 days of start-up, the COD removal efficiencies in both the photoreactor and non-photoreactor could reach a range of 85–90% while such reactors could achieve > 90% of sulfate being removed. Besides, higher illumination time could facilitate the removal of pollutants in the photoreactor. To sum up, the present study can provide technical support for the clean removal of sulfate from wastewater using photoreactors.

Keywords Sulfate wastewater · Upflow anaerobic sludge blanket · Photo · Sulfate-reducing bacteria · Photosynthetic bacteria

Introduction

As important raw materials, sulfur and sulfur compounds have been widely used in industries (e.g., mining, landfill leachate, molasses, papermaking, pharmaceuticals, leather and petrochemicals), which results in the production of wastewaters simultaneously containing rich sulfate and chemical oxygen demand (COD) [1]. In such waters, the content of sulfate can reach hundreds or thousands of mg/L [2]. The discharge of polluted sulfate wastewater is daily increasing since the industrialization process continues to dominate much of the world. High sulfate content may lead to a sequence of serious environmental complications (e.g., pipes and equipment scaling and metal corrosion); for example, the sulfur cycle can be disturbed via changes in salinity and pH of water caused by the presence of sulfate [3] while aquatic organisms which can only sustain limited water salinity may be negatively influenced by water salinization caused by high sulfate concentrations [4]. Besides, sulfate can be converted into H_2S through sulfate-reducing bacteria under anaerobic environment, in which H_2S has detrimental impacts on human health and the environment. More importantly, laxative effects may be caused once prolonged intake of waters containing 500–750 mg/L of sulfate [5]. Due to long incubation periods and handling problems, it is extremely difficult to treat wastewater containing high sulfate concentrations, which has become one of the biggest environmental problems around the world [6]. Thus, it is essential to conduct

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effective removal of high-content sulfate from wastewater prior to its discharge into the water bodies.

The commonly used technologies for sulfate removal include biological, chemical, and physical (e.g., multi-effect evaporation crystallization, adsorption and ion-exchange) methods, among which the chemical and physical processes are subjected to treat wastewaters containing high concentration of sulfate and may lead to secondary pollution with high capital input [7, 8]. Hence, anaerobic biotechnology is deemed one of the most economical and energy-efficient technologies for effective sulfate removal [9]. Sulfate-reducing bacteria (SRB) are often used for treating sulfur-containing wastewater, where their well control can achieve positive treatment of domestic wastewater [10]. However, sulfate could be easily reduced by SRB to S^{2-}, HS^- and H_2S under anaerobic environment, where such substances are not only highly corrosive, but also toxic to humans, animals, plants, and a variety of microorganisms [11, 12]. At wastewater treatment, anaerobic microbes can be inhibited by sulfide in a concentration range of 100–200 mg H_2S–S/L [13]. Thus, further treatment of sulfide should be considered while treating high-concentration sulfate wastewater.

In previous studies, it was found that photosynthetic bacteria (PSB) (e.g., *Rhodopseudomonas*, *Thiopedia*, *Ectothiorhodospira*, *Chromatium*, and *Chlorobium*) can be cultivated using carbon source and sulfide as an electron donor through anoxygenic photosynthesis, as demonstrated by the equation: $CO_2 + H_2S \rightarrow S^0 +$ fixed carbon [14, 15]. In this scenario, PSB can conduct (in) complete oxidation of sulfide within or outside the cell [16]. Besides, the biological removal of sulfate is often conducted in anaerobic sequencing batch reactor (AnSBR), anaerobic membrane bioreactor (AnMBR), and upflow anaerobic sludge blanket (UASB) [17], which has long residence time and produces a large amount of surplus sludge [18]. PBS also has the unique advantages in organic degradation from high pollution-load wastewater; for example, PSB could effectively remove COD, phosphate, and nitrate while it could also be used as fertilizer and fish feed, thus reducing the costs related to sludge disposal [19, 20].

Inspired by the content discussed above, the removal of sulfate from wastewater was carried out using SRB and PSB in a 4.8-L UASB under anaerobic conditions. Through long-term operation, we compared the performance difference between the photoreactor and non-photoreactor to determine whether the photoreactor can achieve better results. Then the impacts of carbon–sulfur ratio (COD/SO_4^{2-}), illumination time, and temperature on the degradation efficiency of SO_4^{2-} were explored to investigate the appropriate conditions. Thus, this study aims to present a novel approach for treating sulfate-containing organic wastewater.

Materials and methods

Experimental setup

As shown in Fig. 1, the main body of the 4.8 L-UASB was made of plexiglass material while the inner ring was made of ordinary transparent glass material, in which the height was 600 mm. Besides, the bottom part and three-phase separator were made of stainless-steel materials, while a fine mesh plastic film was hung on the glass wall of the reactor. In this experiment, the water was fed through the peristaltic pump with a flow rate of 5 mL/min. Four same reactors were employed in the study, in which $1^{\#}$ and $2^{\#}$ reactors (i.e., photobioreactors) were operated using 18 W fluorescent lamps while $3^{\#}$ and $4^{\#}$ reactors (i.e., non-photobioreactors) were considered as the control group without light input.

Apart from this, start-up of the reactor adopted the influent flow rate of 5 mL/min with hydraulic retention time (HRT) of 16 h. At the beginning of the experiment, the influent COD concentration was 1000 mg/L with a volume loading rate of 1.5 kgCOD/(m^3d) while the influent content of SO_4^{2-} was 250 mg/L, in which the COD/SO_4^{2-} ratio was kept in a fixed value of 4 (Table 1).

Apart from this, the reactors were then operated at different parameters including temperatures (25–40 °C), COD/SO_4^{2-} ratios (4, 2.5 and 1.7) and illumination time (6–24 h) to identify the changes in concentrations of sulfate and COD, and pH values. In the present study, a three-phase separator was designed to isolate air by water seal, so that an anaerobic or low oxygen state was formed within the reactor.

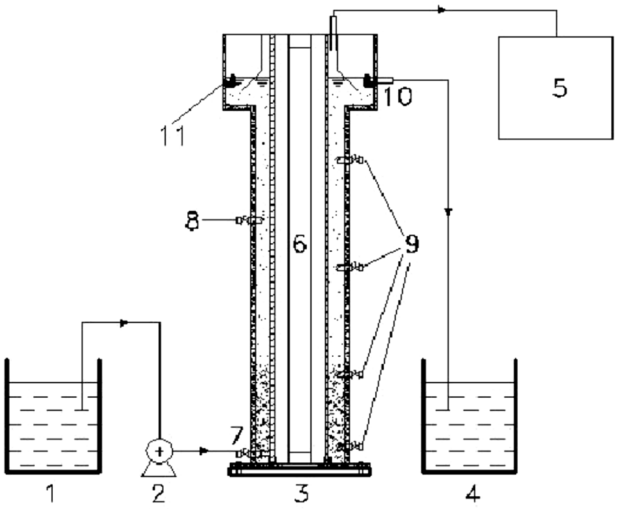

Fig. 1 Diagram of UASB in the present study. 1: influent tank (8 L); 2: peristaltic pump; 3: UASB (4.8 L); 4: effluent collection tank (8 L); 5: air collector bag; 6: daylight tube; 7: water inlet; 8: reserved port; 9: sample connection; 10: overflow port; 11: overflow tank

Table 1 Operating parameters during start-up stage

Operating time (days)	Influent COD (mg/L)	Volume loading rate (kgCOD/(m^3d))	Influent sulfate (mg/L)	Loading rate of SO_4^{2-}($kgSO_4^{2-}$/(m^3d))	HRT (h)
1–15	1000	1.5	250	0.375	16
16–26	2000	3	500	0.75	16
27–40	3000	4.5	750	1.125	16
41–51	4000	6	1000	1.5	16
52–63	5000	7.5	1250	1.875	16

Table 2 Composition of the artificial wastewater

Component	Concentration (mg/L)	Component	Concentration (mg/L)
$C_6H_{12}O_6$	910	Na_2SO_4	370
NH_4Cl	80	$MgCl_2{\cdot}6H_2O$	254
KH_2PO_4	20	Trace element solution	0.5 mL/L

Moreover, the introduction of harmful heavy metals and anions to the reactors was avoided to ensure biological safety.

Influent composition and inoculated sludge characteristics

The inoculated granule sludge in this experiment was derived from an anaerobic reactor in a wastewater treatment plant (Wuhan, China), in which the suspended solid (SS) and volatile suspended solid (VSS) of the granule sludge were 60.1 and 46.9 g/L, respectively. After inoculation, the sludge concentration was 9.77 VSS/L while the initial sludge load was 0.15 kg·COD/kg·VSS in the UASB. Artificial wastewater was used as the influent in the experiment, in which $C_6H_{12}O_6$, NH_4Cl, and KH_2PO_4 were used as the carbon, nitrogen, and phosphorus sources, respectively, with the influent COD:N:P ratio of 250:5:1. Na_2SO_4 was utilized as the sulfate source, while $NaHCO_3$ was employed to adjust the pH to ensure that the influent pH was about 7.5. The concentration of SO_4^{2-} can be altered by adjusting the quantity of sodium sulfate utilized. Some trace elements were also added to the influent, such as Mg^{2+}, Ca^{2+}, Fe^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, and Cu^{2+}, while the composition of the influent is shown in Table 2.

Sampling

10 mL of effluent was daily collected from the four reactors using sterile syringes via dedicated sample ports for testing, after which a 0.45 μm filter membrane was utilized for filtration before measuring the content of COD and SO_4^{2-}, and pH value. The COD concentration and solution pH were measured every day while the content of SO_4^{2-} was tested every 2 days. Besides, gas and sludge samples were collected at various stages for further analysis.

Measurement method

The influent and effluent COD, sulfate, and pH were analyzed during the experiment. COD was determined by potassium dichromate titration [21]. The concentration of sulfate was measured by an ion chromatograph (ICS-90A; Dionex, USA) equipped with the AG18 column (Dionex Ion-Pac™ AG18, 4 × 50 mm, USA). The portable biogas detector (BM14097; Geotechnical Instruments, UK) was used to detect the gas composition, thus detecting the percentage content of CH_4, CO_2, O_2 and the instantaneous concentration of H_2S. The properties of sludge were analyzed, including sludge settling velocity (SV), sludge concentration (MLSS), volatile sludge concentration (MLVSS), sludge volume index (SVI), and sludge ash content. Furthermore, the properties of granular sludge were analyzed by field emission scanning electron microscopy (FESEM; Nova NanoSEM450, FEI, Netherland) and environmental scanning electron microscopy (ESEM; Quanta 200, FEI, Netherland) [22].

Results and discussion

Start-up of UASB

At the start of the experiment, the COD and SO_4^{2-} loads were incrementally increased while maintaining a controlled COD/ SO_4^{2-} ratio of 4. During Stage I, the influent COD concentration was 1000 mg/L and the reactor was operated for 15 d. After the stabilization of the system, the COD load kept gradually increasing to 5000 mg/L with the overall start-up period of 64 d.

As depicted in Fig. 2, in the first 15 d, when the influent COD content was 1000 mg/L, the effluent COD values in the two reactors were nearly the same, showing a downward trend. This indicates that the microbial community structure of the anaerobic system was the same at the beginning in the photoreactor and non-photoreactor, in which no significant difference caused by light conditions was achieved. From 28 d, the COD removal efficiency of each reactor tended to

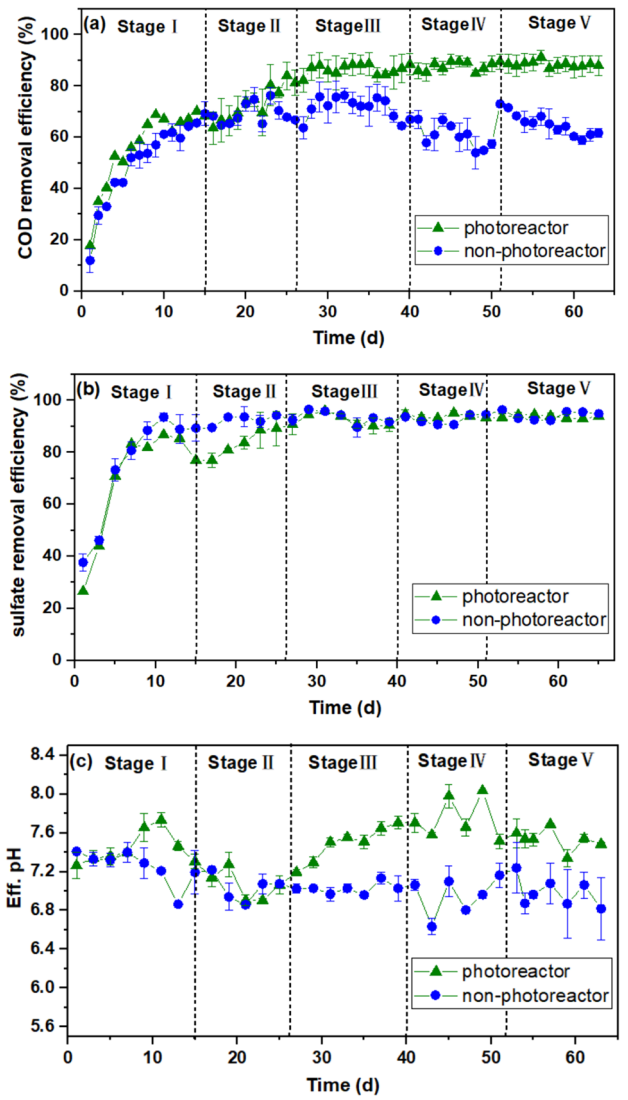

Fig. 2 Time course of the removal efficiency of COD **(a)** and sulfate **(b)**, and effluent pH **(c)**. The influent COD concentrations of Stage I–V were 1000, 2000, 3000, 4000, and 5000 mg/L, respectively, with the COD/ SO_4^{2-} ratio of each stage at 4

be stable, indicating that the UASB was started successfully [23]. Besides, on 38 d, there was a significant difference in effluent COD concentration between the photoreactor and the non-photoreactor, where the former reactor could obtain much higher COD removal efficiency (> 90%) than that in the latter (60–70%). This may be attributed to the sulfide production caused by the sulfate reduction. In the photoreactor, sulfur-oxidizing photosynthetic bacteria can effectively remove sulfide from the reactor [24], thus reducing its toxic effect on other heterotrophic microorganisms. As a result of this, the COD removal by heterotrophic microorganisms in the photoreactor was negligibly affected.

Since the inoculated sludge used in the present work was derived from a mature and acclimated sulfate wastewater reactor, the SO_4^{2-} content in the effluent was rapidly stabilized and maintained at a low concentration at the initial stage of operation. As the influent SO_4^{2-} concentration increased, its removal efficiency increased from 30 to 90%, indicating that SRB in the inoculated sludge were abundant and showed high bioactivity. After 28 d, the SO_4^{2-} concentration in the effluent of the two reactors was relatively stable with a value of less than 100 mg/L, suggesting that the SRB flora of the two reactors had reached a stable state. Besides, the effluent SO_4^{2-} concentration and removal rate of SO_4^{2-} in the photoreactor and non-photoreactor were the same, indicating that the presence of light may have no significant influence on the activity of SRB. However, light may have some indirect effects on methanogens and acetogenic bacteria because the effluent COD/SO_4^{2-} ratio in the non-photoreactor was higher (20) than that (10) in the photoreactor. In the two reactors, the SRB community remained stable, while the activity of methanogens and acetogenic bacteria gradually decreased. As the experiment proceeded, the accumulation of PSB under light conditions made the system more stable, which could facilitate the COD removal. Simultaneously, for the non-photoreactor, methanogens and acetogenic bacteria were negatively affected to some extent, so the system stability was lower than that under the light condition.

It can be seen from Fig. 2 that the effluent pH value (Eff. pH) of each reactor fluctuated to a certain extent. From 1 to 30 d, the effluent pH value of the two reactors was lower than the inlet value, ranging from 6.7 to 7.5. On 31 d, the effluent pH value of photoreactor and non-photoreactor showed an obvious difference, in which the effluent pH value of photoreactor was higher (7.5–7.8) than that (6.5–7.2) derived from the non-photoreactor. This indicates that the UASB under light conditions was in a more stable state. The low pH of the effluent in the non-photoreactor may be attributed to the accumulation of sulfide. Sulfate reduction produces biotoxic sulfides. However, in non-photoreactors, there were fewer sulfur-oxidizing photosynthetic bacteria, which can lead to sulfide accumulation [24]. Sulfides can be toxic to methanogens, hindering metabolic processes and making fatty acid degradation ineffective, which can lead to a decrease in pH.

Each reactor can achieve a stable operational state, providing a foundation for further investigation into the effects of various factors. Furthermore, the COD removal efficiency and effluent pH value of the photoreactor were significantly higher than those of the non-photoreactor, indicating the superior performance of the photoreactor.

The influence of different conditional parameters

COD/SO_4^{2-} ratio

Theoretically, the complete reduction of SO_4^{2-} to sulfide through SRB requires COD/SO_4^{2-} ratio of 0.67 [25]. There

is competition between SRB and other bacteria such as PSB, methanogens, and acetogens for organic carbon sources, so COD/SO_4^{2-} ratio is always higher than the theoretical value to ensure the complete reduction. In the experiment, the impacts of COD/SO_4^{2-} ratio (4, 2.5 and 1.7) on the UASB performance were explored under light conditions at the influent pH of 7.5, temperature of 35 ± 1 °C, and HRT of 16 h. The influent COD concentration was 5000 mg/L while the SO_4^{2-} content was increased to achieve the desired COD/SO_4^{2-} ratios.

As shown in Fig. 3b, the removal efficiency of SO_4^{2-} decreased from 90 to 75% as the COD/SO_4^{2-} ratio reduced from 4 to 1.7. The possible reason for this is that the growth of SRB was restricted because there were not enough electron donors for sulfate reduction, thus declining the sulfate removal efficiency [26]. The removal efficiency of SO_4^{2-} still maintained a relatively high removal efficiency and was relatively stable during the process of COD/SO_4^{2-} decreasing from 2.5 to 1.7. It could be seen that SRB may have sufficient electron donors (i.e., organic carbon source) as COD/SO_4^{2-} > 2.5, in which the reactor maintained a relatively stable state and had higher sulfate removal efficiency. In contrast, the SO_4^{2-} concentration was relatively high and organic concentration was insufficient at COD/SO_4^{2-} ≤ 2.5, so the sulfate reduction process through SRB was subjected to unavailable carbon source. As a result of this, the removal efficiency of SO_4^{2} declined. Interestingly, as the COD/SO_4^{2-} decreased from 2.5 to 1.7, the SO_4^{2-} removal efficiency had a slight increase because low COD/SO_4^{2-} could facilitate the competition of SRB for substrates with other bacteria, thus improving the SO_4^{2-} removal [27]. Some previous studies also confirmed that sulfide-induced inhibition may result in lower sulfate removal rate at low COD/SO_4^{2-} < 2 [28–30].

Besides, the COD removal efficiency was higher in the photobioreactor when compared to the reactor in the absence of light, since both SRB and PSB could contribute to the degradation of COD in the presence of light, thus increasing the COD removal efficiency. More inhibition of COD removal was observed in the non-photobioreactor as COD/SO_4^{2-} ratio was reduced. The possible reason for this is that SRB may predominate at COD/SO_4^{2-} ratio of less than 2.0 when compared to methanogens [31]. This may also be attributed to the increase in the sulfide content of the intermediate product of sulfates [6]. In this scenario, the physiological metabolism of SRB for organic removal was suppressed by the increase in the sulfide concentration while the photoreactor could utilize PSB to oxide partial sulfide to mitigate such inhibition effects. It should be also noted here that the inhibition derived from sulfide may also negatively affect the methanogens [32] and lower their ability to decompose fatty acid, thus decreasing the pH.

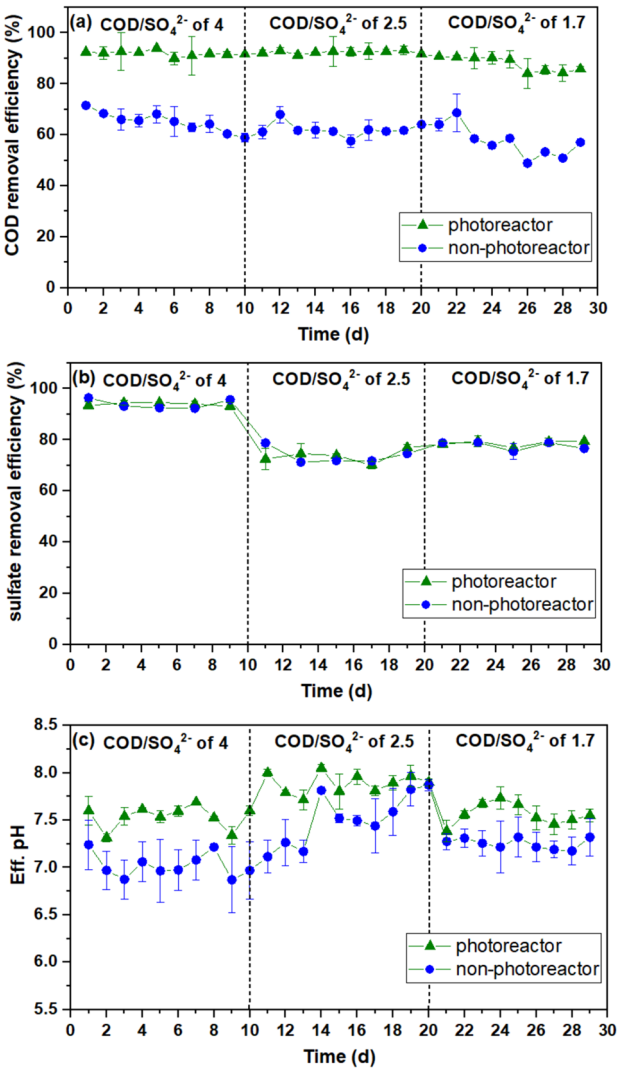

Fig. 3 The removal efficiency of COD **(a)** and SO_4^{2-} **(b)** and the changes of Eff. pH value **(c)** of each reactor under different COD/SO_4^{2-} ratios

Consequently, the effluent pH values were much lower in the non-photoreactor.

Temperature

SRB can be divided into strict psychrophilic, moderate psychrophilic, cold-tolerant mesophilic and moderate mesophilic bacteria, and the optimal temperature is generally 28–35 °C [33]. Most sulfur-oxidizing bacteria and methanogenic archaea metabolize under medium temperature. In the present study, the effect of temperatures (i.e., 25, 30, 35, and 40 °C) on the UASB performance was studied. The influent COD value of each reactor was 5000 mg/L, the influent SO_4^{2-} concentration was 1250 mg/L, the influent

pH was controlled at 7.5, and the illumination time of the photoreactors was 24 h.

As shown in Fig. 4b, the SO_4^{2-} removal efficiencies of each reactor at four temperatures were all over 90% with negligible difference. This indicates that the temperature range may have insignificant impacts on the SRB metabolism. However, Dong, Wang, Gao, Di, Wang, Guo, Hu, Gao and Wang [34] and Kushkevych, Dordević and Vítězová [35] determined the temperature suitable for SRB growth while Wang, Peng, Fan and Li [36] also reported that the reducing efficiency of SRB for sulfate increased with increasing temperature. Moreover, the removal efficiencies of COD in the two reactors were higher at 35 °C while the lowest COD removal efficiency was obtained at 40 °C. This is because higher temperature may inhibit bacterial metabolism and thus negatively affect the degradation of COD [37]. The effluent pH of each reactor was relatively stable in the temperature range of 25–40 °C. Furthermore, the effluent pH values of the two reactors were increased at 40 °C when compared to the other temperatures, which may be attributed to the evaporation of partial volatile fatty acids out of the reactors [38].

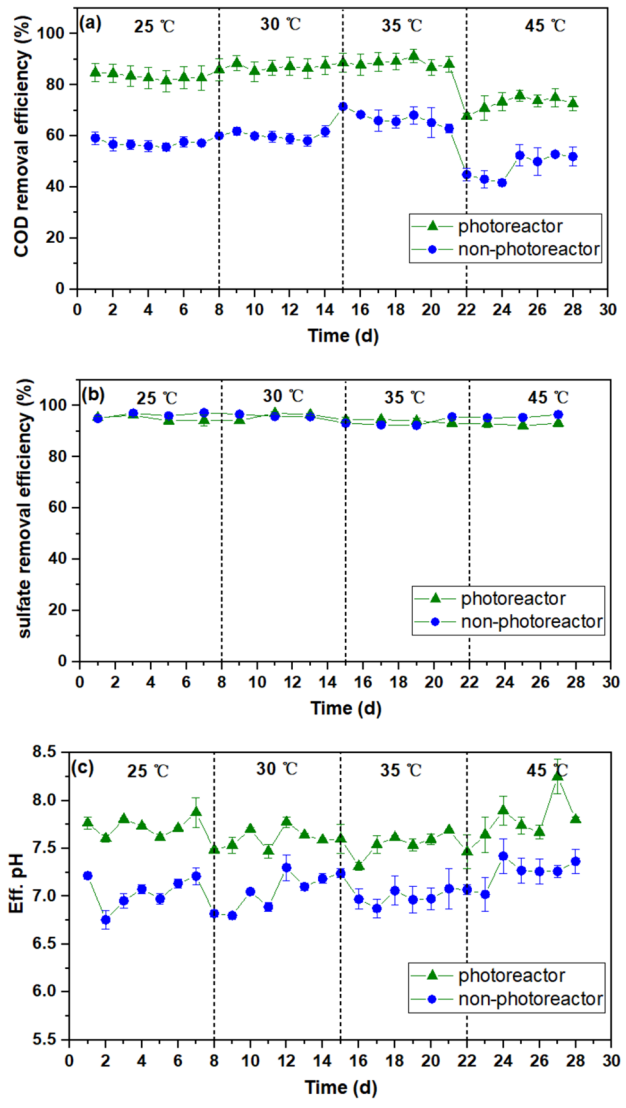

Fig. 4 The removal efficiency of COD **(a)** and SO_4^{2-} **(b)** and the changes of Eff. pH value **(c)** of each reactor under different temperatures

Illumination time

Under the condition of controlled light intensity, it is important to study the influence of light time because the PSB could effectively remove sulfide to reduce the toxic effect of sulfide on methanogenic archaea [39], thus improving the removal efficiency of COD. Therefore, photosynthetic bacteria are the key to the effective removal of COD and stable operation of anaerobic system. This experiment used 18-W fluorescent lamp as the light source with the light intensity of about 6500 lx and illumination time of 6–24 h. Besides, the influent COD and SO_4^{2-} concentrations of each reactor were 5000 and 1250 mg/L, respectively, while the influent pH was about 7.5 and the incubator temperature was 35 °C.

As shown in Fig. 5a, as the illumination time decreased from 24 to 6 h, the removal efficiency of COD in the photoreactor decreased from 90 to 85%. It could be also seen that the illumination duration was proportional to the COD removal efficiency. The possible reason for this is that light directly affects the growth and metabolism of PSB [40], which plays an active role in COD degradation. In this scenario, as the illumination time was shortened, the metabolic capacity of PSB was weakened and the COD removal efficiency was, thus, reduced. The removal efficiency of SO_4^{2-} ions decreased as the illumination time reduced from 24 to 6 h, in which the removal efficiency of SO_4^{2-} in the $2^{\#}$ reactor decreased to $< 85\%$ at the illumination time of 6 h. This is because the metabolic activity of PSB could be weakened and harmful substances may be, thereby, accumulated in the reactor, which affects the degradation of sulfate by SRB [41]. Furthermore, the effluent pH value was decreased at lower illumination time and the illumination duration was proportional to the effluent pH value. As discussed above, the activity of PSB decreased at lower light time, which may lead to the accumulation of sulfide. In this scenario, the bioactivity of bacteria such as methanogenic archaea was inhibited during the utilization of decomposition products of glucose (e.g., fatty acids). As a result of this, such acidic substances would be kept within the reactor and thereby reduce the effluent pH values. San Martín, Puyol, Segura, Melero and Martínez [42] also found similar results in their study of photoanaerobic system.

It should be noted that the COD removal efficiency of the photoreactor was significantly higher than that of the non-photoreactor under different operating conditions. However,

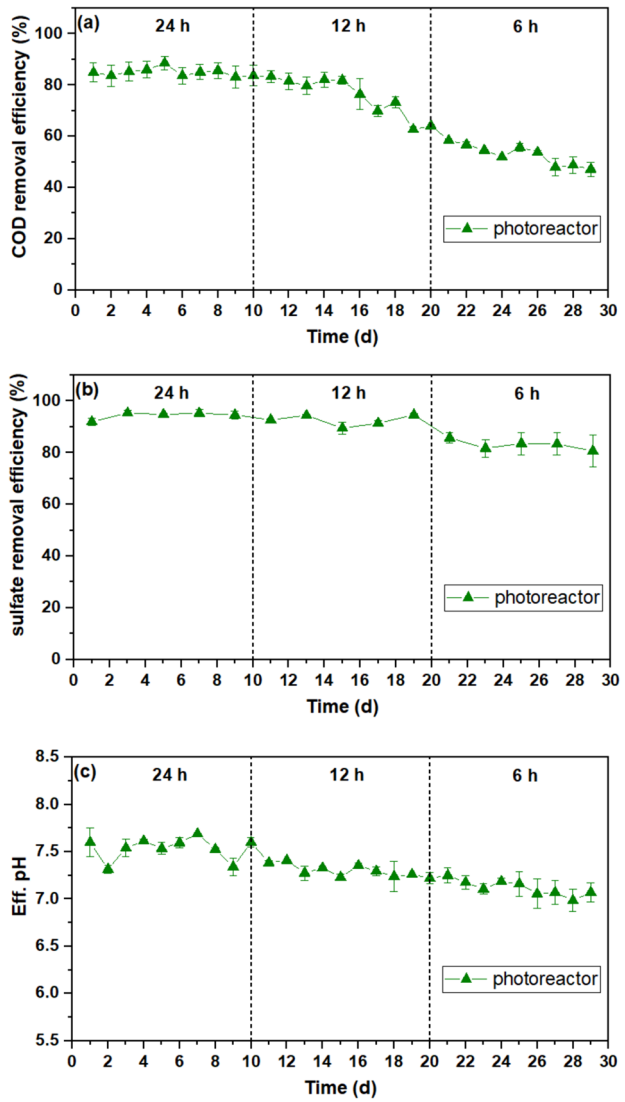

Fig. 5 The removal efficiency of COD **(a)** and SO_4^{2-} **(b)** and the changes of pH value **(c)** of each reactor under different illumination time

there was no significant difference in the sulfate removal efficiency. The efficiency of the photoreactor in treating sulfate-containing organic wastewater was demonstrated, where it was found that effective removal of COD and sulfate can be obtained at the COD/SO_4^{2-} ratio of 4, 24-h illumination time and operating temperature of 30 °C.

Gas composition analysis

The gas derived from the UASB was collected at Time I (start-up period, Stage V) and Time II (COD/SO_4^{2-} ratio of 1.7), respectively, after which they were measured by portable biogas detector. As shown in Table 3, the percentage of CH_4 content in the reactors was the highest, followed by CO_2 and O_2. The high O_2 content may be ascribed to the lax sealing of the three-phase separator, leading to the entry of external oxygen. Jing, Hu, Niu, Liu, Li and Wang [43] reported that HRT and organic loading rate can highly affect the gas production and they achieved the methane percentage in the range of 70–80% in the biogas. The percentages of CH_4 and CO_2 in the photoreactor were significantly higher than that in the non-photoreactor. This indicates that the light condition could facilitate the degradation of organic matter in the anaerobic reaction system, and the activity of methanogenic archaea and acetic acid bacteria in the photoreactor was higher than that in the non-photoreactor. Besides, the H_2S flow rate measured by the biogas detector indicates H_2S may account for a large proportion of other compositions of the gas. H_2S is poisonous to methanogens since it can penetrate into cell membranes to inactivate proteins and enzymes, which seriously influences organic degradation and sulfate removal [44]. Thus, rational control of H_2S emission can facilitate the further use of biogas and mass metabolism. Moreover, it was found that the gas production rate of the photoreactor is significantly higher than that of the non-photoreactor, suggesting that the light can maintain higher activity of the microorganisms in the whole reaction system [45].

Energy spectrum analysis of reactor products

Photos for the reactor were recorded at Time I (start-up period, Stage V), Time III (COD/SO_4^{2-} ratio of 2.5) and Time IV (temperature of 25 °C), respectively. As depicted in Fig. 6, it can be seen that at Time I, the photoreactor showed a pink color, and there were black attachments on the reactor wall. At Time III, the color in the photoreactor was deepened to reddish-brown with a wide range of red distribution, while the attachment of the photoreactor wall also increased significantly. At Time IV, after the light time was 6 h, the color in the photoreactor became lighter, and more objects were attached to the wall of the reactor. In contrast,

Table 3 Gas composition of each reactor

Reactor	Time	CH_4 (%)	CO_2 (%)	O_2 (%)	H_2S (ppm)	Other (%)
Photoreactor	Time I	37.4	16.6	8.0	> 9999	38.0
	Time II	35.5	20.1	7.0	> 9999	37.4
Non-photoreactor	Time I	24.3	11.6	11.6	> 9999	52.5
	Time II	22.8	9.2	10.1	> 9999	57.9

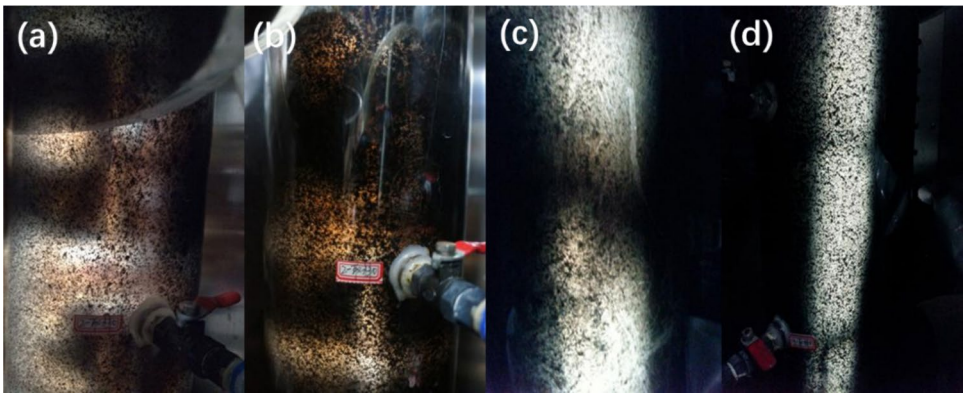

Fig. 6 Characteristics of photoreactor at Time I **(a)**, Time III **(b)**, and Time IV **(c)**, and non-photoreactor at Time IV **(d)**

the non-photoreactor did not appear red or pink and the reactor walls were not attached with thicker black materials. This indicates that the production of red or brown substances in the reactor may be caused by light. It was reported that most of the PSB are red, pink, and reddish-brown, so it is inferred that the red substances in the photoreactor are PSB [46]. Besides, it can be seen that there are black attachments on the walls of each reactor, and they increase with the increase of running time. According to the influent characteristics and reaction conditions of this experiment, it is inferred that the black substance may contain FeS [47].

Light yellow or white substances also accumulated in the three-phase separator of the reactor, as shown in Fig. 7. More specifically, white or light-yellow solid substances appeared in the sludge at the bottom of the photoreactor at Time III while such substances did not appear in the non-photoreactor. Furthermore, there was obvious accumulation of light-yellow solid matter in the photoreactor at Time IV while there was also a layer of light-yellow solid substance floating on the water surface. There was only a very small amount of yellowish solids in the non-photoreactor, and very little yellowish material on the water surface. Overall, light conditions could promote the formation of such white or light-yellow solids. According to the physical properties of the solid, it can be inferred that the substance was sulfur [48]. To further identify the substance, the samples were analyzed by SEM–EDS. It can be seen from Fig. 8 that the energy spectra of the two reactors showed the appearance of sulfur with a high percentage of the solid [49].

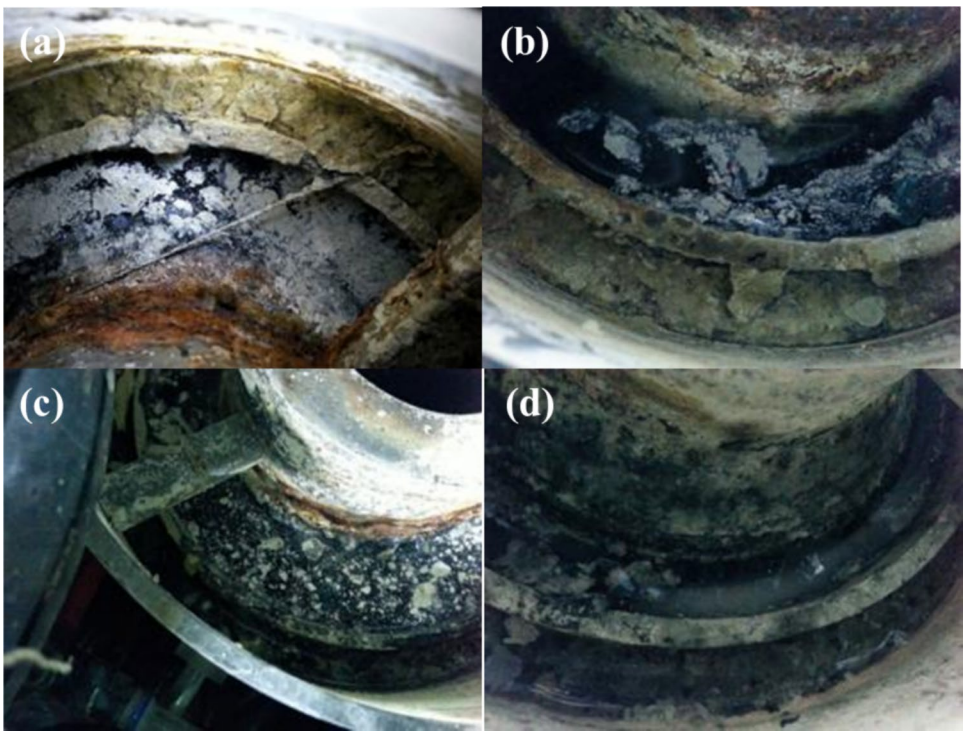

Fig. 7 Solid accumulation in three-phase separator of photoreactor **(a–b)** and non-photoreactor **(c–d)** at Time IV

Sludge characteristic analysis

Sludge biofacies analysis

The sludge collected at Time I and Time III was analyzed by FESEM while the ESEM was used to characterize the sludge collected at Time V (temperature of 40 °C). As can be seen from Fig. 9, the inoculated granular sludge was spherical or ellipsoidal with uneven surface structure. The structure of the inoculated sludge was relatively uniform with high surface porosity, while the individual bacterial morphology was not obvious. At Time I and Time III, the individual bacteria morphology could be observed, in which most bacteria in the photoreactor were short rod-shaped bacteria with a few Micrococcus while the non-photoreactor could detect Bacillus and Sphaerobacterium with some Spirillum. As depicted in Fig. 9, the sludge structure in the photoreactor was tight at Time V, in which a large number of rod-shaped bacteria entangled to form bacteria clusters. In contrast, the sludge structure was fluffy in the non-photoreactor at Time V, where no bacterial micelles were detected with bacillus and globular bacteria mostly existing, and filamentous bacteria co-exiting. To sum up, the light condition had a great influence on the sludge in the reactor, promoting the increase of microorganisms and forming stable bacterial micelles. This also indicates that the light condition could make the microbial community structure in the reactor more stable due to the mitigation of inhibition effects by the presence of H_2S, which facilitates the degradation of pollutants. Similarly, Liu, Wang, Zhang, Zhou and Yan [50] also found that light can promote the enrichment of dominant bacteria and improve community stability and degradation performance.

Sludge proliferation analysis

Yong, Yongzhen and Shuying [51] believed that well control of sludge height can improve effluent quality and operation stability. In the present study, the sludge height in the reactors was measured. It can be seen from Fig. 10 that the amount of sludge in the photoreactors increased with time while the amount of sludge in the non-photoreactor showed a decreasing trend. Furthermore, it can be concluded that the light condition was conducive to the sludge proliferation of the anaerobic system, making the system more stable. The results also confirmed that the light conditions could improve the removal of pollutants in the reactor.

Sludge index analysis

The sludge's SV, MLSS, SVI and MLVSS were measured as well as the ash content of sludge. As shown in Table 4, SV, MLSS, and MLVSS of the sludge in the photoreactor were much higher than those in the non-photoreactor, indicating that the sludge content and concentration in the photoreactor were higher than those in the non-photoreactor. The SVI in the light condition was lower than that in the absence of light, indicating that the sedimentation performance of sludge in the photoreactor was better than that in the non-photoreactor [52]. Furthermore, the sludge ash of photoreactor was significantly higher than that of non-photoreactor, which indicates that the inorganic content of sludge in the photoreactor was higher than that in the non-photoreactor [53]. This is because the light could promote the degradation of substances (e.g., H_2S) that inhibit the growth and metabolism of bacteria in the reactor [54, 55].

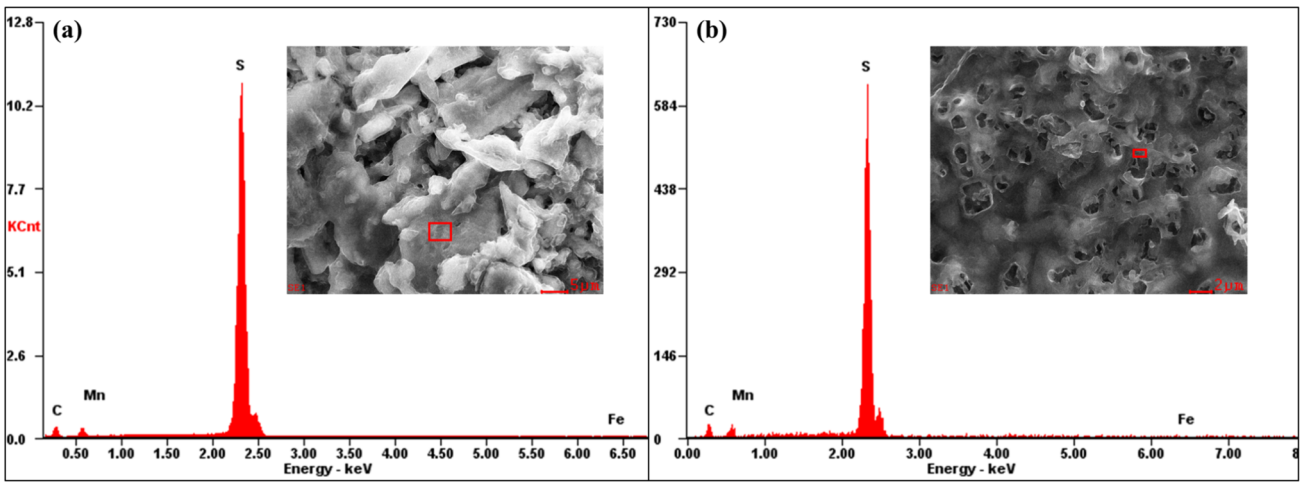

Fig. 8 SEM–EDS images of solids derived from the photoreactor

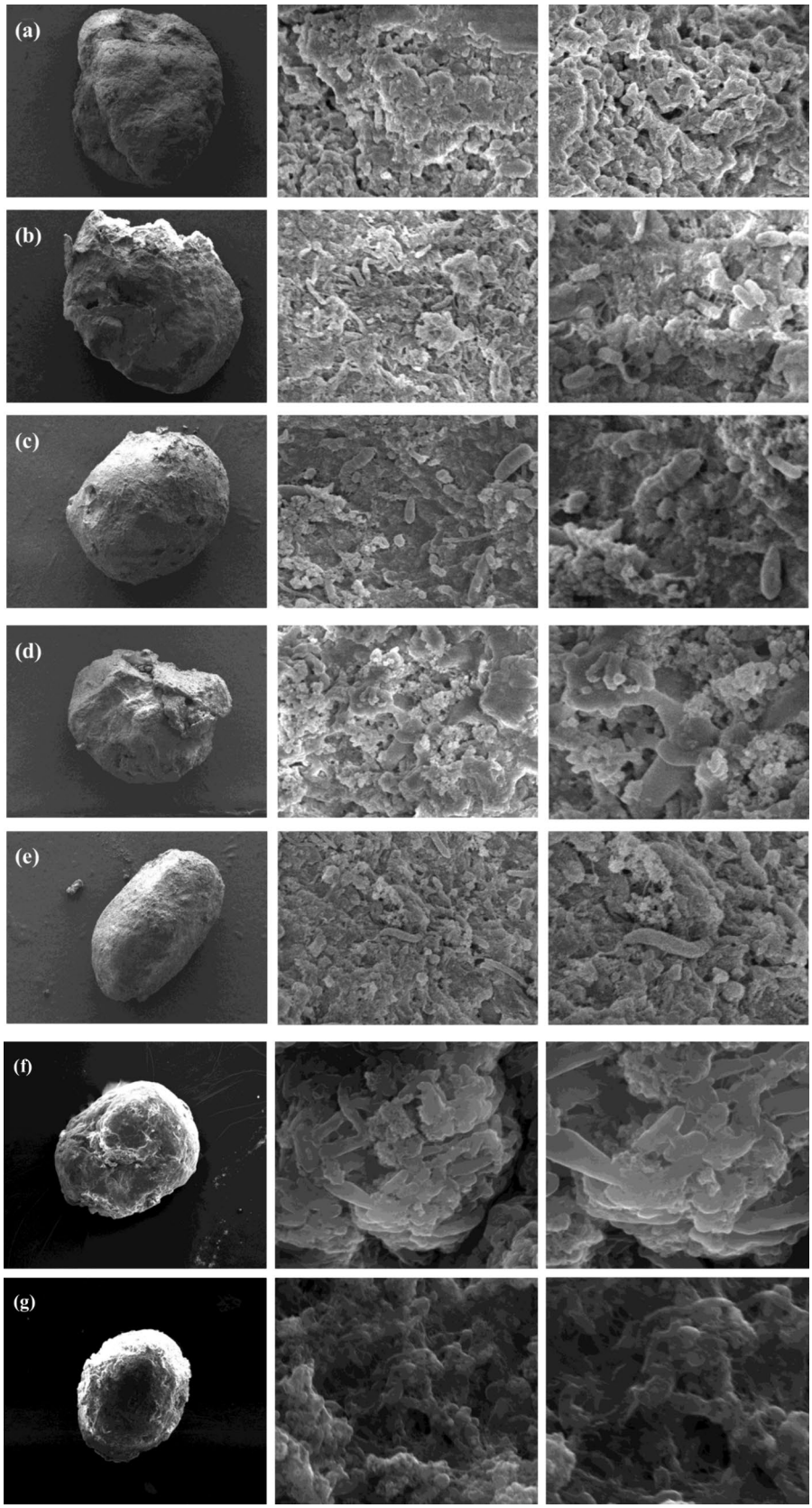

Fig. 9 FESEM images of inoculated sludge **(a)**, sludge of photoreactor **(b)** and non-photoreactor **(c)** at Time I; sludge of photoreactor **(d)** and non-photoreactor **(e)** at Time III; and ESEM images of photoreactor **(f)** and non-photoreactor **(g)** at Time V. (all the images were obtained at different magnifications [65, 10,000, and 20,000 times from left to right])

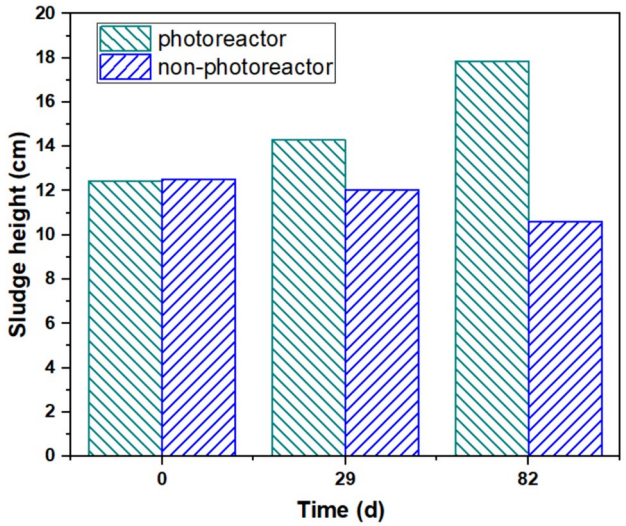

Fig. 10 Sludge height variation over time in each reactor

Table 4 Analysis of sludge index of each reactor

Index	Photoreactor	Non-photoreactor
SV (%)	89	19
MLSS (g/L)	31.59	6.68
SVI (g/L)	2.834	2.844
MLVSS (g/L)	20.89	3.57
Sludge ash (%)	0.341	0.466

Conclusion

In this study, the UASB was first started up and then determined the effect of COD/SO_4^{2-} ratio, illumination time, and temperature on the pollutant removal and sludge characteristics. Photoreactor had better ability to remove COD and sulfate when compared to non-photoreactor, where the concentration of COD and sulfate could be significantly reduced at the COD/SO_4^{2-} ratio of 4, 24-h illumination time and operating temperature of 30 °C. Furthermore, photosynthetic sulfur bacteria were detected in the photoreactor as well as elemental sulfur. The bacteria in the sludge were mostly rod and globular bacteria with a small amount of spiral bacteria and filamentous bacteria. Under the condition of light, the number of bacteria in the granular sludge increased significantly and formed a relatively stable bacterial colloidal group. Further efforts should be made to analyze the microbial community structure and gene expression in the photoreactor, clarify the internal mechanism of photoreactor's excellent performance, and reveal the transformation pathway of sulfur-containing substances in the system. These efforts will promote the industrial application of the system and improve the treatment of sulfate-containing organic wastewater.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Han Y, Wu C, Fu X, Su Z, Liu M (2021) Sulfate removal mechanism by internal circulation iron-carbon micro-electrolysis. Sep Purif Technol 279:119762
2. Hao T-w, Xiang P-y, Mackey HR, Chi K, Lu H, Chui H-k, van Loosdrecht MCM, Chen G-H (2014) A review of biological sulfate conversions in wastewater treatment. Water Res 65:1–21
3. Fernando WAM, Ilankoon IMSK, Syed TH, Yellishetty M (2018) Challenges and opportunities in the removal of sulphate ions in contaminated mine water: a review. Miner Eng 117:74–90
4. Nurmesniemi ET, Hu T, Rajaniemi K, Lassi U (2021) Sulphate removal from mine water by precipitation as ettringite by newly developed electrochemical aluminium dosing method. Desalin Publ 217:195–202
5. Silva AM, Lima RMF, Leão VA (2012) Mine water treatment with limestone for sulfate removal. J Hazard Mater 221–222:45–55
6. Li J, Tabassum S (2022) Synergism of hydrolytic acidification and sulfate reducing bacteria for acid production and desulfurization in the anaerobic baffled reactor: high sulfate sewage wastewater treatment. Chem Eng J 444:136611
7. Hosseini SM, Usefi MMB, Habibi M, Parvizian F, Van der Bruggen B, Ahmadi A, Nemati M (2019) Fabrication of mixed matrix anion exchange membrane decorated with polyaniline nanoparticles to chloride and sulfate ions removal from water. Ionics 25:6135–6145
8. Ao H, Cao W, Hong Y, Wu J, Wei L (2020) Adsorption of sulfate ion from water by zirconium oxide-modified biochar derived from pomelo peel. Sci Total Environ 708:135092

9. Ittisupornrat S, Theepharaksapan S, Krasaesueb N, Phetrak A (2023) Impact of saline valorization in contaminated municipal wastewater on the treatment performance and bacterial community dynamics of a membrane bioreactor. J Chem Technol Biotechnol 98:633–641
10. van den Brand TPH, Roest K, Chen GH, Brdjanovic D, van Loosdrecht MCM (2015) Potential for beneficial application of sulfate reducing bacteria in sulfate containing domestic wastewater treatment. World J Microbiol Biotechnol 31:1675–1681
11. Linssen R, Slinkert T, Buisman CJN, Klok JBM, ter Heijne A (2023) Anaerobic sulphide removal by haloalkaline sulphide oxidising bacteria. Bioresour Technol 369:128435
12. Ahmar Siddiqui M, Biswal BK, Heynderickx PM, Kim J, Khanal SK, Chen G, Wu D (2022) Dynamic anaerobic membrane bioreactor coupled with sulfate reduction (SrDMBR) for saline wastewater treatment. Bioresour Technol 346:126447
13. Omil F, Méndez R, Lema JM (1995) Anaerobic treatment of saline wastewaters under high sulphide and ammonia content. Bioresour Technol 54:269–278
14. Zhang Y, Zhang L, Li L, Chen G-H, Jiang F (2018) A novel elemental sulfur reduction and sulfide oxidation integrated process for wastewater treatment and sulfur recycling. Chem Eng J 342:438–445
15. Liu S, Li H, Daigger GT, Huang J, Song G (2022) Material biosynthesis, mechanism regulation and resource recycling of biomass and high-value substances from wastewater treatment by photosynthetic bacteria: a review. Sci Total Environ 820:153200
16. Tang K, Baskaran V, Nemati M (2009) Bacteria of the sulphur cycle: an overview of microbiology, biokinetics and their role in petroleum and mining industries. Biochem Eng J 44:73–94
17. Deng X, Su C, Luo X, Liu X, Liang Z, Ding F, Yang W, Xu Y, Chen M (2022) Performance and metagenomic analysis of a micro-aerated anaerobic reactor for biological sulfate-rich wastewater treatment. J Environ Chem Eng 10:107769
18. Tan LC, Nancharaiah YV, Lu S, van Hullebusch ED, Gerlach R, Lens PNL (2018) Biological treatment of selenium-laden wastewater containing nitrate and sulfate in an upflow anaerobic sludge bed reactor at pH 5.0. Chemosphere 211:684–693
19. Lu H, Peng M, Zhang G, Song H, Yang A, Zhang Y (2020) Biokinetic and biotransformation of nitrogen during photosynthetic bacteria wastewater treatment. Environ Technol 41:1888–1895
20. Cao K, Zhi R, Zhang G (2020) Photosynthetic bacteria wastewater treatment with the production of value-added products: a review. Bioresour Technol 299:122648
21. Rice EW, Bridgewater L, Association APH (2012) Standard methods for the examination of water and wastewater. American Public Health Association Washington, DC
22. Shi B, Patel M, Yu D, Yan J, Li Z, Petriw D, Pruyn T, Smyth K, Passeport E, Miller RJD, Howe JY (2022) Automatic quantification and classification of microplastics in scanning electron micrographs via deep learning. Sci Total Environ 825:153903
23. Logan M, Tan LC, Nzeteu CO, Lens PNL (2023) Effect of selenate on treatment of glycerol containing wastewater in UASB reactors. Renew Energy 206:97–110
24. Nosalova L, Piknova M, Kolesarova M, Pristas P (2023) Cold sulfur springs—neglected niche for autotrophic sulfur-oxidizing bacteria. Microorganisms 11:1436
25. Lens PNL, Visser A, Janssen AJH, Hulshoff Pol LW, Lettinga G (1998) Biotechnological treatment of sulfate-rich wastewaters. Crit Rev Environ Sci Technol 28:41–88
26. Camiloti PR, Mockaitis G, Domingues Rodrigues JA, Rissato Zamariolli Damianovic MH, Foresti E, Zaiat M (2014) Innovative anaerobic bioreactor with fixed-structured bed (ABFSB) for simultaneous sulfate reduction and organic matter removal. J Chem Technol Biotechnol 89:1044–1050
27. Moon C, Singh R, Veeravalli SS, Shanmugam SR, Chaganti SR, Lalman JA, Heath DD (2015) Effect of COD:SO4 2–ratio HRT and linoleic acid concentration on mesophilic sulfate reduction: reactor performance and microbial population dynamics. Water 7(5):2275–2292
28. Velasco A, Ramírez M, Volke-Sepúlveda T, González-Sánchez A, Revah S (2008) Evaluation of feed COD/sulfate ratio as a control criterion for the biological hydrogen sulfide production and lead precipitation. J Hazard Mater 151:407–413
29. Lu X, Zhen G, Ni J, Hojo T, Kubota K, Li Y-Y (2016) Effect of influent COD/SO42− ratios on biodegradation behaviors of starch wastewater in an upflow anaerobic sludge blanket (UASB) reactor. Bioresour Technol 214:175–183
30. Ai T, Zou L, Cheng H, Luo Z, Al-Rekabi WS, Li H, Fu Q, He Q, Ai H (2022) The potential of electrotrophic denitrification coupled with sulfur recycle in MFC and its responses to COD/SO42− ratios. Chemosphere 287:132149
31. Hu Y, Jing Z, Sudo Y, Niu Q, Du J, Wu J, Li Y-Y (2015) Effect of influent COD/SO42− ratios on UASB treatment of a synthetic sulfate-containing wastewater. Chemosphere 130:24–33
32. Silva AFR, Magalhães NC, Cunha PVM, Amaral MCS, Koch K (2020) Influence of COD/SO42− ratio on vinasse treatment performance by two-stage anaerobic membrane bioreactor. J Environ Manag 259:110034
33. Kushkevych I, Kovářová A, Dordevic D, Gaine J, Kollar P, Vítězová M, Rittmann SKMR (2021) Distribution of sulfate-reducing bacteria in the environment: cryopreservation techniques and their potential storage application. Processes 9(10):1843. https://doi.org/10.3390/pr9101843
34. Dong Y, Wang J, Gao Z, Di J, Wang D, Guo X, Hu Z, Gao X, Wang Y (2023) Study on growth influencing factors and desulfurization performance of sulfate reducing bacteria based on the response surface methodology. ACS Omega 8:4046–4059
35. Kushkevych I, Dordević D, Vítězová M (2019) Analysis of pH dose-dependent growth of sulfate-reducing bacteria. Open Med 14:66–74
36. Wang F, Peng S, Fan L, Li Y (2022) Improved sulfate reduction efficiency of sulfate-reducing bacteria in sulfate-rich systems by acclimatization and multiple-grouting. Alex Eng J 61:9993–10005
37. Payandi-Rolland D, Shirokova LS, Nakhle P, Tesfa M, Abdou A, Causserand C, Lartiges B, Rols J-L, Guérin F, Bénézeth P, Pokrovsky OS (2020) Aerobic release and biodegradation of dissolved organic matter from frozen peat: effects of temperature and heterotrophic bacteria. Chem Geol 536:119448
38. Sun X, Wang Y, Li H, Zhou J, Han J, Wei C (2021) Changes in the volatile profile, fatty acid composition and oxidative stability of flaxseed oil during heating at different temperatures. LWT 151:112137
39. Egger F, Hülsen T, Tait S, Batstone DJ (2020) Autotrophic sulfide removal by mixed culture purple phototrophic bacteria. Water Res 182:115896
40. Qi X, Tian E, Ren Y, Wang X (2018) A weak infrared light strengthens anoxygenic photosynthetic bacteria activated sludge for the anaerobic biodegradation of polylactic acid in microbial fuel cell systems. Polym Degrad Stab 157:44–52
41. Rückert C (2016) Sulfate reduction in microorganisms—recent advances and biotechnological applications. Curr Opin Microbiol 33:140–146
42. San Martín J, Puyol D, Segura Y, Melero JA, Martínez F (2023) A novel photoanaerobic process as a feasible alternative to the traditional aerobic treatment of refinery wastewater. J Water Process ENG 51:103352
43. Jing Z, Hu Y, Niu Q, Liu Y, Li Y-Y, Wang XC (2013) UASB performance and electron competition between methane-producing archaea and sulfate-reducing bacteria in treating sulfate-rich

wastewater containing ethanol and acetate. Bioresour Technol 137:349–357
44. Zhang Y, Zhang L, Yu N, Guo B, Liu Y (2022) Enhancing the resistance to H2S toxicity during anaerobic digestion of low-strength wastewater through granular activated carbon (GAC) addition. J Hazard Mater 430:128473
45. Brzychczyk B, Hebda T, Fitas J, Giełżecki J (2020) The follow-up photobioreactor illumination system for the cultivation of photosynthetic microorganisms. Energies 13(5):1143. https://doi.org/10.3390/en13051143
46. Giraud E, Lavergne J, Verméglio A (2010) Chapter 9—characterization of bacteriophytochromes from photosynthetic bacteria: histidine kinase signaling triggered by light and redox sensing. Method Enzymol Acad Press. https://doi.org/10.1016/S0076-6879(10)71009-0
47. Paepatung N, Boonapatcharoen N, Songkasiri W, Yasui H, Phalakornkule C (2022) Recovery of anaerobic system treating sulfate-rich wastewater using zero-valent iron. Chem Eng J 435:135175
48. Huang J, Zeng C, Luo H, Bai J, Liu G, Zhang R (2022) Enhanced sulfur recovery and sulfate reduction using single-chamber bio-electrochemical system. Sci Total Environ 823:153789
49. Asasian-Kolur N, Sharifian S, Kavand M, Kaghazchi T (2019) Batch and fixed-bed mode mercury uptake by a modified adsorbent. Chem Eng Commun 208:60–71
50. Liu Z, Wang J, Zhang T, Zhou S, Yan K (2019) The effects of microbial fuel cells coupled with solar cells under intermittent illumination on sediment remediation. Environ Sci Process Impact 21(12):2141–2149. https://doi.org/10.1039/C9EM00380K
51. Yong MA, Yongzhen P, Shuying W (2008) Applying the sludge blanket height in the secondary clarifier to control sludge recycle flow rate of A/O process. China Environ Sci 28:121–125
52. Al-Dawery SK, Ahmed A, Reddy SS, Al-Hadhrami O, Al-Lamki K (2020) Cost-effective treatment of sludge conditioning using supernatant fluid polyelectrolyte. Environ Sci Pollut Res 27:10790–10801
53. Chanaka Udayanga WD, Veksha A, Giannis A, Lisak G, Lim T-T (2019) Effects of sewage sludge organic and inorganic constituents on the properties of pyrolysis products. Energy Convers Manag 196:1410–1419
54. Masuda Y, Ito M, Murakami M (2020) Degradation of unprotected aldohexonic acids to aldopentoses promoted by light and oxygen. Chem Lett 49:1309–1311
55. Liu Y, Wen M, Wu L, Cao S, Li Y (2022) Environmental behavior and remediation methods of roxarsone. Appl Sci 12(15):7591