Production of hydrogen in a granular sludge-based anaerobic continuous stirred tank reactor

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Abstract

An investigation on biohydrogen production was conducted in a granular sludge-based continuous stirred tank reactor (CSTR). The reactor performance was assessed at five different glucose concentrations of 2.5, 5, 10, 20 and 40 g/L and four hydraulic retention times (HRTs) of 0.25, 0.5, 1 and 2 h, resulting in the organic loading rates (OLRs) ranged between 2.5 and 20 g-glucose/L h. Carbon flow was traced by analyzing the composition of gaseous and soluble metabolites as well as the cell yield. Butyrate, acetate and ethanol were found to be the major soluble metabolite products in the biochemical synthesis of hydrogen. Carbon balance analysis showed that more than half of the glucose carbon was converted into unidentified soluble products at an OLR of 2.5 g-glucose/L h. It was found that high hydrogen yields corresponded to a sludge loading rate in between 0.6 and 0.8 g-glucose/g-VSS h. Substantial suppression in hydrogen yield was noted as the sludge loading rate fell beyond the optimum range. It is deduced that decreasing the sludge loading rate induced the metabolic shift of biochemical reactions at an OLR of 2.5 g-glucose/L h, which resulted in a substantial reduction in hydrogen yield to 0.36–0.41 mol-H₂/mol-glucose. Optimal operation conditions for peak hydrogen yield (1.84 mol-H₂/mol-glucose) and hydrogen production rate (3.26 L/L h) were achieved at an OLR of 20 g-glucose/L h, which corresponded to an HRT of 0.5 h and an influent glucose concentration of 10 g/L. Influence of HRT and substrate concentration on the reactor performance was interrelated and the adverse impact on hydrogen production was noted as substrate concentration was higher than 20 g/L or HRT was shorter than 0.5 h. The experimental study indicated that a higher OLR derived from appropriate HRTs and substrate concentrations was desirable for hydrogen production in such a granule-based CSTR.

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Keywords: Biohydrogen; Granular sludge; Organic loading rate; Hydraulic retention time; Substrate concentration; Sludge loading rate

1. Introduction

Currently, fossil fuel-dependent economy attracts worldwide concerns ranging from its sustainability to global warming. Hydrogen is a promising alternative to conventional fossil fuels because it has the potential to eliminate all of the problems that the fossil fuels create. However, a major doubt on hydrogen as a clean energy alternative is how it is produced. This is largely owing to the fact that most of the hydrogen gas at present is

currently generated from fossil fuels by thermochemical processes. Because of their sustainable and pollution-free properties, biological processes, such as biophotosynthesis, photodecomposition and anaerobic fermentation routes to harness hydrogen from renewable sources, i.e. water or organic compounds have lately received considerable attention [1]. Among these processes, anaerobic hydrogen fermentation seems to be more favorable, since hydrogen is yielded at a high rate with a concomitant reduction in environmental organic pollutants.

Most recent studies on anaerobic hydrogen fermentation focused on the improvement in systematic hydrogen yield and volumetric production rate. Studies on continuous hydrogen

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fermentation indicated that anaerobic hydrogen-producing cultures are greatly influenced by several operational parameters, such as temperature [2], pH [3], hydraulic retention time (HRT) [4], type of substrate [5] and substrate concentration [6] and organic loading rate (OLR) [7].

OLR is an important parameter dictating the system performance and operational cost of fermentative hydrogen production. As OLR is a function of HRT and influent substrate concentration (ISC), OLR, HRT and ISC are interrelated. However, previous studies on OLR evaluation were mostly carried out by changing one variable while keeping another consistent. The evaluation on HRT–OLR or ISC–OLR is hence incomprehensive, as contribution derived from other variables has been neglected. In fact, it had been reported that hydrogen yield increased consistently with the reduction in OLR, as HRT increased from 2 to 24 h at an ISC of 34 g-COD/L [8], while at a higher ISC of 50 g-COD/L, it increased initially with the HRT increasing from 8 to 18 h, but fell significantly beyond 18 h HRT [9]. It is likely that these contradictory conclusions resulted from the effect of other operational parameters rather than HRT alone, such as substrate concentration and the resulting OLR. Likewise, inconsistent results had been noticed on the effect of ISC on hydrogen production at a different HRT [6,10].

On the other hand, a variety of high-rate bioreactor systems, with a common property of enhanced biomass retention were developed in recent years. These included anaerobic fixed-bed reactor [11], anaerobic fluidized bed reactor [12], and upflow anaerobic sludge blanket (UASB) reactor [13]. Fang and his team [14,15] had successfully cultivated hydrogen-producing granular sludge in a continuous stirred tank reactor (CSTR). The granule-based CSTR exhibited higher hydrogen production rate (0.54 L/L h) than that of conventional CSTR (0.15 L/L h) [16], but the rate was much lower as compared to other immobilized-cell systems (0.93–7.33 L/L h) [11,12,17,18]. While Liu and Fang [15] had studied the effect of HRT and ISC on biohydrogen production of granular sludge at a consistent OLR level, the effect of OLR remained unclear. Furthermore, Van Ginkel and Logan [7] found that hydrogen yield increased with the reduction in OLR from 9.5 to 0.25 g-glucose/L h for suspended cells in chemostat reactors. Whether such an enhanced hydrogen yield could be achieved for immobilized cultures remains uncertain. It follows that a comprehensive evaluation of organic loading through systematically changing the HRT and ISC on the performance of granule-based CSTR is worth pursuing.

The aim of the present study is to investigate the influence of HRT, ISC and hence OLR on hydrogen production performance of granular sludge in a CSTR, which was conducted by examining different HRTs and substrate concentrations and associated OLRs.

2. Materials and methods

2.1. Inoculum and substrate

The seed sludge was originally obtained from a local wastewater treatment plant, which was acclimated with glucose

Fig. 1. Schematics of anaerobic continuous stirred tank reactor system.

(10 g-glucose/L) in an anaerobic CSTR at pH 5.5 for more than 2 months as reported in a previous study [4]. The granular sludge used in the current study was derived by a procedure of acid incubation on the acclimated seed sludge, which had been described in a separate study [19]. The substrate used for hydrogen fermentation consisted of glucose ranging from 2.5 to 40 g/L and nutrients at a constant carbon : nutrient ratio as described in previous studies [4,18].

2.2. Experimental setup and operation

A culture vessel (Biostat B, B. Braun Biotech, Germany) equipped with temperature and pH controllers was used as shown in Fig. 1. It had a working volume of 6 L with an internal diameter of 160 mm and a height of 345 mm. A level sensor and effluent peristaltic pump were used to control the culture volume at a constant level. The reactor was operated at a constant temperature of 37 °C and pH of 5.5 ± 0.05 . The reactor culture was mixed continuously at 160 rpm using two disc impellers, each having 6 flat blades ($\Phi = 64$ mm), which were located at a height of 20 mm above the reactor bottom. A three-phase separator was installed inside the CSTR to promote retention of granular sludge.

After starting up granule-based CSTR, 13 test runs were conducted in series to assess the influence of HRT, ISC and OLR on hydrogen production. The CSTR was operated at five different influent glucose concentrations (2.5, 5, 10, 20, 40 g/L) and four different HRTs (0.25, 0.5, 1, 2 h), resulting in OLRs ranging from 2.5 to 20 g-glucose/L h. The OLRs were computed from the concentration of glucose loaded into the reactor per unit HRT. Starting from an OLR of 20 g-glucose/L h which corresponded to an HRT of 0.25 h and an influent glucose concentration of 5 g/L, the reactor was operated by stepwise OLR reduction. The loading and operating conditions are shown in Table 1. Steady-state conditions were considered attained when the biogas production and glucose conversion rate were consistent within 5% for three consecutive days. Data were collected for a week after the steady-state conditions were reached for each test run.

Table 1
Loading and operating conditions

OLR (g-glucose/L h)	HRT (h)	ISC (g-glucose/L)
20	0.25	5
	0.5	10
	1	20
	2	40
10	2	20
	1	10
	0.5	5
	0.25	2.5
5	0.5	2.5
	1	5
	2	10
2.5	2	5
	1	2.5

2.3. Analytical methods

Biogas flow was measured by a wet gas meter (Ritter TG 05, Germany). The biogas composition was analyzed by a gas chromatography (Varian 4900, USA) equipped with a thermal conductivity detector. Hydrogen was analyzed using a Molsieve 5A Plot column with argon as carrier gas set at 60 °C; methane and carbon dioxide were analyzed using a Propac Q column with helium as carrier gas also set at 60 °C. Soluble metabolic products, viz. ethanol and volatile fatty acids (VFAs), including acetate, propionate, butyrate, valerate and hexanoate were determined by a gas chromatograph (Agilent 6890, USA) equipped with a mass selective detector (220 °C), an injector (200 °C) and a 30 m × 0.25 μm HP-FFAP fused-silica capillary column. The temperature of the oven was initially maintained at 60 °C for 4 min and then increased to 168 °C at a ramp of 6 °C/min and lastly heated to 200 °C at 10 °C/min and maintained for 2 min. Helium was used as the carrier gas. Glucose concentration was determined following the phenol–sulfuric acid method reported by Dubois et al. [20]. Total organic carbon (TOC) was measured by a TOC analyzer (Shimadzu VCSH, Japan). Measurements of volatile suspended solids (VSS) were performed in accordance with the Standard Methods [21].

3. Results and discussion

To examine the influence of HRT, ISC and OLR on hydrogen production of granular sludge, a series of experiments comprising 13 runs were conducted in the CSTR. Influence of the above variables on biogas composition, hydrogen yield, hydrogen production rate, reactor biomass retention and distribution of carbon flow was assessed. The HRT and the influent glucose concentration ranged from 0.25 to 2 h and 2.5 to 40 g/L, respectively, resulting in four different OLR levels from 2.5 to 20 g-glucose/L h.

Fig. 2. Influence of HRT, influent glucose concentration and resulting OLR on (a) hydrogen content, (b) hydrogen yield, and (c) hydrogen production rate.

3.1. Production of hydrogen

Fig. 2 depicts the variation of hydrogen content, hydrogen yield and hydrogen production rate with HRT, and indirectly with ISC under four OLR levels. The biogas produced consisted of hydrogen and carbon dioxide, whereas methane was undetectable throughout the study. As shown in Fig. 2a, hydrogen content was insensitive to the OLR. A general tendency, at a given OLR level, was observed such that hydrogen content decreased with increasing HRT and glucose concentration. For instance, the hydrogen content decreased from 64.1% to 51.2% at an OLR of 20 g-glucose/L h when the HRT was extended from 0.25 to 2 h, corresponding to an increase of glucose concentration from 5 to 40 g/L. The influence of ISC, at a given HRT, on biogas composition can be indirectly evaluated from Fig. 2a, showing that relatively consistent hydrogen content was achieved at each HRT. The hydrogen content varied slightly, generally less than 5% at each HRT as the influent glucose concentration changed. These results suggest that the effect of HRT on biogas composition was much more pronounced than that of the influent glucose concentration and OLR.

As depicted in Figs. 2b and c, a general trend was found such that hydrogen yield and hydrogen production rate decreased

Fig. 3. Hydrogen production rate as a function of OLR (solid line, $R = 0.89$ and $p < 0.0001$; dot line, $R = 0.99$ and $p < 0.0001$).

with the reduction in OLR from 20 to 2.5 g-glucose/L h. Hydrogen yield and hydrogen production rate reached the maximum values of 1.84 mol-H₂/mol-glucose and 3.26 L/L h, respectively, at the highest OLR level, but they both decreased drastically to 0.36–1.41 mol-H₂/mol-glucose and 0.12–0.14 L/L h, respectively, as the OLR was reduced to 2.5 g-glucose/L h. Hydrogen yield was sensitive to HRT, and hence to influent glucose concentration, while hydrogen production rate was less influenced by the HRT and glucose concentration at lower OLRs between 2.5 and 10 g-glucose/L h. At a higher OLR of 20 g-glucose/L h, a substantial reduction in hydrogen production was noted at an HRT of 2 h and a corresponding ISC of 40 g/L. This resulted in a lowered linear response of hydrogen production rate to the overall OLR ($R = 0.89$, possibility (p) < 0.0001) (Fig. 3). In contrast, a very high correlation coefficient ($R = 0.99$) was found existing between hydrogen production rate and OLR, ranging from 2.5 to 10 g-glucose/L h. These findings imply that hydrogen production rate might not essentially increase linearly with the OLR once the impact derived from OLR itself and its variables, i.e. HRT or substrate concentration has not been established.

The respective influence of HRT and ISC on hydrogen yield was assessed and plotted in Fig. 4. For a given HRT between 0.25 and 2 h, hydrogen yield increased with the glucose concentration from 2.5 to 10 g/L, whereas hydrogen yield was found to decrease substantially when influent glucose concentration increased to 20 g/L at 1 h HRT, and to 40 g/L at 2 h HRT. The adverse influence of higher ISC is thus indicated. As hydrogen yield reached the peak at 2 h HRT, the influent glucose concentration corresponded to 20 g/L; such a concentration had resulted in a significant decrease in hydrogen yield at 1 h HRT. These findings thus imply that the magnitude of substrate concentration to suppress hydrogen production was related to the HRT employed. The values obtained are generally in agreement with the reported inhibitory concentrations of 20 g-glucose/L [22] and 35 g-COD/L [6] which were noted at 1.1 h HRT and 12 h HRT conditions, respectively. The shocked

Fig. 4. Hydrogen yield with a matrix of HRT and ISC.

Fig. 5. Glucose conversion rate as a function of organic loading rate.

impact of higher substrate concentration on hydrogen production was probably relieved through extending the HRT. Likewise, the impact of substrate concentration has to be taken into consideration with respect to the influence of HRT on hydrogen yield. At low influent glucose concentrations between 2.5 and 5 g/L, hydrogen yield increased initially as the HRT was shortened from 2 to 0.5 h, and then substantially decreased at an HRT of 0.25 h. As with a glucose concentration of 10 g/L, hydrogen yield increased with the reduction in HRT, while the opposite result was obtained, if the glucose concentration was larger than 20 g/L.

From these experimental results, it is deduced that HRT and influent glucose concentration are interrelated and have an integrated impact on hydrogen yield. These findings could provide a new perspective to some current debatable claims. As indicated by previous studies on the HRT effect, with a relatively low substrate concentration of 20 g-glucose/L, hydrogen yield

Fig. 6. Photograph of granule-based CSTR and granules inside (scale bar =1 mm).

decreased consistently in an HRT range of 12–48 h [23], while at a substrate concentration of 37 g-COD/L, hydrogen yield increased with HRT, from 2 to 24 h HRT [8]. Likewise, with a more concentrated substrate (50 g-COD/L) hydrogen yield increased initially between 8 and 18 h HRT and then fell significantly beyond 18 h HRT [10]. In other studies concerning the effect of substrate concentration, Kim and his coworkers [6] found hydrogen yield to increase with increasing glucose concentration from 10 to 35 g/L at an HRT of 12 h, whereas at shorter HRTs of 2 h [10] and 4 h [8], hydrogen yield decreased consistently as the ISC increased from 6.9 to 27.4 g-glucose/L and 14 to 36 g-COD/L, respectively.

A substantial decrease in hydrogen production might be caused by a shorter HRT or a higher ISC. This could be attributed to deficient fermentation or occurrence of substrate/product inhibition during the hydrogen production [12,24]. Hence, appropriate control of HRT, substrate concentration and the associated OLR is crucial for hydrogen production in the granule-based CSTR system. Ignoring any of these parameters might result in an inferior performance of the biosystems. For example, an OLR of 20 g-glucose/L h was found most favorable for hydrogen fermentation in the present study, but such an optimum OLR condition might have been missed if only influent concentration were examined at a fixed HRT of 2 h.

The optimal operating conditions were found at the OLR of 20 g-glucose/L h, corresponding to 0.5 h HRT and 10 g/L influent glucose concentration where both hydrogen yield and hydrogen production rate reached the maximum values. Glucose conversion rate, however, was found to be $70.9 \pm 4.8\%$ at the highest OLR. In contrast, more than 98% glucose was utilized for OLR in a range of 2.5–10 g-glucose/L h (Fig. 5).

3.2. Biomass concentration

As shown in Fig. 6, a relatively consistent and compact granular sludge layer was formed, which occupied a space of 2.4–3 L under the OLR levels tested (Fig. 7a). The biomass concentrations were found between 19 and 34 g/L-VSS in the granular sludge layer, which was called as granular sludge layer concentration in the context. Extending the HRT resulted in a slight increase in the granular sludge layer concentration, so did glucose concentration. Much more granular sludge was accumulated with the increase in OLR (Fig. 7a). Over the granular sludge layer, fewer granules were suspended in the bulk liquid, with a concentration of 0.9–1.5 g-VSS/L. Therefore, the amount of biomass was mainly regulated by the granular sludge accumulated in the granular sludge layer.

Overall biomass retention in the reactor ranged from 46 to 96 g-VSS, and it was substantially correlated to the OLRs as shown in Fig. 7b, and the corresponding reactor biomass concentrations were in a range of 7.7–16.0 g-VSS/L. Compared to other granular sludge systems, such concentrations of granular sludge were comparable to the values (5.1 and 17.0 g-VSS/L) reported in column-type reactors [25,26], but lower than 20 g-VSS/L [14] and 35.8 g-VSS/L [27] obtained in CSTRs. The retention of biomass might be influenced by the reactor configuration and operational parameters, such as reactor mixing, impeller location, three-phase separator, HRT, OLR, etc. Reducing the stirring rate might increase the level and corresponding concentration of granular sludge layer to a certain extent, but it was not always feasible because the maintenance of culture mixing for those sensors had to be taken care of in such a CSTR equipped with pH and temperature sensors within the reactor. Improvement on reactor control modes may increase

Fig. 7. (a) Effect of HRT on biomass concentration (solid line) and volume of granular sludge layer (dot line). (b) Reactor biomass retention as a function of glucose loading rate ($p < 0.0001$).

the reactor performance. In fact, a biomass concentration up to 35.8 g-VSS/L was reported by Wu et al. [27] using a similar reactor with different control means of temperature and pH. In their study, operating temperature and pH were maintained consistently by external water bath and adding weak alkali buffer in feed, respectively. These might reduce the requirement on culture mixing to a certain extent and hence a much higher biomass concentration and a hydrogen production rate (15.1 L/L h) were achieved at a stirring rate of 120 rpm. Nevertheless, the maximum hydrogen production rate in the present study was 3.26 L/L h, considerably higher than those (0.15–2.36 L/L h) reported by others [10–12,18,22,25], as a result of the high biomass content in the mixed liquor.

3.3. Production of soluble fermentation products

Total concentration of VFAs and ethanol, and the proportions of the individual VFAs and ethanol expressed as the molar percentage of the total are summarized in Table 2. Major soluble products found were butyrate, acetate and ethanol,

Table 2
Soluble product distribution in effluent

HRT (h)	Glucose conc. (g/L)	OLR (g/L/h)	Total (mM)	Ethanol (%) ^a	Acetate (%)	Propionate (%)	Butyrate (%)	Valerate (%)	Hexanoate (%)	(Eth±AA) (%) ^b	BA/AA ^c
1	2.5	2.5	8.27 ± 0.64 ^d	18.67 ± 2.59	50.33 ± 1.75	– ^e	31.00 ± 4.33	–	–	69.00 ± 4.33	0.62 ± 0.11
2	5	2.5	16.54 ± 1.99	11.52 ± 1.70	38.54 ± 1.97	8.10 ± 0.41	37.68 ± 4.52	2.93 ± 0.38	1.26 ± 0.12	50.06 ± 3.65	0.98 ± 0.16
0.5	2.5	5	17.94 ± 0.39	12.72 ± 1.34	22.70 ± 0.72	1.94 ± 3.36	52.35 ± 1.63	7.81 ± 0.46	2.48 ± 0.55	35.42 ± 1.86	2.31 ± 0.06
1	5	5	30.56 ± 0.29	12.15 ± 0.21	22.99 ± 0.47	–	50.00 ± 0.62	6.18 ± 0.26	8.67 ± 0.27	35.14 ± 0.66	2.18 ± 0.07
2	10	5	72.96 ± 1.86	15.59 ± 0.84	22.23 ± 1.16	–	54.95 ± 0.94	2.94 ± 0.19	4.29 ± 0.27	37.82 ± 1.02	2.48 ± 0.16
0.25	2.5	10	18.38 ± 0.61	11.61 ± 0.76	19.59 ± 1.35	–	58.76 ± 1.51	7.62 ± 0.48	2.42 ± 0.56	31.20 ± 2.07	3.01 ± 0.29
0.5	5	10	31.99 ± 0.16	12.20 ± 0.1	22.91 ± 0.28	–	49.70 ± 0.21	6.12 ± 0.12	9.08 ± 0.19	35.11 ± 0.30	2.17 ± 0.03
1	10	10	68.49 ± 1.32	12.81 ± 0.19	25.87 ± 1.69	–	53.68 ± 1.61	3.07 ± 0.19	4.57 ± 0.11	38.68 ± 1.82	2.08 ± 0.20
2	20	10	159.57 ± 7.27	23.54 ± 0.26	16.80 ± 0.95	0.46 ± 0.40	52.63 ± 1.00	2.71 ± 0.24	3.84 ± 0.12	40.35 ± 0.70	3.14 ± 0.24
0.25	5	20	22.38 ± 0.22	22.77 ± 0.31	17.59 ± 0.07	–	36.98 ± 0.17	9.63 ± 0.48	13.03 ± 0.34	40.36 ± 0.25	2.10 ± 0.01
0.5	10	20	51.14 ± 2.31	12.94 ± 0.44	25.67 ± 0.71	–	51.00 ± 0.22	3.79 ± 0.16	6.60 ± 0.57	38.61 ± 0.58	1.99 ± 0.06
1	20	20	137.71 ± 1.62	25.99 ± 0.52	19.55 ± 1.23	0.58 ± 0.51	46.13 ± 2.06	3.08 ± 0.27	4.67 ± 0.16	45.54 ± 1.75	2.37 ± 0.24
2	40	20	190.16 ± 3.09	26.04 ± 0.79	13.73 ± 0.79	0.61 ± 0.02	52.82 ± 0.37	2.85 ± 0.16	3.95 ± 0.27	39.77 ± 0.24	3.86 ± 0.25

^aOf total products (mol/100mol).

^bMolar percentage of ethanol (Eth) and acetate (AA) over total products.

^cMolar ratio of butyrate (BA) to acetate (AA).

^dMean value ± standard deviation, $n = 3$ –6.

^eUndetectable.

accounting for molar percentages of 31.0–58.8%, 13.7–50.3%, and 11.5–26.0% of the total soluble metabolic products identified, respectively. Notably, propionate proportion was very low, and even undetectable in some runs. This is probably owing to the washout of propionate producers as a result of the hydrodynamic selection on the mixed populations during the period of cultivation as noted in a previous study [4]. The amount of overall metabolites increased significantly with the HRT and influent glucose concentration for a given OLR. At an OLR of 5 g-glucose/L h, for example, total metabolites reached 73.0 mM at an HRT of 2 h and an influent glucose concentration of 10 g/L. These decreased to 18.0 mM as the HRT and influent glucose concentration reduced to 0.5 h and 2.5 g/L, respectively. Butyrate was predominated over acetate and ethanol, with an average proportion of $50.8 \pm 5.6\%$ in a range of 37.0–53.7% at the OLRs larger than 2.5 g-glucose/L h, while acetate became more predominant (38.5–50.3%), followed by butyrate (31.0–37.7%) and ethanol (11.5–18.7%) at an OLR of 2.5 g-glucose/L h. These results implied that a shift of metabolic pathways might occur with the reduction of OLR to a certain extend (2.5 g-glucose/g-glucose/L h in the present study). It was interesting to note that the sum of acetate and ethanol was relatively consistent, with an average of 38.0% of the total metabolites as the OLR fell in the range of 5–20 g-glucose/L h. Also the ethanol proportion increased, corresponding to a decrease in acetate with increasing influent glucose concentration. This tendency was especially obvious as the influent glucose concentration was larger than 10 g/L.

High proportion of butyrate revealed that the microbial reaction continued after butyrate fermentation over the OLR range of 5–20 g-glucose/L h. *Clostridium* species are, therefore, considered to be predominant in the reactor cultures because these micro-organisms are responsible for the production of butyrate [26]. The ratio of butyrate/acetate is considered a predictor of microbial yield of hydrogen. In the present study, butyrate/acetate ratio was in the range of 2.1–3.9 at OLRs larger than 2.5 g-glucose/L h (Table 2), which was generally in agreement with values (2.1–5.9) reported in other studies [4,28–30]; the corresponding hydrogen yields (0.8–1.8 mol-H₂/mol-glucose) obtained in the present study were also in line with those reported between 0.7 and 1.9 mol-H₂/mol-glucose. Reducing OLR to a level of 2.5 g-glucose/L h resulted in a significant increase in acetate proportion, indicating that a metabolic shift might occur. As a result, an enhanced hydrogen yield would have been expected because hydrogen production from glucose would increase by one-fold when acetate, instead of butyrate is produced. However, the hydrogen yields were found otherwise. The underlying causes for the inferior yields will be discussed subsequently.

3.4. Carbon balance

A mass balance of carbon was analyzed for 13 runs as tabulated in Table 3. In a strict anaerobic hydrogen fermenter, there was no additional carbon source other than that derived from the substrate. The carbons from the nutrients and dissolved CO₂ in the effluent were ignored and thus they were not included in

the analysis. The composition of biomass was assumed to be C₅H₇O₂N [10,15]. The influent carbon was assumed to be converted into biomass, biogas (CO₂) and effluent TOC in terms of residual glucose, VFAs, ethanol, and soluble microbial products (SMPs). In the present study, SMPs were not monitored separately. The soluble effluent carbon was quantified on the basis of either the overall TOC or its specific effluent components as residual glucose, VFAs and ethanol. This results in two different stoichiometric equations as shown in Eqs. (1) and (2).

Recovery 1

$$= [(\text{effluent TOC}) / (\text{HRT}) + (\text{CO}_2 \text{ production rate}) \cdot (\text{carbon molecular weight}) / (24.45 \text{ L/mol}) + (\text{Effluent VSS}) \cdot (\text{carbon molar ratio}) / 1000] / (\text{OLR}) \quad (1)$$

Recovery 2

$$= [(\text{residual glucose}) / (\text{HRT}) + (\text{VFA} + \text{ethanol}) / (\text{HRT}) + (\text{CO}_2 \text{ production rate}) \cdot (\text{carbon molecular weight}) / (24.45) + (\text{Effluent VSS}) \cdot (5 \cdot \text{carbon molecular weight}) / (\text{carbon molar ratio}) / 1000] / (\text{OLR}). \quad (2)$$

As shown in Table 3, the recoveries of the carbon mass were relatively high according to Eq. (1) (Recovery 1) for the OLRs over 2.5 g-glucose/L h, ranging from 91.1% to 107.9%, while satisfactory recoveries (89.2–107.0%) were only found for OLR in a range of 5–20 g-C/L h based on Eq. (2) (Recovery 2). It was noted that the recoveries at some runs were larger than 100%, probably due to the deviation from the assumed culture formula. Recoveries of glucose carbon in a range of 41.4–47.7% obtained at the OLR of 2.5 g-glucose/L h indicated that unidentified products accounted for more than half of the total carbon flow. These unidentified products were probably contributed by the SMPs and other end products which were difficult to be detected by the present analytical methods [31].

Obviously, the carbon fate was dependent on the OLR. About 26–29% of the influent carbon was converted into fermentation products at the lowest OLR of 2.5 g-glucose/L h, while it increased to 65–79% and 47–66% at the OLRs of 5–10 and 20 g-glucose/L h, respectively. In addition, approximately 4–14% influent carbon was found in the form of biomass.

The effect of OLR on hydrogen yield had been investigated, and the OLR was correlated with hydrogen yield [7,18]. However, since hydrogen yield essentially represents the microbial conversion capability of hydrogen from organic materials, it is likely that biomass concentration affects the hydrogen yield. Sludge loading rate (SLR, g-glucose/g-VSS h) or the food to microorganism (F/M) ratio, defined as the amount of substrate applied to per unit mass of VSS per hour, is expressed as

$$\text{SLR} = \frac{Q S_0}{M_{\text{VSS}}} = \frac{Q S_0}{V X} = \text{OLR} \frac{1}{X},$$

where Q is flow rate (L/h), S_0 , glucose concentration (g/L); M_{VSS} , the amount of VSS (g), V , reactor volume (L); OLR, organic loading rate (g-glucose/L h); and X , biomass

Table 3
Carbon balance for hydrogen fermentation using granular sludge

HRT (h)	Influent glucose (g-C/L)	OLR (g-C/L h)	Effluent TOC (g/L)	CO ₂ production rate (L/L h)	Effluent VSS ^a (mg/L)	Residual glucose (g-C/L)	VFAs ± ethanol (g-C/L)	Recovery 1 (%)	Recovery 2 (%)
1	1	1	0.76 (76.1%) ^b	0.09 (4.6%)	196.67 (10.4%)	0.00 (0.4%)	0.26 (26.0%)	91.09	41.43
2	2	1	1.58 (79.1%)	0.16 (7.8%)	357.78 (9.5%)	0.02 (0.9%)	0.59 (29.6%)	96.39	47.73
0.5	1	2	0.74 (74.2%)	0.49 (11.9%)	265.56 (14.1%)	0.00 (0.5%)	0.73 (73.2%)	100.21	99.66
1	2	2	1.39 (69.3%)	0.70 (17.1%)	472.22 (12.5%)	0.06 (3.0%)	1.30 (64.8%)	98.91	97.41
2	4	2	3.00 (75.0%)	0.83 (20.4%)	261.83 (3.5%)	0.03 (0.6%)	2.94 (73.5%)	98.82	98.01
0.25	1	4	0.79 (78.5%)	1.16 (14.2%)	0.18 (9.5%)	0.02 (2.0%)	0.77 (77.2%)	102.23	102.91
0.5	2	4	1.43 (71.3%)	1.26 (15.4%)	348.15 (9.2%)	0.03 (1.4%)	1.36 (68.0%)	95.99	94.02
1	4	4	3.12 (78.1%)	1.36 (16.7%)	562.73 (7.5%)	0.39 (9.7%)	2.75 (68.8%)	102.28	102.65
2	8	4	6.46 (80.7%)	1.71 (21.0%)	933.33 (6.2%)	0.08 (1.0%)	6.30 (78.9%)	107.89	106.99
0.25	2	8	1.40 (70.0%)	1.52 (9.3%)	502.23 (13.3%)	0.57 (28.4%)	0.95 (47.7%)	92.72	98.69
0.5	4	8	3.21 (80.2%)	1.85 (11.3%)	615.63 (8.2%)	1.40 (35.0%)	2.08 (52.1%)	99.68	106.59
1	8	8	6.96 (87.1%)	2.22 (13.6%)	700.95 (4.7%)	1.86 (23.3%)	5.30 (66.3%)	105.31	107.81
2	16	8	13.87 (86.7%)	1.39 (8.5%)	1055.40 (3.5%)	4.80 (30.0%)	7.54 (47.2%)	98.70	89.16

^aBiomass composition was assumed to be C₅H₇O₂N [16].

^bCarbon distribution of effluent products over the influent carbon.

Fig. 8. Hydrogen yield as a function of sludge loading rate ($p = 0.003$).

concentration (g-VSS/L). Obviously, SLR is a function of OLR and biomass concentration. This understanding may help to regulate hydrogen fermentation. Fig. 8 illustrates that hydrogen yield was a function of SLR. In the present study, the optimum SLR for hydrogen yield was found in a range

of 0.6–0.8 g-glucose/g-VSS h. This is in agreement with the reported maximum hydrogen yield at SLR ranging from 0.8 to 1.2 g-glucose/g-VSS h [2,4,23]. There is a lack of agreement, however, with the work conducted by Van Ginkel and Logan [7], in which maximum hydrogen yield was achieved at an SLR of about 0.1 g-glucose/g-VSS h. The inconsistency indicates that the hydrogen yield might be influenced by culture conditions and other environmental factors.

Reducing OLR at a given amount of biomass, i.e. reducing SLR might result in the culture underfed and hence metabolic shift. It is well noted that influent substrate tends to be converted to energy for microbial maintenance, rather than to end products as the culture remains starved [32]. In fact, it was found that half of the glucose carbon, based on carbon balance analysis of individual products as discussed earlier, shift towards the formation of unidentified products which were unable to be detected by the current analytical methods. These unidentified products, which could be fermentation byproducts, such as ketones and aldehydes, or compounds produced, secreted and released by micro-organisms, were unfavorable or less favorable for hydrogen production [31]. From these experimental results, it was deduced that relatively low hydrogen yield obtained at an OLR of 2.5 g-glucose/L h was possibly attributed to the metabolic shift induced by the reduced SLR.

4. Conclusions

From the results obtained in the present study the following conclusions can be made.

Peak hydrogen yield and hydrogen production rate at a given OLR over a range of 2.5–20 g-glucose/L h corresponded to an increase of OLR. The optimum operating conditions were suggested at an OLR of 20 g-glucose/L h, corresponding to an HRT of 0.5 h and an ISC of 10 g-glucose/L, where both hydrogen yield and hydrogen production rate reached the maximum values of 1.84 mol-H₂/mol-glucose and 3.26 L/L h, respectively. In addition to OLR, hydrogen production is also influenced by HRT and ISC which were interrelated and had a combined influence on hydrogen yield. An appropriate control of HRT, substrate concentration and the associated OLR is crucial. Ignoring any of these parameters might result in an inferior performance of the biosystems. A substantial decrease in hydrogen production was noted as the reactor was operated at an HRT of 0.25 h at any other substrate concentration, or at an HRT of 1 h at 20 g-glucose/L, or at an HRT of 2 h at 40 g-glucose/L.

Butyrate, acetate and ethanol were the major soluble metabolite products. Butyrate was the predominant product over acetate and ethanol, with an average molar proportion of 50.8% of the total volatile fatty acids and ethanol at the OLRs larger than 2.5 g-glucose/L h, while acetate became predominant (38.5–50.3%) at an OLR of 2.5 g-glucose/L h. Carbon balance analysis showed that more than half of the glucose carbon was converted into unknown soluble products at an OLR of 2.5 g-glucose/L h. Metabolic shift of microbial reactions for hydrogen fermentation was likely to occur with the reduction of OLR to 2.5 g-glucose/L h. It was found that high yield of hydrogen coincided with a sludge loading rate of 0.6–0.8 g-glucose/g-VSS h, and substantial decrease in hydrogen yield occurred as the sludge loading rate fell beyond the range. It is deduced that decreasing the sludge loading rate induced the metabolic shift of microbial reactions at an OLR of 2.5 g-glucose/L h, which resulted in a substantial reduction in hydrogen yield to 0.36–0.41 mol-H₂/mol-glucose.

Formation of granular sludge significantly increased the overall reactor biomass concentration to as much as 16.0 g-VSS/L, which enabled the CSTR to operate at an OLR of up to 20 g-glucose/L h and hence enhanced the performance in hydrogen production. The granule-based CSTR in the present study was demonstrated to be a promising biosystem for hydrogen production.

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