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# Performance evaluation of a mesophilic (37 °C) upflow anaerobic sludge blanket reactor in treating distiller's grains wastewater

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**Abstract**

The performance of a laboratory-scale upflow anaerobic sludge blanket (UASB) reactor treating distiller's grains wastewater was investigated for 420 days at 37 °C. After a successful start-up, 80–97.3% chemical oxygen demand (COD) removal efficiencies were achieved at hydraulic retention times (HRT) of 82–11 h with organic loading rates (OLR) of 5–48.3 kg COD $m^{-3}$ $d^{-1}$. The biogas mainly consisted of methane and carbon dioxide, and the methane and carbon dioxide content in the biogas was 57–60 and 38–41%, respectively. The yield coefficient of methane production was 0.3182 l $CH_4$ $g^{-1}$ COD removed until OLR at 33.3 kg COD $m^{-3}$ $d^{-1}$, but afterwards began to decrease. The volatile fatty acid (VFA) in the effluent mainly consisted of acetate and propionate, accounting for more than 95% of total VFA as COD, and other VFA was detected at insignificant concentrations. The mesophilic granules developed in this study showed an excellent specific methanogenic activity (SMA) at 0.91 and 1.12 g methane COD $g^{-1}$ $VSS^{-1}$ $d^{-1}$ using sucrose and acetate as individual substrates on day 200, respectively.


*Keywords:* UASB reactor; COD removal efficiency; Biogas; VFA; SMA

## 1. Introduction

Rural wineries in China generate large quantities of distiller's grains wastewater with high concentration of COD and acidic pH (3.3–4.3). In general, rural wineries often have very little or no treatment equipment for wastewater, and distiller's grains wastewater is usually directly discharged into the aquatic environment and grass fields. Without any treatment, distiller's grains wastewater can result in depletion of dissolved oxygen in the receiving water streams, and poses serious threat to the aquatic flora and fauna. Additionally, if distiller's grains wastewater is directly discharged into the land, it can reduce soil alkalinity, and then inhibits the growth of crops and grass. In order to comply with Chinese environmental protection legislation, it is essential to select an economic and high-performance process for treating distiller's grains wastewater.

Several criteria should be considered before deciding an eco-friendly process for treating distiller's grains wastewater, such as the flexibility to handle various concentration loads, low capital and operating costs [1]. A number of biological systems have been evaluated for high-strength organic wastewaters, such as anaerobic digesters [2–4] and activated sludge reactors [5,6]. Due to low energy consumption, low excess sludge production, enclosure of odours and valuable gas production, the anaerobic treatment has been gaining a wide popularity. In recent years, the upflow anaerobic sludge blanket (UASB) reactor has been recognized as an effective technology for the treatment of high-strength industrial wastewater, and has been successfully used for the treatment of various types of industrial wastewaters [7–11], such as malt whisky distillery pot ale, settled instant-coffee wastewater, slaughterhouse wastewater and dairy wastewater, and it is capable of handling high OLR of 10–25 kg COD $m^{-3}$ $d^{-1}$. However, Considering from commercial interest, it is of great concern whether UASB reactor could stably operate with higher process efficiency in a shorter HRT at a higher OLR (>30 kg COD $m^{-3}$ $d^{-1}$) by process improvement.

In addition, the amount and activity of methanogenic population are very important to improve the process capacity of UASB reactor. Retention of an adequate level of methanogens in the UASB reactor will give, not only a good digester performance in terms of COD removal and methane yield, but also a better quality effluent. The producing methane intensity in UASB reactor

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will be very violent with the increase of OLR. As high methane intensity can make anaerobic sludge leak out from UASB, it is necessary to modify the three-phase (gas–liquid–solid) separator for maintaining a high concentration of biomass in UASB and meet the demand of high OLR.

The objective of this work was to investigate the technical feasibility of a mesophilic (37 °C) UASB reactor with a three-phase separator for the treatment of distiller's grains wastewater. This experiment was conducted for 420 days to describe the reactor performance with respect to COD, along with the sludge characteristics of mesophilic sludge granules in the UASB reactor.

## 2. Materials and methods

### 2.1. Experimental set-up

The schematic diagram of a laboratory-scale UASB reactor is presented in Fig. 1a. The UASB reactor was made of acrylic plastic with an inner diameter of 8.2 cm and a total height of 190 cm, which included a working capacity of 8.18 l with a height of 155 cm in the reaction zone. Six sampling ports were installed along the height of the reactor, starting from a distance of 20 cm at the bottom of UASB reactor. A three-phase separator was located in the upper part of the reactor for separating and collecting the produced gas. Before flowed into UASB reactor, pH of distiller's grains wastewater was adjusted at about 7 with sodium bicarbonate. Distiller's grains wastewater was continuously pumped to the UASB reactor from the feed reservoir with a metering pump (EHC-B220R, IWAKI Co. Ltd., Japan). The liquid effluent and biogas were overflowed into an effluent tank and gas collector by the three-phase separator, respectively, and the sludge was retained in the UASB reactor. The temperature in the reactor was keep at 37 °C in whole experiment by a heat exchanger.

The drawing dimension of the three-phase separator is illustrated in Fig. 1b. The three-phase separator consists of the first separating zone, the second separating zone and the setting zone. A majority of biogas produced can be separated in the first setting zone by the inverted funnel, and residual small air bubble along with wastewater can be re-separated in the second separating zone and the accumulated biogas can be discharged by a connecting pipe. After the second separations, the wastewater flows into the setting zone through a slot between the sloping plate and inverted funnel. As wastewater in the setting zone cannot be disturbed by the biogas, exiguous suspended solids can be also precipitated, which is superior to the conventional UASB reactor. Thus, the three-phase separator allows solids to be retained in the reactor and the produced gases to be separated.

### 2.2. Seeding sludge and distiller's grains wastewater

The seeding sludge, which was harvested from a mesophilic anaerobic digester in a sewage treatment plant, was incubated at 37 °C by an auto-controlled heat exchanger in an airtight glass container for 3 weeks before seeded into the UASB reactor. The UASB reactor was seeded with 5.2 l of the incubated sludge at a concentration of 12.3 g $l^{-1}$ volatile suspended solid (VSS).

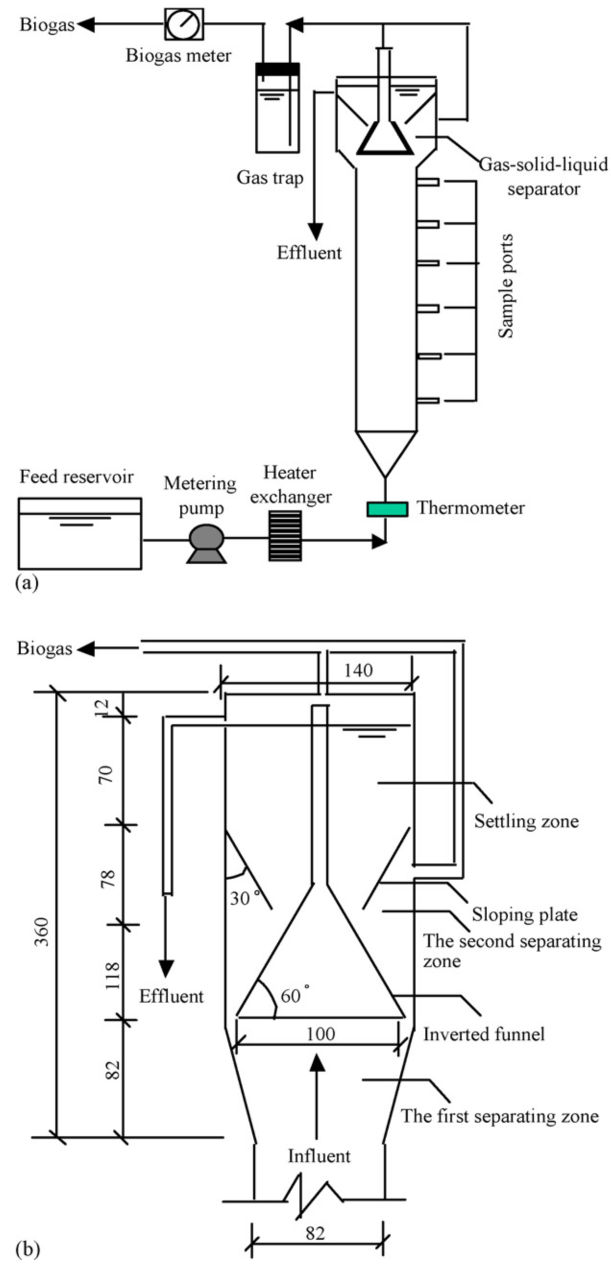


Fig. 1. Schematic diagram of the experimental set-up: (a) flow chart of UASB; (b) three-phase separator (dimensional unit: mm).

Distiller's grains wastewater, which was obtained from a local winery, was used in this study. As distiller's grains wastewater was not continuously discharged from wineries, it was first collected at a large regulating reservoir with a mesophilic temperature. The representative characteristics of the distiller's grains wastewater in rural wineries are presented in Table 1.

### 2.3. Analytical procedures

Suspended solids were determined in triplicate in a convection oven at 105 °C [12]. Volatile suspended solids determination was performed in triplicate as described in Standard Methods [12], and the pH was measured with a portable pH meter (Model HM-14P, TOA, Japan). COD measurement was

Table 1
Characteristics of distiller's grains wastewater

| Component | Concentration range (mg $l^{-1}$) |
|---|---|
| COD | 16500–22520 |
| Volatile fatty acids (VFA) | 3000–3600 |
| Suspended solid (SS) | 250–770 |
| Volatile suspended solid (VSS) | 190–640 |
| Total nitrogen (TN) | 120–150 |
| Total phosphor (TP) | 15–18 |
| pH | 3.3–4.3 |

based on digestion with potassium dichromate in concentrated sulphuric acid for 2 h at 150 °C, and each COD measurement was the average of three analyses of the same sample.

The composition of biogas and VFA was determined according to the procedure reported by Harada et al. [13] with minor modifications. The specific methanogenic activity (SMA) was measured as described by Harada et al. [13]. The number of hydrogen-consuming methanogens and acetate-consuming methanogens were analyzed by using the most probable number (MPN) method [14,15].

## 3. Results and discussions

### 3.1. Start-up of UASB reactor

In order to start up the experiment at 37 °C, 63.96 g of VSS of incubated sludge was added to the UASB reactor. Fig. 2 illustrated the process performance of the mesophilic UASB during the start-up. OLR was increased stepwise from 0.42 to 5.6 kg COD $m^{-3}$ $d^{-1}$ with the increase of influent COD strength at 2.5 day HRT, and there was an increase in the COD removal efficiency from 78.3 to 93.8% along with the volumetric methane production rate from 0.2 to 2.31 $CH_4$ $l^{-1}$ $d^{-1}$. However, with OLR increased extremely from 5.6 to 10.8 kg COD $m^{-3}$ $d^{-1}$ on day 28, a sharp decline occurred in COD removal efficiency and volumetric methane production rate, which also resulted in the accumulation of VFA in the effluent. In order to avoid the acidification of the reactor, 2000 mg $l^{-1}$ sodium bicarbonate solution ($NaHCO_3$) was added to the influent as OLR was reduced to 1.8 kg COD $m^{-3}$ $d^{-1}$ on day 29. The removal efficiency of COD and VFA in the UASB was recovered with a subsequent increment of the loading to 3.2 kg COD $m^{-3}$ $d^{-1}$ after 8 days. With OLR increased in principle from 3.2 to 23.7 kg COD $m^{-3}$ $d^{-1}$ in the following 40 days, the average removal efficiency of COD and VFA was 85.6 and 88.3%, respectively, and the volumetric methane production rate increased from 2.8 to 12 l $CH_4$ $l^{-1}$ $d^{-1}$.

Fig. 3 showed the VSS distribution with the height of sludge bed in the UASB during the start-up on days 14, 59, 78. When OLR was 3.3 kg COD $m^{-3}$ $d^{-1}$ on day 14, the VSS mainly accumulated in the bottom of the reactor due to the lower biogas production, which could not completely mix the wastewater and the sludge. With the gradual increase of OLR, the sustainable growth of biogas production made the water and the sludge completely mixed, however, the higher biogas production resulted in the washout of the dispersed sludge from the UASB reactor on day 50 (Fig. 2c). With the prolong of operational period, dispersed biomass had agglutinated to form granules, which was investigated with an average size of about 1.0–1.5 mm by SEM, and no sludge washout was found on day 78 in the effluent. The sludge specific activity has been achieved at 0.51 l $CH_4$ $g^{-1}$ VSS $d^{-1}$ on day 78, and sludge volume index (SVI) was decreased to 18.5 ml $g^{-1}$ from the initial 59.8 ml $g^{-1}$. From the above results, we concluded that the start-up of UASB and the culture of granule sludge had been completed and the excellent operation would begin in the later study.

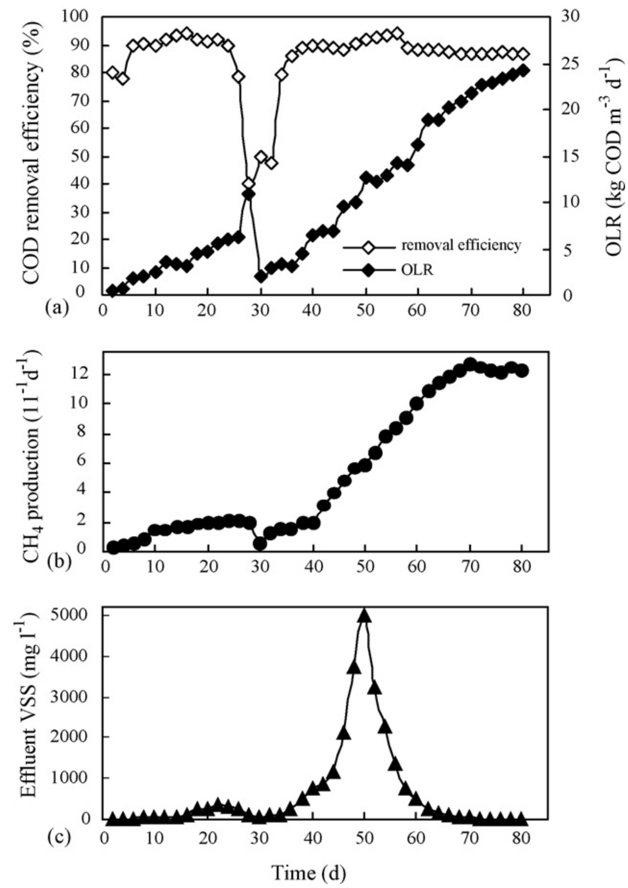


Fig. 2. Process performance of the UASB during the start-up: (a) COD removal efficiency; (b) volumetric $CH_4$ production; (c) effluent VSS.

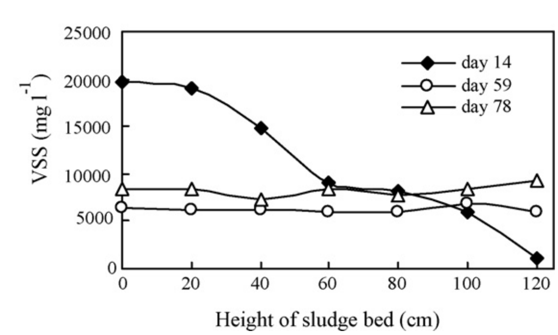


Fig. 3. VSS distribution with the height of sludge bed in the UASB during the start-up on days 14, 59, 78.

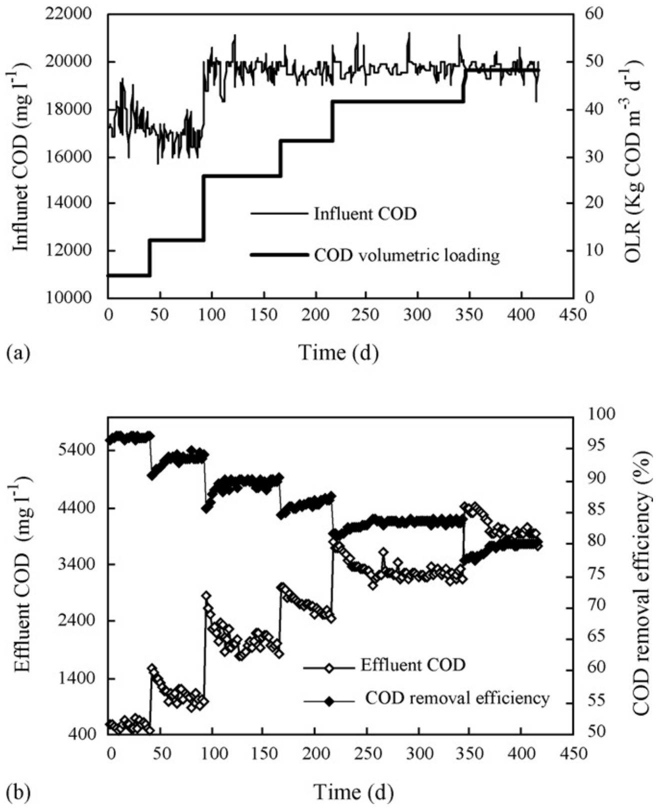


Fig. 4. Performance of the UASB reactor related to COD removal efficiency: (a) influent COD and OLR; (b) effluent COD and COD removal efficiency.

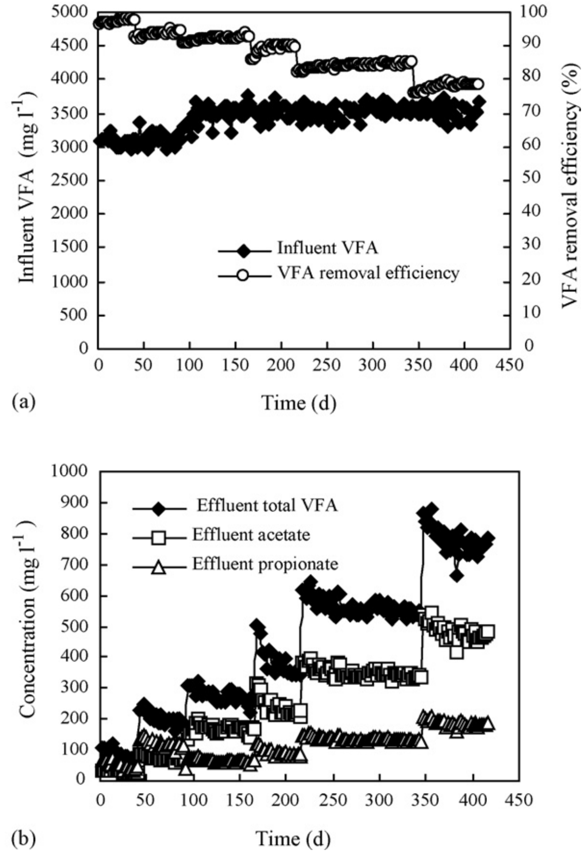


Fig. 5. Performance of the UASB reactor related to VFA: (a) VFA removal efficiency; (b) VFA composition in the effluent.

### 3.2. *Evolution of COD removal efficiency*

After a successful start-up, OLR of the UASB reactor was increased stepwise from 5 to 48.3 kg COD $m^{-3}$ $d^{-1}$ by reducing HRT. The performance of the mesophilic UASB reactor with respect to COD removal efficiency is showed in Fig. 4. When OLR was about 5 kg COD $m^{-3}$ $d^{-1}$, the average COD removal efficiency was over 95% (maximal content 97.3%). However, the average COD removal efficiency gradually decreased with the continuously increasing OLR, which illustrated that 1 kg COD $m^{-3}$ $d^{-1}$ increased in the reactor could make the COD removal efficiency decline about 0.28%, and the average COD removal efficiency was about 83.6% (minimal content 80%) at the OLR of 48.3 kg COD $m^{-3}$ $d^{-1}$. Compared to previous studies [13,16–18], the increase of OLR did not significantly affect the performance of the UASB reactor. The COD average removal efficiency was only decreased 11.4% with the increase of OLR from 5 to 48.3 kg COD $m^{-3}$ $d^{-1}$, and this illuminated that the UASB reactor could stably operate with high COD removal efficiency at high OLR.

Additionally, average content of specific loading rate (SLR), sludge yield rate (SYR) and sludge retention time (SRT) were also investigated as shown in Table 2. With the increase of average OLR from 5 to 48.3 kg $COD^{-1}$, average SYR varied from 0.0476 and 0.0843 g VSS $g^{-1}$ COD removed. In order to prevent excessive sludge from being washed out together with the effluent, the UASB reactor was operated at 6, 7, 8, 9, 17 and 52 days SRT, which correspond to the SLR at 2.02, 1.80, 1.68, 1.39, 0.92 and 0.39 kg COD $kg^{-1}$ VSS $d^{-1}$.

### 3.3. *Evolution of VFA composition*

During the whole experimental period, total VFA in the effluent was gradually increased with the variation of OLR from 5 to 48.3 kg COD $m^{-3}$ $d^{-1}$ by reducing HRT, and the VFA removal efficiency reduced from 96.63 to 79.0% as showed in Fig. 5. Acetate and propionate in VFA of the effluent were major components, accounting for more than 95% of total VFA as COD,

Table 2
Average content of specific loading rate, sludge yield rate and sludge retention time with the variation of OLR in the UASB reactor

| | | | | | | |
|---|---|---|---|---|---|---|
| OLR (kg COD $m^{-3}$ $d^{-1}$) | 5 | 12.35 | 25.65 | 33.7 | 41.6 | 48.3 |
| VSS ($g\,l^{-1}$) | 12.43 | 13.28 | 17.23 | 20.12 | 22.72 | 23.8 |
| Specific loading rate (kg COD $kg^{-1}$ VSS $d^{-1}$) | 0.39 | 0.92 | 1.39 | 1.68 | 1.80 | 2.02 |
| Sludge yield rate (g VSS $g^{-1}$ COD removed) | 0.0476 | 0.0632 | 0.0759 | 0.0748 | 0.079 | 0.0843 |
| Sludge retention time (d) | 52 | 17 | 9 | 8 | 7 | 6 |

Table 3
Specific methanogenic activity (SMA) of granules using acetate and sucrose as individual substrate

| Source of sludge/substrate | Reactor temperature (°C) | SMA temperature (°C) | SMA (g methane COD $g^{-1}$ VSS $d^{-1}$) | | References |
|---|---|---|---|---|---|
| | | | Acetate | Sucrose | |
| Modified UASB | 37 | 37 | 1.12 | 0.91 | Present study |
| Starch | 37 | 37 | 2.26 | 0.99 | Fang et al. [21] |
| Sucrose | 37 | 37 | 1.20 | 0.85 | Fang et al. [22] |
| Brewery | 37 | 37 | 0.40 | 0.32 | Fang et al. [22] |
| Sugar | 37 | 37 | 0.90 | N/A | Dolfing et al. [23] |

N/A: not available.

and other VFA was detected at insignificant concentrations. Until day 94, acetate was the dominant species and contributed 60–70%. After OLR was increased up to 25.6 kg COD $m^{-3}$ $d^{-1}$, it is interesting that propionate in the effluent began to be dominant until the termination of the experiment. So far, several workers have also reported that propionate degradation was the rate-limiting step of the overall methane production in anaerobic digestion of various substrates [19–21]. As the degradation of VFA, both pH and alkalinity in the effluent were consistently higher than the feed values. The alkalinity rise was variable but typically around 0.16 mg $CaCO_3$ $mg^{-1}$ COD removed, and the effluent pH values were about 7.5.

### 3.4. Biogas evaluation

The biogas volumetric production increased with increasing OLR until day 350 (Fig. 6), but afterwards began to decrease, due partly to the deterioration of COD removal efficiency. The biogas mainly consisted of methane and carbon dioxide, and the methane and carbon dioxide content in the gas was 57–60 and 38–41%, respectively. When OLR varied from 5 to 48.3 kg COD $m^{-3}$ $d^{-1}$, the volumetric methane production increased from 1.6 to 11.5 l $CH_4$ $l^{-1}$ $d^{-1}$, and the relationship between OLR and the average methane production rate was given in Fig. 7. The volumetric methane production rate increased linearly with the increase of OLR until reaching a maximum of 33.3 kg COD $m^{-3}$ $d^{-1}$, and the ration of methane production per COD removal was estimated to be 0.3182 l $CH_4$ $g^{-1}$ COD from the slope of linear regression, which was almost equal to the theoretical value of 0.35 l $CH_4$ $g^{-1}$ COD removed.

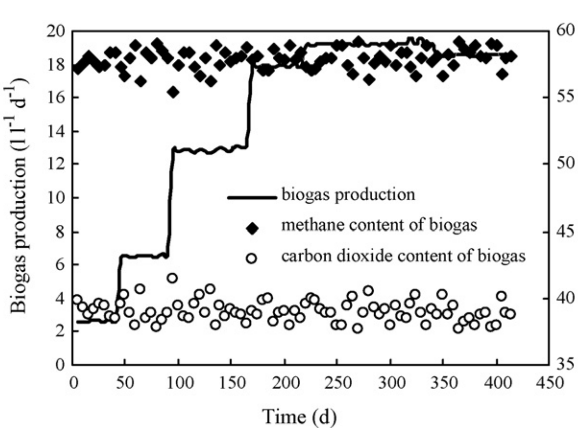


Fig. 6. Biogas production, and methane and carbon dioxide content in the biogas.

### 3.5. Specific methanogenic activity

The SMA of granular sludge sampled from the reactor was determined on day 200 using sucrose and acetate as individual substrates (Table 3), and were also compared with corresponding data in previous studies [21–23]. The mesophilic granules developed in this study showed an excellent mehtanogenic activity at 0.91 and 1.12 g methane COD $g^{-1}$ $VSS^{-1}$ $d^{-1}$ for sucrose and acetate, respectively, due to the retention of methanogenic bacteria by the three-phase separator in the UASB reactor.

In order to adequately analyze the SMA of granular sludge, the number of methanogens was also investigated in per ml mixed liquor and per gram VSS by the most probable number (MPN) method as shown in Table 4. The number of hydrogen-

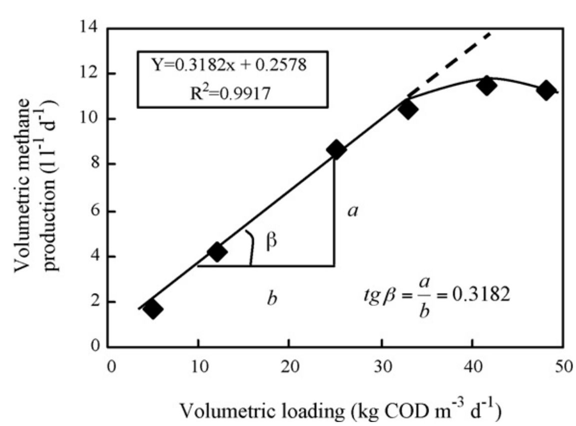


Fig. 7. Relationship between COD volumetric loading and methane production rate.

Table 4
Populations of hydrogen- and acetate-consuming methanogens at different OLR

| OLR (kg COD $m^{-3}$ $d^{-1}$) | Hydrogen-consuming methanogens | | Acetate-consuming methanogens | |
|---|---|---|---|---|
| | $10^7$ $ml^{-1}$ | $10^9$ $g^{-1}$ VSS | $10^7$ $ml^{-1}$ | $10^9$ $g^{-1}$ VSS |
| 5.0 | 2.2 | 1.6 | 1.5 | 0.9 |
| 12.0 | 3.6 | 2.6 | 2.8 | 1.9 |
| 25.6 | 8.3 | 5.9 | 7.2 | 4.8 |
| 33.3 | 9.2 | 6.5 | 7.4 | 5.7 |
| 41.7 | 9.7 | 7.1 | 7.5 | 5.8 |
| 48.3 | 9.6 | 7.3 | 7.8 | 5.6 |

consuming methanogens varied from $2.2 \times 10^7\,ml^{-1}$ in the mixed liquor to $9.7 \times 10^7\,ml^{-1}$ and from $1.6 \times 10^9\,g^{-1}$ VSS to $7.3 \times 10^9\,g^{-1}$ VSS with the increase of OLR, corresponding to the population variation of acetate-consuming methanogens from $1.5 \times 10^7\,ml^{-1}$ in the mixed liquor to $7.8 \times 10^7\,ml^{-1}$ and from $0.9 \times 10^9\,g^{-1}$ VSS to $5.8 \times 10^9\,g^{-1}$ VSS. It is very obvious that the number of hydrogen-consuming methanogens was higher than that of acetate-consuming methanogens.

## 4. Conclusions

A UASB reactor was feasible for the treatment of distiller's grains wastewater at 37 °C. High COD removal efficiency in the range of 80–97.3% was obtained after a successful start-up despite OLR changes from 5 to 48.3 kg COD $m^{-3}\,d^{-1}$. The biogas mainly consisted of methane and carbon dioxide, and the methane and carbon dioxide content in the gas was 57–60 and 38–41%, respectively. The volumetric methane production rate increased linearly with the increase of OLR until reaching at 33.3 kg COD $m^{-3}\,d^{-1}$, and the ration of methane production per COD removal was estimated to be 0.3182 l $CH_4\,g^{-1}$ COD removed, which was almost equal to the theoretical value of 0.35 l $CH_4\,g^{-1}$ COD removed. Considering the availability of biogas, 33 kg COD $m^{-3}\,d^{-1}$ is the optimum organic loading. Acetate and propionate in volatile fatty acid (VFA) of the effluent were major components, accounting for more than 95% of total VFA as COD, and other VFA was detected at insignificant concentrations. The mesophilic granules developed in this study showed an excellent mehtanogenic activity at 0.91 and 1.12 g methane COD $g^{-1}\,VSS^{-1}\,d^{-1}$ using sucrose and acetate as individual substrates, respectively.

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