Surface characteristics of acidogenic sludge in H_2-producing process

Yang Mu, Yi Wang, Guo-Ping Sheng, Han-Qing Yu*
School of Chemistry, University of Science & Technology of China, Hefei, 230026 China
*Corresponding author. Fax: +86 551 3601592; E-mail: hqyu@ustc.edu.cn

ABSTRACT

The surface characteristics, including rheological, fractal characteristics, hydrophobicity as well as surface free energy, of H_2-producing sludge in acidogenic fermentative process were investigated in this study. Both rheological and fractal characteristics of the H_2-producing sludge changed slightly in the acidogenesis. The sludge fractal dimension was larger than those of other microbial aggregates, whereas the affinity of the microbial cells for the hydrocarbon had a peak value in the fermentation process. Both specific H_2 and volatile fatty acids/ethanol production rates of the sludge had a peak of 108 mL-H_2 L^{-1} h^{-1} g-VSS^{-1} and 480 mg L^{-1} h^{-1} g-VSS^{-1}. There was a relationship between the hydrophobicity of the H_2-producing sludge and its specific H_2-producing activity. The surface free energy of the H_2-producing microorganisms had a lowest value in their growth process.

KEYWORDS: Acidogenesis; H_2-producing sludge; Hydrophobicity; Rheological; Surface characteristics; Surface free energy

INTRODUCTION

The surface characteristics of sludge, such as rheology, fractal properties hydrophobicity and surface free energy, are significant factors influencing the performance of a wastewater treatment process (Johnson et al., 1996; Dentel, 1997; Zita and Hermansson, 1997). Rheology is a powerful tool for characterizing the non-Newtonian properties of sludge suspensions, as it can quantify flow behaviors in real processes on a scientific basis (Dentel, 1997). Properties of sludge permeability, density, and porosity can be calculated from the fractal dimension and have important implications for the aggregation kinetics, floc break-up, and settling velocities of sludge as a function of their fractal structure (Johnson et al., 1996). Thus, measurement of the fractal dimension of sludge is of considerable interest. Hydrophobicity of sludge plays an important role in the self-immobilization and attachment of cells to a surface (Zita and Hermansson, 1997; Zheng et al., 2005).

The biological H_2 production from anaerobic fermentation of organic wastes is an economical and sustainable technology for both pollution control and clean energy generation (Chen et al., 2001; Levin et al., 2004). In anaerobic fermentative H_2-producing process, majority of the removed organic matters is converted to H_2, CO_2, and volatile fatty acids (VFA) and alcohols. This fermentative process is greatly influenced by many factors, such as substrate composition, substrate concentration, hydraulic retention time, pH and temperature (Yu et al., 2002; Lin and Jo, 2003;

Zheng and Yu, 2004).

The surface characteristics of H_2-producing sludge might also be a significant factor affecting the fermentative H_2 production. However, little information concerning the surface characteristics of H_2-producing sludge in acidogenic fermentative process is available in literature. Therefore, the main objective of this study was to explore the surface characteristics of H_2-producing sludge, including rheological and fractal properties as well as hydrophobicity, in order to provide useful information for fermentative H_2 production.

MATERIALS AND METHODS

Seed Sludge

The anaerobic seed sludge used in this study was obtained from a full-scale upflow anaerobic sludge blanket reactor treating citrate-producing wastewater. Prior to use, the seed sludge was first washed with tap water five times, and was then sieved to remove stone, sand and other coarse matters. Thereafter, the seed sludge was heated at 102^oC for 90 min to inactivate the hydrogentrophic methanogens and to enrich the H_2-producing bacteria as described by Logan et al. (2002). The image of the H_2-producing sludge is shown in Fig. 1.

Figure 1 Image of the anaerobic H_2-producing sludge

Experiment

Fermentative H_2 production experiments were conducted in a 5-L fermentor (Baoxin Biotech Ltd., China). An 1000-mL heat-treated seed sludge of volatile suspended solids (VSS) of 19.2 g L^{-1} and 3 mL of nutrients solution were added to the fermentor. The working volume of the fermentor was adjusted to 3.0 L with distilled water. The solution in the fermentor was composed as follows (unit in mg L^{-1}): NH_4HCO_3 2025; $K_2HPO_4 \cdot 3H_2O$ 800; $CaCl_2$ 50; $MgCl_2 \cdot 6H_2O$ 100; $FeCl_2$ 25; NaCl 10; $CoCl_2 \cdot 6H_2O$ 5; $MnCl_2 \cdot 4H_2O$ 5; $AlCl_3$ 2.5; $(NH_4)_6Mo_7O_{24}$ 15; H_3BO_4 5; $NiCl_2 \cdot 6H_2O$ 5; $CuCl_2 \cdot 5H_2O$ 5; $ZnCl_2$ 5. Prior to operation, the fermentor was purged with nitrogen gas for 10 min to ensure anaerobic condition. The pH of the mixed liquor was kept constantly by feeding NaOH (4M) or HCl (2M) solutions via respective peristaltic pumps. The agitation rate in the fermentor was kept at 120 rpm. A 20-mL sample including sludge was taken from reactor at each given interval and was analyzed.

Two trials, at pH 5.5, temperature 35.0°C, sucrose concentration of 25.0 g L^{-1} (Run 1) and pH 6.0, temperature 30.0°C, sucrose concentration of 20.0 g L^{-1} (Run 2), were respectively carried out to investigate the time evolution of the sludge surface characteristics, and each of them was replicated at least three times.

Analytical Methods

The rheological characteristics of the H_2-producing sludge were determined using a rotational viscometer (NXS-11A Rotational Viscometer, Chengdu Instrument Co., China), a coaxial cylindrical measurement device with a double gap measuring system. The rheogram of shear stress (τ) as a function of shear rate ($\dot{\gamma}$) was recorded and analyzed, then the apparent viscosity (η_{app}) of the sludge was calculated from $\eta_{app} = \tau/\dot{\gamma}$.

The fractal dimension (D_f) of the sludge was determined using image analysis. An Olympus CX41 microscope (Olympus Co., Japan) equipped with a digital camera (C5050 Zoom, Olympus Co., Japan), connected to a PC via a grabbing board was used. A drop of mixed liquor was carefully deposited and covered with a cover slip. No staining or fixation was done. A series images was grabbed by a systematic examination of the slide: adjacent fields are grabbed by scanning the slide from the top right corner to the bottom left one. The illumination was kept constant for all the samples. The pixel size calibration was done with a stage micrometer. Then the images obtained were analyzed by using the software of Fractal Image Process System (FIPS) developed by the University Science and Technology of China.

The hydrophobicity of sludge was determined by measuring contact angle of sludge (Sheng et al., 2005). A suspension of sludge containing biomass was deposited on a cellulose membrane filter. Samples were washed three times with deionized water, and residual water was removed by filtration. The drop shape of a sessile distilled water droplet placed on the layer of biomass was determined using a contact angle analyzer (JC2000A, Powereach Co., China).

The surface free energy of H_2-producing microorganism was evaluated with the data of contact angle measurement. According to the Young equation (Sharma and Rao, 2002), the surface free energy at liquid-vapour interface, γ_{lv}, solid-liquid interface, γ_{sv}, and solid-vapour interface, γ_{sv}, which in equilibrium, has the following relationship:

$$\gamma_{lv}\cos\theta = \gamma_{sv} - \gamma_{sl} \tag{1}$$

where θ is the contact angle. Considering the surface thermodynamics of a two component three-phase solid-liquid-vapour system, an equation-of-state type relation exists between γ_{lv}, γ_{sv} and γ_{sv} (Sharma and Rao, 2002):

$$\lambda_{sl} = \frac{(\sqrt{\gamma_{sv}} - \sqrt{\gamma_{lv}})^2}{1 - 0.015\sqrt{\gamma_{sv}\gamma_{lv}}} \tag{2}$$

In this study, the surface free energy of the H_2-producing microorganisms, e.g., γ_{sv}, could be estimated with Eqs. (1) and (2).

The amount of biogas produced in the fermentation was recorded daily using water-replace equipment. The H_2 and CO_2 contents were determined using a gas chromatograph (Model SP-6800A, Lunan Co, China) equipped with a thermal conductivity detector and a 1.5 m stainless-steel column packed with 5Å molecular sieve. The temperatures of injector, detector and column were kept at 100^{o}C, 105^{o}C and 60^{o}C, respectively. Argon was used as carrier gas at a flow rate of 30 mL min^{-1}. The concentrations of VFA in the solution were determined using a second gas chromatograph (Model 6890NT, Agilent Inc., USA) equipped with a flame ionization detector and a 30m×0.25mm×0.25µm fused-silica capillary column (DB-FFAP). The liquor samples were first centrifuged at 12000 rpm for 5 min, and were then acidified by formic acid and filtrated through 0.2 µm membrane and finally measured for free acids. The temperatures of the injector and detector were 250^{o}C and 300^{o}C, respectively. The initial temperature of oven was 70^{o}C for 3 min followed with a ramp of 20^{o}C min^{-1} for 5.5 min and to final temperature of 180^{o}C for 3 min. Nitrogen was used as carrier gas with a flow rate of 2.6 mL min^{-1}. Sucrose concentration was measured using enthrone-sulfuric acid method (Dubois et al., 1956), while the VSS concentration was determined according to the Standard Methods (APHA, 1995).

RESULTS AND DISCUSSION

Fermentative H_2 Production

In the fermentative H_2-producing process, sucrose was converted into gaseous and aqueous products as well as biomass. The biogas was mainly composed of H_2 and CO_2, and the mixed liquor was composed of VFA and ethanol. Fig. 2 illustrates the experimental results of Run 1. The H_2 percentage in the reactor headspace gradually increased and reached a maximum value of 0.61 atm after 25-h fermentation, then it declined with the fermentation time (Fig. 2a). The produced biogas increased and reached a maximum of 33000 mL after 35-h fermentation, and remained nearly unchanged afterwards (Fig. 2b). The H_2 yield was calculated as 1.78 mol-H_2 mol-glucose^{-1}.

The formation of H_2 was accompanied by the production of VFA and ethanol (Fig. 2c). After a lag phase, VFA and ethanol increased sharply and maximized of 10000±560 mg L^{-1} at the end of test. Ethanol was the sole alcohol detected. The concentration of VFA and ethanol increased with fermentation time. Among them, butyrate and acetate were the main products, accounting for 97% (W/W) of the total VFA and ethanol, suggesting a butyrate-type fermentation in this trail.

Rheological Characteristics Of Sludge

Figure 3 shows a typical rheogram of the H_2-producing sludge: its apparent viscosity (η_{app}) decreased rapidly as the shear rate increased, but became constant at a higher shear rate, which was called as the limiting viscosity (η_{∞}) at the infinite shear rate (Tixier et al., 2003). The limiting viscosity has been commonly used as a parameter for characterizing sludge rheology (Tixier et al., 2003). The limiting viscosity of the sludge changed slightly with the fermentation time in both trails (Fig. 4), at a level of 38 mPa s, suggesting invariable rheological characteristics of sludge in this H_2-producing process. This might due to the fact that the experimental conditions, such as sludge concentration, pH, temperature and agitation rate, were kept unchanged in both trails (Tixier et al., 2003).

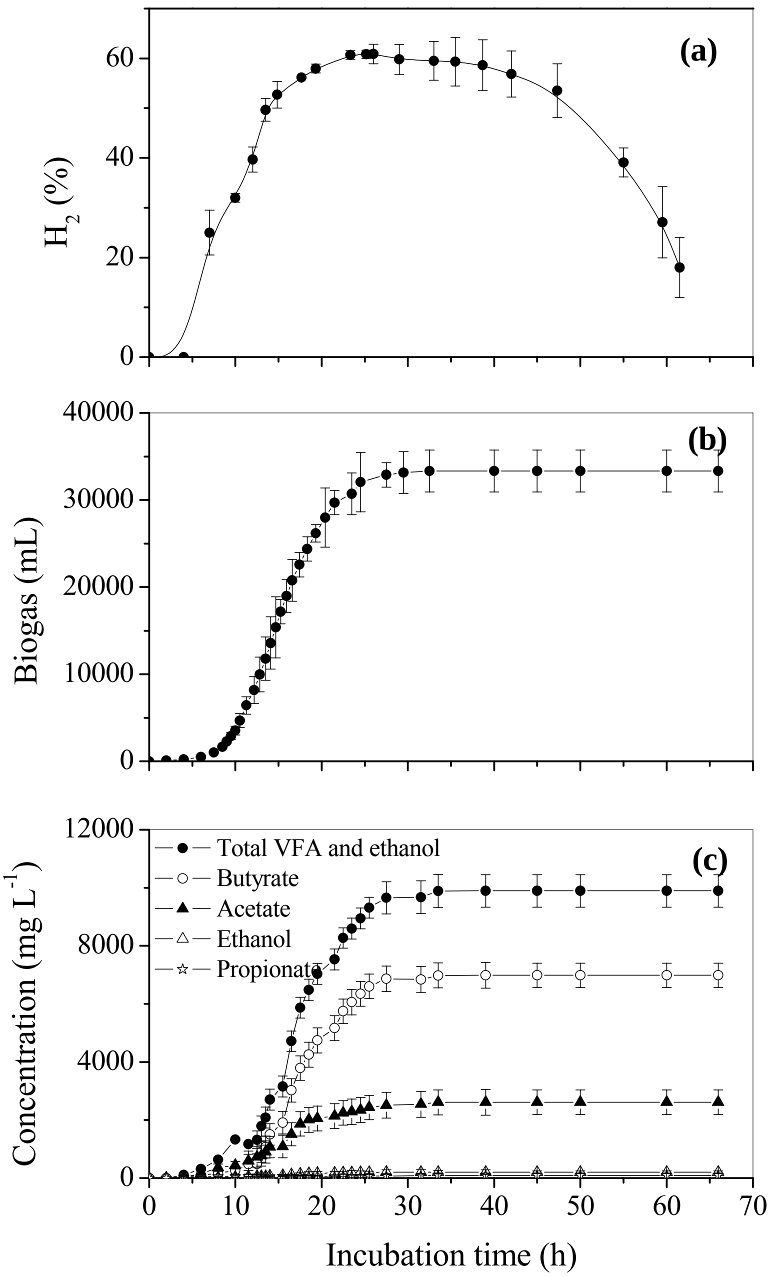

Figure 2 Effect of incubation time on: (a) H_2 concentration in the reactor headspace, (b) biogas production, and (c) VFA and ethanol

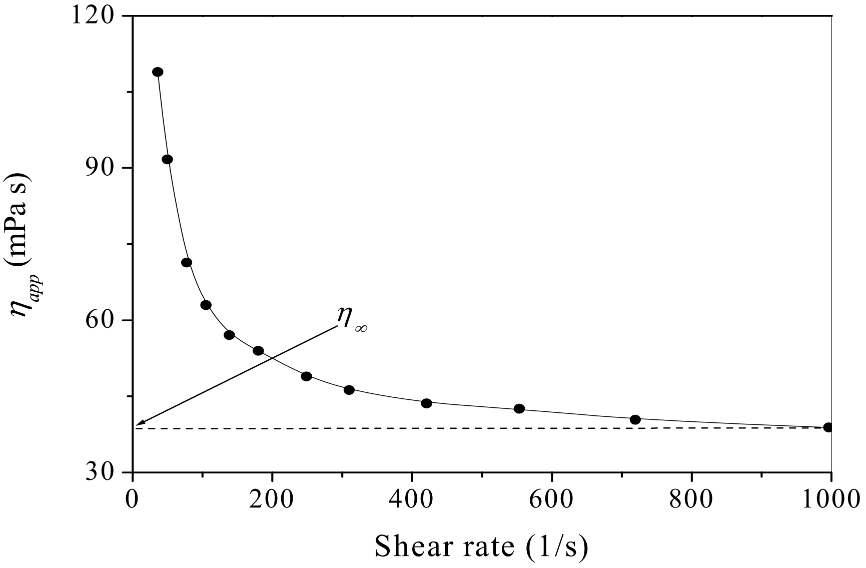

Figure 3 A typical rheogram of the H_2-producing sludge

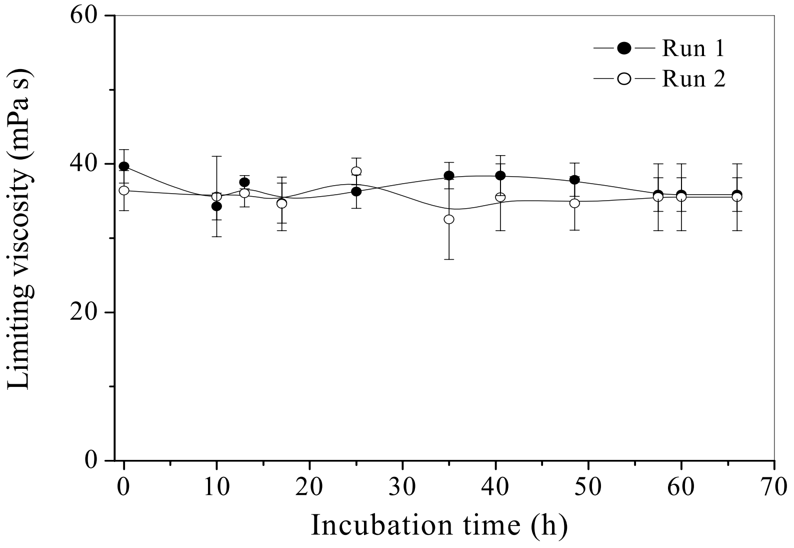

Figure 4 Time evolution of the limiting viscosity

Fractal Characteristics Of Sludge

The most important numerical parameter in fractal theory is the fractal dimension (D_f), which is usually very sensitive to the definition of the contour of the particle. The theoretical values of D_f vary from 1 to 3, which provide an useful index for describing the degree of floc compactness and how the particles are packed (Lee and Hsu, 1994). The high value of the D_f is related to compact and dense sludge (Jin et al., 2003). As shown in Fig. 5, with increasing fermentation time, the fractal dimension of the sludge was altered slightly and at a level of 2.80 in both two trials. This suggests that the sludge contour almost didn't change during the fermentation. The D_f values obtained in this study and various microbial aggregates in literature are listed in Table 1 for comparison. The D_f of the H_2-producing sludge was larger than those of the other microbial aggregates, implying that this sludge was more compact and denser. Recent theoretical work has shown that permeability of sludge drastically decreases when the fractal dimension was greater than 2.0 (Snidaro et al., 1997). It implies that the microorganisms within the H_2-producing sludge were less active than those located at the surface (Snidaro et al., 1997). On the other hand, the ratio of the hydrodynamic radius (R_H) to the sludge radius of (R_A) could be calculated by following equation (Gmachowski, 1996):

$$\begin{aligned}\frac{R_H}{R_A} &= \sqrt{1.56-(1.728-\frac{D_f}{2})^2}-0.228 \\ &= 0.977\end{aligned} \tag{3}$$

With the ratio of R_H/R_A, the dynamic behavior of the sludge, i.e., the velocity ratio between the primary particle and the aggregate (Gmachowski, 1995), could be described. Furthermore, the aggregate structure factor (S) of the sludge, could be estimated as 0.937 by Eq. (4) (Gmachowski, 1995):

$$S = (\frac{R_H}{R_A})^{D_f} \tag{4}$$

The aggregate structure factor could be employed to characterize the space-filling ability of the H_2-producing sludge and thus its compactness. The dynamic behavior of the sludge greatly depends on the compactness, as it has a substantial effect on the fluid flow through the microbial flocs (Gmachowski, 1995).

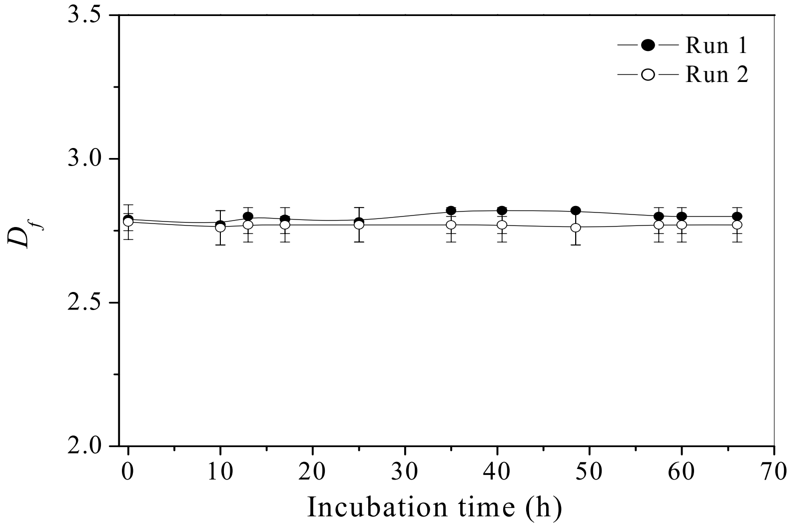

Figure 5 Time evolution of the fractal dimension

Table 1 Comparison of D_f values from this work and literature

Sludge	D_f	References
H_2-producing sludge	2.80±0.01	This study
Activated sludge	2.3-2.5	Li and Ganczarczyk, 1989
	2.34±0.04	Motta et al., 2001
Shear induced aggregates	2.25±0.11	Thill et al., 1998
DLA* aggregates	2.09±0.11	Thill et al., 1998

*Diffusion limited aggregates

Hydrophobicity Of Sludge

The contact angle, which is generally used to evaluate the hydrophobicities of pure bacterial strains and solid surfaces (Daffonchio et al., 1995), was employed to study the hydrophobicity of the H_2-producing sludge. As shown in Fig. 6a, in the Run 1, the sludge contact angle increased from 69.4^o to a peak value, 79.8^o as fermentation time lasted to 15 h, but it then decreased to 67.6^o as the fermentation time was increased to 66 h. A similar trend was observed for the Run 2: the sludge contact angle increased from 71.8^o to a peak value, 85.7^o as fermentation time lasted to 15 h, but it then decreased to 67.6^o as the fermentation time was increased to 66 h. These results indicate that the affinity of the H_2-producing sludge cells for the hydrocarbon had a peak value in fermentation process. Both specific H_2 and VFA/ethanol production rates shared similar trends with its hydrophobicity (Fig. 6). The peak values of 108 mL-H_2 L^{-1} h^{-1} g-VSS^{-1} and 480 mg L^{-1} h^{-1} g-VSS^{-1} were observed after 15-h fermentation. These results suggest that there was a positive relationship between the hydrophobicity of the H_2-producing sludge and its specific H_2-producing or VFA-producing activity.

Apart from the surface charge and hydrophobic or hydrophilic character of the bacterial cells, the surface energy is a very important parameter governing their adhesion on solid surfaces. Lower surface free energy of bacterial suggests means easily adhesion on solid surfaces (Sharma and Rao, 2002). As shown in Fig. 7a, in the Run 1, the surface free energy of the H_2-producing microorganisms decreased from 50 mJ m^{-2} to a lowest value, 39.2 mJ m^{-2}, after 15.5-h fermentation. After that it increased to 51.5 mJ m^{-2} in the subsequent fermentation. A similar trend was observed for the Run 2 (Fig. 7b).

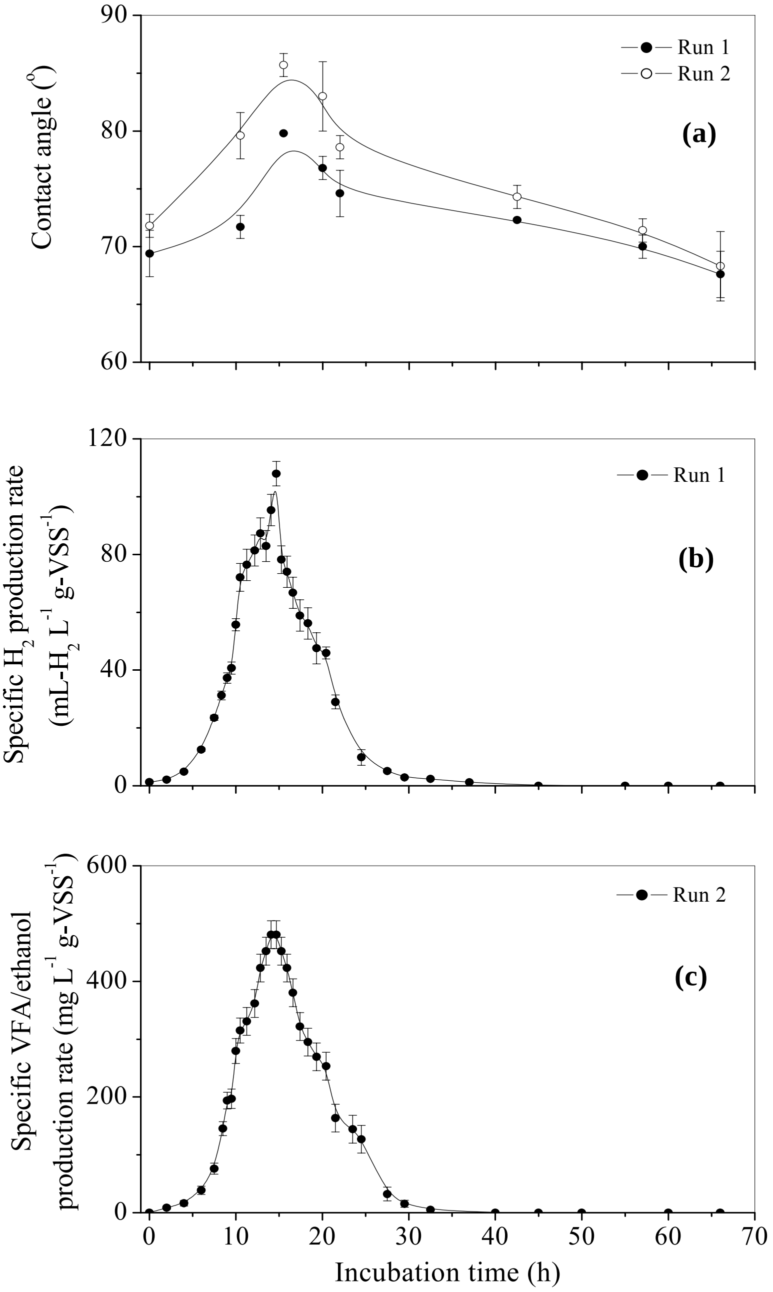

Figure 6 Time evolution of: (a) sludge contact angle of; (b) specific H_2 production rate; and (c) specific VFA/ethanol production rate

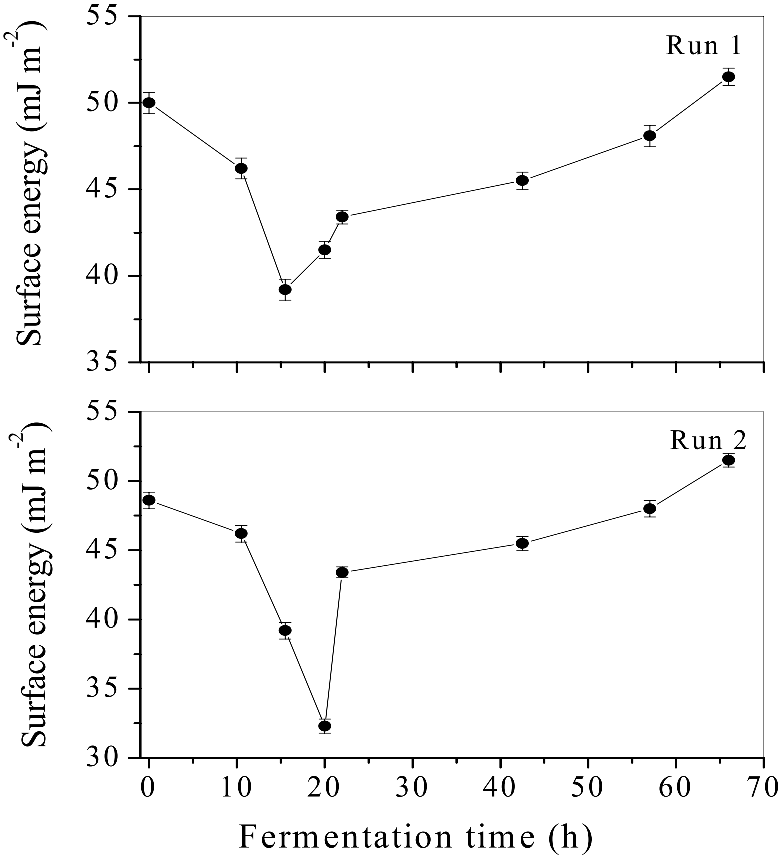

Figure 7 Time evolution of the surface free energy of the microorganisms

CONCLUSIONS

This study shows that both rheological and fractal characteristics of H_2-producing sludge changed slightly with increasing fermentation time in the acidogenic fermentative process. Moreover, the fractal dimensions of H_2-producing sludge were larger than those of some other aggregates, implying that the H_2-producing sludge was more compact and denser. The contact angle of sludge increased to a peak value with the increasing of fermentation time, and then decreased. This indicates that the affinity of the H_2-producing microbial cells for the hydrocarbon had a peak value in the fermentative H_2-producing process. Furthermore, the specific H_2 production rate and the specific VFA/ethanol production rate of H_2-producing sludge have the same trend with its hydrophobicity, suggesting that there was a positive relationship between the hydrophobicity of the H_2-producing sludge and its specific H_2- or VFA-producing activity. The surface free energy of the H_2-producing microorganisms had a lowest value in their growth process.

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