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RESEARCH ARTICLE

Fermentative hydrogen production from beet sugar factory wastewater treatment in a continuous stirred tank reactor using anaerobic mixed consortia

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Abstract A low pH, ethanol-type fermentation process was evaluated for wastewater treatment and bio-hydrogen production from acidic beet sugar factory wastewater in a continuous stirred tank reactor (CSTR) with an effective volume of 9.6 L by anaerobic mixed cultures in this present study. After inoculating with aerobic activated sludge and operating at organic loading rate (OLR) of 12 $kgCOD \cdot m^{-3} \cdot d^{-1}$, HRT of 8h, and temperature of 35°C for 28 days, the CSTR achieved stable ethanol-type fermentation. When OLR was further increased to 18 $kgCOD \cdot m^{-3} \cdot d^{-1}$ on the 53rd day, ethanol-type fermentation dominant microflora was enhanced. The liquid fermentation products, including volatile fatty acids (VFAs) and ethanol, stabilized at 1493 $mg \cdot L^{-1}$ in the bioreactor. Effluent pH, oxidation-reduction potential (ORP), and alkalinity ranged at 4.1–4.5, −250–(−290) mV, and 230–260 $mgCaCO_3 \cdot L^{-1}$. The specific hydrogen production rate of anaerobic activated sludge was 0.1 $L \cdot gMLVSS^{-1} \cdot d^{-1}$ and the COD removal efficiency was 45%. The experimental results showed that the CSTR system had good operation stability and microbial activity, which led to high substrate conversion rate and hydrogen production ability.

Keywords fermentative hydrogen production, continuous stirred tank reactor (CSTR), specific hydrogen production rate, beet sugar factory wastewater, ethanol-type fermentation

1 Introduction

Fossil fuels (i.e., petroleum, natural gas, and coal), which meet most of the world's energy demand today, are being depleted fast [1,2]. Also, their combustion products are causing the global problems, such as the greenhouse effect, ozone layer depletion, acid rain, and pollution, which are posing great danger to our environment and eventually to all lives in our planet [3,4]. Most of scientists think that the solution to these global problems would be to replace the existing fossil fuel system by the hydrogen energy system [5,6]. Hydrogen is a high-value industrial commodity with a wide range of applications. It is a very efficient and clean fuel. No greenhouse gases, no ozone layer depleting chemicals, no acid rain ingredients, and no pollution produce from the combustion of hydrogen. It can be converted into electricity via fuel cells or directly utilized in internal combustion engines [7]. It can also be used for the synthesis of ammonia, alcohols and aldehydes, as well as for the hydrogenation of edible oil, petroleum, coal and shale oil. Generally, there are four available basic processes for the production of hydrogen from non-fossil primary energy sources: 1) water electrolysis; 2) thermochemical process; 3) radiolytic process; and 4) biologic process. Among these various hydrogen production processes, The biologic hydrogen production process is environmentally friendly, cost-effective, and sustainable [8,9].

The anaerobic digestion process has been used for years for energy production and waste treatment [10,11]. During the acidogenesis of organic wastes, hydrogen, carbon dioxide, volatile fatty acids (VFA), and sometimes alcohols, are simultaneously produced. The feasibility of applying acidogenesis of organic wastes to produce hydrogen has been widely demonstrated at various laboratories. As the anaerobic fermentative hydrogen production process plays the dual role of waste reduction and energy production, it has been drawing growing attention in recent years [12,13].

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On the other hand, the beet sugar factories generate large volumes of high strength wastewater that is of serious environmental concern. The main component of sugar factory wastewater is molasses which has a high commercial value due to its use as a carbon source in various fermentations. The wastewater is characterized by extremely high chemical oxygen demand (COD) (5000–10000 mg·L^{-1}) and biochemical oxygen demand (BOD_5) (4000–7000 mg·L^{-1}), apart from low pH, strong odor and dark brown color. Their free disposal presents a serious challenge to the natural ecosystem and can cause considerable environmental problems [14]. Due to the high BOD_5 of the wastewater, application of anaerobic treatment technology has been reported to be highly effective [15,16]. However, the strongly acidic wastewater will inhibit the methanogenic activities due to their high pH sensitiveness. So it is difficult to achieve satisfying treatment efficiency [17].

A low pH, ethanol-type fermentation process is one of the most successful dark-fermentation methods for producing hydrogen gas from sugars [18]. High organic load and the availability of large quantity of wastewater may be considered as potential sources for biohydrogen production by anaerobic fermentation. So acidic beet sugar factory wastewater is a kind of ideal substrate for anaerobic fermentative hydrogen production. However, so far little information is available regarding simultaneous biohydrogen production and wastewater treatment using beet sugar wastewater in the literature. Thus the purpose of this study is to investigate the characteristics of simultaneous H_2 production and wastewater treatment utilizing beet sugar wastewater by continuous experiments using mixed acidogenic culture. In this communication, the feasibility of employing a continuous stirred tank reactor (CSTR) and the individual effects of operating parameters on the hydrogen production of sugar factory wastewater were collectively evaluated.

2 Materials and methods

2.1 Seed sludge

The reactor was inoculated with excess sludge taken from a secondary settling tank in harbin beer wastewater treatment plant. The ratio of mixed liquor volatile suspended solid (MLVSS) to mixed liquor suspended solid (MLSS) was 0.65 in the inoculated sludge. The sludge concentration of the CSTR system after inoculation was 4.90 gMLVSS·L^{-1}.

2.2 Experimental set-up

The continuous fermentative bio-producing hydrogen reactor used in this study is a patent continuous flow stirred-tank reactor (Fig. 1). It was constructed from 10 mm thick transparent Perspex. The cubage of model reactor was 16 L and the effective volume was 9.6 L. The temperature was automatically maintained at 35±1°C. The influent flow rate was controlled by a feed pump to regulate the HRT and organic loading rate (OLR) in the reactor. The evolved biogas was collected and led into a waterlock. The biogas volumes were measured using a wet gas meter (Model LML-1, Changchun Filter Co., Ltd.). The waterlock and wet gas meter were filled with water at pH 3 to prevent dissolution of the biogas.

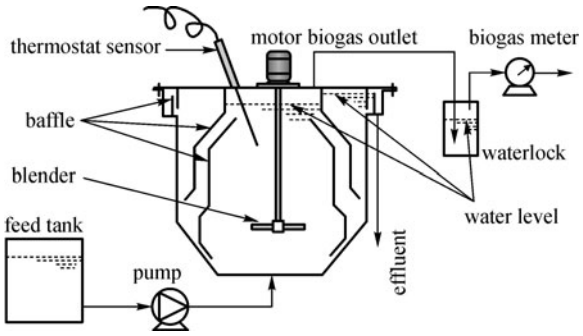

Fig. 1 Schematic diagram of the continuous stirred-tank reactor system

2.3 Feed and medium composition

Beet sugar wastewater, which was used in this investigation, was obtained from botian sugar refinery, and its characteristics are given in Table 1. The raw wastewater shows the following characteristics: COD 6300 mg·L^{-1}; total nitrogen (TN) 53.23 mg·L^{-1}; total phosphorus (TP) 4.77 mg·L^{-1} and pH 5.0. Raw wastewater was diluted by water to a COD of 4000 mg·L^{-1}, with a CODT:NT:P ratio of 100:10:1 to supply microorganisms with adequate

Table 1 Composition of the normal molasses

component	percentage/%
dried materials	78–85
total sugar	48–58
TOC	28–34
TKN	0.2–2.8
P_2O_5	0.02–0.07
CaO	0.15–0.8
MgO	0.01–0.1
K_2O	2.2–4.5
SiO_2	0.1–0.5
Al_2O_3	0.05–0.06
Fe_2O_3	0.001–0.02
ash content	4–8

nitrogen and phosphorus. The following nutrients were added as supplements to provide essential trace elements and nutrient for H_2 producing consortia in bioreactors, respectively: 1.25 $g \cdot L^{-1}$ $NaHCO_3$, 2.5 $g \cdot L^{-1}$ NH_4Cl, 0.25 $g \cdot L^{-1}$ KH_2PO_4, 0.25 $g \cdot L^{-1}CaCl_2$, 0.032 $g \cdot L^{-1}$ $NiSO_4$, 0.32 $g \cdot L^{-1}$ $MgSO_4 \cdot 7H_2O$, 0.02 $g \cdot L^{-1}$ $FeCl_2$, 0.0144 $g \cdot L^{-1}$ $Na_2MoO_4 \cdot 2H_2O$, 0.023 $g \cdot L^{-1}$ $ZnCl_2$, 0.021 $g \cdot L^{-1}$ $CoCl_2 \cdot 6H_2O$, 0.01 $g \cdot L^{-1}$ $CuCl_2 \cdot 6H_2O$, 0.03 $g \cdot L^{-1}$ $MnCl_2 \cdot 4H_2O$, 0.05 $g \cdot L^{-1}$ yeast extract and 0.5 $g \cdot L^{-1}$ cysteine. The pH and alkalinity of the feeding solution were adjusted to 7.0 and 270 $CaCO_3$ $mg \cdot L^{-1}$ by $NaHCO_3$ powder, respectively.

2.4 Analytical methods

COD, MLVSS, pH, alkalinity and oxidation-reduction potential (ORP) were measured according to standard methods of EPA [19]. The hydrogen content was analyzed by a gas chromatogram (Agilent 4890D, GC, USA) with a thermal conductivity detector (TCD) and a 2 m stainless column packed with Porapak TDS201 (60/80 mesh). The concentrations of VFAs (acetic acid, propionic acid, butyric acid and valerate acid) and the ethanol were measured using another gas chromatograph (Agilent 4890D, GC, USA) with a flame ionization detector (FID) and a 2 m stainless column packed with Porapak GDX103 (60/80 mesh). Photomicrographs of dominant bacteria were taken by a scanning electron microscopy (SEM) (Feiquanta-200, FEI, USA).

3 Results and discussion

3.1 Acidogenic fermentation treatment of beet sugar wastewater

After inoculation with selectively enriched mixed consortia, the bioreactor was operated with beet sugar wastewater at OLR of 12 $kgCOD \cdot m^{-3} \cdot d^{-1}$ and temperature of 35°C by adjusting the influent pH value to 7. 23 days later, the CSTR system reached to a stabilized state, then the reactor was operated at higher organic loading rate of 18 $kgCOD \cdot m^{-3} \cdot d^{-1}$ for 17 days. The bioreactor presents satisfactory operation efficiency on COD removal rate as depicted in Fig. 2. In the initial days of the start-up period, the COD removal efficiency was higher due to the activity of inoculated aerobic activated sludge and the absorption of sludge floc. The average COD removal efficiency was 9.3% in the first 5 days and then gradually increased to 40.3% in 6–28 days. The reactor registered a maximum COD reduction of 43% under stable conditions after 28 days. At higher OLR (18 $kgCOD \cdot m^{-3} \cdot d^{-1}$) the system documented a maximum COD removal efficiency of 45% in the CSTR system during this phase of stable operation.

Beet sugar wastewater consists of a variety of sugars, mainly sucrose, which can be converted to methane in a traditional anaerobic wastewater treatment process by a sequence of four reaction steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. After the hydrolysis of complex sugars to simple sugars, the further degradation is known to proceed through simultaneous steps by rapidly growing and pH-insensitive acidogenic bacteria to organic acids (butyric, propionic, and acetic acids), carbon dioxide and hydrogen. In the next step, slowly growing and pH sensitive acetogenic bacteria further oxidize the higher acids to acetic acid, carbon dioxide and hydrogen. Methanogenesis involves the reduction of carbon dioxide to methane, using hydrogen, by relatively fast growing pH sensitive autotrophic bacteria. Methanogens also catalyze the reduction of acetic acid to methane. However, In the CSTR system where acidogenic bacteria were dominant, COD was removed through the cytogenesis and gas releases (mainly CO_2 and H_2), while a significant amount of COD was converted to liquid intermediate products (e.g., ethanol, acetate, butyrate, and propionate) and stayed in the system [20]. Therefore, COD removal efficiency in this system was lower than traditional anaerobic process.

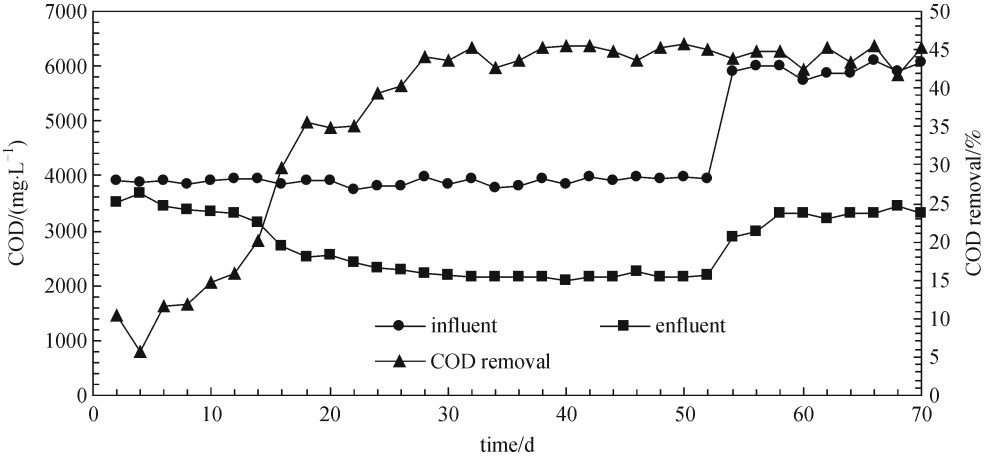

Fig. 2 COD and COD removal rate in the CSTR system

3.2 Biogas and biohydrogen production

The hydrogen yield and specific hydrogen production rate have generally been considered as the important indices to evaluate the bio-hydrogen producing processes [21]. Figure 3 showed the results of biogas and hydrogen yields in the process of startup and sludge acclimatization. Because activated sludge was in this phase of adjusting and acclimatizing itself to the inner environment of the reactor, the biogas productivity and hydrogen content were low at the beginning of the startup (the first three days). As the operation time went on, the activated sludge acclimatized gradually and the biogas productivity increased. When the sludge acclimatization was done by the 28th day, the biogas productivity and hydrogen content reached to stability. The biogas kept at 8.0–10.0 $L \cdot d^{-1}$ in the CSTR system and correspondingly the hydrogen content was 4.5–6.0 $L \cdot d^{-1}$. When the OLR of the system was improved by the 53rd day, the biogas productivity reached to 20.7 $L \cdot d^{-1}$ and the hydrogen content increased to 10.8 $L \cdot d^{-1}$. The produced biogas was found to consist of hydrogen and carbon dioxide, and free of methane. It is apparent from the experimental data that the OLR has shown significant influence on both H_2 production and substrate removal (Fig. 2). The differences in hydrogen production rate can be attributed to the differences in the microbial population and OLR [22]. During the stable operation, the reactor demonstrated stable performance with respect to biogas production and substrate degradation. This indicated that the beet sugar factory wastewater participated as primary carbon source in metabolic reactions involving molecular H_2 generation in this present study.

3.3 Biohydrogen production process evaluation

Several parameters such as VFAs, pH, ORP and alkalinity were investigated in this present study for evaluating the performance of this fermentative bio-hydrogen production process (Figs. 4 and 5).

Figure 4 illustrates the variation in effluent VFAs and ethanol and the relationship between pH and liquid end products concentrations in the CSTR system during the bioreactor operation. The constituents of liquid fermentation products significantly changed before the 28th day. The variation of liquid products suggested that the system had undergone a transition of fermentation types. An obvious fermentation phenomenon occurred in the CSTR system after the bioreactor startup. The concentrations of ethanol, acetic acid, propionic acid, butyric acid and valerate acid were 40.2, 333.2, 148.5, 202.9 and 0.7 $mg \cdot L^{-1}$, respectively, which indicated mixed-acid type fermentation happened, meanwhile the hydrogen production was inactive (Fig. 3). Among these VFAs, acetic acid was a major metabolite formed during H_2 production. On the 13th day, the concentrations of ethanol, acetic acid, propionic acid, butyric acid and valerate acid changed to 168.7, 327.5, 107.1, 283.9 and 36.8 $mg \cdot L^{-1}$, respectively. The total amount of acetic acid and butyric acid was 611.0 $mg \cdot L^{-1}$, 67% of total liquid products, which was typical butyric acid fermentation [18,23]. It indicated that the butyric acid type fermentation microbe community took the dominant position during the operation. On the 28th day, the CSTR system reached a stabile stage, with the concentrations of ethanol, acetic acid, propionic acid, butyric acid and valeric acid of 448.2, 436.4, 254.4, 105.5 and 27.2 $mg \cdot L^{-1}$, respectively. The total amount of ethanol and acetic acid was 884 $mg \cdot L^{-1}$, 69% of the total liquid products, which can be attributed to ethanol-type fermentation [18,23]. When the OLR was further increased on the 53rd day, the total concentration of end liquid products was increased correspondingly, but the concentrations of ethanol and acetate took up an average ratio of 76% of the total liquid end products. It indicated that the ethanol type fermentation microbe community had

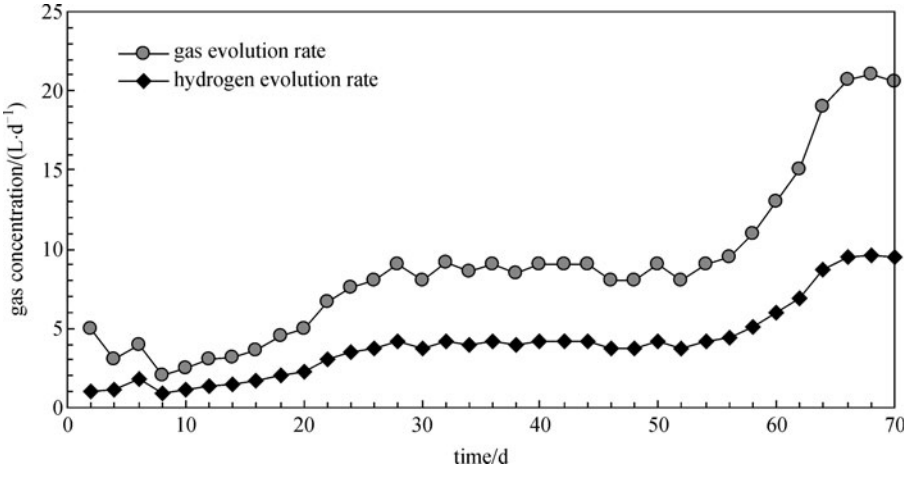

Fig. 3 Biogas and hydrogen yields in the CSTR system

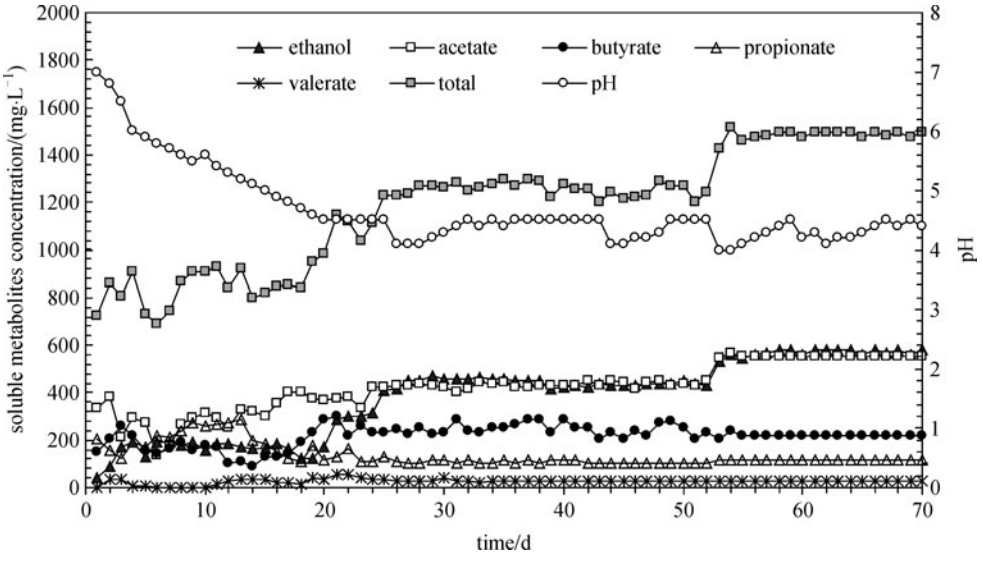

Fig. 4 Variation of soluble metabolites concentrations in the CSTR system

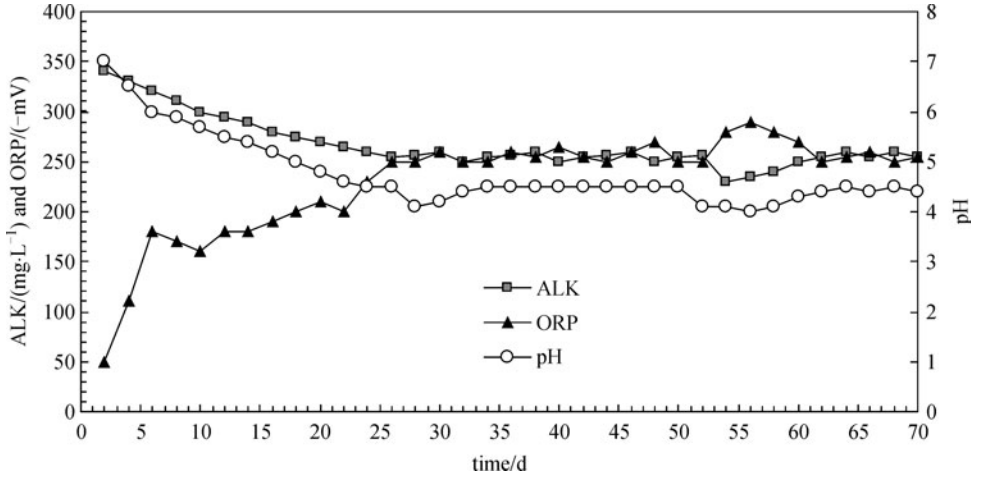

Fig. 5 Variation of pH, ALK and ORP in the CSTR system

been fully established in the hydrogen bio-producing process.

Ethanol-type fermentation process is one of the most successful dark-fermentation methods for producing hydrogen gas from sugars [18,23,24]. This process differs from traditional butyrate-type fermentation of clostridia due to the simultaneous production of high concentrations of acetic acid and ethanol. In addition, the low pH fermentation results in the reduced concentrations of propionic acid in comparison with near neutral pH conditions [21]. Ethanol-type fermentation had higher hydrogen production ability than mixed acid-, butyric acid-, and propionic acid- type fermentations [24], and hydrogen production has been found to occur at pH of 4.0–4.5. Thus, this result suggested that ethanol-type fermentation process is convenient for bio-hydrogen production from acidic beet sugar wastewater treatment.

Figure 5 showed the changes in the pH, ALK and ORP in the CSTR. The changes of the three factors affected not only the anaerobic hydrogen production ability, but also the microbial community and fermentation types. It was found that the bioreactor underwent significant variations of pH, alkalinity and ORP in the first 28 days, which was concurrent with the fluctuation of hydrogen and liquid fermentation productions (Figs. 3 and 4). The pH dropped from 7 on the first day to 4.1 on the 28th day. After 28 days, the pH of the bioreactor stabilized at 4.0–4.4. It was evident that typical anaerobic mixed cultures could not produce H_2 as it was an intermediate for methane formation, and was rapidly consumed by methane-producing bacteria [25]. Most effective ways to enhance H_2 production from the anaerobic culture is to restrict or terminate the methanogenesis process by allowing H_2 to become an end product in the metabolic flow. The

experimental data illustrated that the biogas was composed of hydrogen and carbon dioxide and free of methane (data not shown) in this present study. It can be concluded that the low effluent pH suppressed the methanogenic activity. Methanogenic population in the anaerobic inoculum may be inhibited/killed due to the persistent acidophilic microenvironment maintained during the reactor operation.

Similar to the pH pattern, the alkalinity of the bioreactor dropped from 340 $mg \cdot L^{-1}$ on the first day to 250 $mg \cdot L^{-1}$ on the 28th day. After 28 days operation, the alkalinity of the bioreactor stabilized at 250–270 $mg \cdot L^{-1}$. When the OLR was further increased on the 53rd day, the ALK of the bioreactor still ranged from 230 to 260 $mg \cdot L^{-1}$. Alkalinity is a key parameter that influences greatly on the stability and hydrogen yields of biohydrogen production reactor [24]. The mixed liquor pH in an anaerobic system was determined by volatile fatty acids (VFAs) concentration and alkalinity. Because alkalinity was affected by the balance between $[CO_2]$ and $[HCO_3^-]$, and the majority of alkalinity was $[HCO_3^-]$ at pH lower than 5, low pH and alkalinity were expected at high VFA concentration due to the consumption of HCO_3^-. After 15 days, both biogas production (Fig. 2) and VFAs (Fig. 4) increased, indicating anaerobic bacteria had adapted to the CSTR system. With more CO_2 being produced, $[HCO_3^-]$ became higher and alkalinity increased correspondingly. A higher alkalinity enhanced the system neutralization capability for VFAs and led to a stable pH value. Thereby, pH can be stabilized at 4.0–4.5 even though more VFAs were produced after the 53rd day when OLR was increased in the CSTR system.

As for ORP values, it dropped from −50 mV on the 1st day to −250 mV on the 28 th day in the bioreactor, and then ranged from −250 to −290 mV during the operation. ORP values were mainly affected by pH in an anaerobic system. It could be seen from Fig. 5 that ORP was inversely related with pH in most cases, with low ORP corresponding with high pH.

3.4 Microbiology and biomass concentration

SEM images (×3.0 K; Fig. 6) of the anaerobic mixed culture acquired from experiments visualized slightly bent, scattered and short chain rods. Images of mixed consortia showed the proliferation of morphologically similar group of bacteria. The selective enrichment procedure adopted in this study might result in the enrichment of specific group of bacteria capable of producing H_2.

Figure 7 shows the evolution of the sludge concentration in the fermentative bio-hydrogen production reactor. In the start-up period, the sludge concentration increased with time. The biomass reached at 9.99 $gMLVSS \cdot L^{-1}$ on the 28th day in the CSTR system. The increase in sludge concentration was due to the efficient anaerobic operating conditions (pH, temperature and loading rate) for the anaerobic bacteria. When the OLR was further increased on the 53rd day, the sludge was washed out because of the hydraulic shock, thus the biomass concentration decreased

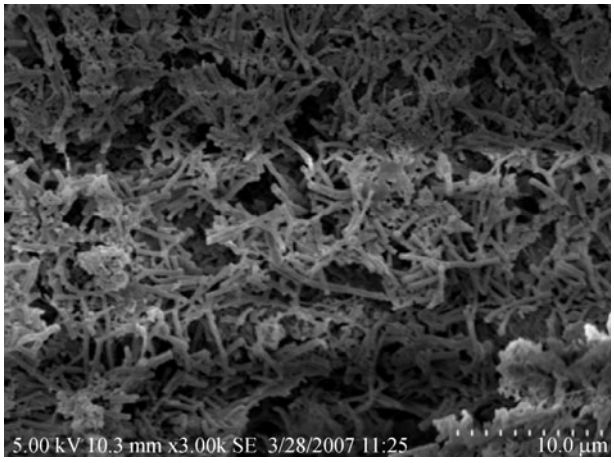

Fig. 6 Scanning electron microscopy of the suspended bacteria in the CSTR reactor (Magnification: × 3000)

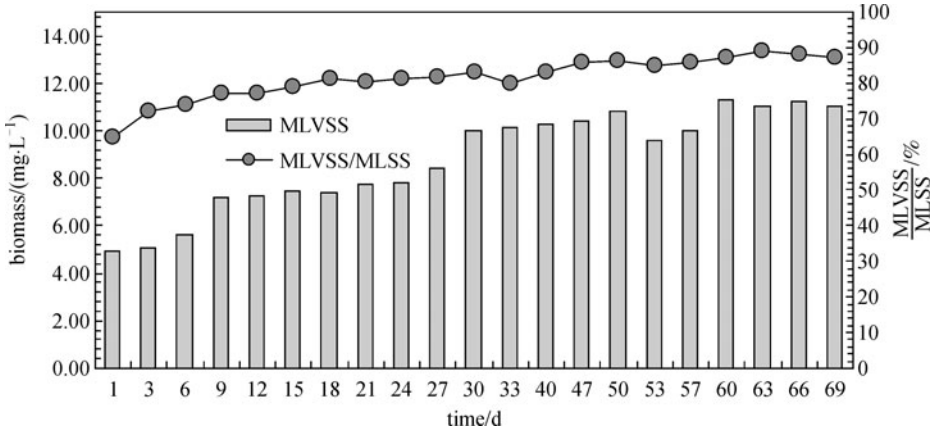

Fig. 7 the variation of biomass concentration (MLVSS) in the CSTR system

slightly. After a week, the MLVSS and MLVSS/MLSS stabilized at 11.2 $g \cdot L^{-1}$ and 88%, respectively. Based on the results at the stable period, the hydrogen productions were 10.8 $L \cdot d^{-1}$. Therefore, the specific hydrogen production rates was 0.100 $L \cdot gMLVSS^{-1} \cdot d^{-1}$ in the CSTR system.

4 Conclusions

The main objectives of this present study are to study feasibility of simultaneous bio-hydrogen production and wastewater treatment using beet sugar wastewater. The CSTR system reached stable ethanol-type fermentation after 28 days of acclimatization, when OLR was 12 $kgCOD \cdot m^{-3} \cdot d^{-1}$ and HRT was 8.0 h. During this period of stable operation, the reactor showed a stable COD removal efficiency of 43% and hydrogen production yields of 8.0–10.0 $L \cdot d^{-1}$ in the system. When the OLR was further increased to 18 $kgCOD \cdot m^{-3} \cdot d^{-1}$ on the 53rd day, the reactor also showed a COD removal efficiency of 45% and a specific H_2 production rate of 0.100 $L \cdot gMLVSS^{-1} \cdot d^{-1}$ in the system during the stable operation (60–70 days). Effluent pH, ORP, and alkalinity ranged from 4.0 to 4.5, −250 to −290 mV and 230–260 $mgCaCO_3 \cdot L^{-1}$, respectively. A low pH, ethanol-type fermentation process is an effective dark-fermentation method for producing hydrogen from acidic beet sugar factory wastewater.

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