

## Effect of COD/SO<sub>4</sub><sup>2-</sup> ratio and alkalinity on COD removal and sulfate reduction in two-phase expanded granular sludge bed

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### Abstract

In the present study, a bench-scale two-phase expanded granular sludge bed (EGSB) reactor was applied for treatment of high-sulfate wastewater. The reactor was operated continuously at 35°C. The EGSB1, as sulfate-reducing phase, and EGSB2, as methane-producing phase, were combined after successful start-up. The treatment characteristics, including the effect of influent COD/ SO<sub>4</sub><sup>2-</sup> ratio and alkalinity in reactor have been discussed for EGSB1 and EGSB2 respectively. When total COD removal was 85%, the influent COD/SO<sub>4</sub><sup>2-</sup> ratio was 2.8. When the ratio was less than 2.8, the COD removal descended quickly. The total sulfate removal then raised to 90% and the ratio exceeded 4.4. When influent alkalinity was kept at 8-10 mmol l<sup>-1</sup>, the total COD and SO<sub>4</sub><sup>2-</sup> removal reached 93.3% and 94.0% respectively. The results presented in the study provide some useful information for optimization of COD removal and SO<sub>4</sub><sup>2-</sup> reduction process in wastewater treatment.

### Key words

Alkalinity, COD/SO<sub>4</sub><sup>2-</sup>, Granule sludge, High-sulfate wastewater, Two-phase anaerobic digestion process

### Introduction

The success of new high-rate anaerobic technology has encouraged environmental scientists to extend its application to treat wastewater of more complex nature. Sulfate is a common constituent of many industrial wastewaters and sulfate reduction may cause several problems in the anaerobic treatment process (Hilton and Archer, 1988). Anaerobic digestion of organic wastes is a complex process involving hydrolysis, fermentative acidogenesis and methanogenesis. Due to high organic waste content, single-phase anaerobic digestion of kitchen waste easily leads to a subsequent accumulation of intermediate

products with a resultant fall in pH, those give rise to unbalanced fermentation, diminishing the stability of the process (O.lnce, 1998). A two-phase anaerobic digestion could optimize the conditions for hydrolytic acidogenic group of bacteria, as well as for the acetogenic-methanogenic group and enhance the stabilization of organics and gasification rates (Yilmazer *et al.*, 2002; Banks and Wang, 1999; Bhattacharya *et al.*, 1996; Qi *et al.*, 2003). Different strategies have been proposed to control the substrate competition inhibition and sulfide toxicity, including sulfide precipitation using iron salts; pH control; use of anaerobic filter in down-flow mode (Speece, 1996). Sulfate reduction in acidogenic phase has also been reported

in a two-phase anaerobic digestion process treating distillery molasses slop effluent (Reis *et al.*, 1988). These reactors provide conditions for operation at high cell retention times even at low hydraulic retention times (Zaiat *et al.*, 1987). For un-acidified wastewater, a two-phase anaerobic treatment process has a number of advantages, as optimum environmental conditions are provided for acid-forming and methane-forming microorganisms (Pohland and Ghosh, 1971; Demirel and Yenigun, 2002).

When sulfate is present in the wastewater, sulfate reduction occurs at the acidification stage. Therefore, sulfide can be removed prior in the methanogenic reactor in a two-phase anaerobic treatment process, resulting in higher methanogenic activities (no sulfide inhibition) and a less (sulfide) contaminated biogas from the methanogenic reactor.

The influent COD/SO<sub>4</sub><sup>2-</sup> ratio and alkalinity is an important parameter affecting the competition between sulfate reducing bacteria (SRB) and other anaerobic bacteria involved in anaerobic wastewater treatment, namely fermentative bacteria for monogenic starting compounds (e.g. sugars or amino acids), syntrophs for intermediate fermentation products (e.g. propionate, butyrate or ethanol), homoacetogens for H<sub>2</sub> and methanogens for H<sub>2</sub> or acetate (Mizuno *et al.*, 1998).

The effect of COD/SO<sub>4</sub><sup>2-</sup> ratio on the performance of an anaerobic baffled reactor (ABR) was investigated. The results of the study showed that when COD/SO<sub>4</sub><sup>2-</sup> ratio was changed with increasing sulfate concentration from 180 to 500 mg l<sup>-1</sup>, COD conversion did not decrease, but a slight increase in COD removal was observed (Vossoughi *et al.*, 2003). Prasad *et al.* (1988) observed that methanogenic bacteria prevailed over sulfate reducing bacteria for COD/SO<sub>4</sub><sup>2-</sup> ratio around one.

The aim of the present study was to study the performance of two-phase anaerobic digestion process by two-phase expanded granular sludge bed (EGSB) reactor. The main objective was to investigate the start-up, effect of alkalinity and COD/SO<sub>4</sub><sup>2-</sup> ratio on the removal of COD and SO<sub>4</sub><sup>2-</sup> in order to provide some useful information for the optimization of COD removal and SO<sub>4</sub><sup>2-</sup> reducing processes.

## Materials and Methods

**Operation of two-phase anaerobic reactor :** A schematic diagram of the pilot experimental apparatus for high-sulfate wastewater treatment is illustrated in Fig. 1.

The EGSB1 reactor was made up of a plexiglass column with an internal diameter of 11cm and an overall height of 100 cm. The total volume was 9.5 l. The column consisted of three parts: bottom, middle and top. The bottom part of the column with a height of 12 cm was influent distribution area. The middle part served as the main biochemical reaction section, with a height of 61 cm. The top part, with a height of 27cm, played the role of a gas-liquid-solid separator, which allows the separation of biogas and washed-out solids from the liquid phase.

The structure and operation form of EGSB2 was similar to EGSB1 with an internal diameter of 11cm and an overall height of 195 cm. The total volume was 20.5l (excluding head space) with height-per-diameter ratio of 1:5. An inverted funnel shaped gas separator was used to measure the volume of the biogas produced. Thirteen sampling ports were placed at suitable distances along the height of the column and the effluent was collected from the top of the reactor. The EGSB2 reactor was operated under mesophilic condition (35°C ) and its temperature was maintained by circulating hot water through the reactor jacket.

**Synthetic wastewater :** The synthetic substrate was prepared daily and the contents of the feed tank were continuously stirred with mechanical agitator. The COD and sulfate concentration were added according to the requirement of experiment. The substrate was continuously injected into the reactor using a peristaltic pump. The synthetic substrate contains glucose, carbamide and KH<sub>2</sub>PO<sub>4</sub> as sole carbon. Nitrogen and phosphorus source were added to yield a COD/N/P ratio of 200:5:1. Na<sub>2</sub>SO<sub>4</sub> was used as sulfate source. Salaratus (NaHCO<sub>3</sub>) was used to adjust the pH of influent. The influent also contained yeast leaching ointment and micronutrients having the following reagents (Mizuno *et al.*, 1998): MgCl<sub>2</sub>·6H<sub>2</sub>O, 125 mg l<sup>-1</sup>; FeSO<sub>4</sub>·7H<sub>2</sub>O, 25 mg l<sup>-1</sup>; FeCl<sub>2</sub>·4H<sub>2</sub>O, 180 mg l<sup>-1</sup>; CoCl<sub>2</sub>·6H<sub>2</sub>O, 2.5 mg l<sup>-1</sup>; MnCl<sub>2</sub>·4H<sub>2</sub>O, 2.5 mg l<sup>-1</sup>; KI, 2.5 mg l<sup>-1</sup>; H<sub>3</sub>BO<sub>4</sub>, 0.5 mg l<sup>-1</sup>; NiCl<sub>2</sub>·6H<sub>2</sub>O, 0.5 mg l<sup>-1</sup>; ZnCl<sub>2</sub>, 0.5 mg l<sup>-1</sup>.

**Experimental design:** Initially, keeping hydraulic residence time (HRT) constant, the organic loading rate (OLR) and sulfate loading rate (SLR) was enhanced by changing the influent substrate concentration. After the adjustment of the influent substrate concentrations, the reactors were operated under this condition until the effluent quality became stable.

The EGSB1 as sulfate-reducing phase were inoculated with anaerobic digested sludge obtained from

Hangzhou Starpro Starch Co., Ltd. The SS and VSS concentration of the sludge was  $41.2 \text{ g l}^{-1}$  and  $19.8 \text{ g l}^{-1}$  respectively and the value of VSS/SS was 0.48. The amount of inoculated sludge was approximately 45% volume of the reaction part. The inoculum was acclimatized to the synthetic substrate containing glucose as a the sole carbon source for 90 days in an anaerobic continuous culture. A two-phase EGSB for simultaneous COD and sulfate removal was operated for more than six months. As the different microorganisms were characteristic of sulfate-reducing phase and methane-producing phase, the EGSB1 and EGSB2 were combined after successful start-up.

**Analytical methods :** Samples were collected and subjected to the analysis of the following parameters: influent and effluent of COD, influent and effluent of  $\text{SO}_4^{2-}$ , effluent TA, effluent alkalinity, VFA and reactor pH. The analyses were carried out following the standard protocol of APHA (2012). Biogas production was measured daily by a wet-test gas meter and making necessary correction for atmospheric pressure and temperature by standard temperature and pressure (STP).

Methane content was determined by GC2010A gas chromatography (Shimadzu, Japan) with a stainless steel column (300cm X 0.3 cm) packed with activated carbon (30-60mesh) using thermal conductivity detection (TCD). Volatile fatty acids (VFA) were analyzed by HP 5890 series II gas chromatograph (HP, USA) equipped with a flame ionization detector (FID).

## Results and Discussion

**Inoculum and start-up; Acidogenic reactor; COD and  $\text{SO}_4^{2-}$ :** During the start-up stage (Fig. 2,3,4), HRT of the reactors was kept for 8 hrs, glucose was the sole carbon source and electron donor in the synthetic wastewater. At first, OLR and SLR was  $21 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$  and  $4.5 \text{ kg}/(\text{m}^3 \cdot \text{d})$  respectively, the influent was at high concentration (approximately  $\text{COD}=7000 \text{ mg l}^{-1}$ ,  $\text{SO}_4^{2-}=1500 \text{ mg l}^{-1}$ ). It was found that the sludge in the EGSB1 expanded badly and washed out at the top of the EGSB1 reactor and the effluent SS was high *i.e.* up to  $300\text{--}400 \text{ mg l}^{-1}$ . So, the influent COD concentration was decreased to  $3000 \text{ mg l}^{-1}$ , with no sulfate till 10 days (*i.e.* from day 4 to 14). From day 4 to 11, the SS of the effluent decreased from  $420$  to  $65 \text{ mg l}^{-1}$ . It was not observed that sludge was washed out from the experiments since the effluent SS concentrations were lower than  $60 \text{ mg l}^{-1}$ . Small bubbles were found attached to the sludge, and the interface between sludge and wastewater was clear.

From 15<sup>th</sup> day, the influent contained sulfate of  $500 \text{ mg l}^{-1}$ . The COD removal was not affected by the low strength of sulfate. The intermediate products from the degradation of glucose by acidogenic bacteria (AB) could be utilized by AB and SRB (Flaherty *et al.*, 1998).

On 40<sup>th</sup> day, COD and sulfate removal were measured 93% and 61% respectively. Increased sulfate concentration lead to more COD removal. The evidences indicate that the sludge had gained its anaerobic activity. In the course of substrate competition for anaerobic bacteria in the bioreactors, MPB mainly used acetate and  $\text{H}_2/\text{CO}_2$  as substrates. However, when the increase in COD and sulfate concentration increased from  $4000 \text{ mg l}^{-1}$  to  $1000 \text{ mg l}^{-1}$  on day 41, the OLR and SLR were measured  $12 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$  and  $3 \text{ kg}/(\text{m}^3 \cdot \text{d})$  respectively. COD removal decreased to 80% and sulfate removal was about 38%. Fig. 9 shows daily biogas, methane and percentage during start-up in EGSB2. On day 50, the COD and sulfate removal was 70% and 57%, respectively. Between 51<sup>th</sup>-70<sup>th</sup> day, when COD and sulfate concentrations increased it raised to  $5500 \text{ mg l}^{-1}$  and  $1500 \text{ mg l}^{-1}$ , the OLR and SLR were measured  $16.5 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$  and  $4.5 \text{ kg}/(\text{m}^3 \cdot \text{d})$ . The COD and sulfate removal in the effluent were 40% and 75%, respectively. When the anaerobic system contained high concentration of  $\text{SO}_4^{2-}$  and lacked preferential substrate, SRB competed against MPB for acetate and  $\text{H}_2$  (Henze and Harremoës, 1983). Between 71-90 day, the COD and sulfate concentration increased from  $7000 \text{ mg l}^{-1}$  to  $2000 \text{ mg l}^{-1}$  respectively. The OLR and SLR were  $21 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$  and  $6 \text{ kg}/(\text{m}^3 \cdot \text{d})$ . The COD and sulfate removal in the effluent were 24% and 92% respectively. When  $\text{COD}/\text{SO}_4^{2-}$  was 3.5, and glucose was used as organic substrate, SRB and AB were found to compete for the intermediate. Also, SRB and MPB were found to compete for acetate and  $\text{H}_2$  which were generated by organic acidification. A substrate chain of co-metabolism was formed among SRB, AB and MPB. As a result, the sludge was acclimated and gradually became the population of sulfidogens. As a matter of fact a new balance was obtained.

**Biogas and methane :** The biogas was composed of hydrogen, methane, carbon dioxide and hydrogen sulfide. Between 1<sup>th</sup>-90<sup>th</sup> day (Fig.5), the percentage of methane enhanced from 20% to 44%. The biogas production rate decreased with increase in sulfate concentration. The production of free- $\text{H}_2\text{S}$  as a strong inhibitor of MPB was facilitated by the equation ( $\text{H}_2\text{S} \rightleftharpoons \text{H}^+ + \text{HS}^-$ ). The acidogens and SRB were less sensitive to free- $\text{H}_2\text{S}$  as compared with MPB.

**Methane reactor; COD and  $\text{SO}_4^{2-}$  :** At the same time, the EGSB2 as methane-producing phase was started (Figs. 6,7,8). Between 1-10, the COD concentration was  $1000 \text{ mg l}^{-1}$ , the OLR was  $1.4 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$ . At first, COD removal was 32%. By day 10, COD removal was increased up to 60%. Between day 11-25, the COD concentration increased up to  $2000 \text{ mg l}^{-1}$ , the OLR was  $2.8 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$ . At 11<sup>th</sup> day, COD removal was up to 45%. By day 25, COD removal was increased up to 60%. Between 26-40, COD concentration was increased up to  $2500 \text{ mg l}^{-1}$ , the OLR was  $3.5 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$ . At 26<sup>th</sup> day, COD removal was 63%. By day 40, the COD removal was increased up to 68%. Between 41-55 day, the COD concentration was increased up to  $3000 \text{ mg l}^{-1}$ , the OLR was  $4.2 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$ . On day 41, the COD removal was up to 69%. By day 55, the COD removal was increased up to 91%. Between 56-60, the COD concentration was increased up to  $3500 \text{ mg l}^{-1}$ , the OLR was  $4.9 \text{ kg COD}/(\text{m}^3 \cdot \text{d})$ . On 56<sup>th</sup> day, the COD removal was up to 92%. By 60<sup>th</sup> day, the COD removal was increased up to 95%.

From 61<sup>th</sup> day, the EGSB1 and EGSB2 were combined. The COD concentration was gradually increased from  $2750 \text{ mg l}^{-1}$  to  $5320 \text{ mg l}^{-1}$ . The sulfate concentration was gradually decreased from  $570 \text{ mg l}^{-1}$  to  $160 \text{ mg l}^{-1}$ . At the end of the start-up, the COD and sulfate removal were 94% and 92%, respectively.

**Biogas and methane :** Fig. 9 illustrates biogas and methane production during the start-up in EGSB2. On comparing, it was found that the gas production rate underwent change when the concentration of COD and sulfate was raised. Between 1-60<sup>th</sup> day, the COD concentration raised from  $1000$  to  $3500 \text{ mg l}^{-1}$ , the effluent COD was consistently less than  $100 \text{ mg l}^{-1}$ . At day 60, the average COD removal was kept as high as 90%. When COD removal was at a high level, the proportion of COD transformed into methane was accordingly bigas, which resulted in high gas production rate.

Between 61-90<sup>th</sup> day, the effluent of EGSB1 containing COD and  $\text{SO}_4^{2-}$  were fed into EGSB2; the methane percentage of biogas was lower. The SRB competed with MPB for acetate and hydrogen which were the most important methane precursors.

**The overall performance of two-phase system :** From 61<sup>th</sup> day, EGSB1 and EGSB2 were combined when the EGSB1 and EGSB2 reactors were in good condition. The influent COD and  $\text{SO}_4^{2-}$  were about  $7000 \text{ mg l}^{-1}$  and  $2000 \text{ mg l}^{-1}$ . The

HRT of EGSB1 and EGSB2 were 8.0 and 17.3h. At last 30 days operation, the total removal of COD and sulfate were kept as high as about 90% and 94% respectively. The start-up of the process was successful.

**Effect of COD/ $\text{SO}_4^{2-}$  ratio :** In this case, the HRT of the EGSB1 and EGSB2 were 8.0 and 17.3, respectively. During this period the influent sulfate concentration was about  $2000 \text{ mg l}^{-1}$  and SLR of the influent was about  $6.0 \text{ kg}/(\text{m}^3 \cdot \text{d})$ .

Keeping other operational parameters at constant level, by varying COD concentration of the influent, the effect of different COD/ $\text{SO}_4^{2-}$  ratios were studied from 0.5 to 6.0. The reactor had 7-14 days operation for each ratio. The main monitoring parameters observed during the eight periods of the two-phase anaerobic digestion process are summarized in Table 3.

The minimum COD/ $\text{SO}_4^{2-}$  ratio was 0.67, if sulfate was reduced completely (Lens *et al.*, 1998). From Fig. 10 it is evident that the total COD removal efficiency steadily raised when COD/ $\text{SO}_4^{2-}$  ratio exceeded 2.8. The COD removal of the EGSB2 and the total process was up to 75% and 85% respectively, and were slightly affected by COD/ $\text{SO}_4^{2-}$  ratio. Inhibition was not observed and the EGSB1 COD removal was approximately 40%, which was chiefly used for sulfate reduction. This behavior can be attributed to the presence of numerous SRB, which can utilize organic carbon for new cell metabolite. Stoichiometrically, 1200 mg COD is required for reducing 1800 mg sulfate (Maree *et al.*, 1987).

For sulfate removal (Fig.5), when COD/ $\text{SO}_4^{2-}$  ratio was raised to 4.4, the sulfate removal of EGSB1 and EGSB2 were raised up to 80% and 50% respectively, and were steady at high level. The total sulfate removal was up to 90%. Nevertheless, when COD/ $\text{SO}_4^{2-}$  ratio was less than 4.4, the sulfate removal of two reactors were decreased noticeably with a drop in COD/ $\text{SO}_4^{2-}$ . The total sulfate removal was less than 75% at COD/ $\text{SO}_4^{2-}$  ratio of 3.6. Comparing Figs.10 and 11, it can be concluded that COD and sulfate removal had different demand of influent COD/ $\text{SO}_4^{2-}$  ratios by two-phase anaerobic digestion process. When total COD removal was raised to 85%, the COD/ $\text{SO}_4^{2-}$  ratio was only required up to 2.8; however, total sulfate removal up to 90% required the ratio exceeding 4.4.

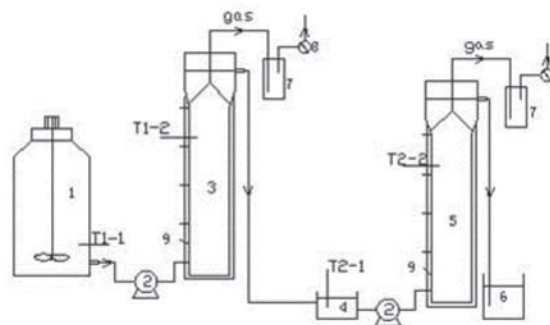
**Effect of alkalinity :** During these periods, the influent COD and sulfate concentration were about  $5600 \text{ mg l}^{-1}$  and  $2000$

mg l<sup>-1</sup> and the COD/SO<sub>4</sub><sup>2-</sup> ratio was 2.8. In EGSB1 and EGSB2, alkalinity and pH condition were monitored and controlled during the experiment. Sulfate reduction directly reduces salinity and protons, producing alkalinity in the form of sulfide (Maree *et al.*, 1987). Alkalinity is expressed as CaCO<sub>3</sub> and was obtained by multiplying the amount of sulfate removal by the factor 100/96 (mass ratio of CaCO<sub>3</sub> to SO<sub>4</sub><sup>2-</sup>) (Ristow and Hansford, 2001). The effluent alkalinity of EGSB1 and EGSB2 were increased with influent alkalinity as illustrated in Fig 6, where Y=0.931 X + 24.70 and Y= 1.058 X + 18.47.

From the illustration in Fig.12, it was found that the effluent alkalinity underwent changes opposite to VFA. The VFA was consumed for neutralizing the influent alkalinity that was generated by acidogenic bacteria (AB) metabolizing organic substrate and SRB reducing sulfate while alkalinity was generated during the process when VFA was oxidized to CO<sub>2</sub> by acetotrophic sulfate reducing bacteria (ASRB) and SO<sub>4</sub><sup>2-</sup> was reduced to HS<sup>-</sup> (Richardl, 2000). Alkalinity generation and consumption, keep the balance so that anaerobic system is in good condition for wastewater.

Fig. 12 explains that with the increase in influent alkalinity from 1 to 30 mmol l<sup>-1</sup>, the VFA (as acetic acid) declined from 30.1 to 19.5 mmol l<sup>-1</sup> in EGSB1. In the reactor, the anaerobic system might eventually lead to acidity failure as VFA concentration was at a high level. The alkalinity and VFA kept the balance in reactors to maintain the treatment process operating smoothly. However, it was steady at 3.0 to 4.8 mmol l<sup>-1</sup> in EGSB2. Performance improvement verified the acidogenic bacteria produce VFA which can be utilized by the acidogenic and methanogenic bacteria and contacted by alkalinity.

When influent alkalinity was changed from 1 to 30 mmol l<sup>-1</sup> and the influent pH was maintained from 6.8 to 8.9, the effluent pH of EGSB1 increased from 5.4 to 6.6, but the effluent pH of EGSB2 was comparatively stable, varying from 7.3 to 7.8 (Fig. 13). However, as influent alkalinity exceeded 8-10 mmol l<sup>-1</sup>, the effluent pH in EGSB2 reactors was comparatively stable which was suitable for methanogenesis. The system had sufficient alkalinity to neutralize organic acids coming from the influent, hydrolysis and fermentation stage. When influent alkalinity was 8-10 mmol l<sup>-1</sup>, the pH effluent in EGSB1 changed about 6.2, which was advantageous for the system.



**Fig. 1 :** Schematic diagram of two-phase anaerobic reactor. 1-influent tank; 2-influent pump; 3-EGSB1 (sulfate reduction reactor); 4-storage vessel; 5-EGSB2 (methanogenic reactor); 6-effluent tank; 7-water seal; 8-wet gas meter; 9-jacket heating controllers; T1-1,T1-2,T1-3,T2-1,T2-2,T2-3: temperature probe.

**Table 1 :** Operation conditions of EGSB1 during the start-up

Date	COD <sub>in</sub> (mg l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	Flow rate (l d <sup>-1</sup> )	HRT (hr)	OLR (kg COD/ (m <sup>3</sup> ·d))	SLR (kg/ (m <sup>3</sup> ·d))
1-3	7000	1500	28.5	8	21	4.5
4-14	3000	0	28.5	8	6	0
15-40	3000	500	28.5	8	9	1.5
41-50	4000	1000	28.5	8	12	3
51-70	5500	1500	28.5	8	16.5	4.5
71-90	7000	2000	28.5	8	21	6

**Table 2 :** Operational conditions of the EGSB2 during the start-up

Date (d)	COD <sub>in</sub> (mg l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> (mg l <sup>-1</sup> )	Flow rate (l d <sup>-1</sup> )	HRT (hr)	OLR (kg COD/ (m <sup>3</sup> ·d))	SLR (kg/ (m <sup>3</sup> ·d))
1-10	1000	0	28.5	17.3	1.4	0
11-25	2000	0	28.5	17.3	2.8	0
26-40	2500	0	28.5	17.3	3.5	0
41-55	3000	0	28.5	17.3	4.2	0
56-60	3500	0	28.5	17.3	4.9	0
61-90	According to EGSB1		28.5	17.3	According to EGSB1	

Note : From the day 61, The EGSB1 and EGSB2 were combined.

**Table 3 :** Main date of the two-phase anaerobic digestion process

Time (d)	COD/SO <sub>4</sub> <sup>2-</sup>	COD <sub>in</sub> (mg l <sup>-1</sup> )	COD <sub>out</sub> (mg l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> <sub>in</sub> (mg l <sup>-1</sup> )	SO <sub>4</sub> <sup>2-</sup> <sub>out</sub> (mg l <sup>-1</sup> )
1~7	0.5	1000	250	2000	1400
15~22	1.2	2400	528	2000	1100
23~31	2	4000	840	2000	940
32~41	2.8	5600	840	2000	600
42~54	3.6	7200	1080	2000	500
55~65	4.4	8800	1056	2000	200
66~80	5.2	10400	1040	2000	140
81~88	6	12000	1440	2000	100

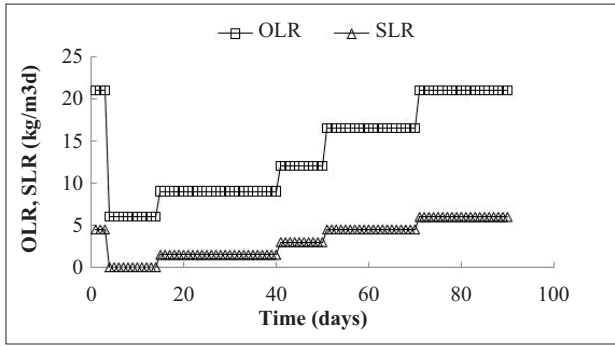


Fig. 2 : OLR and SLR during the start-up stage in EGSB1

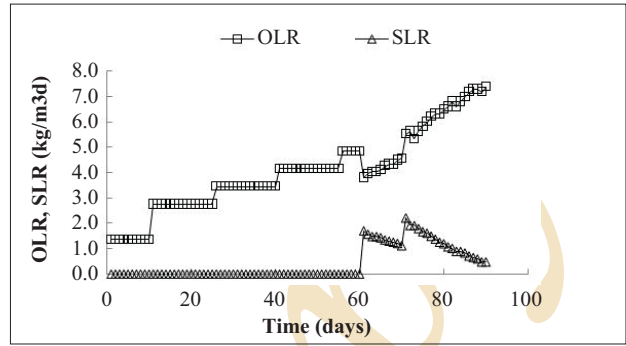


Fig. 6 : OLR and SLR during the start-up in EGSB2

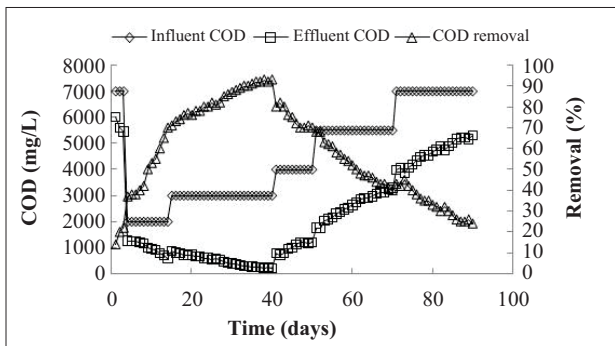


Fig. 3 : COD and COD removal during the start-up stage in EGSB1

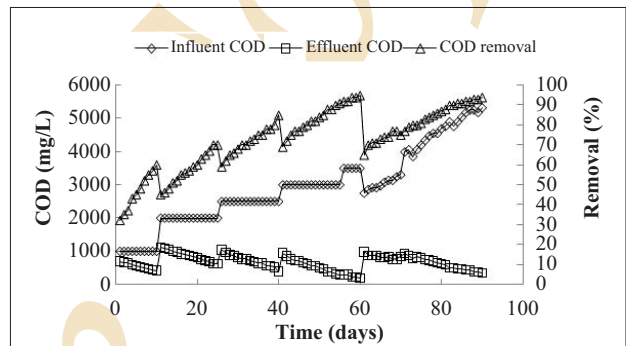


Fig. 7 : COD and COD removal during the start-up stage in EGSB2

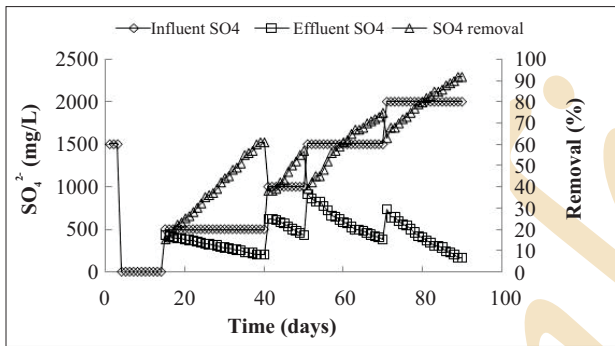


Fig. 4 :  $SO_4^{2-}$  and  $SO_4^{2-}$  removal during the start-up stage in EGSB1

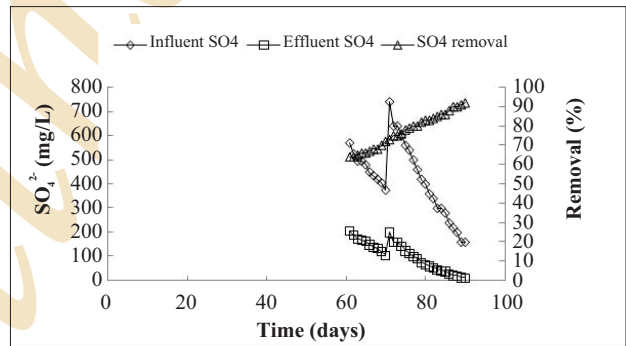


Fig. 8 :  $SO_4^{2-}$  and  $SO_4^{2-}$  removal during the start-up in EGSB2

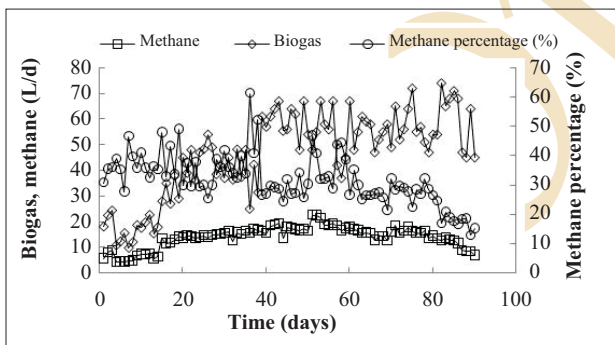


Fig. 5 : Biogas, methane and percentage during the start-up in EGSB1

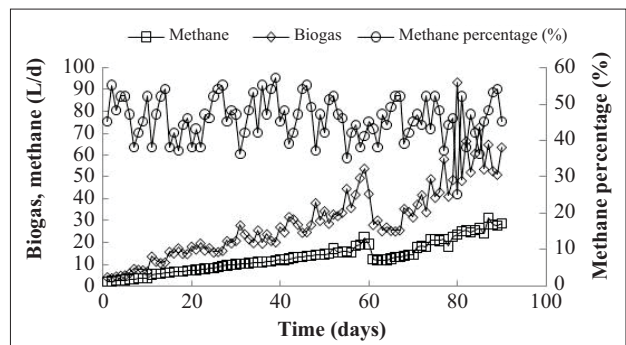


Fig. 9 : Biogas, methane and percentage during the start-up in EGSB2

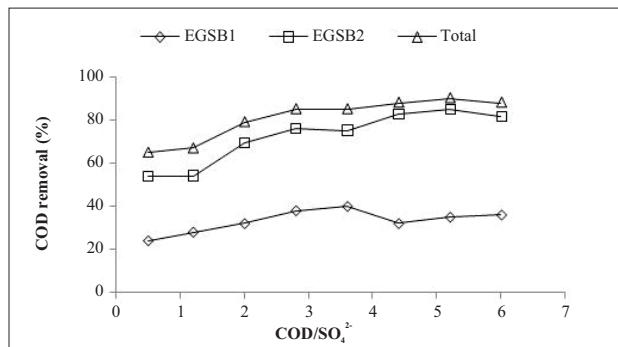


Fig. 10 : Effect of COD/SO<sub>4</sub><sup>2-</sup> on COD removal

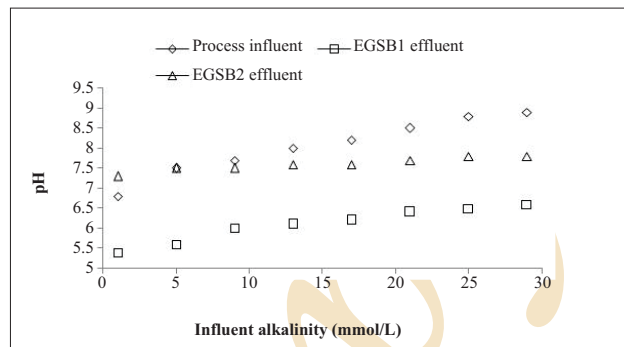


Fig. 13 : Effect of influent alkalinity on pH

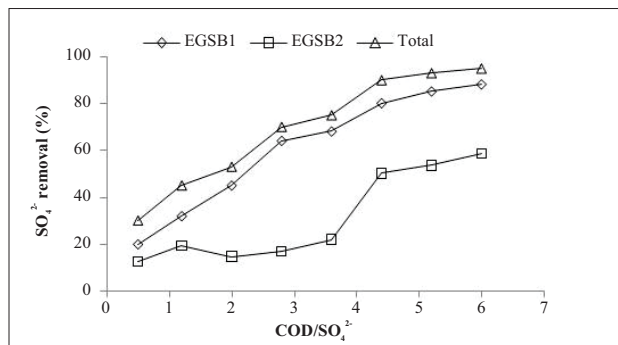


Fig. 11 : Effect of COD/SO<sub>4</sub><sup>2-</sup> on sulfate removal

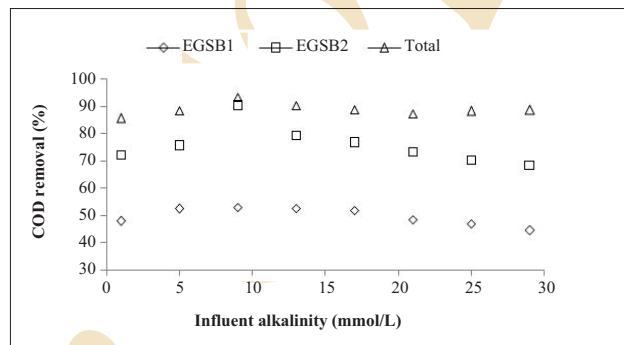


Fig. 14 : Effect of influent alkalinity on COD removal in reactor

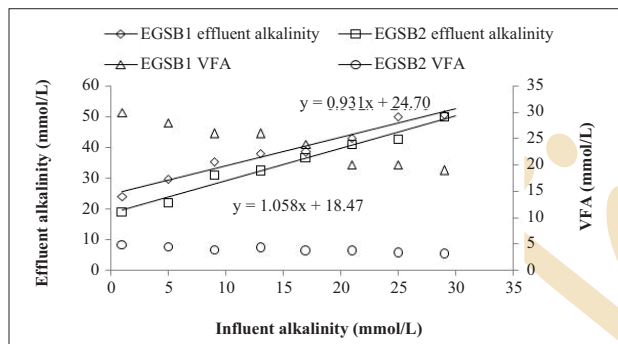


Fig. 12 : Effect of influent alkalinity on effluent and VFA

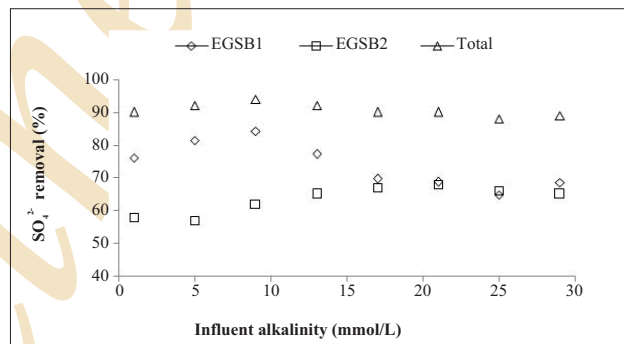


Fig. 15: Effect of influent alkalinity on SO<sub>4</sub><sup>2-</sup> removal in reactor

The effect of influent alkalinity on COD and sulfate removal is shown in Fig. 14 and 15. When influent alkalinity was kept at 8-10 mmol l<sup>-1</sup>, the alkalinity of EGSB1 and EGSB2 were 35.4 mmol l<sup>-1</sup> and 31 mmol l<sup>-1</sup> respectively and the total COD and sulfate removals approached 93.3% and 94.0% respectively. Influent alkalinity of 8-10 mmol l<sup>-1</sup> met the requirement of the process.

**Operational problems and countermeasures**

**Sludge flotation :** Sludge flotation occurred once. Some granular sludge floated on the surface when OLR and SLR

were raised excessively. So, the rise of OLR and SLR should be observed.

**Blockage of pipeline and gas meter :** Pipelines were blocked by sludge or inorganic mineral compound sedimentation. The presence of water vapor, carbon dioxide in biogas caused rusting and blockage of gas meter. Regular cleaning of the sediment from the pipelines and gas meter was required to avoid the blockage.

**Based on the performance of two-phase anaerobic digestion process, the following conclusions were devised :** In two-phase anaerobic digestion process, the COD/ SO<sub>4</sub><sup>2-</sup>

ratios were changed from 0.5 to 6 with increasing COD concentration; slight increase in COD removal was observed. When total COD removal was up to 85%, the influent COD/SO<sub>4</sub><sup>2-</sup> ratio was 2.8 (COD=5600mg l<sup>-1</sup>, SO<sub>4</sub><sup>2-</sup>=2000mg l<sup>-1</sup>). When the ratio was less than 2.8, COD removal descended quickly. When total sulfate removal raised up to 90%, the ratio exceeded 4.4 (COD=8800 mg l<sup>-1</sup>, SO<sub>4</sub><sup>2-</sup>=2000 mg l<sup>-1</sup>).

TA was generated and consumed, keeping balance along the anaerobic process. The effluent alkalinity in EGSB1 and EGSB2 was linearly correlated with the growth of influent alkalinity. When influent alkalinity was kept 8-10 mmol l<sup>-1</sup>, the total COD and sulfate removals approached 93.3% and 94.0% respectively, so influent alkalinity of 8-10 mmol l<sup>-1</sup> met the requirement of the process.

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