Effect of pH Shock on the Treatment of High Concentration Organic Wastewater *via* a Fe^0/GO-Anaerobic System

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The pH is one of the key factors affecting microbial activity in anaerobic systems. In this paper, the pH impact tolerance of Fe^0/GO (zero-valent iron/graphene oxide) mediated anaerobic treatment system for high concentration organic wastewater was studied. The effects of a Fe^0/GO mediated anaerobic system on wastewater treatment, degradation kinetics, and the physicochemical properties of sludge were studied at pHs of 5.5 and 8.5; the separate addition of Fe^0 and GO and a blank system were used as the blank control. The results showed that the pH had adverse effects on the treatment of each system and the physicochemical characteristics of sludge. However, the Fe^0/GO system under pH shock maintained a relatively high COD_{Cr} removal rate and gas production; the effluent volatile fatty acid content was the lowest, the effluent pH value deviation from the normal range was small, the degradation rate constant, and sludge concentration and flocculation performance of the mixed liquid were better than those of other systems. The recovery phase of Fe^0/GO returned to normal in a relatively short time. These results showed that adding Fe^0/GO to the anaerobic treatment of high concentration organic wastewater system can drastically improve the pH shock resistance of the system.

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INTRODUCTION

Anaerobic biotechnology has the following characteristics: a low energy demand, large potential for renewable energy *via* biogas production, low amount of surplus sludge, and low operating costs, so it is widely used in various high concentration organic wastewater treatments (Appels *et al.* 2008; Chan *et al.* 2009; Poh and Chong 2009). Under anaerobic conditions, the organic matter in wastewater is transformed into biogas to purify wastewater under the co-metabolism of bacteria. However, microorganisms under anaerobic conditions have strict requirements in terms of environmental conditions (especially methanogens). When the external environment fluctuates, the microbial activity will be inhibited, and the wastewater treatment efficiency will be considerably lowered. Therefore, it has become a hot research topic to improve microbial activity to resist adverse effects when environmental conditions fluctuate. It was found that the combined addition of iron series and carbon series enhanced the anaerobic fermentation system and achieved good research results (Zhang *et al.* 2020).

The pH is a key factor affecting anaerobic biological treatment technology. The optimal pH range of different microorganisms is different, and the sensitivity of different microorganisms to pH is different. In the process of wastewater treatment by anaerobic fermentation system, the pH value is constantly changing, and the fluctuation range of system pH value is related to wastewater quality. The cost of wastewater treatment is related to the frequency of pH regulation. The adverse effects of improper pH on the growth and reproduction of microorganisms are predominantly manifested in the following aspects: first, the decrease of pH will induce positive and negative charges on the surface of microorganisms, and then it will alter the absorption of nutrients by microorganisms; secondly, too high or too low pH value will induce ionization of organic compounds, which indirectly affects microorganisms; last but not least, an unsuitable pH reduces enzyme activity, which in turn affects biochemical processes within microbial cells. Therefore, it is of great research value to reduce the influence of system pH fluctuation on anaerobic fermentation bacterial community and to ensure the stable operation of anaerobic fermentation system, which can reduce the cost of anaerobic fermentation system to treat wastewater. Braguglia *et al.* (2017) found that the fermentation bacteria could remain active in a pH range of 4 to 8.5, while the optimal pH for methanogens was 6.5 to 7.2. Lu *et al.* (2020) found that the concentration, composition, intermediate products, and metabolic state of volatile fatty acids (VFAs) produced *via* the anaerobic digestion of potato peel waste were different under different pH conditions. In addition, the activities of acetate kinase and butyrate kinase were slightly inhibited at a pH of 5.0 and a pH of 11.0, which resulted in relatively low production of VFAs. It was found that adding ferrous materials and carbonaceous materials can stimulate the growth and reproduction of microorganisms, enhance the activity of related enzymes, and maintain high microbial activity even when the pH is beyond the appropriate range (Liu *et al.* 2012; Teng *et al.* 2017). This alleviates the adverse effects caused by pH fluctuation and provides the system with a certain amount of pH shock tolerance.

A cheap and green reducing agent, zero-valent iron (Fe^0) is converted into Fe^{2+} in an anaerobic system, which does the following: supply pollutant electrons, accelerate the degradation of organic substances, reduce the oxidation reduction potential (ORP), promote electron transfer between species, and create more favorable conditions for anaerobic digestion (Liu *et al.* 2012). In the development of microbial fuel cell sensors, Jia *et al.* (2017) used Fe^0 to promote acetic acid conversion and inter-species electron transfer to delay excessive acidification and reduce the impact of excessive acidification on sensor performance, thus making the system more resistant to pH shocks. At the same time, Fe^0 is also one of the essential micronutrient elements of methanogens and an indispensable component of the prosthetic group of methanogens. Fe^0 can increase the enzyme activity of anaerobic microorganisms, reduce the inhibitory effect of pH shock on an enzyme, and considerably increase the methane production in the anaerobic system (Wu *et al.* 2015). Kong *et al.* (2016) put forward the idea of adding Fe^0 to an anaerobic digestion system to inhibit the over acidification of food waste. During the experiment, it was found that the pH of the reactor without Fe^0 ranged between 5.2 and 5.4, while the pH of the reactor with Fe^0 was maintained between 7.5 and 8.0. These results showed that Fe^0 can effectively inhibit the excessive acidification of the anaerobic digestion process and enhance the ability of the system to resist pH shock. However, Fe^0 can easily accumulate at the bottom of the reactor and therefore is not fully effective. As such, fixing Fe^0 to the supporting medium can prevent it from gathering together and losing its effectiveness (Stefaniuk *et al.*

2016). Carbon materials with large specific surface areas, *e.g.*, activated carbon, carbon nanotubes, mesoporous carbon, and graphene, have been proposed as carriers (Teng *et al.* 2017). Graphene oxide (GO), as a two-dimensional monolayer with abundant functional groups (epoxy, hydroxyl, and carboxyl), is a very promising carrier (Perreault *et al.* 2015). Many oxygen-containing functional groups, *e.g.*, ·OH, -O-, C=O, and more abundant hydrophilic groups, are introduced on its surface. These hydrophilic groups provide a large number of active sites for the connection of various organic molecules, macromolecules, and biomolecules, improving the possibility of GO surface functionalization and contributing to its dispersion in various solutions (Konios *et al.* 2014; Smith *et al.* 2019). At the same time, due to its large specific surface area, layered structure, and good conductivity, GO can effectively adsorb pollutants and promote interspecific electron transfer (DIET) as well as effectively reduce the impact of pH fluctuation on an anaerobic system and improve the methane production in an anaerobic system (Fan *et al.* 2018). Consequently, it is hypothesized here that the addition of Fe^0 and GO in the wastewater treatment process can upgrade the enzyme activity of anaerobic microorganisms and strengthen the pH tolerance of the key enzymes of anaerobic fermentation, that is, sustain a high activity in the non-optimal pH range (6.7 to 7.5). It is expected that Fe^0, particularly the complex combined with GO, can give full play to their respective advantages in the anaerobic fermentation system, make up for each other, enhance the pH shock tolerance of the system, and make the system more secure and more effective in eliminating pollutants.

This paper studied the effect of a Fe^0/GO anaerobic system on treating high-concentration organic wastewater under pH shock conditions in order to explore the growth and reproduction of microorganisms under different pH conditions. In addition, this study explored the impact resistance of an Fe^0/GO anaerobic system under pH fluctuations and provided a basis for the practical application of the new anaerobic system.

EXPERIMENTAL

Wastewater and Inoculated Sludge

Simulated citric acid wastewater was used in the experiments. The chemical oxygen demand based on $K_2Cr_2O_7$testing (COD_{Cr}) was approximately 8000 mg/L, and the pH was 4.0 to 5.0. Ammonium sulfate ($(NH_4)_2SO_4$) and potassium dihydrogen phosphate (KH_2PO_4) were added as the nitrogen and phosphorus sources with a COD_{Cr}:N:P weight ratio of 200 to 5 to 1. The inoculated sludge was obtained from an upflow anaerobic sludge bed (UASB) reactor from a brewery in Qingdao, China.

Experiment Methods

Preparation of the Fe^0/GO

Graphene oxide was prepared according to a modified Hummer's method (Chen *et al.* 2013). Fe^0 and GO at a mass ratio of 5 to 1 (1.0 g of Fe^0 and 0.2 g of GO) were placed in a 100 mL beaker, 20 mL of deionized water was added, and ultrasonic treatment was conducted for 20 min under nitrogen protection. Then, after ultrasound treatment, the material was put into a vacuum drying oven and dried at a temperature of 105 °C to get the Fe^0/GO composite. The amount of composite combined with deionized water was 0.06 g. The activity of the prepared Fe^0/GO composite was stable for at least 6 months, and at the end of use, the Fe^0/GO composite were easily recovered with a magnet. A scanning electron microscopy (SEM) image of the prepared Fe^0/GO material is shown in Fig. 1. The

GO sheets were coated with a large number of small particles. The particles were evenly dispersed, and agglomeration was not obvious, which indicated that GO and Fe^0 were fully and evenly compounded. The average particle size of the iron powder used was 37.4 μm. The BET surface areas of the Fe^0, GO, and the Fe^0/GO composite are shown in Table 1. Compared with Fe^0 and GO, the BET surface area of the Fe^0/GO composite was greatly increased, which was conducive to the adsorption of organic matter in the system and provided growth sites for microorganisms. This promoted the growth and reproduction of microorganisms and improved the microorganism activity, thus enhancing the wastewater treatment effects.

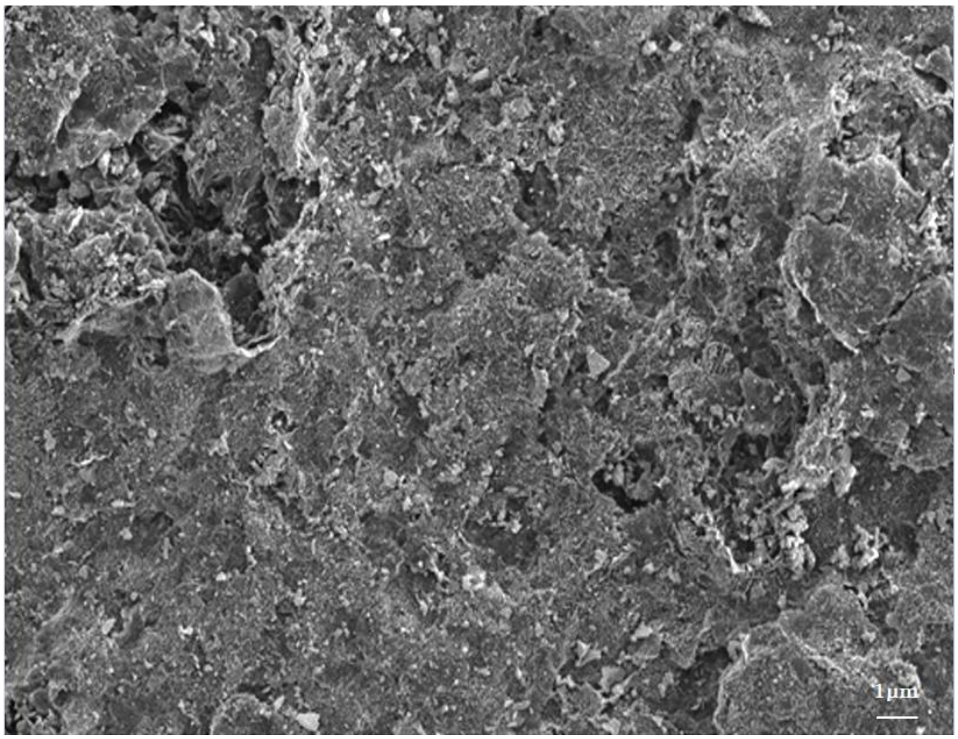

Fig. 1. SEM image of the Fe^0/GO composite

Table 1. The BET Surface Areas

Materials	BET Surface Areas (m^2/g)
Fe^0	1.85
GO	4.89
Fe^0/GO composite	16.72

Four 500 mL anaerobic reactors were taken, and 200 mL of acclimated anaerobic activated sludge was added into each reactor. The four reactors were labeled as follows: the blank group; GO group; Fe^0 group; and Fe^0/GO group. Then, 0.2g/L of GO, 0.1g/L of Fe^0, and 0.12g/L of the Fe^0/GO composite were added into the GO group, Fe^0 group, and Fe^0/GO group, respectively, in addition to the 200 mL of experimental influent.

Four anaerobic reactors were placed in a constant temperature oscillator at a temperature of 37 °C. The optimal pH value for the growth and metabolism of anaerobic fermentation bacteria is between 6.7 and 7.5 (Zhang *et al.* 2020). In actual production operation, the pH value of the anaerobic system will not be lower than 5.5, and some bacteria will lose their removal capability when it is higher than 8.5. Therefore, the low pH value and high pH value were set at 5.5 and 8.5 in the pH shock experiment. The pH value of the four reactors was set at 5.5, the treatment period was 12 h, and the effluent pH, gas production and COD_{Cr} removal rate of the four reactors were measured in each cycle. At the end of the seventh cycle, PN and PS, VFAs, MLSS were determined. Then the shaking table pH value was restored to 7.2, and the above steps were repeated for 7 cycles. After the experiment, set the four reactors pH value to 8.5 and repeat the above steps.

Analysis Methods

The pH values were determined using a pH meter (PHS-3C/501, INESA Scientific Instrument Co. Ltd., Shanghai, China) with composite electrodes. The COD_{Cr} concentrations were determined using a COD analyzer (DR1010, HACH, Loveland, CO). The produced gas volumes were measured *via* the drainage method. The mixed liquor suspended solid (MLSS) levels were measured according to the national standard method (National EPA 2002). The dilute sulphuric acid method was used to extract extracellular polymers (Adav and Lee 2008). The phenol-sulfuric acid method was used to quantify the polysaccharides (PS) using glucose as the standard (Dubois *et al.* 1956). The protein (PN) contents were determined with a modified Lowry method using bovine serum albumin as the standard (Frolund *et al.* 1996). The VFAs (volatile fatty acids) were determined using a GC2014C gas chromatograph (Shimadzu, Kyoto, Japan) with an ID detector and a DB-FFAP capillary column with a specification of 30 mm ID × 0.32 mm ID × 0.25 μm. The capillary column flow rate was adjusted to 75 mL/min, with 2 μm for each injection, and a shunt injection at a ratio of 5 to 1. In addition, the temperature of the inlet, detector, and cylinder were set as 220 °C, 230 °C, and 100 °C, respectively. The temperature of the 6 °C/min program rose to a temperature of 130 °C and remained at this temperature for 1 min, then rose to a temperature of 190 °C at a rate of 10 ℃/min and stayed constant for 2 min.

RESULTS AND DISCUSSION

The Effect of pH Shock on Anaerobic System Performance

When the system was operated under optimal conditions of pH 6.7 to 7.5 and temperature 37℃, the average gas production of the blank, GO, Fe^0, and Fe^0/GO reactors was 488, 496, 505, and 511 mL, respectively. Then the four groups of reactors were subjected to a pH 5.5 and 8.5 shock treatment. The resulting gas production values of the reactors are shown in Fig. 2. When the influent was in the shock stage of pH 5.5, the gas production of blank group, GO group, Fe^0 group, and Fe^0/GO group was 260, 280, 320, and 345 mL, respectively. Gas production increased to 375, 395, 410, and 450 mL, respectively, when influent pH was restored to the optimal range of 6.7 to 7.5. In contrast, the gas production of the four groups reached 250, 275, 290, and 310 mL, respectively, when the influent was in the shock stage of pH8.5, and the gas production increased to 360, 390, 400, and 415 mL when the pH returned to the optimal range of 6.7 to 7.5. By analyzing the data shown in Figs. 2 and 3, it was found that the gas production of the Fe^0 and Fe^0/GO reactors was higher than the gas production of the blank group and GO group. As shown in Figs. 2A and 2B, the gas production treatment effect of the Fe^0 and Fe^0/GO reactors under a pH shock of 5.5 was better than the gas production under a pH shock of 8.5. In addition, the recovery of system gas production after pH 5.5 shock is better than that after pH 8.5 shock. By analyzing the results, it was found that when the pH shock was 5.5, the effluent pH of the system was stable, between 6.7 and 7.5 (as shown in Fig. 3A). At this time, the methanogens were at an optimal pH range and had high activity. By contrast, at a pH shock of 8.5, the water pH was greater than 7.5 (as shown in Fig. 3B), and the methanogen activity for the four groups of reactors was suppressed. It was found that the addition of Fe^0 increased the concentration of Fe^{2+} and Fe^{3+} in the system, thus increasing the methane production rate (Hu *et al.* 2015). Moreover, Fe^{3+} has also been shown to accelerate the electron transfer rate by promoting the direct interspecific electron transfer of anaerobic microorganisms in a fermentation system, thus promoting methane production (Li *et al.* 2015). However, this may also be due to the reaction of Fe^0 with water to produce H_2 and OH^-, as shown in Eq. 1,

$$\mathrm{Fe^0} + \mathrm{2H_2O} \rightarrow \mathrm{Fe^{2+}} + H_2 + \mathrm{2OH^-} \quad (1)$$

This process increased the pH of the Fe^0 and Fe^0/GO anaerobic digestion systems, maintaining the pH of the system within a range of 6.7 to 7.5 for the methanogens, and thus promoting an increase in gas production in the anaerobic system (Ma *et al.* 2018). This explains the high gas production of the Fe^0 and Fe^0/GO reactors, and why the gas production under a pH shock of 5.5 was better than a pH shock of 8.5.

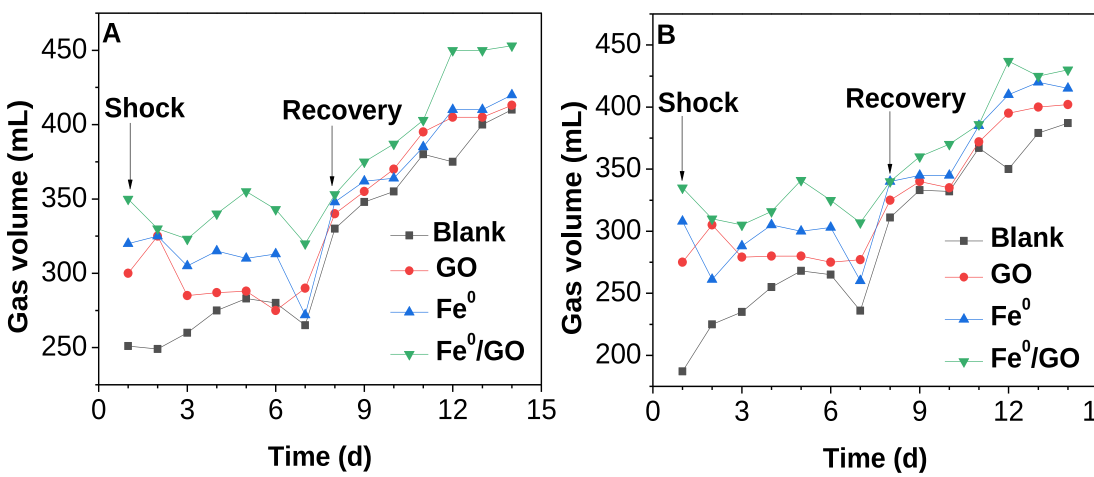

Fig. 2. Effect of the pH shock on the gas volume: A) pH = 5.5; and B) pH = 8.5

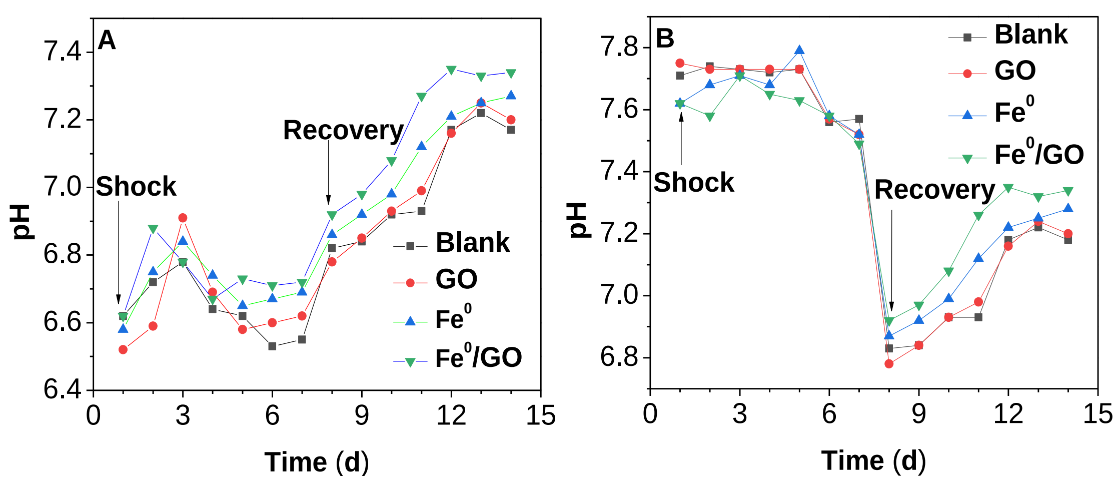

Fig. 3. Effect of the pH shock on the pH of the effluent: A) pH = 5.5; and B) pH = 8.5

When the system was operated under optimal conditions of pH 6.7 to 7.5 and temperature 37 °C, the COD_{Cr} removal rates of the blank, GO, Fe^0, and Fe^0/GO reactors were 82.7%, 85.8%, 88.9%, and 91.8%, respectively, which basically remained above 80%. The COD_{Cr} removal rate in the Fe^0/GO group was the highest, followed by the Fe^0 group and the blank group. The COD_{Cr} removal rates of the four reactors after they experienced pH 5.5 and 8.5 shock are shown in Fig 4. The COD_{Cr} removal rates of the blank, GO, Fe^0, and Fe^0/GO groups were stable at 28.8%, 32.5%, 48.3%, and 49.6%, respectively, under a pH shock of 5.5. After the pH of the reactor returned to the optimal range of 6.7 to 7.5, the COD_{Cr} removal rates were 71.2%, 75.8%, 79.9%, and 82.5%, respectively. When the influent pH was 8.5, the COD_{Cr} removal rate was stable at 35.2%, 37.5%, 40.5%, and 42.3%, respectively. The COD_{Cr} removal rates of the reactor were 69.8%, 74.6%, 77.8%, and 80.5%, respectively, after the reactor returned to the optimal range of 6.7 to 7.5. The COD_{Cr} removal rate of the Fe^0 and Fe^0/GO reactors under a pH shock of 5.5 was higher than the COD_{Cr} removal rate under a pH shock of 8.5, and the recovery of the COD_{Cr} removal rate after an acid shock was also better than the recovery after an alkaline shock, which was consistent with the results of the gas production of the system.

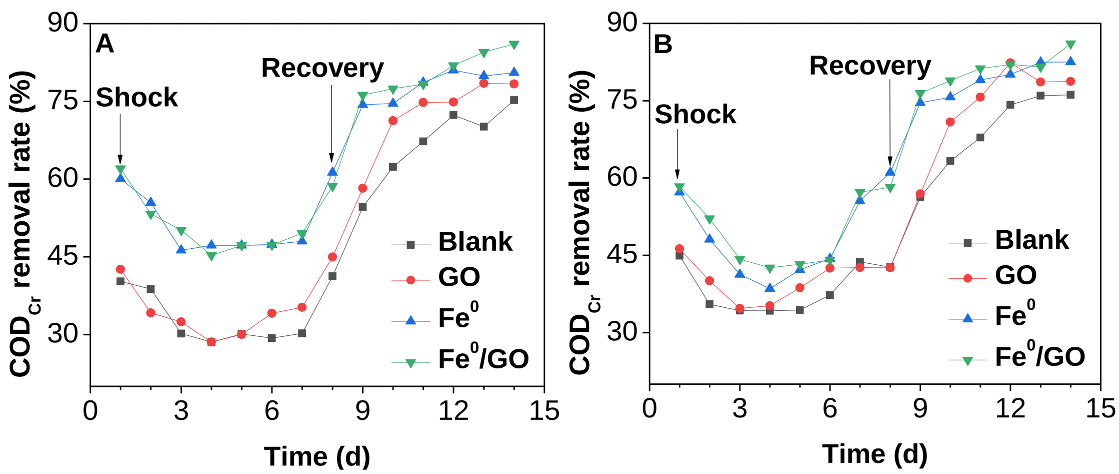

Fig. 4. Effect of the pH shock on the COD_{Cr} removal rate: A) pH = 5.5; and B) pH = 8.5

When the four groups of reactors were subjected to pH 5.5 and 8.5 shock, the removal rate of the COD_{Cr} and gas production in the Fe^0/GO group were the highest, and the gas production and COD_{Cr} removal rate in the Fe^0 group were higher than those in the GO group, which indicated that the promotion effect of Fe^0 on the COD_{Cr} removal rate and the gas production was better than the promotion effect of Fe^0 in the GO group. Although GO has a certain adsorption effect on pollutants in wastewater, it also has a certain inhibition effect on the anaerobic methane production process. Studies have shown that in the anaerobic digestion process of sludge, the activity of corresponding enzymes in methanogens (such as coenzyme F_{420}) will be reduced as the GO concentration increases, which seriously inhibits methane production (Dong *et al.* 2018). The addition of Fe^0 weakened the GO inhibitory effect on the anaerobic microbes, promoted the sludge anaerobic digestion process, and considerably improved methane production (Wei *et al.* 2018). However, it also can effectively remove H_2S, stimulate the anaerobic acidification process and the key enzyme of the methanogenesis process, and effectively reduce the oxidation reduction potential (ORP), so as to provide a comfortable environment for anaerobic digestion (Baniamerian *et al.* 2019). Under pH shock, the Fe^0/GO reactor had the best operating effect, which was the result of the combined action of Fe^0 and GO. The functional groups on the GO surface provided abundant contact sites for Fe^0, which made it more evenly dispersed throughout the system, thus promoting the electron transfer rate of the anaerobic system and making the reaction more sufficient (Ren *et al.* 2018).

Figure 5A and 5B show plots based on the kinetics equations of the COD_{Cr} degradation of the four reactors under pH 5.5 and pH 8.5 conditions, respectively.

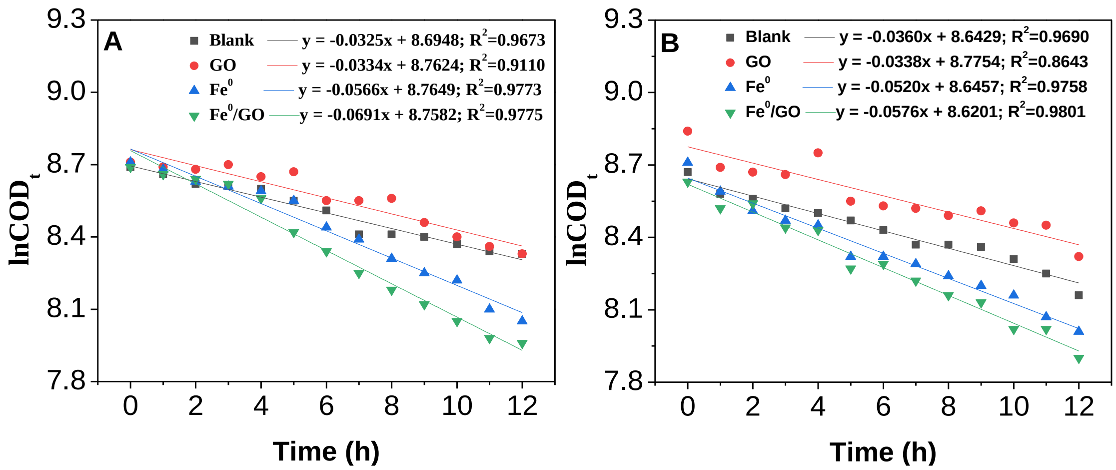

Fig. 5. Fitted curve of the first order kinetics: A) pH = 5.5; and B) pH = 8.5

Table 2. Effect of pH Shock on Degradation Kinetics Equations

	pH Shock Value	
	5.5	8.5
Blank group	$-\frac{dCOD}{dt} = 0.0325COD$	$-\frac{dCOD}{dt} = 0.0360COD$
GO group	$-\frac{dCOD}{dt} = 0.0334COD$	$-\frac{dCOD}{dt} = 0.0338COD$
Fe^0 group	$-\frac{dCOD}{dt} = 0.0566COD$	$-\frac{dCOD}{dt} = 0.0520COD$
Fe^0/GO group	$-\frac{dCOD}{dt} = 0.0691COD$	$-\frac{dCOD}{dt} = 0.0576COD$

By comparing the degradation kinetics equations of each system, it was found that the degradation rate constant (k) of the Fe^0/GO system was the highest for both the pH 5.5 impact and pH 8.5 impact (k = 0.0691 h^{-1} at a pH 5.5 impact and k = 0.0576 h^{-1} at a pH 8.5 impact). In addition, the degradation kinetics of the four groups of reactors was better than the degradation kinetics of the four groups under a pH 5.5 impact. This was consistent with the trend of the COD_{Cr} removal rate and the gas production.

The comprehensive analysis of Figs. 2 through 5 showed that when the system was shocked by pH 5.5 and 8.5, the gas production and COD_{Cr} removal rate of the four reactor systems decreased, and the effluent pH fluctuated. The treatment effect of the reactor adding Fe^0/ Go was obviously better than the treatment effect of the other three reactors. When the pH of the reactor was restored to the optimal range of 6.7 to 7.5, the effluent pH of the four systems gradually recovered to a stable state, while the gas production and COD_{Cr} removal rate of the Fe^0/GO group increased rapidly and tended to be stable faster.

This data and the degradation rate constant of the kinetic equation all indicated that the addition of $Fe^0$and Go made the system more tolerant to pH shock, and the Fe^0/GO reactor had the strongest resistance to pH shock.

The Effect of pH Shock on the Effluent VFAs

The influence of pH shock on VFAs in system effluent is shown in Fig 6.

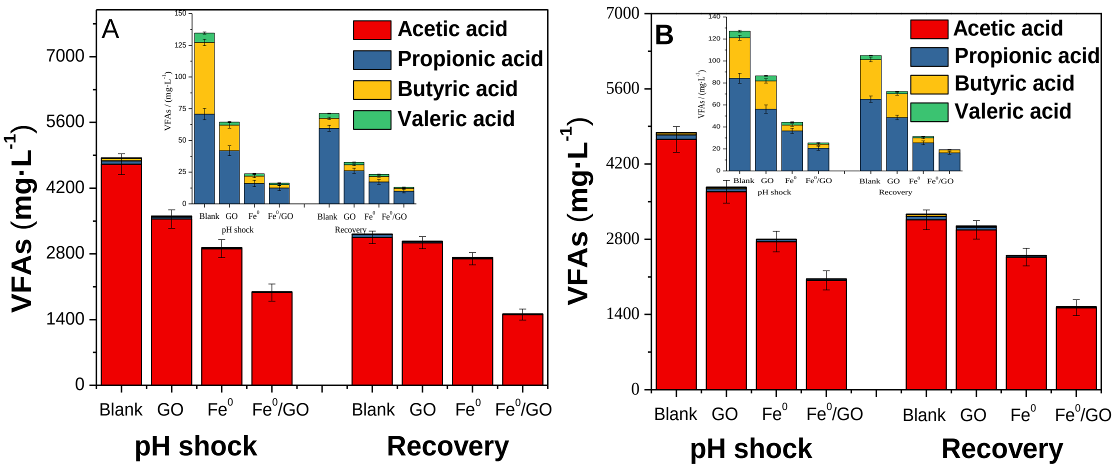

Fig. 6. The effect of pH shock on VFAs: A) pH = 5.5; and B) pH = 8.5

In an anaerobic system, the fermentation type of the microorganisms plays an important role in the process of methane production. Through the determination of the VFAs in the effluent of each system, the influence of Fe^0 and GO on the anaerobic system was studied. There was little difference in the acetic acid content in the same reactor after a pH 5.5 and pH 8.5 shock as well as after recovery. However, the content of the VFAs in each group was considerably different. After the pH shock, the acetic acid content in the effluent of the blank group, GO group, Fe^0 group, and Fe^0/GO group were approximately 4700, 3500, 2850, and 1900 mg·L^{-1}, respectively. After the pH returned to the optimal range of 6.7 to 7.5, the acetic acid content in the effluent of the blank group, GO group, Fe^0 group and Fe^0/GO group were 3000, 2900, 2600, and 1400 mg·L^{-1}, respectively. The acetic acid content in each system always maintained at a high level (greater than 90%), which indicated that the acetic acid type climax community was always the dominant acid production and fermentation climax community in the anaerobic system. By comparing the propionic acid and butyric acid contained in the systems, it was found that under pH shock conditions, the propionic acid content in the Fe^0 and Fe^0/GO groups was considerably lower than the propionic acid content in the blank and GO groups, which may be because the addition of Fe^0 promoted the transformation of propionic acid. Meng *et al.* (2013) found in their study that adding iron powder to an anaerobic digestion system can reduce the free energy required for propionic acid decomposition, increase the activity of enzymes related to acetic acid production, and improve the conversion efficiency of propionic acid to acetic acid. At the same time, the addition of Fe^0 increased the number and diversity of the microbial community, especially the bacteria responsible for propionic acid transformation. Ye *et al.* (2021) found that iron was an important component of some coenzymes in anaerobic bacteria.

During the stage of hydrolytic acidification, the activities of dehydrogenase, acetic kinase, phosphotransacetylase, and butyrate kinase were increased by 47.8% to 88.1% with the addition of Fe^0, which was conducive to the conversion of propionate and butyrate to acetate. Therefore, a large amount of acetic acid produced *via* hydrolytic acidification can be rapidly utilized by methanogens. It can be seen from Fig. 6 that the Fe^0 group was better than the GO group in promoting acetic acid decomposition, and the effluent VFAs content of the Fe^0/GO group was much lower than the effluent VFAs content of the other three groups after pH shock and after recovery. This was primarily due to the addition of Fe^0 and GO, which promoted the synthesis of key enzymes in the process of methane production as well as improved enzyme activity, enabled the anaerobic system to maintain enzyme function under the impact of pH, sped up the conversion of VFAs to methane, and reduced the accumulation of VFAs in the system. At the same time, Fe^0 promoted the transformation of methanogenic dominant bacteria from *Methanothrix* to *Methanosarcina thermophila* (its acetic acid utilization rate was 3 to 5 times higher than *Methanothrix*) in the anaerobic reactor, thus accelerating the utilization of acetic acid (Wei *et al.* 2018). These two effects accelerated the conversion of VFAs to methane in the anaerobic system, while the addition of GO made the Fe^0 dispersion more uniform, which further improved the treatment effect of the Fe^0/GO system on high concentration organic wastewater. Combined with various effluent indicators, the changes in the VFAs content in each system further verified that the system showed a stronger impact tolerance to pH when Fe^0 and GO are added.

Influence of pH Shock on System Mixed Liquor Suspended Solid (MLSS)

The impact of pH 5.5 and 8.5 shock on the MLSS of the system is shown in Fig 7. The charge on the surface of the microorganisms changed as the four reactors were subjected to pH shock, which affected the activity of the enzymes of the microorganisms, which in turn affected the growth of the organisms. After a pH shock of 5.5, the blank, GO, Fe^0, and Fe^0/GO systems was reduced to 7.40, 7.41, 7.80, and 7.85 $g \cdot L^{-1}$; after a pH shock of 8.5, the MLSS was reduced to 7.30, 7.33, 7.70, and 7.75 $g \cdot L^{-1}$. The microbial activity gradually increased while the pH of the inlet water returned to the optimal range of 6.7 to 7.5. At the same time, MLSS concentration in Fe^0 and Fe^0/GO groups increased to slightly higher than that in blank group and GO group, the MLSS in the Fe^0/GO group changed the most. After recovery, the MLSS increased by 0.38 $g \cdot L^{-1}$ (pH 5.5) and 0.29 $g \cdot L^{-1}$ (pH 8.5) compared with the shock. These results were consistent with the COD_{Cr} removal rate and gas production results. The MLSS value of the Fe^0/GO group in the pH shock phase and the recovery phase was the highest among the four reactors, which indicated that the growth and reproduction of the microorganisms in this reactor was the best, which was consistent with the COD_{Cr} removal rate and production of the reactor with Fe^0/GO. This is because iron plays an important role in the metabolic mechanism of a variety of anaerobic microorganisms, which can accelerate the growth and reproduction of anaerobic microorganisms while promoting the conversion of complex organic matter to biogas, and thus shows the growth of MLSS (Yekta *et al.* 2014). However, under the action of Fe^0, a large number of anaerobic microorganisms took advantage of the large specific surface area of GO to enrich and grow, so that anaerobic activated sludge can maintain a dense structure under pH impact and improve the sludge floc and microbial population structure to a certain extent (Liang *et al.* 2017). These results indicated that the Fe^0/GO reactor had strong impact resistance. Under the impact of pH, the degree of damage to the system and the degree of sludge loss were lower than other reactors and the MLSS in the system was higher. In addition, the pollutant degradation effect was better. Therefore, adding Fe^0/GO to the reactor can enhance the anti-pH shock ability of anaerobic microorganisms.

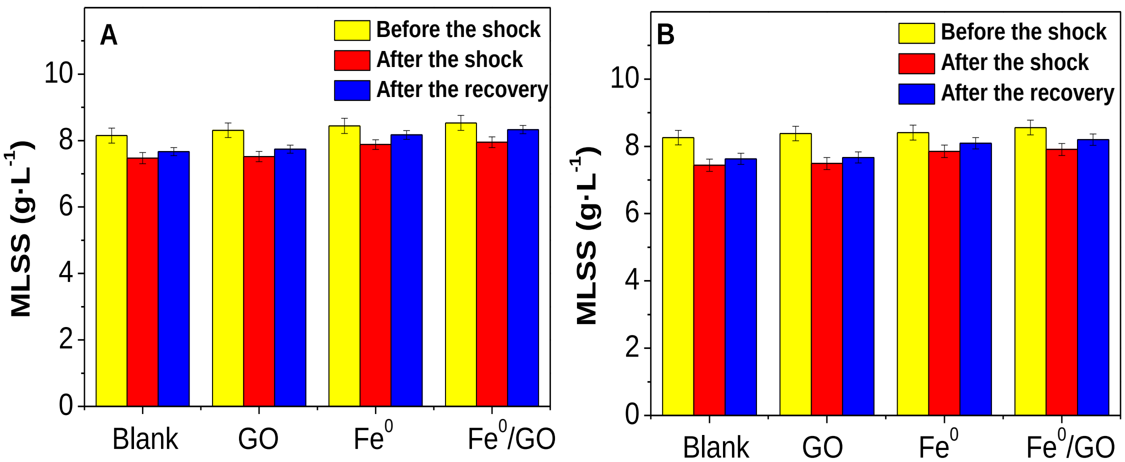

Fig. 7. The effect of pH shock on the MLSS: A) pH = 5.5; and B) pH = 8.5

The Effect of pH Shock on the Extracellular Polymers

The effect of pH 5.5 and 8.5 shock on the extracellular polymers is shown in Fig. 8. Extracellular polymeric substances (EPS) contain three-dimensional, gel-like, highly hydrated, charged characteristics. As the skeleton of microbial aggregates, EPS can considerably promote the aggregation of microorganisms and maintain the stability of microbial aggregates (Zhang *et al.* 2019). Extracellular polymeric substances consist of proteins (PN), polysaccharides (PS), nucleic acids, and humic acid (HA) (Desmond *et al.* 2018). The flocculation performance of sludge usually is reflected by the content and ratio of PN and PS. Proteins can maintain the integrity and stability of the sludge through the three-dimensional structure formed by bridges with cations because the binding capacity of PN and cations is greater than the binding capacity of PS. Therefore, the higher the PN content, the stronger the sludge flocculation ability, which is beneficial to the separation of sludge and water (Campo *et al.* 2018). As shown in Fig 8, at the system was subjected to pH 5.5 and 8.5 shock and recovery to the optimal range of 6.7 to 7.5, the PN and PS contents in the sludge extracellular polymer of each reactor under different pH shock conditions were not much different. However, the PN/PS values of the blank, GO, Fe^0, and Fe^0/GO groups were quite different during the pH5.5 and 8.5 shock and after recovery. Whether during the pH 5.5 and 8.5 shock or after the system recovery, the PN/PS value of the Fe^0/GO system was considerably higher than the other three systems, which indicated the addition of Fe^0 promoted the growth of PN in the EPS, thereby improving the flocculation performance of sludge. The Fe^0/GO group took full advantage of Fe^0 and GO, making the PN/PS value considerably higher than the other three groups. Zhang *et al.* (2018) found that the addition of Fe^0 increased the concentration of Fe^{3+} in the system. Fe^{3+} acts as a chelating agent to promote the production of EPS and contributes to an increase in the PN content in EPS. At the same time, Fe^{3+} also promotes PS secretions from microorganisms. Therefore, the addition of Fe^0 and GO is beneficial for promoting the increase of the PN and PS content of the sludge, enhancing the anti-pH shock ability of the system, and reducing the negative impact caused by the pH shock. Thus, the integrity and stability of the sludge are maintained, the efficiency of sludge-water separation increases, and the wastewater treatment effect is improved.

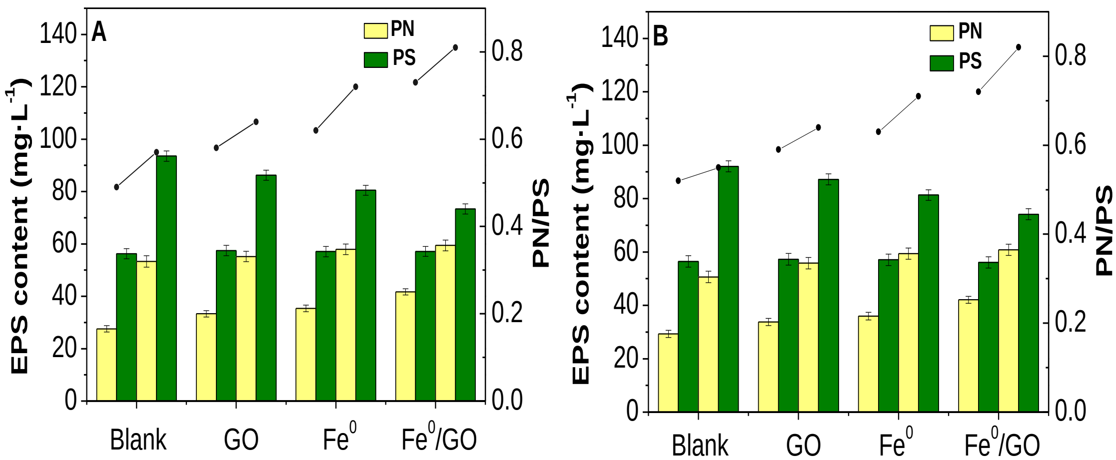

Fig. 8. The effect of pH shock on the EPS: A) pH = 5.5; and B) pH = 8.5 (Note: the bar chart shows the content of the EPS, while the line chart shows the PN/PS; S represents the PN, PS, and PN/PS content after the pH shock; and R represents the content of the PN, PS, and PN/PS content after the pH recovery.)

CONCLUSIONS

1. In the impact experiment, the reactor with Fe^0 was less affected by pH shock. Compared with the reactor without Fe^0, the reactor with Fe^0 had higher gas production, higher COD_{Cr} removal rate and lower effluent VFAs content. Nevertheless, the promotion effect of GO was not evident in the pH impact test.
2. In the recovery experiment, the gas production and COD_{Cr} removal rate of the Fe^0/GO reactor were higher than the gas production and COD_{Cr} removal rate of the other three reactors; the effluent VFAs content was the lowest, and the recovery time was shorter.
3. Through the analysis of the physical and chemical characteristics of anaerobic activated sludge, it was found that the increase rate of MLSS and PN/PS value in Fe^0/GO group were better than the other three groups in both pH shock stage and recovery stage, which indicated that after a pH impact, the synergy of Fe^0 and GO ensured the anaerobic system had relatively good flocculation performance and greater recoverability of the system.

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Competing Interests

The authors declare that they have no conflict of interest.

Author Contribution Statement

Yecheng Lin and Huixia Lan conceived and designed the research. Yecheng Lin and Xiangzhi Wang conducted the experiments. Da Yang contributed new reagents or analytical tools. Yecheng Lin and Longyu Wang analyzed the data. Yecheng Lin wrote the manuscript. All authors read and approved the manuscript.

Consent to Participate

All authors consented to participate in the manuscript.

Consent to Publish

All authors consented to publish this manuscript.

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