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Hydrogen utilization rate: A crucial indicator for anaerobic digestion process evaluation and monitoring

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Hydrogenotrophic methanogens had been considered as key species for the anaerobic digestion (AD) of industrial wastewater and municipal sludge. However, how to evaluate the activity of the hydrogenotrophic methanogens was less studied. In this study, a volumetric device and a test procedure were developed for measuring the specific hydrogen utilization rate (HUR) of anaerobic sludge. Results showed that HUR values were highly influenced by sludge concentrations because of limitation on H_2 mass transfer. The critical value of sludge concentration in the test bottle should not be higher than 1 gVSS/L. Under such condition, the kinetics of HUR would not be limited by H_2 mass transfer and the maximal value of HUR could be obtained. Field survey confirmed that HUR exhibits a good relationship with specific methanogenic activity (SMA) and reactor performance. An anaerobic system with a relatively high HUR was found to be beneficial for maintaining H_2 partial pressure in an appropriately low level. Moreover, such system was thermodynamically favourable for the syntrophic degradation of volatile fatty acids. As a crucial parameter of the anaerobic process, HUR could be used as a key indicator for evaluating and monitoring AD processes.

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[**Key words:** Anaerobic digestion; Hydrogenotrophic methanogens; Hydrogen utilization rate; H_2 partial pressure; Specific methanogenic activity]

Anaerobic digestion (AD) is widely recognised as a sustainable technology for biological wastewater treatment. During this process, at least three groups of microbes, including acidogenic and acetogenic bacteria as well as methanogenic archaea, participate in organic compounds conversion into CO_2 and CH_4 (1). Acetogenesis, in which intermediates such as propionate and butyrate are oxidized to acetate, is a key step in organic methanogenic conversion. In order to keep acetogenesis process thermodynamically feasible, low H_2 partial pressure (<10 Pa) has to be maintained (2–7). H_2 partial pressure has been considered as an indicative parameter for regulating acetogenesis and for evaluating anaerobic digester performance (8). However, studies on full-scale anaerobic systems have found that H_2 determination, combined with thermodynamic calculations, is not sufficient for providing meaningful information on actual AD systems (9,10). The metabolic potentials of anaerobic sludge cannot be assumed to be the same, even if the H_2 partial pressure of different anaerobic systems is maintained on an equivalent level. Therefore, determining other parameters appropriate for evaluating anaerobic degradability of contaminants fully is essential.

In AD process, methanogenic archaea, which catalyze the terminal stage of the process, are generally divided into two main groups, i.e., acetoclastic methanogenes (convert acetate into CH_4) and hydrogenotrophic methanogenes (convert H_2/CO_2 into CH_4), based on their available substrate. Although acetoclastic methanogenes have a major role in CH_4 production (approximately 70% of CH_4 is formed from acetate), hydrogenotrophic methanogenes also play a key role in the process (11) by maintaining low partial pressure of H_2, which is necessary for the growth of intermediate syntrophic bacteria (3,12,13). Schmidt and Ahring (14) reported that the addition of H_2-utilizing methanogenes to disintegrating granules increases the degradation rate of both propionate and butyrate. Accordingly, the enrichment of active hydrogenotrophic methanogens will enhance the degradation efficiency of intermediate volatile fatty acids (VFAs) in an anaerobic system; however, few studies have focused on the development of efficient anaerobic processes by enriching hydrogenotrophic methanogens (15,16). Therefore, determining the activity of hydrogenotrophic methanogens, which can be used to indicate the regulating capacity of H_2 partial pressure by this type of methanogens in reactors, is very essential.

Numerous criteria can be used for evaluating microbial activities of different microbial groups in AD process; however, few studies have focused on determining the activity of hydrogenotrophic methanogens (13,17). Gijzen et al. (18) proposed a hydrogenotrophic methanogenic activity test that uses formic acid as substrate. However, the consumption rate of formic acid is unable to reflect H_2 utilisation potential of hydrogenotrophic methanogens directly. In addition, the test procedure was not elucidated and factors that influence the hydrogen utilization rate (HUR) test were not discussed. Leu et al. (19) developed a kinetic model based on the double Monod relationship for describing H_2/CO_2 utilization rate by the methane-producing archaea FJ10. Although model

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TABLE 1. Average operational data of the anaerobic systems in the study period evaluated in the survey.

		Treatment process	pH	Working temperature (°C)	Flow rate (m^3/d)	Reactor volume (m^3)	MLVSS (g/L)	COD_{inf} (mg/L)	COD_{eff} (mg/L)	HRT (h)	Nv (gCOD/(L· d))	SRT (d)
Lab-scale CSTR (with glucose as the sole carbon source)		CSTR	7.2	35±1	0.0045	0.0045	12.92	4000	110	24	4.0	20.0
Industrial wastewater treatment facilities in Xi'an	GuoWei starch factory	UASB1	7.0	35±1	750	1300	20.0	8000	1000 − 1200	41.5	4.62	27.0
	Hans brewery	UASB2	7.1		2000	820	17.30	1800	500 − 700	10 − 12	4.39	36.0
	Wan Long paper mill	UASB3	7.3		1400	300	13.07	1000	300	15	3.33	/
	Xi'an Coca-Cola Beverages Co.	UASB4	7.1		400	240	15.97	1200	350	10 − 15	5.0	/

prediction agrees well with the experimental value, determination and optimization of parameters, as well as the subsequent model solution and validation are relatively difficult and time-consuming to conduct. Coates et al. (20) developed an assay method by detecting manometric change of headspace pressure for measuring hydrogenotrophic methanogenic activity of anaerobic sludge. Although this manometric test is easy to conduct, it is not sufficiently accurate for reflecting actual activity of hydrogenotrophic methanogens because balance between the dissolution and evacuation of CO_2 cannot be controlled. Bicarbonate in the inoculums may contribute to surplus of CO_2 during the experiment. Moreover, the residual organics in inoculums will also produce excess CO_2, thus resulting in changes in the liquid−gas equilibrium of CO_2 in the test vials (13,21). Therefore, a simple and more reliable test approach for hydrogenotrophic methanogenic activity should be developed.

In the present study, a volumetric test device and a test procedure were developed for measuring HUR of anaerobic sludge. The influences of HUR on specific methanogenic activity (SMA) and reactor performance were also discussed.

MATERIALS AND METHODS

Sludge samples Sludge samples were respectively from five different anaerobic reactors. A summary of the compiled average operational data for the study period is shown in Table 1. To enable the residual matrix to achieve complete consumption, portions of all sludge samples were elutriated with oxygen-free water and reacclimatized for 8 h−12 h at 35°C prior to HUR and SMA determination.

The experimental HUR test device A new volumetric experimental device, shown in Fig. 1, was designed for monitoring HUR of anaerobic systems. The reactor was made of glass and had a volume of 1.0 L. A higher height-to-diameter ratio (H/D: 3) was selected to maintain highly efficient H_2 transmission. Liquid agitation was achieved by employing a magnetic stirrer and an H_2 supplement that uses a gas lift system by recirculating headspace gas through a peristaltic pump. To make pressure within the reactor in equilibrium to atmospheric pressure, a micromanometer was used to monitor gas pressure in the vial headspace and inert gas N_2 served as the balance gas. The reactor sealing test was conducted before HUR testing.

HUR test procedure Using the device shown in Fig. 1, the detailed experimental procedures are as follows. Firstly, an appropriate sludge sample with a buffer solution was deposited into the reactor, and liquor pH was maintained at approximately 7.0. The buffer solution contained 0.2 g NH_4Cl, 0.08 g KH_2PO_4 and 2.0 g $NaHCO_3$ per litre of oxygen-free water. The reactor was sealed with gas-tight rubber septa. Secondly, an appropriate volume of pure H_2 gas was introduced into the reactor to replace the supernatant buffer solution. The peristaltic pump was opened to cycle H_2 gas continuously in the reactor. Meanwhile, the magnetic stirrer was turned on to launch the test. To balance gas pressure in the reactor headspace with atmospheric pressure, a U gauge was used to monitor pressure within the reactor and inert gas N_2 served as a balance gas. Since the test was initiated, headspace gas was sampled regularly (once per 0.5 h) and analyzed by gas chromatography (GC). At the end of the HUR test, the amount of biomass present in the reactors was quantified in terms of volatile suspended solids (VSS) by ashing the sludge pellet obtained via gravimetric method (22).

HUR calculation The decrease in H_2 concentration obtained by GC could be converted into HUR with a unit of mL-H_2/(gVSS·h) using Eq. 1 and a unit of gCOD/(gVSS·d) using Eq. 2, as follows:

$$HUR' = \frac{dc_{H_2}}{dt} \times V \times \frac{1}{X} \tag{1}$$

$$HUR = 0.633 \times 24 \times HUR' = 0.633 \times 24 \times \frac{dc_{H_2}}{dt} \times \frac{V}{X} \tag{2}$$

where dc_{H_2}/dt is the H_2 consumption rate in the reactor (h^{-1}), V is the headspace volume (mL), X is the total biomass in the reactor (gVSS) and 0.633 is the conversion coefficient of hydrogen to oxygen at 35°C.

Gas analyses H_2 and CH_4 were measured using an Agilent gas chromatograph (Agilent 6890N GC, Agilent Technologies, CA, USA), equipped with a TDX-01 packed column (2 m × 0.3 mm) and a thermal conductivity detector (TCD). The inert gas argon was selected as the carrier gas at a flow rate of 49.9 mL/min. The column, injection port and detector temperatures were 100°C, 120°C and 160°C, respectively. The headspace gas in the reactor was sampled using a 500 μL pressure-lock syringe (Unimetrics, CA, USA), followed by direct injection into the column through a septum. The gas volume percentage (C_i) was got from the data-processing software of the GC. H_2 partial pressure was calculated according to the following equation:

$$p_{H_2} = 101325 \times C_{H_2} \tag{3}$$

SMA test To examine the specific maximum anaerobic uptake rate of diverse substrates for generating CH_4, the SMA test was conducted in 250 mL serum bottles at 35 ± 1°C under anaerobic conditions.

The sludge concentration of the serum bottle was approximately 5 gVSS/L. Acetate, propionate and butyrate were used as the substrate for anaerobic microbes generating CH_4, and the initial concentration was prepared in 4000 mg/L. Prior to addition into the test bottles, the substrate solution was adjusted to approximately pH 7.0. CH_4 production was measured at a regular time interval (once every 1 h) after

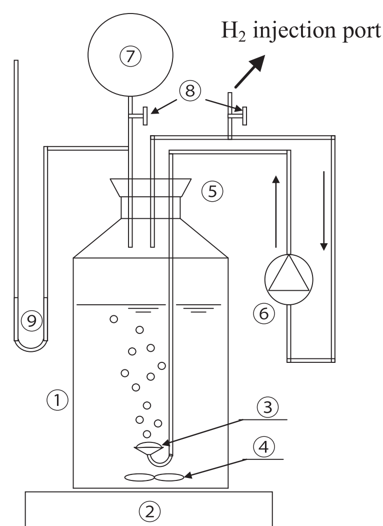

FIG. 1. Schematic diagram of the HUR test device: 1, serum bottle; 2, magnetic mixer; 3, gas diffuser; 4, rotor; 5, sealing plug; 6, peristaltic pump; 7, balance gas bag (N2); 8, valve; 9, micromanometer. The volumetric experimental device was designed for monitoring HUR of anaerobic system.

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